



Forging New Frontiers

# AQUACULTURE AMERICA 2017

INTERNATIONAL CONFERENCE AND EXPOSITION

FEBRUARY 19 - 22, 2017

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# WELCOME

Welcome back to Texas for Aquaculture America 2017! We are delighted to welcome you to America's 7<sup>th</sup> largest city, San Antonio, Texas. We hope you enjoy the conference and trade show sponsored by the United States Aquaculture Society (USAS), National Aquaculture Association (NAA) and the Aquaculture Suppliers Association (ASA). Please take the time to get a flavor of Texas in this historic city where you'll find the Alamo just 3 blocks from the hotel!

The theme "Forging New Frontiers" celebrates U.S. aquaculture's enduring pioneer spirit and determination to push the boundaries by seeking new opportunities, creating new innovations, looking for expansion and being ready to take on necessary battles. This theme also requires us to look to the future of U.S. aquaculture – how is aquaculture perceived by younger generations, how will research be funded and how can we together create a sustainable legacy for aquaculture in the U.S. This path forward must be forged with knowledge, passion and science.

This year our Plenary speakers provide hints to the future of aquaculture from the perspective of the millennial generation, funding partnerships and understanding how we are communicating to others about aquaculture. Our first speaker represents the future of aquaculture as a recent Ph.D. graduate in Aquaculture Economics from the University of Arkansas at Pine Bluff, Dr. Jonathan Van Senten shares his insight as to how the millennial generation sees our industry and the path forward. Our second speaker, Dr. Sally Rockey, is the Executive Director of the Foundation for Food and Agriculture Research and provides a perspective on the future of private-public partnerships as a model for funding aquaculture research. Finally, Dr. Aoife Lyons, Alltech's Director of Educational Initiatives and Engagement, will talk about 'Communication across Generations... Implications for Sustainability'. She will explain how a better understanding of our work force, how we support our industry by funding innovative research and how we communicate the value of our products is essential to "Forging New Frontiers" in aquaculture.

Aquaculture America is the premier Aquaculture Convention in the United States and provides a forum to bring together research and industry, as well as 14 aquaculture organizations from around the country. This year's conference features a Federal Town Hall, producer meetings, diverse technical sessions, educational workshops, a comprehensive tradeshow (one of the largest ever), and numerous opportunities for students and others to network, job hunt and learn about the latest aquaculture research and technologies from around the world. The San Antonio Marriott Riverwalk is centrally located in the iconic Riverwalk District, making it an ideal location to visit with colleagues and friends. We hope you enjoy everything that San Antonio has to offer!

Dr. Michael Denson, Conference Chair  
Dr. Kathleen Hartman and Dr. David Straus, Technical Program Chairs  
Mr. Paul Zajicek, Industry Program Chair

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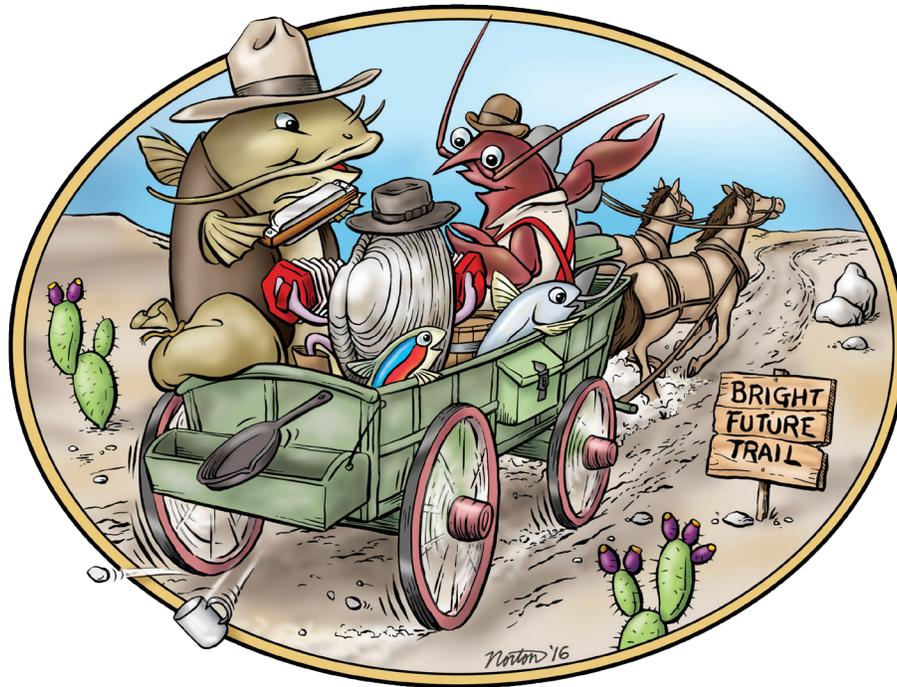
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# AQUACULTURE AMERICA 2017



*Forging New Frontiers*

# ABSTRACTS

## FARM BIOSECURITY – PREVENTION IS BETTER THAN CURE

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Disease causing pathogens pose a formidable threat to intensive aquaculture practices and can potentially cause significant economic losses and jeopardize the expansion of the industry. Biosecurity is a set of practices used to minimize the introduction, establishment, and spread of these pathogens. Since aquaculture works around pathogens and disease prevention is better than cure, it is a sensible approach to adopt biosecurity practices. Again, the practices differ according to the stakeholders. At the fish farm site, practices that reduce the risk of a disease outbreak is required. Farm biosecurity risks include introduction of infected fish, using surface water, vectors, fomites (nets, vehicles, equipment, boots, waders, buckets, pumps) etc. Biosecurity could be applied to individual ponds, farms or to the whole industry. Implementation of biosecurity practices are more effective and easy in small production systems than in outdoor and large-scale operations. It would be beneficial to adopt the biosecurity practices from other successful animal industries. A farm specific biosecurity plan could be prepared which identifies disease concerns, risky areas and implements the best practices. An effective biosecurity plan comprises of disease surveillance, biosecurity awareness, quarantine of incoming fish, appropriate farming practices, vaccination, traffic control, vector control and disinfection methods (Figure 1). Since the occurrence of a regulated disease can have a severe adverse effect on the farm and to the industry, it is almost impossible to ignore the economic benefits of a biosecurity program.

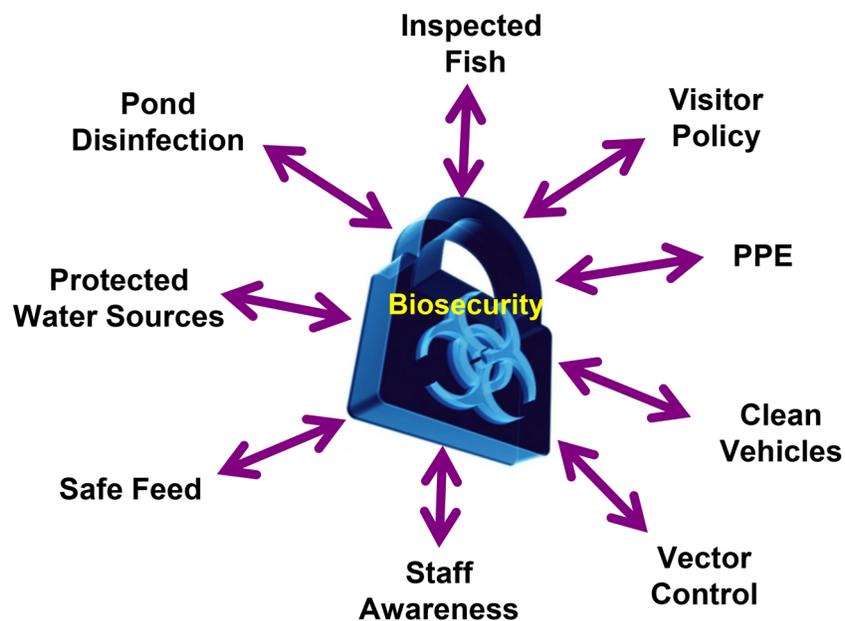


Figure 1: Different components of biosecurity plan.

## SEX EFFECTS ON GROWTH RESPONSE TO GROWTH HORMONE (GH) INSERTION FOR TWO GH CONSTRUCTS IN CHANNEL CATFISH, *Ictalurus punctatus*, AT TWO AGES

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More than 925 million people are suffering from chronic hunger in the world and the majority of hungry people live in developing countries. Fish are an important source of animal protein and nutrition for people worldwide. Gene transfer represents a potential solution to develop the fast-growing, high-performance. Catfish is the primary aquaculture species in the United States. Growth hormone (GH) has been considered as a candidate gene for growth and development in teleost fish.

Channel catfish, *Ictalurus punctatus*, GH cDNA driven by the ocean pout *Zoarces americanus* antifreeze protein promoter (opAFP-ccGH) or rainbow trout *Oncorhynchus mykiss* metallothionein promoter (rtMT-ccGH) were transferred to the channel catfish, *I. punctatus*, via electroporation. GH transgenic ( $P_1$ ) were mated to produce the  $F_1$  generation. The present study was designed to compare the growth performance of male and female transgenic channel catfish GH cDNA of both promoter types and their non-transgenic full-siblings in earthen ponds at different ages.

Body weight among the transgenic individuals and their full siblings was different at all ages. Catfish transgenic for opAFP-ccGH grew 1.67-2.8 fold larger than their full siblings and those transgenic for rtMT-ccGH grew 1.51-3.16 fold larger than their full siblings. Significant differences in body weight between the sexes were found at 12 and 36 months. Body weight of the males was significantly higher compared with those of the females at 12 months of age ( $P < 0.001$ ) However, body weight of the females was significantly higher compared with those of the males at age 36 months ( $P < 0.0001$ ). The body weight of 36-month-old transgenic opAFP-ccGH females (2110±880 g), was 1.20 times higher than that of the males (1760±600 g). However, the body weight of transgenic rtMT-ccGH females (2340±470 g), was 1.31 times higher than that of the males (1780±570 g). In the case of non-transgenic GH siblings males grew faster than females at 12 and 36 months ( $P < 0.001$ ). Apparently, elevation of growth hormone levels or associated epistasis alters sexually dimorphic growth after sexual maturation.

## **EFFECTS OF CLAY AND PHYTOPLANKTON TURBIDITY ON THERMAL STRATIFICATION IN SHALLOW AQUACULTURE PONDS**

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Water temperature is a vital factor controlling the growth rate of all cultured aquatic species. Shallow water aquaculture ponds typically stratify thermally in the day and destratify at night. The severity of thermal stratification in the pond is controlled by the degree of light penetration. The depth of penetration of solar radiation into a small pond is greatly controlled by water turbidity which either caused by suspended clay particles or phytoplankton abundance. This study was conducted to determine the effect of different causes and levels of water turbidity on water temperature profiles in shallow aquaculture ponds.

The experiment was performed at the Auburn University E. W. Shell Fisheries Center Auburn, AL. The study was conducted in two phases. Three, 0.04-ha research earthen ponds with the same dimensions were selected and dedicated for each phase. During the first phase, one pond had clear water, while the second one was treated with fertilizer to maintain phytoplankton turbidity. Kaolin clay added to a third pond to develop turbidity. During the second phase, each pond had a different level of clay turbidity.

In each pond, light intensity and water temperature at the surface, and at 20-cm depth intervals within the water column were monitored at 0.5-h intervals with a Model 64K HOBO Pendant® Temperature/Light Data Logger. Air temperature under shade and in full sunlight also were monitored at 0.5-h intervals. Water turbidity and Secchi disk visibility were measured daily in each pond. Results showed that turbid ponds thermally stratified more strongly than did the clear pond.

## THE PERFORMANCE OF NEW AIRLIFT AERATORS AT DIFFERENT SALINITIES, USING DIFFERENT BLOWER POWERS

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In semi-intensive and intensive aquaculture, aerators are necessary to satisfy the oxygen requirements for respiration of the cultured organisms. There are many kinds of mechanical aerators. One bubbler aerator is the diffused-air aeration system, which releases air bubbles near pond or tank bottoms to rise through water column. This study was conducted to determine the influence of different salinities and different blowers on the performance of two new airlift aerators.

The tests were performed in indoor tanks located at the Auburn University E. W. Shell Fisheries Center, Auburn, AL. The approximate tank volumes were 3.5 and 7 m<sup>3</sup>, but actual water volume was estimated during each test. The tanks were supplied with clean city water. The procedure for conducting the tests followed protocol suggested by the American Society of Civil Engineers. The procedure involved deoxygenating water in the tank with sodium sulfite and cobalt chloride, measuring dissolved oxygen concentration during reaeration by using smarTROLL RDO handheld oxygen meter (In-Situ Inc.), and calculation of the oxygen transfer coefficient ( $K_{LaT}$ ). The value for the  $K_{LaT}$  was adjusted to 20°C. The adjusted value ( $K_{La20}$ ) was then used to calculate the standard oxygen transfer rate (SOTR). The wire power was computed and divided into SOTR to provide the standard aeration efficiency (SAE) in kilograms of oxygen per kilowatt hour. The SOTR and SAE of two airlift devices – Hydro Burst<sup>®</sup> and Hydro Stream<sup>®</sup>– (Aqua Hill Aeration Inc.) were tested at five salinities (0.1, 10, 20, 30 and 40‰). Three different blower power (0.37, 0.75 and 1.5 kW) were tested at all salinities. Three replicate trials were performed with each airlift device with each blower at each salinity.

The results clearly demonstrated a significant effect of salinity on airlift aerators oxygen transfer performance. This is because air bubbles are more numerous and smaller in saline water than in freshwater. Our findings also showed that, the bigger the blower, the higher the SOTR, the smaller the SAE. There is a trade-off between SAE and SOTR. The SAE is an important but not the critical factor for tanks.

## COMPARATIVE GROWTH AND FATTY ACID PROFILE OF *Oreochromis niloticus* FED PLANT AND ANIMAL ORIGIN FAT SUPPLEMENTED FEED

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Nile tilapia (*Oreochromis niloticus*) was examined with 4 % animal (Fish oil) and plant (Soybean oil) origin fat supplemented feed to evaluate the fatty acid profile and growth performance. For ten weeks, 15 fish per treatment were fed three times a day at 7 % of body weight in triplicate. The study of fish growth parameters i.e. body weight and total length revealed a highly significant differences at the end of trial. The percentage weight gain was 18.99%, 51.3% and 31.11% in the three treatments T<sub>0</sub> (control), T<sub>1</sub> (soybean oil) and T<sub>2</sub> (fish oil) correspondingly. The highest SGR value was 0.84 displayed by the soya bean oil and lowest was 0.03 displayed by control. The FCR observed was 8.3 in control 2.76 in soybean oil treatment and 4.2 in fish oil treatment. As the proximate body composition, the higher value of ash (3.8%), lipid content (7.8%) and protein content (14.5%) were observed in fish oil treated treatment, as compared to soybean oil treated samples ash (3.18%), lipid (7.4%) and protein content (14.4%). The gas chromatography analysis showed that fatty acid (FA) profile showed significant differences. Large number of PUFAs was detected in soya bean oil treated fish as compared to fish oil treated fish. The saturated fatty acids detected in soya bean oil treated fish were Stearic acid, Capric acid and Caproic acid ; and monounsaturated fatty acids were Myristolic acid (C14:1, 19.3%) and Oleic acid (C18:1, 3.5%), and polyunsaturated fatty acids were Docosahexaenoic acid (C22:6n-3, 1.9%), C18:2 n-6 Linoleic acid (11.2%), EPA (C20:5n-3, 0.35%), Arachidonic acid (C20:4, 13.8%) and Decosapentaenoic acid (C22:5n-3, 6.6%). Whereas, the fatty acids were detected in the fish oil treated fish were Caproic acid, EPA (C20:5n-3, 0.91%), Docosahexaenoic acid (8.3%), DPA (1.4%) and Oleic acid (C18:1, 1.8%). In conclusion it is revealed that dietary supplementation of plant origin fat (soybean oil) in feed not only increased FCR but also modified the fish fatty acids profile and growth of *Oreochromis niloticus* significantly as compare to animal origin fat (fish oil).

## PREDICTION OF THE LARVAL METATRANSCRIPTOME TO UNDERSTAND THE FUNCTION OF GUT MICROBIOTA UNDER AQUACULTURE CONDITIONS

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High-throughput sequencing of 16S ribosomal RNA (rRNA) gene amplicons has revealed the diversity of microbial communities (MC) from many different environments and animals. In recent years, much attention has been paid to the intestinal MC and the role they play. The MC in fish intestine is rich and diverse and is thought to serve a crucial role in homeostatic and metabolic processes such as gut functioning and development, immune response and nutrition. Despite significant progress in the discovery of the MC residing in the fish gut, much of the functionality of these communities, fish-microbiota interactions and inter-community interactions remain unclear, as 16S-sequencing does not directly reveal the functional capabilities of the community. Recent studies have indicated, however, that the phylogeny of a community and its functionality are sufficiently linked to allow for a predictive metagenomic approach.

In this study, newly hatched Nile tilapia larvae were reared under two different culture conditions, either a recirculating aquaculture system (RAS) or an active suspension (AS) system. At 42 days post first-feeding, tilapia larvae were randomly collected from RAS and AS replicate tanks and gut samples were processed for high-throughput sequencing of the V1–V2 region of the 16S rRNA gene. Sequencing data was processed using the QIIME software for identification of operational taxonomic units (OTUs). To predict the function of identified bacteria, OTUs were further analyzed using the PICRUSt software. Statistical differences were assessed using the STAMP software. Functional data was mapped to KEGG pathways and orthology.

We identified 44 KEGG ortholog groups with different abundances between aquaculture systems (Figure 1). Further mapping to significant KEGG pathways revealed large differences in abundance of MC contributing to ABC transporters, protein kinases and a host of metabolic processes. Understanding these functional characteristics linked to culture systems may help identify solutions to issues affecting intensive production.

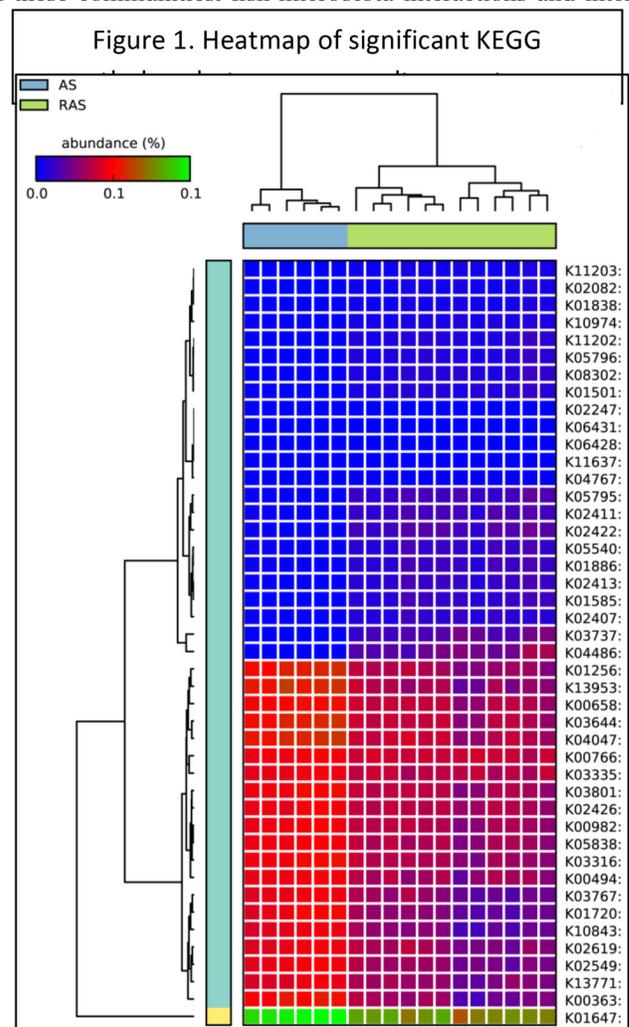


Figure 1. Comparison of microbiota community in commercial strain of rainbow trout reared on either a fishmeal (FM) based diet or a plant protein (PM) based diet.

## **WATER QUALITY PARAMETERS OF AFRICAN CATFISH *Clarias gariepinus* FED COMMERCIAL PELLETED FEED, SUPPLEMENTED WITH LIVE TILAPIA FRY *Oreochromis niloticus* AT 1% AND 2% BODY WEIGHT IN CONCRETE TANKS**

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The study was conducted in Akwa Ibom State, Nigeria to assess the water quality parameters of catfish fingerlings (*Clarias gariepinus*) fed pelleted feed supplemented with live tilapia fry (*Oreochromis niloticus*) in concrete tanks. Nine hundred (900) catfish fingerlings (*C. gariepinus*) of mean weight  $9.68 \pm 0.25$ g were stocked in ninety (90) units of concrete tanks of  $2 \times 2.5 \times 1$ m<sup>3</sup> dimension, at the rate of ten fingerlings per unit. The fingerlings were fed commercial pelleted feed at the rate of 1%, and 2% body weight. Within each pelleted feeding level, three treatment levels and a control were established 0%, 0.5%, 1% and 1.5% as (T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>) and were fed with live tilapia fry (*O. niloticus*). The experiment was replicated three times. Water exchange was continuous at a rate that replaced the entire volume within seven days. The result revealed a highest mean final weight (1437.28g) in T<sub>3</sub> at 2% PFL; Specific Growth Rate (SGR) was 2.17% per day. The hydrogen ion concentration (pH), dissolve oxygen (DO), ammonia and temperature were within the tolerable limits for catfish culture. No significant difference ( $p > 0.05$ ) was observed within treatments for water quality. The study showed that combination of pelleted feed and tilapia had no adverse effect on the water quality during catfish culture.

## THE INFLUENCE OF CAGE CULTURE OF THE MULLET *Lisa haematocheilus* (Temminck & Schlegel, 1845) ON BIOCHEMICAL COMPOSITION OF THEIR EGGS

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The Far Eastern haarder *Lisa haematocheilus* (Temminck & Schlegel, 1845) is considered as one of the most promising aquaculture species in the southern seas of Russia. Despite the fact that this species have become naturalized in the Azov and Black Sea basin, the development and improvement of biotechnologies on artificial breeding and cultivation remain an urgent problem. Not only meat but also roe of haarder is of great commercial interest because caviar is regarded as a gourmet product. It is known that rearing conditions, especially diet, influence the chemical composition of an organism as determined by its adaptation to new conditions. To assess the effect of cage culture with our own compound feed formulation used, a comparative analysis has been done of the biochemical composition of eggs of the Azov Sea haarder raised in cages that were installed in the adjacent estuary.

The studies have shown that the water concentration and lipid content in the eggs of caged fish were, respectively, by 30% and 28% more than in the eggs of wild fish ( $P < 0.05$ ). Differences in protein and mineral fractions were minor.

Greater accumulation of triacylglycerols (by 60% more) and phospholipids (by 19% more) is observed in the eggs of farmed fish than in the wild haarder roe ( $P < 0.05$ ). The levels of other lipid fractions were similar enough. There were no significant differences in the phospholipid spectrum of eggs of both groups.

The main differences in fatty acids were associated with docosahexaenoic acid, its concentration was 1.4 times higher in wild fish, while linolenic and eicosadienoic acid levels were 1.6 times lower than those of the caged fish.

Despite some differences in the relative content of both individual and a group of saturated, monoenoic and polyenoic fatty acids, the sum of the differences in  $\omega 3/\omega 6$  acids and lipids did not exceed 6%. Hence, in total lipids and phospholipids the  $\omega 3/\omega 6$  ratio ranged slightly within respective limits of 2.3-2.4 and 3.0-2.8.

The content of vitamin A amounted to  $0.05 \pm 0.001$  mg% in wild haarder eggs, vitamin C averaged  $5.15 \pm 0.29$  mg%, and it was slightly higher as compared with the eggs of caged fish whose values were  $0.03 \pm 0.001$  and  $4.11 \pm 0.04$  mg%.

Thus, the biochemical composition of eggs obtained from the fish reared in cages and fed with artificial diets did not differ much from the naturally feeding fish, which indicates that adaptive capacities of haarder are high, and it can be reared without losing its nutritional properties. However, the biochemical status of haarder eggs is subject to certain fluctuations, it depends on environmental conditions, including food composition, and these aspects should be also considered in aquaculture practices.

## **FISH SPECIES COMPOSITION, DISTRIBUTION AND ABUNDANCE AND GILLNET FISHERIES IN IKERE GORGE, NIGERIA**

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Overfishing is a major problem in fisheries as fishermen without adequate knowledge of the composition, distribution and abundance of fish species continuously fish in water bodies using various fishing gears thereby depleting the stock. Here, we carried out the ecological study of Ikere gorge between January 2004 and December 2005 to assess the fish resources of the gorge and the gear commonly used by fish farmers in the gorge. We collected data on fish composition, abundance and gear used from four sampling stations. Thirty-four (34) fish species belonging to thirteen (13) families were present in the gorge. Our findings revealed that Bagridae (25.1%), Cichlidae (37.1%) and Mormyridae (14.5%) were most abundant families contributing 76.7% by number and 75% by weight of the total catch followed by Characidae (9.6%), Cyprinidae (7%) and Centropomidae (3%) constituting (19.6%) by number and 8.7% by weight. Schilbedae (1.1%) Clarridae (0.8%), Hepsetidae (0.7%), Mochokidae (0.6%), Channidae (0.5%), Ichthyoboridae (0.2%) and Malapteruridae (0.1%) were least represented. We found out that commonly used gears were cast net, traps, bamboo stems, spears, gura net and gillnet which was the major fishing gears in used. Also, the gillnet fisheries observed showed the sensitivity of experimental gillnets mesh sizes to different fish species. 78.4mm, 112mm, 67.2mm mesh sizes were efficient in catching *Chrysichthys nigrodigitatus* *Lates niloticus* and the cichlids respectively. The study contributed to baseline data on the fish composition and distribution and gear selectivity on fish catch. Thus aiding fisheries policy makers in the management of this water body.

### **Biography**

Adeosun, F.I has completed his PhD at the age of 39 years from the Federal University of Agriculture Abeokuta, Ogun State, Nigeria. He has held different offices within and outside the institution. He has served as Acting Head of Department (HOD) in the absence of the HOD. He has also served in different capacity of the Academic Staff Union of the University: as the Vice to the Chairman and then as the Chairman amongst others. He is currently a member of the Community based farmers Society (COBFAS). He has published more than 25 papers in reputed journals.

## STRUCTURAL DEFORMATION IN THE KIDNEY AND LIVER OF *Clarias gariepinus* JUVENILES EXPOSED TO GRADED CONCENTRATION OF ZINC

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The structural deformation of the kidney and the liver of African catfish, *Clarias gariepinus* exposed to graded concentration of zinc was determine for a period of 96 hours. The stock solution was prepared with zinc and the fish juveniles were exposed to the following concentration in mg/L : 0.5, 0.75, 1.00, 1.25 and 1.50 with a control of 0.0mg/L where the zinc was not introduced. The regression plot of the probit value transformed mortality against the concentration of zinc showed a strong relationship with the death of the fish exposed to the concentration of the metal with  $R^2$  value of 0.8725. All the same no death was observed in the control throughout the 96 hours period of the study while the lowest death of 15% was recorded in the 0.5mg/L and the highest of 60% in the 1.50mg/L concentration of zinc. The  $LC_{50}$  was determined to be 1.25mg/L during the study time. The ultrastructural characteristics showed pathological lesion in the kidney and vacuolation of the hepatocytes of liver of the fish. The DO of the water was below the recommended level for the survival of the fish in the waters that the concentration of zinc was introduced and may cause distress to the fish. The fish showed erratic movement and distressful behaviour where they were exposed to zinc and eventually the ones that could not tolerate the toxicity metal died. The study conclude that the fish should not be exposed to the elevated concentration of zinc as it is toxic and recommend that anthropogenic activities that release zinc into the environment should be controlled.

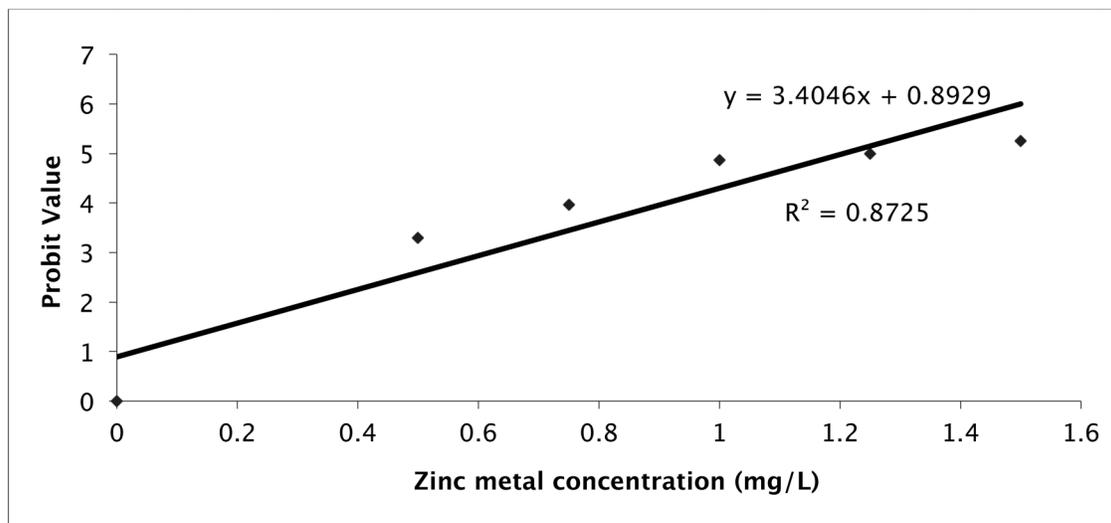


Fig 1: Regression of Probit mortality values and graded concentration of zinc exposed to juveniles of *Clarias gariepinus*.

## GENE SEQUENCING AND SENSITIVITY OF BACTERIA IN *Tilapia zilli* FROM IJAKA-OKE LOCATION ON YEWA RIVER

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Bacteria were identified using the 16S rRNA gene sequencing method, and the antibiotic sensitivity test was done using five different antibiotics. Bacteria were isolated from the gut, gills and skin of *Tilapia zilli* from Ijaka-Oke. Bacteria DNA were extracted using CTAB method, PCR amplification was carried out using 16S universal primer for bacteria, purification of PCR product was done using ethanol precipitation, and thereafter DNAs were sequenced using automated DNA sequencer. Sequence data were analyzed on GenBank database (NCBI) using BLAST search tool to find closest related sequence. Antibiotic susceptibility patterns of isolates were also determined using Kirby-Bauer disc diffusion method. Bacteria strains were identified as *Proteus mirabilis* strain, *Providencia vermicola* strain, *Providencia rettgeri* strain, *Morganella morganii* strain, *Myroides odoratimimus* strain, *Providencia rettgeri* strain, *Morganella morganii* subsp. *Sibonii* strain, *Providencia rettgeri* strain, *Providencia rettgeri* strain, *Morganella morganii* strain, *Morganella morganii* clone, *Proteus mirabilis* strain, *Enterobacter spp.*. Cephalexin recorded highest zone of inhibition (21mm) on *Enterobacter sp.*, followed by Erythromycin on *Enterobacter spp.* (19mm), Lowest inhibition zone was recorded with Erythromycin on *P. vermicola* (10mm). *P. mirabilis* was susceptible to cephalexin and ceftriaxone, *P. vermicola* and *P. rettgeri* were both susceptible to erythromycin, cephalexin, streptomycin and ceftriaxone while it was resistant to cotrimoxazole. *M. morganii* was susceptible to streptomycin and ceftriaxone, *Enterobacter sp.* was susceptible to erythromycin, cephalexin, and streptomycin but resistant to ceftriaxone. *Myroides odoratimimus* was resistant to all antibiotics used. The study therefore showed that diverse bacterial species exist on skins, gills and guts of *Tilapia zilli*.

## FORMULATIONS FOR IMPROVED ORAL DELIVERY OF IMMUNOACTIVE RIBONUCLEOTIDES AND LPS TO RAINBOW TROUT (*Oncorhynchus mykiss*)

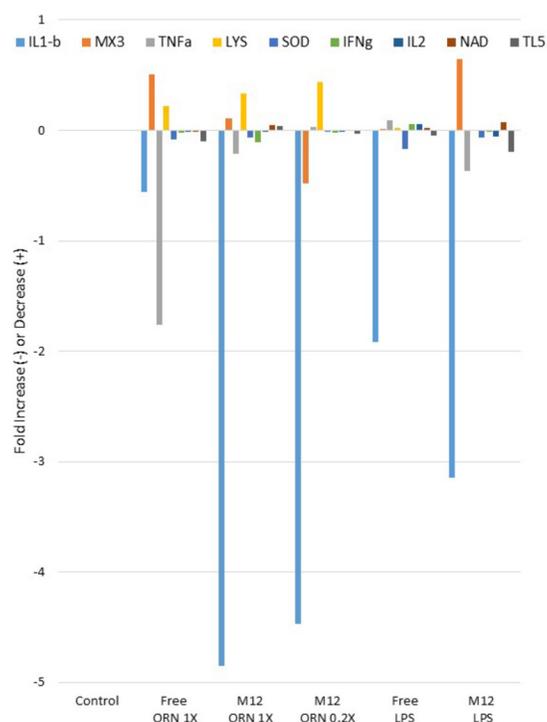
F. C. Thomas Allnut<sup>\*1</sup>, Kennard Roy<sup>1</sup>, Scott LaPatra<sup>2</sup>, Mariam Ashraf<sup>1</sup> and Arun K. Dhar<sup>3</sup>

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Protein and oligonucleotide based products that deliver improved health to aquacultured species face hurdles to their expanded use in aquaculture - particularly high cost of production, ineffective oral delivery, and regulatory burden for their approval. This project is developing inexpensive formulations that provide improved stability and oral delivery while basing the formulations on materials that are generally regarded as safe (GRAS) or approved dietary ingredients to lower the regulatory burden for approval.

Formulations were developed to better deliver a ribonucleotide-based immunostimulant product, LactORN<sup>®</sup>, to rainbow trout. In addition, our formulations were used to better deliver *Yersinia ruckeri* LPS (high molecular weight) as both a control and possible immunostimulant in its own right. Our M12 formulation was used to produce particles containing LactORNs at the recommended dosage as well as 20% of this dosage. LPS was also encapsulated for delivery at rates on the low end of those shown to stimulate the immune system in the literature. Controls were LactORNs & LPS in unprotected form as well as empty M12 particles as a negative control. The efficacy of our formulations for delivery of LacORNs and LPS was evaluated by evaluation of the expression of candidate immune genes by PCR.

Juvenile rainbow trout (~25 g/5 fish per treatment) were weighed and placed 5-gallon buckets containing 15°C UV-treated water. Fish were fed commercial diets supplemented with the appropriate additive (see Figure). Fish were fed three days on the test feeds then on Day 4, head kidney and gill arches (from the left side) were sampled. RNA was isolated using RNazol as described by the manufacturer then cDNA produced. Real-time RT PCR was run using SYBR-Green (Life Technologies) to measure expression of IL-1, TNF $\alpha$ , IRF-1, LYS, SOD, IFN  $\gamma$ , IL2, TL5, NAD and MX3. EF1 $\alpha$  was the housekeeping gene. The results show that the M12 encapsulation provided improved efficiency of delivery of the LactORN and LPS as assessed by the expression data. These data show that less ORN was needed when supplied in the M12 formulation than when not protected for the same gene expression enhancement.



## STATUS OF CAGE FISH FARMING ON THE LAKE VOLTA IN GHANA

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Cage fish farming, carried out mainly on the Lake Volta, is a relatively new development in Ghana with concerns raised on its potential environmental and socioeconomic impacts. The study was carried out in the Volta catchment in the Asuogyaman District of the Eastern Region of Ghana to examine the status and cultural practices of cage fish farming in the Lake Volta. Twenty-three cage farmers with cages located on the Volta were examined through questionnaires, interviews and field observations. Relevant information and data collected included demographic and educational background of farmers, sources of income, age of farmers, farming practices, sources of inputs and level of production. Only 23% of the farm owners depended solely on the fish farm for their incomes. Cage farming remains a male-dominated activity, with ages ranging from 35 to 65 years and about 50% aged 50-65. Fingerlings were obtained from three main sources: the Aquaculture Research and Development Centre, Private small scale, and individual commercial hatcheries. Production system remained intensive with stocking densities of 23 to 96 fingerlings/m<sup>3</sup>. Total fish production for small-medium scale farms varied from 1 tonne to over 5,000 tonnes in 2013; with feed conversion ratio ranging from 1.5 to 2.0. High cost of feed, lack of adequate fingerlings, lack of capital, poaching and water quality problems remain leading constraints to viable operations. Conflicts on space and access between lake artisanal fishermen and cage farmers remain a formidable setback. There is a massive potential for cage fish production with a ready local market, and this is corroborated by Ghana government's annual fish imports of over USD 20 million. Evidence of deleterious environmental impacts remain is low and insignificant.

## TILAPIA GROWTH PERFORMANCE USING TWO TYPES OF EXTRACTED SOYBEAN MEAL

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In many countries, there is limited availability of solvent-extracted soybean meal whereas expeller pressed soybean meal is available. This research sought to compare a moderate fishmeal (6% of diet) and solvent-extracted soybean meal (44% of diet) based diet (Diet 1) to that of fishmeal free diets based on two forms of soybean meal. Diet 2 was developed with commercially available solvent extracted soybean meal (44% of diet) which was obtained locally. Diet 3 utilized extruder-expelled soy meal trade marketed as ExPress® soy meal (45% diet) was produced from whole soy using an Insta Pro press. The diets are described in table 1. Twenty sexed reversed tilapias (mean initial weight 60.2g) were stocked in each of 12 tanks. Fish were randomly assigned to one of three diets and were fed to satiation, twice a day for 16 weeks. Results are showed in table 2, mean weights were different among treatments and no differences are observed in biomass, feed conversion rate and survival.

Table 1. Diet composition (33% protein, 6.0% lipid).

<b>Ingredients</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Diet 3</b>
Fishmeal special select	6.00	0.00	0.00
Soybean meal	44.00	44.00	0.00
ExPress® soy meal.	0.00	0.00	45.00
Cotton seed meal	15.00	15.00	15.00
Empyreal 75®	0.00	5.00	5.00
Fish oil	2.95	3.43	1.13
Corn, yellow	28.90	29.42	30.72
Premix	3.15	3.15	3.15

Table 2. Final means weights, biomass, FCR and survival at 16 weeks of the trial.

<b>Diet</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Diet 3</b>	<b>PSE</b>	<b>P value</b>
Mean Weight (g/fish)	275.8 <sup>a</sup>	235.7 <sup>ab</sup>	218.6 <sup>b</sup>	33.8	0.014
Biomass (g/tank)	5193	3963	4015	1068	0.137
FCR	1.61	2.03	2.00	0.39	0.212
Survival (%)	93.8	84.7	91.7	13.3	0.602

FCR: Feed conversion ratio, PSE: Pooled standard error

## THE EFFECTS OF BROODSTOCK PARAMETERS ON THE SPAWNING BEHAVIOR OF THE CLEANER WRASSE *Labroides dimidiatus*

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The bluestreak cleaner wrasse *Labroides dimidiatus* is native to the Indo-Pacific region and is the 13th most common marine aquarium fish imported into the United States annually. Cleaner wrasses are called the “doctors of the sea”, as their cleaning behaviors decrease the overall load of ectoparasites on reef fishes. The removal of *L. dimidiatus* from reefs can have a detrimental impact on the reefs they inhabit, through decreased diversity and increased ectoparasite levels burdening reef fish. Given this, the number of *L. dimidiatus* taken for use in the marine aquarium trade can greatly influence the reef ecosystem as a whole, and with a lack of commercially available captive-raised individuals, this impact is amplified. Previously, only one successful attempt to culture the species has been recorded.

The objective of this research is to determine the ideal environmental conditions for *L. dimidiatus* to spawn in captivity. In an ongoing effort to characterize and optimize their spawning, experiments were conducted to determine the effects of tank height on spawn quantity and quality. Seven broodstock pairs were kept in two tank types, a 100 cm high “tall” enclosure, and a 52 cm high “short” enclosure. Spawns were collected daily, and the eggs were counted and analyzed for egg diameter (mm), oil globule diameter (mm), and percent viability (neurula stage) at 16 hours post spawn. Although preliminary results illustrate no difference in egg quantity between the two tank treatment types, ongoing data collection will determine any differences in the remaining egg quality parameters. Additionally, experiments are being conducted on the effects of the presence of client fish on spawns, as well as a replication of the tank size experiment. This effort to procure consistently high quantity and quality spawns from broodstock pairs of *L. dimidiatus*, in addition to obtaining spawns in high frequency, is a precursor to the sustainable commercial aquaculture of the species.

## PRODUCTION OF MICROALGAE FOR SHRIMP LARVICULTURE

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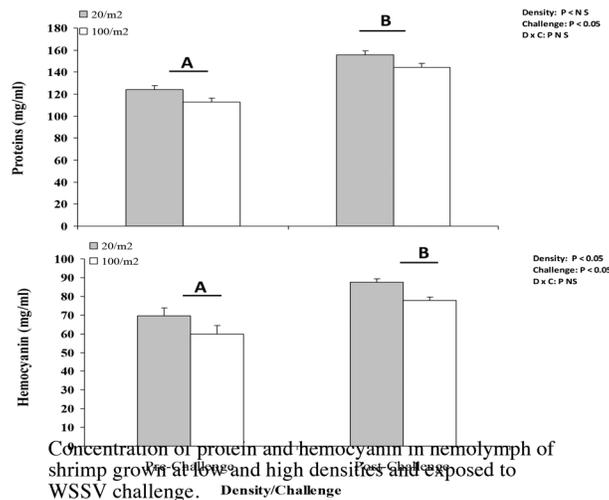
Shrimp culture in Nigeria is not yet well developed despite the abundance of the species in the marine waters. This has been attributed to lack of sustainable production of microalgae both in quality and quantity for shrimp larviculture. A study was conducted at the microalgae laboratory of Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria to determine the culture possibility of four local microalgae, *Chaetoceros*, *Skeletonema*, *Isochrysis* and *Chlorella* species. *Chaetoceros*, *Skeletonema*, and *Isochrysis* were cultured in modified Guillard F medium while *Chlorella* was cultured in Conwy medium. The growth pattern of the four microalgae showed very short lag phases indicating that they adapted well to the culture conditions. The exponential phases of all the microalgae were similar occurring in the third day of culture. *Chaetoceros*, *Skeletonema*, *Isochrysis*, and *Chlorella* species reached their stationary phases from the sixth to the ninth day with the highest densities of 7,750,000; 1,105,000; 6,091,000 and 6,510,000 cells/ ml respectively. *Chaetoceros* specie had the highest cell density of 7,750,000cells/ ml at the 7<sup>th</sup> day while *Skeletonema* had the lowest density of 101,500 cells/ml at the 13<sup>th</sup> day. The study showed that the microalgae species can grow well and be maintained in the laboratory through continuous sub-culturing. Successful mass cultures of these species indicate that hatchery production of shrimp and prawn larvae is feasible in Nigeria.

## INFLUENCE OF STOCKING DENSITY AND EXPOSURE TO WHITE SPOT SYNDROME VIRUS IN BIOLOGICAL PERFORMANCE, METABOLIC, IMMUNE AND BIOENERGETICS RESPONSE OF WHITELEGS SHRIMP *Litopenaeus vannamei*

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The risk of disease in shrimp farming has been suggested to increase with stocking density, although it is not clear if this is related to an increased susceptibility to pathogens based on poor physiological condition or a lower capacity of immune response. This study analyzed the influence of stocking density on the metabolic and immune response to exposure to white spot syndrome virus (WSSV) on whiteleg shrimp *Litopenaeus vannamei*. Juvenile shrimp were submitted to two stocking densities (20 and 100 shrimp m<sup>2</sup>) for eight weeks; water quality, growth, and survival were recorded. Then, some shrimp was sampled for determining biochemical composition, adenylic nucleotides, arginine phosphate (Arg-P), total hemocyte count, and clotting time to determine baseline values. Other shrimp was challenged with WSSV for 48 h and then analyzed for the same variables. Concentration of nitrogenous compounds and phosphorus were significantly higher in tanks with the higher shrimp density. Growth and survival were significantly lower by 42% and 18%, respectively, under high density conditions. No outstanding effect of stocking density on biochemical and immunological variables were observed, except for higher levels of hemocyanin in hemolymph and lactate in muscle in shrimp under low density conditions. In contrast, the WSSV challenge induced several metabolic disruptions, including a higher adenylic energy charge (AEC) in the hepatopancreas and higher content of Arg-P in muscle, higher content of triglycerides in hemolymph, hepatopancreas, and muscle, and higher content of glucose, protein, hemocyanin, and cholesterol in hemolymph. These responses correspond to a transition between increased energy demand caused by exposure to virus and a phase of metabolic depression. Conditions of high stocking apparently do not increase the susceptibility of shrimp to WSSV since no differences were observed in the severity of infection, total hemocyte count, and clotting time.



## **BIOSECURITY AND THE RELEVANCE OF THE PROFICIENCY TEST (PT TEST)**

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One of the critical steps in biosecurity in Aquaculture is to ensure the exclusion of pathogens. For instances, during the last twenty years, several shrimp farms around the world faced a WSSV outbreak that caused high mortalities in grow-out ponds affecting the shrimp production dramatically. The combination of the some factors such as the low water temperatures, the presence of the susceptible population of shrimp and the deficiency in the pathogens-detection system caused the WSSV outbreak.

One of the critical processes in the whole Biosecurity strategy is to determine the pathogens present in the shrimp industry environment. The main diagnostic technique, in which most if not all the shrimp farms rely, is the polymerase chain reaction (PCR) due to its high sensitive and specificity for shrimp-pathogen detection. Three different PCR approach have routinely used: commercial kits, OIE manual procedures and procedures published in a peer-reviewed journals. The shrimp industry trusts on these diagnostic laboratories and takes such an important decisions based on a given result. When a positive results for any of the OIE-listed diseases appears, (even if mortality is not reported) creates a panic in the shrimp industry especially when this pathogen can potentially cause high mortality at farm level and usually have economic consequences for the shrimp producers.

Based on the importance of having a reliable diagnostic lab results, it is important to inter-calibrate the performance with a reliable laboratory. One of the purposes of inter-laboratory performance testing, also known as a ring test, is to assure the clients or regulatory officials that the results provided are accurate, specific and reproducible.

As a World Organisation for Animal Health (OIE) reference laboratory, the University of Arizona's Aquaculture Pathology Laboratory has taken on the role of providing training and assistance to other shrimp diagnostic laboratories for the detection of shrimp pathogens through the use PCR testing. The lab has been routinely implementing ring tests since 2005. Based on the high demand for the diagnostic laboratories, since 2009 this process is being carried out twice per year (February and August). Laboratories from all over the world have actively participated in this inter-calibration ring test process. Participation in the ring testing is completely voluntary. There are no prescribed methods, and each participating laboratory employs the same PCR procedures it routinely uses in the analysis of clinical samples. On 2016, in addition of the OIE listed pathogens, the acute hepatopancreatic necrosis disease (AHPND) was added among the panel set. For the ring test on February 2017, the emerging disease enterocytozoon hepatopenaei (EHP) will be included in addition to the panel set of pathogens including WSSV, TSV, IMNV, IHNV, YHV & NHP-B.

## **TiLV, AN EMERGING NEW VIRAL DISEASE OF TILAPIA SPP.**

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In Summer 2009, massive losses of Tilapia were observed in several fish farms in Israel. Very high mortality of Tilapia was described in all sizes. The disease outbreak was transferred readily from one pond to the next one indicating to be highly contagious. Most important, morbidity and mortality was limited only to Tilapia species and hybrids (*Oreochromis niloticus* X *O. aureus* hybrid). Tilapia that survived the initial outbreak in the same ponds appeared to be more resilient than naïve Tilapia if exposed to further infections.

Gross lesions were characterized mainly by unilateral or bilateral ocular alterations (cataracts). Other lesions, observed in diseased pond-raised Tilapia included skin erosions and moderate congestion of the spleen and kidney.

Eyngor et al. (2014) isolated successfully from diseased fish the etiological agent responsible for massive mortality in Tilapia. The virus was designated Tilapia Lake Virus (TiLV).

Disease outbreaks are frequently characterized by secondary Bacterial and Parasitic infection, leading to mortality rates of over 50% in juvenile Tilapia populations.

The existence of fish that survived the TiLV disease strongly suggests that an effective immune response against this pathogen can be mounted. This has important applications for future disease containment strategies.

Reports of similar clinical symptoms and very high mortality rates have been reported by Tilapia producers in Colombia and Ecuador during these last few months.

This disease process may endanger the rapidly developing Tilapia industry, worldwide.



## MARKET SHOCKS AND DISEASE: THE IMPACT OF ISA ON CHILEAN SALMON

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Chile's production of Atlantic salmon flattened out in 2005 after a decade of rapid production growth. It remained at a plateau of just under 500,000 metric tons (mt) until 2009, when the production was more than halved. In 2010, production was further reduced to about 130,000 mt, before it started to increase rapidly again reaching a 460,000 mt in 2013. The main reason for this dip in production is an outbreak of the disease Infectious Salmon Anemia (ISA). The reduction in Chilean salmon production was so large that salmon prices globally more than doubled. Because of the disease outbreak, the size of the Chilean salmon also changed, precipitating a substantial shift in which markets were served and which product forms were exported, and of particular interest is the development of the Brazilian salmon market.

From the late 1990s, the general literature indicates that there is a global market for salmon. However, the link between the American market and the rest of the world is weaker than between European and Asian markets. Moreover, it is strongest for the same product forms. On the other hand, there is also evidence that the integration is becoming strong with increased market size. Hence, the shocks caused by the disease outbreaks in Chile can provide additional insights with respect to the strength of market integration in the salmon market.

In this paper we investigate the degree of market integration between four product forms of Chilean salmon, fresh and frozen whole and fresh and frozen fillets, and for the fresh product forms we also consider the largest markets, Brazil and the USA. We also investigate the link to the Norwegian salmon price, as Norway is the largest producer. Since all prices are non-stationary, the market integration analysis is conducted using Johansen's cointegration test.

The empirical results indicate a highly integrated market for all Chilean product forms, but where frozen fillets are partially segmented. Hence, the development of the Brazilian market did not in any way disrupted the price determination process in Chile. Moreover, the Norwegian price lead all the Chilean prices, indicating that Chilean salmon prices is determined at the global market. This also implies that Chilean salmon producers did not gain any additional price increase during the disease crises due to an origin premium for Chilean salmon. Rather, all salmon producers benefited to the same degree of the price increase, with the difference that the other producers did not see any reduction in quantity produced.

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

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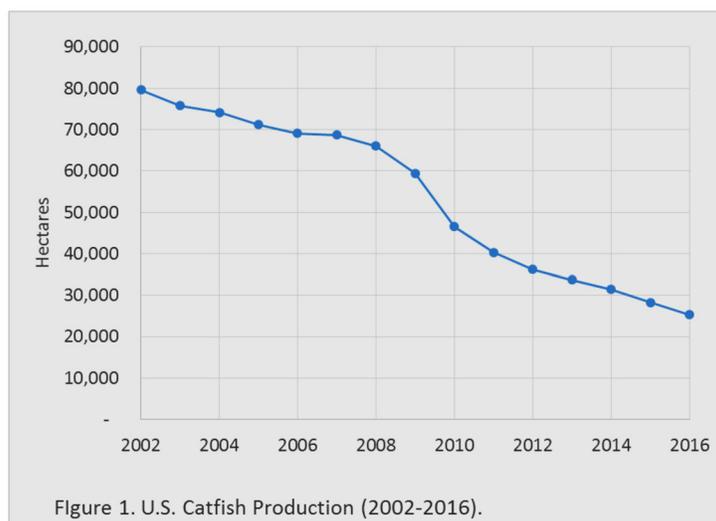
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The U.S. farm-raised catfish industry continues to contract. Since reaching its peak at 79,626 hectares in 2002, the amount of pond space has decreased to 25,309 hectares in 2016 (Figure 1). The annual rate of decrease has slowed dramatically over the last few years with some new ponds being built in 2016. This renewed interest is attributed to record live fish prices (\$2.67/kg) and lower feed prices.

Producers have increased yield per hectare by adoption of new technologies. Increased aeration rates to maintain minimum dissolved oxygen levels above 3 ppm has led to improved feed conversion levels. Hybrid catfish (channel catfish *Ictalurus punctatus* females X blue catfish *Ictalurus furcatus* males) are now estimated to comprise 50% of the catfish produced. Producers continue to switch traditional ponds to improved production systems such as intensively aerated small ponds and split-ponds. The use of hybrid catfish in the improved production systems has resulted in per hectare yield increases of 100% to 200% on some farms.

Despite improvements in fish price and productivity, the industry continues to face several challenges. Opponents of the change of catfish inspection from the U.S. Food and Drug Administration to the USDA Food Safety Inspection Service (FSIS) continue their efforts to have the law repealed or defunded. Although officially still in the transition phase, FSIS inspection has led to increased refusals of imported products and a recall of one lot of U.S. product. Despite claims that the switch to FSIS inspection would be a trade barrier, imports of Siluriformes imports are on pace to approach 2015 import levels.

An environmental, non-government organization has filed a petition with the U.S. Fish and Wildlife Service to list 43 fish and crustacean species as Injurious Wildlife under the Lacey Act. These 43 species have been previously identified as “High Risk” by the U.S. Fish and Wildlife Service. Blue catfish *Ictalurus furcatus* is one of the species proposed. Listing would prohibit the interstate transport of live animals or hybrids and would have a dramatic negative impact on the already declining U.S. industry.



## EFFECT OF BIOFLOC ON INFECTION OF TILAPIA FINGERLINGS BY PARASITES

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Infection of fish by parasites (protozoa and different worms) may be a severe problem for producers. Controlling and confronting infections often involves the use of chemicals, often affecting the pond environment.

We report here on a study comparing triplicated nursery systems: control treatment using high water exchange and relatively clear water and biofloc treatment using minimal water exchange and low protein feed. 51,000 fingerlings, 1 g. each were stocked into 80 m<sup>3</sup> concrete ponds.

A rather severe infection with different parasites (The most common Sessilina, Dactylogyrus, Centrcustus, Trichdina) was detected 12 days following stocking in all ponds. All ponds were treated with Formalin and Bromex.

There-after, the degree of infestation by parasites was followed. Randomly selected samples of fingerlings were sent to the governmental veterinary diagnostic laboratory. Prevalence of the different parasites externally or on the gills was evaluated microscopically. The extent of infections was characterized by 6 grades, from clean to extremely heavy infestation. In order to semi-quantize the results we gave the clean and the extremely low infestation ponds a value of zero while all higher infestation degrees were given values of one. The standardized degree of infestation was defined as the sum of infestation by the individual parasites grade and the sum of external or gills infestations.

The potential effects of biofloc systems was clearly demonstrated (see table below).

More research on the means to use this effect and on the mechanisms involved is needed.

### **Standard infection with parasites:**

<b>Days of culture</b>	<b>12</b>		<b>20</b>		<b>48</b>	
	<b>Average</b>	<b>Standard Deviation</b>	<b>Average</b>	<b>S.D.</b>	<b>Average</b>	<b>S.D</b>
<b>Control</b>	<b>2.3</b>	<b>0.6</b>	<b>3.0</b>	<b>1.7</b>	<b>1.3</b>	<b>0.6</b>
<b>Biofloc</b>	<b>2.0</b>	<b>1.0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>

## USE OF VEGETABLE WASTE AS A SOLE FEED IN THE DIET OF ROHU *Labeo rohita* FINGERLINGS

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A study was conducted by using vegetable waste as sole feed in the diet of rohu (*Labeo rohita*) fingerlings. Vegetable waste was comprised of ten different vegetables including Green gourd, Bitter gourd, Ridge gourd, Egg plant, Edible pea, Okra, Potato, Cucumber, Colocasia and Tomato. These vegetable wastes were dried, mixed and analyzed chemically. As the feed was very dry, therefore to make granules 11% gelatin solution was added to it and granules of 2mm diameter were produced. In a trial period of 45 days, three experimental groups and one control group were maintained each containing 12 rohu (*Labeo rohita*) fingerlings. Control group was fed with aquarium feed while experimental groups were fed with vegetable waste. Feeding at 1% body weight, all the groups showed negative growth. When fed at 2% body weight, some fish started gaining weight and when feeding at 2.5% body weight, all the groups started showing positive growth. It was concluded from the present study that vegetable waste cannot be used as sole feed but it can be used as supplementary diet for providing extra protein, fats and vitamin contents.

## A REVIEW OF MICRONUTRIENT REQUIREMENTS IN JUVENILE AND BROODSTOCK JAPANESE EEL, *Anguilla japonica*

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Japanese eel, *Anguilla japonica*, is an important freshwater food fish cultured in East Asia. Farming of this species requires a nutritionally balanced diet to support its development. While the requirements for macronutrients have been established, the optimum levels for micronutrients of this species are still lacking. This review therefore summarizes the results obtained from a series of experiments conducted to evaluate the optimum requirements for micronutrients, including vitamin C and E, and arachidonic acid in juvenile and male broodstock Japanese eel (*Anguilla japonica*). On the basis of weight gain and feed efficiency, juvenile eels (initial body weight of 15 g) required Vitamin C and E supplementation between 41.1 and 43.9, and 21.2-21.6 mg/kg in the diets, respectively. Arachidonic acid levels at 69-71 mg/kg are essential for maximum growth rate of juvenile eel. For male Japanese eel broodstock (average body weight of 360 g), ascorbic acid (AA) concentrations in both liver and testis suggest optimum inclusion at 410.7-911.7 mg/kg in the diets. In addition,  $\alpha$ -tocopherol should be supplied in the diets from 212.9 to 199.7 mg/kg. Requirement for arachidonic acid to achieve maximum weight gain of male eel broodstock is established at 71-92 inclusion levels.

### Requirements (mg/kg of diet) for micronutrients in juvenile and male broodstock Japanese eel

Nutrients	Juvenile	Male broodstock
Vitamin C	41.1-43.9	410.7-911.7
Vitamin E	21.2-21.6	199.7-212.9
Arachidonic acid	69-71	71-92

## PRODUCTION OF BASIL VARIETIES IN THE UVI COMMERCIAL AQUAPONIC SYSTEM

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Basil (*Ocimum basilicum*) is a fast-growing crop on aquaponics systems. Previous research has indicated that basil is a high cash-value for aquaponics, and the evaluation of specie adaptation in tropical conditions is mandatory to recommend new varieties for the UVI Commercial Aquaponic System. Two trials were conducted to assess different basil varieties for production in aquaponics. On Summer 2015 we evaluated five basil varieties ('Genovese', 'Lemon', 'Purple Ruffles', 'Red Rubin', and 'Spicy Globe'), arranged on a completely randomized block design (CRBD) with four replications. On Fall 2015 we evaluated seven varieties ('Cinnamon', 'Genovese', 'Lemon', 'Purple Ruffles', 'Red Rubin', 'Spicy Globe', and 'Thai'), arranged on a CRBD with three replications. On both trials, 3-week old seedlings were transplanted in net pots at a density of 16.15 plants/m<sup>2</sup>. The UVI Commercial Aquaponics System consists of four fish rearing tanks, two cylindro-conical clarifiers, four filter tanks, one degassing tank, six deep flow culture hydroponic vegetable production troughs), one sump and one base addition tank. The entire system contains 110 m<sup>3</sup> of water and 214 m<sup>2</sup> of plant growing area, and occupies a land area of 0.05 ha. All varieties adapted well to the aquaponic culture conditions. On Summer 2015, 'Genovese' (14.909 kg/m<sup>2</sup>) and 'Spicy Globe' (13.991 kg/m<sup>2</sup>) presented the highest total yield and 'Purple Ruffles' the lowest (4.181 kg/m<sup>2</sup>) ( $P < 0.0001$ ). Fresh weight followed the same trend ( $P < 0.0001$ ), and dry weight was higher on 'Genovese' (0.890 kg/m<sup>2</sup>) ( $P < 0.0001$ ). As expected, leaf anthocyanin was higher on the red varieties 'Red Rubin' (28.35 ACI) and 'Purple Ruffles' (34.36 ACI) ( $P < 0.0001$ ). Chlorophyll content was higher on 'Genovese' (48.59 CCI) ( $P < 0.0001$ ). On Fall 2015, 'Cinnamon' (6.596 kg/m<sup>2</sup>), 'Genovese' (6.701 kg/m<sup>2</sup>), and 'Spicy Globe' (6.349 kg/m<sup>2</sup>) showed the highest total yield and 'Purple Ruffles' the lowest (1.677 kg/m<sup>2</sup>) ( $P < 0.0001$ ). Fresh weight followed the same tendency ( $P < 0.0001$ ), and dry weight was higher on 'Cinnamon' (0.328 kg/m<sup>2</sup>) ( $P < 0.0001$ ). Leaf anthocyanin differed in all varieties over time, with larger values on 'Purple Ruffles' (80.5 ACI) and 'Red Rubin' (36.5 ACI) ( $P < 0.0001$ ). Chlorophyll content was a response of variety and plant growth, ranging from 12.06 ('Lemon') to 17.99 CCI ('Cinnamon') ( $P = 0.0002$ ). Values increased overtime ( $P < 0.0001$ ). Plant growth index was higher on 'Genovese' and 'Lemon' (Summer 2015) on DAT 58 ( $P < 0.0001$ ), and higher on 'Cinnamon' (Fall 2015) on DAT 87 ( $P = 0.0014$ ). The total yield reflected the measured plant morphology. Yield was greater during Summer 2015, which was conducted May-August. Yield declined for the fourth harvest, indicating that a farmer may want to terminate production after the third harvest and replant the crop. Fall 2015 trial was conducted September-November and shortening day length ( $\approx 1$  h) and changes in environmental conditions could have impacted yield. Based on our results, we recommend 'Genovese' and 'Spicy Globe' during Summer and Fall, were Cinnamon' is also an alternative. 'Purple Ruffles' was the smallest variety. Varieties with smaller plant size can be spaced closely to increase total yields.

## IMPROVING CANTALOUPE FRUIT SUGAR CONTENT IN THE UVI COMMERCIAL AQUAPONIC SYSTEM

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Controlled water stress imposed during the reproductive stage of fruit crops are well-known for increasing flowering and fruit quality. However, deficit irrigation is challenging to apply on recirculating aquaponics systems due to the use of deep water hydroponic troughs for vegetable production. Our study evaluated the effect of partial root and canopy cut performed before two different harvest schedules on cantaloupe fruit sugar content. The UVI Commercial Aquaponic System used consisted of three main components: fish rearing, solids removal and hydroponic vegetable production troughs. The hydroponic troughs were 30×1.2×0.3 m with a volume of 11.3 m<sup>3</sup> and a surface area of 214 m<sup>2</sup>. The water flow rate on the troughs was 125 L/min for a retention time of 3 h. Fish waste products were the source of nutrients for plant growth. Three-week old cantaloupe ‘Goddess’ seedlings grown on peat-based substrate were transplanted into 1.2×2.4-m (2.97 m<sup>2</sup>) Styrofoam® rafts on the aquaponics system on Oct 2, 2015 (day after transplanting, DAT 1). We planted 2 plants/raft spaced every 1.2×1.2 m in a density of 1.485 plants/m<sup>2</sup> and used 12 rafts/trough. Our treatments were the combination of partial root and canopy cuts (0%, 25%, 50% and 75%) in two harvest schedules (10 days after cutting or at fruit maturation), resulting in 20 treatments, in a CRD with two replications. The root and canopy cutting was performed on DAT 37. The first and last harvest were performed on DATs 47 and 64. Sugar content increased only on the treatments with no root cut, 75% and 25% of canopy cut and fruits harvested at the maturation (9.1 and 8.4°Brix), with a negative effect on the treatment with 75% of root cut, no canopy cut and fruits harvested at the maturation (3.7°Brix) ( $p=0.0060$ ). The treatment with no root or canopy cut and fruits harvested at the maturation promoted the highest yield (30,227 kg/ha), indicating a negative effect of the partial cutting on fruit total yield ( $p=0.0072$ ). No treatment response was found on fruit length and width, fruit hardness, fruit pulp thickness, leaf chlorophyll and anthocyanin content, root and shoot dry weight and shoot fresh weight ( $p>0.05$ ). Even though our results indicated an increase in fruit sugar content, more research is necessary to develop an alternative cultural practice for increasing cantaloupe fruit sweetness in aquaponics without compromising total yield.

## HEMATOLOGICAL AND BIOCHEMICAL RESPONSE OF *Clarias gariepinus* (AFRICAN CATFISH) JUVENILE EXPOSED TO THERAPEUTIC CONCENTRATIONS OF TETRACYCLINE

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Bacterial disease outbreak in fish is a bane to aquaculture development in Nigeria. Fish farmers engage antimicrobial agents therapeutically and prophylactically to abate economic loss resulting from mortality often experienced in cultured infected with bacteria disease. Prolong bath of tetracycline concentration ranging between 50 – 100mg/l was recommended for treatment of fish infected with bacteria from temperate region. Fish farmers in Nigeria have limited information on the dosage of microbial agents for indigenous fish species thus, rely on recommended dosage for exotic fish species. Since the toxicity of chemicals is specie dependent, it is indispensable to provide information on the response of *Clarias gariepinus* to therapeutic concentrations of tetracycline.

In a 5 x 3 complete randomised experimental design and a static renewal method of toxicological study, *Clarias gariepinus* juvenile of average weight of  $11.77 \pm 0.57$ g stocked at 15 fish/20 liters of water were exposed to 0, 25, 50, 75 and 100mg/l of tetracycline in a 30 days trial. The 0mg/l of tetracycline exposure served as the control treatment. At the end of the exposure period, fish sampled from each treatments were bled through caudal puncture to obtain blood sample for haematological and biochemical analysis. Data obtained were subjected to one-way analysis of variance and significant difference tested at  $p < 0.05$ . Duncan multiple range test was used for post-Hoc analysis.

There were no significant difference ( $p > 0.05$ ) in the haematological and biochemical parameters measured when compared with the control group except the White Blood Cell (WBC) count. There was significant increase ( $p < 0.05$ ) in the WBC count of fish exposed to 25 and 75 mg/l of tetracycline when compared with the control. The WBC of fish exposed to 25 and 75mg/l of tetracycline were not significantly different ( $p > 0.05$ ) from those exposed to 50 and 100mg/l of tetracycline.

Remarkable alteration in the health status assessed from haematological and biochemical parameter of *Clarias gariepinus* exposed to therapeutic concentration of tetracycline was not evident in this study. The use of tetracycline concentration ranging from 25 to 100mg/l as a therapeutic treatment on *Clarias gariepinus* would not inflict negative effect on their physiology.

## THE EFFECTS OF SUPPLEMENTARY LED LIGHTS ON THE FUNCTION OF BIOFLOC SYSTEMS AND GROWTH OF TILAPIA

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Biofloc-based aquaculture is becoming more common due to low rates of water use, enhanced biosecurity, and low feed conversion rates. Biofloc systems focus on cultivating a dense microbial community which may include autotrophic bacteria, heterotrophic bacteria, and photosynthetic algae. The microbes may recycle nutrients into biomass that animals can consume, thereby decreasing the amount of feed that must be added. Microbes can also convert ammonia into much less harmful nitrate, reducing the need for external filtration or water exchange. Algae add oxygen to the water through the process of photosynthesis and often contain beneficial fatty acids and proteins. Increased oxygen production and greater algal abundance can lead to improved water quality which may, in turn augment fish production. Artificial lighting may be used to supplement natural sunlight and improve algal health and abundance; some of the most efficient lighting available is LED technology.

To assess the effect of supplemental LED lighting in biofloc-based aquaculture systems we stocked tilapia (*Oreochromis niloticus*) with a mean weight of 36.7g into twelve 13 m<sup>3</sup> tanks at 88 fish/m<sup>3</sup>. Four tanks were contained in each of three high tunnel greenhouses. Two of the tanks in each high tunnel were randomly assigned to an extra light treatment (EL) with LED light arrays, and two received only natural light (NL). The light arrays consisted of 15 individual, 15W LED bulbs hung from a wooden frame twelve inches above the water surface. Each bulb contained 126 LEDs (90 red, 36 blue) that create light in the red and blue spectrums. The LED arrays were operated twenty-four hours a day to maximize the effect they have on the systems.

Although final data are still pending, results indicate that the LED arrays had a positive effect on water quality. In EL tanks ammonia concentrations were generally lower and algal concentrations tended to be higher, as indicated by chlorophyll measurements. However, an increase in foam on the water's surface of all tanks began to block the light. The abundant surface foam corresponded with an increase in ammonia levels and a drop in algae concentrations. In response foam fractionators were constructed and installed on all tanks which drastically reduced the surface foam. In addition, pH and DO were generally higher in the EL tanks, especially before and after the development of surface foam.

This project helps to refine some of the management tactics necessary for establishing tilapia biofloc systems and indicates that supplemental lighting may benefit system performance. Forthcoming results will indicate whether these changes may translate to improvements in fish production.

## **EVALUATION OF EFFLUENT WASTE WATER FROM SALMONID CULTURE AS A POTENTIAL FOOD AND WATER SUPPLY FOR CULTURING LARVAL PACIFIC LAMPREY *Entosphenus tridentatus***

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Pacific lamprey populations have declined in abundance from historic levels in the U.S. Pacific Northwest. The decline of this species has raised concern among tribal, state and federal management agencies. Conservation efforts involving this native species include habitat restoration, improvements to fish passage systems, and development of culture techniques. The present research focused on refining the culture methods for the larval stage of the species. The first objective of this study was to test the viability of waste water from salmonid culture as a potential food source and water supply for raising the ammocoete (larval) stage of the Pacific lamprey. Larval lamprey filter feed on detritus and cohabitate with juvenile salmonids in the wild, therefore the effluent water from rearing salmonids may be a viable source of water and food for culturing lamprey. Rearing lamprey in this manner could be a sustainable method for raising lamprey at existing salmonid hatcheries. The second objective was to determine if the quality of the effluent water is improved by running through lamprey culture tanks before it leaves the facility. Release of nutrients in effluent water could have negative impacts on wild systems, and the filter feeding action of the lamprey may sequester nutrients from the water thus improving the quality of the effluent stream.

The experimental unit in this study was a combination of a steelhead trout (*Onchorynchus mykiss*) tank containing 150 juveniles and a lamprey tank containing 90 fish. Flow through well water entered the steelhead tank and then flowed into the lamprey tank for a second use. To complete the first objective, three treatments with three experimental units each tested the use of the effluent, a conventional lamprey diet, or both combined as a means for rearing lamprey. For the second objective a fourth treatment with three replicate systems where lamprey were excluded served as a control to test the effect of lamprey presence on effluent quality. After nine weeks of study, growth, survival, proximate composition and fatty acid profile of the remaining lamprey were assessed. The quality of the effluent water as it left the lamprey tanks was also analyzed. The results of this research may provide additional methods for producing quality lamprey and to potentially reduce effluent concerns from salmonid aquaculture.

## DIETARY INCLUSION OF A *Saccharomyces cerevisiae* FERMENTATION PRODUCT TO A COMMERCIAL RATION IMPROVES IMMUNE READINESS AND COLUMNARIS DISEASE RESISTANCE IN HYBRID CATFISH *Ictalurus punctatus* X *Ictalurus furcatus*

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Columnaris disease, caused by *Flavobacterium columnare*, continues to be one of the most detrimental bacterial diseases of catfish commercially grown in the US. There are currently few effective weapons available to combat this disease. Catfish producers are eager to gain protection against disease in a more natural and cost-effective manner, i.e. through a diet supplemented to provide for mucosal health as well as performance. In the present study, we evaluated a *Saccharomyces cerevisiae* fermentation product called Diamond V XPC. The trial featured four levels of XPC at rates of 0, 2.5, 5, and 10 lb per ton which were added to a commercial 32% protein floating catfish ration. Following six weeks of feeding, there were no differences in growth except in fish fed the 5 lb/ton dose, which were significantly larger ( $80.7 \pm 1.6$  g) than control fish ( $75.2 \pm 2.2$  g) at the end of the 6 week trial ( $P < 0.05$ ). There were no significant differences between the feed conversion ratios (FCR) of any treatment. Hematological analyses revealed differences in the cellular composition of the blood in XPC fed animals as compared to the control group. White blood cell numbers were significantly higher ( $P < 0.001$ ) in the 5 and 10 lb/ton treatment. Differences were also evident in the red cell compartment, with the absolute number of red blood cells being significantly greater in the 2.5 and 5 lb/ton treatments. At the end of the feeding trial an *F. columnare* challenge was performed to examine the influence of Diamond V XPC on columnaris disease susceptibility. The challenge revealed that animals fed XPC showed significantly better survival; however, the overall mortality was relatively low with 16.3%, 0%, 0%, and 1.25% mortality observed in the 0, 2.5, 5, and 10 lb/ton treatments respectively. Trials are ongoing to repeat this work in channel catfish *Ictalurus punctatus*.

## MICROFLUIDIC DEVICE FOR MOTILITY AND OSMOLALITY ANALYSIS OF ZEBRAFISH SPERM

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An increasing number of laboratories are evaluating sample quality via motility analysis by means of computer-assisted sperm analysis (CASA) after sperm activation by manual dilution and mixing. Even with the use of CASA, due to user variation, there is a lack of control over the activation process, resulting in inconsistent motility analysis. Low sample volume ( $\sim 1\text{-}2\mu\text{L}$ ), and a short motility duration (burst motility of less than 15s) add to the complexity of these difficulties.

The objectives of this study were to develop a microfluidic device with the capabilities to (1) standardize the method of activation for zebrafish sperm so that all cells in a sample are subjected to the conditions needed to activate in a reproducible way, (2) reproducibly enable motility analysis of the activated sample within 5 s after activation without the interference of bulk fluid flow, and (3) facilitate the generation of activation curves by relating osmolality of the sample solution to percent motility at the time when motility analysis was performed. The device described here is a three-inlet microfluidic platform fabricated from polydimethylsiloxane (PDMS) bound to a glass substrate with a microfabricated gold floor electrode for osmolality detection. A passive micromixer is utilized to activate sperm samples, and a novel flow control system was designed to aid with the demands of sample analysis.

The device demonstrated consistent zebrafish sperm activation and osmolality detection. The device was also able to consistently reach flow cessation in under 1s, allowing for rapid analysis of the sample. This device represents a pivotal step in streamlining methods for consistent, rapid assessment of sperm quality for zebrafish and other aquatic species. The capability to rapidly activate sperm and consistently measure motility with CASA using the microfluidic device described herein will help improve the reproducibility of studies on aquatic species germplasm physiology.

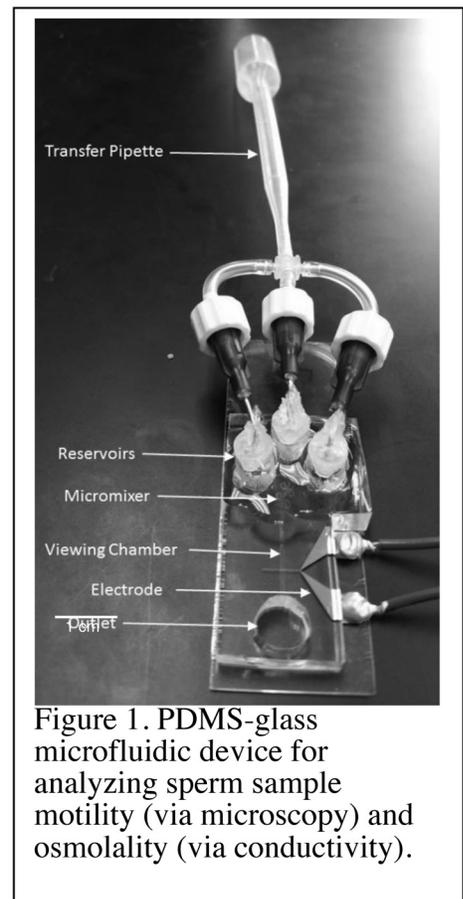


Figure 1. PDMS-glass microfluidic device for analyzing sperm sample motility (via microscopy) and osmolality (via conductivity).

## STATUS OF FISH FARMING IN SHIKTAHAN VDC OF RUPANDEHI DISTRICT OF NEPAL

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A survey was conducted in 30 households to study the status of fish farming in Shiktahan VDC of Rupandehi district in 2014. It was found that 26.7% of respondents have pond area between 84 and 169 m<sup>2</sup>, whereas 43.3%, 16.7% and 13.3% have pond area between 170-253 m<sup>2</sup>, 254-338 m<sup>2</sup> and above 338 m<sup>2</sup> respectively. Most farmers (86.7%) were dependent on rainfall because fish farmers in this area are poor and unable to afford a motor pump. Only 6.7% of the farmers owned motor pump to pump water for their pond while 6.7% were using irrigation canal as a source of pond water. Silver Carp (*Hypophthalmichthys molitrix*) was cultivated in 100% of the surveyed household. Common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), big head carp (*Hypophthalmichthys nobilis*), rohu (*Labeo rohita*) and naini (*Cirrhinus mrigala*) were cultivated by 86.6%, 93.3%, 40%, 63.3% and 6.7% households respectively. Of the total stocked fingerlings in the pond, 39.6% were silver carp, 17.7% common carp, 16.4% grass carp, 9.7% big head carp, 13.8% rohu and 2.7% naini. Comparing the total harvest, 44.8% was obtained from silver carp, 18.8% from common carp, 14.2% from grass carp, 11.3% from rohu, 8.5% from bighead carp and 2.3% from naini. Similarly while evaluating the total income of pond harvest, 36.3% of total income was contributed by silver carp, 20.1% by common carp, 19.5% by grass carp, 12.2% by bighead carp, 10.1% by rohu and 1.8% by naini.

100% of the farmers used IAA based carp polyculture technology for fish farming. This is incorporation of agriculture with aquaculture. In this technology different vegetables like pumpkin, cucumber, sponge gourd, bitter gourd, ridge gourd, okra etc. are cultivated on pond dikes depending on the season. This helps to generate the secondary income and also minimizes the risk of fish farming as vegetable production may compensate if there is economic loss from fish farming. The study concluded that fish farming has helped to improve economic development of rural VDC members. There is great potential for fish farming in this area because fish demand is higher than supply. Despite of this fact farmers are unable to meet the demand because of lack of knowledge, source of pond water, and threats from disease.

## LARVICULTURE DEVELOPMENT FOR THE AFRICAN RIVER PRAWN *Macrobrachium vollenhovenii*

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The African river prawn *Macrobrachium vollenhovenii* is a large decapod crustacean endemic to the west coast of Africa from the Senegal River to Angola. Historically, the Senegal River basin supported an artisanal prawn fishery extending from the coast to more than 200 miles inland. Due to its relatively large size (>100g) and high market value, *M. vollenhovenii* has been proposed as a candidate for commercial aquaculture in the region. The development of species specific larviculture technologies is the primary bottleneck to commercial production. To date very little research has been done related to the propagation of this particular species. Larval development in Palimonidae crustaceans is characteristically complicated by a relatively long hatchery phase where larvae undergo several stages of transformational development before reaching the post-larval stage when they are ready for freshwater. Several attempts using established protocols for the commercially important Asian prawn *M. rosenbergii* have proven unsuccessful with survival to post-larvae consistently <1%.

Initially, a series of static assays were conducted to provide basic information of optimal salinity and safe levels of ammonia for larviculture. The iso-osmotic salinity was estimated to be 16-18 ppt after a 5 day starvation test with freshly hatched stage one larvae where tested salinities ranged from 0-24 ppt. To determine safe levels of ammonia, a 96 h static ammonia assay was performed on stage one larvae. The nominal concentrations tested ranged from 2 to 16 mg NH<sub>4</sub>/L. Water temperature was maintained at 26°C by use of a single water bath and a submersible heater. The salinity of the test water was 16 ppt and the pH was 8.3. The 96 h LC50 value was calculated to be 7.4 mg/L. These results suggest that *M. vollenhovenii* may require higher salinity and exhibits a lower tolerance to ammonia compared to *M. rosenbergii*. Over a two year period many trials were conducted using different feeding protocols with continued poor survival to PL in recirculating systems where ammonia concentrations routinely spiked to over 0.5 mg/L during the >90 day larval cycle. A recent trial evaluated an outdoor “greenwater” system which resulted in 25% faster progression to stage 7; while maintaining ammonia concentrations <0.1 mg/L. Greenwater systems may provide more efficient removal of nitrogenous waste compounds and may also provide a nutritional contribution through direct feeding on algae and/or continued enrichment of *Artemia*.

## COMPARISON OF POND AND RACEWAY PRODUCTION METHODS ON TEXTURE OF CHANNEL CATFISH (*Ictalurus punctatus*) FILLETS, SHOWING A DEPENDENCY ON SIZE AND FILLET POSITION

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The objective of this study was to compare the effect of the production environment (pond vs in-pond raceway) on the chemical composition, color, and textural properties of channel catfish fillets. Compositional analysis consisted of percent moisture, lipid, protein, and ash content. Additional samples were baked in foil for presentation to ten trained sensory panelists or subjected to a mechanical texture analyzer.

Although sensory analysis showed no significant differences, mechanical texture analysis was able to show a dependency of the measured hardness value on the fillet thickness, with a slight divergence between pond and raceway as the fillet thickness increased. The fillet thickness was also related to the size of the catfish, therefore an increase in catfish size would show an increase in mechanical hardness, or firmness. Since the fillet shows an increase in thickness from tail to head, the firmness is also seen to increase in this same direction.

Because the sensory panel analysis was based on the average of all catfish from each sample, their inability to distinguish a difference in firmness would be related to the average thickness of raceway samples (7.5 mm) and pond samples (8.3 mm) having a corresponding calculated hardness value of 105.6 g and 105.8 g. These values are not significantly different.

Color analysis determined catfish fillets from ponds had a greater red color than those from raceways. But no significant differences were found for proximate composition. Overall results imply that either pond type can be used to produce catfish without adversely affecting composition or texture.

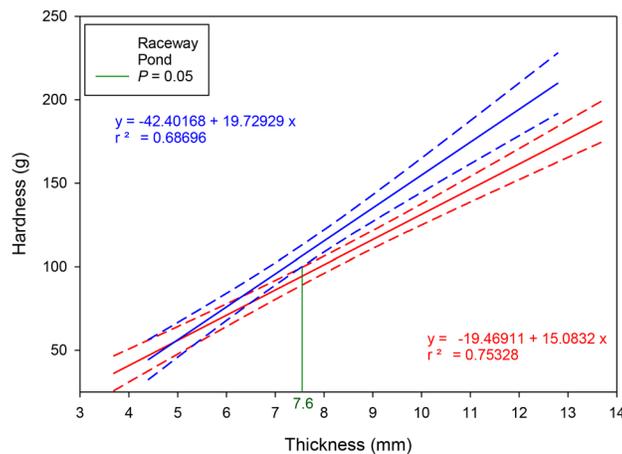


Figure 1. Relationship of mechanical hardness to fillet thickness.

## **BEAD FILTER APPLICATIONS IN MARINE AQUACULTURE AT THE UNIVERSITY OF SOUTHERN MISSISSIPPI**

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Established in 2006, The Thad Cochran Marine Aquaculture Center (TCMAC) transformed the Gulf Coast Research Laboratory's aquaculture program into a modern state-of-the-art 100,000 ft<sup>2</sup> marine hatchery. Because of the lack of a consistent supply of quality seawater and restrictions on effluent discharge, the hatchery was designed as a closed system, recirculating facility operating primarily on artificial salt water. As such, primary design criteria were to achieve minimal discharge and maximal retention of valuable artificial salt water. Other design criteria for our budget-conscious operation included low maintenance, small footprint, efficient filtration of fine particles, longevity and resistance to corrosion and low pressure operation which allows for the use of cheaper high flow/low head pumps. Bead filters are ideally suited to meet these criteria.

All TCMAC finfish filtration designs are variations on a single plan and include mechanical filtration, a moving bead bioreactor, foam fractionation, appropriate sanitization (ozone or UV), and oxygenation. Our designs have successfully employed Polygeyser Drop Bead filters, Bubble Bead filters, and Propeller-washed Bead filters for mechanical filtration in a variety of applications. Polygeysers accomplish the least water loss per backwash and, depending on which bead design is used, can provide some biofiltration, which we have found to be sufficient in some systems. In our experience they are most suited for broodstock applications with low biomass and a relatively low level of fine particles. Bubble Bead filters lose more water per backwash than Polygeysers, but they allow control over backwash frequency, and they require no external inputs (i.e., air or electricity). Bubble bead filters are ideally suited for broodstock and larval rearing applications where a variety of particle sizes must be filtered. Propeller-washed Bead filters, although they can lose more water per backwash than either Polygeysers or Bubble Bead filters, provide maximum backwash flexibility and are ideally suited for high density, high biomass nursery and growout systems where feed rate and amount changes require frequent adjustment to backwash schedules.

Possible disadvantages include the potential for returning some fine particles to the system following backwashes, the low pressure thresholds of the units which require the use of a pressure bypass valve to avoid damage due to pressure increases, the time required for the bead bed to "mature" to achieve maximum filtration efficiency, and complexity of automation.

Overall, bead filters have made it possible for the TCMAC to use a common design to achieve substantial production capabilities while meeting our goals of salt water conservation, low discharge, and a budget-conscious operation.

## THE USE OF DIETARY ADDITIVES AS A MEANS OF COUNTERACTING ELEVATED TEMPERATURE IN RAINBOW TROUT: GROWTH, EFFICIENCY AND PHYSIOLOGICAL PERFORMANCE

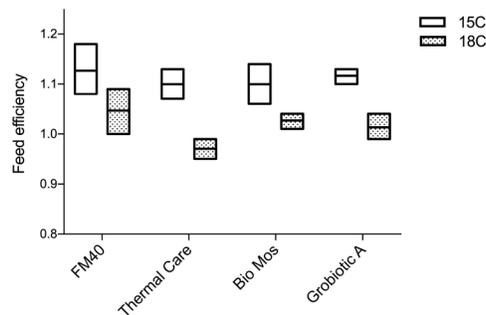
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Global surface temperatures are projected to rise 2-4°C by the year 2100. The consequences of this environmental change on physiological function will alter production efficiency in the rainbow trout (*Oncorhynchus mykiss*) industry with concomitant regional and economic impacts. Here, we examine the effects of warming on growth, feed efficiency, and performance, and whether dietary additives can ameliorate the metabolic costs associated with elevated temperature.

Rainbow trout (44 g) were stocked into one of two recirculating systems maintained near trout's thermal optima of 15°C or at an elevated temperature of 18°C. Each dietary treatment was stocked into triplicate 440 L polyethylene tanks with 15 fish per tank. Individuals were fed a control fishmeal-based diet or one of three treatment diets including either a prebiotic (Bimos and Grobiotic A) or a metabolic modifier (Thermal Care). Diets were randomly assigned to triplicate tanks and fed twice daily to apparent satiation for 12 weeks. Fish were weighed every three weeks to assess growth and feed efficiency. At termination of the trial, three fish per tank were collected for whole-body proximate analysis and nutrient retention efficiencies. Blood from an additional three individuals was analyzed for acid-base balance and various metabolites. Five individuals per treatment were used to determine changes in maximum swimming speed. Data were compared using two-way ANOVA. When a significance level of  $p \leq 0.05$  was found, Dunnett's contrast was used to compare treatments to fish fed the control diet at 15°C.

Elevated temperature had a significant effect on survival. Further, there was an interactive effect of temperature and diet on growth (gram gain per fish). Feed efficiency was higher and feed intake lower for individuals at 15°C than individuals at 18°C. There was a drop in plasma  $\text{HCO}_3^-$  from 15°C to 18°C and this effect was suppressed in fish fed Grobiotic A but not the other dietary additives. Other plasma metabolites also showed changes with temperature and diet. There was no change in maximum swimming speed between treatments; however, plasma  $\text{PCO}_2$  was lower at exhaustion in 18°C fish fed a control diet than those at 15°C and this drop was suppressed in fish fed the dietary additives. Efficacy of these dietary constituents for physiological enhancement and implications for future productivity will be discussed.



## DEVELOPMENTS IN CAPTIVE BREEDING AND REARING OF *Selene vomer*

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The Atlantic Lookdown, *Selene vomer*, is a popular fish in both the private and public aquaria trade. *S. vomer* belongs to the Carangidae family and has a wide distribution ranging from the northern Atlantic to the Caribbean. It is considered to be one of the most desirable roving, predatory fish in the aquarium trade. Thousands of *S. vomer* are taken from the wild each year by public aquaria alone. Breeding *S. vomer* in captivity will reduce strain on wild populations and increase popularity of the fish in private and public aquaria. Here we present detailed methodology on the captive broodstock conditioning, egg collection, and larval rearing of this species.

A mature population of *S. vomer* broodstock (two males, eight females) is kept at the New England Aquarium's offsite holding facility in Quincy, MA in a 3,000 L recirculating seawater system. Broodstock were cannulated to determine gender and gonadal development. Females were injected with the slow release sGnRH $\alpha$  hormone implant OvaPlant (Syndel) and spawning ensued 36 hours post injection. The eggs were collected and transported to Roger Williams University, where they were sanitized, enumerated (N=30,000) and distributed among four isolated 200 L tanks and 12, 70 L tanks contained within two (6 tanks per system) 600 L recirculating systems. All tanks were outfitted with 40  $\mu$ m outflow screens and subjected to light aeration. The four 200 L tanks were initially non-recirculating, but converted to a recirculating system with mechanical and biological filtration by day 30. The eggs hatched 24 hours post fertilization. Larvae hatched without eyes, mouth, or gut, and transitioned to exogenous feeding by day 3. On day 3, limited by space, we reduced the larval density by 75% (~8,000 larvae remained) and fed *Parvocalanus* sp. copepods (5 nauplii and 2 adults/mL). Flexion occurred at day 8-10, and post-flexion larvae were transitioned onto L-strain rotifers (*Brachionus plicatilis*; 5-10/mL) and instar I brine shrimp (*Artemia* sp.; 5-10/mL). 90% water changes were performed three times daily. By day 14, larvae were transitioned onto adult brine shrimp (10/mL; enriched with microalgae Tahitian *Isochrysis galbana*) and frozen copepods, and by day 33 exclusively ate Skretting marine pellets. Larvae settled by day 20, and juveniles reached marketable size (2-3 inches) by day 60, at which point they were relocated to four 1,000 L flow-through seawater tanks. Each tank housed 500 fish; water changes were performed twice daily and fish were fed three times daily. In total, raised over 2,500 fish from this one spawn, which were shipped to US-based wholesalers for distribution into public and private aquaria across the country.

The techniques gleaned from the rearing of this cohort are the first detailed procedures reported for this species. Due to the robustness of the larvae, with adequate space and proper filtration, we predict high yields of cultured *S. vomer*, upwards of at least 75%. The technology can be transferred to commercial farmers to increase cultured production of this and similar species to meet the growing demand of the aquarium trade.

## **RESEARCH INVESTMENT IN AQUACULTURE FEED TECHNOLOGY DEVELOPMENT AND TRANSFER FOR GLOBAL FOOD SECURITY**

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Investment in science and innovation is fundamental to developing effective solutions to many of the social, economic, and ecological challenges associated with global food security. Transferring new and improved technologies to farmers and industry is essential for scaling up farming technologies and best practices for broad adoption. The AquaFish Innovation Lab develops sustainable aquaculture technologies and systems, with a research portfolio that includes a focus on improving feed efficiencies. Supporting and partnering with international research institutions allows AquaFish researchers to customize technologies for local conditions and on-the-ground needs as part of this effort to create sustained impacts and effective technology transfer.

The development of sustainable and affordable feeds and feed strategies for small- and medium-scale aquaculture in Asia and Africa is a major focus of AquaFish research. Quality fish feeds are critical for optimal fish growth and production; however, commercial fish feeds are often expensive, lack reliable ingredients, and typically are not environmentally sustainable as they commonly contain fish meal as the main protein source. The greatest production cost for farmers is feed, comprising about 70-90% of total costs in grow-out systems. The high cost and variability of commercial fish feeds remains a barrier to the profitability of aquaculture operations and is exacerbated by the common practice of overfeeding.

The use of alternative feed sources and feeding strategies can decrease production costs, reduce reliance on expensive feeds, increase overall fish yield, and decrease environmental impacts associated with aquaculture effluents. Innovative research on feed formulations is resulting in improved low-cost, alternative feeds that incorporate the use of locally produced, high quality protein sources such as earthworms and maggots. Research on reduced feeding strategies, such as feeding fish every other day, can successfully decrease input costs without decreasing production. Similarly, research on enhancing periphyton growth in ponds has shown increases in naturally-occurring food sources for fish that allows for a 50% reduction in feed use and an increased gross margin.

## DEVELOPING BASELINE POPULATION AND DIVERSITY STATISTICS FOR ASSESSING AQUACULTURE'S ROLE IN RESTORATION OF EASTERN OYSTERS *Crassostrea virginica* IN DELAWARE INLAND BAYS

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Delaware is currently the only state on the Northeast Atlantic seaboard without commercial shellfish aquaculture. As the push for legalized aquaculture grows, legislation is developing policy and protocols for implementation. Neighboring states have shown the economic and cultural benefits of functioning industry. Three inland bays in Southern Delaware, due to protection from open waters and ease of access for workers, offer promising future locations for bottom leases. Oysters are functionally extinct within the Bays and with the rapid development of the local watershed, the ecological services oysters contribute are more important than ever. Oyster aquaculture can help restore depleted wild populations of oysters while filtering the water, providing structural habitat, and creating a new sources of jobs. There is a unique opportunity to study directly how aquaculture facilitates restoration, but baseline statics are essential. This research aims to further understand the current oyster population by 1) developing baseline population locations and standardized survey methods to be used as a management to measure changes over time and 2) investigating population dynamics by analyzing genetics of spatfall within the Delaware Inland Bays.

Spat collectors were designed using PVC and upright ceramic tiles in a design modified from the University of North Carolina. After five weeks in the field they were removed to be examined for recruitment. Genetic analysis is intended to see if a disease resistant oysters, from Rutgers University Haskins Shellfish Laboratory, supplied to local oyster gardening programs are spawning and populating the Bay.

Current results show consistency in spat sets for Indian River Bay waters while significant differences are recorded in spat sets in Rehoboth Bay during three years. Little Assawoman Bay oyster sets is also consistent but the second season yielded no oysters possibly due to genetic variance in spawning. (Fig. 1).

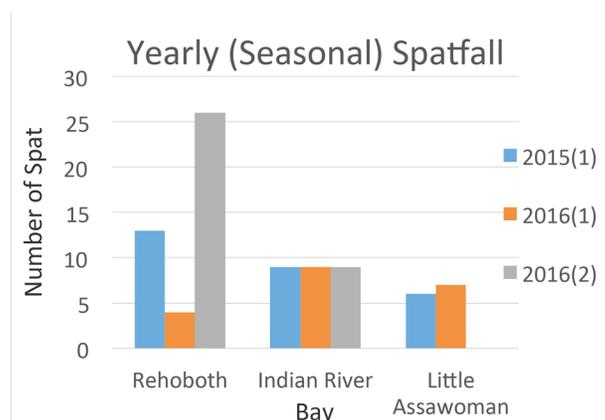


Fig. 1. Natural spat settled on ceramic tiles placed in various locations in the bays. Season (1) occurred from June 13-July 18 and season (2) occurred from July 18-August 22.

## EVALUATING THE POTENTIAL OF DETRITUS AS A SUPPLEMENTAL DIET FOR BIVALVE AQUACULTURE USING STABLE ISOTOPES AND FATTY ACID BIOMARKERS

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There has been increasing evidence that bivalves are not solely herbivorous and are indeed omnivores. One of the many alternative food resources available to bivalves besides phytoplankton is detritus. Detritus has been found to be important for cultured bivalves which have been shown to be able to incorporate detrital material into their diets. For example, *Mytilus edulis* cultured off the shores of Ireland have been found to utilize a mix of phytoplankton and detritus in their diet.

As a commercially important aquaculture species *Mytilus edulis* is generally reared in areas selected for high phytoplankton abundance with little consideration of other potential food sources such as detritus. With increasing evidence that bivalves do utilize detritus understanding which detrital source is utilized and to what degree by bivalves becomes an important question for aquaculture.

This study will characterize the biochemical composition of detritus along the coast of Maine using stable isotope, lipid and fatty acid analysis. Samples were collected from six locations throughout Saco Bay, ME, from April until Nov. 2016. Stable isotopes and fatty acid biomarkers will be used to trace detrital material back to its source while nutritional quality will be determined by the percent organic content and C:N of detritus as well as its lipid and fatty acid composition. Comparison of the stable isotope and fatty acid signature of bivalves in the field with that of local detritus will inform if ingestion of the material occurs and what proportion of the diet it comprises. Areas suspected to be dominated by a single type of detritus, i.e. salt marsh, macroalgae or phytoplankton, will be selected in order to ascertain if there is any effect of detrital source on nutritional quality and contribution to bivalve diets. Nutritional quality, likelihood of ingestion, presence in the environment as well as bioavailability will be used to evaluate detrital types for their potential to be used as bivalve diets for aquaculture.

The goal of evaluating the potential of detritus to be used as a supplemental diet for bivalve aquaculture is to create another criterion that could be used for site selection. Better site selection criteria will allow more informed aquaculture placement as well as help avoid use conflicts by allowing aquaculture to move out of crowded estuarine systems. By using stable isotopes and fatty acid biomarkers to evaluate detritus as a supplemental diet for bivalve aquaculture we will also be informing the role detritus plays within coastal foodwebs.

It is anticipated that preliminary results from the stable isotope analysis from the collected bivalves will be available for presentation.

## QUALITY CONTROL IN AQUACULTURE WATER ANALYSES

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Water analysis required to verify the effectiveness of water quality management procedures is becoming an important aspect of aquaculture. Thus, many producers make analyses on site, and samples often are sent to commercial or institutional laboratories for special analyses. Recent assessments have revealed large errors in analytical results for samples sent to laboratories, and there no doubt are many opportunities for errors in analyses done on site. There appears to be a general lack of quality control to assure that precision and accuracy are being achieved. Moreover, there is considerable reliance – especially at aquaculture facilities – upon water test kits. Some types of kits can produce reliable results; others may be notoriously inaccurate. This presentation will provide methods for assessing the precision and accuracy of water analyses. Simple methods of quality control that can be used for tracking precision and accuracy of water analyses made at aquaculture facilities will be explained. In addition, methods for assessing the probable accuracy of samples sent to outside laboratories will be discussed.

## SELECTIVE PRECIPITATION REACTION: A NOVEL DIAGNOSTIC TEST FOR TISSUE PATHOLOGICAL IN ATLANTIC SALMON (*Salmo salar*) INFECTED WITH SALMONID ALPHAVIRUS (SAV3)

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While investigating biomarkers for infection with salmonid alphavirus (SAV), the cause of pancreas disease (PD), a selective precipitation reaction (SPR) has been discovered in serum which could be an on-farm qualitative test and an in-laboratory quantitative assay for health assessments in aquaculture. Mixing serum from Atlantic salmon, *Salmo salar*, with SAV infection with a sodium acetate buffer caused a visible precipitation which does not occur with serum from healthy salmon. Proteomic examination of the precipitate has revealed that the components are a mix of muscle proteins, for example enolase and aldolase, along with serum protein such as serotransferrin and complement C9. The assay has been optimized for molarity, pH, temperature and wavelength so that the precipitation can be measured as the change in optical density at 340 nm ( $\Delta_{340}$ ). Application of the SPR assay to serum samples from a cohabitation trial of SAV infection in salmon showed that the  $\Delta_{340}$  in infected fish rose from undetectable to a maximum at 6 weeks post-infection correlating with histopathological score of pancreas, heart and muscle damage.

Through manipulation of protein stabilities in aqueous solutions a novel diagnostic tool has been identified in identifying the pathological damage elicited during pancreas disease (PD) in Atlantic salmon. This novel assay destabilises proteins, which during homeostasis are intracellular, from plasma/ serum samples thus serving as a useful non-destructive health screening tool. The assay has potential platforms as a quantitative in lab (Figure 1) and qualitative point of care (Figure 2) test. The cause of this differential precipitation reaction has been found through proteomic investigation to be due to the difference in stabilities to normal circulatory proteins and that of intra-cellular and some immune response proteins. This study details the discovery, optimisation, application, and potential for this novel assay

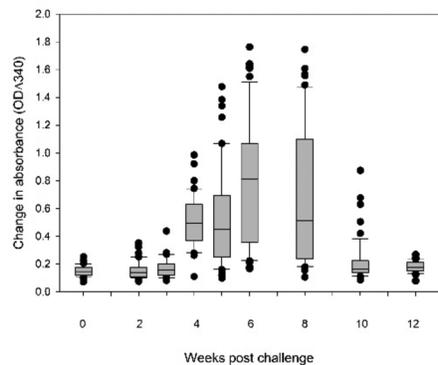


Figure 1. Quantified delta OD (340nm) from sequential sampling during pancreas disease challenge

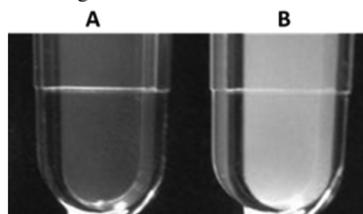


Figure 2. Observed difference in turbidity action elicited when adding either samples with no (A) or pathological damage to tissues during PD (B)

## DEVELOPING MARKETS FOR IMTA GROWN SEA VEGETABLES

Gabriela M. Bradt\* and Michael Chambers

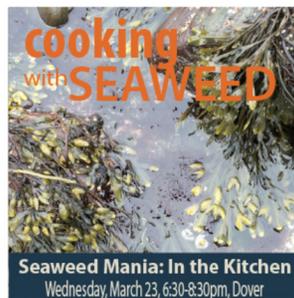
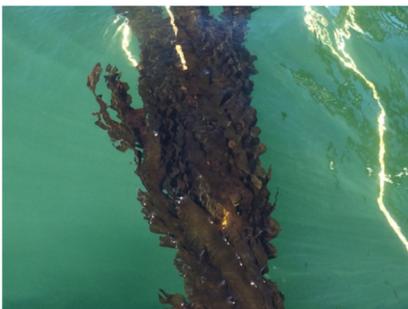
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*Saccharina latissima* and *Alaria esculenta* are two species of cold water kelps that are being grown as part of New Hampshire Sea Grant's Integrated Multi-Trophic Aquaculture project in New Castle, NH. With the increasing consumer interest in seaweeds/ sea vegetables for their inherent health benefits we have been developing workshops and partnerships with local restaurants and breweries to develop viable markets for the product in New Hampshire. Additionally, with the precarious nature of New England's iconic groundfish fisheries, we are also working to aid local fishermen to pursue seaweed aquaculture and or IMTA as alternative revenue streams.

We have observed an increase in awareness and interest regarding many aspects of these seaweeds from the health benefits, to the ecology to the use of sea vegetables in every day diets, based on our efforts through "learn and dine" workshops at local restaurants, foraging and identification workshops and presentations to local elementary and middle schools and other K-6 groups. Our recent efforts with local breweries to make sugar kelp beer has also brought to light the need for seaweeds in other aspects of brewing other than just flavor. With our continuing outreach efforts and development of partnerships in the food and beverage industry, we hope to develop a stable sustainable market for locally grown seaweeds for future seaweed farmers in the state.

### FIGURE 1A-C:

Examples of different outreach efforts for developing seaweed markets.



## **ASSESSING THE IMPACT OF ECOLABELS ON CONSUMER PREFERENCES FOR SEAFOOD: THE ROLE OF PRODUCTION METHOD, CERTIFICATION AND ORIGIN ON WILD HARVEST AND AQUACULTURE PRODUCTS**

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The production of seaweed and bivalve shellfish represents a substantial and growing sector of the U.S. and global seafood industry (NOAA, 2016; Watson, 2016). While the increase in production of bivalve shellfish and seaweed has been matched by heightened seafood demand, consumers often experience confusion over the characteristics of products during the purchasing process. At the same time, producers and retailers struggle to determine optimal methods of production and marketing to maximize profits. Seafood labels play an important role in the communication of product characteristics from the producer to the consumer and vice versa.

This paper explores the impact of ecolabels on consumer preferences for seafood products. We examine three key attributes that may impact consumer choice: (1) preferences for production method: wild harvest or aquaculture products; (2) preferences for organic (aquaculture) or sustainably harvested (wild harvest) products; and (3) whether a product is labeled as imported, from the U.S., or from a consumer's home state. We use data from a 2016 online consumer experiment (n=2000) and perform a conjoint analysis to improve our understanding of the role these three attributes may play in decision making. This work fills a key knowledge gap by examining consumer preferences for coastal shellfish (oysters, mussels, scallops and clams) and seaweed products across the nation.

Preliminary findings indicate that consumers prefer products from their home state regardless of production method or certification.

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## **STOCK ENHANCEMENT AS A SCIENCE-BASED TOOL FOR FISHERIES MANAGEMENT DECISION-MAKING: CASE STUDIES FROM 10 YEARS OF RED DRUM, STRIPED BASS, SPOTTED SEATROUT AND COBIA STOCKING IN COASTAL SOUTH CAROLINA**

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Over the past 10 years, the integration of the hatchery-based aquaculture, population genetics, and independent fisheries monitoring scientific disciplines within the South Carolina Department of Natural Resources has allowed the SCDNR Marine Stock Enhancement Research Program to follow “A Responsible Approach” in designing fisheries enhancement projects that address management objectives for stock enhancement (red drum), re-introduction (striped bass), restocking (spotted seatrout), and stock supplementation (cobia) of local species. With the application of genetic parentage analysis and progressive hatchery management, researchers have been producing genetically unique experimental units within each yearclass to serve as treatments for optimizing release strategies, increasing effective population size of hatchery releases, and answering specific scientific questions related to life history, population dynamics, and habitat utilization. The author will present case studies for each species that demonstrate how data developed by the program have improved our understanding of ecosystem function and informed management action at the state and federal level.

## SUSTAINABLE RECIRCULATING AQUACULTURE SYSTEMS (SRAS) UTILIZING A VACUUM AIRLIFT (VAL) FOR FOAM FRACTIONATION, CARBON DIOXIDE STRIPPING, OXYGEN REPLENISHMENT AND WATER CIRCULATION

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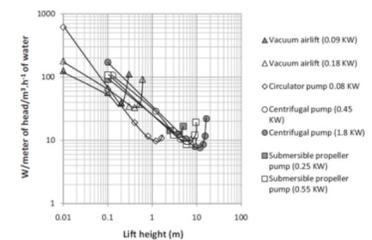
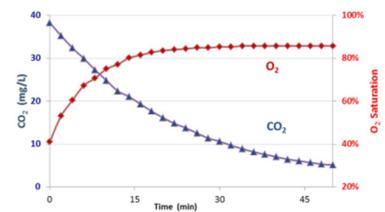
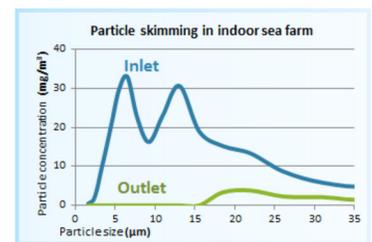
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In Sustainable Recirculating Aquaculture Systems (SRAS) there is a continual accumulation of micro particles from unconsumed food, feces, parasites and bacteria. In addition there is a continuous depletion of oxygen and a buildup of carbon dioxide in the water. These factors reduce the water quality causing stress that leads to slower growth and reduced yields. The fouling of the water is compounded by the high density of reared organisms (fish or shellfish) in a SRAS environment. Without corrective action to remove these contaminants, they will accumulate to the point that they create a toxic environment for the organisms. This study summarizes research directed toward the advancement of a new technology that will enhance aquaculture production through improved animal health, improved aquaculture production yield, reduced water usage, reduced capital costs and increased energy efficiency. These improvements are accomplished through the application of Vacuum Air Lift (VAL) technology to SRAS.

The accumulation of particulate organic matter (POM) in SRAS has become an important issue with the intensification of finfish production. The objective of this study was to assess the foam fractionation efficiency of a VAL in different conditions (POM concentrations, airflow rates, bubble sizes, water renewal rates and feed addition). In sea water, the vacuum airlift allowed removing 20% of the initial POM concentration per hour (foam fractionation efficiency), corresponding to a 20.7-fold concentration factor between the tank and the foam. Calculated POM production by fish ranged between 15.9 and 23.5 g h<sup>-1</sup> and was equivalent to estimations based on feed conversion ratio (FCR). This indicated that the entire POM produced was extracted by the vacuum airlift.

In aquaculture, oxygen transfer and carbon dioxide stripping are the first limiting factors to fish rearing intensification. This study measured the O<sub>2</sub> and CO<sub>2</sub> mass transfer coefficient (KLa) for a vacuum airlift in fresh (<1‰ salinity) and sea water (35‰ salinity) SRAS. Several types of air injectors were tested, delivering different sizes of bubble swarms depending on their porosity and functioning conditions (low or high injection pressure), with air flows varying from 0 to 80 L min<sup>-1</sup>. For SRAS, the vacuum airlift provides a Standard Aeration Efficiency (SAE) of 1.13 kgO<sub>2</sub> kW h<sup>-1</sup> and a Standard Stripping Efficiency (SSE) of 1.8 kgCO<sub>2</sub> kW h<sup>-1</sup> or 0.023 kgCO<sub>2</sub> kW h<sup>-1</sup>.

A study was undertaken to measure the water flow (Q<sub>w</sub>) delivered by a VAL designed for SRAS in fresh (<1‰ of salinity) and sea water (35‰ of salinity). The VAL consists of two concentric tubes connected at their top to a chamber at partial vacuum. When working with low head SRAS (under 0.3 m), the VAL could save up to 50% of the energy required for centrifugal pumps.



## **SUSTAINABLE SEAFOOD FROM AQUACULTURE AND WILD FISHERIES: INSIGHTS FROM A DISCRETE CHOICE EXPERIMENT IN NORTHERN GERMANY**

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There is an increasing focus on sustainable seafood that creates a potential for segmentation in the seafood market. Several recent studies demonstrate a consumer preference for wild seafood that is labeled as sustainable relatively to unlabeled seafood. In addition, there is increasing evidence of a preference for wild fish relatively to farmed fish despite an increasing aquaculture production and market presence. Recently, ecolabels have been introduced also for farmed fish. An interesting question is if the preference for wild fish primarily is related to the perceived sustainability for aquaculture or whether it is a perceived quality difference. A choice experiment is used to investigate these issues in Germany for salmon. Using a mixed logit model, the random parameter specification indicates substantial variation in consumer preferences beyond demographic variables. In particular, the ecolabel makes up for the negative association of aquaculture, indicating that environmental concerns and not quality differences are the major issue.

## **HOW MARKET NEEDS INCLUDING GOVERNMENTAL RULES AND REGULATIONS MAY AFFECT THE DESIGN AND USAGE OF AQUAPONIC SYSTEMS**

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Freshwater Integrated Multi-Trophic Aquaculture aka Aquaponics is an innovation. As suggested by Geoffrey Moore in his book “Crossing the Chasm,” for an innovation to survive it must meet the needs of the market. Most importantly the needs of those markets do not remain the same over time. This is the place where aquaponics is today. The needs of the mainstream/early majority such as cost effectiveness and compliance with food safety protocols are different than from those of the early adopters whose work has driven much of aquaponic development so far. This paper investigates how the needs of the burgeoning mainstream market are affecting how aquaponic systems are being designed and applied.

## **DEVELOPMENT OF A REPLICATED LABORATORY SCALE SYSTEM FOR SHRIMP LARVAL NUTRITION RESEARCH**

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The mass cultivation of penaeid larvae has been the foundation for rapid growth of global shrimp farming. Successful cultivation of shrimp larvae requires careful management of a variety of prepared feeds along with live algae, live or frozen *Artemia* nauplii and/or live feed replacements. Despite the overwhelming importance of proper nutrition and feed management, limited information is available for hatchery managers on precise nutritional requirements and comparative commercial feed performance by larval stage.

Nutrition research is typically conducted in small scale, highly replicated systems. Few systems like this exist for shrimp larval nutrition due in part to the complexity and inherent variability of larval culture systems. Highly skilled labor requirements are intensive, more a function of the number of tanks, than tank volume, making operations prohibitively expensive. Because of this, much of the larval nutrition research to date has been run in small or medium scale systems where replication was very low and/or the research systems bore limited resemblance to commercial scale systems. The present research was designed to develop a commercially relevant, controlled, and highly replicated small-scale larval culture system that can be successfully operated by a relatively low number of technicians.

The ZARC larval culture system consists of 64, 18-L LDPE cylindro-conical larval rearing tanks. The larval rearing tanks are mounted on 8 racks. Each rack of 8 tanks has independently controlled lighting which can be adjusted to match desired intensity. Temperature in the tanks is controlled by controlling room temperature. Typically a randomized block experimental design is used, with all treatments represented on each rack of tanks. This allows for statistical compensation for differences in performance related to tank position. With independent aeration, screening and carefully engineered plumbing, racks of tanks are designed for precise and convenient management and water exchange. Seawater from a wellpoint is adjusted to a salinity of 31 ppt, treated with 5 ppm of EDTA, and filtered through activated carbon, 10 micron and 5 micron filter bags and a 180 watt UV sterilizer for 12 hours prior to filling of the LRTs. The temperature of makeup water is closely controlled by circulating the water through a 1-hp heat pump. Software for system and data management has been developed to streamline data collection and management for large numbers of tanks. Macroscopic observations of shrimp larvae and conditions in the larval rearing tanks are recorded tank-side on tablet computers. The data is then uploaded into a computer spreadsheet that precisely adjusts feeding rates based on the conditions in individual tanks. All data are archived for subsequent analysis. Initial experiments demonstrate growth and survival rates typical of commercial systems. Results are replicable between studies and relatively small differences in performance between diets can be demonstrated statistically.

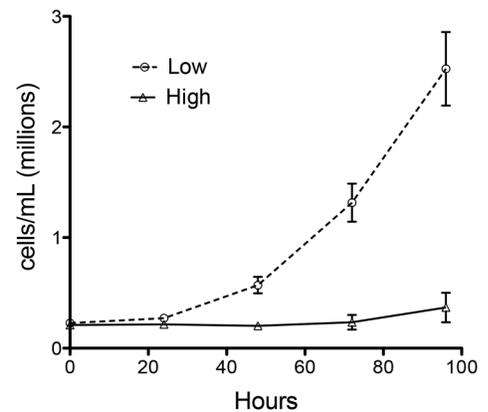
## OPTIMIZATION OF LIGHT AND AIRFLOW ON MICROALGAE *Tisochrysis lutea*

Jessica F. Brown\*, Andrew L. Rhyne, Joseph T. Szczebak, Bradford D. Bourque

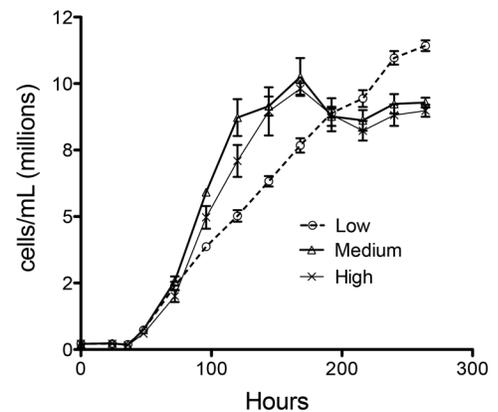
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Microalgae is the most important and operationally expensive component of a hatchery. Although, culture methods have been established for decades, little effort has been focused on continuously updating culture protocols as technology advances. Recent advances in lighting technology (LEDs) require a reexamination of these culture protocols. Here, we demonstrate the need to update culture parameters and protocols as culture technology improves. The effects of light and airflow were explored to optimize the growth rate of *Tisochrysis lutea* (T-ISO, CCMP 1324), a common microalgae, high in polyunsaturated fatty acids, in aquaculture hatcheries. We tested the effects of different light sources (fluorescent tubes and LEDs) as well as aeration rates (airflow) on algal growth rate and concentration.

Light intensity is important during lag phase when optical density is the lowest, as photo inhibition was observed in high light cultures (Figure 1). Photo inhibition does not allow the cultures to reach the densest capacity. Equally self-shading limits density of the culture as optical density increases. Shades (smoked acrylic lens) are used to shade the algae during the lag phase and removed once culture is in exponential growth phase. The amount of shading and time for shading allows for the microalgae to past the lag phase without reaching photo inhibition. Airflow (sheer and mixing) was tested to determine the optimal aeration during both lag and exponential phase (Figure 2). Airflow is tested on three different flow rates. These flow rates were controlled by the pressure and volume of various mechanisms that the air was allow flowing into the research vessels. By using these mechanisms, it allowed the flow rate be the same for each airflow observed. Optimal airflow and light are fundamental for high-density microalgae cultures, and ultimately effect efficiency production in hatcheries. With this research, it will allow hatcheries not just to grow microalgae efficiently; also, it will allow hatcheries to grow various types of microalgae beneficial to their projects. Our findings demonstrate the need to update and provide open source culture protocols for 21<sup>st</sup> century microalgae production.



**Figure 1. The effect of light intensity on cell growth.**



**Figure 2. The effect of aeration on cell growth.**

## **AQUACULTURE 101: SUSTAINABLE SEAFOOD PRODUCTION IN THE MIDWEST**

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An in-service education (ISE) module will be offered at the University of Missouri's Bradford Research Center in July 2017 on indoor, greenhouse covered, and in-pond shrimp and fin-fish production techniques targeted for farmers in the Midwest. This training is designed to provide an overview of shrimp and fish production ranging from low intensity ponds (at 500 lb/acre-season) to super-intensive, clear-water, green-water and brown-water closed-system aquaculture processes (at 20,000-35,000 lb-acre/cycle). Lectures will cover fundamentals of water treatment using algal, biofloc and biofilter technology, as well as, aeration/oxygenation and waste solids management. Educators, farmers, investors, and other stake-holders will increase their knowledge and understanding of aquaculture production of large-mouth bass, crappie, catfish, marine and freshwater shrimp, as well as, economics of aquaculture systems, including required capital investment, fish transport, marketing and sales, cash flow, and profitability. Participates will be provided field trips to view pond production of freshwater shrimp, in-pond raceway production of crappie, and catfish, and indoor recirculating culture of large-mouth bass, and super-intensive zero-discharge production of marine shrimp. In addition, all participates will be afforded a hands-on opportunity to experience system feeding, waste solids handling and water quality analysis including,  $\text{NH}_4$ ,  $\text{NO}_2$ , salinity, pH, dissolved oxygen, temperature, alkalinity and hardness, as well as, animal sampling/measurements, microbial identification, and system-wide measurement of photosynthesis and water treatment capacity.

This training will increase knowledge and understanding of the current "state of the art" of limited and zero discharge aquaculture production and environmental benefits, enhancing the potential for Midwestern farmers to successfully incorporate environmentally compatible seafood production into their existing farming and pond management activities, enhancing income and quality of life. Outreach activities will target two principle groups and to a lesser extent the general public. The majority of outreach activities will be targeted at educators and persons interested in adopting the new seafood production technology into their farming operations. The extension component will focus on two main objectives. The first objective will promote this new technology to individuals interested in adapting to commercial scale. The second objective will focus on technology transfer via publications, on-site demonstrations, trainings, and power-point presentations and videos. Post-adoption surveys will be used to monitor real and potential economic impacts. Using techniques presented and discussed in this ISE, aquaculture production could be provided with no discharge of pollutants to U.S. surface or ground water and without the need to import expensive fish-meal as a component in aquaculture feed. Midwestern farmers could potentially provide a 100% American-grown seafood supply eliminating a trade deficit of \$11.5 billion/year, using 100% American produced soy, wheat and corn meals as feed ingredients.

## **PRODUCTION OF SOFT-SHELL AUSTRALIAN RED CLAW CRAYFISH *Cherax quadricarinatus* AT A SMALL SCALE**

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The push for locally sourced, sustainable food production has grown in recent years; as a result, there is some interest in locally sourced soft-shell crustacean products. In Kentucky, most aquaculture operations are on a small scale; therefore, unique products that draw premium pricing are necessary to have a profitable business. Studies have been conducted on the feasibility of native crayfish (*Procambarus clarkii* & *Orconectes rusticus*) for soft-shell production. However, the relatively short time (as little as 1 hour) between molting and the hardening of the shell equate to a high labor investment that can diminish profitability.

Anecdotal evidence suggests that Australian Red Claw Crayfish (*Cherax quadricarinatus*) take up to 12 hours to fully harden after molting. An experiment was performed where Australian Red Claw Crayfish, from 5 cm to 10 cm in length, were cultured in individual strawberry baskets submerged in a recirculating aquaculture system. A high protein commercial shrimp diet was fed at 3% body weight twice daily and water quality parameters were monitored daily (Temperature & Dissolved oxygen) or weekly (pH, ammonia, nitrite). It was expected that these crayfish would molt as they consumed feed and grew in size. The crayfish were checked at 12 hour intervals and molted crayfish were removed, weighed, and frozen. Weight gain and number of days between molts were recorded to determine the average number of days to molt for various size ranges.

Most crayfish with starting weights < 30g molted between 30 and 40 days after their previous molt, while crayfish with starting weights >30g often exceeded 40 days.

**THE EFFECTS OF THE MASOU SALMON DELTA 5-DESATURASE TRANSGENE ON N-3 FATTY ACID PRODUCTION IN COMMON CARP *Cyprinus carpio* AND CHANNEL CATFISH *Ictalurus punctatus***

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This study aims to determine the effectiveness of the masou salmon delta5-desaturase transgene driven by a beta-actin promoter for improving N-3 fatty acid production in common carp, *Cyprinus carpio*, and channel catfish, *Ictalurus punctatus*. F2 generation common carp and F1 generation channel catfish were spawned from confirmed transgenic parents. Muscle samples were taken from these progeny and lipid extraction was performed. Barbel samples were tested as well to determine their effectiveness for observation of lipid profiles. Quantification of N-3 fatty acid levels was done through gas chromatography-mass spectrometry. N-3 fatty acid production was higher in transgenic individuals than in controls.

## ATLANTIC SALMON (*Salmo salar*) SMOLTS FED DIETS CONTAINING PROPLEX, A YEAST PROTEIN PRODUCT

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Atlantic salmon (*Salmo salar*) is an important cultured carnivorous species and the feed for this species has increasing amounts of alternative proteins. Atlantic salmon have been shown to have slightly reduced, but not significantly lower, performance when fed diets that were fishmeal-free and contained wheat gluten, corn gluten, fish solubles, and crystalline amino acids. Amino acid supplementation is often needed to maintain growth performance of fish fed plant protein-based feeds. This study examined the effects on the yeast protein Proplex on the growth performance of Atlantic salmon.

A digestibility study was conducted using ~500-g Atlantic salmon. The apparent digestibility coefficients (ADCs) of macro nutrients in Proplex yeast product was higher for organic matter and dry matter, but lower for protein compared to menhaden fishmeal. The lysine ADC for Proplex was lower compared to fishmeal while the methionine ADC was higher compared to fishmeal. After the conclusion of the digestibility trial, a 16-week growth trial was conducted. Atlantic salmon smolts were grown in a recirculating aquaculture system. The Proplex diets did not affect salmon growth when included in the diet up to 15% (Table 1). Average final weight ranged from 395g to 446g, weight gain ranged from 237% to 292%, feed conversion ratios ranged from 0.85 to 1.1, and specific growth rate ranged from .93%/day to 1.08%/day. The feed conversion ratios was in the range we normally see for fish of this size with most gaining more weight than feed consumed. The proximate composition of the fish was not affected by the yeast product, except for the ash content of fish fed the 15% inclusion diet. Percent ash did vary significantly and ranged from 1.8% to 2.5%. The proximate composition did not differ from previous experiments. Overall including Proplex at 15% of the diet did not affect Atlantic salmon performance during this sixteen week study.

**Table 1. Performance data of Atlantic salmon fed an experimental yeast protein product. (average  $\pm$  se)**

Diet	Avg Day 120 Weight (g)	FCR (g fed/g gain)	Weight Gain (g)	% growth	SGR (%/day)
Reference	395.61	0.96	237.46	174.70	0.98
5% Proplex	426.35	0.89	292.14	218.56	0.97
10% Proplex	446.22	1.10	285.15	214.89	1.08
15% Proplex	426.61	0.85	287.79	209.82	0.93
P values	0.54	0.56	0.54	0.53	0.63

## **AQUACULTURE DEMONSTRATIONS IN A FARM PROGRESS SHOW FORMAT: UPDATE ON THE FISH POND AREA AT THE SUNBELT AGRICULTURAL EXPOSITION**

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The Sunbelt Exposition is a farm progress show that attracts over 100,000 visitors each year to view agriculture and rural living exhibits that showcase technological progress. Aquaculture has been a part of that show for over 30 years in various exhibit formats. Early exhibits were part of University tent exhibits or sponsored by aquaculture associations. Now, two permanent ponds, two tents, and a greenhouse are used to exhibit vendor and extension technology and information. A committee of aquaculture extension specialists from Alabama, Florida, and Georgia has manned the exhibit and developed plans to reach clients and to attract vendors. After additions, the fish pond area contacts rose from an average of 9,115 between 2006 and 2009 to 12,330 in 2015. Pass through activity increased from 1,800 to 3,100 after the change. The number of extension faculty involved increased from 9.25 between 2006 and 2009 to 10.0 from 2011 to 2015. Expendable resources allotted to the activity were valued at 13,200 or \$1.07 per contact. Estimated impact of the show in sales of catfish and vendor products was \$820,800. Plans for future exhibits at the farm progress show were developed from this experience.

## EFFECTS OF MULTISTRAIN PROBIOTICS ON THE GROWTH, BEHAVIOR, AND GUT HEALTH OF JUVENILE RED DRUM *Sciaenops ocellatus*

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Probiotics are defined as a “good bacteria.” Important to a functional digestive system, the use of probiotics has become more common in the last 10 years to help re-establish the gut flora after a treatment of antibiotics. Red drum (*Sciaenops ocellatus*) a commercially important carnivorous marine fish have a short gut and fast transport. Rapid digestion and assimilation rates are important to rapid growth. The current study was conducted to determine the effects of probiotics on growth rates, behavioral patterns, and evaluate gut health, and will in the future include assessing the composition of gut microbiome. Together these investigations will provide an index of health of the red drum while being treated with probiotics, in order to determine if probiotics is a viable sustainable alternative to antibiotics as a growth enhancer.

A 28 day feeding trial was conducted in 20 gallon tanks inside a temperature controlled room at 27°C. Juvenile red drum from APEKS Aquaculture® (Palacios, Texas) were measured and weighed at the start of the experiment. Experimental tanks were divided into: 1) control, 2) probiotics recommended dosage, and (3) 2x the recommended dosage. Probiotics selected was Primilac® a multi strain mixture containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifido bacterium bifidium*, *Enterococcus faecium*. Probiotics was added to treatment tanks daily along with the fish meal, while controls were only fed fish meal. Once per week, three fish were sampled, weighed, and measured. Their overall body condition was assessed and catalogued. The fish were then sacrificed for gut microbiome analysis.

After 28 days, the fish fed with the recommended dosage of probiotics showed a growth increase of 18% compared to controls (Fig. 1). All fish in the 2x treatment fish died by day 16 (Fig. 1). Several interesting behavioral and physical appearance observations were noted; 1) the fish in the probiotics groups rarely nipped at each other, and caudal fins were undamaged. In contrast the control group (no probiotics), 1) nipped each other frequently, 2) often reducing other fish’s caudal fins to nubs and 3) red bite whelps occurred at the dorsal fin. The mediating effects of probiotics on aggressive behavior will be tested in future work, as these behavioral modifications may be significant in aquaculture where aggression is common due to overcrowding.

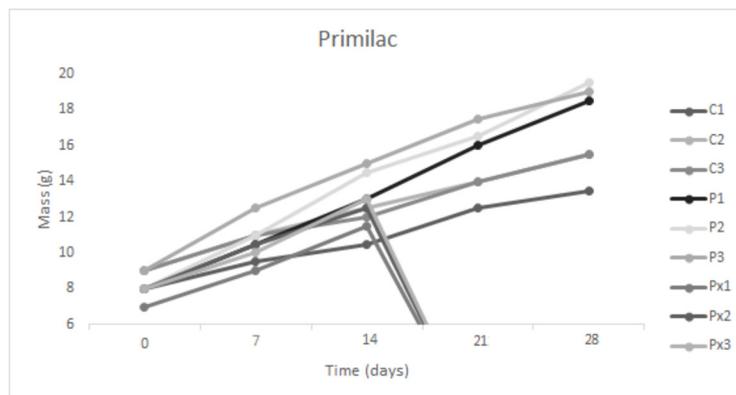


Figure 1: Graphical representation of the growth data obtained from the 28 day trial with Primilac® on *Sciaeniops ocellatus*.

## **PONDS FOR PEACE: RESURRECTION OF AQUACULTURE IN LIBERIA**

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Liberia was established in 1822 on the west coast of Africa for freed slaves from the United States. Prior to the U.S. Civil War, hundreds of former slaves were relocated to Liberia. They did not intermingle well with indigenous Africans which resulted in many conflicts that culminated in a two-phase, protracted civil war that devastated the country (1989-1996 and 1999-2003). A fragile peace has been established, which requires access to education, health care, job opportunities, and food security to become sustainable.

Aquaculture can play an important role in achieving a sustainable peace. While a tradition of fish culture exists in Liberia, much infrastructure and expertise was lost during the civil war. To help resurrect and expand aquaculture in rural Liberia as a route to sustainable peace, Salem State University (SSU) and the United Methodist University (UMU) in Liberia developed a Memorandum of Understanding in 2011. While aquaculture efforts in developing regions are not uncommon, the long-term, approach which brings together rural farmers with educational networks and administrators is unusual. This personalized approach, facilitated by SSU faculty and UMU personnel has brought people together that share a common goal, possess compatible personalities, and provide needed skills. The team approach has made pond construction and fish production possible in a manner consistent with cultural ethos and extremely limited resources. Immediate objectives are to expand production and availability of fish, to increase assistance to subsistence farmers, to train a cadre of Liberian aquaculture extension specialists, and to rejuvenate the aquaculture concentration within the School of Agriculture. These objectives are being realized (Figure 2). The goal is to establish a viable and sustainable aquaculture capability to increase supply of critically needed animal protein, to create jobs and generate revenue in rural Liberia, and to promote food security. A viable aquaculture industry will reduce the need to import 80% of seafood consumed and reverse the unsustainable rural migration to Monrovia. It will also reduce consumption of bush meat thereby promoting conservation, ecotourism, and avoiding the threat of Ebola. The long-term commitment and personalized approach employed in Liberia, may prove broadly transferable.

## TRYPTOPHAN ENRICHED DIETS INCREASE THE SEROTONIN LEVEL IN THE BRAIN OF THE TELEOST *Totoaba macdonaldi* AND ACTIVATES THE NEGATIVE FEEDBACK MECHANISM AFTER AN EVENT OF ACUTE STRESS

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Central serotonergic system plays a pivotal role in the regulation of HPI axis in fish<sup>1</sup> and could be involved in neuroendocrine loops adjusting body homeostasis and improving ability to cope stress in fish through an inhibitory effect on HPI activity, under stress conditions. The aim of this study was to determine the effect of dietary tryptophan level on serotonergic system and the acute stress response in juvenile of *Totoaba macdonaldi*.

A group of totoaba juveniles ( $90 \pm 5.0$  g) were distributed in twelve 500 L tanks in a recirculating system. Four diets with different tryptophan levels (0.5, 1.09, 1.69 and 2.28%) were formulated. At the end of experiment fish from each tank were divided into three groups and each group was exposed to one of following stress conditions: Control (unrest) Handling (persecution) and hypoxia ( $1 \text{ mg L}^{-1}\text{O}_2$ ). After stress, each condition fish were anesthetized and bled. Later, they were euthanized by decapitation and telencephalon and diencephalon were extracted. Plasma cortisol level (EIA 1887 DRG), 5-HT and 5-HIAA (HPLC-Electrochemical detector) were determined. A two way ANOVA was performed using the Sigma Plot 12.5 packet with a significance level  $\alpha = 0.05$  to evaluate the possible effects.

A significant increase ( $P = 0.001$ ) of 5-HT was observed in telencephalon of undisturbed fish fed with D1.69 and D2.28 compared against D0.5. However, after both stress exposure, no differences were observed. In contrast 5-HIAA levels were significant increase in fish fed with D0.5 and D1.09 ( $P < 0.001$ ) by handling compared against to undisturbed and then, 5-HIAA levels were restored with D1.69 and D2.28. This data suggest that higher 5-HT content in fish fed with higher level of dietary trp could mediate a negative feedback after acute stress<sup>2</sup>. Cortisol response was significant higher in D1.09 compared to D0.5 ( $P < 0.001$ ) and then decreased with D1.69, and D2.28.

Our data demonstrate that serotonergic activity in telencephalon and plasmatic cortisol are modulated by stress, and dietary tryptophan could play an important role as a modulator in totoaba for induced hatchery stressors like handling. Likewise other captive bread fish could respond in a similar way.

### Acknowledgements:

The authors would like to thank staff from both the Nutrition Lab and the Fish Biotech Unit (FCM-UABC). Also to Dr. Veronica M. Rodriguez from the Institute of Neurobiology (UNAM-Juriquilla) for their collaboration during the experimental phase and analytical work.

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<sup>1</sup>Medeiros and McDonald. Comparative Biochemistry and Physiology, Part A. 164 (2013) 612-621. <sup>2</sup>Jacobs and Formal. Seminars in the neurosciences. 7. (1995) 401-408.

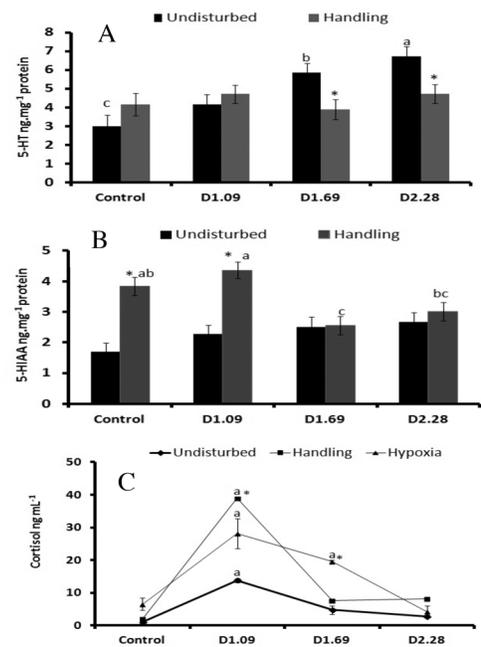


Figure1. 5-HT (A) and 5-HIAA (B) levels in telencephalon and plasma cortisol (C) of totoaba juveniles fed with different tryptophan levels. The differences within basal each treatment are represented by "a,b,c" or letters respectively, and asterisked represent the difference against control within each diet.

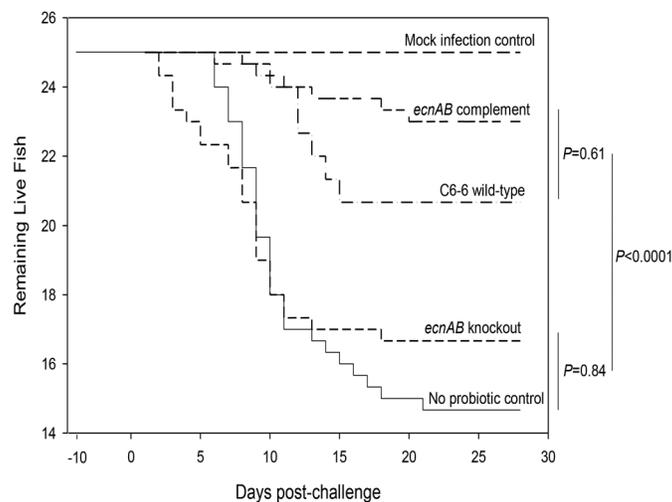
## CHARACTERIZATION OF A PUTATIVE PROBIOTIC ENTEROBACTER STRAIN (C6-6) AND POTENTIAL MECHANISMS ASSOCIATED WITH PROTECTION OF RAINBOW TROUT CHALLENGED WITH *Flavobacterium psychrophilum*

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An autochthonous *Enterobacter* strain (C6-6) provides protection against coldwater disease/rainbow trout fry syndrome (CWD/RTFS) in fish when administered as a microbial feed additive, and directly inhibits the *in vitro* growth of *Flavobacterium psychrophilum*. This putative probiotic works equally well with or without microencapsulation when top coated onto the diet, and appears to be a potential alternative strategy for managing CWD in aquaculture. It was originally isolated from healthy rainbow trout and appears to colonize the gastrointestinal (GI) tract and exist as part of the normal flora. Continued investigation of this *Enterobacter* strain has suggested that growth inhibition of *F. psychrophilum* and CWD related protection in fish is directly linked to a low-molecular-mass (< 3kDa) antimicrobial fraction found in bacterial culture supernatant. The mechanisms associated with health benefits of probiotics are seldom investigated; however, SDS-PAGE, and subsequent tandem mass spectroscopy of this < 3kDa fraction identified an entericidin (EcnB), a small membrane lipoprotein considered a putative pore-forming toxin. By creating *ecnAB* knockout strains of *Enterobacter* C6-6, inhibition of *F. psychrophilum* was negated, while *ecnAB*-complemented knockouts showed recovery of the inhibitory phenotype. In fish experiments, the engineered C6-6 strains and the wild-type (C6-6) strain were added to the diet and fish were fed for 38 days. On day 11, the fish were challenged by injection with a virulent strain of *F. psychrophilum* (CSF 259-93). Fish that were fed C6-6 had significantly greater survival than fish fed the *ecnAB* knockout strain ( $P < 0.0001$ ), while fish fed the complemented knockout strain recovered the probiotic phenotype (Fig 1). This demonstrates that this antimicrobial peptide is the predominant molecule associated with the probiotic activity of *Enterobacter* C6-6, and may present new therapeutic and prophylactic treatment opportunities for *F. psychrophilum* or other similar pathogens.

Figure 1. Survival of fish fed engineered or wild type *Enterobacter* (C6-6) strains and challenged with *F. psychrophilum*.



## **CONTRIBUTION OF AMAZONIAN FISH TO BOLIVIAN LIVELIHOODS AND FOOD SECURITY, AND QUALITY CONTROL IN BOLIVIA MARKET**

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Bolivia is a landlocked country in the middle of South America, in the headwaters of the Amazon with substantial floodplain regions and associated tropical Amazonian fisheries. While overall fish consumption is not high, local consumption can be quite substantial and current government policies are promoting increased use of local fish resources. Artisanal fisheries and small-scale aquaculture supply these markets, sold in spaces ranging from informal street-side open air structures to more substantial formal infrastructure or directly to hotels and restaurants. Value chains vary from farm-gate sales, through small-scale local intermediaries, to larger scale intermediaries that serve urban centers. Fish quality under these conditions can be precarious, and improving practices is challenging. This paper provides an overview of Bolivian markets for Amazonian fish and their contribution to livelihoods and local food security. Current fish handling practices are examined – from the boat to the plate - and efforts to improve fish consumption and quality from legislative and practical perspectives are described. Experiments on decomposition rates and Torres-inspired species-specific characteristics for assessing freshness of several representative Amazonian fish species are also described.

## DIETARY LEUCINE AND ISOLEUCINE REQUIREMENTS OF RED DRUM *Sciaenops ocellatus* AND THE EFFECTS OF EXCESS LEUCINE ON BRANCHED-CHAIN AMINO ACIDS UTILIZATION

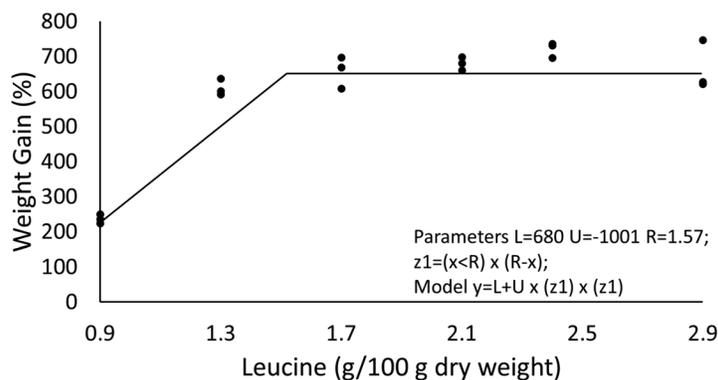
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A current priority in aquaculture is the potential replacement of fishmeal with alternative feedstuffs to ensure sustainability. However, most alternative protein sources are deficient in  $\geq 1$  indispensable amino acids (IAAs). Thus, there is a critical need to establish refined estimates of IAA requirements of fish. Although the red drum's dietary requirements for some IAAs have been determined, the need still exists to quantify all IAA requirements for this species. Therefore, the objective of this study was to quantify the dietary leucine (Leu) and isoleucine (Ile) requirements of juvenile red drum *Sciaenops ocellatus* and elucidate a possible antagonistic interaction of leucine with the other branched-chain AAs (BCAAs), which include valine (Val) and isoleucine (Ile).

Two separate feeding trials were conducted where a basal diet containing 35% crude protein [CP] and 3.2 kcal/g diet was prepared by combining lyophilized red drum muscle (RDM) as an intact protein (10.5% of dietary protein) and supplemented with crystalline L-AAs to simulate the pattern found in 35% CP from RDM. Leu and Ile contributed by RDM in the basal diet was analyzed at 0.89% and 0.50% of dry diet, respectively. In the first feeding trial, six experimental diets were supplemented with L-Leu in increments of 0.40% (0.90, 1.30, 1.70, 2.10, 2.50 and 2.90% dry diet) and kept isonitrogenous by adjusting the inclusion level of an aspartate/glutamate premix. In the second feeding trial, six experimental diets were supplemented with L-Ile in increments of 0.30% (0.50, 0.80, 1.10, 1.40, 1.70 and 2.00% dry diet); furthermore, a leucine-deficient diet (0.80%) and a diet with excess Leu (6.80%) were prepared, where the levels of Val and Ile were kept close to the estimated dietary requirements of red drum, 1.20% and 1.00% respectively. In both feeding trials, red drum juveniles were stocked in 38-L glass aquaria (15 fish/aquarium), and diets were fed to fish in triplicate aquaria at a rate approaching apparent satiation, twice daily, for 7 weeks.

Final values of weight gain and feed efficiency significantly improved as Leu level increased from 0.90% to 1.70% of diet; at higher inclusion levels of Leu there were no significant differences among treatments in any of the evaluated responses. Analysis of the weight gain data using a quadratic broken-line regression model estimated the Leu requirement of juvenile red drum to be  $1.57\% \pm 0.17\%$  of dry diet (Fig 1). The Ile feeding trial is currently ongoing; however, the dietary Ile requirement appears to be approximately, 1.05% of dry diet. The finalized Ile requirement, together with the effect of excess leucine on BCAAs' utilization will be included in the final presentation.



**Figure 1.** Quadratic broken-line model showing the leucine level at which weight gain reaches a plateau (breaking point).

## MEAT YIELD AND PROXIMATE COMPOSITION RELATIONS OF SEA BREAM (*Sparus aurata*) AND SEA BASS (*Dicentrarchus labrax*) IN DIFFERENT SIZES

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In this study, determination of differences in fresh meat yield and proximate compositions of different weight groups of sea bream and sea bass grown in cages in Izmir region of the Aegean Sea were aimed.

For this purpose, the length and weight of five different weight groups of sea bass (I: 175.8±5.2, II: 227.3±10.2, III: 293.3±21.3, IV: 404±9.9, V: 508.7±46 g) and sea bream (I: 146.6±13.6, II: 239.8±21.7, III: 279.2±20.8, IV: 400.9±10.5, V: 546.8±0.8 g) were measured and the amount of edible and non-edible parts were determined. Besides this, protein, lipid, dry matter, ash, condition factor, HSI and VSI values were compared according to different weight groups for each species.

According to the results of analysis, while the absolute meat yields of sea bream was between 69-294 g, it was between 71-252 g for the sea bass and the highest meat yields were found in fifth (V) weight groups of fish for both species. The relative meat yield (%) was determined in weight group II for sea bass and in the IV. group in sea bream with 51.9%. However, the amount of muscle tissue lipids in I. and V. weight groups of sea bream ranged between 3.6 to 11.9 % and ranged between 6.2 to 9.0 % for sea bass respectively. Protein, fillet and ash content increased in direct proportion to the weight.

As a result, it can be speculated that when the meat yield and lipid rates were considered, IV. Group (400.9±10.5 g) in sea bream and II. group (227.3±10.2 g) in sea bass are the most advantageous groups for the consumers.

## **LESSONS LEARNED IN STARTING A COMMERCIAL AQUAPONICS OPERATION: GREENER SCENES, LLC**

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Starting a commercial aquaponics operation is a challenge. They are capital intensive, require specialized technical expertise, and are supported by primarily niche markets. Success rates are improving but some sources suggest up to a 2/3 failure rate of commercial aquaponics operations, arising from excessive start-up debt, lack of experience, poor market assessment, or a combination thereof. This paper presents lessons learned by Greener Scenes, LLC of Brazil Indiana USA as their operation went from a hobby to several small proof-of-concepts to a commercial proof-of-concept. Infrastructure and operational challenges (and occasional successes) are presented. Financial variances during build-out and ramp up are summarized and production models and results are compared. The paper concludes with expansion plans as Greener Scenes moves from commercial proof-of-concept to actual commercial operation.

## INTEGRATED MULTI-TROPHIC AQUACULTURE OF SUGAR KELP (*Saccharina latissima*)

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The University of New Hampshire (UNH) and New Hampshire Sea Grant has recently designed and deployed a floating integrated multi-trophic aquaculture (IMTA) raft for research, outreach and training. This stable, robust platform was designed and demonstrated to culture steelhead trout (*Oncorhynchus mykiss*), blue mussels (*Mytilus edulis*) and sugar kelp (*Saccharina latissima*) simultaneously. State and federal permitting agencies favor this type of aquaculture because the lower trophic, shellfish and seaweed species, extract nutrients from the fish reducing nitrogen input to the ecosystem. The 3D structure, allows farmers to grow multiple species that utilize the 3D water column in a productive manner for greater economic value. Mature kelp from the raft is spawned in captivity and cultured for 6 weeks in a nursery at the UNH Coastal Marine Laboratory. Kelp seed line is then set on the cage mooring lines and vertical lines suspended from the raft in early winter. Harvest initiates in the spring at 2 m length and continues until late July. Fresh kelp has been sold to restaurants and breweries in Portsmouth, NH for a premium price.



## MOLECULAR CHARACTERIZATION OF THE MOLT INHIBITING HORMONE GENE FROM THE BANANA SHRIMP *Fenneropenaeus merguensis*

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To develop a technique for molt stimulation in shrimp, the Molt Inhibiting Hormone of the banana shrimp *Fenneropenaeus merguensis* (FmMIH) has been cloned and characterized. The mature peptide of FmMIH consists of 76 amino acid residues, a glycine residue at position 10 of the mature peptide, 6 cysteine residues located in the relative identical conserved position of the mature peptide. In addition to the eyestalk, high level of FmMIH transcript can be detected in the intestine. During the molting cycle, FmMIH transcript level was low throughout the post-molt, early to mid-intermolt and premolt stages. However, a sharp increase was observed in late intermolt (C3 stage). Both alignment and phylogenetic tree analysis revealed that FmMIH is most similar to MIH of other shrimp species. To perform the functional assay, double stranded RNA for FmMIH was prepared and injected into juvenile shrimp. A significant reduction of 2.3 days ( $P<0.05$ ) in molt cycle duration was observed in shrimp receiving dsMIH injection. Interestingly, injection of recombinant FmMIH also caused a significant ( $P<0.05$ ) reduction in the molt cycle duration. We have hypothesized that the recombinant protein is biological inactive but it can compete with the endogenous MIH for carrier protein binding and consequently reduce the amount of MIH that could reach the targets. The molt cycle shortening is probably in response to the increase in ecdysteroid titer after MIH inhibition is relieved. It is then followed by an increase in ecdysone receptor expression for molt advancement. In conclusion, the result of this study will provide new insight for the manipulation of molting in decapod.

Supported by the National Science Foundation of China (NSFC# 31572606)

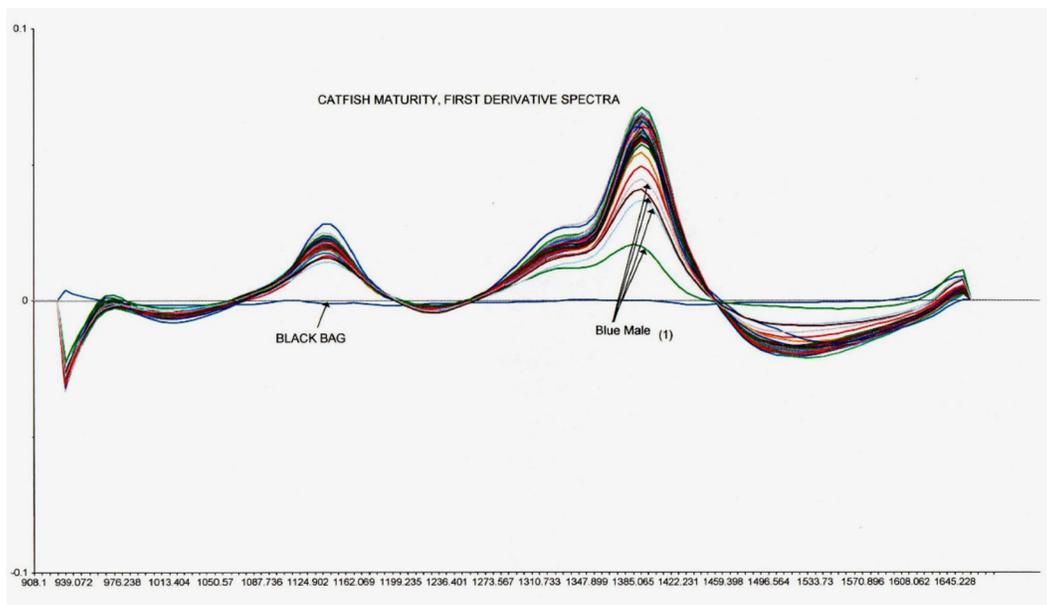
## EVALUATION OF PORTABLE NEAR INFRA RED SPECTROPHOTOMETER TO STAGE MATURITY IN CHANNEL CATFISH

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Gonadal maturity of channel catfish varies within the same cohort of fish. Female channel catfish with superior maturity need to be identified and staged for higher success to induce spawn with ovulating hormones to produce channel x blue hybrid catfish fry in hatcheries. Maturation is not synchronized in channel catfish, hence fish are handled more frequently to determine the stage of maturity. Known methods to stage maturity in catfish are time-consuming, un-reliable, subjective, expensive, and invariably need expertise. The only method adopted by producers is to select gravid or fully mature fish based on superior secondary sexual characteristics. Hence, the feasibility of portable hand held Near Infra Red (NIR) spectrophotometer to objectively determine the stage of maturity in channel catfish is evaluated in this study.

A preliminary study was conducted to stage maturity in 35 catfish. Fish were unanesthetized and held in individual soft mesh bags to obtain four abdominal scans per fish. Distinct NIR spectral characteristics, absorbance and first derivative were observed for individual catfish and not for the soft mesh bag. NIR spectral profiles of the soft black bag had minimal impact on individual catfish NIR spectral profiles. The results of this study will be discussed for possible application of NIR spectrophotometer in US farm-raised catfish aquaculture.



## EFFECTS OF CALCIUM AND MAGNESIUM HARDNESS ON THE FERTILIZATION AND HATCHING SUCCESS OF HYBRID CATFISH EGGS

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Hybrid catfish are exclusively produced by strip spawning of channel catfish females, fertilizing stripped eggs with blue catfish sperm, and hatching the fertilized eggs.

As egg development takes outside the fish's body, water hardness is one abiotic parameter, suggested to have a major effect on egg development and embryo survival.  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contribute to water hardness, and are important for ionic regulation of freshwater fish. These ions influence the permeability of egg membranes, preventing diffusive flow and high ionic loss to surrounding water. Previous research has conclusively demonstrated that water hardness is an important environmental factor affecting hatching success of hybrid catfish eggs.

Calcium alone or in combination with Magnesium hardness in hatching waters facilitate fertilization and hatching success of hybrid catfish eggs was evaluated in this study.

A total of 10 different type of waters were tested in this study: Four levels of magnesium hardness (0, 6, 15 or 30 mg/L) with fixed (60 mg/L of calcium hardness); Four levels of magnesium hardness (0, 6, 15 or 30 mg/L) with varying levels of calcium hardness (60, 54, 45 or 30 mg/L of calcium hardness), 1 type of natural water that had calcium and magnesium hardness and 1 type of prepared water that matched the natural water. Desired level of hardness in prepared water was achieved by adding stock calcium chloride ( $\text{CaCl}_2$ ) and magnesium sulphate ( $\text{MgSO}_4$ ) solutions.

Multiple hatching trails were evaluated in four types of hardness waters and mean percent hatch of hybrid catfish eggs did not differ (Figure 1). Results of the study suggest calcium is the critical component of hardness and that magnesium has no noticeable effect on egg-hatching success.

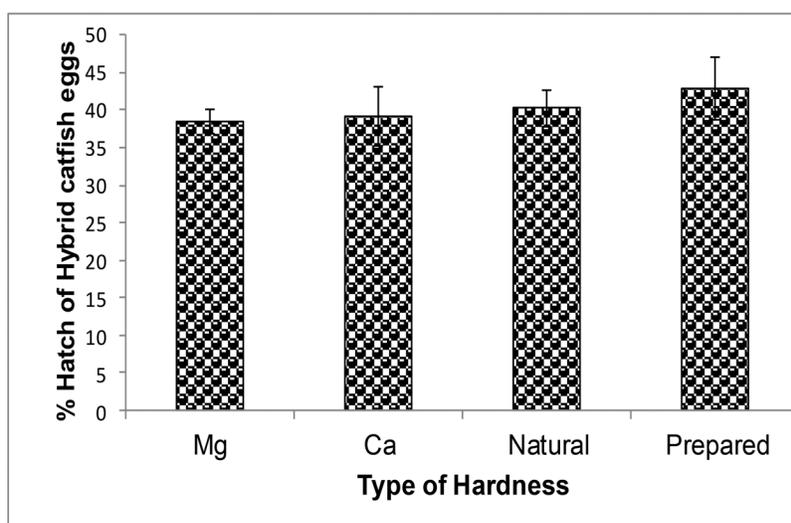


Figure 1. Mean % hatch of hybrid catfish eggs incubated in 4 types of hardness waters did not differ ( $P > 0.05$ ) in multiple hatching trials

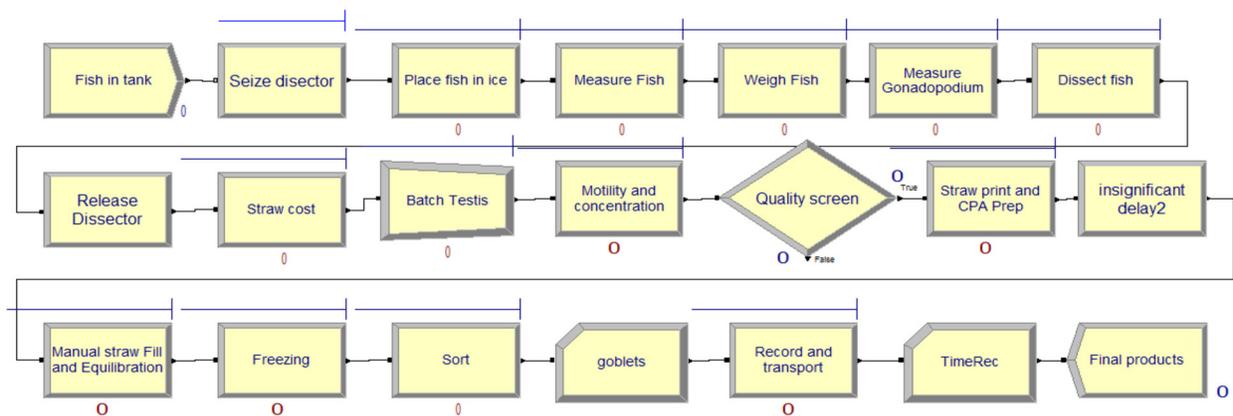
## SIMULATION MODELING OF THE CRYOPRESERVATION OF *Xiphophorus* GERMPLASM

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To date, there has only been one published study that applied simulation modelling to cryopreservation in an aquatic species (blue catfish). Catfish must be dissected to remove the testis which is crushed to obtain sperm, and generally testes from different males are not pooled. Sperm from other fishes, such as salmon can be dry stripped which is less labor intensive than dissection. Small-bodied fish such as *Xiphophorus* must be dissected, and the testes pooled to create a single sample. These examples of differences in processing and labor demonstrates the need to develop simulation models for various species. The goal of this project was to model the cryopreservation processes of the live-bearing genus *Xiphophorus*. The objectives were to: 1) develop a list of specific activities during cryopreservation and record processing times, personnel involved, supply costs, and sample quality for each activity; 2) predict the maximum output for an 8-hr day with 2 dissectors and 1 freezing operator, and 3) analyze how increasing the number of dissectors affects labor costs and output. ARENA software (Rockwell Automation, Inc.) was used to create the model (Fig. 1). After processing times for each step were recorded, distributions were generated using ARENA Input Analyzer. After 50 simulation replications, the model predicted that 224 fish could be processed in an 8-hr day with 2 dissectors and 1 freezing operator. This resulted in a labor cost of \$165 and supply cost of \$113. Increasing the number of dissectors resulted in 330 fish processed at a labor cost of \$247 for 3 dissectors and 400 fish processed at a labor cost of \$319 for 4 dissectors. After 4 dissectors, only 410 fish were processed at a labor cost of \$389 for 5 dissectors and 415 fish were processed at a labor cost of \$450 for 6 dissectors. These simulations can play a major role in planning the distribution of labor among different processes, estimated how long it will take to complete the process for a range of sample numbers, and the costs vs. benefits associated with labor and supplies. Mimicking real working situations can also help identify bottlenecks otherwise not foreseeable. Trips can be better planned when traveling to fish farms or hatcheries to cryopreserve onsite, saving time, money, and enabling of economies of scale.

Figure 1. Schematic overview of the simulation model developed for *Xiphophorus*.



## EFFECTS OF ASSOCIATED BACTERIA ON *Phaeodactylum tricornutum* BIOMASS AND GROWTH RATE

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Bacteria are known to have beneficial (e.g., production of signaling/inducer molecules, nutrient release) and adverse (e.g., competition and pathogenic interactions) effects on algal growth rates and metabolite production. The potential for enhancing biofuel production using bacteria prompted this assessment of the impact bacterial-algal associations on algal biomass production.

An axenic strain of *P. tricornutum* and the same algal strain co-incubated with bacterial isolates were grown in 1L flasks in triplicate and sampled for 20 days. Growth rates of the different co-cultures of *P. tricornutum*/bacteria and the axenic samples were measured using OD, HPLC pigment analysis, and cell counts. Correlations were made between all of the proxy measurement and cell counts. Bacterial identification was accomplished by 16S rRNA gene sequencing. HPLC-TOF was used to identify compounds associated with axenic and mixed cultures.

Growth rates were calculated from cell counts (Figure 1). Enhanced growth occurred in some bacterial-algal cultures relative to axenic cultures in both exponential and linear phases ( $p < 0.05$ ). Correlations of proxy and direct cell counts indicated strong linearity for optical density at both 655nm and 750nm (Table 1). Two different species of bacteria were identified through bacterial 16S sequencing; the most common was *Oceanicaulis alexandrii*. *O. alexandrii* has previously been isolated from dinoflagellate and coral cultures. Eicosapentaenoic acid (EPA) was found in higher quantities in bacterial:*P. tricornutum* isolates than in axenic isolates. Ongoing work will use HPLC-TOF to identify regulated bioactive metabolites in bacterial-algal cultures.

Supported by: Part of this work performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 and the TAMUCC Center for Coastal Studies by DOE-BER's Genomic Sciences Program under the LLNL Biofuels SFA FWP SCW1039-02.

	cellcount	OD655	OD750	Chla
r: cellcount	1	0.96135	0.96443	0.59689
Prob >  r		<.0001	<.0001	<.0001
r: OD655	0.96135	1	0.97974	0.6094
Prob >  r	<.0001		<.0001	<.0001
r: OD750	0.96443	0.97974	1	0.56663
Prob >  r	<.0001	<.0001		<.0001
r: Chla	0.59689	0.6094	0.56663	1
Prob >  r	<.0001	<.0001	<.0001	

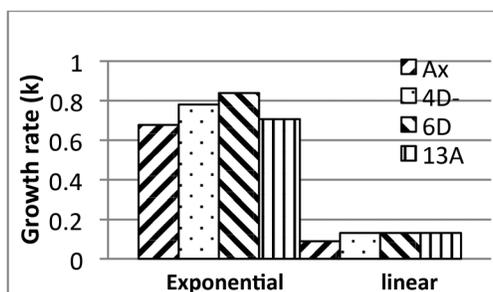


Table 1: Correlation for measurements. N= 96

Figure 1: Growth rates for exponential and linear phases

## 21<sup>st</sup> CENTURY CONSUMER BEHAVIORS - WHY WE ARE INTERESTED IN VALUE-ADDED “GUCCI” TILAPIA

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The tilapia aquaculture industry has been enjoying continual increases in seafood market share not only through increased production volume, but via new and innovative product developments. However, the tilapia industry has seen a setback due to the misconceptions by the consumers. The quote, “worse than bacon,” was brought up in 2008 and has been stuck with tilapia in online health food blogs ever since. Highly influential blogs are affecting the consumer behaviors more than ever since. This change in consumption patterns must be addressed to improve the image of tilapia and to develop a healthy relationship between the aquaculture industry and consumers. Also the high consumption of arachidonic acids (ARA; 20:4*n*-6) have been associated with cardiovascular diseases with detrimental effect on heart health recently. Although controlling for ARA in the diet is important, overall *n*-6:*n*-3 ratio is very important factor that must be evaluated. Current US consumers are consuming *n*-6:*n*-3 ratios at 10:1 or greater. As cardiovascular disease has been number one cause of death throughout the world, it affects are in global scale.

In order to understand the claims about poor lipid quality of tilapia, fatty acid composition (FAC) of tilapia fillets in US supermarkets were analyzed. The fatty acids of interest that were quantified were the oleic acid (OA; 18:1*n*-9) at 158.9 ±47.6 mg/g oil, long chain *n*-3 fatty acids (LC*n*-3; includes 20:5*n*-3, 22:5*n*-3, and 22:6*n*-3) at 23.5 ±16.3 mg/g oil, and ARA at 13.1 ±4.6 mg/g oil. The variation in samples from different origin of country resulted in high variation within the samples. Regardless, *n*-6:*n*-3 ratios values were relatively low at 4.7 ±2.1 compared normal US diets.

Our market study indicated healthy FAC in tilapia fillet lipids. However, to meet the changing demands of consumers for more premium products, we conducted two separate 8 week growth trials to improve FAC through enhanced diets. First trial developed enhanced diets through algae meal or fish oil with corn oil as base. 1, 3, and 5% algae meal or fish oil inclusion in the diets resulted in tilapia fillet lipids with *n*-6:*n*-3 ratios of 2.1 ±0.6. Although we addressed improving *n*-3 fatty acid quality, we still observed high levels of ARA and low levels of OA. Thus, in the second study, we utilized algae oil, fish oil, high oleic sunflower oil and soybean oils at various concentrations. 7% lipid diets that consisted of 15:85 fish or algae oil to high oleic sunflower oil provided high levels of OA (194.9 ±22.0 mg/g oil) and *n*-6:*n*-3 ratio of 1.6 ±0.2. Therefore, we observed improvement of reduction in ARA as well as excellent overall *n*-6:*n*-3 ratios especially compared to current American diet of 10:1.

The results from these studies show that tilapia products with FAC that are healthy improvement can be produced. More importantly, we can customize the diets to create products with specifically designed lipid content and/or FAC profile. Consumers have shown high interest in these premium value-added products, and tilapia has huge potential in value-added segment of the market place.

## LEUCINE AND METHIONINE DEFICIENCY INDUCE CATABOLISM THROUGH NON-OVERLAPPING MECHANISMS IN RAINBOW TROUT *Oncorhynchus mykiss* PRIMARY MYOBLASTS

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Amino acids (AA) have anabolic effects on protein accretion in muscle. In fish it is unknown if this anabolic response is directly attributed to a single AA or a specific AA profile. Therefore, our experimental objective was to determine if AAs or AA profiles regulate protein turnover and growth-related mechanisms in rainbow trout myocyte cultures. The overall goal of this research is to identify the functional roles of AAs and identify nutrient-gene interactions that may affect growth performance in rainbow trout.

Myogenic precursor cells were isolated from rainbow trout skeletal muscle and incubated in media containing 10% fetal bovine serum for 7 days, thereby differentiating into myoblasts. On day 7 cells were incubated with treatment media varying in AA concentrations, and the proteolytic or gene expression response was analyzed after 6 or 24 hours, respectively. Incubation of cells in minimal essential media (MEM) lacking essential AAs (EAA) increased protein degradation by 1.5-fold ( $P < 0.05$ ) compared to MEM containing a complete AA profile. This response was attenuated 50% and 75% by addition of leucine and branch chain amino acids (BCAA), respectively ( $P < 0.05$ ). Further addition of lysine decreased protein degradation to levels comparable to cells in complete media, suggesting that BCAAs and lysine are central regulators of protein degradation in muscle. Therefore, dietary deficiencies of these amino acids, particularly leucine, may be most detrimental to muscle accretion in salmonids. Further supporting this observation was that cells treated with leucine-less media exhibited protein degradation rates equivalent to cells treated with media lacking all EAA, indicating that a leucine deficiency mimics the effects of an EAA deficiency on muscle proteolysis.

While media lacking methionine did not affect protein degradation rates, it did affect expression of several genes associated with muscle growth, reducing expression of follistatin (*fst*), myogenin (*myog*), growth hormone receptor (*ghr2*), insulin-like growth factor binding protein-5b1 (*igfbp5b1*), and the signaling protein, *smad7* ( $p < 0.05$ ). In contrast, media lacking leucine reduced expression of *fst* only ( $P < 0.05$ ). Collectively, these findings suggest that a dietary deficiency of leucine and methionine produce catabolic effects directly in muscle, although through different mechanisms (Figure 1).

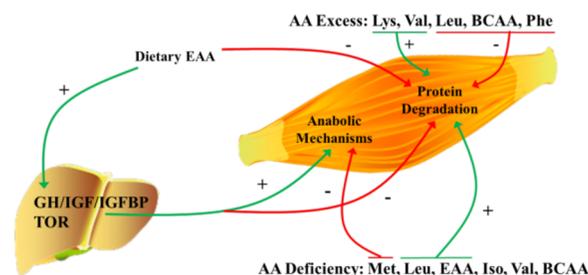


Figure 1. Effects of specific AA on growth-related mechanisms in rainbow trout liver and muscle

## CREATING BETTER EDUCATIONAL VIDEOS FOR EXTENSION AND OUTREACH

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Welcome to the video age. The spread of high speed internet access and simple video editing software makes the creation and delivery of online educational videos easier than ever. YouTube videos provide an excellent mechanism for Extension specialists to share information and reach a large potential audience. Putting a Power Point presentation in video format does not make the most of this medium. The creation of good video content requires careful planning and practice. The average attention span of most adults is 15 minutes and students approximately 7-10 minutes. Most lecture presentations, however, last 30 minutes to an hour. How can you get your point across as quickly as possible? We must learn to provide entertainment in addition to education. YouTube suggests that the first 15 seconds of a video are critical for engagement and retention of the audience.

A central figure or “hero” and a story with a beginning, middle and end will go a long way towards retaining the attention of the audience. Join us for a discussion these and other simple tips and techniques that can improve your ability to create watchable or perhaps even enjoyable educational Extension videos.

## IMPACTS OF CHILLER FAILURE ON TEMPERATURE CHANGE IN ISOLATION INCUBATORS FOR SALMONIDS

John Colt\* and Desmond Maynard

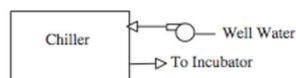
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In salmon recovery programs it is commonly necessary to chill incubation and early rearing temperatures to match wild development times. Chiller failure can result in rapid temperature changes and increased deformities and mortality during the early development stages of sockeye salmon. Two common types of chiller systems are shown in Figure 1. The most common failure modes are (a) chiller failure due low voltage, failure of compressor or internal pumps, and low refrigerant levels) or (b) failure of external recirculation pumps. The magnitude of the temperature change depends strongly on both the type of chiller system and the failure mode. The objective of this research is to document the temperature variation of the two types of chiller systems and develop design criteria for chiller systems used in salmon incubation/early rearing. Three types of chiller failure were studied: (a) Type 1, chiller failure (NR), Type 2, chiller failure (CF), and Type 2, pump failure (PF).

Representative temperature changes are presented in Figure 2 for the three failure modes. The most rapid temperature in the first 120 minutes occurred for NR followed by PF and CF. The maximum temperature for CF was significantly higher than well water supply. The maximum temperature changes for 30-, 60-, and 90-minutes were estimated. The temperature response of incubation systems was modeled using tracer analysis. It was assumed that the reservoirs and incubators could be considered ideal continuous-flow stirred-tanks reactors (CFSTR), the volume of the connecting pipes could be ignored, and heat transfer could be ignored. The Type 1 system was modeled as a single CFSTR while the Type 2 system was modeled as two unequal sized CFSTR in series. The models were evaluated in terms of theoretical and measured mean hydraulic residence times. The models could be used to accurately predict temperature results as a function of system characteristics.

Figure 1. Typical chilling systems.

Type 1



Type 2

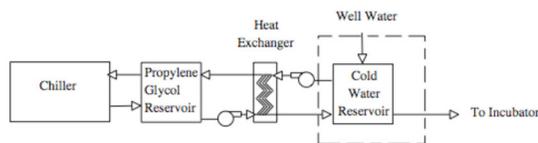
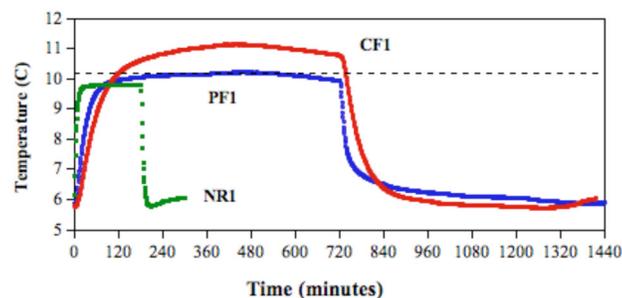


Figure 2. Representative temperature changes.



## **AQUABOUNTY'S AQUADVANTAGE SALMON: CLIMATE-SMART AQUACULTURE**

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In 1989, a transgenic Atlantic salmon was developed using recombinant DNA technology by a research team working at Memorial University of Newfoundland. The transgene enabled the salmon to grow year-round and thus to achieve market size in half the time of conventional Atlantic salmon. It soon became apparent that farming these salmon in conventional sea pens would not be possible due to concerns regarding escape and possible negative impacts on wild Atlantic salmon populations. Consequently, land-based farming in tanks using either flow-through or recirculating water was proposed. The development of recirculating aquaculture systems (RAS) has come a long way since 1989 and the current state-of-the-art of RAS technology enables full-cycle production from egg to market-size (4-5kg). The bottleneck for land-based salmon farming to market size has been slow growth using conventional Atlantic salmon. AquaBounty's AquAdvantage Salmon, with its fast growth rate, eliminates the bottleneck and enables the financial viability of full-cycle production.

This presentation will discuss the benefits of farming AquAdvantage Salmon in land-based farms with reference to climate change and the increasing need to have total control of the farming environment in the face of an increasingly unpredictable natural environment.

## REPRODUCTION, DEVELOPMENT, AND GROWTH OF REDHEAD CICHLID (*Cichlasoma synspilum*) AND ITS HYBRID WITH MIDAS (*Amphilophus citrinellum*)

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The reproduction and early life history of the blood parrot, one of the most popular hybrid cichlids, has received surprisingly little attention in the scientific literature despite world trade estimated at \$10 million. We performed two spawnings of both *C. synspilum* (Cs) and hybrids (Cm). Juveniles began consuming exogenous live food after 7 days post-fertilization (dpf) at 28-30°C, prior to complete yolk absorption (Fig. 1). Following 10 days of feeding with *Artemia* nauplii, fish were transitioned to formulated feed (Otohime, B2) and then Otohime S1 at 28 dpf. We did not observe differences in growth of hybrids and Cs during juvenile stages.

In parallel, a feeding study was carried out with Cs on the acceptance of the formulated commercial diets in comparison to live feed. Progenies from one pair of Cs were divided into 12 glass aquaria (156 fish per replicate) and fed one of three commercial starter feeds or *Artemia* nauplii for 21 days of growth (Fig. 2). Fish fed *Artemia* were significantly larger ( $0.29 \pm 0.03$  g) than other treatments and differences were also significant among commercial diet groups. However, survival was  $97 \pm 3\%$  in all diet groups and so we conclude that in this species formulated feeds can completely replace live *Artemia* during early life.

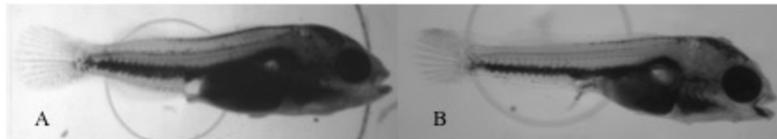


Fig. 1 Juvenile hybrid redhead cichlid (female) x midas (male) (A) prior to first exogenous feeding (120 hours post-fertilization (hpf), 28.5°C) and (B) redhead cichlid (192 hpf). Inner circle diameter = 3mm

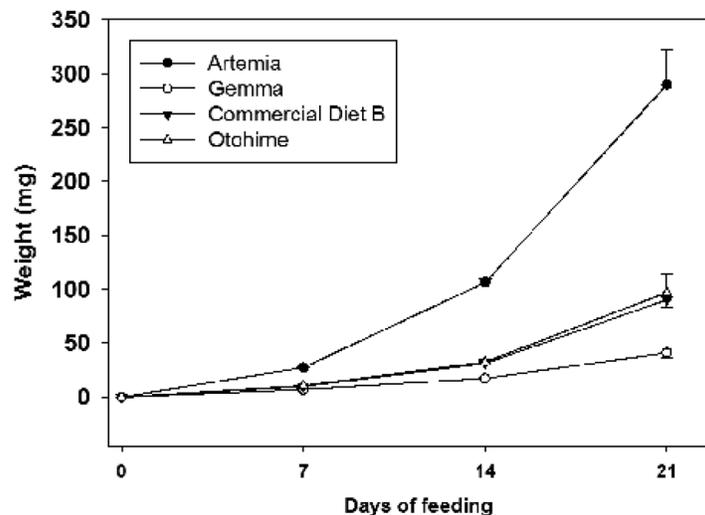


Fig. 2 Growth of redhead cichlid juveniles on 3 commercial diets and *Artemia* nauplii.

## VOLATILITY SPILLOVER IN AQUACULTURE AND FISHERIES MARKETS

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Several papers study market integration between farmed and wild fish prices, and many find that the output from two production technologies are substitutes. Consequently, a competitive aquaculture industry will increase the total supply at reduced prices. To further study this market relationship, this paper considers volatility spillover between aquaculture and wild fisheries markets. Previous studies show that aquaculture has a relatively low price volatility compared to wild fisheries, partially due to better opportunity to smooth supply by adjusting production and timing. With varying seasonal output from wild fisheries, quantity, quality and timing cannot be controlled in the same manner.

The paper considers a set of fish prices from 01.1990 to 11.2015 using monthly trade data for EU, Japan and US markets. We consider volatility spillover for a set of species where products from both technologies occur per region. In particular, we consider salmon for all three regions, trout in EU and US, seabream in EU and smelt in the US market. For invertebrates, we consider prawns in EU and crab for Japan and US. Finally, for molluscs, we have data for clams, mussels and oysters in the US market. Volatility spillover is calculated using Diebold and Yilmaz (2012), allowing us to estimate both directional and total volatility spillover between markets, and measure market integration with regards to price volatility over time.

The results suggest that there is considerable market interaction in price volatility between farmed and wild fish prices. Moreover, spillover originates from the wild fish prices to farmed fish prices, which may be a result of uncertainty from landings and seasonal output in fisheries. To accommodate a constant demand for seafood, the aquaculture industry can profit from adjusting according to expected supply from the fisheries. Consequently, it is intuitive that the uncertainty originates from wild seafood supply.

For a commodity like salmon, aquaculture has steadily increased its supply share from about 50% in the 90s to 80% in 2015 for Europe and US. For Japan, it has also increased, from only 13% in 1990 to 56% in 2015. The European (Figure 1) and US market has common characteristics: volatility spillover occurs from captured to farmed fish prices, although at a lower rate as the aquaculture supply increases. The Japanese market does not experience the same, as spillover in volatility since 2002 has occurred from farmed fish prices.

### Reference:

Diebold, F. X., & Yilmaz, K. (2012). Better to give than to receive: Predictive directional measurement of volatility spillovers. *International Journal of Forecasting*, 28(1), 57-66.

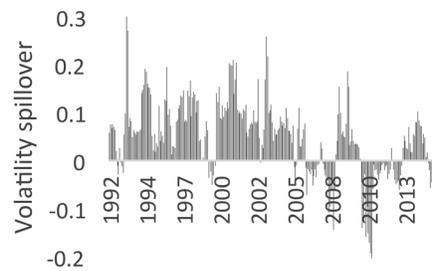


Figure 1 – Volatility spillover from captured to farmed salmon prices

## **SPONTANEOUS VOLITIONAL SPAWNING, FRY CULTURING, AND SPAWNING INDUCTION VIA WATER HARDNESS MANIPULATION OF *Otocinclus vittatus***

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*Otocinclus vittatus* are small, South American armored catfishes belonging to the family Loricariidae and are popular in aquaria. Currently, all *O. vittatus* are collected from the wild and captive culture methods have proven elusive for commercial producers. Little is known about spawning cues or fry and juvenile culture. To date methods have not been defined to reliably spawn or culture *O. vittatus*. Other South American loricariids have successfully been triggered to spawn via a simulation of a dry season followed by a wet season as found in their native range. Water hardness and temperature are two of the water quality parameters commonly manipulated to trigger this simulated change in seasons. In addition, the little information available on fry and juvenile culture suggests that they consume the same plant and algae based diet as the adults. Several commercial diets with varying nutritional composition are available but have never been compared in research with loricariids.

We will present the results of the manipulation of water hardness and temperature on spawning induction, results of spawning data from wild caught and the F1 generations, and various diets on juvenile growth and survival.

## **USING INDIVIDUAL-BASED MODELS TO DETERMINE OPTIMAL HARVEST REDUCTION AND STOCK ENHANCEMENT TO MINIMIZE GENETIC IMPACTS DURING RESTORATION OF WILD COBIA POPULATIONS**

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Cobia is the target of a well-developed recreational fishery in South Carolina. Research from our lab has shown that the inshore spawning aggregation of cobia in Port Royal Sound is genetically distinct from offshore groups, and recent estimates of inshore abundance are indicative of steep declines. Concerns over the status of the inshore cobia population in SC have been raised by a diverse group of stakeholders, including state resource managers, recreational fishermen, and charter boat captains. The combined evidence for declining abundance necessitates conservation action, potentially including both changes in regulations and stocking. Little information is available to guide the relative roles of stock enhancement and harvest reductions to facilitate recovery of this population. From a genetic perspective, care is needed to insure that recovery efforts maintain both genetic diversity and effective population size, as both are important for the long-term adaptability of the population. Thus, guidance is needed to identify the best responsible conservation and restoration strategies for SC's inshore cobia population. Our project has specifically addressed this need by modeling the demographic and genetic outcomes of a range of possible management alternatives using both harvest reduction and stock enhancement strategies. The incorporation of quantitative predictions derived from our simulation model to guide comprehensive restoration activities constitutes a significant advancement in fisheries management.

## **WALLEYE *Sander vitreus* PERFORMANCE, WATER QUALITY, AND WASTE PRODUCTION IN RECIRCULATION AQUACULTURE SYSTEMS WHEN FEEDING DIETS WITH AND WITHOUT FISHMEAL**

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Walleye represent a niche-market species that has potential for profitable production in recirculation aquaculture systems (RAS). Walleye are a popular sport and food-fish species in states and provinces bordering the Great Lakes; however, walleye are mainly provided as food-fish by limited capture fisheries. Walleye are cultured to fingerling size for stock supplementation, but less is known regarding the intensive culture of walleye to food-size, particularly in RAS. In addition, no information is available regarding the effects of feeding alternate ingredient, fishmeal-free diets to walleye. Therefore, a study was conducted to compare the effects of feeding a fishmeal-free diet versus a traditional fish-meal based diet on walleye performance, water quality, and waste production in replicated RAS.

Six replicated RAS (9.5 m<sup>3</sup>) were used. Specially formulated fishmeal-free (FMF) and fishmeal-based (FM) diets were fed to walleye in triplicate RAS. The FMF diet used proteins derived from poultry meal, wheat flour, soy protein concentrate, and corn protein concentrate, and lipids from menhaden oil and poultry oil. The FM diet used proteins from fishmeal, poultry meal, soybean meal, wheat flour, and blood meal, and lipids derived from menhaden oil. Each diet was formulated with a protein/ fat ratio of approximately 42/18. The study began when fish were 85 g and continued for 9 months. Each RAS was operated with a 99.9 % recycle rate on a flow basis and a mean system hydraulic retention time of 135 days or 4.5 months.

Differences in fish performance between the FMF and FM diet treatments were not significant ( $P > 0.05$ ): mean weight of walleye at study's end was  $589 \pm 15$  and  $571 \pm 26$  g; thermal growth coefficient was  $0.83 \pm 0.02$  and  $0.82 \pm 0.01$ ; condition factor was  $1.03 \pm 0.02$  and  $1.05 \pm 0.02$ ; and feed conversion ratio was  $1.27 \pm 0.03$  and  $1.32 \pm 0.02$ , respectively. Survival over the trial duration was  $\geq 98.5$  % for both treatments. With the exception of total and dissolved phosphorous, total nitrogen, and watercolor index values, differences in culture tank water quality between diet treatments were small ( $P > 0.05$ ). Both forms of phosphorous, however, were more than two times greater in RAS where walleye were fed the FM diet. Total nitrogen was also greater ( $P < 0.05$ ) in RAS associated with the FM diet. Measurements of true color and UV transmittance indicated slightly clearer water in RAS associated with the FMF diet. Waste production (kg waste produced/ kg feed) was similar between treatments with the exception of phosphorous, which was greater ( $P < 0.05$ ) in effluents associated with the FM diet. Reduced phosphorous discharge associated with feeding the FMF diet could increase the feasibility of meeting stringent discharge standards and could reduce the capital investment required for waste treatment. Product quality and fillet attributes were also assessed and will be presented.

## THE AQUACULTURE DEVELOPMENT PARK AT HARBOR BRANCH OCEANOGRAPHIC INSTITUTE: 20 YEARS OF OPERATION

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enterprise has led the way in advancing aquaculture in Florida, the United States and internationally. In 1996 Harbor Branch broke ground on the 30-acre Aquaculture Development Park (Park) that continues to host a mix of research and industry and fosters expansion of aquaculture through workforce training, production development and technology transfer. The Park includes 8 buildings totaling 39,000 square feet of space for marine and freshwater species such as fish, clams, shrimp, algae and seaweeds. In addition, the Park has an Aquatic Animal Health Lab, nutrition lab and recirculating systems.

Harbor Branch's workforce development impact has included a retraining program focused on clam farming for displaced fishermen in the 1990s. The Park housed a large-scale hatchery to produce the seed for the field-based program, and clam farming now is a prosperous business sector in Florida. The Park's Aquaculture Center for Training, Education and Demonstration is used to facilitate vocational workshops and a degree program with Indian River State College.

Harbor Branch developed some of the first systems to grow warm water marine shrimp in low-salinity waters and developed similar techniques with other species such as Florida pompano. This latter work was part of a 10-year partnership with the USDA Agricultural Research Service, which produced advances in recirculating culture technology, nutrition for low-salinity culture of marine species including reduction of fish meal use, and husbandry techniques spanning egg to product. These and other research studies led to the present focus on land-based integrated multi-trophic aquaculture (IMTA). Now in its fifth year of operation at the Park, the IMTA system has enabled testing of several combinations of fed (e.g., fish, shrimp), extractive (e.g., urchins, sea cucumbers) and assimilative (e.g., macroalgae, *Salicornia*) culture species. Developing viable species and techniques for restoration and stock enhancement is another primary research focus, and currently includes seagrass culture for habitat restoration in the Indian River Lagoon and culture of prized sportfish for stock enhancement. The Park also serves as a commercial feasibility incubator, such as with the Zeigler Aquaculture Research Center, a Harbor Branch-Zeigler Bros., Inc. partnership in the Park.

The activities in the Harbor Branch Aquaculture Development Park are helping to meet one of the most significant long-term challenges we face: the need to expand aquaculture to help satisfy an expected doubling in the global demand for food over the next 40 years.

## VARIATION OF VELIGER HATCHING, DEVELOPMENT, AND GROWTH FROM FIVE *Strombus gigas* QUEEN CONCH EGG MASSES: FOR RESTORATION IN THE BAHAMAS

Megan Davis\*, Laura E. Issac

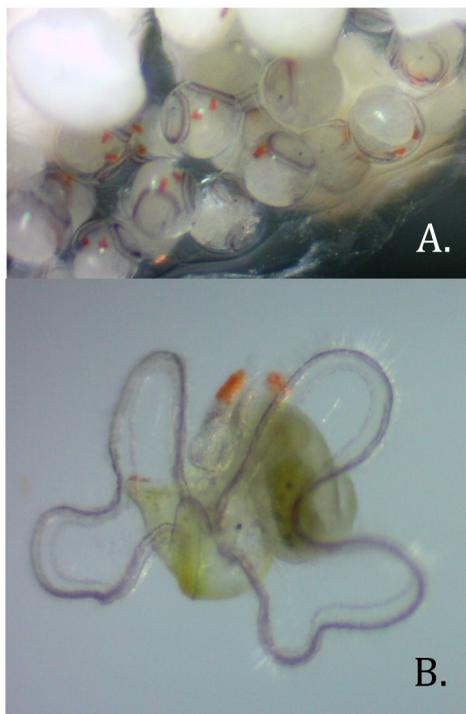
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The Queen Conch, *Strombus gigas*, is a marine gastropod that lives in the warm waters of southern Florida, the Caribbean and Bermuda. Due to the high demand for this species there has been a steep decline in the population numbers in the past 40 years. To help manage the fisheries, the queen conch was listed as a CITES II species (Conservation of International Trade of Endangered Species of Wild Fauna and Flora) in 1992.

Since the 1970's many laboratories and one commercial conch farm developed cultured techniques to grow conch for food and restoration. In 2016, a new experimental hatchery was built on Hummingbird Cay, Great Exuma, Bahamas with the purpose to culture conch for restoration in the Bahamas. The initial experiments investigated the viability of the new hatchery by conducting studies that observed conch egg mass hatch rates and veliger (larval) growth and development. A total of five egg masses were collected from two locations.

The conch egg masses hatched 4 to 6 days after they were laid. The embryos were well developed with velar lobes, shell, and pigmentation on the day of hatch (see photo A.). Hatching occurred in the early evening hours and depending on the egg mass, 35-100% of the eggs hatched on the first night. Average growth rate of veligers varied depending on the source of phytoplankton fed and the egg mass. The range for the first four days was 25 – 50  $\mu\text{m}$  per day. In the first four days the veligers develop from the two velar lobe stage to four velar lobes with the beginning of the sixth set of lobes showing (see photo B.).

Culturing queen conch in the new hatchery demonstrated that there is variation between egg masses and veliger growth and development from different egg masses. Since restoration of a species requires this type of genetic variation, it is recommended that several conch egg masses from nearby locations should be used in a conch restoration program.



## PRODUCTION OF HYBRID CATFISH USING IN-POND RACEWAY SYSTEM

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With the US Catfish industry's declining production since 2003, increased competition from imported substitute products and higher feed prices, technological advances have to be made in order to increase production while lowering costs to keep the industry competitive.

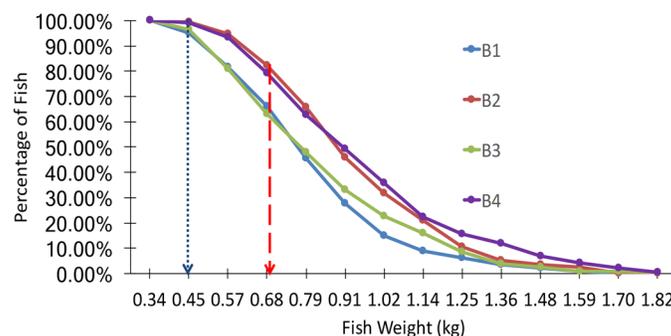
Beginning in March of 2016 the first year of a four-year study has been set out to assess the feasibility of In-Pond Raceways Systems for production of market size catfish compared to other current conventional pond production used by the catfish industry. The In-Pond Raceway System (IPRS) was first developed at Auburn University in the early 1990s. IPRS has been experimentally proven to improve survival and feed conversion (FCR) and allows for a high standing crop of catfish in production units that are only a small fraction of the pond's total area. However IPRS has not consistently produced market size catfish in past trials. Thus, there is still a lack of sound production data needed to demonstrate and encourage catfish farmers to adopt this technology.

IPRS allowed us to produce a marketable catfish (>454g) in one growing season. The percentage of catfish that made it to marketable size (>454g) B1-95.2%, B2-99.4%, B3-96.5%, B4-99.1%. The percentage of catfish above 680g (1.5 lb) B1-66.0%, B2-82.4%, B3-63.0%, B4-79.3% meaning that well over 50% of the fish population made it to the first year's goal. The two larger IPRS units doubled the yields (~16,237 kg/ha) of conventional catfish ponds (7,800 kg/ha). The two smaller IPRS units (~13,720 kg/ha) nearly doubled the pond standard yield. FCR values calculated in this IPRS study (1.5-1.64) were far better than those of conventional catfish pond production systems (2.2-2.4). This significantly reduced feeding cost in the IPRS. The weighted total cost of production was US\$2.08/kg (US\$2.01 - 2.15/kg). Fish were sold to processor at US\$2.53/kg yielding a profitability index of 24.0% above total costs.

**Table 1 - Production data from the first year study on farming hybrid catfish in IPR (Av. stocking weight = 41 g; 268 days of growout).**

	Ponds			
	B1	B2	B3	B4
Pond area (ha)	0.40	0.40	0.40	0.40
Raceway volume (m <sup>3</sup> )	63.5	63.5	45.3	45.3
Number of fish stocked	11,030	11,086	8,083	7,821
Stocking biomass (kg)	456	463	345	322
Feed applied (kg)	9,699	9,817	8,200	7,733
Mean weight (g)	671	794	712	817
Biomass (kg)	6,388	6,601	5,510	5,467
FCR	1.64	1.60	1.59	1.50
Avg. feeding rate (kg/ha/day)	90.5	91.6	76.5	72.1
Standing crop (kg/m <sup>3</sup> )	100.7	104.0	121.6	120.7
Standing crop (kg/ha)	15,971	16,502	13,774	13,666
Survival (%)	86.4%	75.0%	95.7%	85.6%

**Weight Distribution of fish at Harvest (kgs)**



## EVALUATION OF SODIUM HYPOCHLORITE IMMERSION PROTOCOLS FOR THE DISINFECTION OF *Acartia tonsa* AND *Parvocalanus crassirostris* CULTURES

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Contamination of live feed cultures by unwanted organisms can have major negative impacts on hatchery production. The calanoid copepods *Acartia tonsa* and *Parvocalanus crassirostris* are major candidates as live feeds for marine aquaculture. Cultures of these two copepod species are commonly contaminated by a variety of bacteria, protists, algae, and rotifers, and protocols for their easy disinfection/re-isolation are essential for maintaining consistent production and reducing manpower and costs.

Previous work has demonstrated the effectiveness of a variety of antibacterials on copepod eggs, however protozoa also can reduce the productivity of copepod cultures by outcompeting the copepods for food, attaching to the copepods (occasionally being parasitic), and fouling culture tanks. To evaluate the effectiveness of sodium hypochlorite (bleach) for the control of protozoa in cultures of *Acartia tonsa* and *Parvocalanus crassirostris*, we exposed eggs of the two species to a range of bleach concentrations (25, 50, 100, 200 and 400 ppt total chlorine, Cl<sub>2</sub>) over a range of exposure durations (15 sec, 5, 15 and 30 min). Each treatment was replicated three times. For each treatment, copepod eggs were mixed with a concentrated protozoa culture containing *Euplotes* sp. and *Euglena* sp., exposed to each treatment, then rinsed three times with filtered saltwater. Water from the last rinse was tested for residual chlorine to confirm that all chlorine had been removed. Protozoa were visually observed after the rinses and then 40 hours post-exposure. Copepod egg hatch percentage was evaluated 40 hours after exposure. Results show that a bleach concentration of 50 ppt and above is effective in killing protozoa for all durations tested. For both species of copepods, egg hatch was negatively related to bleach concentration, and the correlation was stronger for the longer exposure durations. Eggs of both copepod species were able to survive exposure to a relatively high level of bleach (400 ppt) for a short period of time (15 sec.), but almost complete mortality (> 90%) was observed when exposure to high concentrations lasted 15 min or more. Differences in percent egg hatch between the two species at many of the treatment levels indicates that *P. crassirostris* is more sensitive to the exposure duration and bleach concentrations tested than *A. tonsa*.

This study showed that a 15 sec exposure to 50 ppt bleach was sufficient to control protozoa. This protocol would allow cost effective disinfection of large numbers of copepod eggs simultaneously, which can be useful when starting fresh cultures. However, because of the differences in bleach tolerance noted in the eggs of the two copepod species, the optimal bleach concentration and exposure duration may need to be adjusted in order to apply this method to other copepod species.

## EVALUATION OF MICROALGAE CONCENTRATES AS FISHMEAL REPLACEMENTS FOR HYBRID STRIPED BASS *Morone sp.* INCLUDING EFFECTS ON NUTRIENT DIGESTIBILITY, GROWTH AND UTILIZATION OF FEED

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The rapid growth of aquacultural production has resulted in an increasing demand for aquafeeds. Currently, the feed industry is progressively finding alternative ingredients especially to replace fishmeal [FM] due to its steadily increasing price due to heightened demand as aquaculture continues to grow. The use of processed microalgae concentrates in aquafeeds in recent years have been considered to be potentially viable alternatives to FM and fish oil. However, considering FM has an ideal amino acid profile for most cultured species, it is more feasible to partially replace FM with microalgae concentrates. The nutrient digestibility and utilization of microalgae ingredients have not been determined for hybrid striped bass. Therefore, the objectives of this study were to evaluate several microalgae concentrates in hybrid striped bass diets and their effects on weight gain, feed utilization and nutrient digestibility.

A comparative feeding trial was conducted to evaluate the replacement of FM with different types of microalgae in the diet of juvenile hybrid striped bass (average initial weight of 15.1 g/fish). The microalgae evaluated included dried products from monocultures of *Phaeodactylum tricornutum* and *Nanochloropsis salina*, as well as mixed cultures of those two species. In addition, several preparations of *Chlorella sp.* extracted by various means and bluegreen algae biomass were evaluated. All diets were kept isonitrogenous at 40% CP and isocaloric at 3.3 kcal digestible energy g<sup>-1</sup>. The reference diet was formulated to contain an equal amount of protein from Special Select menhaden FM and soy protein concentrate. The various algae products were substituted to replace either 10 or 20% of the protein in the reference diet. The feeding trial was conducted in a recirculation system where 15 fish were stocked into 110- L glass aquaria. All treatments were triplicated and fish were fed twice daily at a rate approaching apparent satiation for 8 weeks.

No mortality was observed during the feeding trial, but growth performance of hybrid striped bass was affected by dietary treatments. Fish fed the reference and bluegreen algae diets has similar weight gain which was significantly greater than that of fish fed diets in which the algae products were included. Substitution of the other algae meals tended to reduce weight gain by 10 to 17% compared to that of fish fed the reference diet. There are many other analysis currently underway such as protein and amino acid digestibility which will be presented.

## **BRAND EQUITY IN SHRIMP ACROSS METRO MARKETS IN USA: AN ANALYSIS OF STORE-BASED SCANNER DATA**

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Recent studies on frozen seafood products (Chen 2016, Asche et.al., 2015; Asche et.al., 2013; Ahmad and Anders, 2012; Roheim et al., 2007; Roheim et al., 2011; Larkin et al., 2012) using scanner data revealed that brand and fish species were the main factors in retail prices. Other factors which add distinct value to seafood products were package size, product and processed forms. The present study investigates the extent of brands equity for shrimp in the United States at a disaggregated product and market level. It attempts to answer the following questions: Does brand matters to the shrimp consumers in USA? If so, what is the strength of various brands? How do brand strengths vary by shrimp products, markets and time, and why? To answer the above questions, this paper has quantified price premium for different brands, shrimp products and sizes, and measured brand loyalty of shrimp consumers in different market locations. It has analyzed market prices, purchase volume of shrimp products under promotional schemes and regular price situations, and market share of different brands. The study is based on data collected for the period 2009 to 2013 from Walmart by the A.C. Nielsen Company under the Expanded All Outlets Combined (xAOC) ScanTrack data programs. Ten metro markets, namely, Atlanta, Boston, Chicago, Detroit, Los Angeles, New York, Philadelphia, Pittsburgh, San Francisco and Washington D.C. are studied.

## GYNOGENESIS IN ZEBRAFISH RESULTS IN ALL MALE PROGENIES

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Zebrafish *Danio rerio* has rapidly become the model vertebrate of choice for developmental and biomedical research. Despite rigorous investigation, the sex determination system of zebrafish remains poorly understood. Current understanding suggests the system to be polygenic, and mapping studies only identify markers weakly associated with sex. Gynogenesis, which is inheritance of only maternal chromosomes, is a widely used technique for investigating the sex determining systems of fish. However, the literature describing gynogenesis in zebrafish is contradictory and marred by poor growth and yields of gynogens as well as the use of irradiated zebrafish sperm, not heterologous sperm, to activate oocytes.

We showed that common carp *Cyprinus carpio* sperm is capable of fertilizing zebrafish oocytes, and the resulting hybrids are inviable past the embryonic stage. Three meiotic and three mitotic gynogenetic progenies (defined based on the time of shock after insemination, 1.5 and 13 min, respectively; Streisinger et al. 1981) were obtained by applying physical shocks to oocytes after insemination with UV-irradiated common carp sperm. Using a novel larval rearing method, growth of the gynogens was comparable to controls (Fig. 1). All gynogens which reached reproductive maturity (n=46) were male. The fertility of gynogen males was first assessed by performing natural spawns; 60% of meiotic and 40% of mitotic gynogen males successfully fertilized oocytes compared to 100% of biparental males. *In vitro* fertilization (sperm collected by dissection and maceration of testes) was attempted with two meiotic and five mitotic gynogens which failed to fertilize oocytes by natural spawns. Sperm from both meiotic gynogens and three mitotic gynogens successfully fertilized oocytes *in vitro*. Fertilization rate was significantly lower among these fertile (*in vitro*) mitotic gynogens (50%±33) than in biparental controls (n=3, 75%±17) (paired t-test, p>0.05).

Sex ratio in progenies produced by meiotic gynogens and their biparental siblings was investigated. The distributions of progeny sex ratio were found to be markedly different with meiotic gynogens giving a multimodal distribution of both highly biased and balanced progenies, while biparental males gave a unimodal distribution centered around a balanced ratio. The results of these studies confirm that the sex determination system is polygenic but suggest that an additive model of inheritance is not accurate.

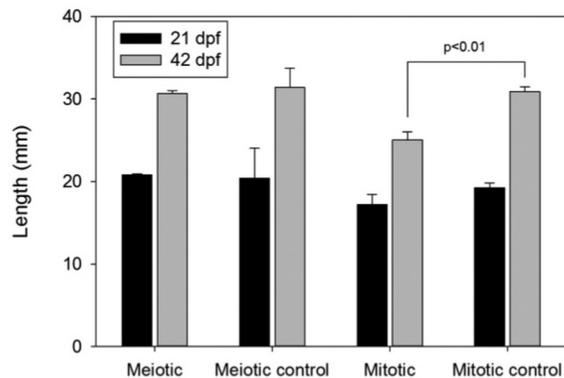


Fig. 1 Growth of gynogens and controls

## A NEW PROTOCOL FOR EXPLORING THE POTENTIAL GROWTH RATE OF ZEBRAFISH LARVAE AND JUVENILES

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Over the past twenty years, zebrafish have become a widely popular model organism. However, husbandry techniques, particularly in the early life stages, require further development and are lacking standardization. We describe a novel larval culture protocol that results in high survival and rapid growth during the larval and juvenile stages. Larval fish were reared in static, 3ppt saline water with a 24L:0D photoperiod from 5-21 days post-fertilization (dpf). Turbidity was maintained at 10-20 NTU with *Nannochloropsis* algae paste. For the first five days of feeding (5-10dpf) marine rotifers *Brachionus plicatilis* were maintained in the fish tank at a concentration of 200 rotifers/mL. At 11dpf, density of fish was decreased by 1/3. Fish were then fed *Artemia* nauplii, which were maintained at a concentration of 10/mL until 21dpf. The effect of stocking density on growth during this period was investigated. Twenty-five progenies from individual breeding pairs were stocked at various densities and reared using this method. A density of approximately 16.7 fish/L (25 fish/L from 5-10dpf) was determined to be the highest density achievable in this stagnant water system without compromising growth (Fig 1). Higher densities decreased growth but not survival.

At 21dpf, 22 progenies were transferred to a freshwater recirculating system with a 13L:11D photoperiod and stocked at a density of 20 fish/L. Fish were fed *Artemia* nauplii supplemented with dry feed (Otohime B1/B2). At 42dpf, fish were measured (Table 1) and density was decreased to 1.6 fish/L. Once all fish were clearly identifiable as male or female, three pairs from three progenies (total of 9 pairs) were spawned (Table 2). Sex ratio was determined in 16 progenies and the average percent male was 58±24%. These results demonstrate that the described protocol for larval rearing results in high survival and rapid growth without any negative impacts on fertility or sex differentiation.

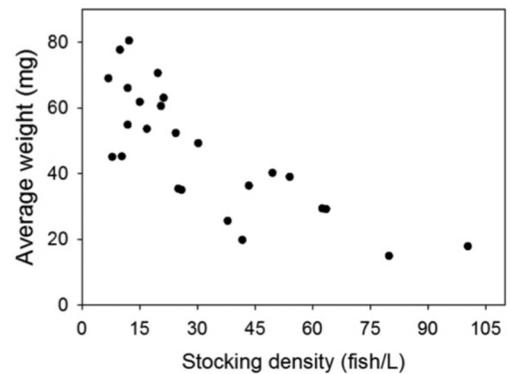


Fig 1. Dependence of size at 21dpf on density (11-21dpf)

Table 1. Mean growth and survival in progenies with stocking density of 12-22 fish/L (11-21dpf, n=8)

Age (dpf)	21	42
Weight (mg)	64±9	245±28
Length (mm)	19.4±1	30.4±1.5
Survival (%)	87±9	98±3

Table 2. Fertility of fish produced with this method (62-72 dpf)

Parameter		Mean±SD
Weight (mg)	Male	358±30
	Female	476±45
Length (mm)	Male	35.6±0.7
	Female	37.1±1.2
Age at spawning (dpf)		66±3
Fertilization rate (2-4 cells)		72±16%
Clutch size		232±124

## WEST COAST AQUACULTURE PERMITTING: TRIALS OF A CONVOLUTED REGULATORY ENVIRONMENT

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Permitting allows for review of potential beneficial and negative impacts in order to balance protection and utilization of important resources. While permitting generally serves important objectives, the current regulatory framework for aquaculture is frequently inefficient and overly complex, creating significant uncertainties and disincentives for siting new farms. This framework stymies national and state goals to expand the country's aquaculture production and provide nutritious seafood for a growing population. We provide three examples of challenges currently faced by West Coast shellfish farmers in order to illustrate inefficiencies with the current regulatory system and identify potential solutions.

First, aquaculture operations are subject to review and approval by numerous agencies, many of which analyze the same issues but require different methods for analysis. For example, there are currently numerous and evolving methods for delineating native eelgrass (*Zostera marina*). The level of detail and approach currently required by different agencies can result in significantly different calculations, and some methodologies do not scale the level of effort with the potential to actually impact the eelgrass bed. Depending on the size of the project area, this can result in extensive cost for projects that ultimately avoid eelgrass habitat. Projects reviewed by federal, state, or local authorities should have one standardized approach to habitat surveys.

Second, regulatory attention sometimes strays from the big picture. Regulatory programs typically have key, overall objectives, such as protecting water quality or ensuring no net loss of ecological functions. Frequently, however, regulatory attention on aquaculture projects has increasingly focused on whether there will be any changes to a single habitat type (e.g., eelgrass). Extensive monitoring and mitigation is required, regardless of whether changes to that habitat would be a significant or even meaningfully impact to protected species. In addition, potential ecological functions and services provided by shellfish beds have largely been disregarded because there is no accepted method to evaluate these services in relation to baseline habitat. Refocusing attention on the net environmental impacts of the proposal can save significant time and resources, streamline the regulatory process, and increase certainty.

Third, the regulatory framework for aquaculture as a whole is overly complex and duplicative. Aquaculture is not simply regulated by numerous agencies, but many of the same agencies are analyzing the same impacts and sometimes have conflicting opinions. Since approval is required from all agencies, a single "no" can prevent an entire proposal from moving forward. Even when all approvals are obtained, this framework encourages appeals that can add hundreds of thousands of dollars in costs and multiple years of delay. Consolidating and streamlining the permitting process can significantly reduce unnecessary obstacles, costs, and uncertainties.

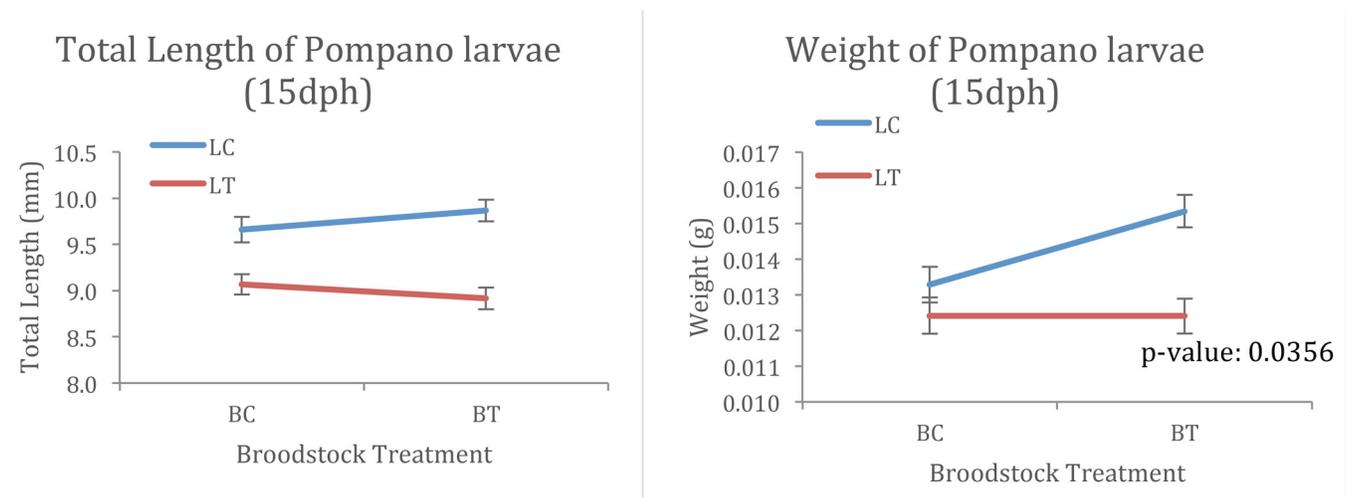
## SUPPLEMENTING BROODSTOCK AND LARVAL DIETS FOR FLORIDA POMPANO *Trachinotus carolinus* WITH TAURINE TO IMPROVE EGG, LARVAL, AND WEANED JUVENILE QUALITY

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Florida Pompano have been identified as an ideal species for aquaculture in America. As with many cultured marine finfish species there is a production bottleneck between egg and weaned juveniles. Florida Pompano have the potential to produce up to 1.5 million eggs but it is not uncommon to have survivability of 5% to 15% from eggs to weaned juveniles. Recent research shows that amino acids supplemented in broodstock diets were passed onto the eggs. Taurine, a 2-aminoethanesulfonic acid, is a common organic compound and has been suggested to help enhance egg quality. To evaluate the potential of taurine supplementation for Florida Pompano broodstock and larvae, a 2x2 factorial experiment was conducted, where two groups of adult fish received formulated gel diets with or without taurine supplementation, and the resulting larvae were divided to receive taurine-supplemented or unsupplemented live prey. Broodstock received experimental diets three times a day for 3 weeks prior to spawning, while the larvae were raised on an otherwise standard protocol based on rotifers (in green water) and *Artemia* enriched with a commercial emulsion and weaned on a dry feed at 15 days-post-hatch when the trial was terminated. Results show that supplementation of the broodstock diet could be beneficial while larval supplementation may not be warranted. Further research is being conducted to strengthen and complement these results.

Graphs 1 & 2. Interaction plots for length and weight results of the trial at termination (15 dph) (Legend: BC- Broodstock Control, BT- Broodstock Taurine, LC- Larval Control, LT- Larval Taurine)



## **ANALYSIS OF SCANNER DATA ON SEAFOOD PRODUCTS IN THE UNITED STATES: REVIEW OF RECENT STUDIES AND IMPLICATIONS FOR AQUACULTURE/FISHERIES INDUSTRY**

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The United States (U.S.) is a major net importer of seafood, and its demand for imported seafood has continued to grow over time. Seafood retailing in the U.S. is carried out via two chief outlets: restaurants and grocery stores. The recent availability of commercial scanner data allows significant advances in understanding seafood grocery sector in the U.S. A number of recent studies used store-based as well as household-based scanner data from the Nielsen Company, and analyzed market trends and estimated demand and hedonic price models for various seafood products in the United States. This article reviews recent studies that used Nielsen scanner data, and provides some implications for global aquaculture and fisheries industry.

The number of seafood species sold in the U.S. markets has shrunk over the last five years. On average, the top-10 species accounted for approximately 94 percent of all the seafood sold in individual metro markets. Not all the species in the top-10 list were able to maintain their position in the markets. Over the last 5 years, only 20 percent of the total products launched in the markets lasted for more than 4 years. Tilapia continued to maintain its strong position in the U.S. markets for the past 5 years. While mixed results were observed for salmon. Swai is another seafood commodity that has shown a steady improvement in market share.

Estimated hedonic models reveal that the implicit price of form is species and market specific. Results indicate that consumers are willing to pay higher prices for private brands relative to national brands and that small package size (below 16oz = 4 servings) carries a price premium. Convenience is also an important attribute valued by costumers. Results show that the responsiveness of seafood demand to changes in its own and substitute product prices vary over seasons and U.S. census divisions. The review reveals that the seafood market environment in the U.S. is market and product specific. The seafood industry needs to develop market specific strategies to gain further market share in retail stores.

## RECENT ADVANCES IN THE CULTURE OF THE PACIFIC BLUE TANG *Paracanthurus hepatus*

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It is estimated that over 11 million marine ornamental fishes, representing approximately 1,800 unique species, are sold annually; with the preponderance of specimens resulting from wild capture. Growing interest in marine ornamental aquaculture has served as an impetus for efforts to commercialize new species for the industry and develop novel culture protocols. The Pacific blue tang, *Paracanthurus hepatus*, is consistently among the top twenty species imported into the United States by volume, with all specimens sourced from wild stocks. Captive culture of this species through metamorphosis has not been previously documented and fundamental information regarding reproduction, larval culture, and production techniques is scarce. This study aimed to elucidate methods that would advance our understanding and success with captive propagation of this species.

A total of 50,000 eggs were collected from a single broodstock population (1 male, 2 females) over a three day period in May 2016. The eggs were successively stocked in a 1000 L larval tank for a final density of 50 eggs/L. Beginning at 3 days post hatch (DPH) larvae were fed 3 times daily a diet comprised exclusively of the copepod nauplii ( $<75 \mu\text{m}$ , mean  $\pm$  SD =  $5.1 \pm 2.3 \text{ mL}^{-1} \text{ day}^{-1}$ ) of *Parvocalanus crassirostris*. At 12 DPH, enriched rotifers *Brachionus plicatilis* ( $6.8 \pm 3.2 \text{ mL}^{-1} \text{ day}^{-1}$ ) were first fed to the tank. At 20 – 21 DPH, powdered feed and first instar *Artemia* nauplii ( $1.1 \pm 0.7 \text{ mL}^{-1} \text{ day}^{-1}$ ) were also added to the diet. Live microalgae (~3:1 of *Tetraselmis chuii* and *Symbiodinium microadriaticum*) were added daily to the culture tank throughout the rearing trial. Large mortality events were observed at 7 and 20 DPH corresponding with starvation and flexion, respectively. By 41 DPH, a behavioral change was noted with the majority of the remaining larvae associating with the bottom of the tank. On day 50, the first signs of blue pigmentation marked the beginning of metamorphosis. A total of 27 juvenile blue tangs were cultured during this trial. This effort represents the first successful rearing of this species in captivity and provides important information for future production studies.

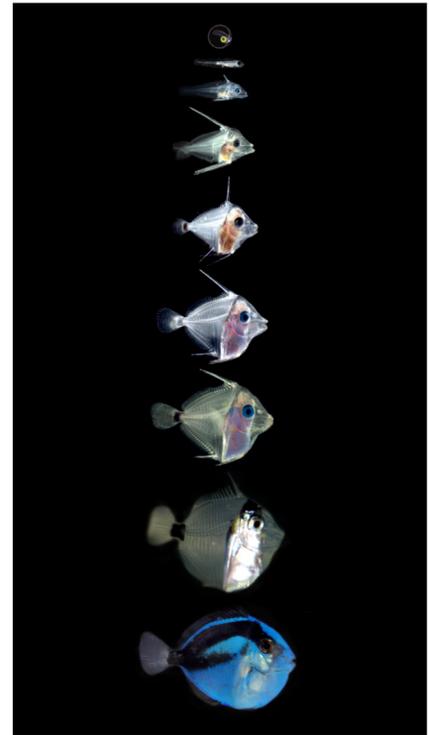


Figure 1: Larval development of the Pacific blue tang, *Paracanthurus hepatus*

## WASTE PROCESSING AND COMPOSTING WORM PRODUCTION IN A MEDIA-BASED AQUAPONICS SYSTEM

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In aquaponics, the use substrate-based plant culture is common for its substantial root support and allowance for the farmer to regulate root moisture. This benefits crop yields and creates efficiency for both the aquaculture and hydroponic components of the system. An issue that occurs in these systems is the deposition of feed waste, fecal material, and decomposing roots in the substrate. The accumulation of waste causes water quality and disease issues for the plants, fish, and beneficial bacteria. Removal of these sediments is beneficial to plants but is labor intensive. Due to their biological processing capability, composting worms are used to transform waste into plant-available nutrients. Additionally, the production of worms (Vermiculture) is a potential revenue source for aquaponic producers. Formalized research in this area is lacking despite the popular use of worms to manage the organic matter. The goal of this experiment is to analyze the effects of worm species for processing waste, as well as production characteristics for the plants, fish, and worms in a substrate-based aquaponics system.

Barramundi (*Lates calcarifer*) and plant seedlings (lettuce, cilantro and basil) were stocked into one of three replicated 720-L aquaponic systems, each containing four plant culture units. The plant culture units measured 75 cm x 80 cm x 20 cm and were filled to a depth of 17 cm with rounded river pebbles (pea gravel) measuring 0.5 to 1.5 cm diameter. Water was pumped to the plants hourly from 06:00 to 18:00 for 15 minutes intervals (controlled via timer). Plant production data (i.e. number and biomass) was collected throughout the culture period. Composting worm treatments consisted of a control (no worms), Red Wigglers (*Eisenia fetida*) and Red European Night Crawlers (*Eisenia hortensis*). Sediment deposition was analyzed in each plant culture unit between crops. At harvest, the remaining worms were enumerated and weighed to assess biomass gain.

This experiment evaluates the viability of worms as a waste processing vehicle. Potential value to the farmer will be discussed in terms of worm production rates and economic potential of vermiculture enterprise.

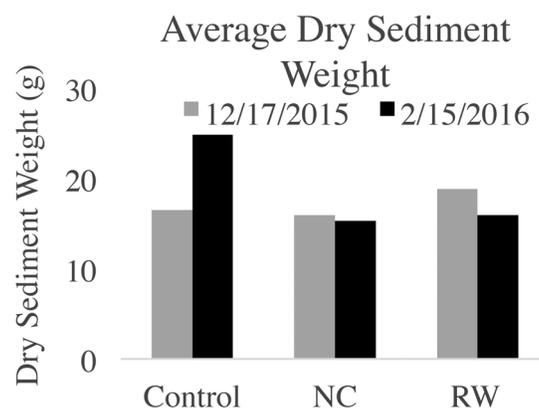


Figure 1. Average dry sediment weight of subsamples collected from the control (no worms), night crawlers (NC), and red wigglers (RW) culture units over the culture period.

**COMBINING ABILITY OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALES AND BLUE CATFISH (*Ictalurus furcatus*) MALES FOR RESISTANCE TO ENTERIC SEPTICEMIA OF CATFISH (*Edwardsiella ictaluri*) AND TOLERANCE OF LOW OXYGEN**

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Catfish are the backbone of the aquaculture industry in the United States accounting for the majority of finfish production. The main species raised is channel catfish but that is rapidly changing as many farmers are adopting the hybrid catfish due to its increased performance and disease resistance. However, there are large gaps in knowledge regarding disease resistance of hybrid catfish. Enteric septicemia of catfish (ESC) is one of the two most common bacterial infections in catfish industry which results in large economic loss annually. Full-sib and half-sib families were compared for resistance to *Edwardsiella ictaluri* the causative agent of ESC and for tolerance to low oxygen. Family variation was observed and general and specific combining abilities are being calculated. These results will guide catfish genetic improvement programs as this will determine whether selection for channel catfish dams and/or blue catfish sires will allow production of better performing hybrids.

# EFFECTS OF DIET AND REARING UNIT SIZE ON GROWTH AND SURVIVAL OF NEWLY-HATCHED WALLEYE *Sander vitreus* GROWN UNDER INTENSIVE CONDITIONS AT ONTARIO'S WHITE LAKE FISH CULTURE STATION AND PROGRESS ON DEVELOPING A CAPTIVE BROODSTOCK

Timothy D. Drew\*, Ryan W. Zheng, Jennifer L. Smith, Kyle A. Reynolds, and Kevin K. Loftus

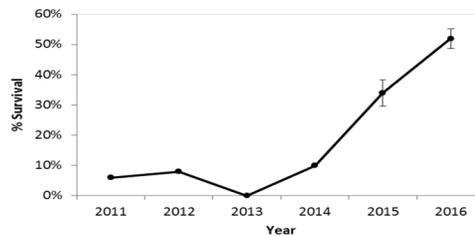
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Staff from White Lake Fish Culture Station have been extensively culturing Walleye larva in fertilized ponds for more than 20 years to support stocking efforts. These Walleye are stocked as either summer pond fingerlings (SPF) in mid-July when they typically weigh 0.5 – 1.0 gram (about six weeks post hatch), or as fall fingerlings (FF) when they typically weigh 20 grams (about 21-22 weeks post hatch). In recent years, the demand for both SPF and FF has increased beyond White Lake’s production capacity. In response, staff began to learn about and apply the intensive rearing practices that were pioneered by Summerfelt and colleagues. The success of these methods relies on a balance of turbidity, light, rearing unit, temperature, diet, and feeding regime. The most critical component of this methodology is the addition of clay during the first few weeks post hatch to maintain turbidity. When applied correctly, the technique can routinely result in survival rates to the SPF stage in excess of 50%.

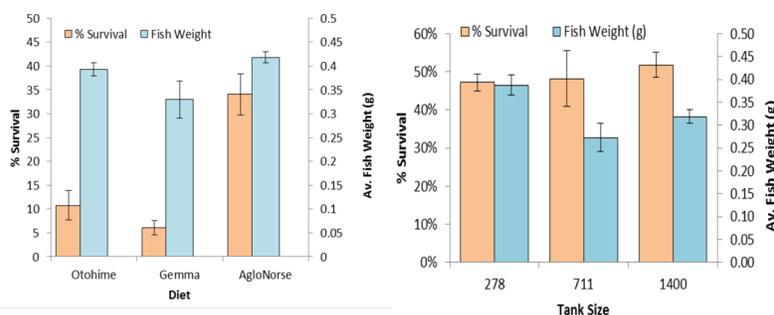
In 2011 and 2012, White Lake staff attempted rearing Walleye intensively without the use of turbidity modifiers. Survival rates were low. In response, staff identified experts in Iowa and Wisconsin who had expertise in the application of Summerfelt’s methodology (or modifications thereof). Staff’s first attempt to apply the methodology in 2013 failed; however they have seen steady improvements in survival and growth since that time (Fig. 1). This presentation describes the results of those efforts and summarizes the findings from diet and tank size trials.

The presentation also describes the effects of diet and tank size on growth and survival (Fig. 2) and of efforts to develop captive broodstocks.

**Figure 1.** Survival of walleye to summer pond yearling phase in the best treatments at White Lake Fish Culture Station



**Figure 2.** Survival and growth of walleye to summer pond yearling phase during a feed study (left) and tank volume study (right)



## EFFECTS OF DIET AND REARING UNIT SIZE ON THE INTENSIVE CULTURE OF NEWLY-HATCHED WALLEYE *Sander vitreus* IN A RECIRCULATING AQUACULTURE SYSTEM AT ONTARIO'S BLUE JAY CREEK FISH CULTURE STATION

Timothy D. Drew\*, P.F. Methner, R.W. Zheng, J.L. Smith, S.V. Krause, and K.K. Loftus

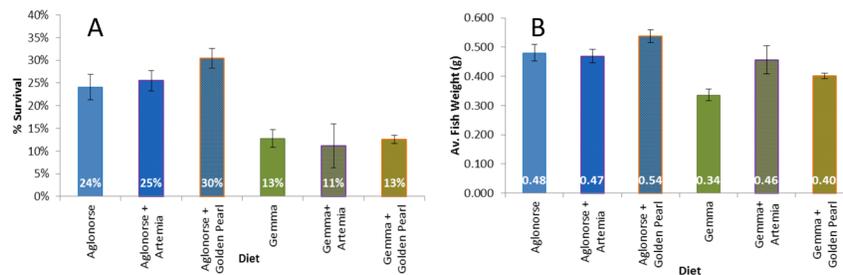
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Staff from Blue Jay Creek Fish Culture Station have been extensively culturing walleye larva in fertilized ponds at their substation for more than 20 years in order to support stocking efforts. The station stocks its walleye as either summer pond fingerlings (SPF) in mid-July when they typically weigh 0.5 – 1.0 gram (about six weeks post hatch), or as fall fingerlings (FF) when they typically weigh 20 grams (about 21 weeks post hatch). In recent years, the demand for both SPF and FF has increased beyond Blue Jay's production capacity. In response, staff began to explore the intensive culture methods pioneered by Summerfelt and colleagues. The success of these methods relies on a balance of turbidity, light, rearing unit, temperature, diet, and feeding regime. Of particular importance is the addition of clay during the first few weeks post hatch to maintain elevated turbidity levels. When applied correctly, the methodology can routinely result in over 50% survival to the SPF stage.

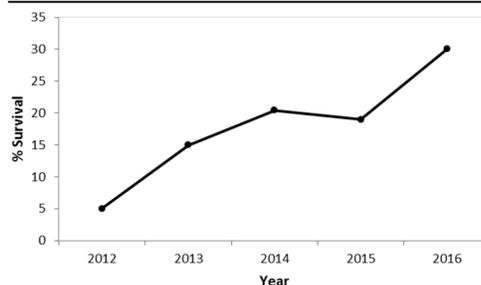
Due to space and temperature constraints at Blue Jay Creek, staff developed one of the first recirculating aquaculture systems for the intensive culture of newly-hatched Walleye in North America. This presentation summarizes some of the challenges faced, and progress made, over three years of effort. The challenges included difficulties maintaining optimum temperatures and turbidity levels, low oxygen levels, ammonia spikes, system clogging and fish health issues possibly related to the challenge of disinfecting the water between passes during the high turbidity phase.

Over a three year period, staff overcame many of these challenges while also evaluating the effect of tank size and diet on growth and survival (Fig.1). Results to date are promising (Fig. 2).

**Figure 1.** Average survival (A) and weight (B) of juvenile Walleye to six-weeks post hatch. Fish were fed either Aglonorse (blue); or Gemma (green) either alone or with supplements



**Figure 2.** Overall survival of juvenile Walleye to six-weeks post hatch in the intensive recirculating system at Blue Jay Creek Fish



## **DETERMINATION OF METHIONINE REQUIREMENT AND EFFICACY OF METHIONINE SUPPLEMENTS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei* FED WITH SOY-BASED DIETS**

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Soybean meal and other plant based protein sources have been studied as alternatives to fishmeal. However, the amino acid profile of many of these meals is less than ideal. Methionine is usually the first limiting amino acid in many diets, especially those containing high level of soy protein. To improve the acceptance of high soy feed formulations for Pacific white shrimp *Litopenaeus vannamei*, two growth trials were conducted and one growth trial is ongoing exploring the methionine requirement and efficacy of different methionine supplements. In the first growth trial, the basal diet was formulated to contain 38% protein, 9% lipid and 0.54% methionine. Two sources of methionine were supplemented to basal diet to produce diets containing graded dietary methionine levels of 0.6%, 0.63%, 0.73% and 0.83% with DL-methionine supplementation, and 0.61%, 0.63%, 0.75% and 0.81% with micro-capsulated methionine supplementation. Additionally, the tenth diet was formulated by using corn gluten meal as a replacement for gelatin, dietary methionine level of this diet was 0.62%. Four replicate tanks of 15 juvenile shrimp (initial weight  $0.22\pm 0.02$ g) were randomly fed one of the diets for six weeks. At the end of the growth trial, shrimp were counted and weighed, whole shrimp body samples were collected for proximate and amino acids analysis. In the second growth trial, the basal diet was formulated to contain 35% protein, 9% lipid and 0.49% methionine. Coated methionine and DL-methionine were supplemented to basal diet to produce diets containing dietary methionine level of 0.67%. Additionally, the fourth diet was formulated by replacing gelatin and some level of soybean meal with corn gluten meal, dietary methionine level of this diet was 0.64%. Triplicate aquaria of 10 juvenile shrimp (initial weight  $0.55\pm 0.04$ g) were randomly fed one of the diets for six weeks. At the end of the growth trial, shrimp were counted and weighed, tail muscle and hepatopancreas samples were collected for amino acids and protein analysis. There were no significant differences with regard to final biomass, final meal weight, percent weight gain and feed conversion ratio among treatments for the two trials. Analysis for whole shrimp body samples and tissue samples is ongoing. The third growth trial exploring the potential of intact protein for meeting the methionine requirement of Pacific white shrimp is ongoing.

## OXYGEN DEPLETION IN A HIGH INTENSITY HYBRID CATFISH POND AFTER A POWER OUTAGE: HOW MUCH TIME IS TIME ENOUGH?

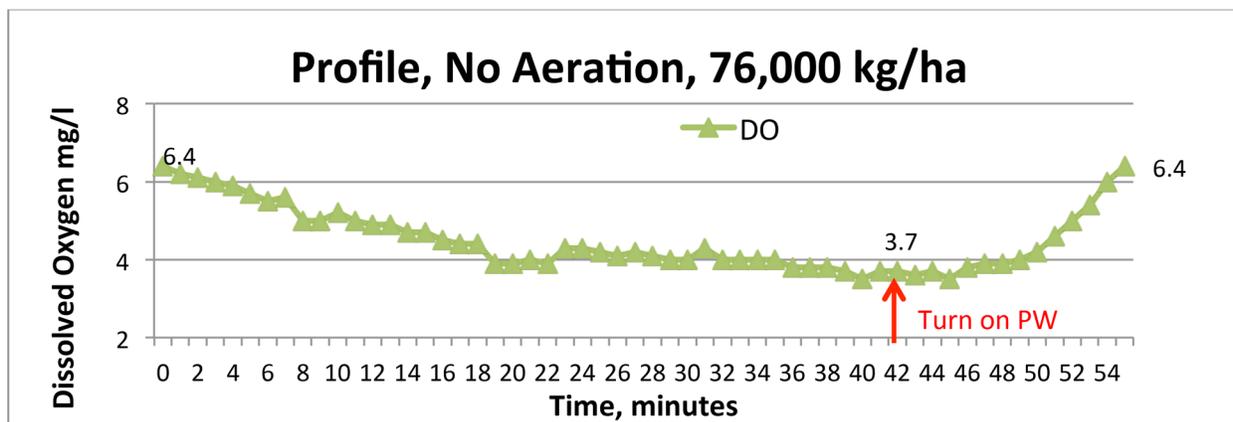
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A 0.04 ha clay-bottomed catfish pond accumulated biomass over 165 days due to periodic manual addition of biomass and growth from feeding. Due to the small size of the pond and the large biomass in the pond, paddlewheels were turned off during feeding to prevent feed moving into the grass before it could be eaten. Likewise, fish were fed slowly to prevent feed loss and feedings could extend beyond 30 minutes. This simulated a pond experiencing oxygen depletion due to a power outage.

Simulations occurred from standing crop levels of 19320 kg/ha to 128656 kg/ha. Readings began at time 0, immediately after paddlewheels were turned off and recorded every minute until feeding ceased. Paddlewheels were turned back on and readings continued every minute until Time 0 DO was regained. DO was not allowed to drop below 3.0 mg/l. This reveals the amount of time needed to deplete the pond to a low, but safe level. Times varied depending upon starting DO. An example of the process is shown in the graph above. The table below shows the time needed drop from a DO of 6 to 3 under different biomass rates.

This suggests that while there is little time to react to loss of aeration, there may be more than previously thought, depending on the DO at Time 0 and pond temperature.



Kg/ha hybrid catfish	Time, minutes, to drop from DO 6 to DO 3
28,000	108 (am) 63 (pm)
71,149	27 (T > 31C)
101,945	53
128,656	30

## IS THERE A DIFFERENCE GROWING LETTUCE WITH TILAPIA *Oreochromis niloticus* OR BLUEGILL *Lepomis macrochirus*

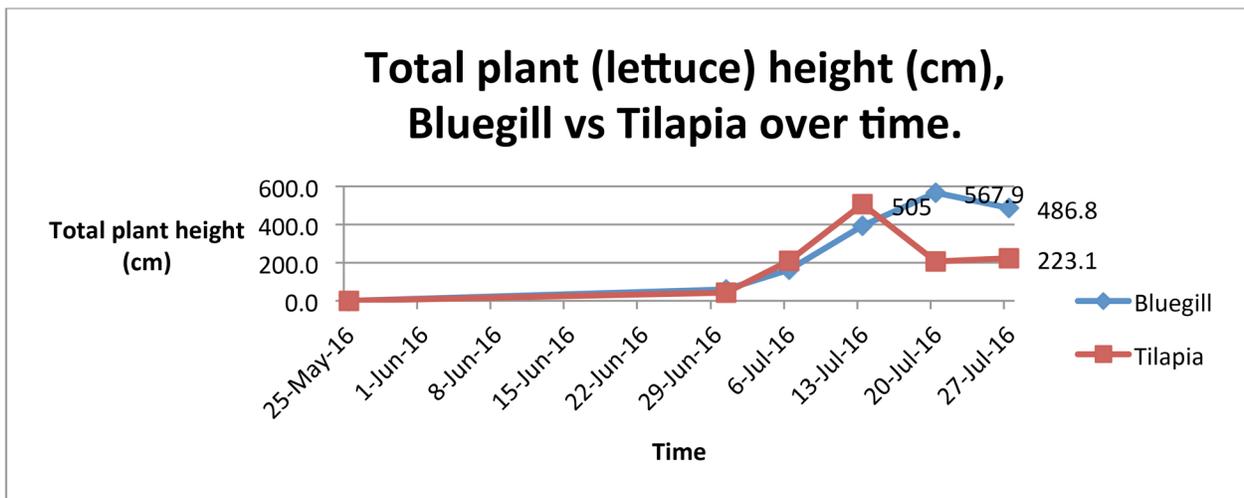
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Two identical aquaponics systems were designed to grow buttercrunch lettuce in a nutrient film technique (NFT) format placed in a greenhouse. Fish portions of the systems were stocked with equal biomass of either tilapia or bluegill. NFT portions of the design were constructed of plastic rain gutters with foam board covers. Holes were drilled in the covers sufficient to hold 5 cm diameter net pots in place. Rockwool was placed in the net pots and seeded with 1-2 lettuce seeds per pot. The plant portion of each system was composed of 6 gutter lines with 19 holes per line for a total of 114 net pots per system. Plant height was measured every 7 days and all heights summed for comparison between the two units. At the end of a trial, fish and plant total weights were recorded.

Three trials were completed. In one of the trials, fish species were switched between NFT systems. From trial to trial fish results were variable, but in each case, the Bluegill NFT system created taller lettuce, although in Trial 2 case the overall mass was greater with Tilapia.

These trials suggest bluegill are as productive as tilapia in aquaponics systems and may reduce problems with thermal requirements and non-native species aspects of raising tilapia.



Trial	Bluegill (g)	Lettuce (g)	Tilapia (g)	Lettuce (g)
1	1772	149	2000	43
2	2264	1350	2718	2057
3	1000	1411	891	1156

## HYBRID CATFISH *Ictalurus punctatus* x *Ictalurus furcatus* RAISED AT HIGH DENSITY WITH INTENSIVE AERATION IN A TRADITIONAL CLAY-BOTTOM CATFISH POND

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Channel catfish have been raised by traditional means in clay-bottom ponds for decades and produced yields up to 13,440 kg/ha, although in Arkansas a yield of 7,840 kg/ha is considered average. In recent years, the split-pond system has increased yields to 28,000 kg/ha. These systems often use hybrid catfish. Elsewhere, channel catfish raised in high-aeration cement-bottom ponds have attained yields greater than 155,000 kg/ha. Can intense aeration in a clay pond approach yields found in cement-bottom ponds? This pilot study was performed in a 0.04 ha pond initially stocked with 200 kg of hybrid catfish. Growth was simulated by weekly addition of fish to the pond, in addition to feeding.

The trial began on May 6 and ended October 18, 2016 (165 days). The goal was to accumulate biomass until the pond could no longer support additional inputs. Maximum water quality levels are shown in the table below. Chloride level was maintained at 0.5 ppt. Dissolved oxygen minimum, outside of feeding, was 4 ppm with 99 hp/ha aeration. Our model predicted a harvest of 5432 kg and the actual harvest was 5746 kg (143,650 kg/ha). Calculated FCR was 1.93. This information will be used to develop further studies and set goals for catfish pond potential yields in both intensive aeration and split ponds.

Maximum levels for measured water quality parameters: level recorded, day of study, and kg/ha of fish estimated in the pond.

Maximum levels for measured water quality parameters: level recorded, day of study, and kg/ha of fish estimated in the pond.

Test	High	Study Day	Kg/ha
Total ammonia	9.0	84	70830
Nitrite	20.3	150	130656
Nitrate	158	158	133741
Kg feed/ha/day	328	108	82574

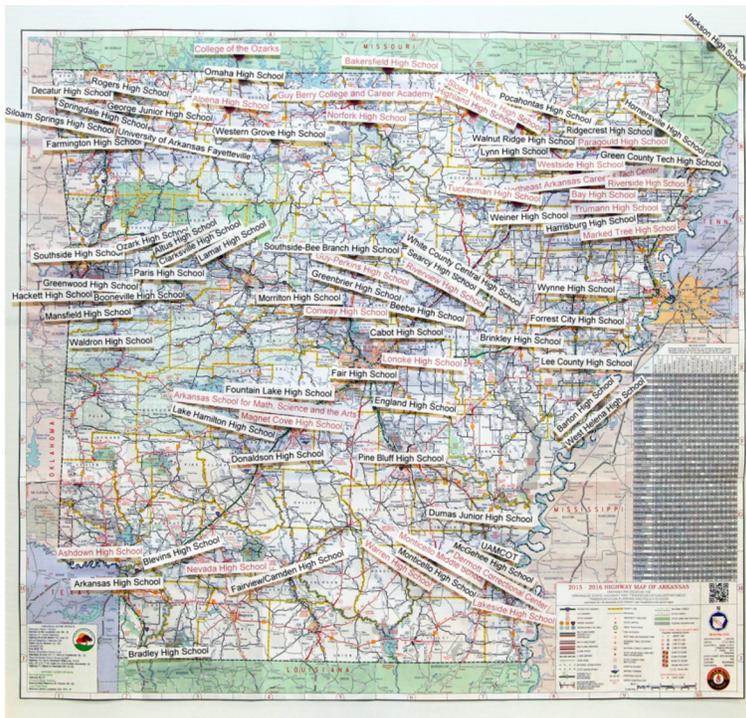
## AQUACULTURE IN THE CLASSROOM: A REVIEW OF TEACHER TRAINING IN ARKANSAS

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The University of Arkansas at Pine Bluff began supporting high school vocational/agriculture teachers in 1992 and to date has worked with nearly 100 elementary, secondary, and higher education institutions. UAPB provides free fish, technical assistance, systems operation education, disease diagnostics, in-service training, and support by phone, text, email and house calls free of charge to any educational institution. Although originally geared to agriculture teachers, biology and physics teachers have also used aquaculture in the classroom.

Different strategies have been implemented and new approaches are tested annually. Interesting outcomes noted by teachers across the state: provides a field trip without getting on a bus; a useful hands-on approach to convey math and science principles; excellent method for students to take ownership of their studies; cheaper method of keeping livestock; and a useful tool for managing anger and frustration in at-risk youth in special education situations.



## MAD BEAST

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The University of Arkansas at Pine Bluff supports High School teachers who use recirculating aquaculture systems to teach agriculture and science principles. UAPB also offers gardeners and homeowners support and advice concerning water gardens. These two areas overlap in the field of aquaponics. The MAD BEAST (Mobile Aquaponics Demonstration for Beginning Engineering, Agriculture and Science Training) is a mobile unit that is used at schools, garden shows, master gardener training seminars and other events in Arkansas. The unit is mounted on a ten foot long trailer. The foundation is permanently mounted to the trailer, and all components are removable so the system can be built in front of the audience in a short period of time. The unit demonstrates the raft, flood and drain, and nutrient film technique systems.



Early prototype

## TOWARD THE DEVELOPMENT OF SOYBEAN-TOLERANT ATLANTIC SALMON *Salmo salar* LINES

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Soybean meal (SBM) stands as viable replacement for fishmeal in aquaculture because of the relatively high protein content, amino acid profile and digestibility, as well as its global availability and low cost. Unfortunately, to date, SBM inclusion in salmon feed has been rather limited due to poor performance and negative physiological impacts in fish fed SBM-containing diets. However, studies have shown that there is significant individual and family variation in the ability to use SBM derived protein in salmonids, and that this trait has moderately high heritability. Since adaptability to SBM utilization is heritable, it is possible to develop lines of salmon with increased efficiency in utilizing SBM as a protein source through the use of traditional selective breeding strategies. Marker assisted selection, (MAS) using genetic markers such as single nucleotide polymorphisms (SNPs) associated with traits of interest, can be used to compliment traditional breeding and reduce the time required to achieve genetic gains.

Results will be presented here on a study aimed at using genome-wide association studies (GWAS) to identify Atlantic salmon SNPs associated with increased tolerance to or efficiency in utilizing SBM as a protein source. A 60-day feeding trial is being conducted to evaluate the effects of two different SBM inclusion levels (Control and Test diets with 5 and 30% SBM, respectively) on weight gain, feed efficiency, body composition, nutrient deposition, and intestinal histology. Thirty full-sib families (initial body weight:  $12.3 \pm 1.0$  g) of Atlantic salmon (St. John River strain) were utilized for the trial. Six fish per family were allocated to six different 325-liter tanks so that all families were represented equally in each tank. Each fish was tagged with passive integrated transponders (PIT); and each diet was allocated to three tanks.

At the end of the feeding trial, the eight largest and eight smallest fish from both the top five and bottom five performing families in terms of weight gain in each treatment will be selected for whole-body composition analysis, and histological examination of mid and hindgut. Samples from each fish will subsequently be subjected to high density SNP genotyping. Nutrient deposition and efficiency of utilization as well as intestinal inflammation are phenotypes that will be described quantitatively and correlated with SBM inclusion levels and genotype results. The expected outcome of this project is a set of SNPs associated with increased tolerance and/or efficiency in utilizing SBM as a protein source. These novel markers will play a key role in the development of improved genetic lines that will allow for a significant increase in SBM inclusion in aquafeeds for Atlantic salmon.

## OPEN SOURCE ENGINEERING DESIGNS FOR AQUACULTURE/AQUAPONICS SYSTEMS PRODUCING 500, 5,000 AND 50,000 LBS OF FISH PER YEAR

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Demand for locally grown, farm-raised fish is rising as the public becomes increasingly aware of the health benefits of eating safe, nutritional, seafood. For the past decade or more, aquaculture has been the fastest growing segment of agriculture, providing consumers with a locally grown, healthy product, produced utilizing sustainable methods. In addition, aquaponics, the integration of aquaculture and hydroponics, has recently gained increasing attention, because it utilizes the nutrient-rich water from aquaculture production of fish as a source of nutrients for growing plants and vegetables.

Over the past several years, a family of open source engineering designs have been crafted by the authors to promote aquaculture and aquaponics covering a range of production goals, operating systems and resource availability. Three of these aquaculture designs are presented covering production goals for home/schools (500 lbs per year), small farm/ urban (5,000 lbs per year) and commercial aquaponics systems (50,000 lbs per year). In addition, each can be combined into an aquaponics systems utilizing a raft hydroponic system, NFT system or media bed system for plant and vegetable production, i.e. fancy lettuces, tomatoes, basil and other leafy greens. Currently each of the systems have been build and have successfully been operated for several years. The open source designs include an engineering design spreadsheet for component sizing and selection, details drawings of system layout and construction details, equipment lists and specifications, and estimates of equipment costs.

The three systems presented in detail include Tanque Verde High School, Tucson AZ, ([www.facebook.com/Tucson-Aquaponics-in-Schools-and-Homes-753814521389793](http://www.facebook.com/Tucson-Aquaponics-in-Schools-and-Homes-753814521389793)), Merchant's Garden, Tucson AZ ([www.merchantsgarden.com](http://www.merchantsgarden.com)) and Ripple Rock Fish Farms, Frazeyburg, OH ([www.ripplerockfishfarms.com](http://www.ripplerockfishfarms.com)). In addition, these open source designs include several other sizes, both smaller and larger. All of the detailed designs, equipment specs and cost estimates will be available from Aquaculture Systems Technologies, LLC web page, [www.ASTFilters.com](http://www.ASTFilters.com).



## ENGINEERING DESIGN AND CONSTRUCTION OF A 10,000 SQ FT AQUAPONICS GREENHOUSE, MERCHANT'S GARDEN

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**Aquaculture** has been the fastest growing segment of agriculture for the past decade, providing consumers with a locally grown, healthy product, produced utilizing sustainable methods. In addition, **aquaponics**, the integration of aquaculture and hydroponics, has recently gained increasing attention, because it utilizes the nutrient-rich water from aquaculture production of fish as a source of nutrients for growing plants and vegetables. More recently, we have seen an incredible interest in the concept of urban farming, vertical farming and 'plant factories' for the production of healthy, nutritional plants and fish.

Merchants Garden represents the integration of a well-engineered aquaculture system with a well-managed hydroponic system, yielding a highly productive, sustainable and economically viable system for plants and animals. Indoor production of fish, plants and vegetables in a controlled environment has the ability to guarantee both the safety and the quality of the fish, plants and vegetables produced throughout the year. An integrated, engineered design allows for construction of "turn-key" systems at relatively low capital costs, creating jobs and small businesses through easier entry of urban farmers, horticulturalists and other interested parties in the localized aquaponic production of fish and produce.

The primary goal of this presentation is to walk you through the engineering design steps: from selecting the species to grow, setting the yearly production goals for both fish and plants, determining the BioPlan from fry to growout, estimating biomass densities, determining tank size and daily feeding rates. The engineering design specification for each of the unit operations are reviewed, including solids capture, biofiltration, aeration, degassing, and disinfection. Equipment options are explored and some rough estimates of capital costs are presented. Finally, knowing the feed rate per day, an estimate can be made of the size and production capacity of the hydroponics systems.

Finally some of the engineering experiences constructing the system will be shared highlighting both what worked really well and what the authors really don't want to talk about.



## PROTEIN AND LIPID COMPOSITION OF INDIGENOUS OILSEEDS IN GHANA AND THEIR POTENTIAL FOR USE IN ANIMAL FEEDS

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Environmental and cost considerations have led to increased research to find alternative feed ingredients for use in the production of animal feeds. In West Africa and Ghana to be specific, there are various indigenous plants that have potential for use in animal feeds. However, little is known about their nutritional value due to limited research in this area. This study sought to identify and determine the nutritional composition of some indigenous seeds and their potential for use in animal feeds.

A survey was conducted in four regions in Ghana and a total of 20 oilseeds were identified with seven selected for the study. They were; *Cucumeropsis mannii*, *Citrillus lanatus*, *Parkia biglobosa*, *Cucumis metuliferus*, *Cucurbita spp.*, *Sesamum indicum* and *Lagenaria siceraria*. Protein content for the selected seeds ranged from 21-39% and a total of 22 amino acids were detected; the essential amino acid profiles were good and comparable to soybean meal -particularly that of *Cucumeropsis mannii*, *Curbita spp* and *Cucumis metuliferus*. The seeds had relatively high levels of arginine (2.5-5%) but rather lower levels of methionine (0.2-0.8%). The lipid levels ranged from 17-53%; the seeds were rich in oleic acid (n-9) (14-41%) and linoleic acid (n-6) (34-64%). However, linolenic acid (n-3) levels were less than 1%.

The results show that the seeds have relatively high levels of protein and lipids and have the potential for use in animal feed. However, there is a need for further studies on the availability (digestibility) of the essential nutrients, and content of anti-nutritional factors.

Table 1: Protein and lipid contents of selected ingredients (n=3)

Ingredient	Protein (%)	Lipid (%)
<i>Lagenaria siceraria</i>	28.9 ±1.1 <sup>b</sup>	48.0 ±0.7 <sup>b</sup>
<i>Cucurbita spp.</i>	37.5 ±0.2 <sup>a</sup>	46.0 ±0.6 <sup>b</sup>
<i>Sesamum indicum</i>	20.9 ±0.2 <sup>c</sup>	52.5 ±0.7 <sup>a</sup>
<i>Cucumis pepo</i>	38.7 ±0.8 <sup>a</sup>	16.7 ±0.2 <sup>c</sup>
<i>Parkia biglobosa</i>	30.6 ±0.3 <sup>b</sup>	13.0 ±0.2 <sup>c</sup>
<i>Cucumeropsis mannii</i>	36.4 ±0.3 <sup>a</sup>	49.0 ±0.5 <sup>b</sup>
<i>Citrillus lanatus var neri</i>	26.3 ±1.2 <sup>b</sup>	48.0 ±0.5 <sup>b</sup>

Table 2: Fatty acid profile of the selected ingredients

Ingredient	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3
<i>Lagenaria siceraria</i>	13.8	11.0	22.1	51.8	0.1
<i>Cucurbita spp.</i>	15.7	10.2	35.4	37.4	0.2
<i>Sesamum indicum</i>	10.1	-	41.5	40.8	0.4
<i>Cucumis pepo</i>	15.9	9.8	31.1	42.3	<0.1
<i>Parkia biglobosa</i>	19.2	17.4	18.2	47.4	<0.1
<i>Cucumeropsis mannii</i>	15.1	7.7	16.0	60.4	0.1
<i>Citrillus lanatus var neri</i>	9.8	12.5	14.0	63	<0.1

## GENETIC DIVERSITY AND POPULATION STRUCTURE IN NATIVE AND INTRODUCED POPULATIONS OF THE *Artemia franciscana* (Crustacea, Anostraca) IN AUSTRALIA

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Recent studies revealed two distinct invasion pathways of *Artemia franciscana* from its native range in North America to Eurasia. The potential source of the newly established populations in Eurasia mainly derived from two commercially sources in USA; Great Salt Lake (GSL) and San Francisco Bay (SFB). In the present study, we are determined the phylogenetic relationships, haplotype network and population genetic structure of *Artemia* from native (North America) and introduced regions in Australia using sequence variation in a mitochondrial gene (cytochrome *c* oxidase subunit I) *COI* marker. Phylogenetic trees (ML/BI) reveal the colonization of *A. franciscana* in four geographical regions whereas, parthenogenetic lineages were observed only in two regions of Australia. Genetic diversity for all introduced Australian populations reveals a higher value ( $HE = 0.737 \pm 0.038$ ) than those in GSL ( $HE = 0.649 \pm 0.065$ ), suggesting multiple introductions with founder effects. The analysis at the regional level shows that the invasion of *A. franciscana* is associated with a loss of genetic diversity, indicating bottleneck effects. Tajima test shows a non-significant value, indicating that the populations are under equilibrium without selection. The genetic partitioning reveals that most of genetic variations occurred among populations (70.87%). The distribution of genetic diversity and pattern of genetic differentiation suggests the colonization of *A. franciscana* from native regions in North America into Australia (pairwise  $F_{ST} = 0.7$ ). Phylogenetic network points to multiple invasions from several genetically different sources in Americas and Eurasia, which seems to facilitate the widespread colonization success of *A. franciscana* into Australia. This study signifies extensive human-mediated introductions which influence the pattern of genetic diversity throughout Australia.

## **DEVELOPMENT OF SITING GUIDELINES, MONITORING STANDARDS, AND ENVIRONMENTAL MODELS COASTAL FINFISH AQUACULTURE IN MOROCCO**

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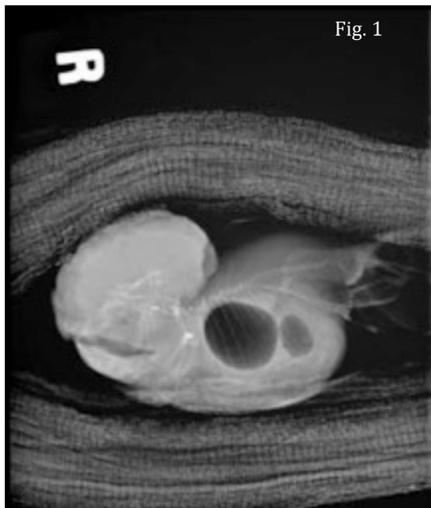
Morocco is well-positioned to grow a strong coastal aquaculture industry given its available suitable ocean space, investor interests, and proximity to high demand seafood markets. For sustainable coastal aquaculture to occur, management decisions must be based on reliable scientific data and an advanced knowledge of the local marine environment. Planning and implementing aquaculture based on best management practices reduces relative risks to the environment, increases chances of successful continued operations, and allows Moroccan coastal managers to work towards development of resilient aquaculture in the near future. With the aim to increasing the capacity of the Moroccan government to utilize environmental management tools for finfish aquaculture, a partnership was established between the National Agency for the Development of Aquaculture (ANDA) and NOAA's National Ocean Service. This collaboration was created by the U.S. Department of State in support of the current US-Morocco Free Trade Agreement. The goal of the collaboration is to develop three written guidelines focused on siting, modeling, and environmental monitoring of marine finfish aquaculture along the Mediterranean and Atlantic coasts of Morocco, applying the most current management frameworks and tools available.

## WEN TRIMMING TO CORRECT BUOYANCY ISSUES IN AN ORANDA GOLDFISH *Carassius auratus auratus*

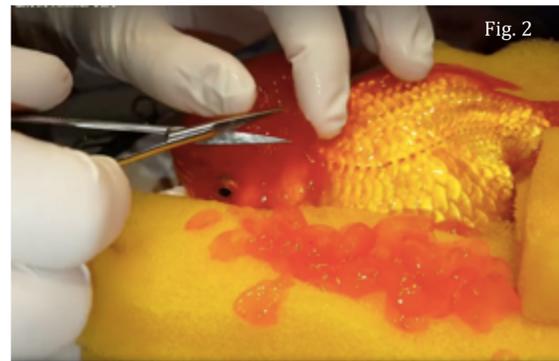
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Remarkable diversity in pet goldfish (*Carassius auratus auratus*) has developed over centuries of domestication. Similar to other commonly kept pets, this process of trait selection has led to a development of distinctive and breed-specific morbidities. Oranda goldfish can be particularly hindered by the overgrowth of the large fleshy hood on their rostrum, termed the wen. The following presentation outlines the clinical and radiographic findings, surgical procedure, and postoperative care used for treatment of wen overgrowth in a 1-year-old female Oranda goldfish with a month-long history of buoyancy abnormalities.



Right lateral (Fig 1) radiographic projection of a 1-year-old female oranda. Cranial swim bladder distension and wen overgrowth noted.



Wen trimming procedure with patient fully anesthetized (Fig 2).

## EFFECT OF FEEDING DIFFERENT MICROALGAE ON SURVIVAL, GROWTH AND BIOCHEMICAL PERFORMANCE OF ARTEMIA

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The successful culture of marine fish species is mainly based on live prey quality during larval culture period. It is obvious that nutritional value of artemia is lower than that of copepods in marine natural environment. Therefore, artemia must be fed with microalgae which contain high amounts of essential nutrients such as essential fatty acids and amino acids. *Artemia metanauplii* is also important for alternative cultured species such as rockfish, paralarvae of octopus and sea horses. During the growth phase of marine fish species, as mouth gets bigger, larger size of artemia is also needed. Therefore, the aim of this study was to evaluate the effect of four microalgae species on the survival, growth and biochemical composition of artemia.

Four microalgae species (*Amphora coffeaeformis viridis*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Dunaliella salina*) were maintained and cultured at the Phytoplankton and Zooplankton Culture Laboratory, Istanbul University, Faculty of Fisheries.

Artemia were fed with 5 microalgae diets; four of them contained a single one of the four microalgae species and the fifth diet contained a mixture of four species during 15 days of feeding. Microalgae were daily counted before giving to artemia. Each group was designed as triplicate and each erlen (working volume 250 ml.) contained 22 artemia ml<sup>-1</sup> before starting the experiment. Total length was measured at day 3, day 6, day 9, day 12 and day 15 of the experiment. For that purpose, 30 artemia were removed from each group and put in 5 % formaldehyde solution.

Final survival was determined by counting all alive artemia in each experimental group then stored in -80°C in refrigerator before biochemical analysis. The results showed excellent survival (average 70%) in artemia exclusively fed with *Amphora coffeaeformis viridis*, *Dunaliella salina* and Mix microalgae diet with better values than *Chlamydomonas reinhardtii* and *Chlorella vulgaris* fed artemia (Fig. 1). Proximate and fatty acid compositions of both microalgae and artemia were analysed.

Despite the utilization of commercial diets in live prey cultivation, microalgae seem to be still adequate for culture performance of artemia. In this case, *Amphora coffeaeformis viridis*, *Dunaliella salina* and Mix diet of microalgae seem to sustain good growth performance and biochemical composition.

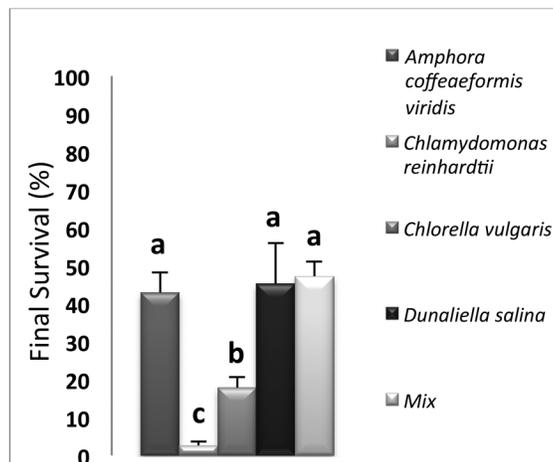


Figure 1. Survival of artemia fed five microalgae diet

## EFFECT OF LIGHT LIMITATION ON THE WATER QUALITY, BACTERIAL COUNTS, AND PERFORMANCE OF *Litopenaeus vannamei* POSTLARVAE REARED WITH BIOFLOC AT LOW SALINITY AND ZERO-WATER EXCHANGE

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The aim of this study was to evaluate the effect of light limitation on the water quality, bacterial counts, and performance of *Litopenaeus vannamei* postlarvae reared with biofloc at low salinity and zero-water exchange. Two treatments were designed: T<sub>1</sub> = Culture with natural sunlight and T<sub>2</sub> = Culture in darkness. After 28 days, in both treatments, the final weight of shrimp was over 0.6 g with a specific growth rate (SGR) over 7.4%/d, and a survival rate over 70%. In both treatments, *Vibrio* sp. concentration presented low values (culture with natural sunlight = 0.1 to 9.9 × 10<sup>2</sup> CFU/mL, culture in darkness = 0.4 to 11.7 × 10<sup>2</sup> CFU/mL) and *Bacillus* sp. had high values (culture with natural sunlight = 0.7 to 66.0 × 10<sup>4</sup> CFU/mL, culture in darkness = 0.7 to 65.8 × 10<sup>4</sup> CFU/mL). All water quality parameters remained within the ranges suitable for shrimp culture, except for alkalinity during the first stage of the study. Although in some sampling periods, some significant differences were found in bacterial counts and water quality parameters, shrimp productive performance under culture with biofloc at low salinity (≈ 9 ‰) and zero-water exchange was not significantly affected by light limitation.

## EFFECT OF WATER EXCHANGE AND STOCKING DENSITY IN A BIOFLOC-BASED SYSTEM ON SALINITY STRESS TOLERANCE AND PERFORMANCE OF *Litopenaeus vannamei* POSTLARVAE

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The effect of water exchange and stocking density was evaluated on the salinity stress tolerance (by low and high salinity) and performance of *Litopenaeus vannamei* postlarvae reared in a biofloc-based system at nursery level. A bioassay was conducted with four treatments in triplicate:  $T_1 = 8250$  orgs/m<sup>3</sup> without water exchange,  $T_2 = 16,500$  orgs/m<sup>3</sup> without water exchange,  $T_3 = 8250$  orgs/m<sup>3</sup> with 50% water exchange per week, and  $T_4 = 16,500$  orgs/m<sup>3</sup> with 50% water exchange per week. At 0, 15, 30, and 45 days of culture, a group of shrimp from each treatment was exposed to acute stress by low (27 to 2 ‰) and high salinity (27 to 60 ‰). After 49 days, the water exchange rate and water exchange rate  $\times$  stocking density interaction affected shrimp survival, but shrimp growth was affected only by stocking density. The mean weight of shrimp in the treatments with the lowest stocking density was higher than that with the highest stocking density, independently of the water exchange rate. In the salinity stress tests, shrimp survival was only affected by the interaction between the salinity stress tests and treatments ( $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ ). Generally, shrimp showed a greater tolerance when were subjected to low salinity (27 to 2 ‰). In the culture with biofloc at nursery level, apparently, shrimp from the treatments with water exchange improved their tolerance to stressful events caused by changes in salinity, but this response was not related to growth and survival.

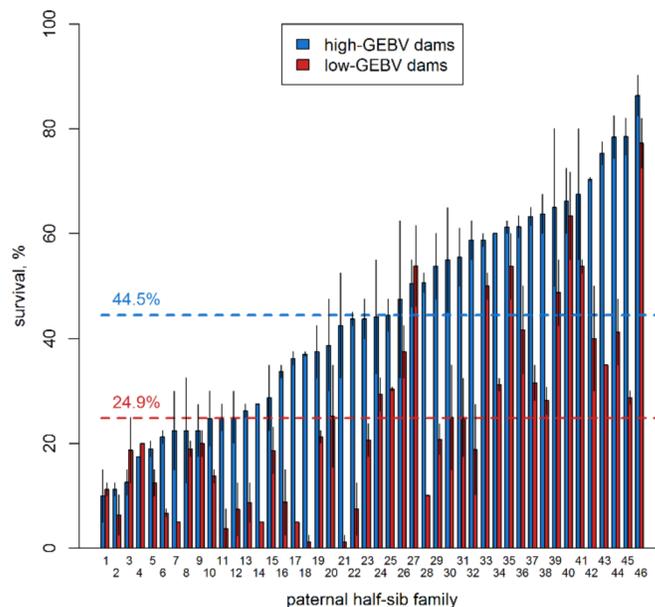
## GENOMIC SELECTION EXPLOITS WITHIN-FAMILY GENETIC VARIATION FOR DISEASE RESISTANCE IN RAINBOW TROUT

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*Flavobacterium psychrophilum* is the etiological agent causing bacterial cold water disease (BCWD) in salmonid fish. Previous breeding strategies to reduce losses due to BCWD involve testing and determining phenotypic differences between multiple families of rainbow trout. These results were used to determine an estimated breeding value based on a resistance phenotype. This study used genotypic markers, generated from a training population and associated with improved BCWD survival, to identify individual fish within a family to generate a genomic-estimated breeding value (GEBV). A single sire was crossed with high-GEBV and low-GEBV dams from the same family of rainbow trout. Progeny were then challenged with *F. psychrophilum* to determine the improvement made due to differences in the GEBV. Mean survival of progeny from high-GEBV dams after experimental *F. psychrophilum* challenge was 19.6 percentage points greater compared to progeny from low-GEBV dams. After adjusting the data for non-genetic effects, progeny from high-GEBV dams had a greater ( $P < 0.0001$ ) probability of survival ( $0.43 \pm 0.03$ ) than their half sibs from low-GEBV dams ( $0.22 \pm 0.02$ ). Mortality kinetics were similar between high- and low-GEBV progeny groups, with peak mortalities occurring between 4 and 9 days post-infection in both groups. However, during this 6-day period of peak mortality, cumulative mortality was smaller in high-GEBV progeny (38.8%) than in low-GEBV progeny (57.5%). Between 10 and 21 days post-infection, cumulative mortalities were nearly identical between high- and low-GEBV progeny (16.1 versus 16.7%, respectively). Thus, mean days to death, with a value of 21 used for fish surviving the challenge, was greater ( $P < 0.0001$ ) in high- ( $14.0 \pm 0.4$  days) versus low-GEBV progeny ( $11.1 \pm 0.4$  days). Accuracy of GEBV predictions made on the non-phenotyped parents was 0.72 (95% CI = 0.60 – 0.81). These results demonstrate that using genomic selection, improved BCWD survival can not only be achieved by identifying more resistant families of trout, but also by identifying superior individual fish within a family.



## **DO WHITE WORMS *Enchytraeus albidus* HAVE COMMERCIAL POTENTIAL AS A FEED IN THE ORNAMENTAL INDUSTRY?**

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White worms, *Enchytraeus albidus*, an oligochaete, are an attractive live feed for many cultured organisms including fresh and saltwater fishes. Historically they were mass cultivated in the former U.S.S.R. to feed juvenile sturgeon. Now they mostly are grown by aquarium hobbyists as a live feed for ornamental fishes or by biological supply companies for toxicology studies. At the University of New Hampshire, we have been exploring the potential of commercial scale cultivation of white worms and their market within the aquaculture industry. Advantages of using live white worms include: the low input costs and simplistic techniques for feeding and maintaining worm cultures; white worms have tested pathogen-free of all viral, bacterial, and parasitic assays screened so far; white worms are euryhaline and will survive in both fresh and full saline water, wriggle and attract predators, and do not impair water quality as they remain alive; their residency time in aquaculture systems is longer as they are bottom-dwelling and are not easily flushed out like other traditional live feeds; and in the majority of small-scale hatchery trials, ornamental fishes have readily consumed white worms. While these results are promising, there still are some limitations to commercial scale white worm culture. Amongst those confines are automating the white worm harvesting process, and overcoming upper limit thermal conditions of white worms encountered in aquaculture facilities in southern climes. In this talk, we will present the latest ornamental industry feedback generated from testing live white worms in commercial facilities.

## SURVEY OF TRACE ELEMENTS CONTENT OF TILPIA, CATFISH AND SALMON IN THE VIRGINIA MARKET: RISK, NUTRITIONAL ASSESMENT AND SELENIUM HEALTH BENEFIT VALUES (Se-HBV)

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Elemental food composition data are important to both consumers and health professionals because they are useful for improving dietary guidelines. The aquaculture industry has experienced rapid growth in recent years with health and nutrition trends that emphasize the importance of dietary seafood. Fish has been acknowledged as an essential component of a well-balanced diet and is an important source of minerals. However, determination of trace elements in complex matrices, such as food, often requires extensive sample preparation and/or extraction regimes prior to instrumental analysis. Traditional techniques for sample preparation are time consuming and require large amounts of reagents, which are expensive, generate hazardous waste, and might contaminate the sample with the analytes. In this study, a total of 12 fresh and frozen samples of the commercial catfish, tilapia and salmon available in the Virginia market were analyzed for levels of As, Ca, Cd, Cu, Fe, Mb, Mn, Na, Pb, Se, and Zn. The potential human health risks for the consumers and nutritional values of the minerals were assessed. Since Se has high binding affinity to Mercury (Hg) and many researchers have demonstrated the antagonist interaction of Se and Hg in different animal models, the mercury-selenium ratios and the selenium Se-HBVs were also calculated for each species. Se-HBV shows both absolute and relative amount of Se an Hg in the diet. Positive Se-HBV is indication of no health risk.

Microwave assisted acid digestion method was optimized for digestion of fish fillet. Samples were analyzed for aforementioned minerals using ICP-MS. Methodology was verified by analysis of fish protein standard reference material for trace element analysis (DORM-4). Potassium (K) presented the most remarkable concentrations of these elements. The highest concentration of the essential metals analyzed was found for catfish. All species analyzed showed beneficial Hg:Se ratios and Se-HBVs (Table1) the contribution of these fish to the Recommended Daily Allowance (RDA) and adequate intakes of these elements for adults ranged from 0.06% (Se) to 20% (Mg). Intake of these elements through these fish would not pose any risk for the average consumer. Information regarding the trace metal content of fish from this study can be used in developing new aqua feeds to enhance fish production while providing consumers a product that is healthier to consumer.

**TABLE 1.** Hg:Se molar ratios and Se-HBV

Species	<i><b>Molar ratio</b></i> <i><b>Hg:se</b></i>	Se-HBV
Catfish	0.04±0.02	0.12±0.05
Salmon	0.06±0.03	0.15±0.06
Tilapia	0.03±0.03	1.08±1.22

## THE EFFECT OF PHOTOPERIOD MANIPULATION UPON MATURATION AND SEX STEROIDS PROFILES OF BROOK TROUT (*Salvelinus fontinalis*)

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Maturation is the major constraint towards the commercial production of brook trout pre harvest maturation which affects growth, flesh quality and immunocompetency. Photoperiod manipulation is relatively used to inhibit or reduce the incidence of maturation in salmonids, so an experimental adaptation of this approach was trialed for brook trout. Mixed sex fish (age =14 months) were subjected to simulated natural photoperiod (NP), advanced photoperiod (AP) and continuous photoperiod (CP) to investigate the response of this species endogenous circannual rhythm of sexual maturation. Light treatments commenced on August 1<sup>st</sup>, 2011 until May 25, 2012. Maturation was observed in all females and 96% of total males held under NP. Fish exposed to AP corrected their maturation cycle by advanced phase shift of their endogenous rhythm for 8 weeks. These fish achieved final maturation during March-April and could be manually stripped instead of natural spawning season of June-July. Plasma profiles of testosterone (T) and estradiol-17 $\beta$  (E<sub>2</sub>) were also adjusted according to advancement of photoperiod and were at similar concentration levels as observed in fish exposed to NP during specific developmental phases. Exposure to AP inhibited maturation by 6% and 8% in males and females, respectively, however most of the fish successfully attained maturation. Similarly, treatment of CP for 10 consecutive months failed to inhibit the onset of maturation in brook trout however did inhibit the final stage of maturation i.e. spermiation and ovulation. Although photoperiod regimes applied in present study could not successfully inhibit the maturation in brook trout but data suggest that manipulation of maturation is possible in this species if technical approach or regime is refined.

## OPTIMIZING REARING CONDITIONS FOR LARVAL PIGFISH, *Orthopristis chrysoptera*

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Pigfish are a popular marine baitfish in the southeastern United States, and commercial production could increase availability and reduce pressure on wild populations. Research on optimal rearing conditions for pigfish is needed for production to be feasible. We investigated the effects of temperature and weaning protocols on larval pigfish growth and survival.

First, larvae were reared on live feed to 20 days post-hatch (dph) at 5 temperatures from 22-30 °C (Table 1a). Larval growth was slowest at 22 °C and final size was significantly smaller compared to higher temperatures. In contrast, survival was highest at 22 °C, decreasing with increasing temperature.

We quantified the ontogeny of digestive enzyme activity in larvae, and the results suggested that larvae could be weaned at approximately 11 mm SL, or 30 dph when reared at 24 °C. To test this estimate, we conducted a weaning study at 24 °C where larvae were co-fed live prey and a dry microdiet (MD) beginning 15 dph (~5 mm SL). Live prey was eliminated from the diet at 24, 28, 32, or 36 dph corresponding to approximate SLs of 7, 10, 12, and 15 mm. Controls were co-fed with live prey and MD throughout. On 43 dph, larval size was not significantly different across treatments, but survival was lower when the fish were weaned at 24 dph (Table 1b).

In order to maximize both growth and survival, we suggest a rearing temperature of 24 °C. At this temperature, larvae were efficiently weaned to a MD at 28 dph, corresponding to 10 mm SL. Producers rearing larvae at lower or higher temperatures to match production goals, could schedule weaning once larvae reach this size.

**Table 1. Mean standard length (SL) and survival ± S.E. of larvae reared at different temperatures through 20 dph (a) or weaned to dry feed at different ages (b).**

a

Temperature (°C)	SL (mm)	Survival (%)
22	6.0 ± 0.6 <sup>a</sup>	69.1 ± 10.1 <sup>a</sup>
24	7.3 ± 0.6 <sup>b</sup>	48.2 ± 8.6 <sup>ab</sup>
26	7.9 ± 1.3 <sup>b</sup>	39.9 ± 10.9 <sup>ab</sup>
28	8.9 ± 1.5 <sup>b</sup>	23.5 ± 17.9 <sup>b</sup>
30	---	0

b

Age at weaning	SL (mm)	Survival (%)
24	21.7 ± 0.8	4.7 ± 1.4 <sup>a</sup>
28	21.6 ± 2.7	10.5 ± 2.3 <sup>ab</sup>
32	21.3 ± 1.5	13.4 ± 0.3 <sup>b</sup>
36	23.2 ± 3.0	11.4 ± 5.2 <sup>b</sup>
control	19.7 ± 3.3	15.5 ± 4.9 <sup>b</sup>

## **A UNIQUE PROTEIN PLATFORM TECHNOLOGY TO MEET THE NEEDS OF AQUACULTURE**

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To meet the expanding needs of a growing global population, innovative technologies that can lead to production efficiencies, reduce stresses to wild fisheries and increase overall animal welfare are in high demand. Aquaculture's potential for growth is significant but only if done both environmentally & economically sustainably. Feed represents a significant portion of the operating expense for a given farm and is a critical component for an animal's health. By improving animal nutrition, we have the potential to lower disease while decreasing our use of antibiotics and ultimately provide great tasting product.

In this presentation, we will discuss a novel single cell protein platform technology, its versatility and recent findings that are expected to support producer operations worldwide. A first-of-its-kind development will be shared with the Aquaculture America audience. Our industry-leading technology is uniquely applied to tackle some of aquaculture's biggest problems. With the goal of reducing the need for fishmeal, lowering the cost of high-value biomolecules and lowering the rates of mortality on the farm to make operations more profitable, an update to the efficacy of this proprietary technology will be provided.

# TECHNICAL EFFICIENCY OF THE EUROPEAN FISH AND SEAFOOD PRODUCTION INDUSTRY

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The purpose of this research is to evaluate the technical efficiency of the European (EU28) fish and seafood production industry over the period 2008-2013. To estimate technical efficiency, we adopt time-invariant SF models of Translog and Cobb-Douglas production functions for panel data in which the inefficiency effect ( $u_i$ ) has a normal distribution truncated at zero. Maximum likelihood (ML) estimates of the parameters of the Translog and Cobb-Douglas functions as well as the technical efficiency estimate for each country were obtained simultaneously using the *xtfrontier* command of the statistical package STATA. Technical efficiency scores for each country and production system (fishing and farming) were estimated using industry-level data from EUMOFA to get yearly values of fish and seafood production by each type of production system and from STECF to get yearly values of total employment (number of employees), vessel power (Kw), and total production assets (constant €) for each country.

Maximum Likelihood (ML) parameter estimates of the European fish and seafood production (fishing and farming) industry inefficiency frontier models are presented in Table 1. Regarding the fishing production system, the likelihood ratio test value for the Translog production function was significant at the 1% level (chi-square = 223.31). On the other hand, the validity of the Translog specification over the Cobb-Douglas one in the case of the aquaculture production system was strongly rejected (chi-square = 5.69). Moreover, the sum of the estimated parameters of the factors in the Cobb-Douglas fishing production function indicates a value above the unity ( $0.0976 + 1.2792 > 1$ ) what enables us, therefore, to say that the situation of increasing returns to scale is established in the European fish and seafood fishing production system. Conversely, the sum of the estimated parameters of the factors in the farming production function indicate a value below the unity ( $0.3478 - 0.0185 < 1$ ) whereby it can be inferred that the farming production system presents decreasing returns to scale.

We report estimation results of *TE* using the Translog SF production function for the fishing production system and the Cobb-Douglas SF production function for the farming production system in Table 2. These results show that the mean values of *TE* in the European production (fishing and farming) industry are relatively low being the fishing production system mean value (44.5%) much larger than the farming production system mean value (24.6%). In addition, according to these figures, countries

**Table 1.** Maximum Likelihood (ML) estimation results for the European fish and seafood stochastic frontier production models

Variable	Fishing		Farming	
	Translog function	Cobb-Douglas function	Translog function	Cobb-Douglas function
Constant	-30.7374-	4.1655	50.0584	16.7572-
Ln(Labour)	1.3514	0.0976	6.0696	0.3478-
Ln(Capacity)	6.5008-	1.2792-	-	-
Ln(Capital)	-	-	-5.5868	-0.0185
Ln <sup>2</sup> (Labour)	-0.2820-	-	-0.0796	-
Ln <sup>2</sup> (Capacity)	-0.4802	-	-	-
Ln <sup>2</sup> (Capital)	-	-	0.4090	-
Ln(Labour) × Ln(Capacity)	0.0600	-	-	-
Ln(Labour) × Ln(Capital)	-	-	-0.2922	-
$\mu$	0.6405	1.9032-	2.3673-	1.9168 <sup>*</sup>
$\sigma$	2.4579	1.9797	2.4871	3.2277
$\gamma$	0.9843	0.9772	0.9906	0.9907
Log-likelihood	-20.7557	-30.5806	-12.0623	-14.5235
Number of observations	102	102	27	27
Number of countries	17	17	9	9
Wald test (chi-square)	223.31***	48.72***	20.13***	15.78-
Test: all $\beta_j = 0$	46.41***	-	5.69	-

Source: Authors' elaboration using STECF database.  
 Note: Normal truncated model.  
<sup>\*</sup> Significance at the 10% level  
<sup>\*\*</sup> Significance at the 5% level  
<sup>\*\*\*</sup> Significance at the 1% level

**Table 2.** Technical efficiency (%) of European seafood production industry by country and production system (period 2008-2013)

Country	Production system		Country	Production system	
	Fishing	Farming		Fishing	Farming
Austria	NA	NA	Italy	33.7	NA
Belgium	29.9	NA	Latvia	61.6	NA
Bulgaria	NA	NA	Lithuania	13.1	NA
Croatia	NA	NA	Luxembourg	NA	NA
Cyprus	NA	4.3	Malta	4.7	NA
Czech Republic	NA	NA	Netherlands	43.6	NA
Denmark	91.6	29.3	Poland	46.8	NA
Estonia	91.6	1.3	Portugal	37.7	4.1
Finland	25.7	11.2	Romania	NA	4.7
France	32.0	NA	Slovakia	NA	NA
Germany	33.2	80.6	Slovenia	39.2	2.0
Greece	NA	NA	Spain	91.2	NA
Hungary	NA	NA	Sweden	37.7	NA
Ireland	NA	NA	United Kingdom	43.2	83.6
			<b>Average EU28</b>	<b>44.5</b>	<b>24.6</b>

Note: NA = Not available.



This research has received financial help through the European Union's Horizon 2020 research program, Grant Agreement 635188.

(Continued on next page)

with the most efficient fishing industry are Denmark, Estonia, and Spain with technical efficiency scores over 90% of the potential level of output. By the contrary, countries with the less efficient fishing industry are Malta, Lithuania, Finland, and Belgium with values lower than 30%. On the other hand, British and German farms are, on average, among the most technically efficient with scores over 80%, whereas Estonia, Slovenia, Portugal, Cyprus, and Romania are the countries with the less efficient farms in Europe with scores below 10%.

Therefore, these results show significant differences of inefficiency among the countries under study so that there is a room for improvement in the European fish and seafood production industry to be more efficient technically.

This research has received financial help through the European Union's Horizon 2020 research program, Grant Agreement 635188.

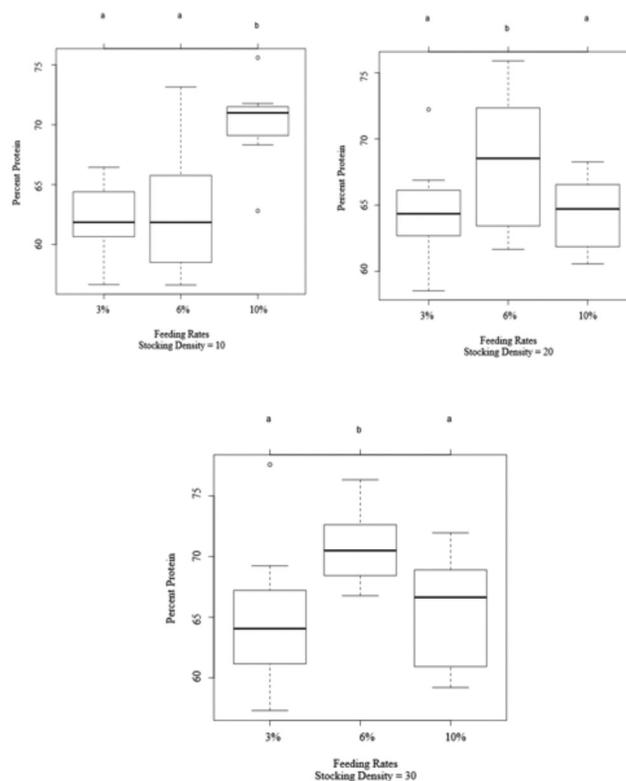
## EFFECTS OF STOCKING DENSITY AND FEED RATE ON THE GROWTH OF HATCHERY-REARED JUVENILE SPOTTED SEATROUT, *Cynoscion nebulosus*

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The spotted seatrout, *Cynoscion nebulosus*, is a highly sought after recreational fish in the Gulf of Mexico. Fisheries managers in Texas use stock enhancement as one of the management tools to protect the fishery. Hatchery production of juvenile spotted seatrout has been successful in Texas; however, to increase post-release survival it is thought that the size of fish upon release should be increased from 35 mm to > 40 mm total length. To address this question, a baseline study on the effects of stocking density and feed rate on juvenile fish was investigated. Spotted seatrout were stocked into indoor 44-L aquaria at 10, 20, and 30 fish/tank (equivalent to 0.226, 0.453, and 0.680 fish/L) and fed at rates of 3, 6, or 10% biomass per day. In order to evaluate variation in population performance, two trials were conducted (Trials 1 and 2). Mean initial wet weights of fish in treatments for Trial 1 were  $0.91 \pm 0.13$  g (stocking density of  $0.020$  kg/m<sup>3</sup>) and  $0.82 \pm 0.12$  g (stocking density of  $0.019$  kg/m<sup>3</sup>) for Trial 2. Mean final weight was  $5.51 \pm 1.43$  g for Trial 1 (30 day duration) and  $4.952 \pm 1.386$  g for Trial 2 (27 day duration).

Survival of fish at the highest stocking density significantly ( $P < 0.05$ ) increased with increased feed rate. Fish at the lowest stocking density showed significantly ( $P < 0.05$ ) higher whole-body protein content when fed the high feed rate. Fish fed at higher feeding rates had significantly ( $P < 0.05$ ) higher specific growth rate and, Fulton's condition factor (K), as well as whole-body energy, but reduced whole-body ash content. Treatment combinations of low stocking density and high feed rate resulted in highest survival and weight gain. The results of this study show that there is potential to rear spotted seatrout to larger sizes (> 40 mm) under hatchery conditions.



## **INTENSIVE PRODUCTION OF HYBRID WALLEYE *Sander vitreus* x *S. canadense* IN A RECYCLE WATER SYSTEM**

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Past research efforts have investigated seasonal advancement of spawning, intensive indoor fry-fingerling production, and hybridization as potential ways to improve walleye (*Sander vitreus*) aquaculture, but the commercial advantages of these technologies have not been proven. Scientists at the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility (UWSP-NADF) have successfully adapted and modified these technologies to produce purebred walleye, purebred sauger (*Sander canadensis*) and hybrid (*Sander vitreus* x *S. canadensis*) (saugeye) fingerlings utilizing a commercial scale 23°C water recycle system. Field trials conducted by UWSP-NADF scientists showed remarkable growth of hybrids fed commercial diet of up to 1.0kg within 12 months. Preliminary results with these fish from food processors and aquaculture marketing indicate a high dollar potential for purebred walleye and hybrid walleye for stocking and food fish uses.

## UNDERSTANDING THE MECHANISMS OF WINTER SURVIVAL THROUGH BIOCHEMICAL ADAPTATIONS IN INVASIVE SPECIES OF ASIAN CARP

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Bighead carp, *Hypophthalmichthys nobilis*, are a warm water species native to Asia, present in the Mississippi River Basin and were labelled as an injurious species by the US Fish and Wildlife Service in 2011. Concerns exist about how Asian carp may impact the multibillion-dollar annual sport fishery if they are able to invade the Great Lakes. However, a gap in scientific data exists regarding survival of young-of-the-year (YOY) Bighead carp in Great Lakes winter water conditions. An experiment was conducted from Nov. 2014 to Apr. 2015 which examined the survival, growth, and biochemical adaptations of YOY Bighead carp of different sizes subjected to winter water temperatures similar to those in Lake Erie.

Fifteen YOY fish from each of four categories, small (SK 0.18g) and large Koi carp (LK 0.49g), small (SBH 0.2g) and large (LBH 1.8g) Bighead carp were randomly distributed to six tanks. Two treatments were applied to simulate the presence or lack of winter food availability. Mortality and temperature data during the experiment were collected.

The current study specifically examined lipids, fatty acid composition, mineral levels, and crude protein, first prior to simulated winter onset, second when 50% mortality of the stock (LC50) was achieved, and finally following extended exposure to winter water conditions. LC50 was reached for two groups during the experiment, small Koi carp and small Bighead carp. Comparisons of polyunsaturated fatty acids (PUFA) in neutral lipid (NL) relative % at LC50 reveals significant differences between fasted SBH (22.51±0.24%) and SK (11.85±0.64%) while the ratio of n-3:n-6 fatty acids among groups are similar (SBH: 0.85±0.14; SK: 0.84±0.03). The polar lipid (PL) profile at LC50 follows a similar trend with fasted SBH (47.72±0.61%) conserving greater relative % of PUFA than SK (30.78±11.1%) while maintaining comparable n-3:n-6 fatty acid ratios (SBH: 2.87±0.17; SK: 2.52±0.89). Increased relative % conservation of PL PUFA by Bighead carp may well be an adaptation which preserves membrane fluidity allowing them to survive longer exposure to low temperatures than Koi carp. These results demonstrate that there is potential for YOY Bighead carp to survive Lake Erie short winter water temperatures (4 months).

## SLOWING SALES OF TILAPIA IN THE US: WHY AND WHAT CAN BE DONE TO RETURN TO RAPID GROWTH

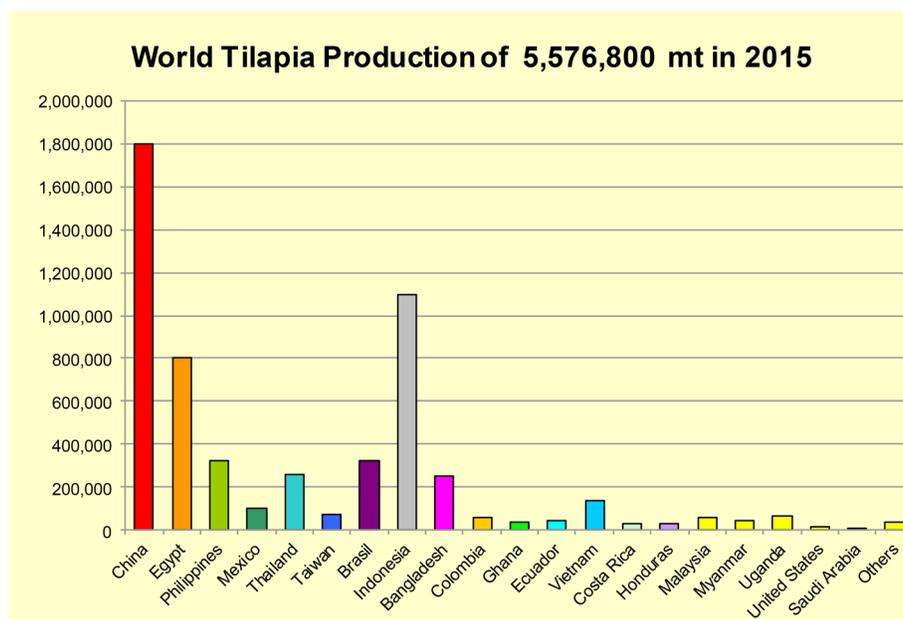
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Tilapia sales in the US have stalled during 2015 and 2016. Sales of live and fresh whole fish have been static, with prices soft, especially when compared to increased sales of higher priced salmon and shrimp. Supplies have increased slightly on a global basis with a global production estimate for 2015 of 5,576,800 mt. China continued its position as the single largest producer (1,800,000 mt in 2015). China's exports to the US slowed in 2015 and 2016, but increased significantly to sub-Saharan Africa and to the Middle Eastern Gulf States. Indonesia continues as the second biggest producer with over 1,100,000 mt of production. Indonesia's exports did not increase much but domestic demand grew significantly.

The weakness in the US markets seems to be most attributable to a vast increase in social media questioning of quality and nutrition of tilapia. Three story lines seem to be driving these offerings on the web. First, is a rebirth of the discredited journal article claiming that tilapia was less healthy than bacon or donuts, and a local newspaper headline repeating the claim while trying to grab attention. Second, is a story of unknown origin, reporting that tilapia in East Asia are fed animal wastes, poultry or hogs in most versions. Third, was a 2011 New York Times article titled, Tilapia, the Perfect Factory Fish.

The first article was widely refuted by most nutrition experts at time it was first published, and a year later by Young (2009), but of course little of this has picked up by the media. The second story has been harder to trace. Brown et al. (2014) published an article pointing out that animal wastes and manures are frequently used as fertilizers in ponds and that even if fish swim up and grab these materials, they almost instantly spit them out. The racist tone of most of these web stories ignore the fact that American or European farmers using the same materials are applauded as organic farmers. The third story from the New York Times was relatively even in tone and mostly accurate, but the headline and a few comments have been widely reported out of context. A description of other efforts to combat these representations will be presented.



## **TILAPIA (*Oreochromis niloticus*) PERFORMANCE IN CLEAR-WATER RAS, BIOFLOC, AND HYBRID NURSERY SYSTEMS**

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Biofloc-based production systems convert waste to microbial biomass, contained largely on small, suspended “biofloc” particles, which can be consumed by tilapia. Fingerling and adult tilapia growth has been shown to increase in biofloc systems when compared to clear-water recirculating aquaculture systems (RAS) due to their consumption of biofloc, however the effects of biofloc on tilapia fry growth are unclear. Both clear-water and biofloc systems have production advantages and disadvantages; hybrid systems combining features of both have recently been proposed in an attempt to optimize animal performance. This study compared the growth rate of tilapia fry in clear-water RAS, biofloc, and hybrid systems.

Twelve 0.19 m<sup>3</sup> tanks were stocked with 55 tilapia fry at an average weight of .17g per fish in the KSU Production Technologies Building. Three treatments were created: 1.) clear-water RAS (CW), 2.) Biofloc (BF), and 3.) Hybrid (HY) systems, these were each randomly assigned to four replicate tanks. The CW tanks had a settling chamber, a foam fractionator, and an external biofilter containing plastic biomedial. The BF tanks only had a settling chamber to control solids concentration, and the HY systems used a settling chamber and an external biofilter. All systems were fed the same amount of feed 3 times daily. Ammonia, nitrite, nitrate, turbidity, and TSS/VSS were measured weekly and fish weights were sampled biweekly.

We found no significant difference between tilapia growth in the three systems. Fish in the hybrid treatment had the highest average weight and the highest specific growth rate (SGR). HY tanks had an average weight of 10.5g/fish, CW averaged 10.1g/fish, and BF tanks averaged 10.1g/fish. SGR was 18.2 in the HY systems, 17.3 in the CW systems, and 17.3 in the BF systems. The variability between tanks in both average weights and SGR was significantly higher in the BF systems than clear-water and hybrid systems. Ammonia, nitrate, and TSS/VSS levels were not significantly different between treatments. Nitrite and turbidity levels were significantly higher in the BF systems versus the other system types, although nitrite never exceeded tilapia tolerance levels. Although some previous research indicates that tilapia fingerlings and adults have higher growth in biofloc, the results of this study indicate that tilapia nursery system design may have little impact on overall growth. System managers may therefore choose the lowest cost system for tilapia nurseries. This would likely be biofloc systems, as they have the least amount of filtration; however, the oxygen consumption and potential need for greater aeration in biofloc systems should be explored further.

## THE CARBON BUDGET AND ENERGY CONTENTS IN THE CULTURE OF THE AMAZON RIVER PRAWN AND TAMBAQUI FARMED IN MONOCULTURE AND IN INTEGRATED MULTITROPHIC SYSTEMS

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This work aimed to determine the inputs, outputs and accumulation of carbon and the energetic content in each of the ecological compartments of the monoculture and polyculture systems with the Amazon River prawn, *Macrobrachium amazonicum* and tambaqui, *Colossoma macropomum* in earthen ponds, using hypereutrophic water. In addition, we tested the hypothesis that the integrated multitrophic aquaculture system (IMTA) is more efficient in using the input of carbon than monoculture.

A completely randomized experiment was designed with four treatments and three replications: MP - monoculture with 30 prawns/m<sup>2</sup>, MT- monoculture with 1 fish/m<sup>2</sup>, PF- polyculture (IMTA) with 30 prawns/m<sup>2</sup> and 1 fish/m<sup>2</sup> free, PH - polyculture (IMTA) with 30 prawns/m<sup>2</sup> free and 30 fish/m<sup>3</sup> in hapa nets. Samples of water, gas, sediment, feed, and animals stocked and harvested, were all collected throughout the experiment to determine the total carbon content in each ecological compartment and the carbon balance. Energetic content was determined in the feed and the animals.

Results showed that the carbon supplied was mainly accumulated inside the ponds as sediment and effluent water, with a small quantity of carbon being lost to the atmosphere (Table 1). The use efficiency of carbon by the animals ranged from ~10 – 27%. Data did not confirm the hypotheses that IMTA is more efficient than monoculture to accumulate carbon in the biomass produced.

**Table 1. Concentration of carbon as observed in the various compartments of the four production systems.**

System Compartments	Treatments			
	PM	FM	IMTA-FREE	IMTA-HAPA
<b>Inputs (Kg.Ha<sup>-1</sup>)</b>				
Prawn	5.5E-4 ± 9.0E-8		5.5E-4 ± 1.4E-7	5.5E-4 ± 1.6E-7
Fish		0.05 ± 1.3E-5 b	0.05 ± 3.6E-5 b	0.01 ± 1.5E-3 a
Diet	993.7 ± 199.7 b	2290 ± 324.5 a	1793 ± 333.2 a	390.8 ± 52.7 b
Supply Water TC	569.1 ± 133.8	532.1 ± 82	445.5 ± 96.2	433.6 ± 42.9
CO2 Gas Dif	692.1 ± 186	529.2 ± 93.7	751.4 ± 199.6	593.8 ± 60.3
CH4 Gas Dif	4.25 ± 2.8	8.2 ± 4.1	5 ± 2.1	5.5 ± 2.8
<b>Total (Kg.Ha<sup>-1</sup>)</b>	<b>2474.8</b>	<b>3576.8</b>	<b>3247.6</b>	<b>1631.1</b>
<b>Outputs (Kg.Ha<sup>-1</sup>)</b>				
Prawn	152.01 ± 30.48 a		91.65 ± 14.39 b	77.28 ± 23.49 b
Fish		853.09 ± 203.3 b	571.7 ± 99 b	114.6 ± 33 a
Sediment	1931 ± 453	1101 ± 785	1028 ± 442	1139 ± 379
Effluent Water TC	328.3 ± 63.4	336.3 ± 71.9	453 ± 113	291.6 ± 63.8
CO2 Gas Dif	28.4 ± 17	67.5 ± 29.4	74.3 ± 35.6	68.3 ± 54.3
CH4 Gas Dif	3.7 ± 2.7	2.3 ± 2.1	2.5 ± 2.5	0.8 ± 0.9
CO2 Bubbles	2.1 ± 1.5 b	11.1 ± 7 ab	22.8 ± 7.3 a	8.5 ± 6 ab
CH4 Bubbles	13.2 ± 15.7	5.6 ± 5.8	44.8 ± 26.2	120.9 ± 124.1
<b>Total (Kg.Ha<sup>-1</sup>)</b>	<b>2507.6</b>	<b>2429.5</b>	<b>2346.7</b>	<b>1907.5</b>
<b>Unaccounted (Kg.Ha<sup>-1</sup>)</b>	<b>32.8</b>	<b>1147.3</b>	<b>900.9</b>	<b>276.4</b>

Means followed by letters in same line indicate significant difference between the treatments according to the tukey test ( $P < 0.05$ ).

**IDAHO - MORE THAN FAMOUS POTATOES - SITE OF THE NATIONAL AQUACULTURE EXTENSION CONFERENCE JUNE 2017**

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We hope you will come to Boise, Idaho in June 2017 to participate in the National Aquaculture Extension Conference. Come visit Idaho – home to hundreds of miles of mountain bike trails and more whitewater than any other state in the continental United States. There are mountains, canyon lands and sand dunes to explore, waters to fish, resorts for summer fun, plenty of state parks and the largest wilderness area in the lower 48 states.

The U.S. is a leading nation in aquaculture research and development. Therefore, it is critical that new discoveries and knowledge reach potential beneficiaries as quickly as possible. Effective networking of extension professionals, with a common vision of benefiting private sector development, is an important element for continued and future success. Networking can be initiated by extension professionals in partnership with others in research and development. Today's issues and challenges require new, improved ways to move industry forward and educate the public. Growing interest in vocational education with aquaculture as a component has led to establishment of numerous programs across the U.S.

This conference offers participants new contacts, knowledge about new topics, information about new tools to enhance productivity, appreciation of experience and perspectives on issues, a chance to replicate model programs, gain in expanding professional networks, opportunities to contribute to the development of others, insights into participating in regional and national initiatives and growth in skills development relevant to responsibilities. The conference provides a forum for professionals from the Sea Grant Marine Advisory Service, the Cooperative Extension Service, and other outreach programs to seek collaboration for mutual benefit to the aquaculture industry and the public.

Come enjoy two information filled days punctuated with a tour of the nation's leading trout producing area located in the scenic Magic Valley. We will visit the University of Idaho Hagerman Fish Culture Experiment Station. We will tour commercial farms that grow trout, tilapia, and sturgeon. Come experience the National Aquaculture Extension Conference in Idaho; it will bring together both Sea Grant and Land Grant educators to explore common program bonds and ways to collaborate professionally to enhance the program activities of both.

## **MICROALGAE BIOMASS IN AQUACULTURE FEEDS - A ROADMAP TO COMMERCIALIZATION**

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The aquaculture industry has a strong interest in developing aquatic feeds which require greater diversity of protein- and lipid-based ingredients to meet the needs of an expanding global demand. A major concern for the aquaculture industry is the use of costly, environmentally unsustainable marine animal-sourced feed ingredients such as fishmeal. Current efforts at Texas A&M AgriLife Research (Corpus Christi, Texas) with development of mixed-species outdoor cultures of *Nannochloropsis salina* and *Phaeodactylum tricornutum* have yielded productivity levels exceeding 15 g afdw/m<sup>2</sup>/day with dry matter protein and lipid levels approximating that of fishmeal or other semi-purified ingredients. This presentation examines nutritional and economic considerations for the commercial use of marine microalgae in aquaculture production feeds. The economic and environmental sustainability of various biological product-based pathways to commercialization are explored from the viewpoint of the aquaculture feeds and algae biomass and industries. A roadmap to sustainability is proposed.

## **ENGINEERING TECHNIQUES FOR THE DESIGN OF OPEN OCEAN MACROALGAE FARMING SYSTEMS**

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Our future depends upon the development of high value marine products that have positive ecological impacts and macroalgae (kelp) aquaculture has enormous potential for human foods, fuels, feed supplements, fertilizers, hydrocolloids, pharmaceuticals and nutraceuticals. Evidence also exists that large-scale kelp aquaculture may provide ecosystem services that offset many anthropogenic impacts including bioremediation of excess nutrients and carbon sequestration. Expanding kelp aquaculture in populated coastal regions will be important, however, large-scale growth will likely be limited by social carrying capacity due to multi-stakeholder issues. Therefore, development is expected to occur in exposed, open ocean regions. This will require a detailed design approach to ensure system integrity.

The objective of this presentation is to describe engineering techniques necessary for the design of kelp farms in high energy coastal and open ocean areas. We report on our use of numerical models to represent large deformations, compliancy and contact between elements of kelp farms in the ocean. To have confidence with our computer models, input parameters must accurately represent the farm system so our approach incorporates the geometric and material properties of kelp and harvest densities. Full-scale physical models are then designed and built by matching dimensions, mass densities and flexural rigidity characteristics of a section of long-line at harvest. We have designed and performed some tow, wave and planar motion experiments in a large tank with full-scale models to obtain drag and inertia coefficients. Hydrodynamic characteristics can then be incorporated into numerical models so that a wide range of kelp farming systems can be analyzed.

## **DEVELOPMENT OF A SNP MARKER PANEL FOR PARENTAGE, DIVERSITY AND RELATEDNESS ANALYSES IN LARGEMOUTH BASS, *Micropterus salmoides***

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Largemouth bass (*Micropterus salmoides*) are an extremely popular gamefish species, that has evolved in to a multi-billion dollar sportfishing industry in the United States. Largemouth bass as a foodfish additionally represent a growing sector of the US aquaculture industry with production estimates exceeding 1 million pounds annually. As stock enhancement and breeding programs for this species continue to expand, there is an increasing need for genetic tools that can be used for various applications including parentage analysis and assessments of population structure, genetic diversity, and relatedness. We report here on the development and characterization of a single nucleotide polymorphism (SNP) panel for largemouth bass. A panel of ~ 150 SNP markers was established and used in a study to genotype broodstock samples (n~200) from Kansas Department of Wildlife, Parks, and Tourism's (KDWPT) hatchery program and perform parentage analyses on genotyped samples (n~600) collected from stocked reservoirs. Overall genotype call rates averaged 98.5% (+/- 2.7%), and >99 % of the samples collected from reservoirs were assigned as either hatchery-sourced (to single parent-pair crosses), or as wild-sourced. Additional results will be presented on the use of the SNP panel in providing information on diversity and relatedness of hatchery broodstock populations.

## MODELING OF DAILY WEIGHT GAIN, FEED CONVERSION RATIO, NITROGEN RETENTION AND DIGESTIBLE ENERGY AND PROTEIN REQUIREMENTS OF GROW-OUT CAGE-FARMED NILE TILAPIA FED HIGH DENSITY DIET

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Nile tilapia is one of the most important farmed fish species in Brazil. In recent years, tilapia culture has been expanded from earth pond to intensive culture in net cages. High density diets have been proposed to grow-out Nile tilapia to enhance weight gain, feed efficiency of cage-farmed Nile tilapia. Digestible energy (DE) and digestible protein (DP) have been proposed to elaborate diets based on precision nutrition concept for Nile tilapia. The present work was designed to elaborate modeling of growth performance and dietary DE and DE requirements of grow-out cage-farmed Nile tilapia using linear or second-order regression analysis.

Nile tilapia (n=6,000;  $29.9 \pm 4.9$  g initial weight) were distributed into six hexagonal cages of 12 m<sup>3</sup> each. The fish were hand-fed to extruded diet containing 320.2 g kg<sup>-1</sup> of DP, 15.6 MJ kg<sup>-1</sup> of DE (dry matter basis). Fish were hand-fed fed at 3.5 to 2.5% of fish biomass, twice daily, seven days per week, during 100 days. Dietary digestible energy and digestible protein were determined by *in vivo* digestibility assay. Twenty fish per cage were weighed at beginning and every 20 days to access the growth performance and adjust the feeding rate. The survival of fish was calculated from daily mortality and recorded in each cage. The relationship between body weight and daily weight gain, feed conversion ratio and nitrogen retention and the relationship between daily weight gain and digestible energy intake and digestible protein intake were determined by linear ( $Y = a + bx$ ) or second-order ( $Y = a + bx + cx^2$ ) regression analysis. The relationships between body weight and daily weight gain of cage-farmed Nile tilapia was best expressed by second-order regression analysis. However the relationship between daily weight gain, feed conversion ratio and nitrogen retention were best expressed using linear regression analysis (Table 1). Coefficients of determination ( $R^2$ ) ranged from 0.753 to 0.859.

Nitrogen retention decreased linearly according to increases in body weight of grow-out Nile tilapia fed high-density diet. The relationship between daily weight gain and DE intake ( $Y = 7.1045x - 11.422$ ,  $R^2 = 0.892$ ) and DP intake ( $Y = 0.6098x - 0.9804$ ,  $R^2 = 0.8924$ ) were best fit by linear regression analysis. Digestible energy and digestible protein requirements increase linearly according to increases in daily weight gain of cage-farmed Nile tilapia.

Table 1 – Relationship between body weight (BW) and daily weight gain (DWG), feed conversion ratio (FCR), nitrogen retention (NR) of grow-out cage-farmed Nile tilapia fed high-density diet

BW	DWG (g) <sup>1</sup>	FCR <sup>2</sup>	NPU (%) <sup>3</sup>
61.8	2.9	0.9	48.2
136.4	4.9	1.1	41.3
246.6	6.4	1.3	37.2
367.2	6.7	1.7	36.0
509.3	7.4	1.7	34.9

<sup>1</sup> $Y = -0.0003x^2 + 0.1003x - 2.0355$ ,  $R^2 = 0.959$   
<sup>2</sup> $Y = 0.0062x + 0.5682$ ,  $R^2 = 0.8019$   
<sup>3</sup> $Y = -0.1306x + 55.802$ ,  $R^2 = 0.753$

## CHARACTERIZATION OF EARLY LIFE HISTORY AND SEXUAL SYSTEM OF THE YASHA GOBY *Stonogobiops yasha*

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The yasha goby, *Stonogobiops yasha*, has remained largely unstudied since its first description in 1997, despite its high demand in the marine aquarium trade. In 2016, the Roger Williams University (RWU) Marine Lab successfully bred this species in captivity. Facilitated by this success, our study aimed to (a) characterize the embryonic and larval development of *S. yasha* from fertilization to juvenile metamorphosis, and (b) determine the sexual system employed by *S. yasha* (i.e., gonochorism or hermaphroditism). This research will constitute the first examination of the full life history of this genus, providing essential data for commercial-scale production of *S. yasha* in hatcheries.

Embryology was documented using video and camera lucida sketching, and larval development was documented using microphotography to measure body length and characterize onset of key morphological changes. Embryos and larvae were collected from 4 separate nests of eggs, and approximately 10 embryos or larvae were used for each developmental stage analyzed. Embryonic development lasted 6 days, with larval hatching occurring the morning of day 6. Flexion occurred between day 8-12, settlement morphology was evident at day 15-20, and metamorphosis took place at day 27- 50.

Based on culture trends observed in the RWU Marine Lab and literature on other reef-dwelling gobies, this study tested the hypothesis that *S. yasha* are protogynous sequential hermaphrodites. An attempt to determine the sexual system of the specie was performed on recently settled juveniles (N=41). The juveniles were collected and separated into 13 pairs and 15 individual arrangements. Half of the pairs and individuals (N=7, N=8, respectively) were provided PVC pipe burrows; the other half were maintained in bare-bottom tanks. Total length (mm) was measured monthly, and juveniles were visually observed daily for the display of secondary sexual traits (i.e., presence/absence of black-tipped pelvic fins). At 70 days post hatch (dph), 50% of the juveniles from each of the four treatments were euthanized and processed histologically to assess gonad development, comparing results to previously processed adult specimens. The remaining juveniles were euthanized and processed at 200 dph.

This research aims to expand the understanding of Gobiid sexual system diversity, and provide essential data for *S. yasha* and culture methods. These results are the first characterization of this genus, and reveal broader implications for survival at key developmental stages, as well as the role of culture environment on developing juveniles into highly socially dominated dynamics.

## EFFECTS OF FISH DENSITIES ON GROWTH, SURVIVAL AND HEAVY METAL ACCUMULATION IN FISH AND PLANTS IN A RECIRCULATING AQUAPONIC SYSTEM

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An experiment was conducted to determine the optimum fish density and investigate heavy metal accumulation in fish and plants grown in a recirculating aquaponic system. The aquaponic system consisted of 6 fish tanks and 6 plant tanks (80 x 40 x 40 cm) each containing 100 L of tap water. Based on the previous experiment that showed better growth and survival of fish at 20 fish per tank compared with 10 and 15 fish per tank, the present experiment tested higher densities of 20, 25 and 30 fish per tank. Seedlings of lettuce, beans, and the ornamental plant *Petunia* were planted on a styrofoam raft with holes for the roots to extract water and nutrients they need to grow. Tilapia (*Oreochromis niloticus*) with initial weight of 40 g were grown in combination with either lettuce and *Petunia* or lettuce and beans and were fed a commercial feed three times a day. Fish and plant sampling for heavy metals and measurement of fish weight and length were conducted every two weeks. Lettuce weight was measured during harvest at the end of the 6-week experiment. Growth and survival of fish at different densities were not significantly different but highest density of 30 fish/tank is recommended based on economic consideration. Final weight of lettuce was highest among those grown in combination with 20 fish/ tank. Concentrations of heavy metals (lead, cadmium, arsenic and mercury) in fish were very much below the maximum limits and are therefore safe for human consumption. There was low concentration of lead (Pb) in all three plant species grown in combination with fish at different densities. Cadmium (Cd) concentration was low in the beans and lettuce, thus, they are safe for human consumption but the concentration of Cd in the ornamental plant *Petunia* was high. Mercury was not detected in the samples.

**EVALUATION OF THE EFFECTS OF AN ALTERNATIVE FEEDING REGIME APPLIED IN THE HATCHERY ON GROWTH AND BODY COMPOSITION OF JUVENILE CHINOOK SALMON *Oncorhynchus tshawytscha***

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The environment of hatchery raised fish is significantly different from the conditions in the wild with regard to food/ feeding rates and water temperature. Some hatcheries have very little temperature fluctuation during the fishes' rearing period. Even if the hatchery used the available creek water, the feeding regimes and the food used is quite different from the wild fish diet. The conditions under which the fish are raised could have a great effect on their growth/morphology.

A feeding trial was conducted at a National Fish Hatchery to determine the effects on the fish of alterations in the level of feed provided at a key developmental period. Test fish were fed one half of normal rations from July through November while control fish were fed normal rations. The reduced ration treatment did significantly reduce the whole body lipid level, fork length and percent weight gain in the fish. When the full ration was resumed and fed until April, the fish gained whole body lipid to equal the full fed treatment fish. However, the fork length and percent weight gain remained significantly less.

## DEVELOPMENT OF INTERMUSCULAR BONE IN TELEOSTS

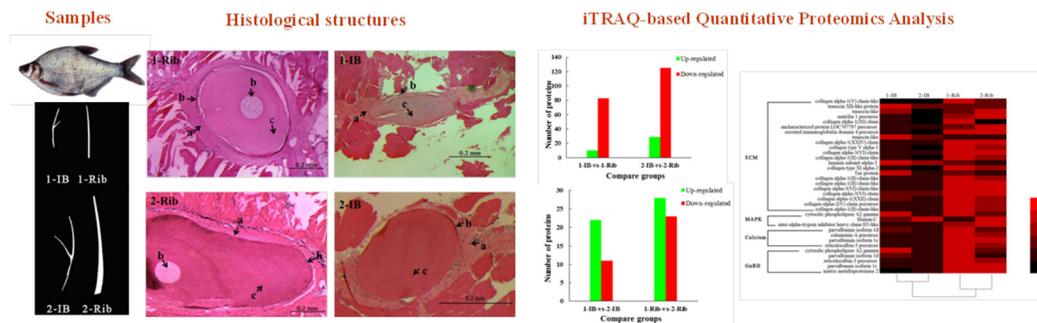
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Intermuscular bone (IB), which occurs only in the myosepta of lower teleosts, is attracting more attention because they are difficult to remove and make the fish unpleasant to eat. By gaining a better understanding of the genetic regulation of IB development, an integrated analysis of miRNAs and mRNAs expression profiling was performed on one typical Cyprinidae species, blunt snout bream (*Megalobrama amblycephala*). Four key development stages related to IB's development were selected for transcriptome and small RNA sequencing. A number of significantly differentially expressed miRNAs/genes associated with bone formation and differentiation were identified and the functional characteristics of these miRNAs/genes were revealed by GO function and KEGG pathway analysis. These were involved in TGF- $\beta$ , ERK and osteoclast differentiation pathways known in the literature to affect bone formation and differentiation. MiRNA-mRNA interaction pairs were detected from comparison of expression between different stages.

Besides IBs, ribs are also a part of skeletal system in teleosts, but IBs and ribs have different developing process. The chemical composition of fish IBs and ribs as well as the underlying mechanism about their development have not been investigated. In our study, histological structures showed that one bone cavity containing osteoclasts were existed in ribs, but not in IBs of *M. amblycephala*. We constructed the first proteomics map for fish bones including IBs and ribs, as well as to identify the differentially expressed proteins between IBs and ribs through iTRAQ LC-MS/MS proteomic analysis. The proteins extracted from IBs and ribs were quantified 2342 proteins, with 1451 proteins annotated with GO annotation in biological processes, molecular function and cellular component. A number of bone related proteins as well as pathways were identified in the study.



Our study provided the first comprehensive transcriptome and proteomics resources of intermuscular bone for teleosts.

## GEODUCK AQUACULTURE MONITORING IN NEAH BAY AND SEAWATER ISOTOPIC ANALYSES

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Makah Fisheries Management (MFM) has conducted a monitoring program at the Neah Bay geoduck aquaculture beds since 2013, and obtained water samples and environmental parameters (e.g., temperature, salinity, pH, dissolved oxygen and pressure) *in situ* on a weekly basis. The water samples were analyzed for dissolved inorganic carbon (DIC) for  $\delta^{13}\text{C}$  and  $^{18}\text{O}/^{16}\text{O}$  ratios. The two-year water quality data reflect patterns of natural variation: a clear seasonal shift in both temperature and salinity. The DIC ranged from 0.7‰ to 4.2‰ while the  $\delta^{18}\text{O}$  ranged from -4.5‰ to -0.6‰. The pH showed a range from 7.5-8.4 in the first year, but much lower (6.4-7.5) in the second winter. The pH variation appeared consistent with the seasonal change of temperature. In contrast, the DIC variations were correlated with temperature and the  $\delta^{18}\text{O}$  correlated with salinity. There were no clear patterns of variation for dissolved oxygen, or significant correlations with environmental parameters. These results indicated that our sampling scheme is viable and can provide valuable input for the MFM projects with geoduck, mussels, and scallops. Once stable isotope data from shell material (mainly for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) have been obtained, the shell and seawater  $\delta^{18}\text{O}$  allow us to create a predictive model of past temperatures that the animal has been exposed to; whereas the shell and seawater  $\delta^{13}\text{C}$  can allow us to track the animal's sources of carbon that are changing through time.

## THE APPLICATION OF CULTURE TECHNIQUES TO RESTORE LAKE WHITEFISH *Coregonus clupeaformis* AS AN INTEGRAL COMPONENT OF THE COLDWATER FISH COMMUNITY OF OTSEGO LAKE, NY, USA

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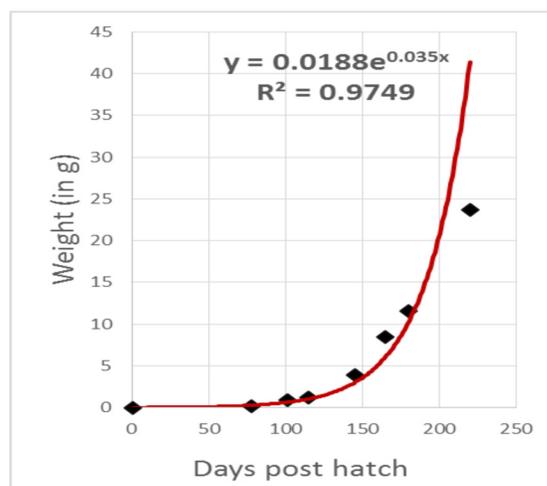
Lake Whitefish (LWF), a key component of the coldwater fish fauna of Otsego Lake was decimated by the introduction of alewives in the 1980's. With the recent collapse of the alewife population, the restoration of the native coldwater ecology of Otsego Lake is now feasible. A collaborative effort to enhance LWF in Otsego Lake is now underway, involving the State University of New York (SUNY) at Cobleskill, New York State Department of Environmental Conservation, and the SUNY Oneonta Biological Field Station. The goal of this project is to spawn and propagate offspring of local LWF to provide ~6,000 LWF annually for stocking back into Otsego Lake. LWF stocking is expected to jump start the recovery of the LWF population and balance the coldwater ecology, as has been done in other lakes (Gerdeaux 2002).

Boat electrofishing, trap netting and fry emergence traps were used to discover three LWF spawning shoals in Otsego Lake. In December 2015, 18 ripe LWF were captured at Sunken Island and processed to record length, weight, sex, and age via scale samples. The LWF captured ranged from 6-13 years old, were 521-629 mm long and weighed 1.7-2.9 kg, respectively.

A total of three females and 10 males LWF were field spawned on 9-10 December, 2015 producing over 70,000 eggs for incubation. Milt from a minimum of three males was used to fertilize the eggs of each female. Eggs were disinfected with 50 ppm iodine following USFWS protocols, and incubated in three McDonald jars for 350 degree days at 8.6°C. Eggs were treated with formalin three times a week and picked daily to remove mortalities. Fertilization rate was ~80%, eye-up rate ~60% and hatch rate was ~35%.

LWF fry were fed a live diet of rotifers and brine shrimp, supplemented by semi-moist starter diet and Otohime fry diet following OMNR 2010. Fingerlings were fed Otohime, Ziegler trout diet supplemented by brine shrimp, live wild plankton, vinegar, grindal and micro-worms. Growth and condition of fry and fingerlings were monitored by length-weight measurements. Growth was 0.6 mm/day similar to Raisanen & Behmer (1982). Fry reached 1g for theoretical spring stocking in 100 days. Fingerlings are expected to reach 40g for a potential fall stocking, 200 days post hatch.

### Whitefish Fry-Fingerling Growth



## ENHANCING SEAFOOD QUALITY AND SAFETY BY REDUCING RELIANCE ON ANTIBIOTICS: APPLYING A NOVEL ANTIBODY IN TILAPIA

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Aquaculture has been the fastest growing animal-production sector in the world increasing at an average annual growth rate of 9% over the last forty years. Despite this growth, the rapid expansion of this industry has not been without issues. For example, disease outbreaks have overwhelmed certain sectors of the industry and have been catastrophic for many nations. To minimize disease outbreaks, efforts are underway to enhance animal health and disease resistance to pathogens without the use of antibiotics.

Recent work by a number of investigators has demonstrated that a conserved mechanism by which gastrointestinal pathogens avoid immune detection is through the up-regulation of interleukin-10 (IL-10), an immune cytokine that suppresses immune responses. This study will focus on the use of anti-IL-10 antibody to prevent disease in tilapia (*Oreochromis niloticus*) caused by pathogen *Aeromonas hydrophila*. The oral antibody to an IL-10 peptide derived from chicken egg yolk, has been successfully used in the prevention of growth rate depression in chickens due to infection. Anti-IL-10 has also been shown to improve immune protection while fed simultaneously with a vaccine rather than feeding the vaccine alone. This success leads to the implication that inhibition of IL-10, an anti-inflammatory cytokine in the digestive tract may be a target to control pathogenic diseases. The antibody's ability to disrupt the invasion of pathogens into host cells, suggests potential disease protection.

Studies will be conducted in the Virginia Tech aquaculture Biosafety Level-2 laboratory. There will be 4 treatments in the study; control feed (no disease challenge), control feed (with disease challenge), anti-IL-10 peptide-based feed (no disease challenge), and anti-IL-10 peptide-based feed (with disease challenge). The disease challenge will be with a clinical isolate of *A. hydrophila*. Tilapia from each treatment group will be monitored for clinical signs of disease by the observation of clinical signs, histopathological examination, and growth rate evaluation. This data will be analyzed to determine if anti-IL-10 can significantly protect fish from disease.

## EFFECTS OF BLACK SOLDIER FLY (*Hermetia illucens*) MEAL IN STURGEON (*Acipenser baerii*) JUVENILES FEEDS: PRELIMINARY RESULTS

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Recent investigations have highlighted that insect-based protein meals can be used as a more sustainable alternative to conventional protein (fish or plant protein meals) used so far in aquaculture (Henry et al., 2015; Gasco et al., 2016). *Hermetia illucens* (HI) is a good candidate due to its valuable nutritional properties. The aim of this research was to investigate the effects of the inclusion of a defatted HI larvae meal in sturgeon feeds on performances, somatic indexes, and condition factor.

Three hundred fifty two *A. baerii* of about 24g were weighed and allocated to 16 fiberglass tanks. Four diets were formulated (Table 1). Diets were isonitrogenous and isoenergetic. Trial lasted 118 days.

At the end of the trail weight gain (WG), feeding rate (FR), feed conversion ratio (FCR), protein efficiency ratio (PER), and specific growth rate (SGR) were calculated using the tank as experimental replicate. Hepatosomatic (HSI), viscerosomatic (VSI) indexes, and condition factor (K) were calculated. Data were statistically analyzed by ANOVA (post-hoc test: Tukey).

Preliminary results (Table 2) indicate that the inclusion of HI significantly affected fish performances and K. Generally, up to 25% of FM substitution, fish performed as well as CF or CV.

Henry et al., 2015. Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol., 203, 1-22.  
Gasco et al., 2016. *Tenebrio molitor* meal in diets for European sea bass (*D. labrax*) juveniles: growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34-45.

**TABLE 1. Ingredients (g/kg) of experimental diets**

	CF	HI25	HI50	CV
<b>Fish meal</b>	700	525	350	320
<b>HI</b>	0	185	375	0
<b>Wheat meal</b>	140	120	100	0
<b>Fish oil</b>	60	70	75	90
<b>Starch gelatinized</b>	80	80	80	80
<b>Soia protein concentrate</b>	0	0	0	200
<b>Soia Bean meal (48%)</b>	0	0	0	140
<b>Gluten meal</b>	0	0	0	150
<b>Mineral mix</b>	10	10	10	10
<b>Vitamin mix</b>	10	10	10	10

**TABLE 2. Growing performance (n=4), Hepatosomatic and Viscerosomatic indexes (n= 12), and condition factor (n= 24) of sturgeons fed experimental diets**

	CF	HI25	HI50	CV
<b>WG (g)</b>	2932 A	2648 AB	2520 B	2916 A
<b>FR (%/day)</b>	1.90 b	1.97 ab	2.05 a	1.93 b
<b>FCR</b>	1.04 B	1.15 AB	1.23 A	1.06 B
<b>PER</b>	1.99 a	1.80 ab	1.69 b	1.91ab
<b>SGR (%)</b>	1.59 A	1.51 AB	1.48 B	1.58 A
<b>HSI (%)</b>	2.69	3.02	3.39	3.41
<b>VSI (%)</b>	8.04	8.50	8.91	8.76
<b>K</b>	0.256 ab	0.246 b	0.260 a	0.261 a

A, B:  $p < 0.01$ ; a, b:  $p < 0.05$

## USING VETERINARY FEED DIRECTIVE DRUGS IN AQUACULTURE

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A veterinary feed directive (VFD) is a written statement issued by a licensed veterinarian that authorizes the use of a VFD drug in animal feed. In 2000, the US FDA issued the 1<sup>st</sup> VFD Rule and finalized regulations for distribution and use of VFD drugs. In 2005 Aquaflor® became the first VFD drug used in aquaculture. Unlike previous antibiotics, orders for VFD drugs were available to fish producers (clients) only through licensed veterinarians.

Use of antibiotics in food animals has been debated for decades, and in response to continued concern over antimicrobial resistance in humans, FDA has issued several guidelines. In 2012 FDA finalized the “Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals” (Guidance #209) which represented FDA’s thinking on antimicrobial drugs that are medically important in human medicine and also used in food-producing animals. The two main principles covered by this guidance were to (1) limit medically important antimicrobial drugs to uses in animals that were necessary for assuring animal health, and (2) include veterinary oversight on medically important antimicrobial drugs used in animals. This guidance would gradually eliminate the use of medically important antimicrobial drugs for production purposes and phase in veterinary oversight of therapeutic uses of these drugs.

Another FDA guideline was Guidance #213 “Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209” finalized in 2013 to provide pharmaceutical sponsors with recommendations to voluntarily modify the use of their medically important antimicrobial drug products to support the two principles in Guidance #209. This guideline changed the marketing status of antibiotics from over the counter to either a prescription (if dispensed in water) or VFD (if dispensed in feed) effective January 2017 and to withdraw production uses. This marketing shift requiring veterinary oversight was an important step to decrease microbial food safety risks of antimicrobial animal drugs.

The FDA also revised the 1<sup>st</sup> VFD rule of 2000 based on Guidances #209 and #213, and in 2015 published the 2<sup>nd</sup> (Final) VFD rule (USFDA CVM 2015). This detailed what information must be included on the VFD order. The Final Rule went into effect October 1, 2015 for Aquaflor® because it was already a VFD drug. The target date for conversion of Romet® and Terramycin® from over-the-counter drugs to VFD drugs is January 1, 2017.

The major provisions of the Final VFD rule cover revised definitions, information required on a VFD order, changes to veterinary client patient relationship (VCPR) requirements, VFD expiration dates and duration of use, VFD refills, number of animals to be treated, affirmation of intent statements, extra-label use of VFD drugs, transmitting VFD orders and recordkeeping. All of these topics will be discussed in detail in the presentation.

## EFFECTS OF SALINITY AND DENSITY ON GROWTH AND SURVIVAL OF SPOTTED SEATROUT *Cynoscion nebulosus* REARED IN RECIRCULATING AQUACULTURE SYSTEMS

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The spotted seatrout (*Cynoscion nebulosus*) is the most recreationally targeted fish in the northern Gulf of Mexico. Aquaculture of this species is being developed both for stock enhancement and for commercial production intending to supply local and regional markets. Aquaculture in recirculating aquaculture systems (RAS) presents several potential advantages over traditional pond-culture including the possible use of higher stocking density and reduced environmental impacts. Currently, culture of seatrout in RAS for stock enhancement is performed at a salinity of 25 psu and density of 20kg/m<sup>3</sup> or less. Culture at higher density would contribute to improve the cost effectiveness of the production process. In addition, culture at low salinity would be beneficial in inland areas where saltwater is not easily accessible. This study aimed to document the effects of rearing density and salinity on spotted seatrout zootechnical performance during grow-out in RAS in order to assist in the evaluation of protocols involving high density and/or low salinity for this species.

The first experiment examined the effect of density on growth and survival of juveniles. Twenty-five day old fish were stocked into two systems featuring two 10 m<sup>3</sup> tanks each. One tank in each system was stocked at 200 fish/m<sup>3</sup> and the other tank was stocked at 300 fish/m<sup>3</sup>. Each system was equipped with a propeller-washed bead filter, a moving bed bioreactor, a protein skimmer, an oxygen cone, and an auto-controlled heater/chiller unit. Salinity, temperature, dissolved oxygen, pH, alkalinity, ammonia, and nitrites were monitored daily. pH and alkalinity were adjusted as necessary using sodium bicarbonate. After 180 days, 200-300 fish from each tank were harvested, counted, weighed to the nearest g, and measured (total length mm). Survival was similar among tanks and averaged 69.8% during the experimental period. Density at the end of the experiment ranged between 8-12kg/m<sup>3</sup>. Growth differed between systems but not between the two tested densities. Differences in growth rate between the two systems may have been due to periodic degradation of the water quality recorded in one of the two systems.

The second experiment examined the effect of salinity on growth and survival. Twenty-five day old and 180 day old fish were stocked into four systems featuring four 1 m<sup>3</sup> tanks each. Each of the systems was run at a different salinity (10, 15, 20, and 25ppt). Two tanks in each system were stocked with twenty-five day old fish at a density of 280 fish/m<sup>3</sup> based on the results of the previous study. The other two tanks in each system were stocked with 180 day old fish at a slightly lower density (210 fish/m<sup>3</sup>) because higher survival was expected in that group. Fish were fed 1.5-5% body weight per day and samples were collected from the entire population every 75 days. After the first 75 days, the mean weight of the 15psu group (20.98g and 105.13g) was higher than that in the three other treatments but corresponding differences in length were minimal (.1 - 4.5%). The 15psu fish were 4% heavier than the 10psu fish, 6.5% heavier than the 20psu fish, and 14.5% heavier than the 25psu fish. This trial will continue until fish in each group reach approximately 365 days old at which point tanks will be harvested to compile data regarding product quality and yield.

## MOLECULAR ASPECTS AND TRANSCRIPTIONAL ANALYSIS OF SH2-DOMAIN CONTAINING LEUKOCYTE PROTEIN FROM BLACK ROCKFISH (*Sebastes schlegelii*)

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Adapter molecules, which can be found in every cell type, contribute vitally in signal transduction. These are multi-domain proteins which are lacking with intrinsic catalytic activity. However, their function is facilitating *via* nucleating molecular complexes during signal transduction. The SLP-76 family of adapters has three members, each expressed exclusively in hematopoietic cells—SH2 domain containing leukocyte phosphoprotein of 76 kDa, B cell linker protein (BLNK/BASH/BCA/SLP-65) and cytokine-dependent hemopoietic cell linker (Clnk/MIST). Here in, we have characterized the molecular aspects of SH2-domain containing leukocyte protein where it is also known as Lymphocyte cytosolic protein 2 (LCP2) from black rockfish (RfLCP2). Web based bioinformatics tools were used for the analyzing of domain architecture of the SH2-domain containing leukocyte protein. According to the *in silico* analysis this protein has a molecular weight of 55.66 kDa and a theoretical pI value of 7.29. Domain architecture analysis revealed that this protein has a conserved SH2 domain which is important for the cell signaling transduction process. Moreover there are conserved polypeptide binding sites including; phosphotyrosine binding pockets and hydrophobic binding pockets. Moreover, two *N*-glycosylation sites were observed in the putative protein sequence. Absence of signal peptide suggested that this protein does not have any secretory properties with it. Multiple sequence alignment with its known orthologs revealed highly conserved residues in the RfLCP2 protein. Identity similarity analysis confirmed that RfLCP2 shared the highest identity percentage with *Larimichthys crocea* with a percentage of 78 %. Ubiquitous expression was observed in *RfLCP2* transcripts in healthy rock fish tissues with different magnitudes. However, the immense expression was observed in the blood tissue. Immune responsive function of RfLCP2 was evaluated with immune challenged tissues. According to the transcriptional analysis, the highest expression of *RfLCP2* was observed in 24h post infection against *Streptococcus iniae*. However, at the 12 h time point *RfLCP2* was highly up-regulated against LPS. Upon viral infection (against Poly I:C) *RfLCP2* was possessed the highest up-regulation at 12h pi. Collectively, we can suggest that RfLCP2 play an important role in the immune responsive function in black rockfish.

## FUNCTIONAL CHARACTERIZATION OF BIG-BELLY SEAHORSE *Hippocampus abdominalis* NATURAL KILLER CELL ENHANCING FACTOR-A (NKEF-A/Prx1)

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Natural killer cell enhancing factor (NKEF) has been remarked as a cytosolic protein that enhances the natural killer cell cytotoxic activity. Apart from the cytotoxic activity of the NKEF, it has shown potential antioxidant function similar to that of peroxiredoxin members. Hence, natural killer cell enhancing factor A (NKEF-A) is also referred as peroxiredoxin 1. It protects the host cells from different oxidative damages such as hydrogen peroxide, alkyl hydroperoxide and heavy metals. In the present study, molecular features, functional properties and immune responses of *Hippocampus abdominalis* NKEF-A (HaNKEF-A) were assessed. Putative open reading frame encoded 594 amino acids with 29.9 kDa polypeptide and a *pI* of 6.43. Two conserved domains (Prx\_typical\_2Cys and Thioredoxin\_like) and several active sites including, catalytic triad, dimer interface, decamer, peroxidatic and resolving cysteines were identified through bioinformatics tools. It shared the highest identity (93.4%) and similarity (98%) with the *Cyprinodon variegatus* Prx1. Multiple sequence alignment revealed the conservation of functionally active peroxidatic and resolving cysteines among the other NKEF-A/Prx1 counterparts suggesting the common peroxidase activity. Metal catalyzed oxidative (MCO) stress, cleaved the pUC19 DNA from supercoiled state into nicked state where, rHaNKEF-A protein could protect the pUC19 DNA cleavage by MCO system in a concentration dependent manner. The rHaNKEF-A catalyze the insulin reduction activity with the presence of 1,4-Dithiothreitol (DTT) in a time dependent manner. The results of the MTT assay revealed that the presence of the rHaNKEF-A increased the cell viability% against the H<sub>2</sub>O<sub>2</sub> oxidative stress. Moreover, the activity was dose dependent and the highest cell viability percentage was gained with the 100 µg/mL of rHaNKEF-A. The same concentration of the rHaNKEF was given the highest reduction of the ROS level in the human LNCaP cells against the H<sub>2</sub>O<sub>2</sub> oxidative stress. The *HaNKEF-A* transcripts were ubiquitously expressed in all examined tissues with highest expression in liver. Bacterial (*Edwardsiella tarda*, *Streptococcus iniae* and LPS) and viral (poly I:C) immune stimulated liver tissue showed significant *HaNKEF-A* mRNA expression after the post infection. Collectively, HaNKEF-A is belonging to the teleostean peroxiredoxin family member with its antioxidant function and potential immune responses upon bacterial and viral challenges. Also, it could be suggested that HaNKEF-A is an active member of seahorse antioxidant defense system.

## **BUILDING HUMAN CAPACITY AND GENDER EQUITY AMONG GLOBAL AQUACULTURE STAKEHOLDERS**

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The development and strengthening of human capacity in partner countries is a central objective of the AquaFish Innovation Lab and one success of the many years of AquaFish is the development of an expansive collaborative network of international researchers and stakeholders dedicated to the sustainable development of aquaculture and fisheries. AquaFish provides support, mentoring, and academic guidance for students in post-secondary and graduate-degree programs in a wide array of disciplines, helping early-career scientists build and strengthen their professional networks. Since 2006, AquaFish has supported nearly 600 students in pursuit of post-secondary degrees, creating a pipeline of educated professionals who move on to careers in government, academia, and private enterprise upon graduation.

Short-term training courses and workshops are another way in which AquaFish partners teach end-users about innovative aquaculture technologies and provide farmers, extension agents, producers, and policy makers with knowledge and expertise to implement and adapt strategies to their conditions. More than 9,000 aquaculture and fisheries stakeholders have been trained by AquaFish partners since 2006 on a variety of topics, including fish harvesting and processing, record keeping, marketing, best management practices, value chain analysis, and business skills development.

Women have long been underrepresented in science, engineering, and technology careers and face marginalization within the aquaculture value chain. Although improved in recent years, collective quantitative data shows that women do not remain in science at the same rate as men and they lack access to resources and training opportunities, trapping them in vulnerable and poorly paid positions with little prospect of getting ahead. To address this, AquaFish sets a benchmark of women comprising at least 50% participation in both short- and long-term trainings, and designs interventions to engage women throughout the sector including research in universities, high level management in organizations, and private operators in lending, fish marketing, and farming. AquaFish has successfully increased the proportion of women's participation in short-term trainings from 37% in 2008 to 50% in 2016. Furthermore, 50% of all AquaFish-supported long-term students were women in 2016.

## PROXIMATE COMPOSITIONS OF FARMED AND WILD-CAUGHT STRIPED SEA BREAM (*Lithognathus mormyrus*)

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The objective of this study was to determine and compare the proximate composition of wild and farmed striped sea bream (*Lithognathus mormyrus*).

The present study was carried out on farmed (group I) and wild-caught (group II) striped sea bream (*Lithognathus mormyrus*) from the sparidae family. Eight fish samples for each group directly obtained from the fisherman and a fish farm in Antalya, Turkey. Fish were individually measured for their total body weight and length and filleted before analysis. While the average total length of group I was 26,14±0,88 cm and the weight was 252,75±36,78 g, in group II, the average total length of the samples was 24,70±1,49 cm and the weight was 198,00±31,33 g. After homogenisation of the fillets, crude protein, lipid, ash and moisture were determined and compared. Condition factor also for both groups were found. Date analysis was carried out using Analysis of variance (ANOVA) according to the statistical analysis system and the calculation was performed with SPSS 11 for windows.

At the end of the study, condition factor and proximate values consisted of crude protein, lipid, ash and moisture were determined. Condition factor values were found as 1,41±0,16 and 1,31±0,10 for group I and II respectively and there was no statistical difference between the groups. However, crude protein, lipid, ash and moisture were statistically different when the two groups were compared. While the crude protein was higher (23,49±0,15) in group I (farmed) than the group II (21,80±0,18), lipid level in wild-caught samples statistically higher (2,52±0,07) than that of the farmed (1,55±0,08) group. Farmed fish group has a higher ash (1,41±0,03) and moisture (73,91±0,26) compared to ash (1,31±0,03) and moisture (75,67±0,30) of other group.

## GONAD DEVELOPMENT IN ANEUPLOID ORNAMENTAL KOI CARP OBTAINED BY CROSSING TRIPLOID FEMALES WITH DIPLOID MALES

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Recent studies showed that some triploid ornamental koi carp (*Cyprinus carpio*) females were able to develop large ovaries and produce mass aneuploid progenies when crossed with diploid koi males. Aneuploid fish had very low survival; only 248 4-month-old juveniles were collected from outdoor tanks in which about 32,000 swim-up larvae obtained from triploid females were stocked. For further rearing, fish were stocked in 400-l tanks in indoor recirculating systems with water temperature maintained at 24-26°C. Under these rearing conditions, normal diploid koi males and females reach maturity at 2 years of age.

Reproductive ability and gonad development of aneuploid fish obtained from triploid females were investigated at fish age of 28 months; only 33 fish survived to this age. Preliminarily, fish were individually marked with PIT tags and their ploidy determined by flow cytometric analysis. All investigated fish were injected with carp pituitary extract (CPE) at a dose of 3 mg/kg. No fish released sperm or eggs upon stripping after hormonal injection. Fish were then dissected and their gonads were investigated.

The data on fish ploidy and gonad development are presented in Table 1. All investigated fish were aneuploid with ploidy range from 2.31n to 2.78n. No dependence of sex or stage of gonad development on fish ploidy was detected. Similar to triploid males, aneuploid males had pinkish (brownish) testes which did not contain sperm. Ovaries of most aneuploid females were undeveloped and contained oocytes at the beginning of vitellogenic growth. However, one investigated female (with gonadosomatic index 9.6%) had relatively large ovaries filled with fully grown oocytes. In general, results of this study showed that reproductive ability of aneuploid koi was similar to that of triploid koi investigated earlier. Males are functionally sterile while some potentially fertile aneuploid females can be found.

Table 1. Gonad development in 28-month-old aneuploid koi.

Sex group	No. of fish	Fish ploidy, n		Fish weight, g		GSI, %	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Females	12	2.55 $\pm$ 0.10	2.31-2.71	434 $\pm$ 176	210-811	3.0 $\pm$ 2.6	0.5-9.6
Males	15	2.59 $\pm$ 0.11	2.44-2.78	361 $\pm$ 239	52-904	1.2 $\pm$ 1.0	0.3-3.5
Intersex	1	2.65	-	1,028	-	2.4	-
Sterile	5	2.55 $\pm$ 0.12	2.38-2.76	367 $\pm$ 207	188-730	-	-

## EFFECTS OF PROBIOTICS *Pediococcus*, *Bacillus subtilis* AND *Lactobacillus acidophilus* ON FOOD SAFETY INDICATORS IN AQUAPONIC SYSTEMS

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Foodborne pathogens are a source of concern in ready-to-eat foods such as leafy greens. Aquaponic systems combine recirculating aquaculture and soilless vegetable production to produce a wide variety of vegetable produce. Food safety concerns stem from the direct integration of fish culture water with plant grow beds. Typically, pathogens affecting fish are not a risk to human health; however, vectors such as systemic water, birds, mice, and humans may introduce pathogenic organisms. The addition of probiotics into aquaponic system water may competitively exclude pathogenic bacteria by actively altering the composition of microbial communities.

Researchers evaluated the effects of a probiotic mix containing equal parts *Pediococcus* spp., *Bacillus subtilis*, and *Lactobacillus acidophilus* in aquaponic systems. Six, 500 gallon replicate aquaponic systems were each stocked with six kg (~50 fish) of Nile tilapia (*Oreochromis niloticus*) and 71 Nevada heat tolerant lettuce (*Lactuca sativa*) plants. Randomized probiotics treatments were inoculated weekly with the probiotic cocktail at a concentration of  $10^6$  colony-forming units (CFUs/ml). System water, plant leaves, and plant root samples were collected weekly. Samples were processed, plated onto selective media, and enumerated. Colonies of interest including potential *Salmonella* spp., *Escherichia coli*, *Enterobacter* spp., *Vibrio* spp., and *Listeria monocytogenes* were isolated and identified using the bioMérieux Vitek 2 bacterial identification system.

This study was run in two lettuce cycles to reproduce a typical production cycle. A two sample t-test will be used to analyze data between the control and treatment.

## EFFECT OF DIETARY LIPID LEVEL ON THE PRESENCE AND ACTIVITY OF LIPASE IN THE GASTROINTESTINAL TRACT OF RED DRUM, *Sciaenops ocellatus*

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Knowledge of the nutritional requirements of red drum, *Sciaenops ocellatus*, is considerable, since its commercial culture development started in the 1980s. Dietary lipid requirement for this species has been estimated to be around 10%, and lipase, the gastrointestinal (GI) tract enzyme digesting this nutrient, is apparently not stimulated by increasing dietary fat content, at least not in larvae, where a plateau in lipase activity is reached at around 12% dietary neutral lipid, suggesting this is the limit for maximal capacity in lipase synthesis at this developmental stage. It would be interesting to verify if the same holds true for other developmental stages. Thus, the present work further investigated the effect of dietary lipid level on the presence and the activity of lipase in the GI tract of *S. ocellatus* during the growout stage, as well as on its growth performance, and body composition.

An 8-week feeding trial was performed in an indoor clear-water recirculating culture system at the Texas A&M University Aquacultural Research and Teaching Facility, consisting of 110-L aquaria provided with biological and mechanical filtration, a UV light chamber, and water aeration. Juvenile fish were fed different diets with a fixed dietary crude protein (CP) level of 40% and increasing levels of dietary crude fat (CF), from 2 to 20% of dry diet. Each diet was randomly assigned to four replicates. Fish were fed to apparent satiation twice daily, with pre-weighed rations based on a percentage of total fish weight per aquarium (3–5% of total body weight) and visual feeding cues. Total ammonia nitrogen, nitrite, and pH were monitored weekly, while dissolved oxygen, salinity and temperature were monitored daily.

At the end of the feeding trial, the GI tract of fish subjected to the different dietary lipid treatments were dissected and homogenates in a 50 mM Tris-HCl buffer solution (pH 7.5) were obtained. The molecular mass of native lipase and its content in the fish GI tract were determined by SDS-PAGE using 10% polyacrylamide gels. Lipase activity was measured by using Na cholate hydrate, 50 mM Tris-HCl buffer and  $\beta$ -naphthyl-caprylate as substrates, followed by incubation and addition of 100 mM Fast Blue BB salt, with the reaction stopped after 5 min. by using 0.72 M trichloroacetic acid and ethanol-ethyl acetate (1:1, v/v). The samples were read at 540 nm against DI water as a blank. Body proximate composition and growth performance indexes such as final weight, weight gain, percent weight gain, daily weight gain, thermal growth coefficient, and specific growth rate were also determined for fish in each dietary treatment.



## **PARTIAL CHARACTERIZATION AND QUANTIFICATION OF PANCREATIC LIPASE IN THE GASTROINTESTINAL TRACT OF TOTOABA, *Totoaba macdonaldi***

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Understanding the utilization of dietary lipids in marine fish is crucial for understanding their energy metabolism. Nevertheless, our knowledge of the biochemical mechanisms that allow marine fish the use of this particular nutrient is rather basic. Pancreatic lipase (PL) is the main digestive lipolytic enzyme in higher vertebrates and is secreted by the exocrine pancreas. A pancreatic lipase has been reported in top minnow (*Tripurtheus* sp.), sardine (*Sardinella longiceps*), cod (*Gadus morhua*), and turbot (*Scophthalmus maximus*). *Totoaba macdonaldi* is a marine sciaenid of the Gulf of California with great potential for aquaculture. Development of aquafeeds for its culture is progressing as quantitative nutritional requirements are established, but lipid levels from 8 to 22% do not seem to affect its growth performance, similarly to red drum, *Sciaenops ocellatus*, another sciaenid for which lipase is apparently not stimulated by increasing dietary fat content. The present work investigated the molecular mass of pancreatic lipase and quantified its presence along the gastrointestinal tract of *T. macdonaldi*.

Forty adult totoabas spawned in captivity were donated by the Center for Reproduction of Marine Species of the State of Sonora (CREMES); their mean average weight and total length ( $\pm$  S.D.) were  $1477.25 \pm 145.37$  g and  $52.86 \pm 1.99$  cm, respectively. After obtaining some additional biometric data for the determination of the viscerosomatic index (VSI), hepatosomatic index (HSI), and condition factor (K), the GI tract of fish were dissected and homogenates in a 50 mM Tris-HCl buffer solution (pH 7.5) were obtained. The molecular mass of native lipase and its content in the fish pyloric caeca, as well as in the anterior, middle and posterior GI tract were determined by SDS-PAGE using 10% polyacrylamide gels resolved at a constant voltage of 115V. Lipase activity was determined with a lipase activity assay kit (Sigma-Aldrich, MAK046, St. Louis, MO, USA) using a coupled enzyme reaction resulting in a colorimetric (570 nm) product proportional to the enzymatic activity present. One unit of lipase was the amount of enzyme that generated 1.0 mmole of glycerol from triglycerides per minute. Activity of pancreatic lipase was determined at different pH (6-10) and temperatures (20-60°C).

## ASSESSING PERACETIC ACID AS A MEANS TO CONTROL POST-VACCINATION SAPROLEGNIASIS IN ATLANTIC SALMON *SALMO SALAR* PARR IN RECIRCULATION AQUACULTURE SYSTEMS

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Land-based closed containment facilities, utilizing recirculation aquaculture system (RAS) technologies, can reduce or eliminate the introduction of obligate fish pathogens. Regardless, the presence of opportunistic pathogens must be assumed, and these agents can cause disease during unfavorable conditions. One such disease, saprolegniasis (caused by *Saprolegnia* spp. oomycetes), is associated with enormous losses in aquaculture; it is estimated that 10% of all hatched farmed Atlantic salmon *Salmo salar* die from saprolegniasis. This disease is often observed subsequent to vaccination. Because Atlantic salmon smolt production is increasingly being carried out in RAS, control strategies must be designed to minimize impacts on biofiltration. We investigated daily peracetic acid (PAA) treatments to determine their efficacy in reducing post-vaccination saprolegniasis while assessing biofilter performance in replicated RAS. Twelve replicated experimental-scale RAS were stocked with Atlantic salmon parr (200 fish per RAS, 94g mean weight); fish were subsequently vaccinated with a commercial salmon vaccine via intracoelomic injection. Daily pulse treatments with PAA – i) 0.2 mg/L, ii) 0.5 mg/L, iii) 1.0 mg/L, or iv) deionized water (control) – were administered to culture tank water for six weeks post-vaccination. During this period, data were collected on mortalities and incidence of clinical saprolegniasis, *Saprolegnia* spp. colony counts from RAS water samples, histopathology of gill, spleen, and kidney tissues, and biofiltration as measured by total ammonia nitrogen (TAN) removal efficiency. Welfare was assessed by examining fish for gross lesions and fin erosion, hemorrhage, and visible *Saprolegnia* spp. infection. Ultimately, no major post-vaccination *Saprolegnia* spp.-associated mortality was observed in this study; however, survival was statistically ( $p < 0.05$ ) lower in control parr, and clinical saprolegniasis was significantly more prevalent in the control group. Conversely, PAA treatment was associated with significantly lower fish weight by study's end. Biofilter TAN removal efficiency was not impacted by PAA administration at all dosages. Water sample plate counts of *Saprolegnia* spp. colonies increased over time in all treatment groups during the post-vaccination period. Welfare assessments indicated that PAA treatment was significantly protective against observable pectoral fin saprolegniasis and hemorrhage; however, fin erosion in general was not associated with PAA treatment. Histopathology data are currently forthcoming. Overall, results suggest that daily low-dose PAA application can be effective in reducing post-vaccination saprolegniasis in Atlantic salmon while not significantly impacting RAS biofiltration. Further research, however, is necessary to refine PAA dosage and to assess its effectiveness in controlling saprolegniasis in commercial settings.

## **TRAINING WOMEN AND YOUTH TO ENHANCE SUSTAINABLE AQUACULTURE AND INCREASE FOOD SECURITY**

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Successful aquaculture development depends upon building and sustaining a gender-balanced community of students, professionals, and community members. However, women's potential to contribute to agriculture sector growth and improved nutrition is limited by restricted market opportunities, lack of access to education, and by inequalities in economic participation and decision-making power. Likewise, the world's 1.8 billion youth, most of whom live in developing countries, encounter similar obstacles due to inadequate access to employment, education, and decision-making. Not only are women and youth tied by their barriers to participation in the agriculture sector, they also are intrinsically linked to household nutrition by maternal health through early childhood development.

Undernutrition in the first two years of a child's life can have permanent consequences on their well-being. Mothers, as gatekeepers of household nutrition, have the power to improve children's health — if given the opportunity. As such, the Feed the Future Innovation Lab for Collaborative Research on Aquaculture & Fisheries (AquaFish Innovation Lab) is conducting research and training activities that engage women and youth on sustainable aquaculture practices. Since 2006, AquaFish has engaged over 3,300 women in short-term trainings on sustainable aquaculture and household nutrition. In 2016, 50% of degree-seeking students supported by AquaFish were women.

In Nepal, school ponds were established to train 8 teachers and 120 students about carp aquaculture and best management practices. Associated women's fish farmer groups, totaling 44 women, received additional training on how to improve household food security by incorporating small indigenous fish. AquaFish researchers in Kenya conducted trainings with 51 youth on establishing cage culture systems. Participants were trained on several aspects of pond culture from site selection and construction to production, management, and fish farming as a business. Empowering women and youth with knowledge and skills on aquaculture can positively impact household nutrition, food security, and income.

## THE DEVELOPMENT OF INNOVATIVE ENGINEERING STRUCTURES FOR OFFSHORE MACROALGAE FARMING

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The industrial-scale production of macroalgae (seaweeds, kelps) in oceanic waters is a branch of offshore aquaculture that has the potential to dwarf other forms in terms of both food production, environmental benefit, and energy production. Ocean-based biomass production can be accomplished without displacing land-based food crops or impacting present uses. The photosynthetic efficiency of aquatic biomass is 6 to 8% compared to terrestrial efficiency at around 2%. The US has a vast Exclusive Economic Zone (EEZ), and combined with the additional continental shelf areas over which it has rights, the US controls more ocean area than any other nation. In spite of these holdings, the US currently produces only 1.6% of global macroalgae harvests. One reason for this is that other than a narrow strip of ocean along the coast and within protected waters, most of this area is too deep and too exposed for the use of conventional macroalgae culture techniques.

With support of the NOAA SBIR program, the project team set out to develop engineered systems that will facilitate the farming of macroalgae in the open ocean. This presentation will report the findings from our 6-month Phase 1 effort that included the design, model construction, and wave tank testing of a prototype culture array aimed at enabling dense cultures of kelp (*Saccharina latissima*) and other species positioned in waters of any depth using a single-point mooring.

In addition, a geospatial model of the oceans was developed that includes relevant biological and physical growth factors to determine location-based growth rates. Combined with other economic factors related to harvest, transport, and processing, a model has been developed to identify optimal locations for industrial-scale macroalgae farming. This model and our wave-tank findings will guide our further development of affordable and robust systems for the production of algal biomass in sufficient quantities to support biofuel production and carbon sequestration.

## EVALUATING DIFFERENT LEVELS OF $\alpha$ -TOCOPHEROL SUPPLEMENTATION FOR LIVE FOOD PUFA ENRICHMENTS AND THEIR INFLUENCE ON THE SURVIVAL, GROWTH, AND CONDITION OF YELLOW PERCH *Perca flavescens* LARVAE

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Yellow perch (*Perca flavescens*) is an important commercial and recreational finfish species in the Great Lakes region with great potential for aquaculture expansion. The optimization of feeding and nutrition, especially in early life stages, is necessary for the commercial success of yellow perch production. Live food enrichment with polyunsaturated fatty acids (PUFA) is a commonly used technique for increasing the growth and survival of larval fish, but increased dietary PUFA can also be a major source of oxidative stress. This investigation examined the intensive culture performance of yellow perch larvae during the first ten days of feeding with rotifers (*Brachionus plicatilis*) and *Artemia* nauplii enriched with PUFA and three levels of  $\alpha$ -tocopherol ( $\alpha$ -T) antioxidant supplementation.

Larvae were reared in a specialized recirculating system with nine 50-L conical tanks equipped with surface spray. Water was kept at  $22.6 \pm 0.6^\circ\text{C}$  and evaporated salt and marine microalgae were continuously added to maintain a salinity of  $2.3 \pm 0.3$  ‰ and a turbidity of  $9.3 \pm 1.1$  NTU. Enrichment emulsions contained 754 mg PUFA and 0, 155, or 260 mg  $\alpha$ -T  $\text{g}^{-1}$  dry weight (Control PUFA, 25%  $\alpha$ -T, and 50%  $\alpha$ -T, respectively). The fatty acid and tocopherol composition of live feeds and yellow perch were assessed using gas chromatography and high-performance liquid chromatography.

The treatments correlated with significant differences ( $\alpha \leq 0.1$ ) in fish survival and final weight at the end of the feeding period (Figure). The  $\alpha$ -T content of both live foods and the fish were significantly influenced by treatment, with the supplemented groups having greater concentration than the Control PUFA group ( $p < 0.001$ ). The only significant difference between the 25%  $\alpha$ -T and 50%  $\alpha$ -T groups was in yellow perch  $\alpha$ -T concentration ( $p = 0.038$ ). These data suggest that  $\alpha$ -T supplementation to live food PUFA enrichments is beneficial to the intensive culture of yellow perch larvae, and that 260 mg  $\alpha$ -T  $\text{g}^{-1}$  has no significant benefit over 155 mg  $\alpha$ -T  $\text{g}^{-1}$ .

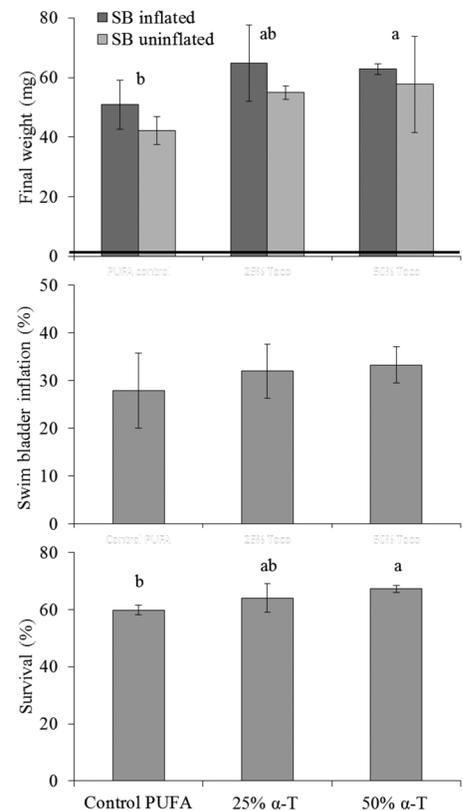


Figure. Survival, swim bladder (SB) inflation, and final weight of yellow perch after 10 days of feeding on rotifers and *Artemia* nauplii enriched with PUFA and 0, 155, or 260 mg  $\alpha$ -tocopherol  $\text{g}^{-1}$  dry weight (Control PUFA, 25%  $\alpha$ -T, and 50%  $\alpha$ -T, respectively). Black bar represents initial size and letters correspond to significant differences at  $\alpha < 0.10$ .

## IMPROVED SITING OF KELP AQUACULTURE USING N15 STABLE ISOTOPE ANALYSIS

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The time has come to gain a better understanding of the opportunities and limitations for seaweed harvesting along urbanized coastlines. Interest in wild harvest of seaweed, and farming it using aquaculture techniques, has skyrocketed in the Americas and Europe. As such, more research is needed to determine how land use along these coastlines could influence decisions regarding optimal siting of seaweed farms or the identification of best areas for wild harvest. The objective of this study was to test if nitrogen isotopes measured in the tissue of sugar kelp (*Saccharina latissimi*) grown in strategic locations could be used to improve siting of future kelp farms. The small curved embayment of Saco Bay, Maine was selected as the field site for this study because it is akin to many developed coastlines. It has a strong tourism industry, historic industrial operations, and it receives water from a major river, effluent from 6 wastewater treatment plants (WWTPs), water from tidal pools and salt marshes, and runoff from residential developments.

Buoys were deployed along two perpendicular transects in Saco Bay and mature kelp was attached to each buoy, along with temperature, light, and salinity sensors. Tissue samples were collected from the kelp every two weeks, and following rain events greater than 1 in. Samples were excised from the meristem, mid part, and distal tissue of the kelp and analyzed for their N15/N14 ratio. These results were compared with effluent data from the area's wastewater treatment plants, discharge measurements from the Saco River, and ocean current velocity hindcast in a spatial analysis tool to identify relationships between predicted dispersion of anthropogenic nitrogen and observed nitrogen isotope ratios in kelp grown on the buoys. Results from this and other parallel efforts may help to inform a management plan for wild or farmed seaweed harvest in the region.

## PRODUCTION OF HYBRID STRIPED BASS STOCKERS IN A BIOFLOC SYSTEM

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High yields can be obtained from an outdoor biofloc technology (BFT) production system in response to high stocking and feeding rates because a complex of living organisms, including phytoplankton and bacteria, closely associated with particulate organic matter that is maintained in suspension in the water column by continuous aeration metabolizes nitrogenous waste excreted by the intensively fed fish. Currently, Published research demonstrates that hybrid striped bass (*Morone chrysops* x *M. saxatilis*; HSB) can be reared intensively in recirculating aquaculture systems and in-pond raceways, but HSB have not been grown in a BFT production system. We report for the first time on the production of HSB advanced (or stocker-sized) fingerlings in an outdoor BFT production system. Study objectives were to quantify the relationship between HSB stocking rate and growth, yield, and water quality. Fingerling (1.2 g/fish) HSB were stocked at 50 – 250 fish/m<sup>2</sup> in 50-fish/m<sup>2</sup> increments in continuously aerated 2.4-m diameter HDPE-lined tanks in late June and grown for 105 days. Each tank was equipped with a 76-L settling chamber to remove solids. Water was added to tanks only to replace losses to evaporation and settling chamber draining. Fish in each tank were fed a formulated ration (45% protein, 12% lipid) to apparent satiation twice daily. Water samples were analyzed weekly for dissolved inorganic carbon, nitrogen, and phosphorus, solids concentrations, and algal biomass. Fish production and water quality relationships will be discussed.

## RICE HULL BIOFILTER DESIGN: LAB AND COMMERCIAL SCALE RESULTS

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Rice hulls have been explored for use as a biofriendly and economical biofilter media. Lab scale studies suggest they are not significantly different from EN (plastic) media in their ability to host bacteria in biofilters at levels of total ammonia nitrogen (TAN) from 3-6ppm. Two factors are critical in differentiating this media and engineering has been done to adapt to the media. First, their density is different from that of the EN plastic media, so reactors must be designed to accommodate their density. Second, they do biodegrade slowly over time and small pieces can break off and must be removed to reduce BOD. Hence, both solids reduction techniques and correct flow design in biofilters are necessary.

Rice hulls are available in many developing countries, so use of rice hulls as media in biofilters could be advantageous and economically viable in these areas. A separate project focused on the economics of this technology and possible low cost applications for more sustainable aquaculture.

Removal rates ranged from 70-84% in laboratory studies up to 6 ppm. A commercial scale vortex reactor was designed and built using rice hulls as biomedium. This reactor worked up to 60ppm TAN, but design improvements were required to keep the biomedium moving with an airlift driven vortex.

A technique was used to remove about 10% of biodegraded biomedium every 2 weeks and an economic analysis suggests this media may be useful in developing countries where capital costs must be minimized but labor costs may remain low, allowing an economic use of this biobased system. This may especially benefit developing countries to use this more affordable technique for biofiltration in their aquaculture industries.

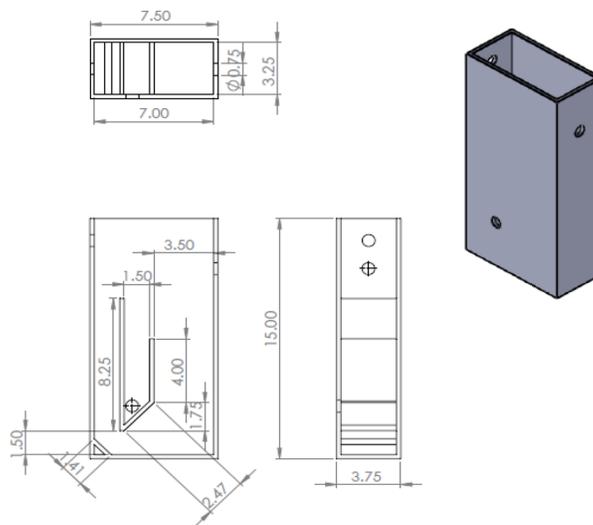


Figure 1. Design for rice hull biofilter.

## **THE FUNCTION AND USE OF GRANULAR FILTRATION IN RECIRCULATING AQUACULTURE SYSTEMS: BASIC PRINCIPLES**

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Recirculating aquaculture systems (RAS) rely on a water treatment process train to perform both physical and biochemical remediation of the process water to enable reuse and minimize the volume of the effluent stream. The first step in the water treatment process train is the removal of the solid wastes produced in the culture vessel. The efficiency of the solids removal process will significantly influence the intensity and degree to which the subsequent, secondary treatment processes must perform. As such, maximizing the efficiency of the primary, solids treatment will improve the efficiency and reduce the cost required for the downstream treatment processes. Of the many options available to system designers and operators, granular filtration has proven to offer robust and flexible options for meeting the needs of the aquaculture industry.

Granular filtration is largely recognized as an effective means for the capture and removal of solid wastes from process water in a variety of water treatment systems. Granular filtration may also be used as an effective means for biological conversion of dissolved constituents. However, effective granular biological filtration requires an effective solids removal process directly upstream. Properly designed, granular filtration devices may even successfully combine the solids capture and biological filtration processes into a single unit, thus reducing space requirements as well as initial costs. Due to the flexibility in design and dynamic performance characteristics, granular filtration is successfully used in a wide variety of nutrient-loading scenarios and industries. Recent developments in the design and utilization of granular filtration in RAS have made improvements in the concentration of captured solids prior to discharge as a means for reducing the overall volume of the effluent stream.

Proper selection of granular filtration devices requires an understanding of the filter design and function of the media used in the filter. An appropriate characterization of the filter influent will play a large role in the selection of the proper filter type. Particle diameter and capture efficiency, hydraulic headloss, media bed backwash frequency, media characteristics (i.e. shape, material, biofilm retention, etc.), backwashing mechanism, and flow rate/velocity must all be considered in the appropriate selection and application of granular filters.

The purpose of this presentation will be to provide a foundation for understanding the form and function of granular filtration and the application thereof in RAS. Filter design, media effects, and filtration (physical and biological) will be discussed.

## **OPPORTUNITIES AT THE LSUAC AQUATIC GERMPLASM AND GENETIC RESOURCES CENTER**

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Cryopreservation and repository development is beginning to move into commercial application for aquatic species. Such activities have been performed at an industrial level in livestock for decades. Livestock germplasm approaches can provide methods, equipment and experience that can be transferred to fish and shellfish; they can also provide facilities. The LSUAC Aquatic Germplasm and Genetic Resources Center (AGGRC) was initiated to convert the LSU Dairy Improvement Center (DIC) into a comprehensive, stand-alone facility for research, technology development, teaching, outreach, training, cooperation, and commercial-scaler processing for biobanking and germplasm repository development in aquatic species. In 2015 the DIC facility became available for transition into dedicated use with aquatic species. The facilities comprise 23,000 ft<sup>2</sup> of laboratory, office, cold-room, and barn space including a conference room and space specifically designed for use as a cryopreservation center. In addition there are several outbuildings (an additional 5,500 ft<sup>2</sup>) that can be converted into fish holding space.

The AGGRC combines 25 years of experience in cryopreservation and repository development with hundreds of aquatic species with facilities suitable for research and commercial-scale cryopreservation. Staffing includes biological and engineering expertise, and projects emphasize standardization of repository development by development of devices for specific activities in the cryopreservation process. Prototyping and testing are performed with 3-D printing and microfabrication capabilities. The facility is located adjacent to the LSU campus and enables easy access and collaboration with students and scientists at the university, including teaching of classes. In addition to separate research and commercial-scale laboratories, we have developed a self-contained mobile laboratory that can perform high-throughput cryopreservation at cooperating facilities. The mobile laboratory can provide comparable quality and throughput as work performed at AGGRC. creating the opportunities for species that are be troublesome to transport. Cryopreserving on-site also allows genetic data (blood or tissue samples) and environmental data (water quality, location) to be collected along with the germplasm. This is especially valuable at cooperating hatcheries or with field studies.

## AN EMPIRICAL ANALYSIS OF LAW OF ONE PRICE IN BANGLADESH FISH MARKETS: A GENERALIZED ADDITIVE MODELING APPROACH

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The Law of One Price has a long history in economics and does provide an important basis for arbitrage behavior thought to characterize movements of commodity prices over time and across spatially-distinct markets. There is a vast literature that has evolved from linear regression models to classes of models that consider nonlinear price linkages investigating this phenomenon. Recently, the literature addressing the Law of One Price has concentrated on methodologies suitable for adapting structural change and mean-shifting behavior with unknown break points and variable speeds of adjustment among regimes that overcomes the problems of nonstandard test statistics and parameters. The regime switching behavior stems from unobservable transactions costs that may result in discrete trade/no trade regimes or smooth, continuous transitions between different states of the market. Among the models that integrated this mean-shifting behavior and the reasons behind these price movements are Goodwin, Holt, and Prestemon (2012), Enders & Holt, 2012; Holt & Terasvirta, 2012; Ng & Voselgang, 2002.

The current article proposes a new class of semi-parametric model for spatial and regional price behavior that accommodates mean-shifting behavior in a vector autoregressive modeling framework to test the validity of Law of One Price for perishable fish products in Bangladesh. These vector autoregressive models adopt the Generalized Additive Models (GAM) estimation procedures proposed by Hastie and Tibshirani (1986) and Linton (2000). In particular, the backfitting and integration algorithms developed for GAM model estimation to incorporate a non-parametric mean shift in the linkages describing individual pairs and larger groups of market prices are utilized. The empirical specification involves simple and vector error correction models that relate price differences to lagged values of prices and price differentials.

The current application is to six major regional fish markets (Khulna, Mymensingh, Rangpur, Faridpur, Barisal, and Jessore) in Bangladesh for seven main freshwater aquaculture species (*Catla catla*, *Labeo rohita*, *Ctenopharyngodon idella*, *Anabas testudineus*, *Oreochromis niloticus*, *Pangasius hypophthalmus*, and *Hypophthalmichthys molitrix*). We employ exhaustive data, collected by the WorldFish Center, consisting of weekly prices of fresh and chilled fishes at the wholesale and retail levels covering the period from October 2010 to June 2015.

Additionally, impulse response functions are used to evaluate the dynamics of regional price adjustments to localized shocks in individual markets. Implications for regional price adjustments are also discussed in the paper. Finally, suggestions for further extensions of the semi-parametric analysis of regime switching behavior are offered.

## **COST-BENEFIT ANALYSIS OF COMMERCIAL AQUACULTURE HEALTH PROGRAM STANDARDS (CAHPS) PILOT PROJECTS**

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Regulations governing aquaculture farms are complex and create a significant financial burden for producers in the United States. Hundreds of Local, State, and Federal regulations govern the production, health, and transport of hundreds of aquatic species currently produced in the U.S. The Commercial Aquaculture Health Program Standards (CAHPS) is a non-regulatory program to verify and improve farm raised aquatic animal health. With the implementation of a new program comes concerns about costs and benefits, which are essential for marketing the program and encouraging participation. Van Senten and Engle presented research in 2016 that showed a positive net benefit to bait and sport fish producers if CAHPS was implemented and regulations requirements were streamlined.

Pilot projects have been established to test and demonstrate how CAHPS would be implemented on U.S. aquaculture farms. One component of these pilot projects includes analyzing the costs and benefits of the activities. Three pilot projects have been approved and funded through USDA's Animal and Plant Health Inspection Service. A five farmer cooperative of tilapia producers in North Carolina have already completed three of the five principles in the first CAHPS pilot project. A second pilot project is working with salmon producers in Washington and Maine to examine how CAHPS will work for more complex industries with fresh and salt water, open and closed, settings. A third CAHPS pilot project is comparing the cost-effectiveness of site specific versus zoning surveillance options to demonstrate disease freedom.

This research will quantify the costs and benefits of implementing components of CAHPS. This should provide a picture of resources required to implement CAHPS and identify the potential benefits (cost savings, market expansion, etc.) to support producer decisions regarding participation in this voluntary effort. Direct costs related to labor, supplies, travel expenses, shipping laboratory fees, etc. will be estimated to show producers the anticipated investment levels they will need to make to implement CAHPS. The benefits of implementing CAHPS will be estimated as well, and will include the potential sales growth from expanding markets and resource savings (direct costs and saved time). The anticipated largest benefit would be the increase in domestic and international sales as a result of CAHPS implementation. A cost savings of implementing CAHPS may occur for those producers who will spend less time ensuring their farms are in compliance with regulations or if CAHPS promotes lower testing levels than what is currently required.



## EFFECT OF IRRADIANCE AND LIGHT WAVELENGTH ON A MIXED MICROALGAL/ CYANOBACTERIAL CULTURE

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Microalgae has been produced traditionally in monocultures. Mixed cultures of microalgae and other species such as bacteria, including cyanobacteria, have some advantages over single-species culture. Among these advantages are a greater resistance to contamination, symbiotic relation among the cultured species and a diversification of the products that can be obtained from the resulting biomass.

Despite the advantages of mixed cultures, there are some issues that need to be considered. Among these issues is the maintenance of a specific species ratio and composition in the culture, to optimize the target bioproducts production. The species ratio depends on a number of factors. In mixed cultures of photosynthetic species, light is an important parameter to consider for this control.

In this work, two sets of experiments were performed to determine the effect of light irradiance and wavelength distribution on the mixed culture. In the first set of experiments, four wavelength distributions (blue – 467 nm, green – 522 nm, red – 640 nm and white – narrow peak at 450 nm and a broad range with a peak at 550 nm) and two irradiance levels (400 and 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were investigated. The wavelength distribution resulting in the highest overall growth rate and productivity of the mixed culture, was used to determine the effect of the irradiance on the growth, productivity and species ratio of the mixed culture. For this second set of experiments, seven irradiance levels (180, 400, 600, 800, 1000, 1200 and 1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) with the wavelength selected on the first set of experiments and white light as control.

The results show that no photoinhibition was observed with irradiances up to 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Lipid content increased at higher light intensities (irradiance), and decrease when the irradiance was lower than 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Red light resulted in the highest productivity, growth rates and photosynthetic efficiency. The culture shifted from a microalgae to a cyanobacteria dominated culture as the light decreased. At 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  this shift was evident after six days of culture. At other irradiances, the shift was observed as the culture density increased. Attenuation curves were prepared to determine the irradiance levels that were related to the shift in species composition.

## EVALUATION OF ULTRA-VIOLET STERILIZATION TO REDUCE POTENTIAL HUMAN PATHOGENS IN AQUAPONIC SYSTEMS

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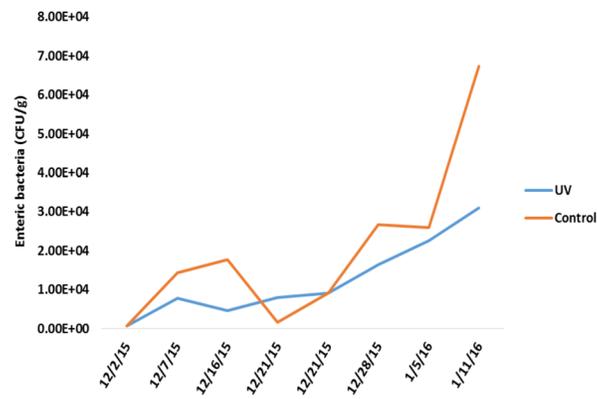
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Food safety is a particular concern in new or emerging industries which often lack the scientific data or history to assess potential risks to consumers. Aquaponics is one such industry. One of the main concerns is that fish culture water is in constant contact with the plants in the system. While pathogens that affect fish are not typically associated with human health issues, other modes of transfer (human contact, birds, rodents, etc.) can introduce pathogens such as coliform bacteria, *Salmonella* spp., and *Listeria* spp. Ultraviolet (UV)-sterilization of water is a common tool used to reduce bacteria and viruses in recirculating aquaculture systems (RAS) and might be effective to reduce bacterial loads and contamination in aquaponic systems.

The objective of this research was to determine if in-line UV-sterilization could reduce potential pathogens in aquaponic systems. Six, 500 gallon replicated systems were stocked with approximately 6,000 grams of tilapia (*Oreochromis niloticus*) and 72 Buttercrunch Bibb lettuce (*Lactuca sativa*) plants per unit. Three systems were fitted with 27 Watt high-output UV-sterilizers while three were operated without UV and served as Controls. Samples of culture water, edible biomass, and plant roots were collected weekly for microbiological analysis. Samples were plated on selective media to enumerate total coliform counts, *Listeria* spp., *Vibrio* spp., and pathogens in the Enterobacteriaceae family. The experiment lasted a total of six weeks over two production cycles of the lettuce.

These data indicate that UV-sterilization of water is an ineffective method to reduce selected bacterial loads in aquaponic systems. Total counts of tested pathogens in water, on plant leaf, and on plant root samples were not significantly reduced ( $P > 0.05$ ). As the edible plant portion poses the greatest risk to consumers, enteric bacteria (which encompass a wide spectrum of potential pathogens) is highlighted (Figure 1). It is likely that reduced water clarity in these systems negatively impacts UV transmittance. Future studies will evaluate increased fine solids filtration and probiotic applications to address food safety concerns with aquaponic produce.

FIGURE 1: Enteric bacteria counts on lettuce leaves from aquaponic systems with and without Ultra-violet (UV) sterilizers.



## **ECONOMIC, ENVIRONMENTAL AND SOCIAL BENEFITS OF IMTM IN THE PERSIAN GULF**

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Large-scale and intensive mariculture of finfish is inevitable along the Iranian coastline of the Persian Gulf. Finfish mariculture at the proposed sites poses a serious threat to the environmental integrity of over 1,800 kilometers of Iranian coastal and marine ecosystems. However, mariculture of finfish is an increasingly critical contribution to the nation's food security, as well as ameliorating public health. This is why Iranian Fishery Organization is promoting finfish aquaculture in offshore cages over the coastline of the Persian Gulf.

Sustainable mariculture implies that operations have a negligible impact on the surrounding environment. When a mariculture operation is destructive to the surrounding environment, this operation may have a lifespan of less than 5-10 years before the operation either becomes self-negating (environmental conditions deteriorate so it can no longer support commercial operations), or governments intercede and severely limit or prohibit operations. The two major mechanisms by which a commercial mariculture operation threatens environmental sustainability are chemical and eutrophic pollution. The most significant pollution from commercial mariculture is eutrophic pollution: the input of nitrogen and phosphorous into the environment, shifting the equilibrium from one that is usually oligotrophic to one that is detrimentally eutrophic. As a measure of Ecological Conservation and protection of coral reefs, sea turtles, and sea grass beds, a feasibility study for the site selection of the cage farms and the development of an Integrated Multi-Trophic Mariculture system (IMTM) is proposed. IMTM is the primary environmental conservation tool in not only mitigating, but preventing the environmental destruction brought on by eutrophic pollution from finfish mariculture operations. IMTM preserves biodiversity by alleviating fishing pressure of wild fisheries, reduces or eliminates the reliance of net-caught "trashfish" as mariculture fish food, reduces the need for destructive fishing practices, and reduces anthropogenic-usage loads for coral reefs. Furthermore, IMTM is a humanitarian effort that provides economic participation of traditional-fishing coastal communities by providing employment and co-localized services and industries. Successful IMTM operations will also allow the government to enact more stringent regulations for the extraction of wild fisheries and protection of coral reefs while having a positive effect on the industries that rely on these natural resources. The application of IMTM is considered the vanguard of environmental sustainability and commercial viability in mariculture. We have run several hydrodynamic models to assess the rate of pollution dispersal from IMTM operations, and results are summarized herein. This paper summarizes the attempts which have done to achieve an Environmentally Sustainable Integrated Multi-Trophic Mariculture System in the Northern Persian Gulf Coastlines.

## COMPARISON OF FOUR DIFFERENT VENUES FOR OUTREACH IN NORTH CAROLINA

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The Marine Aquaculture Research Center in Marshallburg NC provides the opportunity to share with various constituencies, primarily community, commercial and research personnel. This facility is close to the North Carolina coast (a tidal creek allows us to draw marine water for use), but somewhat distant from major population centers. This is a strictly NCSU facility, allowing relatively good control of experiments and demonstrations that occur at the facility. A board of advisors has been constituted to advise with best techniques to perform research and extension at the facility.

The “fish barn” is located in suburban Raleigh, a metro area of over 1 million. This intermediate sized facility is located on the grounds of NCSU’s farm. This allows groups of students, community members, engineers, and other practitioners to visit, but this is largely a freshwater facility of moderate size.

An on-campus facility housed at the Department of Biological and Agricultural Engineering has even less space but is much more accessible to the 34,000 students at NCSU and the 8000 faculty and staff members. Developing small scale demonstrations that convey ideas about aquaculture and also performing controlled experiments at this facility as well as much more controlled space at the College of Agriculture and Life Sciences Grinnell Laboratories tends to be a balancing act between sharing new ideas and performing controlled science experiments in limited space far from the coast.

This presentation will compare and contrast these venues for outreach.

**Figure 1. Diverse audiences have interests in aquaculture.**



## FIELD DETECTION OF PATHOGENS (*Renibacterium salmoninarum*) USING A HANDHELD QPCR SYSTEM

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Bacterial kidney disease (BKD), caused by the bacteria *Renibacterium salmoninarum*, affects both wild and cultured salmonids and can have significant economic impacts in both public and private aquaculture. Screening for the BKD causative agent (*R. salmoninarum*) in broodstock is often done using an enzyme linked immunosorbent assay (ELISA) test. While reliable, immunoassays are substantially less sensitive as a diagnostic tool than quantitative PCR (qPCR), which is rapidly becoming an industry standard for medical diagnostics. In this study, we evaluate the feasibility of using the Biomeme handheld qPCR system for on-site BKD detection. Biomeme can run the same diagnostic tests as benchtop instruments used for *R. salmoninarum* detection, with the added advantage of delivering test results in 40 minutes in resource limited settings. We compare BKD test results between the three alternative diagnostic tools (ELISA, Benchtop qPCR, Biomeme qPCR) using kidney samples from hatchery Chinook salmon (*Oncorhynchus tshawytscha*) in Washington state. Results of our comparative study will be presented, along with diagnostic tool recommendations for alternative BKD testing scenarios. The establishment of a field-based rapid BKD detection platform such as Biomeme would provide necessary information to enable adaptive management practices (e.g. culling eggs) that prevent *R. salmoninarum*-infected progeny from entering production. In turn, this would significantly improve the industry's ability to prevent and control this devastating disease.

## ANTIBACTERIAL ACTIVITIES OF NEEM LEAVE (*Azadirachta indica*) EXTRACTS ON AFRICAN MUD CATFISH *Clarias gariepinus* (Burchell, 1822)

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Large-scale settings of aquaculture have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish. Hot water method was used in extracting the Neem leaves (*Azadirachta indica*) while bacteria (*Aeromonas hydrophila*) was isolated from *Clarias gariepinus*. Twenty post juveniles *Clarias gariepinus* were infected with the isolated bacteria (*A. hydrophila*) and assessed at different concentrations of 25mg/l, 50mg/l, 75mg/l, and 100mg/l to confirm the inhibitory effects of the species and later treated with leave extracts to determine the antibacterial activities. The result shows bactericidal effects of the leave extracts against the *Aeromonas hydrophila*. All the haematological indices i.e. Packed Cell Volume (PCV), Red blood cell count (RBC), White Blood Cell (WBC), Haemoglobin (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) of fishes inoculated with *A. hydrophila* deviated to an abnormal range due to the inhibitory effects of the bacteria on the fish. After treatment with Neem leave extracts, significant differences were noticed at a concentrations of 50mg/l and 75mg/l when compared with the control group as the values rises to normal ranges.

The study was carried out with the purpose of determining the inhibitory activities of *Aeromonas* spp. on post juveniles' stage of catfish, *Clarias gariepinus* and to confirm the antibacterial activities of *Azadirachta indica* leaves extract on *C. gariepinus* infected with the bacteria. The result of the leaves extract on *Clarias gariepinus* post juvenile at the four different concentrations (25, 50, 75, and 100mg) indicates that the leaves extract of *Azadirachta indica* is very active against *Aeromonas* spp on *Clarias gariepinus* post juvenile.

The study reveals that *Aeromonas* spp. is present in the body of *Clarias gariepinus* fish post juveniles and confirmed the inhibitory activities of the bacteria on *Clarias gariepinus*.

The result therefore emphasizes on the usefulness of *Azadirachta indica* leaves extract as an antibacterial agent on *Clarias gariepinus* post juveniles and the needs to enhance its exploitation, because it can be easily located in the different localities and markets across Nigeria and costs lesser than the other antibiotics used which may be scarce in some areas.



Fig. 1: Neem leaf  
(Photos by Dr. A.M Hammed).



Fig. 2: Infected *C. gariepinus*

Table 1: Haematological indices of *Aeromonas* spp. infected adults *Clarias gariepinus*, treated with Neem leaf (*Azadirachta indica*) extracts at different concentrations

Blood indices	Experimental Treatments					Mean
	Ctrlol	A (25mg.l <sup>-1</sup> )	B (50mg.l <sup>-1</sup> )	C (75mg.l <sup>-1</sup> )	D (100mg.l <sup>-1</sup> )	
PCV%	26.9	23(32)	20(37)	25(26)	26(33)	23.5(32)
RBC(10 <sup>12</sup> m <sup>m</sup> )	2.3	1.5(2.75)	1.7(3)	1.9(1.95)	1.8(2.6)	1.725(2.575)
WBC(mm)	7	6000(125)	6000(150)	6100(100)	6300(200)	6100(143.75)
HB(g/dl)	9.6	8.6(11)	8.4(12.3)	8.3(9.3)	8.6(11.4)	8.475(11)
MCHC/100 ml	35.7	34.8(34.4)	35.8(33.2)	35.1(35.8)	36(34.5)	35.425(34.475)
MCH(Pg)	41.7	45.3(40)	44(41)	46(48)	43(43.8)	44.575(43.2)
MCV(F)	116	127(116)	124.5(123)	126(133)	130(127)	126.875(124.75)
Neutrophils %	1.8	3.9(44)	4.8(36)	5.5(36)	3.9(40)	4.525(39)
Lymphocytes%	98.2	95.1(56)	95.8(64)	95.4(64)	96(60)	95.575(61)

Figures in bracket ( ) are results after treatments

## THE CHALLENGES OF USING VISUAL INDICATORS IN MONITORING OF THE BENTHIC IMPACT OF FINFISH AQUACULTURE AT HARD-BOTTOM SITES IN NEWFOUNDLAND (CANADA)

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Finfish aquaculture can be installed over hard and patchy substrates where grab sampling is challenging and use of video can be an appropriate tool to document benthic changes. Video monitoring can show visual indicators of enrichment, namely flocculent matter, bacterial mats, and opportunistic polychaete complexes (OPC). We examined factors influencing presence of indicators using 52 video monitoring reports collected in Newfoundland, Canada.

Visual indicators were present throughout the year on all types of substrates. However, OPC appear to be restricted to depths > 35 m and we observed a tendency for all visual indicators to be less prevalent on hard substrates and at warmer temperatures. In particular, temperature laboratory trials and field measurements confirmed a temperature effect on OPC presence.

The main factor explaining the distribution of indicators is distance to cage (Table 1), with a higher probability of occurrence within 10 m from cages. The low subsurface and bottom current velocities in this region explain the limited distribution of indicators. Of the three indicators, bacterial mats appeared to cover a higher surface and often remained present after one year of fallowing; this period resulted in the reduction of the spatial extent of visual indicators to a surface almost equal to the cage areas. Levels of production explained only a low percentage of the variability of visual indicator presence (Table 1). Higher biomass/wastes might lead to enrichment conditions resulting in the absence of IOE, highlighting the importance of including the number of bare stations in the regulatory framework. The identification of bare stations in baseline surveys (completed at the site licensing application) will allow the proper interpretation of bare stations following aquaculture production. Our results highlight the necessity of amalgamating information related to all the indicators (including bare stations that could indicate anoxia) to establish a more accurate evaluation of aquaculture impact.

Table 1-

Probabilities (P) and proportions effects in the marginal test and using a Forward model on combinations of the 3 visual indicators using DISTLM procedure (N=442).

Predictor	Pseudo-F	P	Prop	
<i>Marginal test</i>				
Distance to cage	94.151	<b>0.0001</b>	17.6%	
Substrate	54.926	<b>0.0001</b>	11.1%	
Median current	34.706	<b>0.0001</b>	7.3%	
Farmed biomass	21.848	<b>0.0001</b>	4.7%	
Depth	11.757	<b>0.0004</b>	2.6%	
<i>Forward</i>				Cumulative prop
<i>DISTLM</i>				
Distance to cage	94.151	<b>0.0001</b>	17.6%	17.6%
Substrate	28.911	<b>0.0001</b>	5.1%	22.7%
Median currents	18.389	<b>0.0001</b>	3.1%	25.8%
Depth	5.460	<b>0.011</b>	0.9%	26.7%

Significant differences are in bold. Prop.: proportions.

## DIAGNOSTIC TECHNIQUES USING FECAL SAMPLES FOR NON-LETHAL SCREENING OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) SHRIMP

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In order to determine the infection level of acute hepatopancreatic necrosis disease (AHPND) in live shrimp, animals need to be sacrificed to get the hepatopancreas, the main organ affected by bacteria. However, most farmers hesitate to victimize valuable animals, especially, in case of broodstock. Here, we describe the diagnostic strategies using fecal samples collected from AHPND infected shrimp, which is useful for non-lethal screening of valuable animals. In this study, AHPND was successfully detected in the tested fecal samples by the PCR assays with either the DNA extraction or the broth enrichment procedures. When comparing the broth enrichment with the DNA extraction, the broth enrichment of the fecal sample is ideal procedure for the PCR, because it shows more sensitive results and even more cost/labor saving technique. Also, these feces samples collected from the AHPND infected shrimp are infectious and able to transfer disease to specific-pathogen free *Penaeus vannamei* shrimp, which is important for the disease control in shrimp farms.

Cumulative mortality, and the AHPND PCR results in the feces from the juvenile shrimp (weights: 0.5-1.0 g) infected with AHPND through immersion in the bioassay 1.

Cumulative mortality, and the AHPND PCR results in the feces from the juvenile shrimp (weights: 0.5-1.0 g) infected with AHPND through immersion in the bioassay 1.

Treatment		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	
6 h immersion (10 <sup>6</sup> cfu/mL) <sup>a</sup>	Tank 1	Cumulative mortality	18/20	20/20	NA <sup>b</sup>	NA	NA	NA
		Feces DNA PCR	++ <sup>c</sup>	++	NA	NA	NA	NA
		Feces broth PCR	++	++	NA	NA	NA	NA
	Tank 2	Cumulative Mortality	19/20	20/20	NA	NA	NA	NA
		Feces PCR	++	++	NA	NA	NA	NA
		Feces broth PCR	++	++	NA	NA	NA	NA
1 h immersion (10 <sup>6</sup> cfu/mL)	Tank 1	Cumulative Mortality	0	0	0	2/20	4/20	3/20
		Feces PCR	+ <sup>d</sup>	++	+	++	++	-
		Feces broth PCR	++	++	++	++	++	++
	Tank 2	Cumulative Mortality	0	0	0	0	0	0
		Feces PCR	+	++	+	++	++	++
		Feces broth PCR	++	++	++	++	++	++
Negative control	Tank 1	Cumulative Mortality	0	0	0	0	0	0
		Feces PCR	- <sup>e</sup>	-	-	-	-	-
		Feces broth PCR	-	-	-	-	-	-

## CAPTURE AND RE-USE OF FISH WASTES FROM IN-POND RACEWAYS

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In pond aquaculture, fish wastes can be a limiting factor in production intensification. If fish manure or any uneaten feed can be removed from the pond environment, then fish production should be able to be increased. This project had several objectives: 1) develop an in-pond raceway system (IPRS) solids capture and removal apparatus and employ it on a floating in-pond raceway system; 2) capture/harvest wastes from catfish grown in the IPRS units; 3) characterize the nutrient “waste” by-product collected; and 4) quantify the amount of dried fish waste collected when guar gum is added to the feed versus when it is not added.

We equipped 8 floating IPRS systems with fish manure harvesting gear and installed solids holding vessels in the pond adjacent to the test IPRS raceway units. Solids removal ports and plumbing were installed and a semi-solids pump was installed for solids collection and removal. This pump lifted the collected water and solids slurry onto truck mounted tanks for transport to the next test arena (settling tanks) where characterization of the nutrient solid “waste” by-product and evaluation of its relative quantity and value with and without guar gum as a binder was conducted. Feed was milled containing the guar gum binder (0.03%) to increase solids capture of fish fecal solids during their culture. There was a control diet (without guar inclusion) as well for comparison. The goal was to determine guar gum effectiveness in consolidating or binding especially small post-digestion feed particles for enhanced efficiency in fish waste/nutrient collection, removal and used in additional value added research, such as its use in plant production and digestion for bio-gas energy production.

Feed trials using guar amended and control (non-guar) 32% protein floating feed were run from August 3 through November 3, 2016. Quantification of the fish waste was conducted through settling tanks and volume measurement followed up with drying a known volume of settled waste and extrapolating that to the total settled waste amount allowed for a total weight of the waste to be calculated. This weight divided by the dry weight of feed fed provided the percent of solids captured. This was done for the regular (control) feed and the guar supplemented feed for comparative evaluation of the guar supplement.

After weekly collection of fish waste ended solids analysis revealed an overall average total solids (TS) content of 38 g/L and an overall volatile solids (VS) content of 32 g/L. Furthermore, after comparing both TS and VS contents for feed with guar added verses feed without guar added, preliminary examination of results indicates that the difference in feed type had no significant impact on solids collected using this particular system. Feeding rate and temperature were shown to have the greatest effect on both TS and VS contents. It was also discovered that due to the nature of the solids collection device (short quiescent zone), actual average solids content of recovered waste may be closer to 10%. This is currently being reevaluated and will be further discussed during the presentation.

## AQUACULTURE EXTENSION FIELD TRIAL EXPERIENCES

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Aquaculture extension field trials are challenging, require much labor, time and money to conduct depending on the complexity and duration. Three extension projects conducted in West Alabama by Alabama Cooperative Extension System – Aquaculture specialists and Auburn University researchers are covered. The three extension projects are: 1) a catfish yield verification project; 2) an in-pond raceway and intensively aerated pond project; and 3) a virulent *Aeromonas hydrophila* field trials experiment.

The goal of the catfish yield verification project was to obtain detailed on-farm production data over three crops in three ponds for three farmers. Data was analyzed to obtain base catfish production data and representative enterprise budgets to better “know” the industry norms and problems. It was funded by a SRAC grant. The three producers agreed to follow some base management procedures but were left to their own decisions that we followed over time. Detailed production data was diligently collected over a four-year period by three researchers. Numerous presentations, several publications and a Master’s thesis resulted from this project. This was a successful project providing much knowledge and gave producers a better understanding of their production and economic outcomes. We learned it was very time consuming and expensive to obtain such good data and we continue to analyze this data.

The goal of the in-pond raceway project was to develop a more controllable production system capable of addressing many of the production problem areas plaguing open pond catfish production in the U.S. It was funded by a number of grants, primarily by USB and Catfish Farmers through a voluntary feed check-off fund. Attempts to generate interest about this new system in West Alabama were difficult due to less than desired research results beforehand. Therefore, research continues on this system in more controlled conditions. In on-farm and at experiment station sites this system routinely generates an FCR of 1.4 to 1.7. In our last experiment, 11,000 to 14,000 pounds of fish were produced in an IPRS located in a one-acre pond. We learned it is difficult to introduce a new concept with different management requirements to farmers who are very busy already. Hopefully, a few farmers will want to pursue this production method because of how it readily resolves many of their production problems.

The goal of the virulent *Aeromonas hydrophila* (vAh) project is to address this disease that is killing millions of pounds of catfish each year in West Alabama. It is being funded by a variety of sources, primarily USDA, Catfish Farmers, AL Department of Agriculture and Industries, and the AL Innovation Fund. A farmer collaborator in West Alabama has allowed us to put three in-pond raceway units into three of his ponds to conduct trials of experimental vaccines, probiotics, hybrids, and phytase treatments to see if they would reduce vAh mortalities compared to control channel catfish treatments. After two years, one vaccine and a probiotic seem to work well but delivery problems are an obstacle. Additionally, regulatory hurdles have been and will have to be met to bring these successful treatments to fruition.

## FINDING THE PROPER PLACE FOR RECIRCULATING AQUACULTURE

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The skewed misperception that recirculating aquaculture plays an outsized role in contributing to global seafood production is rather pervasive among the general public and the environmental NGO community. Many are enamored of the technology and equipment and the idea of a “fish factory” with a high degree of control over production. The environmental NGO community considers the RAS approach to be “eco-friendly” aquaculture. This myopic view is based on the capacity of RAS to address certain environmental impacts associated with shrimp or salmon farming. However, RAS is very energy intensive, resulting in increased carbon footprint per unit production relative to most other production systems.

What are the appropriate roles for recirculating aquaculture? Arguably the best applications of RAS are in hatchery and nursery settings, where control over water quality and the ability to hold fish at high densities are advantageous. Perhaps the best example of this is the now widespread production of salmonid smolts in recirculating systems. Major salmon producing companies are currently making large new investments in recirculating hatchery and smolt operations to support net-pen grow-out operations.

The business success of companies using RAS for grow-out is rather dismal, although investments in RAS technology for grow-out continue. Business success and economic performance of commercial facilities using RAS depends on much more than mastery of the technology. It also depends on a location-specific set of conditions, often linked to being able to obtain a price premium for a high-quality or high-demand product. In many cases, RAS-based operations target specialty or local niche markets because they cannot compete with low-cost commodity producers. Furthermore, isolated and dispersed RAS facilities make it difficult to achieve economies-of-scale with respect to other parts of the value chain, such as feed supply and processing. Regional production from multiple RAS facilities is needed for successful sector development. Initiatives are underway for land-based grow-out of Atlantic salmon in RAS. Analysis of economic performance and environmental footprint suggest that RAS technology is at least competitive and comparable with the net-pen approach.

Another appropriate place for recirculating systems is in so-called “backyard” or hobby aquaculture, including aquaponics. Recirculating aquaculture also has a place in primary and secondary education, where it represents an excellent tool to teach ecology, nutrient cycling, and using waste as a resource.

The community of aquaculture professionals needs to be responsible to investors, environmentalists and the general public in describing the advantages and disadvantages of RAS technology. A balanced and comprehensive perspective about what RAS can and cannot do can foster a realistic outlook and thereby serve to promote further commercial applications of this promising production technology.

## STUDY ON BOTTOM TEMPERATURE CONDITION ASSOCIATED WITH BLOOD COCKLE *Anadara granosa* INDUCE SPAWNING AFFECTED BY THERMAL PLUME DISCHARGE FROM NEARBY THERMAL POWER STATION IN KAPAR, SELANGOR

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Three Acoustic Doppler Current Profiler (ADCP) samplings were conducted at Station KKKL6a (N3.13667, E101.29983) with approximately 1.2Km away from nearby Thermal Power Station (TPS) in Kapar, Selangor. Each sampling covers among three daily major tidal changes known as the Great Diurnal Tide (GT). The sampling were conducted on 6<sup>th</sup> -10<sup>th</sup> May 2016, 19<sup>th</sup> – 26<sup>th</sup> July 2016 and (&) 17<sup>th</sup> – 24<sup>th</sup> August 2016 respectively. Meanwhile a total of 132 sampling stations for temperature (temp) profile i.e., Sea Surface Temp (SST), 1.0m & 3.5m were conducted covering Kapar waters to Sungai Buloh waters. All the data were combined & process for temp profile from surface to the bottom. The aim of this study is to investigate the bottom temp (bt) profile for every water elevation affected by the thermal plume discharge from the nearby TPS. The results (Table 1) suggested that bt trending during 8m water elevation with minimum temp (min) of 29.82°C, maximum temp (max) of 31.31°C & temp difference (t-diff) of 1.49°C respectively. On 7m depth, the min is 29.90°C, max is 31.52°C, & t-diff is 1.62°C correspondingly. While at 6.0m the temp trend is 29.97°C, 31.35°C & 1.38°C respectively. On 5m depth, the result suggest min of 29.89°C, max of 31.41°C & t-diff of 1.52°C. While during 4m depth suggesting min of 29.91°C, max of 31.52°C & t-diff of 1.61°C. The bt start to intermittent (imt) at 3.5m depth proposing min of 29.01°C, max of 31.33°C & t-diff of 2.32°C individually. During 3.0m suggesting min, max and t-diff of 29.15°C, 31.56°C & 1.41°C accordingly. When the tide drop to 1.0m, the bt proposed min, max & t-diff as 29.01°C, 31.84°C & 2.83°C separately. While during the lowest tide (<0.5m), the SST interact with the bt suggesting the high bt sporadic with min, max & t-diff as 28.38°C, 33.64°C and 5.26°C respectively. This physical condition is associated with cockle induce spawning where the bt at KKKL6a is typically at 5-7m depth & t-diff is around 1.52°C - 1.62°C during typical water tides but GT, where it can lowered to 1.5m & some parts of the surrounding areas is exposed. While shallow waters where temp imt is about 2.32°C - 5.26°C. Thus this bt sporadic may rise from 1.52°C to 5.26°C with two interval tide between the lowest tidal during stipulated GT.

Table 1 showing the summary of the bottom temperature profile for every water elevation

Water level (m)	Min temp (°C)	Max temp (°C)	Temp diff (°C)
8.0	29.82	31.31	1.49
7.0	29.9	31.52	1.62
6.0	29.97	31.35	1.38
5.0	29.89	31.41	1.52
4.0	29.91	31.52	1.61
3.5	29.01	31.33	2.32
3.0	29.15	31.56	1.41
1.0	29.01	31.84	2.83
0.5	28.38	33.64	5.26

**NATIONAL VETERINARY ACCREDITATION PROGRAM (NVAP) PRESENTS  
AQUATIC ANIMAL HEALTH MODULES: 15 (“PREVENTING DISEASE INTRODUCTION  
AND SPREAD IN AQUACULTURE”) & 28 (“SIGNIFICANT AND EMERGING VIRAL  
DISEASES OF CARP, KOI AND GOLDFISH”)**

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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 training Modules (<http://tinyurl.com/NVAP-Modules>), four of which specifically cover aquatic animal health regulatory issues. During this conference these modules will be presented:

**Module 15** reviews general biosecurity (disease prevention, control and eradication) for aquatic animal production systems, including biosecurity practices and proper handling of animals and equipment during site visits, and the appropriate use of personal protective equipment for various situations. Attendees will better understand the proper use and dosages of different disinfectants in aquaculture and be able to design an appropriate cleaning and disinfection plan.

**Module 28** (released in early 2017) addresses OIE listed and emerging diseases of carp, koi, and goldfish concern to the aquaculture industry, what is required of producers and accredited veterinarians to investigate, diagnose, respond to, and report, how to meet health requirements for movement, and where to find additional information on carp and goldfish diseases.

\*APHIS Accredited Veterinarians who attend the presentations are eligible for NVAP credit units.



## **H-2B VISA WORKERS AND SEAFOOD PROCESSING INDUSTRIES IN THE U.S.: CURRENT KNOWLEDGE AND FUTURE NEEDS**

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Temporary guest worker programs started in the U.S. with the Bracero program which ended officially in 1965. The H-2B program started as a part of the 1986 Immigration Reform and Control Act. H-2B workers are nonimmigrant seasonal workers coming to the US, working for 10 months a year and up to three years with the same visa. Demand for H-2B workers in the seafood processing industry in the U.S. has been increasing over the last decade. These workers contribute significantly to seafood processing, especially in the crawfish, shrimp, crab, alligator, oysters, and finfish industries. Seafood processing industries have a significant economic impact in the overall economy of Gulf Coast states such as Louisiana, Mississippi, Alabama and Florida. Optimizing the use of H-2B labor in the U.S. seafood processing industry is tied to the nature of production and processing of seafood; processing is labor intensive. With a statutory cap of 66,000 workers per year that number may not meet the demand of the seafood processing industry as other industries such as forestry, hospitality, and produce use this labor. Moreover, a recent policy change advocating an increase in the wage rate also may have an impact on the demand of these workers. It is essential to strengthen understanding and formulate appropriate policy measures on the extent of use of this labor. Particular importance should be given on (i) assessing the impact of wage policy changes in seafood processing; (ii) the preference on labor utilization as domestic vs. H-2B; and (iii) the value and economic impact of this labor along with the full costs of using this labor.

## AQUACULTURE AS A FOLLOWER AND/OR LEADER IN BUILDING AN UNDERSTANDING OF STRESS PHYSIOLOGY IN ANIMALS

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An understanding of the impact of stress on animals has evolved since the initial use of the phrase ‘General Adaptation Syndrome’. Models describing the impact of stress in aquaculture have similarly evolved but the terminology of ‘primary’, ‘secondary’, and ‘tertiary’ responses to stress continues to support the conventional wisdom surrounding fish stress in aquaculture. The paradox of the physiological stress response as being both a necessary adaptation and a source of negative outcomes continues to support integrative research in animal physiology and alter conventional wisdom. Through a review of the literature, a question prompted by this symposium is addressed. “Has investigation into stress physiology in aquaculture species paralleled that of other animal models?”

The search terms ‘Stress Physiology Animals’, ‘Stress Physiology Animals Fishes’ and ‘Stress Physiology Animals Aquaculture’ were used to examine different pools of research. The number of annual citations has increased exponentially over time (Figure 1). Differences in the patterns resulting from the different search phrases may be the result of recent changes to the vernacular used in applied and more general science contexts.

The frequency of terms used in abstracts of a sample of research articles can be used to illustrate differences in how ‘Stress’ is presented by different subsets of scientists over time. As might be expected the glucocorticoids (e.g. cortisol and corticosterone) were common to literature regardless of the search terms used and different biomarkers appear across time (Figure 2). Results of these searches will be discussed in illustrating any paradigm shifts over time or differences among subsets of scientists.

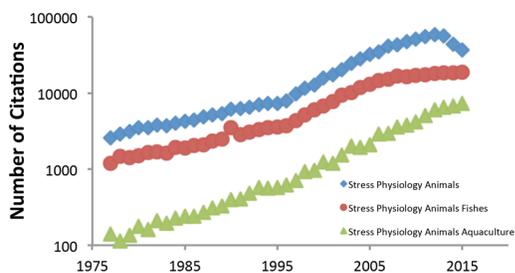


Figure 1. Annual number of citations captured by Google Scholar searches (1977-2015) using search terms shown in the legend. Note that the y-axis is in a log scale.

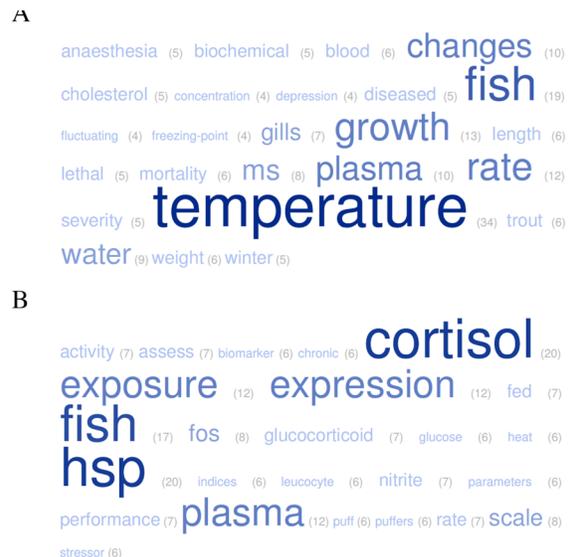


Figure 2. Examples of word clouds generated from abstracts of a random selection of research articles captured by Google Scholar for the year 1977 (A) and 2015 (B) using the search terms ‘Stress Physiology Animals Aquaculture’.

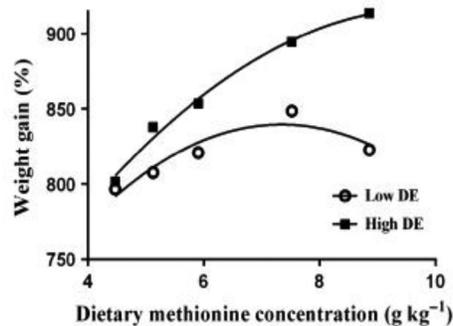
## THE EFFECT OF DIETARY METHIONINE CONCENTRATIONS ON GROWTH PERFORMANCE OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*) FED DIETS WITH TWO DIFFERENT DIGESTIBLE ENERGY LEVELS

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A growth trial was conducted to examine the effect of dietary digestible energy (DE) content on methionine (Met) utilization and requirement in juvenile Nile tilapia (*Oreochromis niloticus*). Ten iso-nitrogenous (288 g kg<sup>-1</sup> protein) practical diets, with two DE levels (10.9 MJ kg<sup>-1</sup>; 12.4 MJ kg<sup>-1</sup>) and five methionine supplementation levels (0, 1, 2, 4 and 6 g kg<sup>-1</sup>), were hand-fed twice daily to triplicate groups of Nile tilapia (initial body weight 8.95 ± 0.06 g) for 8 weeks.

Weight gain (WG) and specific growth rate (SGR) increased significantly with increasing dietary methionine concentration at the same DE content ( $P < 0.001$ ). At the same dietary methionine level, WG and SGR of fish fed high-DE diets were significantly higher than that of fish fed low-DE diets ( $P = 0.0001$ ), although no interaction was found between dietary DE and methionine supplementation. Based on quadratic regression analysis between dietary methionine concentration and weight gain, optimal methionine requirement for maximum growth, expressed as g Met required kg<sup>-1</sup> diet (low- versus high-DE diets), increased as diet DE concentration increased (7.34 versus 9.90 g kg<sup>-1</sup> diet, respectively; with cysteine 4.70 g kg<sup>-1</sup> diet). The results indicated that diet DE content affects methionine utilization and requirement in juvenile Nile tilapia, fish fed high-DE diets required more methionine for maximum growth.



**Figure 1** Weight gain (WG) responses to dietary methionine concentrations (g kg<sup>-1</sup>) at low (10.9 MJ kg<sup>-1</sup> DE) and high (12.4 MJ kg<sup>-1</sup> DE) energy levels using quadratic regression analysis for 8 weeks. The equation that best described the response is follows:  $Y = 527 + 85.2X - 5.81X^2$  ( $R^2 = 0.88, P < 0.0001$ ) at low DE level;  $Y = 546 + 75.1X - 3.79X^2$  ( $R^2 = 0.99, P < 0.0001$ ) at high DE level. Estimates of methionine requirements (g kg<sup>-1</sup> diet, low versus high diet DE diets) were: 7.34 versus 9.90, respectively.

## EFFECT OF METHYLENE BLUE ON DEVELOPING ZEBRAFISH EMBRYOS *Danio rerio*

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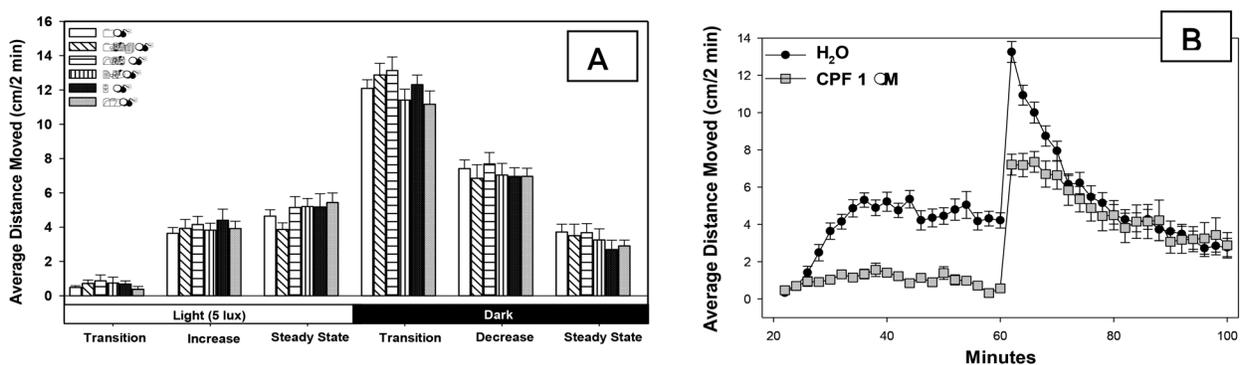
Our laboratory routinely conducts zebrafish (*Danio rerio*) developmental studies on 100s of chemicals, it is therefore imperative that the chemicals we use for animal husbandry are safe. It was brought to our attention that methylene blue, an antifungal agent commonly used in zebrafish embryo rearing, has been reported to affect swim bladder inflation (angelfish), increased fetal death (rats and humans), and produce hyperbilirubinemia, hemolytic anemia, and intestinal atresia (humans). Therefore, we conducted a developmental toxicity and neurotoxicity assessment of methylene blue in zebrafish using concentrations commonly employed by zebrafish researchers.

A 6 day developmental/neurodevelopmental assay was conducted using zebrafish embryos/larvae. At 0 days post fertilization (dpf), methylene blue exposure began in 96 well plates, which were kept in a 26°C incubator on a 14:10 (light:dark) light cycle. Embryos were dosed once daily on 0-4 dpf with 0.6, 1.6, 5.0 or 10.0  $\mu\text{M}$  methylene blue (MEB); 1  $\mu\text{M}$  chlorpyrifos (CPF) was used as a positive control (n=24 per chemical per dose). At 5 dpf embryos were removed from the chemical, and placed in water, and then tested on 6 dpf.

Behavioral testing consisted of assessing the locomotor activity of individual larval zebrafish in a 96-well plate, under a light:dark test paradigm: 20 minute acclimation in the dark, followed by 40 minutes of light (5 lux) and then 40 minutes of dark. Embryos were examined daily on 0-5 dpf and after locomotor testing on 6 dpf for death, hatching, swim bladder inflation, and morphological appearance.

Although the CPF treated larvae showed the expected decrement in locomotor activity (Figure B), no differences were seen between MEB exposed and control larval locomotor activity (Figure A) or any of the developmental aspects (death, hatching rate, swim bladder inflation or dysmorphology). These data indicate that MEB is not developmentally or neurodevelopmentally toxic to larval zebrafish at commonly used concentrations. *This abstract does not necessarily reflect U.S. EPA policy.*

Figure 1: Locomotor behavior in zebrafish after 5 days of methylene blue (A) or chlorpyrifos (B) exposure.



## CORRELATION OF LIPID AND PROTEIN OXIDATION ON COMMON CARP *Cyprinus carpio* FILLET DURING THE STORAGE TIME

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Fish has a high content of polyunsaturated fatty acids (PUFA) and contains also prooxidants such as ferrous iron and is subsequently very prone to oxidation during prolonged storage. Unpleasant off-flavors and formation of volatiles can develop due to oxidation of PUFA. In addition, several investigations have revealed that protein oxidation leads to loss of protein functionality. On the other hand there is an interaction between the primary and secondary products of lipid oxidation with proteins during the storage time. This interaction may additionally affect the functional properties, reduce water holding capacity, odour and change the texture of fish fillet.

Fillets of freshly slaughtered 80 common carp (2.1Kg in weight), were packed into plastic bags, labelled and frozen at  $-20^{\circ}\text{C}$ . Analysis were carried out at the start of the trial and after 1, 2, 3, 4, 8, 16, 24 weeks on six fillets at each time point in order to monitor the development of oxidation. We evaluated peroxide value (PV), level of thiobarbituric acid reacting substances (TBARS) as markers for lipid oxidation and carbonyl concentration as a marker for protein oxidation.

TBARS (Table 1) increased until the 4<sup>th</sup> week and decreased again after that, indicating a further reaction to tertiary oxidation products. However, TBARS results may not show the factual rate of lipid oxidation, because of malonaldehyde (MAD) interaction with proteins and amines. The PV (Table 1) increased constantly during the whole storage time. The increasing of PV and TBARS revealed development of rancidity in the fillet during frozen storage. Carbonyl values (Table 1) were slightly increasing but did not change significantly during the whole storage period, indicating that protein oxidation occurs much slower than lipid oxidation. Protein and lipid oxidation had the same pattern; protein carbonyls and lipid hydroperoxides development were similar and it appears that the reactions between proteins and oxidized lipids occur simultaneously and are linked. Further investigation is needed in order to evaluate the possible reaction products of the lipid oxidation and to link the development of sensory and texture properties to the observed changes due to oxidation.

TABLE 1. Changes in TBARS, PV and Formation of protein carbonyl groups values of samples during 6 months frozen storage at  $-18^{\circ}\text{C}$

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 8	Week 16	Week 24
MAD ( $\mu\text{g/g}$ )	0.03 $\pm$ 0.00 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	0.09 $\pm$ 0.03 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.04 $\pm$ 0.00 <sup>a</sup>
PV (meq/Kg)	1.57 $\pm$ 0.01 <sup>a</sup>	1.58 $\pm$ 0.01 <sup>ab</sup>	1.61 $\pm$ 0.005 <sup>bc</sup>	1.64 $\pm$ 0.01 <sup>cd</sup>	1.67 $\pm$ 0.01 <sup>d</sup>	1.91 $\pm$ 0.02 <sup>e</sup>	1.88 $\pm$ 0.01 <sup>e</sup>	2.12 $\pm$ 0.04 <sup>f</sup>
Carbonyls (nmol/mg)	2.71 $\pm$ 1.23	2.72 $\pm$ 0.06	2.95 $\pm$ 0.15	2.46 $\pm$ 0.20	2.82 $\pm$ 0.16	3.00 $\pm$ 0.52	3.01 $\pm$ 0.55	3.13 $\pm$ 0.44

Different small letters show significant differences in difference time points ( $P \leq 0.05$ )

## *Apocyclops panamensis* – DEVELOPING A PROMISING COPEPOD FOR AQUACULTURE

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Copepods are often considered to be nearly ideal live feeds for many larval fishes because they can provide nutrition superior to rotifers, and the early-stage nauplii of some species are smaller than even the smallest rotifer strains currently in use. Nevertheless, the use of copepods in larviculture has been limited because they are significantly more difficult to culture than rotifers. Common difficulties include limited culture densities, requirements for live algae as feed, and failure of cultures when they are invaded by ciliate protozoans.

The cyclopoid genus *Apocyclops* has not received as much attention from aquaculturists as other copepods such as *Acartia* and *Parvocalanus*. But *Apocyclops* includes several species (e.g., *A. borneoensis*, *A. dengizicus*, *A. distans*, *A. panamensis*, and *A. royi*) that have been cultured. In general, all these *Apocyclops* spp. were capable of rapid growth and development, often demonstrated over a range of salinities. They are rather omnivorous, capable of feeding on a variety of different microalgae and even yeast (*A. dengizicus*), but performing much better on higher-quality microalgae that are good sources of HUFAs, such as *Isochrysis* and *Tetraselmis*.

The best-studied species are *A. dengizicus* and *A. royi*. *A. dengizicus* has N1 nauplii 75 µm wide and 120 µm long, tolerates a wide salinity range, and develops from egg to adult in only 7.4 days at 35 °C, with a population doubling time as short as 2.2 days. Fecundity can be as great as 647 nauplii/female. *A. royi* is widely used for aquaculture in Taiwan. Its N1 nauplii are 110 µm long, it is also euryhaline, and different isolates may have different temperature optima. It is especially noteworthy that *A. royi* has been shown to prey upon and effectively suppress two troublesome invaders of copepod cultures, *Brachionus* rotifers and the ciliate *Euplotes*.

Although *Apocyclops panamensis* has been shown in pond culture to be good first feed for Red Snapper, there is little published information its basic biology, such as optimal salinity, temperature, feeds, growth rates, reproductive capacity, and other life-cycle parameters. At Reed Mariculture we have very encouraging results with production trials. *A. panamensis* has small N1 nauplii measuring 70 x 100 µm, and it is very hardy, tolerating a wide range of salinity and temperature. It thrives on commercial algae concentrates, so no live algae are needed, and ciliates are never detectable in cultures. We are now further developing the potential of *A. panamensis* by selective breeding with the goal of reducing the size of N1 nauplii and increasing its tolerance of high culture densities. The efficacy of selective breeding has been shown in other copepods, in which as few as 5 generations of selective breeding can yield significant changes in body size, fecundity, and resistance to algal toxins. Domestication of rotifer strains and use of concentrated feeds has made it possible to increase culture densities over 1000-fold. The future of aquaculture will depend on such development of domesticated strains of aquatic species that can thrive on commercially-available feeds in intensive culture systems.

## NUTRIENT COMPOSITION OF DIFFERENT LUPIN SPECIES AS INGREDIENTS FOR AQUACULTURE FEEDS IN COMPARISON TO SOYBEAN

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The interest of using lupins as an alternative to conventional plant protein sources in aquaculture diets has been increasing in recent decades, especially in Australia, Chile and some European countries. Lupin seed proteins stand out among other legumes because of their unique chemical composition, low levels of non-nutritional components and bioactivity potential. The aim of the present study was to analyze the nutritional composition of eight different varieties from four main lupin species, namely *Lupinus albus* (Rumbo, Ramses), *L. luteus* (AluPro-CGNA A, AluPro-CGNA B), *L. angustifolius* (Lila, Granotrade) and *L. mutabilis* (Pinta, Baer) distributed and/or produced in the Araucanía region of Chile, in order to compare them with soybean (*Glycine max*) and further explore their suitability for incorporation in fish diets.

The highest protein content (60 %, dry basis) was found in seeds from lupins belonging to *L. luteus*, while *L. mutabilis* varieties contained 52 % protein and approximately 20 % as oil, an amount similar to that found in *G. max* (40 % protein and 20 % lipids). In this sense, *L. mutabilis* can be considered as the strongest potential competitor of soybean since it possess both the nutritional advantage of providing high proportions of protein as well as oil. The lowest contents of protein and lipids were found in all varieties of *L. angustifolius* (40 and 10 % respectively). In terms of phytic phosphorus, the higher concentrations were detected in all varieties of *L. mutabilis*, *L. luteus*, *L. albus* and *G. max* (0.24-0.38 %) and the lowest amounts were observed in all varieties of *L. angustifolius* (0.11-0.15 %). Based on these results, the possibility of using different lupin varieties as a protein source in aquafeeds is discussed. Taking into consideration that nutritional evaluation of alternative ingredients for aquaculture should be based not only on the concentration of components further research is required to elucidate the relative bioaccessibility and bioavailability of nutrients from these lupins in carnivorous cultured fish species.

## GENOME-WIDE ASSOCIATION STUDY OF SOYBEAN MEAL TOLERANCE IN ATLANTIC SALMON

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The utilization of Fish Meal (FM) as a protein source in the Aquaculture industry has been shown not to be sustainable. Soybean meal (SBM) stands as viable replacement for fishmeal in aquaculture because of the relatively high protein content, amino acid profile and digestibility, as well as its global availability and low cost. However, SBM inclusion in salmon feed is hindered by poor performance and negative physiological impacts (e.g. enteritis). Studies in trout have shown that there is significant individual and family variation in the ability to use SBM derived protein in salmonids, and that this trait has moderately high heritability. Therefore, there is potential for the selection of lineages with higher tolerance to SBM inclusion. Marker assisted selection, (MAS) using genetic markers such as single nucleotide polymorphisms (SNPs) associated with traits of interest, can be used to compliment traditional breeding and reduce the time required to achieve genetic gains.

Fin samples from families presenting poor and good growth when fed a high SBM diet were selected and DNA was extracted from fins. Genotyping-by-Sequencing (GBS) was used to obtain genotypes for a total of 291 animals. In total, over 90,000 putative markers were identified, and 46,333 SNPs were selected for GWAS analysis (MAFs > 0.01 and rate of genotyping > 85%). Missing data was imputed using the LDkNNimp algorithm was implemented in the TASSEL v5 pipeline. Association analysis was performed using a weighted mixed linear model with kinship (measured by centered IBS) and population structure (measured by PCA). The thermal coefficient growth (TGC) data was fitted to the model and the effects of each SNPs estimated one by one using both a dominant and an additive model. The Bonferroni approach was used to control for family-wise error (FWER) (i.e. the obtained p-value was divided by the number of tests performed –  $0.05/46,333$  – p-value cutoff of  $1.08 \times 10^{-6}$ ).

Using this approach, seven SNPs (in 5 different chromosomes) were identified as being significantly correlated with TGC after FWER correction. These markers explain had an average R-squared of 0.09 and explain roughly 9% of the estimated heritability of TGC of Atlantic salmon when fed diets containing high levels of soybean meal. The markers, if validates, can be added to breeding programs and facilitate higher inclusions of soybean meal in commercial diets for Atlantic salmon.

## CHARACTERIZATION OF I $\kappa$ B POST-MODIFICATION DURING NODAVIRUS INFECTION IN ORANGE-SPOTTED GROUPER *Epinephelus coioides*

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Orange-spotted grouper is one of the important aquaculture fish in Asia, but the industry faces serious losses of groupers dying of nervous necrosis virus (NNV) infection. NNV infection may induce the post-translational modification of inhibitor of kappa B and then activate the transcription factor, NF- $\kappa$ B, which can transfer into nucleus to bind to the DNA sequence, and regulate pro-inflammatory cytokine gene, like TNF $\alpha$ , to modulate immune responses and defense pathogens.

In this study, we isolated the full-length cDNA of the orange-spotted grouper p65 and I $\kappa$ B $\alpha$  named *osgp65* and *osgI $\kappa$ B $\alpha$* . Both gene and protein levels of *osgp65*, *osgp50* and *osgI $\kappa$ B* followed by the NNV copy numbers are elevated, which is most significant in brain tissue compared with healthy groupers. Moreover, we transfected GF-1 cell with *osgp65* and observed that *osgp65* translocated into nucleus in NNV infection. To investigate the function and activity of *osgp65* to TNF $\alpha$  promoter, we cotransfected *osgp65* and *TNF $\alpha$  promoter-Luc* or *TNF $\alpha$  promoter  $\Delta$ NF $\kappa$ B-Luc* into GF-1 cell, the over-expression of *osgp65* stimulated NF- $\kappa$ B-dependent reporter ( $\kappa$ B-luc) activity but deletion mutant. In addition, we also infected with NNV and discovered that NNV activated *TNF $\alpha$  promoter* more than *TNF $\alpha$  promoter  $\Delta$ NF $\kappa$ B-Luc*. Together, these results describe that *osgp65* and *osgI $\kappa$ B $\alpha$*  are evolutionarily conserved in orange-spotted grouper with mammals and play important roles in regulating TNF $\alpha$  promoter in NNV infection. These may provide clues to the detailed molecular mechanism underlying immune response regulation in groupers.

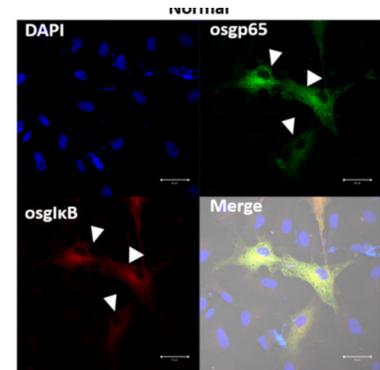


Figure 1. The distribution of *osgp65* and *osgI $\kappa$ B $\alpha$*  in GF-1 cells.

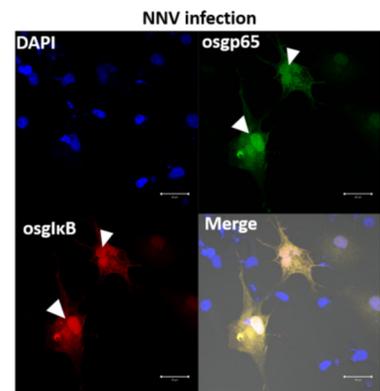


Figure 2. The distribution of *osgp65* and *osgI $\kappa$ B $\alpha$*  in GF-1 cells under NNV infection.

## IMPROVED SOYBEAN MEAL (EnzoMeal) ALTERNATIVE OF FISHMEAL FOR SUSTAINABLE PRODUCTION OF PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

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Marine shrimp production has expanded by the nearly 15% growth per year over the last decade, representing almost two-fold the global aquaculture growth observed for the same period. Within the crustacean group, the Pacific white shrimp (*Litopenaeus vannamei*) is the most popular cultured shrimp globally. The great majority of its production relies on high protein feeds typically containing around 20-30% fishmeal (FM), which leads to high production costs directly affecting the profitability and sustainability of farmed shrimp. To fulfill the high demand of shrimp feed, recently, Ohio Soybean Council and its research partners Kentucky State University and Battelle, Memorial Institute (Columbus, Ohio) have developed a new technology to reduce the anti-nutritional factors and enhance the protein content from conventional soybean meal without compromising the amino acid composition. It is called improved soybean meal (ISBM) or EnzoMeal. EnzoMeal removes many antinutritional factors, such as trypsin inhibitors, lectin and oligosaccharides (100% removed), phytic acid (78% decreased) and also decreases total carbohydrate content (31%) and enhances the protein content (22%) without compromising the amino acid composition.

The overall aim of the present work was to increase the usage of ISBM in feed for commercial shrimp production. Shrimp juveniles (2400; average weight 3.1g) were randomly distributed and stocked into twelve large tanks (1000 L each) in a closed recirculating system. Shrimp were fed four iso-nitrogenous (38% crude protein) diets for 12 weeks. A **control diet** (30% fishmeal) and three experimental diets wherein 33, 66 and 100% fishmeal protein replaced by ISBM (**ISBM<sub>33</sub>**, **ISBM<sub>66</sub>** and **ISBM<sub>100</sub>** respectively).

Results from this study reveals similar ( $P \geq 0.05$ ) growth performance, nutrient utilization parameters and survival rate of EnzoMeal-fed groups compared to fishmeal-fed group. Final body mass gain, survival rate, feed conversion ratio and protein efficiency ratio were in the range from 588 – 688%, 82 – 88%, 1.74 – 1.82 and 1.24 – 1.36 respectively. EnzoMeal inclusion in shrimp diets did not influence ( $P \geq 0.05$ ) the total omega-3 and omega-6 fatty acids, EPA, DHA and cholesterol content in tail meat of shrimp. Physiological parameters including albumin, globulin, total protein, creatinine, glucose concentration and glutathione peroxidase activity in hemolymph of shrimp did not differ significantly ( $P \geq 0.05$ ) among the groups. Overall, this study suggests that there is potential for total replacement of fishmeal with an alternative, sustainable, plant protein such as improved soybean meal (EnzoMeal) for aquafeed.

## BUGS & MORE BUGS: USING PROBIOTICS AND THE FISH GUT MICROBIOME TO PRODUCE HEALTHY FISH FOR HUMAN CONSUMPTION

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An urgent need exists in aquaculture of domestic seafood to develop microbial control strategies without jeopardizing productivity or encouraging the development of antibiotic resistance. An alternative to antimicrobials in disease control and production enhancement may be probiotics. Probiotics are viable cell preparations that have beneficial effects on the health of a host, by improving its intestinal balance via improved feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activities, growth-promoting factors, and an increased immune response. In contrast to terrestrial animals, gut microbiota of aquatic species are dependent on food, as well as water flowing through the intestine. The majority of gut bacteria can be transient and change as a function of feed type, or food/water quality.

Over five years we have investigated the effects of different types of probiotics (single strain vs multiple strain) on the growth, metabolism and gut health in juvenile stages of the freshwater, Mozambique Tilapia (*Oreochromis mossambicus*) and the marine fish, Red Drum (*Sciaenops ocellatus*). For tilapia, a single strain of probiotic was used *Lactobacillus rhamnosus* IMC 501 in a dose response experiment; 0, 5, 10, 15, & 20 x 10<sup>11</sup>CFU/g fishmeal. Results for growth showed the greatest (33.9%) increase at 10 x 10<sup>11</sup>CFU/g fishmeal compared to control over the 28-day experiment. Results for metabolic rates (mgO<sub>2</sub> l<sup>-1</sup>g<sup>-1</sup>h<sup>-1</sup>) showed an increase in metabolic rate with increasing probiotic concentrations. This may have been due to the changing composition of the gut microflora with time. QPCR and deep sequencing microbial techniques are being carried out to determine if growth and metabolic efficiency are governed by changes in gut microfloral complexity. Work on red drum has shown similar increases in growth rate (18%) in juveniles grown using a multi-strain probiotic (See poster by Busby & Hunt von Herbing.)

## SELECTION OF MICROALGAE CULTURES BASED ON AMINO ACID PROFILES AND PROTEIN CONTENT TO BE USED AS FISH MEAL REPLACEMENTS IN AQUACULTURE DIETS

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As world supplies of fish meal dwindle and prices of fish meal rise, microalgae has been proposed as a replacement for fish meal and oil in aquaculture diets. Various fish and crustacean species have been shown to successfully digest and grow with differing percentage fish meal replacements with microalgae. This presentation will focus on the basic course of action we employ in identifying microalgal species that can be utilized to create a favorable fish meal replacement with good amino acid profiles and percent protein in the aquaculture industry.

Amino acid and protein components of *Nannochloropsis salina* and *Phaeodactylum tricornutum* from outdoor trials will be presented. As a comparison, three incubator trials utilizing five different species of microalgae (*Tetraselmis* sp., *Platymonas* sp., *Cylindrotheca* sp., *Cyclotella meneghiniana*, and *Thalassiosira* sp.) were conducted to attempt to identify possible microalgal species that could be utilized in conjunction with *N. salina* and *P. tricornutum* to create a more suitable amino acid profile for the aquaculture feed industry.

Three incubator trials (*Tetraselmis* sp., *Platymonas* sp., *Cylindrotheca* sp., *Cyclotella meneghiniana*, and *Thalassiosira* sp.) were ran under controlled conditions in a constantly illuminated and temperature controlled (22°C) incubator. Cultures were supplied with 24hr. atmospheric aeration at ~0.2 L/min. Two different nutrient formulations were used for growth: f/2 (Bigelow Laboratories) and our traditional ammonium sulfate and phosphoric acid based nutrients with matched molar levels to f/2. Absorbance growth curves (750nm) were built based on AFDW measurements for each individual species.

Flasks (1L) were inoculated to ~0.04 g/L AFDW based on absorbance using chlorinated and DE filtered natural SW salinity adjusted to ~30ppt. After initial nutrient supplementation, each flask was placed into incubator randomly. Trial duration was 10 days to allow for complete nutrient utilization. Each flask was sampled daily for absorbance and nutrient levels.

Results will be presented showing the amino acid profiles and protein content of the five tested strains of microalgae. This presentation will also discuss the possibility of using the selected species in mixed culture systems to enhance the overall viability of using microalgae as a fish meal replacement in aquaculture diets.

## **AQUAFISH RESEARCH INFORMS POLICY TO LIFT CAMBODIAN SNAKEHEAD FARMING BAN THROUGH SUSTAINABLE AQUACULTURE TECHNOLOGIES**

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Fisheries of the Lower Mekong Basin provide the means to create food security and nutrition for 60 million people in Cambodia and Vietnam. While aquaculture of snakehead (*Channa striata*) in Vietnam has been gaining in popularity because of its high market value, snakehead farming was banned in neighboring Cambodia in 2005 in an effort to alleviate pressure on wild populations of small, low-value, freshwater fish that were harvested for snakehead feed. Unsustainable snakehead seed collection practices also were of concern.

AquaFish Innovation Lab researchers — at the Inland Fisheries Research and Development Institute in Cambodia; Can Tho University in Vietnam; and the University of Connecticut–Avery Point, University of Rhode Island, and Oregon State University, all in the US — have been working in the region since the ban to develop technologies and strategies to create a sustainable snakehead aquaculture program, including successful hatchery technology and formulation of commercial feeds that reduce fish content without decreasing growth performance and marketability.

Research conducted in Vietnam first developed a pelleted snakehead feed that contained 40% plant protein, a far more sustainable ingredient than that of typical fish meal. Once the feed was successfully implemented in Vietnam, AquaFish researchers compared the weaning and grow-out performance of wild, indigenous snakehead in Cambodia to that of domesticated snakehead from Vietnamese hatcheries on formulated or pelleted diets. The Vietnamese hatchery fish showed a higher growth rate than that of wild Cambodian snakehead.

This AquaFish-supported research played a substantial role in lifting the snakehead farming ban in Cambodia in 2016 by informing the design and implementation of a successful and sustainable snakehead aquaculture program. Cambodia's Fisheries Administration sought information from AquaFish researchers regarding their investigations of snakehead domestication and breeding, weaning, and grow-out. A key tipping point in lifting the ban, besides the need to increase income opportunities for farmers, was the development of processed fish feeds, which AquaFish researchers helped to develop.

This success in helping to end the ban not only opens the door for improved economic impacts and opportunities for Cambodians but also aims to alleviate the environmental impacts of overfishing.

## **ARTIFICIAL BREEDING AND HYBRIDIZATION OF AFRICAN MUDFISH *Clarias gariepinus* AND *Heterobranchus longifilis* UNDER INDOOR RECIRCULATORY HATCHERY CONDITION**

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Hybridization studies carried out on the offspring of two genetic crosses derived from two *Clariid* catfishes; *Clarias gariepinus* and *Heterobranchus longifilis*. At the hatchery of Ibrahim Badamasi Babangida University, Lapai. In this present study breeding of *Clarias gariepinus* and *Heterobranchus longifilis* was carried out, specimens of *Clarias gariepinus* female and *Heterobranchus longifilis* male fishes were obtained from Federal College of Fisheries, New Bussa, Niger State. The brood stock were acclimatized for one week in plastic container and fed with Vita feed. The female fish sample was injected with Ovaprim hormone (0.5ml), and allowed to stay for a latency period of 12hours after which the eggs were stripped into a dry sterile plastic bowl. The male was sacrificed through dissection and the testes were removed from the lobes into a dry petri dish, little physiological saline solution was added, the milt obtained from the male was then poured on the collected eggs from the female, and mixed with a dry clean feather for fertilization to take place. After fertilization, the growth (Length and weight) were taken weekly. The comparative growth and survival at the end of the ten (10) weeks indoor rearing showed that interspecific pure breeding of *C. gariepinus* male and female had the best survival. The comparison of weight, weight gain and specific growth rate showed that the intergeneric cross of *C. gariepinus* female and *H. longifilis* male were more outstanding with a specific growth rate ( SGR), when compared to the other at the end of 10 weeks growth studies.

## **ATLAS OF FISHERIES, FISH CRAFT AND FISHING GEARS OF SOME SELECTED WATER BODIES IN NORTHERN NIGERIA**

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Survey of fishing gears, fishing crafts and fishes of Kontagora Reservoir, Bakolori Reservoir and River Yauri were carried out between April 2016 and July 2016. Catch and frame assessment survey was used for this study. Gillnet, cast net, hook and line were commonly used as gears. Giwa and clap nets were peculiar to River Yauri, Malian trap (gura) to Kontagora Reservoir and River Yauri. Apart from plank canoe used in the three water bodies, motorized canoe, gourd and ferry were also used in River Yauri. Traditional fishing gears and crafts are still in use in these water bodies. Twenty-six fish species belonging to thirteen families were recorded overall with highest diversity from River Yauri (13) followed by Bakolori Reservoir (10) and the lowest from Kontagora (8). Similarly, River Yauri recorded the highest family (11) followed by Bakolori (9), while the least was Kontagora Reservoir (8). It is recommended that more study need to be done to ascertain if there are other gears and crafts in used and possibly been extended to other water bodies within the northern part of the country.

## **LENGTH-WEIGHT RELATIONSHIP OF *Hepsetus odoe* IN LAPAI-AGAIE RESERVOIR, NIGER STATE, NIGERIA**

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Study on the Length - Weight Relationship (LWR) of *Hepsetus odoe* in Lapai - Agaie Reservoir, Niger State, Nigeria was carried out between April 2016 and August 2016. One hundred and twenty samples, which comprised of 70 males and 80 females were used for this study. These were collected from fishermen that use gillnets. Females were bigger than males though did not show any significant difference ( $P < 0.05$ ). Length - weight ( $b$ ) values showed that males ( $b = 2.6$ ), females ( $b = 2.7$ ) and pooled ( $b = 2.7$ ) exhibited negative allometric growth pattern hence, length and weight did not grow at the same proportion. There was positive correlation ( $r$ ) between length and weight of males ( $r = 0.74$ ), females ( $r = 0.78$ ) and pooled ( $r = 0.85$ ). It is recommended that similar study should be done for dry season, other aspects of the biology of the fish should be studied and water quality assessment through determination of physico - chemical parameters to know the pollution status of the reservoir should also be carried out. This would go a long way to provide better management for *Hepsetus odoe* and other fish species in the reservoir.

## **WATER QUALITY ASSESSMENT OF SELECTED BOREHOLES IN DABBAN COMMUNITY, NIGER STATE, NIGERIA**

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Study on the water quality assessment of five selected boreholes in Dabban Community, Niger State, Nigeria was carried out from April, 2016 to July, 2016. Turbidity (2.30 NTU) recorded was below WHO limit but greater than that of FEPA (0-1NTU) for domestic use. Dissolved oxygen (8.43mg/l) was greater than 6mg/l recommendation for drinking purpose. Temperature (30.26°C) was greater than WHO recommendation (25°C). Chloride ion (27.02mg/l) was below WHO permissible limit but greater than FEPA limit (2.5mg/l). Water from the boreholes were classified as soft water (<0.75mg/l) with corrosive properties. Chloride ion was significantly different ( $P < 0.05$ ) in the boreholes, BH-1(35.15mg/l) and BH-4 (22.75mg/l) though fall within WHO limit but higher than that of FEPA (2.5mg/l). There were fluctuations in monthly water quality parameters.  $DO_2$ , total alkalinity, total hardness and conductivity decreased across the months. The water from these boreholes could be said to have traces of pollution. It is recommended that these parameters be assessed in dry season to check for likely changes, regular monitoring of water quality parameters of these boreholes is encouraged, and also microbial analysis of the water should be carried out.

**NUTRITIONAL QUALITY OF SMOKED *Clarias gariepinus* IN LAPAI, NIGER STATE, NIGERIA**

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This study examined the nutritional quality of smoked *Clarias gariepinus* in three different markets in Lapai, Niger State, Nigeria within a duration of three month. Proximate compositions of smoked *Clarias gariepinus* were determined. The percentage of moisture, Ash, Protein, Fiber, and Fat contents were determined using appropriate methods. The highest protein content ( $49.27 \pm 2.59$ ) was recorded in smoked fish obtained from Baddegi market and was not significant ( $P < 0.05$ ), while the least ( $46.68 \pm 0.52$ ) recorded from Central market also shows no significant differences ( $P < 0.05$ ). Similar results were obtained for the Fat, these differences in proximate composition of smoked fish in Lapai markets were not significantly ( $P < 0.05$ ). The ash content of smoked fish in Efugwaja, Central and Baddegi markets showed significant differences ( $p > 0.05$ ) and these were also exhibited within the months. Sensory evaluation of smoked fish revealed that the taste was significantly different ( $P > 0.05$ ) in all the three markets, while that of colour, texture and appearance were not significant ( $P < 0.05$ ). There was a significant different ( $P > 0.05$ ) in two notable minerals Potassium and Sodium at Baddegi and Efugwaja markets. This result indicates that, the nutritional qualities of smoked *Clarias gariepinus* in the three sampled Lapai markets are not significantly different.

## EXPLORING CLIMATE-RESILIENT ADAPTATIONS OF FARMED FISH FOR CLIMATE-SMART AQUACULTURE

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Exploring climate-resilient adaptations in farmed fish for climate-smart aquaculture is one strategy for helping to improve food security for the world's growing population under changing environmental conditions. People worldwide rely on fish as a primary source of protein and income, supporting a rapidly growing aquaculture industry that provides roughly half of the global fish supply. In an era of global climate change and high demand for animal protein, increasing the production of fish through sustainable and environmentally sensitive practices is critical. The development of climate-smart aquaculture can provide responsible management strategies to the aquaculture industry. One aspect of this effort involves optimizing the culture of fish species that are adaptable with characteristics such as a tolerance for hyposaline conditions, wide temperature ranges, and the ability to breathe air. Incorporating the culture of air-breathing species like the *Pangasius* catfish into climate-smart aquaculture not only provides the potential to grow local economies, it also addresses some of the concerns about environmental threats and takes advantage of the evolutionary ecology of these species in their natural environments. However, it is also necessary to fully understand the positive and negative tradeoffs associated with increasing fish production to ensure that practices remain environmentally and socially responsible.

## HISTOPATHOLOGICAL EFFECTS OF PARAQUAT DICHLORIDE ON THE KIDNEY, LIVER, GILLS AND TISSUES OF TILAPIA *Oreochromis niloticus* FINGERLINGS

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Paraquat, a non-selecting organic herbicide is used to control weeds in areas where labour is in short supply and finds its ways to aquatic systems through run-offs or its usage in integrated fish culture systems. It is quick acting and care must be taken during application. This study investigates the effects of varying concentration of the herbicide on the water quality parameters and on the kidney, gills, liver and tissue of *O. niloticus* fingerlings.

Fingerlings of average length and weight of 4.2cm and 0.4g were gotten from the Departmental fish farm and stocked randomly at 2L<sup>-1</sup> in 43 x 20cm plastic aquaria. They were acclimatized for a period of two weeks and fed twice daily at 3% body weight with 40% CP diets with feeding stopped 24 hours before commencement of bioassay. The fish were exposed to varying concentrations of herbicide at 0mgL<sup>-1</sup>(control), 3.33mgL<sup>-1</sup>, 6.66mgL<sup>-1</sup>, 10mgL<sup>-1</sup> and 13.3mgL<sup>-1</sup> (table 1). The Lethal concentration (LC<sub>50</sub>) after 96 hrs exposure was 4.80mg/L. The mean daily water quality parameters analysed were temperature 26 ± 0.12°C; Dissolved oxygen 6.2 ± 0.16 mg/L, pH 6.8 ± 0.11, Conductivity 176 ± 0.18 us/cm, COD 24.5 ± 0.19 mg/L and Alkalinity 33.1 ± 0.17 mg/L. Analysis of Variance observed significant differences (P<0.05) between Dissolved oxygen, COD and pH between the various concentrations and significant differences (P<0.05) in Conductivity, COD and Alkalinity between the concentrations and the control.

The Histopathological results of the exposed fish showed erosions, lesion, necrosis, malignancy, pigment and inclusion bodies in the gills, periportal hepatic degeneration, necrosis and periportal connective tissue was very prominent in the liver, severe diffuse tubular necrosis in the kidney and no visible lesions in the tissues. Varying the concentration of the toxicant resulted to respiratory stress, sudden quick movements and erratic swimming. Mortality was identified by absence of movement in fish and it increased with increase in concentration of herbicide (table 1). Dead fish were removed immediately.

Paraquat dichloride is toxic to *O. niloticus* and its usage on farms should be in the recommended dosage most especially in integrated fish culture systems.

Table 1: Mortality and Probits of concentration of Paraquat dichloride

Mortality (%)	Probits Units	-Probability	Concentration (mg/l)	Log of concentration
0		-	0	-
10		3.72	3.33	1.20
90		6.28	6.67	1.90
100		7.37	10.00	2.30
100		7.37	13.30	2.59

## NANOMETRIC NEEM OIL EMULSIFICATION THROUGH HIGH-PRESSURE HOMOGENIZATION AND ITS THERAPEUTIC POTENTIAL AGAINST AQUACULTURE PATHOGENS

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Increase in the mortality rate due to the disease outbreaks in this aquaculture sector leads to hindrance in the economic development of a nation. Several strategies has been developed to control the infections caused by the pathogenic microbes such as the use of antibiotics. The excessive application of these antibiotics against the pathogens have resulted in the development of resistance among the pathogens. These problems in the aquaculture sector urge for a better antimicrobial compound with more efficacy against the pathogens also with less eco-toxic property. An alternative biocontrol method that helps in the prevention of pathogenic infection is through application of nanoemulsion which is formulated using an essential oil. The bioactive compounds present in essential oils exhibits low toxicity towards the environment and have high potent inhibitory role against pathogenic bacterial and fungal pathogens. Among several strategies carried out for nano-emulsification process, high-pressure homogenization is one of the new and efficient methods. The present study emphasises on the neem oil nanoemulsion formulation that can act as an alternative to control the pathogenic infection in aquaculture using high-pressure homogenization. The effect of different concentrations of surfactant (tween 20, tween 80), the applied pressure and the number of cycles on the droplet size of neem oil nanoemulsion was investigated. The optimized pressure and cycle for the neem oil nanoemulsion formulation was found to be 20,000 psi at 25th cycle. A significant decrease in the particle size of neem oil nanoemulsion was observed as the pressure, cycle and concentration of surfactant increase. The stability studies of neem oil nanoemulsion formulated using both the surfactants were investigated based on the physicochemical parameters like turbidity, pH, different water matrices (sea water, lake water), and temperature. In all the physicochemical stability studies, the neem oil nanoemulsion prepared using the tween 80 (ratio 1:3) showed a good physicochemical stability with a droplet size of  $20.1 \pm 1.98$  compared to all other formulated ratios. The concentration of the active ingredient, azadirachtin was checked using reverse phase high-performance chromatography and LC/MS technique, which was found to be  $13.54 \pm 1.23$  mg/L and  $21.187$   $\mu$ g/mL respectively. The stable ratio of 1:3 prepared using neem oil and tween 80, showed an efficient antibacterial activity against *Aeromonas culicicola*, *Vibrio alginolyticus* and *Pseudomonas aeuroginosa*, which was confirmed by MIC study. The SEM analysis and cell membrane integrity test confirmed the damage caused to the bacterial cells by the formulated nanoemulsion. Further, the *In-vivo* study of neem oil nanoemulsion on *Penaeus monodon* and *Cyprinus carpio* confirmed its antibacterial potential against *Aeromonas culicicola*, *Vibrio alginolyticus* and *Pseudomonas aeuroginosa*.

Sl.no	Different Pathogens	MIC value of Neem oil nanoemulsion
1.	<i>Aeromonas culicicola</i>	468.75 mg/L
2.	<i>Vibrio alginolyticus</i>	234.3 mg/L
3.	<i>Pseudomonas aeuroginosa</i>	117.18 mg/L

Table 1: Antibacterial activity of neem oil nanoemulsion was analysed using MIC

## MOLECULAR AND FUNCTIONAL INSIGHTS INTO DUPLICATED GOOSE-TYPE LYSOZYMES FROM *Sebastes schlegelii*

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Lysozymes are bactericidal enzymes, ubiquitously distributed from bacteria to human. The major role of the lysozymes is to catalyze the hydrolysis of the  $\beta$ -1,4-glycosidic bond between 2 alternative sugar residues (N-acetylmuramic acid and N-acetylglucosamine) in peptidoglycan layer of bacterial cell wall, thereby causing bacterial cell lysis. In addition, lysozymes play an important digestive role in some animals. Lysozymes of the animal kingdom have been classified into 3 classes: chicken-type (LysC), goose-type (LysG) and invertebrate-type (LysI). LysG was first identified in egg whites of Embden goose and subsequently in other vertebrates. In this study, two isoforms of *LysG* were identified from black rockfish transcriptome database and designated as *RfLysG1* and *RfLysG2*. The cDNA sequences of *RfLysG1* and *RfLysG2*, were of 1514 bp and 900 bp, respectively. Open reading frame (ORF) of *RfGLys1* comprised of 567 bp encoding 188 amino acids with a molecular mass of 20.11 kDa, while ORF of *RfGLys2* consisted of 600 bp encoding 199 amino acids of 22.19 kDa. Isoelectric points were 6.82 and 6.83 respectively. Both sequences possess a soluble lytic trans-glycosylase domain. No signal peptides were detected in both. Predicted three-dimensional structures share the similar structure with other LysGs. Homology studies indicated that the RfLysG1 shown highest identity (84.6%) with LysG-B of *Oplegnathus fasciatus* while RfLysG2 shown highest identity (74.4%) with LysG of *Siniperca chuatsi*. Multiple sequence alignment revealed that both LysGs are evolutionarily conserved. Transcriptional analysis of both genes shown constitutive expression with the highest level in blood under normal physiological conditions. Immune challenges: lipopolysaccharide (LPS), *Streptococcus iniae* and poly I:C injections significantly upregulated the expression of *RfLysG1* and *RfLysG2* in blood and spleen tissues in a time-dependent manner. In comparison, the level of induction was high in both tissues upon LPS than other stimuli. Both Maltose-binding protein (MBP) tagged recombinant proteins exhibit potent bacteriolytic activity against several Gram-positive and -negative bacteria, where no activity was observed with MBP. Optimum temperatures for the recombinant RfLysG1 and RfLysG2 were 40 °C and 50 °C, respectively. Both were highly active at pH 3.0. Results from the present study suggest the vital role of RfLysG1 and RfLysG2 against microbial invasion.

## **EFFECTS OF AERATION ON WATER QUALITY IN WASTE-TREATMENT SECTIONS OF SPLIT-POND AQUACULTURE, A THREE YEAR SYNOPSIS**

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Split ponds have a fish cell and a waste cell, approximately 20% water surface area and 80% water surface area respectively, in which water recirculates to improve water quality as a more intensive management system than traditional ponds. This is a continuation of a study that focuses on the possible benefits of using mechanical aeration in the waste-treatment section of the split-pond culture system.

Work was conducted on a commercial catfish farm in west Alabama. The farm currently has eight split-ponds, each with a fish-holding section of about 8,000 m<sup>2</sup>. Two, 10-hp floating, electric paddlewheel aerators were placed in the waste treatment section of each of four ponds; while four ponds – the controls – had un-aerated waste treatment cells. Water samples were collected biweekly at the inflow and outflow of the waste-treatment cells; once the water became cooler in the fall and winter, the samples were collected monthly. Analyses that were gathered include: pH, dissolved oxygen (DO), temperature, secchi disk visibility, Chlorophyll *a*, total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, total nitrogen, soluble and insoluble nitrogen, total phosphorus, soluble reactive phosphorus, chemical oxygen demand (total and soluble), biological oxygen demand, acidification, water circulation, aeration hours, and sediment carbon/nitrogen concentration. Year 1 (2014) produced no significant results. Year 2 (2015) produced differences between control and aerated ponds for secchi disk visibility, total ammonia nitrogen, total nitrogen, chemical oxygen demand (soluble and total) and DO. Year 3 (2016) will conclude in October. Data for all three years will be presented and interpreted.

## WATER QUALITY AMONG DIFFERENT FEED MANAGEMENT TREATMENTS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Feed management applications can dictate the water quality within a pond. Increasing feeding rates provide an increase in production, thus nutrients such as nitrogen, phosphorus, and organic matter will also increase, respectively. These nutrients promote a greater oxygen demand and concentrations of toxic metabolites which can lead to frequent problems with low dissolved oxygen and an abundance of blue-green algae. Four feed management techniques were used among sixteen ponds culturing Pacific White Shrimp (*Litopenaeus vannamei*) at Gulf Shores, Alabama. The different feeding strategies included two treatments with hand feeding using the Standard Feeding Protocol (SFP) which is calculated with a predicted growth of 1.3 g/wk, and an estimated feed conversion ratio (FCR) of 1.2, assuming a survival rate of 75% over 16-wk culture period, and SFP plus 15% from 8-16 weeks. Two automatic feeder treatments included a solar timer feeding which fed SFP divided into 6 feedings per day and AQ1 acoustic demand feeding allowing up to 12 kg/day/pond based on shrimps feeding response.

These management practices were evaluated by a series of water quality analyses over a 16-week grow-out season; with feeding treatments starting at week 4. Samples were analyzed at weeks 0, 4, and 8-16 for the following parameters: chlorophyll *a*, total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, total nitrogen, total phosphorus, soluble reactive phosphorus, total suspended solids, total suspended volatile solids, turbidity, conductivity, salinity, and biological oxygen demand. Samples were collected and shipped overnight to Auburn, Alabama for off-site analysis. On-site water quality was also obtained at the farm. This included weekly levels of total ammonia nitrogen (probe), and daily measurements of temperature, dissolved oxygen, pH, feed inputs as well as final production. No differences were found between treatments as of week 10 (Table 1). However, characterization of water quality over the 16-week production cycle and possible correlations with production and performance will be discussed.

**Table 1. Example average values for water quality parameters among different feed management treatments for Pacific white shrimp up to 10 weeks.**

Parameter	Hand Feeding	Hand Feeding (+15%)	Solar Timed Feeder	AQ1 Acoustic Demand Feeder
Turbidity (NTU)	10.20	7.31	8.84	9.34
Secchi Disk, on-site	62.00	72.26	62.25	72.75
Conductivity (mS/cm)	19.71	19.28	19.85	19.63
Chlorophyll <i>a</i> ( $\mu\text{g/L}$ )	240.315	282.76	162.840	206.466
Total Nitrogen (mg/L)	3.166	2.941	2.762	2.942
Total Phosphorus (mg/L)	0.672	0.598	0.564	0.743

## DETERMINATION OF CHLORAMPHENICOL AND TWO METABOLITES IN CRAB BY LC-MS/MS

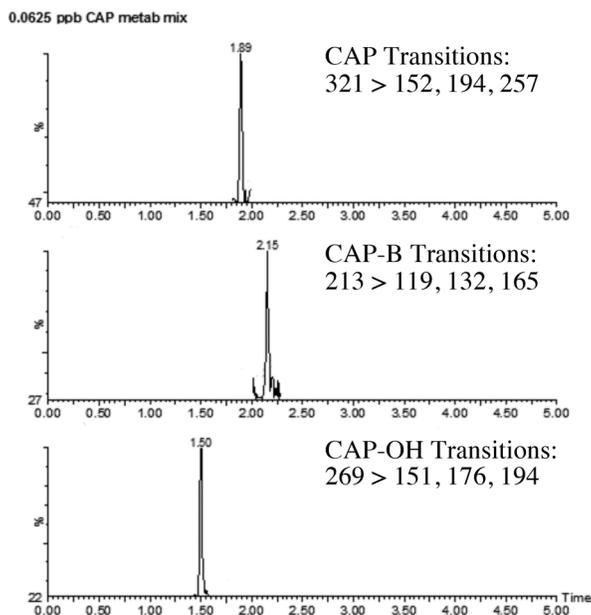
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Chloramphenicol (CAP) is a highly effective, broad-spectrum antibiotic, with a history of usage in food animals, including aquaculture species. Its use has been associated with numerous toxic effects in humans, including bone marrow depression which can cause potentially fatal aplastic anemia. Due to safety issues, CAP is banned from food animal use in the United States (U.S.), and many other countries. The presence of CAP residues in aquaculture products is a major concern for the U.S. Food and Drug Administration, particularly with increases in imports, since it continues to be used for food animal disease treatment in some countries.

CAP is rapidly eliminated following exposures in animals. Depletion and metabolism studies of CAP in seafood have been limited. Currently, validated confirmatory analytical methods are available for monitoring CAP, but not its biomarkers. For the purpose of identifying an appropriate marker residue for CAP exposure, we exposed crab to CAP, and identified the metabolites CAP-base (CAP-B) and CAP-alcohol (CAP-OH) as viable biomarkers.

We present a liquid chromatography tandem mass spectrometric (LC-MS/MS) method for CAP, CAP-B, and CAP-OH in crab muscle. A single LC-MS/MS polarity switching, ESI ( $\pm$ ) method for simultaneous analysis of CAP, CAP-B, and CAP-OH has been developed on a Waters Xevo TQ MS. Separation of analytes was achieved in reverse phase mode on a Phenomenex Kinetex 1.7  $\mu$ m PFP column (50 x 2.1 mm) in 5 min. Retention times for CAP-OH, CAP, and CAP-B are 1.5, 1.9, and 2.2 min, respectively. External standard calibration curves (0.125 to 4 ng/mL) were prepared from CAP, CAP-B, and CAP-OH reference material, with obtained  $R^2$  values of 0.9989, 0.9999, and 0.9999, respectively. Interday repeatability of CAP, CAP-B, and CAP-OH standards ( $n = 7$ ) ranged from 1 to 18%, with average repeatability of 12, 4, and 7%, respectively.



## PROXIMATE COMPOSITION AND TRACE METALS CONCENTRATION OF *Macrobrachium vollenhovenii* (HERKLOTS, 1857) FROM BADAGRY CREEK, LAGOS NIGERIA

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Shellfishes have long been recognized as a valuable source of high quality nutrients in the human diet and also play significant role in income generation, employment creation and foreign exchange earnings of the country. Prawns and shrimps are known to consume sand and mud along with detritus during feeding, and the sediment act as sink for trace metals in the aquatic ecosystems. Hence, these metals can be taken up by these shell-fishes during feeding. These metals are essential for growth and development. The African river prawn, *Macrobrachium vollenhovenii* (Herklots, 1857), found in rivers, creeks and lagoons, and with a spread across the West African sub-region is one of the largest species of *Macrobrachium* known. Consequent upon its nutritional importance and wide spread, this study was carried out to assess the proximate composition and trace metals concentration of this prawn, in order to report the nutrient composition of this prawn from the public health point of view.

One hundred and sixty (160) samples of *Macrobrachium vollenhovenii* and water samples were collected over four months, July - August (rainy season) and February - March (dry season) from Badagry creek. The prawns were washed in flowing water to remove adhering dirt. Total length and body weight were recorded to the nearest 0.1cm and 0.01g respectively. Samples were oven dried at a temperature of 80°C for 72 hours to constant weight and milled separately to powder. The samples were analysed in triplicates. Temperature and pH were determined *in situ* while dissolved oxygen, salinity, total alkalinity and total hardness were determined by titration. The moisture, fat, protein, ash, carbohydrate and crude fibre contents were estimated. Trace metals concentrations were determined using the nitric acid digestion technique. Levels of metals (Fe, Mg and Zn) were expressed in mg/g dry weight. Data were analyzed using SPSS 20 statistical programme at five per cent level of significance. Analysis between seasons was done using the T-test method.

Length and weight of the prawns varied from 15.0cm-17.6cm and 72.76g-130.65g respectively. The mean values for protein, lipids, ash, crude fibre, moisture and carbohydrate were relatively high. Protein, ash, moisture and lipids were significantly different ( $P < 0.05$ ) between seasons. The Zn and Fe contents between the seasons were not significantly different ( $P > 0.05$ ), while Mg was significantly different ( $P < 0.05$ ). The values of these metals are within the recommendations set by the Food and Agriculture Organization and World Health Organization. Consequently, from the nutritional point of view, *Macrobrachium vollenhovenii* can be used as an alternative source of high quality protein, energy and mineral supply for human consumption and even animal feed formulation.



Fig. 1: *Macrobrachium vollenhovenii*

Table 1: Proximate composition of *Macrobrachium vollenhovenii* from Badagry Creek, Lagos, Nigeria

Parameters	Mean (Rainy Season)	Mean(Dry Season)
Protein (%)	52.18±1.11 <sup>a</sup>	43±1.13 <sup>a</sup>
Moisture (%)	4.08±0.44 <sup>a</sup>	31.25±5.23 <sup>b</sup>
Ash (%)	23.39±1.06 <sup>a</sup>	12±1.11 <sup>b</sup>
Lipids (%)	9.65±0.65 <sup>a</sup>	3.5±0.55 <sup>b</sup>
Carbohydrate (%)	1.58±0.07 <sup>a</sup>	1.35±0.17 <sup>a</sup>
Crude Fibre (%)	9.15±0.42 <sup>a</sup>	9.35±6.08 <sup>a</sup>

Values with same superscript in the row are not significantly different ( $p < 0.05$ ).

Table 2: Trace metals concentration of *Macrobrachium vollenhovenii* from Badagry Creek, Lagos, Nigeria

Metals	Rainy Season	Dry Season
Zinc(Zn) (mg/100g)	5.10±0.64 <sup>a</sup>	5.16±0.27 <sup>a</sup>
Magnesium(Mg) (mg/100g)	20±1.02 <sup>a</sup>	18±1.10 <sup>b</sup>
Iron(Fe) (mg/100g)	3.03±0.40 <sup>a</sup>	2.59±0.39 <sup>a</sup>

Values with same superscript in the row are not significantly different ( $p < 0.05$ ).

## TRANSCRIPTOMIC STUDIES OF LIPID METABOLISM IN DIFFERENT INTESTINAL SECTION OF SALMON FRY FED VEGETABLE OIL

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The aim of this study was to understand the transcriptomic development of lipid metabolism in salmon fry fed fish oil (FO) or vegetable oil (VO). RNA-seq was done on stomach (SM), pyloric caeca (PC) and hindgut (HG) of 0.16g salmon (before first feeding), and of 2.5g and 10g salmon fed FO or VO diets. We compared expression of duplicated homolog genes involved in biosynthetic pathways of several lipid classes such as phospholipid (PL), fatty acids (FA) and cholesterol (CH) between diets, tissues and developmental stages.

Gene duplicates can have very different expression levels between diets, tissues and developmental stages. The diverged expression of *pcyt1* duplicates was a representative example (Figure 1). The *pcyt1ab\_1* and *pcyt1ab\_2* duplicates were expressed higher than the two others in SM, while *pcyt1bb\_1* was the highest expressed duplicate in MG and HG. Dietary inclusion of VO reduced the expression of *pcyt1bb\_1*, while other duplicates were not influenced as much. Like *pcyt1* duplicates, we found in most cases one or two gene duplicates had much higher expression than others in each tissue, suggesting these genes to be the key regulators in the pathways. Moreover, the key regulatory genes in PC were mostly expressed higher than the regulators in SM and HG, proving the former to be more important for lipid biosynthesis.

By analyzing the key regulatory genes, we found up-regulation of biosynthetic pathways of phosphatidylcholine, phosphatidylethanolamine, cholesterol and fatty acids during development, while the biosynthesis of other lipid classes was not changed. CH biosynthetic pathway was the most significantly (FDR < 0.05) changed pathway influenced by diet. Almost all regulatory genes in CM biosynthetic pathway were significantly (FDR < 0.05) up-regulated in PC of VO-fed salmon. In relation to lipid transport, lipoprotein formation pathway was also up-regulated in VO-fed salmon. Similar changes of lipid pathways were also identified in HG, though the overall expression levels were much lower in the tissue. The lipid metabolic pathways in SM were mostly unchanged between diets and development.

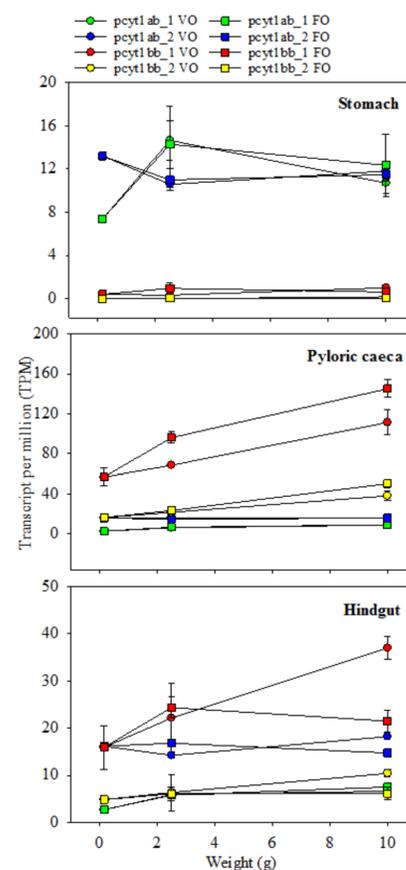


Figure 1: *pcyt1* gene duplicates expression

## POND ALGAE CONTROL BY USING FIXED FILM BIOREACTOR

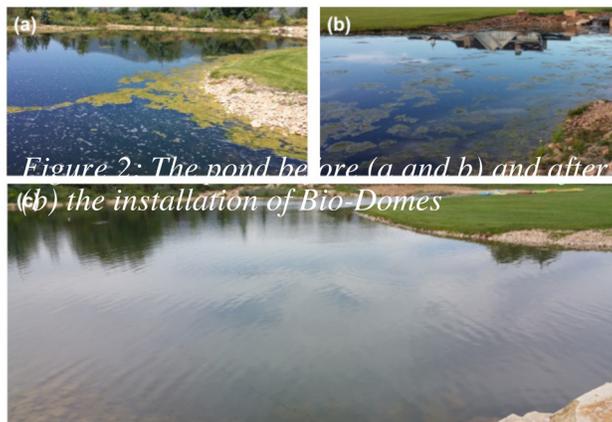
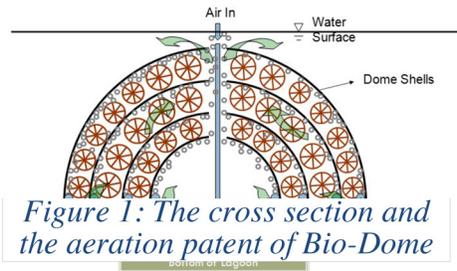
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When the pond contains adequate nutrients, warm temperature, and enough light, algae will grow. Algal blooms can result in anaerobic conditions at night. Once the dissolved oxygen concentration drops to a low level, aquatic organisms such as fish become oxygen starved and die. The water with algal blooms will become unsuitable for other animals and recreational activities. So it is necessary to remove algae to maintain a healthy pond. Biological nutrients reduction is the most reliable long-term algae management. An overabundance of nutrients (nitrogen and phosphorus) from agriculture and urban runoff, dead and decaying plant material, and fish waste are the food for algae. Nitrogen is of particular concern, and different forms of nitrogen must be controlled to prevent algae growth. The biological control usually costly and take many years for significant improvement in water quality, so it is usually not the first option to control algae.

Recently, a system contains numbers of dome-shaped aerated submerged fixed film bioreactors (with the trademark of Bio-Domes, Figure 1) achieved a significant algae reduction in a one-acre recirculating pond (Heber, UT) without the massive capital and O&M expenses due to its unique design. In 2015 summer, the recirculating pond had a serious algae problem (Figure 2a and b). The owner tried different methods (include a bacteria injector) to control the algae, but they all did not work. Within a few weeks of placing the Bio-Domes in the pond, the algae had greatly subsided (Figure 2c). Lab results indicated that the conventions of all nitrogen species are almost completely eliminated. After one year of installation, a sustainable ecosystem had developed, and the algae situation was under control in that pond. This summer, the owner is very happy because the kids are back in the pond because the water is maintained clear and healthy.

The Bio-Dome system can reduce algae for the following reasons: 1) the airlift action in the Bio-Dome can circulate the algae into areas of less efficient sun whereupon the algae dies off and become the organic source for the bacteria inside the Bio-Dome. 2) The fixed-film bacteria in the Bio-Dome out-compete with the algae for nutrients, which supply for cell growth. (3) The mixing action of Bio-Dome prevents the single cellular algae from maintaining optimal equilibrium in the water column where it can reproduce rapidly.



## DIQUAT AND COLUMNARIS DISEASE NEW ANIMAL DRUG RESEARCH AND STATUS

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Use of Diquat dibromide (REWARD ® Herbicide; Syngenta) to control mortality of fish due to *Flavobacterium sp.* infections was first reported by Bullock et al. (1990) and has been available for investigational purposes under the U. S. Fish and Wildlife Service (FWS) Investigation New Animal Drug (INAD) exemption since the early 2000's. Because Diquat does not have an active sponsor, there has been limited research toward a U. S. Food and Drug Administration approval. Recently, the FWS Aquatic Animal Drug Approval Partnership (AADAP) Program developed a drug research plan for Diquat including trying to find a sponsor to lead the approval effort for this drug. In 2016, researchers with the Iowa Department of Natural Resources and AADAP conducted a trial at Rathbun Fish Culture Research Facility to contribute to the Diquat drug approval.

Walleye fingerlings (116 mm, 12.1 g) held in a 1457 L raceway displayed skin lesions consistent with infection of *F. columnare* bacteria. Low fish mortality indicated the disease was in the initial stage of infection. Fish were transferred into eight test tanks (93.9 L; 85 fish/tank) and tanks were randomly designated as treated or control (N=4). Treated tanks received 18 mg/L Diquat dibromide for two hour static bath treatment on three consecutive days whereas control tanks received a static bath sham water treatment for the same duration and frequency. Fish mortality was documented for 14 d posttreatment, and at the end of the study, mean percent cumulative mortality in treated tanks (6.6%) was significantly different ( $P=0.0376$ ) than that in control tanks (38.8%).

## **WALLEYE PRODUCTION CHALLENGES AND SOLUTIONS FOR SPORT FISH AND FOOD FISH**

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Walleye culture for sport fish enhancement is well established in US and Canada with 1 billion stocked annually and almost all walleye are stocked as newly hatched fry with relatively few advanced size fingerlings produced. The vast majority of governmental agencies that stock walleye use wild broodstock sources spawned that spawn in early spring, as a domestic broodstock does not exist. Cultural technology for a completely closed lifecycle on pelleted diets has been developed but has rarely been practiced by agency or commercial aquaculturists. In contrast, commercial food fish production of the European Pikeperch in Europe has developed regardless of that species requirement for live feeds during larval stages. This presentation will describe the cultural “standard” methods as well as research needs for walleye culture from broodstock to advanced or food size production such as out-of-season spawning, larvaculture, diets, disease, and recirculation technologies for both sport and food fish.

## **FACTS ABOUT SHRIMP AND CHOLESTEROL**

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Many people, including health-conscious consumers, are concerned about the cholesterol content of foods such as meat, eggs and dairy products.

It has long been promoted by many in the health community that shrimp is bad for your health due to the high level of cholesterol. This has been challenged through a number of excellent scientific studies but unfortunately the myths continue to be raised.

Our presentation will put the evidence on display and we will highlight why in the case of shrimp, the cholesterol story is different.

Many people are denying themselves the enjoyment and nourishment that a 'feed of shrimp' can give and we will emphasize the need to promote this important information.

## EFFECTS OF DIETARY PREBIOTIC AND PROBIOTIC SUPPLEMENTATION ON GROWTH PERFORMANCE AND DISEASE RESISTANCE OF HYBRID STRIPED BASS *Morone chrysops* X *Morone saxatilis* IN PONDS

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Prebiotics and probiotics have been shown to have a number of positive effects on various aquatic species based on laboratory studies. However, more research is needed to evaluate both additives under conditions similar to commercial production. Therefore, a feeding trial over a full production cycle was conducted with hybrid striped bass to investigate commercially available prebiotic and probiotic supplements on growth performance, immunological responses and disease resistance under conditions simulating commercial culture. Juvenile hybrid striped bass averaging 76.7 g were stocked into 12, 0.04-ha replicate ponds in May of 2015. Each of four diets supplemented with either 0 or 2% prebiotic (GroBiotic®-A, International Ingredient Corporation) and 0 or 10<sup>7</sup> CFU/g probiotic (Aquablend, BIO-CAT Microbials) in a 2x2 factorial arrangement was fed to three replicate ponds. An intermediate sampling (4 months of feeding) and a terminal sampling (11 months of feeding) were conducted to measure production characteristics, histological features of the gastrointestinal (GI) tract, intestinal microbiota and non-specific immune parameters. In addition, 40 fish per treatment were challenged by handling stress under high heat (~32 C) conditions after which mortality was recorded for 7 days.

Results to date showed: (1) no significant differences in growth performance (average final fish weight was 429.1 g); (2) the prebiotic showed a stronger effect on immunological parameters such as intracellular and extracellular superoxide production ( $p < 0.01$ ) (Fig. 1) compared to the probiotic; (3) fish fed the diet with prebiotic alone and both additives had significantly ( $p < 0.001$ ) higher survival compare to the basal group after the handling stress challenge (Fig. 2). Analysis of this experiment is ongoing and more results will be evaluated such as histology and microbiota composition of the GI tract. The goal is to have a clearer understanding of how these prebiotic and probiotic supplements perform under conditions similar to commercial production.

Figure 1. Extracellular Superoxide Anion Production of Phagocytes

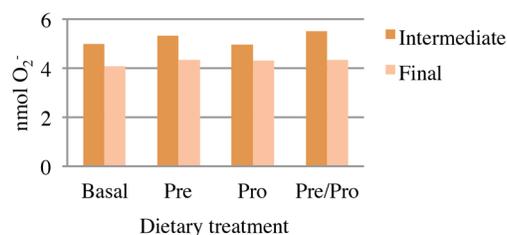
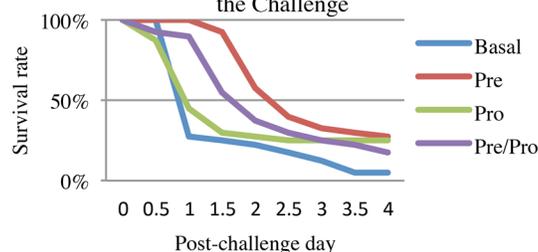


Figure 2. Kaplan-Meier Survival Analysis of the Challenge



## ASSESSMENT OF *Flavobacterium columnare* FROM GOLDEN SHINERS *Notemingus crysolucas* SUBJECT TO CROWDING STRESS

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Intensive aquaculture practices and exposure to environmental stressors can trigger outbreaks of *Flavobacterium columnare*, a bacterial pathogen that causes columnaris disease in commercially important fish including Golden Shiners. A rapid assessment of the bacterial load is essential to prevent outbreaks and subsequent economic losses. Classical methods using bacterial plates and Polymerase Chain Reaction (PCR) is time consuming and requires sample preparation.

In this study we present a technique to enumerate *F. columnare* using flow-cytometry. Two 5-d trials were conducted at 22 and 28° C to determine the effect of temperature and crowding stress on the survival of Golden Shiners infected with *F. columnare*. Both trials consisted of four treatments: (1) low density (600 fish/m<sup>3</sup>) and no bacterial challenge (control), (2) low density and bacterial challenge, (3) high density (2400 fish/m<sup>3</sup>) and no bacterial challenge, and (4) high density and no bacterial challenge. Golden Shiners, weighing 2.62 (±0.78) g each, were stocked in 40, Ultra Low Flow (ULF, after Mitchell and Farmer 2010) tanks containing 10 L of water. The tanks were inoculated with a strain of *F. columnare*, MS94-081. The study was considered complete 48 h post bacterial challenge. Mortality was recorded. Bacterial pellets were formed by centrifuging 50 mL of tank water and the resulting pellets were resuspended in 2 mL of the supernatant. An antibody labelled with fluorescein dye (2µL) was mixed with 100 µL of the concentrated tank water to be analyzed by flow cytometry. The bacterial counts were validated by qPCR. Results from the study showed significantly high mortality in fish exposed to bacterial challenge and fish held at 28°C. No significant differences were found between the high density and low density treatments. Bacterial counts from water samples obtained by flow cytometry and qPCR showed significantly higher numbers of *F. columnare* in tanks exposed to bacterial challenge. The counts did not vary in the density or temperature treatments. Bacterial counts using flow cytometry is a rapid and accurate for assessing bacterial load in a water body.

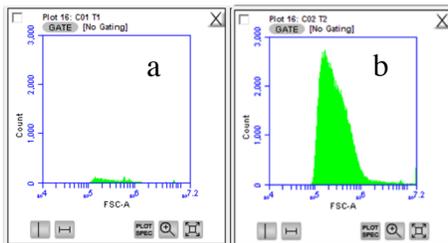


Figure 1. Cytogram showing *F. columnare* counts from a) tank receiving no challenge b) tank receiving challenge.

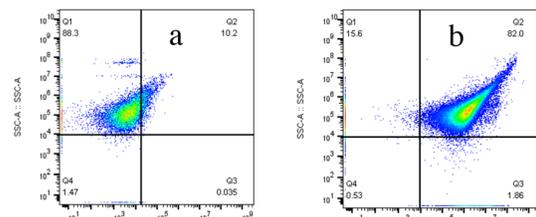


Figure 2. Cytogram showing *F. columnare* sample a) unstained b) stained with fluorescein labelled antibody.

**SPAWNING OF PIGFISH *Orthopristis chrysoptera* FOR THE MARINE BAITFISH INDUSTRY**

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Unlike the well established production of freshwater baitfish, commercial scale marine baitfish culture in the US is still very much in its infancy as an industry. In addition to providing a consistent supply of high quality product to consumers, marine baitfish culture would also reduce pressure on wild stocks of these particular species. Currently, the only live baitfish available in coastal areas is natural production harvested from bays and inshore areas. Captive year-round spawning could provide anglers with live bait outside the natural season of availability.

Several marine species have been identified as viable candidates for culture, one of which is the pigfish *Orthopristis chrysoptera*. During the last five years, our researchers have collected wild broodstock, cultured F1 broodstock, conducted larval and juvenile grow-out studies, and observed volitional spawning of pigfish both during and outside the regular season induced by photoperiod and temperature manipulations. A summary of the observations and data generated during that time will be the topic of the presentation.

## ECONOMIC EVALUATION OF ORAL ESC VACCINATION

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Enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri*, is the most problematic bacterial disease affecting U.S. catfish aquaculture. Prior commercial ESC vaccines were largely unsuccessful due to difficulties in delivering the vaccine to immunocompetent fish and lack of vaccine potency. However, oral vaccination of catfish fingerlings using attenuated live ESC vaccine developed at Mississippi State University through successive passage of virulent *E. ictaluri* isolate (S97-773) on media containing increasing concentrations of rifamycin showed promise under both research and commercial settings over the years.

Research and commercial trials demonstrated that channel catfish can be successfully immunized against *E. ictaluri* with a single oral dose of live attenuated, *E. ictaluri* vaccine. Vaccination significantly improved survival, growth, feed fed, feed conversion, and yield resulting in lower cost of production of fingerlings. Economic analysis found the benefits of vaccination offsetting the cost of administration. With increased demand for vaccinated fingerlings, these results could increase net returns in the range of \$2000 to \$3000/acre on fingerling ponds. Results from whole farm mathematical programming models showed additional economic benefits on farm as a result of benefit diffusion to stockers and foodfish production phases. Food fish production phase could benefit either by increase stocking densities and intensify production or by appropriating more of the available fingerling ponds to profit maximizing foodfish production strategies. Commercial adoption of oral vaccination technology could greatly enhance industry profitability and economic efficiency.



**Figure 1.** The initial catfish vaccine delivery system, designed by Mississippi State University (Photo by David Ammon)

## **PIT FALL AND HONEY TRAPS IN AQUACULTURE FARM MANAGEMENT**

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Aquaculture is a management-intensive business with enterprise success often solely relying upon the on-farm management strategies. High level of investment in facilities and equipments and high operating capital requirements often demand competent managers/owners who is well versed to changing economic, market and regulatory conditions. This presentations is intended to draw the attention of aquaculture managers/owners and potential entrepreneurs to critical management areas of business planning, production strategies, economic feasibility, and technology choices affecting aquaculture business. Common miscalculations leading to overestimation of profits and long term profitability are addressed. Several luring aquaculture ventures, products, and technologies that targets investors without providing all the associated latent costs prove costly to entrepreneurs. Managers should take extra precautions to follow scientifically proven technologies and practices before relying on “snake-oil research and results” provided without enough scientific backing. Sound business planning and reliable extension supports are becoming increasing key for improving farm efficiencies.

## **AMERICAN ASSOCIATION OF FISH VETERINARIANS –YEAR FIVE**

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The American Association of Fish Veterinarians (AAFV) was formed in 2012. As of September 2016 it had 156 members, including 24 students. In April 2016 the third annual AAFV Conference was attended by 75 veterinarians and featured 14 presenters from throughout the U.S. The presentations ranged from case presentations to practical and economic discussions of delivering fish veterinary service. In 2016 there were improvements to the process of directing the increasing number of inquiries by the public for fish veterinary services to AAFV members. Fish farmers, and other potential clients, can find a veterinarian in their state through the FIND A VET tab on AAFV website. The year also saw a dramatic increase in the collaboration with several aquaculture affiliated organizations including the National Aquaculture Association, the American Veterinary Medical Association, the World Aquatic Veterinary Medical Association, and the American Fisheries Society- Fish Health Section. The AAFV was active in distributing current information to its members on the Veterinary Feed Directive. For first time, in 2016, AAFV awarded a veterinary student scholarship and plans to continue and expand this effort in the future.

## UNDERSTANDING INTERSPECIES COMPETITION WITHIN PROBIOTICS AND DEBUNKING THE POND SIDE GROW OUT MYTH

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Although highly susceptible to disease which can result in huge economic losses, shrimp farming has become one of the fastest growing food production areas. Probiotics have become an attractive alternative to chemotherapeutics for use in aquatic disease management and there are numerous scientific references to probiotic use in shrimp aquaculture. Probiotics can improve disease resistance and survival of shrimp due to antagonism to disease causing agents, stimulate the immune system, support digestive activities, and reduce stress by improving water quality. Most commercial products are combinations of bacteria that have been selected for their individual activities. However, there has been increasing speculation that probiotics in combination are capable of either working together or against each other in the ponds. Questions regarding probiotic interactions, dosing strategies and grow out practices need to be answered to improve disease management and water quality in shrimp farming operations. Therefore, we examined interactions among probiotics commonly used in shrimp farming by testing different combinations of bacteria in agar well diffusion assays. Bacteria from overnight cultures (100 µl) were spread on TSAII agar plates. Cell free supernatants (CFS) were prepared from overnight cultures of different *Bacillus subtilis* strains (A-D). Zones of inhibition were observed and measured (Table 1).

Interestingly, all four *B. subtilis* strains react differently in the inhibition assay. Three of the four strains are antagonistic to *B. amyloliquefaciens* and *B. methylophilicus*. *B. subtilis* (A) is antagonistic to *B. pumilus* and possibly to *B. licheniformis*, while *B. subtilis* (C) appears to improve the growth of *B. methylophilicus*. To further examine these interactions, equal amounts of *B. subtilis* (A) and *B. pumilus* were grown in liquid culture and the combined culture was examined overtime with *B. subtilis* (A) overtaking *B. pumilus* within 6 hours. These data suggest that pond side grow outs could reduce the efficacy of the commercial product.

**Table 1. Agar well diffusion results.**

CFS	Bacteria				
	<i>B. licheniformis</i>	<i>B. pumilus</i>	<i>B. subtilis</i> (A)	<i>B. amyloliquefaciens</i>	<i>B. methylophilicus</i>
<i>B. subtilis</i> A	*	14mm	-	16 mm	17 mm
<i>B. subtilis</i> B	-	-	11mm	12 mm	10 mm
<i>B. subtilis</i> C	-	-	-	*	**
<i>B. subtilis</i> D	-	-	-	-	-

\*Diffuse zone, \*\* enhancement

## ANESTHETIC AND HAEMATOLOGICAL RESPONSE OF TOBACCO (*Nicotiana tabacum*) EXTRACT ON AFRICAN CATFISH (*Clarias gariepinus* BURCHELL 1822) FINGERLINGS

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Anesthetic and Haematological investigation was made on the effect of tobacco extract on *Clarias gariepinus* fingerlings (4.03±2.20g) using bioassay tests over a period of 96 hours. Water quality parameters like Dissolved Oxygen and PH decreased significantly while alkalinity increases and temperature was almost at the same level. There were no similarities in the time of anesthesia and time of recovery at different concentrations of ethanol extract of tobacco for *Clarias gariepinus*. Anesthesia time decreases on increase of anesthetic dose. Fish showed marked behavioural changes like erratic swimming, aggression, jumping, air gulping, hyperventilation and settling at the bottom which indicates anesthesia time. There was increase in White Blood Cell (WBC) counts with increase in tobacco concentration, Packed Cell Volume (PCV) and Red Blood Cell (RBC) counts decreases with increase in tobacco concentration. Haematologically, there was destruction of Red Blood Cell and low concentration of Haemoglobin (HB) in the exposed fish compared to fish in the control tank.

**Table showing the mean values of haematological indices of *C. gariepinus* fingerlings exposed to different concentrations of tobacco leaf extract for 96 hours.**

Parameters	control	1.0 mg/l	1.5mg/l	2.0 mg/l	2.5 mg/l
PCV (%)	18.00±0.00	15.20±0.10	10.00±0.04	8.20±0.08	7.20±0.05
Haemoglobin	7.05±0.00	5.82±0.10	4.20±0.10	1.40±0.34	0.64±0.10
WBC (mm <sup>3</sup> )	4.10±0.02	5.25±0.10	6.16±0.08	6.24±0.03	6.40±0.04
RBC (mm <sup>3</sup> )	1.73±0.04	1.50±0.03	1.34±0.66	1.24±0.08	1.18±0.02

PCV-packed cell volume, HB -haemoglobin, WBC- white blood cell, RBC- red blood cell

**Table showing the mean values of water quality parameters for the different concentrations of tobacco leaf extract for 96 hours**

Parameters	control	1.0 mg/l	1.5mg/l	2.0 mg/l	2.5 mg/l
Temperature (°C)	23.34±0.0	22.3±0.2	22.3±0.1	22.3±0.2	22.3±0.1
Dissolved Oxygen (mg/l <sup>-1</sup> )	8.5	8.0	6.7	5.1	4.9
PH	6.9	6.6	6.2	5.4	5.0
Alkalinity (mg/l <sup>-1</sup> )	74.1	84.4	103.7	129.3	142.7

**Dosage, Recovery and Mortality time of *C.gariepinus* fingerlings anesthetized with tobacco leaf extract**

Concentration (g/5l)	Anesthesia time (mins.)	Recovery time (mins)	Mortality (%)
Control (0)	-	-	0
1.5	54	24	0
2.5	51	27	0
3.5	48	30	15
4.5	42	40	40

## **COLLABORATION BETWEEN EXTENSION AND INDUSTRY: COORDINATION AND ASSESSMENT OF BACTERIA TO CONTROL HYDROGEN SULFIDE IN AQUACULTURE PONDS**

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Many times researchers and Extension personnel deal with projects that are difficult to determine how to solve a problem. Collaboration between industry partners, researchers, and Extension personnel can help solve these problems. In Arkansas, an example of this principle is the disappearance of Golden Shiner *Notemingus crysolucus*. Fish farmers reported losses ranging from 20-80% annually. Interviews with the producers determined that losses were not from theft, low dissolved oxygen, depredation or disease. Most farmers did not observe dead fish in the pond, but rather noticed losses when harvesting. Based on this information, an approach was designed to determine the cause and then provide a solution. This approach included research projects targeting water quality parameters and collaborative research projects with industry partners. These studies included laboratory toxicity studies, bacterial application rates, and water quality testing. Results have been disseminated to stakeholders through a number of different venues and are still on-going.

## SULFUR – OXIDIZING BACTERIA AS A POTENTIAL METHOD TO IMPROVE WATER QUALITY IN AQUACULTURE PONDS

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Hydrogen sulfide ( $H_2S$ ) is produced naturally in aquaculture ponds due to the conditions found in the bottom soils but there is still a need for an effective way to reduce  $H_2S$  concentrations. Previous laboratory studies have shown that the use of the bacteria species *Paracoccus pantotrophus* at 500 g/ha and 1000 g/ha significantly reduced the  $H_2S$  concentrations within study aquaria. Twelve ponds on a commercial Golden Shiner *Notemigonus crysoleucas* farm were treated with a *P. pantotrophus* of 500 g/ha and compared to ten untreated control ponds. Treated and control ponds were monitored for one year, weekly when water temperatures were at or above 23°C, then biweekly when the temperatures fell below 23°C, recording measurements of water temperature, pH, alkalinity,  $H_2S$  concentration and phytoplankton abundance. Preliminary results suggest that the  $H_2S$  concentrations in the treated ponds were significantly lower than the concentrations within the control ponds (Fig. 1). Alkalinity levels in the control ponds had significantly more variation than the treated ponds, which remained closer to the desired 100 ppm level. *P. pantotrophus* was detectable from the soil for only 10 days after treatment. The results indicated that the use of bacterial treatment reduced the presence of the harmful blue-green algae compared to the control ponds and a significantly higher abundance of zooplankton was found in the treated ponds. Overall, the use of *P. pantotrophus* at the rate of 500 g/ha seems to have a beneficial effect of reducing  $H_2S$  concentrations and blue-green algae abundances while buffering the alkalinity and enhancing the zooplankton abundance in Golden Shiner ponds. Ponds need to be treated every 10 days when water temperatures are between 21 and 24°C. Further research is needed to determine the concentration of *P. pantotrophus* needed in sportfish ponds.

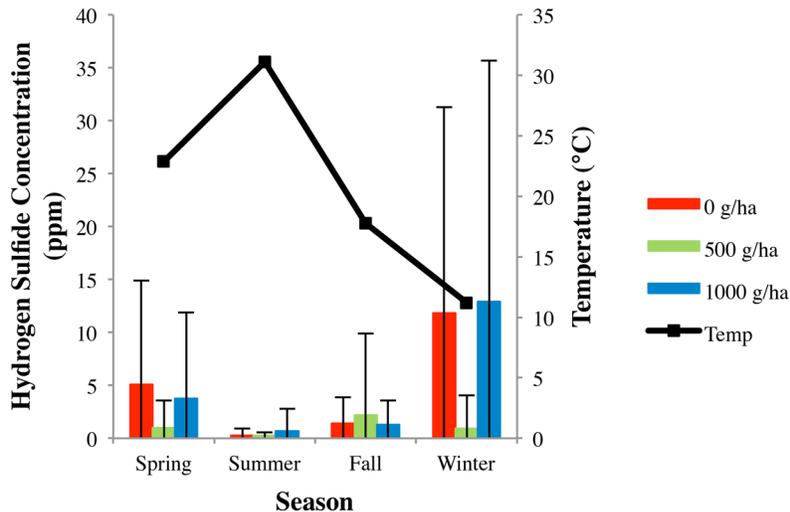


Figure 1. The mean  $\pm$  SD hydrogen sulfide ( $H_2S$ ) concentrations in ponds treated with 500 g/ha or 1000 g/ha of *Paracoccus pantotrophus* compared to control ponds (non-treated) by season. Average seasonal temperatures are also presented. Different letters within a season indicate significant differences ( $P < 0.10$ )

## **APPLIED INTENSIVE CULTURE TECHNIQUES OF WALLEYE *Sander vitreus* USED IN FISHERIES RESTORATION EFFORTS FOR LAKE CHAMPLAIN**

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Walleye (*Sander vitreus*) culture for fisheries restoration on Lake Champlain can be documented as far back as 1899. A cooperative was established in 1986 between the Vermont Fish and Wildlife Department and the Lake Champlain Walleye Association (LCWA) to provide fingerling production using early rearing intensive culture combined with extensive pond culture techniques. Today's program has placed a greater emphasis on intensive culture techniques that can be developed to provide a consistent output of quality fingerlings (38-51mm) in an effort to maximize production efforts and enhance the walleye fishery. The application of recirculation technology for fingerling production began in 2011, allowing for the control of environmental parameters critical for successful development and transition of walleye fry, advanced fry and fingerlings.

Early intensive rearing techniques have been reported in NCRAC Walleye Culture Manual by Summerfelt et al (1996). Using this initial work as a guide along with collaboration with other researchers, the conception of an intensive culture program was put into place. With currently six production cycles completed, overall production survival to distribution has increased from 6% in the initial year to over 30%, while days post hatch (dph) to target size (38mm>) has decreased from 41dph to 30dph. These advances have been achieved by applying various changes to aspects of culture such as fry density, turbidity, temperature and lighting. We expect to continue our refinements to various features and aspects of the production cycle and recognize the advances that have been made to date are now showing positive results that are contributing to the fishery.

## NUTRITIONAL PROGRAMMING OF YELLOW PERCH *Perca flavescens* INCREASES SECOND GENERATION'S ABILITY TO UTILIZE SOYBEAN MEAL-BASED DIETS

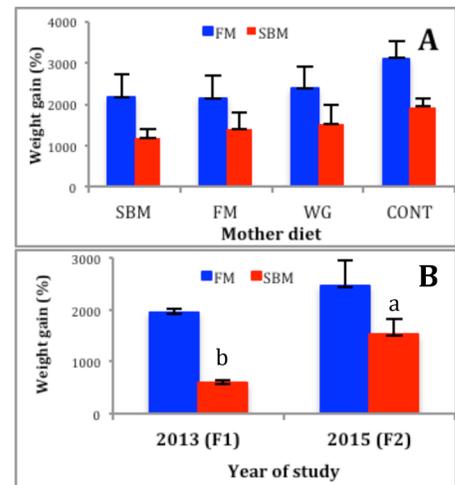
Megan M. Kemski\*, Macdonald Wick, Konrad Dabrowski

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Dietary fish meal protein replacements with plant proteins have been studied for decades, yet there are still limitations surrounding these formulations at higher inclusions levels. In more recent studies, nutritional programming has been used to alter the animal response to their diet. Nutritional programming refers to the changes that occur very early on in life, following initial exposures that can have permanent, long-term effects. We examine nutritional programming across two generations of yellow perch to determine if this approach can enhance dietary soybean utilization. Each generation of fish went through 4 phases. **F1 generation;** phase 1 consisted of four diets where plant proteins replaced 75% fish meal protein: 1) fish meal control (FM), 2) wheat gluten meal (WG) and 3) and 4) two soybean meal (SBM) varieties all fed to juvenile yellow perch for 2 months. In Phase 2, fish were fin-clipped and combined in triplicate tanks and all fed a FM diet for 9 months. In Phase 3, the “challenge” phase, all fish were transitioned to a SBM diet for 7 months. In Phase 4, fish were fed FM diets for 6 months during winter months when gametogenesis occurs and there is minimal somatic growth.

Eggs from 4-programmed females from each initial dietary group (4) were fertilized with the same males (n=3) from an outside source to minimize variability. Females solely fed a commercial control (CONT) diet were also fertilized with the same males. The F2 juveniles were reared for ~30 days on live feed prior to beginning phase 1. **F2 generation; Phase 1** consisted of two programming diets, fish meal (FM), and 75% soybean meal (SBM). Each group of juveniles (61±12mg) was equally divided, and fed either FM or SBM for 2 months. In **phase 2**, juveniles were fin-clipped, combined with siblings and fed a FM diet for 9 months. **In Phase 3**, which is currently ongoing, fish are being fed SBM diet for 6 months. All fish will then be transitioned back to FM diet in **Phase 4**, for 6 months over the winter.

Results from the **F2 generation in Phase 1** showed no significant differences in weight gain (%) within groups fed FM or SBM (**Fig. 1A**). **Figure 1B** depicts the differences in the F1 and F2 generation from Phase 1. Weight gain between fish fed FM showed no significant difference between generations. However, the SBM fed fish in the second generation had a significantly higher weight gain than those in the F1 generation.



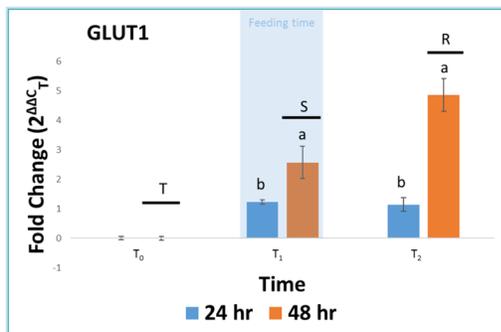
**Figure 1A:** Weight gain (%) over 2 months (Phase 1). Offspring were divided between FM/SBM and were traced back to mother's diet.  
**Figure 1B:** Weight gain (%) following phase 1, comparing F1 and F2 generation. Data was analyzed by both ANOVA and Tukey Kramer statistical tests. Comparisons were done between same diets.

## TRANSCRIPTOMIC EFFECTS OF FEEDING SCHEDULE ENTRAINMENT ON RAINBOW TROUT *Oncorhynchus mykiss* HYPOTHALAMIC APPETITE-REGULATING HORMONES, INTESTINAL TRANSPORTERS, AND CIRCADIAN RHYTHM-REGULATING HORMONES

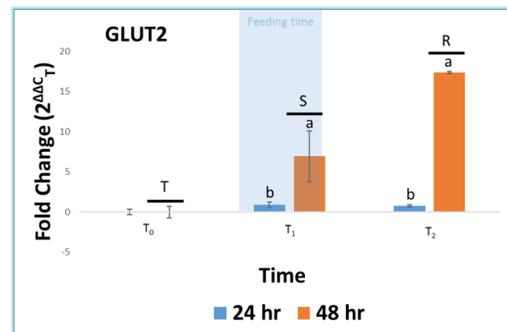
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Anecdotal evidence suggests fish may become physiologically entrained to a feeding schedule and upregulate intestinal transporters in anticipation of a meal. Rainbow trout were fed for two weeks at the same time every day, either by hand or belt feeder, and liver, intestine, and hypothalamic tissues were sampled two hours before, during, and two hours after established feeding times following a 24- or 48-hour fast. We measured relative quantities of mRNA transcripts for appetite-regulating hormones and intestinal transporters to assess molecular response in anticipation of feeding, and we also measured expression of circadian rhythm genes to assess effects of natural diel rhythm on anticipation of feeding. In general, appetite regulators were upregulated in belt-fed fish compared to hand-fed fish after a 24-hour fast, remained upregulated through two hours post-feeding, but displayed reduced expression after a 48-hour fast compared to the 24-hour fasted fish. Feeding method and feeding time did not, in general, affect regulation of intestinal transporters, with some exceptions. Duration of fasting affected most transporters, with expression after 48-hour fast being significantly upregulated compared to expression after 24-hour fast (Figures 1 and 2).



**Figure 1.** Expression of intestinal GLUT1 in rainbow trout entrained to a feeding schedule, fasted for 24 or 48 hours, and sampled before ( $T_0$ ), during ( $T_1$ ), or after scheduled feeding time ( $T_2$ ).



**Figure 2.** Expression of intestinal GLUT2 in rainbow trout entrained to a feeding schedule, fasted for 24 or 48 hours, and sampled as in figure 1.

## **MICROINJECTION OF CRISPR/CAS9 PROTEIN INTO CHANNEL CATFISH, *Ictalurus punctatus*, EMBRYOS TO KNOCKOUT GENES**

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Channel catfish is the most popular catfish species in the United States. The genome of channel catfish has been sequenced, however, there are still many genes in which gene function is not clearly understood. There is an emerging importance to study gene function in channel catfish. Understanding gene function can assist in selecting the best fish for commercial production as well as sport activities. Channel catfish also could be used as a model for research since there is extensive information on the biology of channel catfish. Gene knockout has been used to study gene functions in vivo. Clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) system is a powerful and versatile tool used to edit genomic DNA sequences including gene knockout. Microinjection is the first and widely used method to transfer genes and various constructs in aquaculture species.

In this work, we describe a detailed protocol for microinjection of channel catfish embryos with CRISPR/Cas9 protein as an example for large aquaculture species. Briefly, eggs and sperm are collected then artificial fertilization is performed. Fertilized eggs are transferred to a petri dish with Holtfreter's solution. A microinjection needle is loaded with the injection solution then connected to a microinjector. Injection volume is determined with a hemocytometer by injecting into a drop of mineral oil. Once the needle is adjusted to inject the required volume, fertilized eggs are injected within the first cell stage of embryonic development. The needle is introduced into the yolk and injection material is expelled. Embryos are then incubated in Holtfreter's solution with antibiotic treatment until hatch. To demonstrate the efficiency of the microinjection protocol, sgRNAs were designed and injected to target some channel catfish genes.

## EFFECT OF VARIOUS PROTEIN FEEDS ON THE GROWTH, BODY COMPOSITION, HEMATOLOGY AND ENDOGENOUS ENZYMES OF CATFISH (*Pangasius hypophthalmus*)

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The study was conducted to evaluate the effect of various concentrations of crude protein on the growth, body composition, hematology, and endogenous enzymes of catfish, *Pangasius hypophthalmus*. The trial was carried out in HAPAS (10 x 10 x 5 feet) for 90-days. Fish was fed at 3% of wet body weight twice daily with the experimental feeds having crude protein (CP) levels 44% (T<sub>1</sub>), 35% (T<sub>2</sub>), and 40% (T<sub>3</sub>), each having three replicates. Fish fed on 40% protein feed (T<sub>3</sub>) showed significantly (P<0.05) higher growth (1378.57±53.20g) compared to T<sub>2</sub> and T<sub>1</sub>. The percent weight gain was also highest in T<sub>3</sub> (718.81±43.27) followed by T<sub>2</sub> (69.83±10.83) and T<sub>1</sub> (61.69±1.41). The FCR of fish in T<sub>3</sub> was significantly lower (2.51±0.095) than T<sub>1</sub> (4.00±0.06) and T<sub>2</sub> (3.75±0.42). Proximate analysis values remained uniform among treatments. Concentration of amylase were significantly higher (P≤ 0.05) in T<sub>1</sub> and T<sub>2</sub> than T<sub>3</sub> while phytase and lipase enzymes showed non-significant differences among all the treatments. Values of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in blood of *P. hypophthalmus* increased with where 35%-40% protein was present. The overall performance of fish fed 40% CP feed remained significantly better with no adverse effects on nutritional value of fish.

**Table 1. Growth performance and proximate composition of *Pangasius hypophthalmus* under different dietary treatments**

Parameters	T <sub>1</sub> (44% CP)	T <sub>2</sub> (35% CP)	T <sub>3</sub> (40% CP)
Initial weight(g)	703.56±27.59 <sup>a</sup>	685.23±42.52 <sup>a</sup>	659.75±29.59 <sup>a</sup>
Final weight(g)	1137.38±34.86 <sup>b</sup>	1160.95±6.44 <sup>b</sup>	1378.57±53.20 <sup>a</sup>
weight gain(g)	433.81±7.56 <sup>b</sup>	475.71±39.87 <sup>b</sup>	718.81±43.27 <sup>a</sup>
% weight gain	61.69±1.41 <sup>b</sup>	69.83±10.83 <sup>b</sup>	109.09±8.04 <sup>a</sup>
Initial length(cm)	43.11±0.49 <sup>a</sup>	42.89±1.71 <sup>a</sup>	42.58±3.28 <sup>a</sup>
Final length (cm)	47.16±0.83 <sup>a</sup>	46.89±0.54 <sup>a</sup>	48.25±1.23 <sup>a</sup>
Increase in length (cm)	4.11±1.33 <sup>a</sup>	3.92±2.21 <sup>a</sup>	5.67±2.83 <sup>a</sup>
FCR	4.00±0.06 <sup>a</sup>	3.75±0.42 <sup>a</sup>	2.51±0.09 <sup>b</sup>
SGR%	0.22±0.005 <sup>b</sup>	0.25±0.03 <sup>b</sup>	0.34±0.01 <sup>a</sup>
<b>Proximate composition</b>			
Moisture %	10.9±0.40 <sup>b</sup>	11.5±0.2 <sup>b</sup>	11.3± 0.1 <sup>b</sup>
Crude Protein%	59±1.65 <sup>a</sup>	59.7±1.5 <sup>a</sup>	59.9± 3.0 <sup>a</sup>
Crude Fat%	8.23±0.11 <sup>a</sup>	8.4± 0.2 <sup>a</sup>	8.7± 0.4 <sup>a</sup>
Crude Fiber%	0.86±0.05 <sub>a</sub>	0.83±0.1 <sup>a</sup>	0.9±3.3 <sup>a</sup>
Ash %	17.6±0.57 <sup>a</sup>	17.1±0.6 <sup>a</sup>	17.4±1.7 <sup>a</sup>

## NOVEL NON-ESTROGENIC ENDPOINTS OF PHENOLIC METABOLITES TOXICITY IN FISH: USING ZEBRA FISH

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Evidence of the severe effects of phenolic metabolites on different fish species exists in a number of axes including endocrine and non-endocrine as well. Regardless the classical issues of such metabolites as endocrine disrupting chemicals, novel non-estrogenic points are poorly studied. This article demonstrates the non-estrogenic attribution of certain phenolic metabolites in fish species. Confirming this non-estrogenic action of such compounds, zebrafish (*Danio rerio*) embryos were subjected to different doses of Polybrominated diphenyl ethers (BDEs) to study the effect of such class of chemicals on the fish embryogenesis during the first 120 hours of fertilization.

## GROWTH AND YIELD COMPARISON OF LETTUCE, BASIL AND TOMATO GROWN IN AQUAPONICS AND HYDROPONICS SYSTEMS

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Aquaponics is a production system that integrates aquaculture and hydroponics by recirculating residual nutrients resulted from fish waste for crop production. Although many different crops have been tested in aquaponics systems, limited information is available on the growth and yield of a crop grown in aquaponics compared to hydroponics. The objective of our research was to evaluate growth and yield of vegetable crops grown in aquaponics in comparison to hydroponics. Two leafy vegetables, lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*), and one fruity vegetable, cherry tomato (*Solanum lycopersicum*), were grown in both aquaponics and hydroponics systems. Each aquaponic unit consisted of a 378L fish tank with stocking density of 26kg/m<sup>3</sup> Nile tilapia (*Oreochromis niloticus* L.) and a 378L growth bed with vegetable crops at the recommended planting density. The pH was monitored daily and adjusted to target levels (pH 7 in aquaponics and pH 6 in hydroponics). Measured EC averaged at 0.7 dS/cm in aquaponics while it was maintained at 2.0 dS/cm in hydroponics. The growth, yield, SPAD values and other morphological parameters were assessed during a 5-month production period. The results showed that marketable yield of hydroponically grown lettuce was significantly higher ( $P < 0.05$ ) compared to aquaponically grown one, while there were no significant differences in marketable yields of basil and tomato grown in both systems. Interestingly, aquaponic tomato produced significantly lower ( $P < 0.05$ ) fresh weight of stem and leaves but larger roots compared to hydroponic tomato, indicating that limited nutrients in aquaponics reduced shoot growth without affecting yield of tomato fruits. The SPAD values were higher in aquaponically grown vegetables although leaf parameters were not significantly different from hydroponically grown ones. It is concluded that the growth and yield of different vegetable crops are differently affected by production system, and therefore, a careful consideration is needed in determining vegetable crops suitable for the production in aquaponics system.

## EFFECT OF PLANT SPECIES ON NITROGEN AND PHOSPHORUS REMOVAL IN AQUAPONIC SYSTEM

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Aquaponics is a hybrid production system that integrates aquaculture with hydroponics to grow various crops with fish wastewater in a recirculating system. A well-managed aquaponics can improve nutrient retention efficiency, reduce water usage and waste discharges to the environment, and enhance profitability by simultaneously producing two cash crops. By practicing aquaponics systems in non-traditional locations for agriculture or on marginal lands, aquaponics can function as a sustainable model for food production. Although different types of crops have been tested in aquaponics, the selection of plant species has relied mainly on experience. Nitrogen (N) and phosphorus (P) are major nutrients available in aquaculture effluents causing environmental issues. Thus, improving nutrient use efficiency through proper crop choice is a critical task for better performance of aquaponics system. Since different plants have different growth characteristics, different cultural strategies need to be developed to achieve the best production efficiency. However, limited studies have been conducted to systematically investigate the effect of plant species on the performance of an aquaponics system. The objective of our research was to evaluate the effect of plant species on N and P removal in aquaponics system in order to improve nutrient recovery by utilizing aquaculture effluents as sustainable mineral nutrient sources for vegetable production.

Three vegetable crops (lettuce, basil, and tomato) were separately grown in aquaponic systems. Water quality parameters were monitored daily and N and P concentrations were measured every other day. The results showed that plants effectively removed N and P during their production cycle with increasing removal rate as their biomass increased. Although there were no significant ( $p > 0.05$ ) differences in daily removal efficiency among plant species when the plants were young, tomato showed significantly ( $p < 0.05$ ) higher N removal rate compared to other plant species as they grew mature. However, N accumulated over time in aquaponics systems while P level was maintained throughout production period. Our results indicate that N and P removal and recovery efficiencies are varied by plant species and production stage. It is concluded that, in combination with proper crop choice, reducing N and P inputs in the system is critical to effectively manage aquaponic system.

## **AN OVERVIEW OF THE CHANGES FOR VETERINARY FEED DIRECTIVE (VFD) DRUG USE & IMPLICATIONS FOR VETERINARIANS, PRODUCERS, & FEED MILLS / FEED DISTRIBUTORS IN THE AQUACULTURE INDUSTRY**

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The Food & Drug Administration (FDA) published a new Final Rule in 2015 detailing the requirements for antimicrobials intended to be used in animal feed under veterinary supervision with a Veterinary Feed Directive (VFD). A VFD drug is an antimicrobial intended for use in or on animal feed which is limited to use under the professional supervision of a licensed veterinarian. The feeding of an animal feed containing a VFD antimicrobial must be authorized by a written, lawful VFD. A written, lawful VFD authorizes a producer to obtain and use animal feed containing a VFD antimicrobial to treat the animals listed on the written VFD in accordance with the VFD drug / feed label. A VFD is specific to drug, dose, species, indication, animal location, age class, & other limitations that may be placed by the issuing veterinarian. Deviations from a written VFD are considered to be a violation of federal law.

Aquaflor® was approved as a VFD drug in 2005 with a number of indications for the control of mortality in fish. The FDA's Final Rule changed several aspects of VFD requirements for obtaining and feeding feeds medicated with Aquaflor. Some of these changes include amount of medicated feed required calculations, expiration date definition change, possible inclusion of additional information on the VFD by the issuing veterinarian, and completed VFD form distribution changes.

There are 3 participants in the VFD process: the producer, the veterinarian, and the feed mill or feed distributor. Each participant has responsibilities to accomplish judicious antimicrobial use and to achieve compliance with the new VFD requirements. This presentation will review select aspects of each participant's responsibilities in the VFD process as well as highlight the changes to the VFD process by the implementation of the new Final Rule.

The new VFD Final Rule requires veterinary involvement with fish health problems and programs when a medicated feed needs to be used. Veterinarian, producer, and feed mill/distributor understanding and cooperation will help ensure proper antimicrobial usage which will help optimize antimicrobial performance as well as increase safety for humans and the environment.

## A SEASONAL COMPARISON OF PROXIMATE COMPOSITION OF MIXED CULTURES VERSUS MONOCULTURES OF *Nannochloropsis salina* AND *Phaeodactylum tricorutum* IN SEMI-CONTINUOUS OUTDOOR RACEWAYS

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Aquaculture is continually growing in its contribution as a provider for human-consumed seafood. Diets containing large amounts of fish meal and oil are used for aquacultured shrimp and fish. There is a growing demand for these limited marine feed ingredients, which has not only increased feed costs but has also added more pressure to the world's fisheries. Alternatives to fish meal and oil must be developed in order to lessen the dependence on these finite resources. Previous research has demonstrated that proteins and lipids extracted from microalgae can replace a significant amount of fish meal in aquaculture diets. Mixed cultures of microalgae have shown potential benefits in biomass production for biofuel research. This production technology also has the potential to produce cultures with optimum amino acid and fatty acid levels (profiles) as an aquatic animal feed grade ingredient. Seasonal fluctuations in temperature, salinity, and radiation can affect the lipid and protein content and composition of the microalgae. As many cultures are maintained outdoors, the impact of these variations must be determined in order to accurately predict culture yields of proteins and lipids.

The current study was designed to compare production characteristics and protein and lipid content between monocultures and a mixed culture of the microalgae *Nannochloropsis salina* and *Phaeodactylum tricorutum* produced in outdoor systems. Twelve (n=4/treat.) outdoor 600L raceway systems (surface area 2.8m<sup>2</sup>) were inoculated with cultures at similar ash-free dry weight/L and then maintained over a 90-day period (Nov. 2015 to Feb. 2016). The microalgae culture in each system was circulated by a rotating paddlewheel and pH maintained between 7.6 and 8.0 by CO<sub>2</sub> injection controlled with a pH-activated solenoid. Temperature, salinity, and pH of each system were measured twice daily with a portable meter. Cultures were harvested (25-75% of volume depending on microalgae density) every 3-7 days (i.e., after cultures reached stationary growth). Before harvesting, culture samples from each system were collected and stored for protein and lipid analysis.

The proximate composition of the biomass over the course of the trial will be presented and correlated to weather parameters. This research was supported by a grant issued by the Department of Energy.

## **CRYOPRESERVATION OF REPRODUCTIVE CELLS OF FISH: THE DEVELOPMENT OF RESEARCHERS OF SOUTH OF RUSSIA**

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Since 2004 the staff of the Southern Scientific Center, Russian Academy of Sciences jointly with the Astrakhan State Technical University are conducting research on low-temperature preservation and long-term storage of genetic material of valuable fish species.

The aim of the work is development of technology for cryopreservation and storage of reproductive cells of fish, in order to ensure their structural and functional integrity, as well as the development of techniques of using frozen material in aquaculture.

Received 3 patents for invention:

- «Method of increase the survival rate of sexual cells of sturgeons during the cryopreservation»
- «Method for reducing low-temperature jump cryoprotectant solutions»
- «Method of cryopreservation of fish egg».

Know-how (trade secrets): «Methods of removing a cryoprotectant from sperm defrosting fish».

Work was done to produce offspring and determining the physiological usefulness of juveniles produced using cryopreserved semen. Fish obtained using defrost sperm had the best survival rate, growth rate, fertility, physiological and biochemical characteristics, compared with the traditional methods, due to the selective action of low temperature of liquid nitrogen.

Years of research to develop methods of cryopreservation of eggs of fishes of the South of Russia, allowed to pick up a new cryoprotectant, the basic component of which is a mixture of unrefined vegetable and animal oils containing linoleic, linolenic acids and phosphatides, which is a glycerol esters of fatty acids and a substituted phosphoric acid.

For several years in Southern Scientific Center of the Russian Academy of Sciences jointly with the Astrakhan State Technical University, work is underway on the creation of the Cryobank. The basis of his activities laid the accumulation, conservation and use of genetic material for the shortfall of manufacturers and the correction of existing technologies of artificial reproduction of rare and endangered fish species of the Volga-Caspian and Azov-Black seas basins.

## CATFISH PRODUCTION IN FLOATING RACEWAYS

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In 2016, a four-year study was initiated to establish production protocols, safe standing crop levels and economic feasibility analyses for culturing hybrid catfish in In-Pond Raceway Systems (IPRS). One floating IPRS was placed in each of the four 0.4-ha ponds used in the study. Two IPRS' have a fish growing volume of 64 m<sup>3</sup> (4.9 m wide, 10.7 m long and 1.22 m deep) and two have 45 m<sup>3</sup> (3.05 m wide, 12.20 m long and 1.22 m deep). Sides and bottom of IPRS were made with 3-mm HDPE liner and the inlet and outlet screens used to enclose the fish were made of 1/2" x 1" PVC coated steel mesh wire. Each IPRS has an air-lift water moving device (a grid made with 12 m of diffuser hose placed under a directional hood) positioned just before the inlet screen. A 1.5 HP air blower propels each water moving device, supplying a constant water flow through the IPRS unit and providing supplemental aeration to increase the oxygen levels within the IPRS during night and early morning hours. IPRS units are covered with a 25 mm net to prevent bird predation.

The first year of this project was aimed at assessing whether a commercial size catfish (680 g up) could be produced in one growing season and if ponds fitted with IPRS units could yield a fish biomass twice as much as the fish yield attained in conventional catfish ponds (7,800 kg/ha/yr). Hybrid catfish juveniles (mean wt 41 g) were stocked into the IPRS units in March 2016 and fed 32% CP commercial catfish pellets once or twice a day, according to water temperature and dissolved oxygen conditions. Feed allowance was adjusted every two weeks, by estimating how much feed fish were able to eat in an "up to five minutes" period. Fish were sampled at monthly intervals to estimate average weight, specific daily growth and FCR. Raceways were inspected daily for the presence and removal of dead fish. Results are summarized in Table 1. A sequence of bacterial infections in the second month of the study (first *Flavobacterium columnare*, second *Edwardsiella ictaluri*) caused significant losses of fish in all raceways, especially in B1, B2 and B4. Fortunately, fish were still small at the onset of those infections, causing minor losses of biomass. FCR ranged from 1.52 to 1.75. Final standing crop nearly equaled or surpassed mean catfish yield in conventional ponds and mean weight of fish ranged from 670 to 820 g. Estimated production costs ranged from US\$ 2.01 to 2.35/kg of fish. More information on IPRS and economic data on this trial will be presented at WAS Conference.

	Ponds			
	B1	B2	B3	B4
Pond area (ha)	0.40	0.40	0.40	0.40
Raceway volume (m <sup>3</sup> )	63.5	63.5	45.3	45.3
Number of fish stocked	11,030	11,086	8,083	7,821
Stocking biomass (kg)	456	463	345	322
Feed applied (kg)	9,699	9,817	8,200	7,733
Mean weight (g)	671	794	700	818
Estimated biomass (kg)	6,388	6,601	5,030	5,393
Estimated FCR	1.64	1.60	1.75	1.52
Avg. feeding rate (kg/ha/day)	90.5	91.6	76.5	72.1
Standing crop (kg/m <sup>3</sup> )	100.7	104.0	111.0	119.1
Standing crop (kg/ha)	15,971	16,502	12,574	13,484
Survival (%)	86.4%	75.0%	88.8%	84.4%
<sup>1</sup> Pond B3 - data after first partial harvest				

## CHARACTERIZING THE CARBONATE CHEMISTRY ENVIRONMENT IN THE CHESAPEAKE BAY: FOCUS ON SHELLFISH HATCHERIES

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Oysters and clams are an essential part of our near-shore ecosystems and a vital part of life in coastal communities. Over the last few years Virginia hatcheries have experienced suppressed larval production for intermittent times during a production season. A water quality monitoring program was implemented between academic and State agency partners in Virginia and New Hampshire along with six shellfish hatcheries. This extension and outreach effort, initiated in 2012, provided the training and resources for each hatchery to discretely monitor numerous water quality parameters. In 2014, the program was expanded to include the monitoring of carbonate chemistry. More specifically, a parameter that is particularly useful for the shellfish industry is the carbonate mineral saturation state for aragonite ( $\Omega$ ). Low  $\Omega$  values can suppress larvae production success at our hatcheries. A pilot project was implemented with a robust state-of-the art system that continuously monitors the partial pressure of carbon dioxide ( $p\text{CO}_2$ ). This system was developed in partnership with personnel from the University of New Hampshire and implemented at one of the Virginia shellfish hatcheries.  $p\text{CO}_2$  data was used in conjunction with salinity and temperature to model  $\Omega$  at the hatcheries. This program was expanded in 2016 to include five hatcheries. From our data it is apparent that factors other than ocean acidification are very important for understanding carbonate chemistry in dynamic estuarine environments.  $\Omega$  fluctuates diurnally and seasonally and are influenced by factors such as algae activity, weather events, and likely sediment interactions.

## IMPROVING TILAPIA HEALTH AND INCREASING DISEASE PROTECTION WITHOUT THE USE OF VACCINES OR ANTIBIOTICS

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Tilapia is one of the most important food fish produced by the aquaculture industry because it can tolerate adverse water quality conditions and other stressors during production, and as a result occupies a very large segment of the global seafood market. The tilapia industry grew rapidly in the 1990's. With rapid growth and increased competition, tilapia producers intensified production to remain economically viable and to supply the growing market. Intensification has led to numerous disease outbreaks around the world. Diseases can be caused by bacteria, fungi, parasites and viruses. The most important pathogens of current concern include but are not limited to the following: *Streptococcus* spp., *Flavobacterium columnare*, *Aeromonas hydrophila*, *Mycobacterium* spp. and the new orthomyxo-like tilapia lake virus.

There are two major biosecurity techniques that should be employed in preventing disease at a tilapia farm. The first biosecurity method is a plan that will minimize the introduction of the pathogen into the facility or pond. The second biosecurity method is to minimize production stressors by providing fish with good water quality and nutrition. Unfortunately, commercial vaccines/bacterins have not been developed or are available for minimizing the risk of disease in tilapia. And the use of antibiotics to treat a disease comes with increased regulatory constraints and negative consumer acceptance.

Though the use of vaccines or antibiotics will continue to be an important strategy for disease intervention at finfish farms, many researchers, biotechnology firms, and feed manufacturers are investigating alternative methods for improving fish health and increasing disease resistance without the use of vaccines or antibiotics. Research and development efforts at Virginia Tech are currently underway to investigate some of these alternative strategies such as dietary micronutrients, probiotics, and antibodies. Specific micronutrients and strains of probiotics have been successfully used in tilapia to alter the physiology of the fish and to stimulate the innate immune system. In addition, studies are underway to evaluate the effects of an anti-interleukin-10 antibody on disease resistance to bacterial pathogens in tilapia. Challenge studies in the biosafety laboratory level-2 (BSL2) and other molecular tools are used to confirm improved health and increased disease resistance. These alternative methods will offer the tilapia industry options for minimizing economic loss due to pathogen-based biosecurity risks.

## **GOOD AQUACULTURE PRACTICES (GAqPs) FOR HARVEST AND POST-HARVEST HANDLING**

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Good Aquaculture Practices (GAqPs) are activities, procedures, or considerations optimizing production systems and management protocols to maximize environmental and economic sustainability, final product quality and safety, animal health and worker safety, while concurrently minimizing the likelihood of a disease outbreak. GAqP considerations generic to all aquaculture production systems include considerations for: Regulatory and non-regulatory compliance, facility siting and design, source water, facility security, animal health, feed management, record keeping and employee training. The key to providing your market with safe and high quality product is to understand and follow GAqP recommendations for proper pre-harvest and post-harvest guidelines. GAqPs for pre-harvesting and harvesting activities focus upon maximizing the quality of the product, as well as minimizing stress on the animal. Product that is consistently safe and of the highest quality is paramount for maintaining sales and maximum value for your product in the market place. Food safety is the most important aspect of your product. Pathogenic bacteria, chemicals, and mechanical items are all food hazards that compromise a safe product from entering the market place. Pathogenic bacteria presence can be minimized by ensuring all that you use clean and sanitized surfaces, equipment, personnel and any other item that comes into contact with your product. Any pathogenic bacteria that ends up on your product will multiply rapidly if your product especially if it undergoes temperature abuse. You must, according to federal regulation, develop a hazard analysis and critical control points (HACCP) plan for all of your processing efforts. HACCP is a systematic preventative approach for ensuring that each of your processing, packaging, and storage steps do not compromise your product in regards food hazards.

## DIETARY EFFECTS OF ALGAL MEAL (*Schizochytrium* sp.) ON LIPID METABOLISM AND OXIDATIVE STRESS ENZYMES ACTIVITY IN SHRIMP (*Litopenaeus vannamei*)

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Micro-algae are primary producers of omega-3 fatty acids, and algal-meal made from *Schizochytrium* sp., is rich source of docosahexaenoic acid, DHA. Currently, pacific white shrimp, *Litopenaeus vannamei*, is the most valuable single aquaculture commodity. The present study conducted a 12-week feeding trial to investigate the potential of replacing fish oil with algal-meal (65% lipid, 27% of which is DHA) in practical diets of pacific white shrimp (*Litopenaeus vannamei*), and its effects on the growth, antioxidant enzymes activity and fatty acids level, with the ultimate effects on sustainable shrimp production. For this purpose, three aquaria (110-L per aquarium) were randomly arranged and assigned to each experimental diet. 600-juvenile shrimp (average initial weight, 3.15 g) were fed with 8 iso-nitrogenous (38% protein), iso-lipidic (8% lipid), and iso-energetic diets: a control diet (Diet 1) containing 25% fishmeal (FM), 40% soybean meal, and 6% fish oil (FO) as a reference diet; and FO of diets 2-7 were replaced partially or completely with different combinations of linseed oil (LO), soy oil (SO), and AL-G Rich (*Schizochytrium* sp.) algal-meal (AO). FO and plant-based oil in Diet 8 were completely replaced with AO (Table 1).

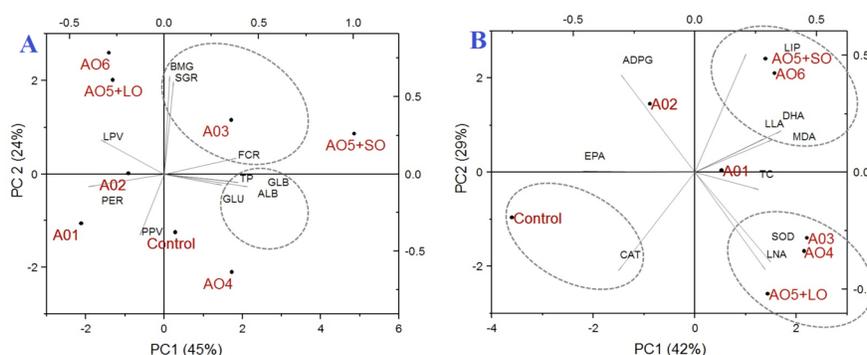
At the end of the feeding trial, the results indicated that no significant differences were found for parameters such as growth and feed utilization ((body mass gain (BMG), specific growth rate (SGR), and feed conversion ratio (FCR)), total hepatic cholesterol malondialdehyde and fatty acids among treatments (Figure 1).

Dietary treatment did not affect survival rate (85 – 99%). However, fish oil replacements by algae meal influenced the activities of enzymes ((catalase (CAT), superoxide dismutase (SOD) and lipase (LIP)), and levels of adipocytes and muscle fatty acids (palmitoleic acid, oleic acid, linoleic acid, linolenic acid, EPA, eicosenoic acid, DHA, and palmitic acid) in shrimp. Notably, significant health-related functions (related to antioxidant and fatty acids capacity) were observed in the group fed Diet 6 (a combination of AO and LO) compared to others, complete replacement of FO with AO. The high level of LO in Diet 6 have produced high EPA in shrimp muscle (Figure 1).

In conclusion, algal-meal made from *Schizochytrium* sp., can totally replace fish oil in shrimp practical diets, without compromising the growth, survival and health of shrimp. This study is fundamental in developing cost-effective, nutritionally balanced and environment-friendly shrimp feed.

**Table 1 :** Ratio of different source of lipid in shrimp feed (fish oil, FO; algal oil, AO; soy oil, SO; and linseed oil, LO)

Diet	1	2	3	4	5	6	7	8
	FO	AO <sub>1</sub>	AO <sub>2</sub>	AO <sub>3</sub>	AO <sub>4</sub>	AO <sub>5+LO</sub>	AO <sub>5+SO</sub>	AO <sub>6</sub>
FO	6.0	3.0	1.5	--	--	--	--	--
LO	--	--	--	0.5	1.0	1.0	--	--
SO	--	2.0	2.5	2.5	1.0	--	1.0	--
AO	--	1.0	2.0	3.0	4.0	5.0	5.0	6.0



**Figure 1:** PCA analysis in PC 1 × PC 2 coordination plane representing the contribution of (A) growth performance, feed utilization and blood protein and (B) muscle fatty acids profile and oxidative stress enzymes under testing conditions

## ENZOMEAL (IMPROVED SOYBEAN MEAL): POTENTIAL PROTEIN SOURCE FOR AQUAFEED

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Currently, aquaculture is the fastest-growing sector of food production. According to the Food and Agriculture Organization (FAO), almost half of the world's fish supply for human consumption derives from aquaculture production. Ongoing intensification of aquaculture has made it essential to develop suitable diets for fish using alternative protein sources. Therefore, the Ohio Soybean Council and its research partners Kentucky State University and Battelle, "the world's largest nonprofit research and development organization," have developed a new technology to increase the protein content and diminish the antinutritional factors from regular soybean meal (RSBM) i.e., improved soybean meal (ISBM) or EnzoMeal.

Using fish (yellow perch, *Perca flavescens*) fingerlings, a 10-week experiment was conducted to evaluate the nutritional quality of the ISBM to compare with that of fish meal and RSBM. Growth performance and nutrient utilization parameters of fish fed with ISBM based diets were comparable to fishmeal fed group. Overall, fish fed with ISBM grew 21% faster than fish fed with RSBM.

Five isonitrogenous (45% crude protein) and isolipidic (16% lipid) were formulated, control diet (fishmeal based), four experimental diets containing two inclusion level 18 and 33% of each ISBM and fermented soy protein concentrate. These diets were fed to for rainbow trout (*Oncorhynchus mykiss*) for 10 weeks. At the end, there were no significant differences with respect to growth performance and feed utilization. Histological evaluation of specific important metabolic tissues, such as the liver, kidney, and spleen indicated no morphological abnormalities related to dietary treatment. Interestingly, histological examination of the gastrointestinal tract provided no evidence of detrimental impacts of high soy inclusion, specifically as related to distal enteritis, a common malady in salmonids fed high levels of soy protein.

A 12-week feeding trial was conducted for Pacific white shrimp (*Litopenaeus vannamei*) and found that 100% fishmeal protein can be replaced by ISBM without compromising the growth and health of the shrimp.

In a continuation of a series of previous projects we focused on the long term (1.5 years) effects of ISBM on carnivorous fish species (largemouth bass, *Micropterus salmoides*) in pond study. Based on interim-sampling (after 3 months) there were no significant differences in terms of growth performance among fishmeal, ISBM and RSBM fed groups. Overall, the results of those studies enlarge the portfolio of EnzoMeal for use in aqua feed.

## HYDRAULIC IMPACT ON FISH MIGRATION IN SARIAKANDHI FISH PASS OF BANGLADESH

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The importance of open water fish in our socio-economic regime has recently drawn the attention of the policy makers of the country. FCD/FCDI projects mainly serve the agricultural interests, but it interfere fish migration. This inevitably affects the open water fisheries sector as migratory routes. Nursing grounds of many species of fish are hampered and disturbed for these projects also. In order to permit fish migration in rivers, it is necessary to maintain conditions that help migrants reach their spawning grounds. To overcome obstacles, such as hydraulic structures, placed in the path of migrating fish, structures must be designed to assist the fish to pass them. The periodic and directed travel of fish mainly for feeding, breeding and over coming adverse climatic conditions is called migration. Fish passes are constructed to allow normal breeding migration and to ensure natural route of fish movement.

The concept of a fish passes is relatively new in Bangladesh. At present, two Fish passes and two fish friendly structures are constructed. These are Fish Pass in Jamuna to Bangali River at Sariakandi in Bogra, fish Pass in Kawadighi Haor of Monu river in Moulovibazar, fish friendly structure in Lohajong river of Tangail and fish friendly structure at Morichardanra in Chapainawabganj. Fish fry, spawning and hatchling movement from Jamuna to Bangali River was the main objective of Sariakandi Fish Pass Project. The Fish Pass Project of Sariakandi is necessary for the development of the dominant fishes like catfish and small fishes. The structures will also aid in efficient development of the carp fishes. Spawning migration, mainly in carp fish, in the study area was found to begin at the 2<sup>nd</sup> week of May and continue up to the 3<sup>rd</sup> week of July. Catfish migrations began at the last week of March and continue up to the 2<sup>nd</sup> week of June.

Fish fry and hatching movement from Jamuna to Bangali river was the main objective of Sariakandi fish pass project. The study also found that there were seven major category migratory species in the project area and the fish pass is contributing positively for growth of fishery resources in then study area. During the monsoon carp fish is the dominating migratory species. Carpfish migrates in a higher velocity, whereas, catfish migrates in a lower velocity. Some problems were found in the operation and management of fish pass.

## FUNCTIONAL ANALYSIS, SPATIAL AND TEMPORAL MRNA EXPRESSION OF THE KAZAL TYPE PROTEASE INHIBITOR FROM *Sebastes schlegelii*

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Kazal-type serine protease inhibitor (KSPI) is one of the serpins, which plays a vital role to regulate and control the proteases as well as development for cellular component and defense. In this study, we have molecular characterized the KSPI cDNA sequence and discovered the spatial and temporal mRNA expression profile from *Sebastes schlegelii* (*Ss*). *In silico* study was conducted using various bioinformatics tools. The full-length of the Kazal-type protease inhibitor from *Sebastes schlegelii* (*SsKSPI*) was 532 bp, including open reading frame (ORF) of 330 bp. The ORF encoded a polypeptide of 110 amino acids with a signal peptide of 21 amino acids. Predicted molecular weight and the theoretical *pI* of *SsKSPI* were 12.33 kDa and 5.52 respectively. The greatest value of the Identity (42.9%) and similarity (50.9%) was observed with *Channa striatas* KSPI. Quantitative real time PCR (qRT-PCR) results showed that *SsKSPI* ubiquitously expressed in many tissues at different levels. Remarkably, *SsKSPI* transcripts in liver showed the highest expression ( $P < 0.05$ ) followed by spleen and head kidney. The biotic and abiotic challenges were conducted and the liver tissue was assessed for the gene modulation by qRT-PCR. The mRNA expression of *SsKSPI* was significantly up-regulated throughout the challenge in response to *S.iniae*. The LPS challenge showed a gradual increment from 3h to 24h and going down at the late phase of the injection. The *SsKSPI* mRNA expression infected with Poly I:C has no significant point without 6h. Overall, the results showed that the *SsKSPI* is related to the immune system of black rockfish potentially.

## CONTROLLED LABORATORY CHALLENGE DEMONSTRATES SUBSTANTIAL ADDITIVE GENETIC VARIATION IN RESISTANCE TO *Streptococcus iniae* IN NILE TILAPIA

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*Streptococcus iniae* is an etiologic agent of streptococcal disease in tilapia and is one of several *Streptococcus* spp. that negatively impact worldwide tilapia production. Methods for the prevention and control of *S. iniae* include vaccines, management strategies, and antibiotics. An alternative and complimentary approach may include selective breeding for increased disease resistance, but the potential for this is unknown in tilapia. Therefore, this study was initiated to phenotype Nile tilapia (*Oreochromis niloticus*) families for disease resistance to *S. iniae* and determine heritability estimates. Fish from the third generation of the Spring Genetics Nile tilapia breeding program with nucleus operations in Homestead, Florida, US, were used for this study. A total of 143 full- and half-sib families (avg. 176 g, sd = 50 g) were challenged with *S. iniae*, and fish were divided into two groups with each containing on average 9 individually PIT tagged fish per family. The challenge was designed with the intent to utilize fish injected with *S. iniae* (Group 1) as shedder fish to transfer the bacterium to cohabitated fish (Group 2) and determine the mortality of each family by both injection and cohabitation. To accomplish this, tilapia from Group 1 were challenged by intraperitoneal injection with a volume containing  $1.15 \times 10^8$  cfu *S. iniae* per fish, and then cohabitated with tilapia from Group 2 in a single 5,550 L tank. Accumulated mortality at the end of the experiment was only 6.4% for fish challenged by cohabitation (Group 2) and was 60% for the fish challenged by injection (Group 1). The results revealed high variation between the mean survivals of the families injected with *S. iniae* (range from 0% to 100%, CV 69%). The estimated heritability of post-challenge survival in Group 1 was 0.42 on the observed binary scale and 0.58 on the underlying liability scale, derived from fitting a linear animal model and a sire-dam threshold model, respectively. The results demonstrated substantial additive genetic variation in resistance to *S. iniae* when fish are challenged by injection, and suggest promise in genetic improvement of tilapia for resistance to *S. iniae* by using selective breeding.

## **DUCKWEED AS A PLATFORM FOR THE PRODUCTION OF VALUABLE PHARMACEUTICALS: THE STORY OF BIOLEX THERAPEUTICS**

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Biolex Therapeutics was a biotech company involved in the development and commercialization of complex proteins and monoclonal antibodies using duckweed, a floating aquatic plant, grown under sterile conditions. The company was successful in culturing genetically modified duckweed to produce interferon- $\alpha$ , a pharmaceutical used to treat hepatitis C. Other drugs came onto the market better able to treat hepatitis C. Interferon produced by genetically modified duckweed was unable to compete with these other pharmaceuticals so Biolex became insolvent and its patents were purchased by other biotech companies.

## **MASS CULTURE OF LIVE FEED ORGANISMS FOR FISH AND INVERTEBRATES**

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Most larvae of fish and invertebrates are carnivorous. Fish larvae must be fed live foods which have the nutrients and (exogenous) enzymes they need, until their own digestive systems mature enough to eat formulated feed. Different animal species are consumed by larval fish and invertebrates depending on the size of the larval fish or invertebrate. Zooplankters, Rotifers, Cladocreatans and Artemia are among the most common animals cultured to feed larvae in commercial fish and shrimp hatcheries. After the fish has developed its own digestive system it can switch to dry formulated feed. This presentation illustrates different organisms cultured as live feed for fish and invertebrates.

## ELECTRONIC SOFTWARE SOLUTIONS ENABLE VFD COMPLIANCE FOR VETERINARIANS

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### Introduction

With the implementation of Guidance Document 213 on January 1, 2017, many veterinarians, producers, feed mills and pharmaceutical distributors are developing or changing their processes for managing Veterinary Feed Directives (VFDs), as well as veterinary prescriptions (scripts).

Many current over-the-counter (OTC) drugs have changed to VFD or script status effective January 1, 2017, so whether or not an animal health practitioner has been familiar with writing VFDs and/or scripts in the past, there is a need for understanding the changes and adjusting management strategies moving forward.

GVL has provided the industry with an electronic VFD system, FeedLINK®, for over 11 years. This platform offers a software solution for food animal practitioners that enables compliance with the new antimicrobial regulations.

This presentation will highlight GVL's electronic VFD system, FeedLINK®.

### FeedLINK

FeedLINK Electronic VFD System is a web-based software solution, developed to assist in the streamlined fulfillment and tracking of VFDs. GVL launched FeedLINK in 2005 and continues to invest in further developments and enhancements to ensure full regulatory compliance. Another major objective of the system is streamlining the communication responsibility of veterinarians by automatically transmitting VFDs to both the feed distributor and producer. By subscribing to GVL, veterinarians, producers, and feed distributors gain access to their VFDs in a secure, CFR Part 11 compliant database, thus fulfilling the two-year storage requirements in Guidance 120.

### Built-in GVL SmartEngine™ Technology

The simplified process provided by GVL's platforms is driven by the patent pending GVL SmartEngine™ technology. SmartEngine technology guides the user through the labelled selections for a specific product to provide assurance to veterinarians, producers and feed mills that each VFD is consistent with label instructions.

VFD drugs can be searched by company, product, or indication of use by species. Once a species and product is selected, SmartEngine only allows legal options for 1) stage of production, 2) indication of use, 3) dosage, 4) duration of use, 5) combination products, and 6) expiration date. SmartEngine also auto-populates all required cautionary statements including target animal and human safety statements, special instructions, and withdrawal times. With the addition of more VFD products to manage in January 2017, SmartEngine technology is designed to help simplify the creation and management of electronic VFDs. By providing VFD expiration date reminders to all stakeholders as well as other features, the system provides compliance assurances to the veterinarian, client and feed distributor.

### Mobile friendly

The FeedLINK systems is mobile friendly. Whether on smartphone or a tablet device, the GVL system is ready to go anywhere that has an internet connection. This mobile connectivity allows veterinarian users to create and send a VFD while on a farm visit, reducing the time it takes to start treatment.

### Data integrity

GlobalVetLINK's objective is to be the world's most trusted, secured and independent repository of animal health data, providing clients the confidence in the security of their information. As an independent third party company, GlobalVetLINK understands the high importance of a client's data security as well as the significance to their operation, and GVL ensures client data is secure.

*(Continued on next page)*

### Summary

GlobalVetLINK's software solutions provide food animal practitioners with a "one-stop-shop" of herd health management solutions to aid in compliance with the new antimicrobial regulations and support better decision making when prescribing interventions for clients, all while helping practitioners save time and resources.

### References

Guidance #213 - Guidance for industry on new animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food producing animals. Available at [www.fda.gov](http://www.fda.gov).

GlobalVetLINK's initial experiences following the VFD and prescription changes post-January 1, 2017

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## THE CARBOHYDRATE L-RHAMNOSE PROMOTES BIOFILM FORMATION WHICH ENHANCES *Flavobacterium columnare* VIRULENCE

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*Flavobacterium columnare*, the causative agent of columnaris disease causes substantial mortality worldwide in numerous freshwater finfish species. Due to its global significance and impact on the aquaculture industry, continual efforts to better understand basic mechanisms that contribute to disease are urgently needed. The current work sought to evaluate the effect of L-rhamnose on the growth characteristics of *F. columnare*. While we initially did not observe any key changes between the *in vitro* growth or in the colonization of gill tissue by *F. columnare* when rhamnose- treated; it soon became apparent that the difference lies in the ability to form more robust biofilms when under the influence of the carbohydrate, L-rhamnose. *In vitro* biofilm assays demonstrated enhanced biofilm formation in a dose dependent manner when using either L-rhamnose or D-galactose but not with D-glucose. Quantitative PCR analyses of gliding motility genes early after L-rhamnose stimulation revealed a pattern of upregulation in planktonic cells to facilitate increased movement and attachment to the host; then during biofilm formation their initial downregulation and later upregulation once again to facilitate the different stages of development. The overall mortality observed in the *in vivo* bacterial challenges indicates that biofilm formation has a direct association to virulence. Our results allow for some conclusions to be made about the formation of *F. columnare* biofilms and their likely mechanism of pathogenicity.

## DEVELOPMENT OF TECHNIQUES TO PROMOTE GAS BLADDER INFLATION OF WALLEYE (*Sander vitreus*) LARVAE IN INTENSIVE RECIRCULATING AQUACULTURE SYSTEM

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A preliminary study about walleye intensive culture showed that non-inflation of the gas bladder remains an issue in a recirculating aquaculture system (RAS) and new techniques need to be assessed. Thus, four treatments were selected for their potential ability to promote GBI of walleye larvae. (i) A low outflow ( $0.6 \text{ L min}^{-1}$ )  $90^\circ$  surface water spray, generally used in flow-through tank culture to emulsify oil film induced by intensive feeding. (ii) An higher outflow ( $1.2 \text{ L min}^{-1}$ )  $90^\circ$  surface water spray. (iii) A commercial oil absorbent sock combined with a low outflow  $90^\circ$  surface water spray. (iv) An in-tank micro-diffuser to provide air bubbles for larvae under the surface, combined with a low outflow  $90^\circ$  surface water spray. Treatments were organized in a randomized block design, a total of 12 semi-square 120 L tanks having 21 larvae per liter from 0 to 26-day post hatch. Three times/week, 10 larvae per tank were sampled to monitor their growth and gas bladder development. Water quality and other parameters were monitored to ensure optimal conditions. At the end of the experiment, all the larvae were individually evaluated for gas bladder inflation using a visual backlight method.

The experimental treatments did not affect the water quality nor the larvae growth. At 26-dph, larvae total length was  $28 \pm 2$  mm. A higher proportion of larvae with a fully inflated gas bladder were observed in tanks with micro-diffusers ( $67 \pm 9 \%$ ) and oil absorbent sock ( $66 \pm 5 \%$ ) versus high ( $58 \pm 4\%$ ) or low ( $39 \pm 7 \%$ )  $90^\circ$  surface water sprays (Figure 1).

These results demonstrate that a low outflow ( $0.6 \text{ L min}^{-1}$ )  $90^\circ$  surface water spray is not effective in RAS compared to the other treatments employed in this study. In RAS, the water tends to accumulate oil and may reduce the effectiveness of this treatment. The positive effect of the micro-diffusers on GBI suggest that walleye larvae may be able to capture air bubbles under the surface of the water to inflate their gas bladder and this finding should be investigated. The commercial oil absorbent sock was also effective in this experiment and represents an inexpensive and simple approach to enhance GBI in intensive walleye culture. Combinations of these treatments should be considered in further studies.

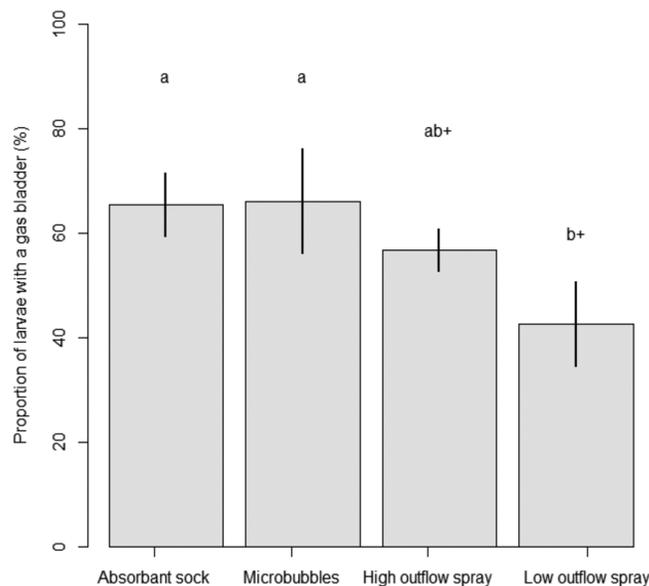


Figure 1 : Proportion of larvae with a fully-inflated gas bladder at 26 dph for each experimental treatment (ANOVA;  $n = 12$ ;  $p = 0.058$ ). Different letters represent significant differences (HSD Tukey;  $^+ : p < 0,1$ ) and vertical lines standard deviation.

## HEALTH OF OYSTER (*Crassostrea virginica*) POPULATIONS IN NATURAL AND RESTORED REEFS ALONG A NORTH TO SOUTH GRADIENT IN THE INDIAN RIVER LAGOON (IRL), FLORIDA

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As a sessile, sentinel species, oysters are used to evaluate ecosystem health. Due to the ecosystem services they provide oyster restoration is of prime importance in the Indian River Lagoon (IRL). The aim of this ongoing study is to investigate the seasonal health (Summer, Fall, Spring) of natural and restored oyster reefs in the IRL along a north to south gradient (Mosquito Lagoon to the Jupiter Inlet). A total of 540 oysters were collected from three natural and three restored sites ( $n = 30/\text{site}$ ), within three regions (north, central, south,  $n = 180/\text{region}$ ) in summer of 2016. Health indices compared included size, physiological condition, sex, prevalence of shell abnormalities, pests and parasites.

Oyster size did not differ significantly between region or reef type, although tissue weight was lower and oysters were in poor physiological (watery, digestive tubule atrophy) condition in the north. Sex ratio was approximately 2:1 females to males with restored and northern reefs having higher percentages of males and indeterminate sex. Pea crabs and mud blisters were more prevalent in the south, and boring sponge in the north. Restored southern reefs had significantly more pea crabs (28%) than did natural reefs (6.7%). *Perkinsus marinus* was more prevalent in north and central regions and in natural reefs. *Bonamia* spp. was only detected in natural reefs in the south. Initial assessment (summer 2016) indicate that oysters in the central IRL are healthier and that this region has the greatest potential for successful restoration efforts.

## GROWTH AND SURVIVAL OF SHRIMP CULTURED IN WATER WITH DIFFERING IONIC PROFILES

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Interest in low salinity shrimp culture has existed for a number of years due to the possibility of rearing shrimp inland. In the late 1990s, production of the Pacific white shrimp, *Penaeus vannamei*, was successfully accomplished at HBOI using fresh well water. Since that time, low-salinity culture of marine shrimp has been successfully accomplished in the Southeast U.S., Mexico and elsewhere. Groups in Florida and the Southeast U.S. have undertaken low-salinity culture of marine shrimp; however, unexplained low survivals still persist. It was soon realized that successful production of shrimp at low salinity was dependent on the ionic profile of source water and that all source water was not equal (i.e., different ionic composition). Previously reported research compared the short-term (5-7 d) survival of PL 12-20 *P. vannamei* exposed to various ionic profiles in an effort to determine minimum and maximum concentrations for Ca, Mg, and K and the effect of interactions of these ions at various concentrations. Concentrations and combinations that resulted in excellent short-term survival ( $\geq 90\%$ ) were then evaluated for a longer term (i.e., 6 weeks).

Growth and survival was assessed bi-weekly and at experimental termination, respectively, of *P. vannamei* exposed to different ionic treatments (n=5 reps/trt) at salinities of 2 or 4. Fifteen shrimp (0.3-0.75 g) were stocked per tank (60 L for 2, 4 ppt and 80 L for 4 ppt) in a clear water RAS system for each ionic treatment. The treatments were: Instant Ocean (control), and 4 ionic profiles (Ca:Mg:K, H=high, L=low for each ion; HLL, LHL, HHL, LLL). Final weight was significantly ( $p < 0.0001$ ) lower at 2 ppt for both the control (2.7 g) and HLL treatment (4.8 g) compared to the other three treatments (5.4-5.75 g). In both of the 4 ppt experiments, growth was significantly ( $p = 0.0008$ ,  $p < 0.0001$ ) lower for both the control (6 g, 5 g) and LHL (6 g, 4 g) treatment compared to the other three treatments (6.8-8 g, 6.4-6.6 g). Survival at 2 ppt was lowest for the control (70%) and LHL treatment (68%) compared to the other treatments (90-100%). Survival at 4 ppt was lowest for the HLL treatment (50%) compared to the other four treatments (68-95%) in the first experiment and for the HLL (68%) and HHL (72%) treatments compared to the other treatments (80-95%) in the second experiment.

These results suggest that regardless of the ionic profile, overall growth is higher at 4 ppt compared to 2 ppt. These results also suggest that waters with either high Ca or high Mg coupled with low concentrations of the other two ions (i.e., K and Mg or K and Ca, respectively) may result in lower growth or survival than waters containing high concentrations of both Ca and Mg or lower than recommended levels of all three major ions. As suggested previously, the ratios of Ca, Mg, and K are as important as merely making sure that minimum ionic concentrations are present. Ratios and minima should be considered when evaluating the ionic composition of potential culture water. (Supported by the FL Aquaculture Research Council.)

## SYNERGETIC EFFECTS OF DIETARY *Bacillus subtilis* WB60 AND MANNAN OLIGOSACCHARIDE (MOS) IN JUVENILE JAPANESE EEL, *Anguilla japonica*

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The current experiment was conducted to evaluate the synergetic effects of dietary *Bacillus subtilis* WB60 and mannan oligosaccharide (MOS) in juvenile Japanese eel, *Anguilla japonica*. Seven diets were formulated to contain three different *B. subtilis* levels (0,  $0.5 \times 10^7$  and  $10^7$  CFU/g  $\text{kg}^{-1}$  diet, BS<sub>0</sub>, BS<sub>0.5</sub> and BS<sub>1</sub>) with two different MOS levels (0 and 5g  $\text{kg}^{-1}$  diet, M<sub>0</sub> and M<sub>0.5</sub>) and oxytetracycline at 5 g  $\text{kg}^{-1}$  diet (OTC). Seven diets, in a 3×2 factorial design (BS<sub>0</sub>M<sub>0</sub>(CON), BS<sub>0</sub>M<sub>0.5</sub>, BS<sub>0.5</sub>M<sub>0</sub>, BS<sub>0.5</sub>M<sub>0.5</sub>, BS<sub>1</sub>M<sub>0</sub>, BS<sub>1</sub>M<sub>0.5</sub>) and OTC, were fed to triplicate groups of fish averaging  $9.00 \pm 0.11$  g (mean±SD) for 8 weeks. Fish were fed one of the experimental diets at 1.5 ~ 2.0% of wet body weight per day. At the end of 8 weeks of feeding trial, weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER) of fish fed BS<sub>0.5</sub>M<sub>0.5</sub> and BS<sub>1</sub>M<sub>0.5</sub> diet were significantly higher than those of fish fed CON, BS<sub>0.5</sub>M<sub>0</sub> and OTC diet. Whereas, on WG, FE, SGR and PER, there were no significant difference among the fish fed BS<sub>1</sub>M<sub>0</sub>, BS<sub>0.5</sub>M<sub>0.5</sub> and BS<sub>1</sub>M<sub>0.5</sub> diet. Nonspecific enzymatic activities including lysozyme and myeloperoxidase (MPO) from fish fed BS<sub>0.5</sub>M<sub>0.5</sub>, BS<sub>1</sub>M<sub>0.5</sub> and OTC diet were significantly higher than those from fish fed CON, BS<sub>0.5</sub>M<sub>0</sub>, BS<sub>0</sub>M<sub>0.5</sub> diet ( $P < 0.05$ ). Parameters for intestinal morphology and histology suggested a healthier gut for the fish fed BS<sub>0.5</sub>M<sub>0.5</sub> and BS<sub>1</sub>M<sub>0.5</sub> diet as compared to fish fed CON and OTC diet. Whereas, results from the disease challenge test with bacteria *Vibrio anguillarum* showed significantly lower survival rate for fish fed CON diet than those of fish fed other experimental diets. Therefore, these results indicated that *B. subtilis* at  $0.5 \times 10^7$  CFU/g  $\text{kg}^{-1}$  diet with mannan oligosaccharide (MOS) could have synergetic effects on the growth performance, immune responses and intestinal condition in Japanese eel.

## EVALUATION OF SOYBEAN MEAL AS PROTEIN SOURCE FOR NORTHERN AND SOUTHERN LARGEMOUTH BASS

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Fish meal (FM) is the first choice as a protein material in aquafeed production for its high quality of protein with a well-balanced amino acid profile. With rapid expansion of aquaculture industry resulting in the limited supply and high price of FM, the alternative protein sources have attracted attention of many researchers and developers. Due to high protein content, balanced amino acid profile and low price, soybean meal (SBM) is widely used as the most cost-effective alternative for FM in many aquatic animals. Several studies showed that SBM could be used well in diets with limiting amino acids supplemented for herbivorous and omnivorous fish. However, the utilization of SBM for carnivorous fish is still limited because of high anti-nutritional factors and lack of sulfur amino acids and micronutrients.

Largemouth bass *Micropterus salmoides*, typical carnivorous fish, is one of the most important commercial aquaculture species in North American and other countries. Commercial farming has relied primarily on trout diets, which contain approximately 60% of FM with expensive price. Little is known regarding nutritional information for the species, especially on the utilization of plant protein sources. Thus, alternative sources for dietary FM protein urgently need to be verified.

The most largemouth bass currently cultured are from the northern subspecies (N) (*M. s. salmoides*) and southern subspecies (S) (*M. s. floridanus*). A 12-week feeding trial in indoor round tank (45 L) was conducted to investigate the effects of dietary SBM levels on growth, survival, feed utilization and body composition of the two types of largemouth bass with initial mean weight of 6.8 g and 5.2 g, respectively.

Four isonitrogenous (crude protein 46%) and isolipidic (crude lipid 11%) diets were formulated with 0, 12, 25, and 40% of SBM, respectively. Each diet was randomly fed to fish in triplicate tanks (each having 20 fish) for each treatment. Fish were fed twice daily (9:00 and 17:30) to visual satiation. Temperatures were kept at 22-25°C. No differences were detected on survival between diet and strain ( $P>0.05$ ). Significant effects of strain on final weight, daily weight gain and specific growth rate were found ( $P<0.05$ ). The growth of fish from "N" strain was significantly higher than those from "S" strain ( $P<0.05$ ). The growth performance of fish decreased with increasing dietary SBM level and no significant differences were observed among all treatments ( $P>0.05$ ) within strain. There was no interaction of strain by diet ( $P>0.05$ ) on growth of the two type of largemouth bass. The results indicated that northern subspecies have superior growth compared to southern subspecies in current experimental setup.

## EFFECT OF POLYPHOSPHATE TREATMENT ON THE QUALITY OF BAKED OR MICROWAVED CATFISH FILLETS

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Frozen fish fillets designed to be baked in the home oven have been one of the major ways fish are consumed in the US. Examples include frozen salmon, tilapia, pollock, and cod with different types of pre-treatment such as precooked, marinated, or breaded and par-fried products. However, frozen catfish fillets are a relatively small portion of the baked and/or microwaved market.

The first objective of this study was to compare the properties of raw frozen catfish fillets baked in a convection oven or cooked in a microwave. The second objective was to evaluate changes in properties as a consequence of treatment with a commercial polyphosphate blend (IQF).

Both fresh and IQF catfish samples were purchased from a commercial Mississippi catfish processor and stored frozen. Fillets (5-7 oz) were trimmed and cut vertically into three pieces, each weighing approximately 50 g. For each treatment, six fillet pieces were used. Sample analysis included weight loss, proximate content, color (CIE L\*a\*b\*), pH, mechanical texture (hardness), and lipid peroxidation (TBARS) measurements.

Significantly greater moisture retention properties were observed for IQF samples, regardless of cooking treatment, relative to the comparable fresh samples, which did not contain polyphosphate. A large cooking loss of ~30% correlated to a reduced moisture content was observed for microwaved samples, with 9.4% moisture loss relative to 4.5% for baked fresh samples. All fresh fillets had a significantly greater amount of red and yellow color, on both surface and cross-section surface, relative to the comparable IQF samples. All cooked fresh fillets were significantly harder (1.2-1.8 times) than IQF fillets, with microwaved samples showing greater hardness than baked samples.

This study will be used to develop frozen catfish products that can be cooked in a home oven or microwave oven.

## INTERACTIONS BETWEEN TIDES, CURRENTS, AND WATER QUALITY IN THE DAMARISCOTTA ESTUARY

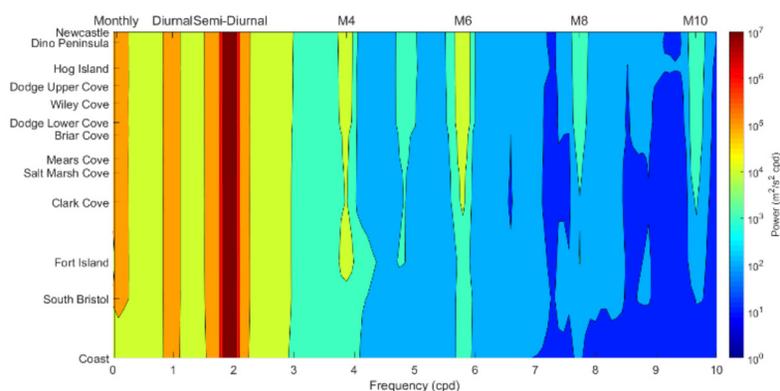
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The Damariscotta estuary in downeast Maine houses over one hundred acres of American oyster aquaculture, as well as several acres of blue mussel and seaweed aquaculture, and it is of economic importance to the region. The ecosystem of Damariscotta could be significantly altered by climate change as increasing temperatures, increased precipitation, and rising sea levels affect the transport of particulate matter. This project explores the spatial and temporal variability of water level and current velocity cycles of the estuary, and how they are correlated to measures of water quality such as turbidity, salinity, pH, and oxygen concentration. Over a period of time from July to November 2016, pressure data were collected from twelve points along the river, which were used to compute the tidal elevation time series. In addition, velocity current, water quality, and wind were obtained from a network of buoys.

A statistical analysis was used to study tidal phase propagation and amplification along the river. It was found that constrictions near Fort Island and Clark Cove cause reflecting overtides that contribute to tidal asymmetry, and that bottom friction from shallow water in the northern region of the river is responsible for higher frequency resonance waves. The overtides result in a net volumetric flow that distributes particulate matter throughout the river. The enclosed figure shows how these overtides become larger in amplitude near the head of the river.

The overtides and tidal asymmetry have an effect on measures of water quality in the river. For example, turbidity is highest during the low tide of the day and salinity is highest during high tide. Low water levels and slow currents near the head of the river result in greater subtidal variability of particulate concentration, with high and low frequency oscillations carrying equal magnitude. In addition, severe rainstorms have a flushing effect on the river, reducing turbidity and salinity but increasing acidity for several days after the storm event. A thorough understanding of Damariscotta's material transport cycle will enhance prediction of the effects of climate change on the health of the aquaculture industry.



Spectral density map of Damariscotta

## INTEGRATING MUSSEL *Mytilus edulis* AND KELP *Saccharina latissima* LONGLINE CULTURE STRUCTURES AND MANAGEMENT

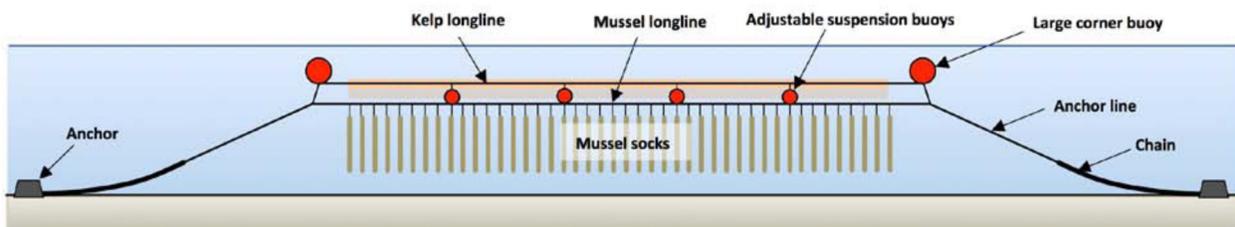
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Mussel (*Mytilus edulis*) farming and sugar kelp (*Saccharina latissima*) farming have been two of the fastest-growing sectors of marine farming in the Northeastern U.S. over the past ten years. Given that both of these crops are individually being grown on the same basic longline structures on private leases in public waters, it makes sense to integrate the cultivation of these crops for several reasons; (1) better space utilization of limited permitted sites – “3D farming”, (2) shared use of the capital costs of expensive anchors, lines, buoys, (3) better risk management via crop diversification, (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 (i) improved water quality, (ii) provision of structure resulting in nursery and foraging habitat for other species, and (iii) a sustainable seafood supply.

We describe the research that started in October 2016 and is intended to advance marine aquaculture over the next two-years by developing (i) innovative gear designs that integrate two different crops into an offshore lease area (Figure 1), (ii) new engineering and *in-situ* trials to make offshore longline aquaculture more efficient and safer for protected species, (iii) methodology for efficient management and harvest of a dual-crop culture system. We are particularly interested in forming a working group (a community of stakeholders) with an interest in determining the most effective outreach materials, and in helping to design a workshop dedicated to resolving technical and regulatory issues.

**Figure 1. A double longline design for integrating kelp with mussel culture.**



## CRYOPRESERVATION OF SPERMATOZEUGMATA (SPERM BUNDLES) FROM ENDANGERED VIVIPAROUS GOODEID FISHES

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The family Goodeidae comprises 38 viviparous fishes distributed throughout Mexico and 4 oviparous species that inhabit the southwestern United States. More than 22 of these species have been reported as endangered. Sperm cryopreservation is an effective tool for conserving genetic resources of imperiled populations, but is especially challenging for use with live-bearing fishes. Sperm of goodeids are usually packed in spermatozeugmata (bundles), which are difficult to dissociate without damaging the sperm. In this study we evaluated cryopreservation of spermatozeugmata. *Xenotoca eiseni* was used as a research model to develop a protocol, which was subsequently tested with two other goodeids, *Goodea atripinnis* and *Ataeniobius toweri*. Sperm quality was evaluated with an activating solution (NaCl-NaOH solution at 300 mOsmol/kg and pH 11.8) and counting of activatable bundles (AB) expressed as percentage AB (%AB) and motility duration of individual sperm at 200-x magnification. Post-thaw %AB was tested by using as cryoprotectants methanol, dimethyl sulfoxide (DMSO), and glycerol at 5, 10, and 15%, equilibration exposure times of 10, 20, 40, and 60 min, and cooling rates of 5, 10, 20, 30, and 40 °C/min. Sperm bundles maintained their packed form (Figure 1) after thawing (before activation). A combination of 20 min equilibration in 10% DMSO with cooling at 10 °C/min yielded the highest post-thaw %AB ( $91 \pm 3\%$ ) and duration ( $140 \pm 18$  sec). Spermatozeugmata concentrations of  $4 \times 10^6$ /ml,  $2 \times 10^6$ /ml,  $4 \times 10^5$ /ml, and  $4 \times 10^4$ /ml yielded no significant differences in post-thaw %AB or duration. Extender solutions at 300 mOsmol/kg had significantly higher post-thaw %AB and duration than did those at 200, 250, 350, or 400 mOsmol/kg. Of these extenders, Hanks' balanced salt solution (HBSS) had the highest %AB ( $71 \pm 2\%$ ) and duration ( $177 \pm 33$  sec) at 72 h after thawing compared to phosphate-buffered saline (PBS) ( $28 \pm 9\%$ ,  $99 \pm 19$  sec) and NaCl (0%, 0s). A specific protocol (10% DMSO, 20-min equilibration, 10 °C/min cooling,  $2 \times 10^6$  ml spermatozeugmata, and 300 mOsmol/kg HBSS) yielded post-thaw values of  $96 \pm 9$  %AB with  $814 \pm 14$  sec duration for *Goodea atripinni*, and  $66 \pm 2$  %AB with  $726 \pm 25$  sec duration for *Ataeniobius toweri*. This is the first study of cryopreservation of sperm within spermatozeugmata for viviparous fishes and provides a basis for establishment of germplasm repositories for goodeids and other livebearers.

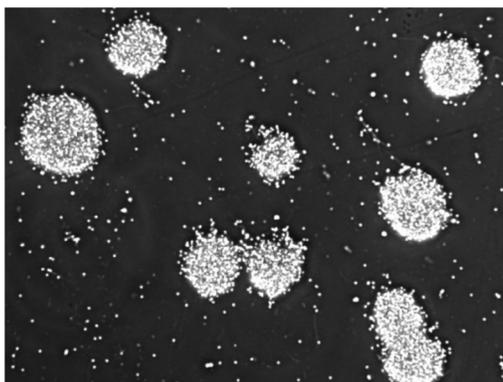


Figure 1. Post-thaw spermatozeugmata of *Xenotoca eiseni* at 200-x magnification.

## ESTABLISH A FLOW CYTOMETRY ANALYSIS OF GROUPEL LYMPHOCYTE DIFFERENTIATION UNDER IMMUNE STIMULATION

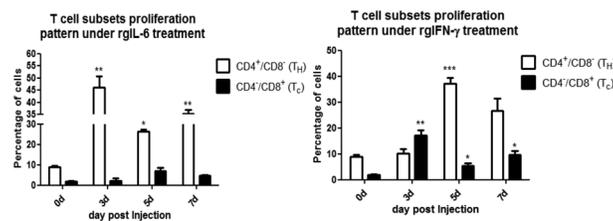
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Grouper (*Epinephelus* sp.) are high economic value aquaculture species around subtropical regions especially in Southeast Asia. However, the disease control is still a serious problem in the recent years. To control the disease, using immunoprophylaxis method, such as vaccine is considered as an effective method to prevent disease outbreak and transmission. An ideal immune protection is contributed by humoral or cell-mediated immune responses mediated by helper T lymphocyte ( $T_H$ ,  $CD4^+/CD8^-$ ) and cytotoxic T lymphocyte ( $T_C$ ,  $CD4^+/CD8^+$ ), respectively. Humoral immunity predominately triggers neutralization of extracellular pathogens whereas cell-mediated immunity is mainly responsive to kill extracellular pathogens and virus. However, in teleost few tools were developed to evaluate the differentiation of these lymphocytes, so the effectiveness of vaccine is hard to detect. Flow cytometry analysis for immune cell differentiation is already used in mammals for years. It can detect multi-parametric cell characteristics simultaneously.

This project is aim to setup this technique for groupers, thus the antiserum for CD4 and CD8 were prepared and the specificity and sensitivity were also evaluated. Cytokines which induce  $T_H1$  and  $T_H2$  pathway, such as interferon- $\gamma$  and interleukin-6 were used as standard to evaluate efficiency of established flow cytometry system in this project; finally, apply this system to the fish inoculated with different adjuvant formula and indicated that cytokine additives can control the direction of immune response and might increase the efficacy of fish vaccine.

FIGURE 1. Grouper lymphocytes treated with rgIL-6 and rgIFN- $\gamma$  were double stained with CD4/CD8 and analyzed by flow cytometry.



## ANALYSIS OF MEETING ABSTRACTS AS AN INDICATOR OF EMERGING ISSUES IN STRESS AND CULTURED AQUATIC SPECIES

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New ideas in science are often introduced at society meetings months or years before those ideas appear in published literature. While not peer-reviewed, meeting abstracts reveal concepts that dominate the conversation in knowledge areas at society meetings. Meeting abstracts from recent WAS and AFS meetings were reviewed in an attempt to determine what concepts dominate the discussion of stress in cultured aquatic species. Generally, “stress” appears in the title of about 1.5% of abstracts from a society meeting, but the word “stress” appears in about 6.8% of abstracts. The word “stress” appears only once in 57% of abstracts where the word appears at least once (Figure 1).

In all but one case ( $n=32$ ), when the word “stress” was included only once in an abstract, the presentation was not about stress. In 79% of abstracts that included the word stress, the subject of stress was introduced as a justification for the work, not as the focus of the presentation. In only 39% of abstracts that included the word stress, was there a comparison of a quantitative stress response among treatment and control groups.

Although plasma cortisol was a common quantitative stress response reported in presentations, other common quantitative stress responses included upregulated miRNAs, glutaredoxins, upregulation of immune response genes, upregulation of genes involved in oxidative stress (e.g. SOD), serum glucose, and HSP70. Concepts most frequently associated with stress, in descending order, included temperature, diet, immunology, the environment, water quality, and oxygen level. While the sources of stress in cultured fish addressed in society presentations are as one might expect, new molecular and cellular techniques are leading to the quantification of stress responses beyond simple measurements of serum cortisol, glucose, or chlorides. Measuring upregulation and downregulation of genes known to code for proteins involved in stress responses appears to be an emerging technique for understanding the role of stress in cultured aquatic organisms.



**Figure 1. Frequency distribution of the number of occurrences of “stress” in abstracts where the word appears**

## **GROWTH AND HEALTH RESPONSES OF JUVENILE LARGEMOUTH BASS *Micropterus salmoides* FED PRACTICAL ANIMAL-BASED AND PLANT-BASED DIETS WITH DIFFERENT LEVELS OF VITAMIN A**

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Largemouth bass (LMB; *Micropterus salmoides*) are economically important as sport fish as well as food fish for ethnic markets in the United States. Interest in commercial culture of this species is driven by high demand and market prices. Like other carnivores, LMB perform well on diets high in fish meal. However, environmental and cost concerns of using fish meal have led to diets with reduced levels of fish and other animal proteins. When protein sources are changed, fish performance may be affected by changes in amino acid composition and availability. However, other nutrient requirements may also be affected. For instance, vitamin A has well-known metabolic interactions with protein in vertebrates. The vitamin A requirement of LMB is unknown, so we identified a range of concentrations that bracket the known requirements of related species. We supplemented these 5 levels of vitamin A into five animal-based diets and five plant-based diets, and tried to equalize the levels of all other nutrients in the diets.

Feed-trained LMB (4 g) were stocked at 20 fish per 4 replicate tanks per treatment in a recirculating system. Fish were fed to satiation twice daily for 8 weeks. Although the goal was to run the trial until the fish had a 500% weight increase, the study had to be terminated at 8 weeks due to low survival in some groups so that we would have enough fish to analyze health parameters and body composition indices. Growth, survival, cannibalism index, body composition indices, and immune parameters were measured to assess diet effects.

Survival (93-100%), feed intake, FCR (1.1-1.2) and cannibalism index did not differ among treatments for the animal-based diets. Weight gain, hepatosomatic index, and hematological parameters were higher in fish fed diets with at least 6060 IU vitamin A/kg diet compared to diets with the basal level (940 IU). Lysozyme was higher in fish fed a slightly higher level (6349 IU/kg diet) of vitamin A compared to the control. In the plant-based diets, weight gain of fish fed diets with 3190 IU or more of vitamin A was higher than that of fish fed diets with 1010 IU or less. Cannibalism was also higher and survival was lower in fish fed diets with higher levels of vitamin A. Feed intake was lower in diets with 3190 IU or more vitamin A compared to fish fed diets with 1010 IU or less. However, feed conversion (1.1-1.6) was similar among treatments. Blood parameters were generally higher in fish fed diets with higher levels of vitamin A. Lysozyme was higher in fish fed the diet with 1010 IU vitamin A/kg diet compared to the control (740 IU/kg diet).

Overall, supplemental vitamin A above basal levels had a few beneficial effects in LMB fed the animal-based diets. However, the more pronounced growth stimulation associated with vitamin A supplementation in fish fed the plant-based diets was offset by increased cannibalism and lower survival. It must be noted that on a commercial farm, LMB of the size we used in this study would have been graded several times over an 8-week period to maintain uniform sizes and reduce cannibalism. Therefore, further studies are needed to determine whether higher levels of vitamin A in plant-based diets would be economically beneficial under industry conditions.

## AN OVERVIEW OF BINATIONAL EFFORTS TO USE CULTURE AND STOCKING TO RESTORE AN EXTIRPATED SPECIES *Coregonus hoyi* TO LAKE ONTARIO

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Bloater, *Coregonus hoyi*, is one of four species of deepwater cisco that was extirpated from Lake Ontario in the last century. Historically, these four species formed the backbone of the lake's deep water prey fish community. A bi-national team consisting of the Ontario Ministry of Natural Resources and Forestry (OMNRF), the New York State Department of Environmental Conservation (NYSDEC), the U.S. Fish and Wildlife Service (USFWS), the U.S. Geological Survey and the Great Lakes Fishery Commission, believes that conditions in the lake have evolved to the point that re-establishment of the species is now possible. Restoration efforts are being guided by the following strategies: develop a reliable source of gametes, develop husbandry practices, increase culture capacity, assess and optimize stocking effectiveness, understand ecological role, and increase public awareness. This presentation provides an overview of progress on several OMNRF-led strategies.

The OMNRF Fish Culture Section staff agreed to co-lead efforts to develop husbandry practices because it had 30 years of experience culturing Lake Whitefish, *C. clupeaformis*, a related species. Since it first began culturing Bloater in 2011, much has been learned, and survival rates during both the incubation and early rearing phases have increased dramatically. To date, the effects of diet and temperature on growth and survival have been examined.

In 2011, OMNRF Fish Culture Section staff also began developing a captive broodstock. The decision to do so stemmed from the challenges being experienced by the USFWS collecting sufficient numbers of gametes from wild populations in Lake Michigan during the winter months. To date, two year classes of broodstock have matured and produced viable gametes. Unfortunately, fewer fish matured than expected and a lack of synchrony between the sexes limited the number of fertilized eggs collected. Staff have partnered with the University of Windsor to investigate the potential of hormone induction using LHRHa to stimulate spawning as well as cryopreservation to preserve sperm until females reach peak spawning. Preliminary results from hormone injection show promise.

In 2014, OMNRF science staff, in collaboration with NYSDEC staff and others, initiated the use of acoustic biotelemetry to describe post-release movement, behaviour, and survival of stocked Bloater. Initial results are encouraging.



## AN ANALYSIS OF ONE BILLION DOLLARS OF AQUACULTURE GRANTS MADE BY THE UNITED STATES FEDERAL GOVERNMENT FROM 1990 TO 2015

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While the United States (U.S.) contributes only a small portion to global aquaculture production, it has made significant public investments over the past few decades into aquaculture research and extension, which support industry development. Federal programs and policies have played a significant role in moving the field of aquaculture forward, in part by awarding internal and external research grants to federal agency staff, academic institutions, companies, and non-governmental organizations. Examples of federal grants programs include the National Oceanic and Atmospheric Agency (NOAA) Saltonstall-Kennedy and Sea Grant grants, U.S. Department of Agriculture National Institute of Food and Agriculture grants, National Science Foundation grants, and Small Business Innovation Research grants.

This presentation will discuss the findings of an analysis of nearly 3,000 aquaculture grants awarded by 10 federal agencies from 1990 to 2015. Adjusting for inflation, federal agencies have awarded over one billion dollars to aquaculture projects over the past quarter century, with the USDA and NOAA as the lead agencies overseeing aquaculture. After reviewing summary statistics such as federal aquaculture grants by aquatic organism class (Table 1), we will discuss patterns in domestic aquaculture funding over this time period, by federal agency, location, discipline, and aquatic organism family. We will put these trends into context by comparing funding patterns with federal agencies' strategic plans that describe priorities and focus research funding. Finally, by analyzing past research activities, we hope to identify opportunities for future research, research funding, and policy.

Table 1. US federal aquaculture grants by aquatic organism class from 1990 to 2015.

Organism Class	Percent of total grants (n = 2957)	Total grant funding (million 2015 US dollars)
Fish	45%	452
Shellfish	17%	116
Algae	12%	340
None listed <sup>a</sup>	11%	126
Crustaceans	6%	155
Other <sup>b</sup>	4%	39
Multiple classes <sup>c</sup>	4%	40

<sup>a</sup> "None listed" means not enough information was provided to assign a category.

<sup>b</sup> "Other" category includes alligators, non-algae aquatic plants, bryozoan, coral, echinoderms, limpet, jellyfish, marine invertebrates, snails, sponge, squid, and whelk.

<sup>c</sup> "Multiple classes" means that more than one organism class was listed in the grant.

## AQUACULTURE, FOOD SYSTEMS, AND PUBLIC HEALTH

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This presentation will address how the aquaculture industry and NOAA can help meet public health goals for seafood consumption by working with supply chain partners and other federal agencies that focus on seafood access and use across the food system.

Americans purchase seafood based on taste preference, cost, and health, and a variety of social factors such as age, income, and gender also influence purchasing. In the U.S., health professionals recommend adults eat 2-3 seafood meals per week of a wide range of species, (Dietary Guidelines for Americans 2015). Research indicates that eating fish-meals improves childhood development and reduces the risk of cardiac death in individuals with pre-existing coronary heart disease. Americans, however, consistently eat much less seafood than recommended, with the lowest rates of consumption among pregnant and breastfeeding women, children, the elderly, and individuals with low incomes (Table 1).

The aquaculture industry and NOAA play important roles in increasing aquaculture production, however production alone is not sufficient to meet goals for seafood consumption. Aquaculture producers and NOAA need to work across the supply chain and in other parts of the food system to advocate for improving consumer access and use of seafood. For example, supplemental nutrition assistance programs funded by the Farm Bill and managed by the US Department of Agriculture can be better leveraged to improve food access among at risk groups. To date, only the domestic catfish industry has engaged in these programs, and only sporadically. Other opportunities include better connection with the White House “Local Food, Local Places” initiative, farm to school programs, engaging with institutional food procurement programs, and more interaction between aquaculture producers, consumer groups, public health professionals, and civil society organizations. These concepts and ideas will be discussed in the presentation.

Table 1. Per capita mean intake (g/wk) of seafood by Americans from 1988 to 2010.

Study	Men (+19y)	Women (+19y)	Year range
NHANES estimates:			
NCI 2015	138.9	99.2	2007 - 2010
Papanikolaou et al 2014	140.1	101.2	2003 - 2008
Tran et al 2013	128.7	86.3	1999 - 2006
Wang et al 2010	115.5	84.7	1999 - 2004
Wang et al 2010	141.4	100.8	1988 - 1994
Dietary Guidelines for Americans*	255 - 284	227 - 255	2015 - 2020

\* <https://health.gov/dietaryguidelines/2015/>

## EFFECTIVENESS OF LARGE SCALE LARVAL SEEDING AND PARENTAGE ANALYSIS FOR BAY SCALLOP *Argopecten irradians* RESTORATION IN CHARLOTTE HARBOR, FLORIDA

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The bay scallop *Argopecten irradians* is a filter feeding bivalve that lives in seagrass beds and has cultural significance for generations of Floridians. Bay scallop population levels can also be indicative of water quality. Florida's bay scallops once supported a statewide commercial fishery. However, populations declined due to various factors. In 1994, commercial harvest was prohibited and recreational harvest was severely restricted. In the late 1990s researchers placed cultured scallops in benthic cages to form large spawning aggregations in Crystal River/Homosassa, Anclote Estuary, and Tampa Bay. By 2002, these restoration efforts had contributed to increased scallop populations, recreational harvest area expansion, and a lengthened season. Restoration efforts are ongoing in many locations. In Charlotte County, bay scallop restoration work has been conducted for the last 4 years by Florida Sea Grant, local government, and the West Coast Inland Navigation District. Aquaculture was used for larval seeding, juvenile production and release, and dock caging of spawning aggregations. These restoration efforts are increasing, and the current project is examining the effectiveness of large scale larval seeding as a restoration method in Charlotte Harbor. Part of this effort is the development of a method for parentage analysis to document restoration success and genetic diversity of cultured scallop larvae.

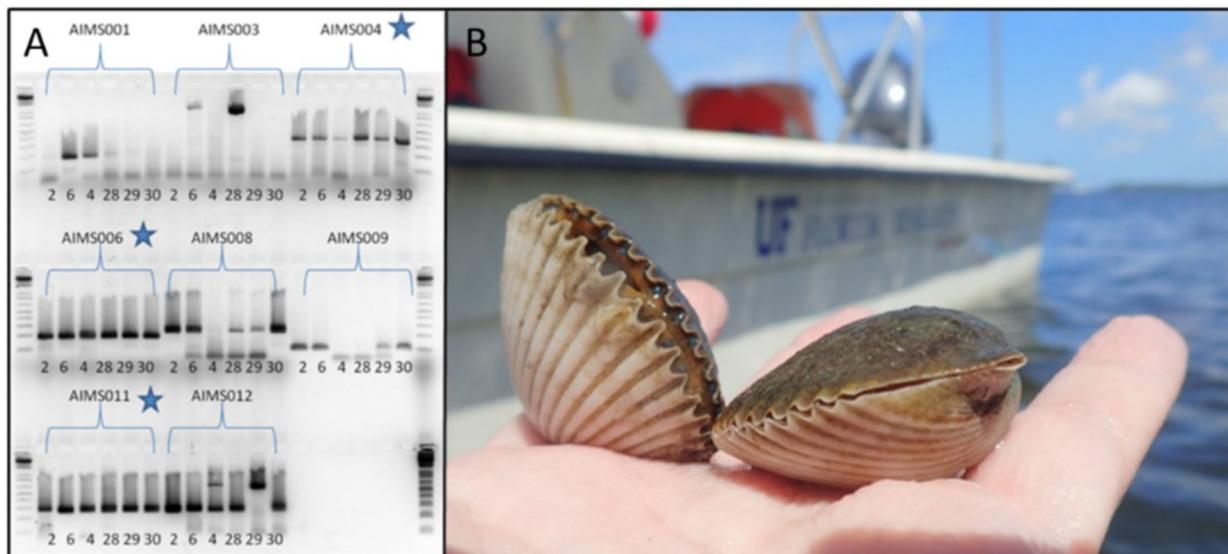


Figure 1: (A) Initial primer screening results for DNA samples from 7-day post fertilization bay scallop larvae. Primers denoted as AIMS### and individual larvae identified as numbers below lanes; (B) Two live adult scallops of eight found during a post-restoration monitoring event.

## DIETARY PROTEASE IMPROVES PRODUCTION PERFORMANCE OF COMMERCIALY FARMED PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Use of dietary protease in aquaculture has been gaining increasing attention. Recently, several studies have shown to improve performance and immune response in Pacific white shrimp when a dietary protease is added. These studies were mostly conducted in Asia where culture conditions, feeds and feeding management are starkly different than those in Americas. Therefore, there is a serious need to evaluate the effects in Latin American conditions, more specifically, under commercial farming conditions. To address this, two trials were conducted in Ecuador. The first trial was conducted for 89 days with four diets, in 12 earthen ponds (400-m<sup>2</sup>) stocked with 4,000 PL in each pond (Table 1). The second trial was conducted for 90 days in four 2-ha earthen ponds, where each pond was stocked at a density of 10 PL-m<sup>-2</sup>. Two ponds were fed with a commercial feed with 35% of crude protein. The other two ponds were fed with 28% crude protein diet supplemented with Jefo protease at 175 g MT<sup>-1</sup>.

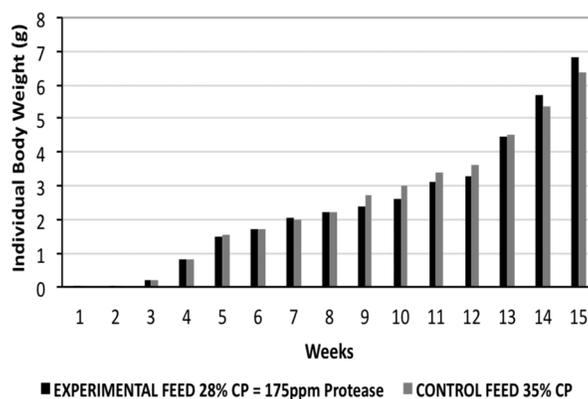
In the trial 1, despite no differences in survival (52%-71%), final biomass (kg ha<sup>-1</sup>), FCR, and SGR among the treatments, weight gain was significantly higher in shrimps fed high marine protein diets with protease (diet 2, 10.0 g ±1.2) than those fed the same diets without the protease (6.4 g ±0.4). As expected, a significantly higher protein efficiency ratio (PER) was observed in shrimps fed the low protein diet with protease (diet 4, 2.4 ±0.2) than those fed diets 1, 2 or 3 (1.7 ±0.1, 1.9 ±0.1 and 1.9 ±0.2, respectively).

In the trial 2, despite no difference in growth (Figure 1), there were large differences in size distribution at harvest between the treatments ( $P<0.05$ ) that significantly impacted the financial outcomes. It showed importance of dietary protease in commercial farming conditions to bring significant cost savings and profit to the farmers even in low-density farming as practiced in Americas. Our findings show that either decreasing the digestible protein or the crude protein level in the diet can bring significant cost savings for both feed manufacturers and farmers when supplemented with Jefo protease.

Table 1. Diets of the trial 1

Diets	Diet A	Diet B	Diet C	Diet D
CP	35,5%	35,5%	35,5%	<b>29,4%</b>
Digestible CP	32,7%	32,7%	32,4%	26,9%
Marine CP	27,7%	27,7%	<b>22,7%</b>	<b>20,8%</b>
Vegetal CP	67,7%	67,7%	72,0%	74,6%
Protease (mg kg <sup>-1</sup> )	0	175	175	175
Cost (\$ MT <sup>-1</sup> )	919.00	923.00	886.00	830.00

Figure 1. The growth of Pacific white shrimp in two treatments



**DEVELOPMENT OF FORMULATED FEED FOR MANGROVE CRAB *Scylla spp.***

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Although the soft shell crab industry has been going for more than 20 years, little research has been done regarding formulated feeds and there is an urgent need to replace “trash fish” with manufactured diets. The present study evaluated a compounded feed for its acceptance and attempted to understand the preferred physical form of formulated feed by mangrove crabs, also commonly known as mud crabs, for better feed utilization. Wild caught crabs, sized 60-80g, were collected from one estuary and delivered to a farm in Ranong, Thailand. A total of 600 crabs were randomly selected and placed into individual boxes with 150 boxed crabs stocked into each of four PVC pontoon rafts. Three experimental diets and one control diet were randomly assigned to the crabs across the four rafts. One group of 150 crabs were fed with a high fish meal content formulated diet, another 150 with high soybean meal diet, 150 with a commercial shrimp feed and 150 crabs were fed a traditional “trash fish” diet as the control group. Crabs were fed 3% of body weight split between two feedings a day in a 45 day feeding trial.

The commercial shrimp diet provided significantly better growth than the other diets, with an average growth rate of 0.89 g/day. The trash fish control was not significantly different from the high fishmeal content diet, 0.80 g/day. The high soy content diet was significantly lower, 0.72g/day. However, the soy based diet had the highest survival rate (95%) versus shrimp feed at 91% and trash fish and high fishmeal at 85% each.

Additional trials are planned in hopes of further increasing growth rates and survival. Results may be available in time for presentation at the conference.

## CULTIVATION OF *Lophiosilurus alexandri* JUVENILE UNDER DIFFERENT LIGHT CONDITIONS

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The survival of several species in captivity depends on their adaptation to environmental conditions, it is essential to adapt the parameters as the biological characteristics of the species. The objective of this study was to evaluate the influence of different luminosities on growth performance, plasma cortisol level, behavior and pigmentation of skin in juvenile pacamã, *Lophiosilurus alexandri*.

The fish were submitted to the following light intensities: 0, 218, 278 and 459 lux. The experiment was conducted for 75 days in recirculation system with temperature control, aeration and controlled photoperiod. The pacamãs were distributed in aquariums with a volume of 35 L, a density of approximately 0.28 individuals of L. To assess the weight is measured-performance, standard length and total weight gain, feed intake, feed conversion, biomass, specific growth rate and survival. Also, was evaluated the swimming behavior, skin pigmentation and plasma cortisol level.

The different luminosities not interfere in growth (Table1), kept low the cortisol levels in plasma (Table 2), and not influence in pigmentation (Table 3). However, it is recommended cultivation in no light environment because it reduces feed intake and improves feed conversion, which are characteristics desirable production.

Table 1 - Performance of pacamã juveniles submitted to different levels of luminosity

Parameters	Luminosity levels (lux)				CV (%)
	0	218	278	459	
Final weight (g)	18.45±4.54	18.65±6.38	18.13±6.11	17.73±5.88	2.00
Weight gain (g)	12.91±4.76	13.16±6.43	12.30±5.82	11.51±5.75	4.37
Standard length (cm)	9.36±0.85	9.49±1.11	9.36±1.07	9.17±1.06	1.41
Total length (cm)	11.02±0.94	11.15±1.32	10.96±1.16	10.78±1.28	2.20
Feed consumption (g) <sup>1</sup>	124.00±5.87	142.7±4.20	175.1±9.50	167.0±12.21	15.31
Feed conversion (g g) <sup>2</sup>	0.96±0.25	1.16±0.34	1.33±0.10	1.45±0.37	17.39
Biomass (g)	184.50±2.51	186.50±1.71	181.30±1.70	177.30±3.76	5.91
SGR (%)	17.37±6.38	17.65±8.67	16.91±7.65	16.24±7.66	3.61
Survival (%)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	0.00

<sup>1</sup>Polinomial effect (p<0.05):  $Y = 12.252 + 0.0199x - 0.7007x^2$ ;  $r^2 = 0.77$

<sup>2</sup>Linear effect (p<0.05):  $Y = 1.0059 + 0.049x$ ;  $r^2 = 0.92$

Table 2 - Means values of cortisol in pacamã juveniles submitted to different levels of luminosity.

Parameters	Luminosity levels (lux)				CV (%)
	0	218	278	459	
Cortisol ( $\mu\text{g dl}^{-1}$ ) <sup>1</sup>	3.14±1.06	2.49±0.85	2.43±0.89	2.78±0.81	11.99

Table 3 - Mean values of a \*, b \* and L \* (luminosity), C \* ab (Chroma), Hab (tone) and W \* (whiteness) of the skin of the juveniles of pacamã submitted to different levels of luminosity.

Parameters	Luminosity levels (lux)				CV (%)
	0	218	278	459	
<b>Head</b>					
a*	4.24±2.23	3.39±0.64	3.33±0.64	4.24±0.71	11.39
b*	7.23±2.97	6.93±2.14	6.90±2.11	7.23±2.18	6.64
L*	45.11±6.47	47.08±1.76	46.02±1.64	45.11±1.69	1.75
C* <sub>ab</sub>	8.58±3.21	7.55±1.84	7.75±1.90	7.38±1.78	6.83
H* <sub>ab</sub>	1.02±0.19	1.06±0.19	1.08±0.15	0.99±0.25	11.62
W* <sub>ab</sub>	45.21±1.52	46.67±1.65	45.44±1.67	44.38±6.88	1.40
<b>Dorsum</b>					
a*	2.27±0.72	1.24±0.88	1.61±0.69	1.86±0.67	16.99
b*	11.63±3.48	11.27±3.55	10.19±3.35	11.63±3.53	7.53
L*	52.29±3.49	53.51±3.01	52.45±3.35	52.29±3.07	1.11
C* <sub>ab</sub>	11.94±3.21	11.15±3.79	10.35±3.22	11.88±3.44	8.06
H* <sub>ab</sub>	1.33±0.20	1.40±0.09	1.43±0.13	1.86±0.10	16.18
W* <sub>ab</sub>	51.29±2.49	51.94±2.67	51.23±3.21	50.72±3.12	0.98
<b>Tail</b>					
a*	2.04±0.66	1.94±0.93	2.73±2.52	2.34±0.65	15.69
b*	8.76±3.15	8.76±3.37	8.88±3.31	8.56±3.32	6.37
L*	45.04±2.20	45.53±2.19	44.12±2.31	45.04±2.36	1.61
C* <sub>ab</sub>	10.25±3.13	8.77±3.05	9.50±3.57	8.95±3.01	7.10
H* <sub>ab</sub>	1.50±0.03	1.49±0.06	1.44±0.23	1.48±0.19	15.35
W* <sub>ab</sub>	44.41±2.14	45.02±2.10	43.21±2.25	44.24±2.05	1.70

## LARVICULTURE OF *Lophosilurus alexandri* IN DIFFERENT COLORS OF AQUARIUM AND LUMINOSITIES

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The well-being promoted by the environment, in particular those that interfere with visual perception, are of paramount importance in fish culture optimization. Knowing that environmental parameters outside the ideal range may increase the negative effect on the performance of production, the objective of this study was to determine the effect of aquarium color and brightness in the larvae of pacamã *Lophosilurus alexandri* rearing.

The larvae were subjected to two aquarium colors (black and white) and two luminosities: no incidence of light (0 lux), when the tanks were covered with black canvas and incidence of light (88 lux), when the tanks remained open. The experimental design was completely randomized with five replications in a factorial 2 x 2. The experiment was conducted for 25 days. The pacamãs were distributed in aquariums with a volume of 08 L, a density of 60 fish of aquarium. We evaluated weight, standard length and total biomass and survival, and skin pigmentation.

It was observed that black tanks are not recommended for creating pacamã larvae therefore adversely influenced survival rates (Table 1). The interaction between black and aquariums without light reduced the biomass and the Fulton's condition factor (Table 2). It is indicated for post larvae cultivation pacamãs the use of white aquariums, and luminosities between 0 and 88 lux, it does not affect the performance of the larvae.

Table 1 - F values, coefficient of variation, means and standard deviation obtained from the pacamã performance at the 25th day of experiment. C = Aquarium Color; L = Luminosity; SL = Standard length; TL = Total length; W= weight; BIO= biomass; SUR = survival; K = Fulton's condition factor.

F values	SL (mm)	TL (mm)	W (mg)	BIO (g)	SUR (%)	K (%)
Aquarium color	0,15 <sup>ns</sup>	0,19 <sup>ns</sup>	1,56 <sup>ns</sup>	7,71*	7,21*	6,98*
Luminosity	0,90 <sup>ns</sup>	0,24 <sup>ns</sup>	0,17 <sup>ns</sup>	0,00 <sup>ns</sup>	0,01 <sup>ns</sup>	3,79 <sup>ns</sup>
Interaction C x L	0,23 <sup>ns</sup>	0,15 <sup>ns</sup>	2,76 <sup>ns</sup>	8,64*	4,46 <sup>ns</sup>	10,72*
CV (%)	4,61	4,22	15,91	30,60	36,40	14,60
Aquarium color						
White	18,50±0,77	23,80±0,61	159,50±12,83	7,44±0,68	86,83±4,99a	2,50±0,37
Black	18,70±1,04	23,60±1,42	153,10±35,64	5,48±2,59	61,04±34,94b	2,27±0,31
Luminosity						
0 lux	18,40±0,98	23,50±1,12	150,70±29,04	5,71±2,69	62,37±33,95	2,37±0,52
88 lux	18,80±0,79	24,00±0,92	161,70±22,42	7,23±0,86	85,49±12,88	2,41±0,13

Means followed by equal letters in the lines do not differ from each other, by Tukey's test, at 0.05 probability. ns= Not significant; \*= Tukey test at 0.05 significance.

Table 2 - Means and standard deviations of the interactions between aquarium color and brightness of the variables biomass and Fulton condition factor.

Luminosity	Aquarium color	
	Black	White
<b>Biomass</b>		
0 lux	3,64±1,20Bb	7,41±0,91Aa
88 lux	7,00±1,19Aa	7,47±0,34Aa
<b>Fulton's condition factor</b>		
0 lux	2,03±0,26Bb	2,68±0,47Aa
88 lux	2,48±1,19Aa	2,33±0,13Aa

Means followed by the same letters, lowercase in the lines, and uppercase in the columns do not differ by Tukey test at 0.05 probability.

## **BUILDING ON STOCK ENHANCEMENT SUCCESS IN SOUTHERN CALIFORNIA: CASE STUDY OF A DEVELOPING CALIFORNIA HALIBUT *Paralichthys californicus* REPLENISHMENT PROGRAM**

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Hubbs–SeaWorld Research Institute (HSWRI) has been involved in the stock enhancement of marine finfish in southern California, particularly white seabass (*Atractoscion nobilis*), for over three decades. In recent years, there has been increasing interest in applying the expertise gained from this to other depleted species. Here, the development of a new angler-funded replenishment program in this region is outlined.

Eighteen candidate species were initially assessed based on management agency and stakeholder input, and a selection procedure involving a broad set of stocking suitability criteria was developed. California halibut (*Paralichthys californicus*) emerged as a clear front-runner, and HSWRI began focusing research efforts on the technical and regulatory requirements of stocking halibut. An extensive review of flatfish stock enhancement, including large-scale programs for the congeneric Japanese flounder (*Paralichthys olivaceus*) and experimental releases of species in the US and Europe, was undertaken to identify global stocking practices, successes and information gaps. A quantitative model was also parameterized to represent halibut life-history and fishery characteristics in southern California, to explore population responses to various release strategies and scenarios (e.g. size-at-release, direct release vs. conditioning, economic considerations).

The availability of such diverse background research provides a unique basis for development of the current program—both the literature and theoretical analyses suggest that stocking of California halibut has the potential to be successful. The next phase of our research is to empirically test these findings through a series of experimental releases, and a preliminary broodstock management plan (conservatively assuming spatial population structure) has been put in place in support of this.

## STUDY ON EFFECT OF POLLUTION ON GENOTOXIC DAMAGE IN *Cirrhinus mrigala* and *Catla catla* FROM RIVER CHENAB

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Industrial development, expansion of urban populations and increased coverage of industrial, domestic water supply and sewerage give rise to larger quantities of municipal wastewater. Disposal of toxic sewage wastes with large volume of water could reduce biological oxygen demand to the lethal level by removing entire oxygen from the water body. Some very toxic chemicals are released into the lakes, streams and rivers e.g., compounds of mercury, Zinc, Lead and Copper etc. causing death of aquatic populations even at very low concentrations. This can cause metabolic activation giving rise to toxic metabolites in the nervous tissues. Comet and Micronucleus assays have been used to assess DNA damage in *Catla catla* and *Cirrhinus mrigala* collected from polluted areas. Heavy metals Cd, Cu, Mn, Zn, Pb, Cr, Sn and Hg were detected by atomic absorption spectrophotometry in River Chenab water. All physicochemical parameters and heavy metals were found beyond the tolerable limits. Comet assay showed significant ( $p < 0.05$ ) DNA damage in *Catla catla* as  $17.33 \pm 2.42$ ,  $11.53 \pm 2.14$  and  $14.17\%$  DNA in tail. Tail moment was  $10.06 \pm 2.71$ ,  $3.11 \pm 0.74$  and  $14.70 \pm 1.89$ , Olive moment was  $8.85 \pm 1.84$ ,  $3.83 \pm 0.76$  and  $7.11 \pm 0.73$ , respectively. Highly significant ( $p < 0.01$ ) damage was reported in *Cirrhinus mrigala* as  $37.29 \pm 2.51\%$ ,  $34.96 \pm 2.53\%$  and  $38.80 \pm 2.42\%$  DNA in comet tail, tail moment was  $23.48 \pm 3.90$ ,  $19.78 \pm 4.26$  and  $14.30 \pm 1.82$ , olive moment as  $16.22 \pm 2.04$ ,  $13.83 \pm 1.96$  and  $10.99 \pm 0.90$  from three different sites of the polluted area of the River Chenab. Significant ( $p < 0.05$ ) differences were reported polluted and farmed fish but non-significant ( $p > 0.05$ ) differences in farmed and non-polluted upstream. Micronucleus assay showed similar findings for single and double micronucleus induction as  $23.20 \pm 4.19$ ,  $2.80 \pm 1.07$  in *Catla catla* and  $44.80 \pm 3.73$ ,  $06.20 \pm 0.97$ /thousand cells, respectively in *Cirrhinus mrigala*. Nuclear abnormalities were found as  $6.00 \pm 0.84$  and  $09.60 \pm 1.72$ /thousand cells, respectively in both species. These findings infer that these novel fish DNA damage assays to detect genotoxicity, could be used as expedient toxicity screening of aquatic environments

## EFFECTS OF LIGHT CONDITIONS ON THE LARVAL DEVELOPMENT OF TWO CENTROPOMID SPECIES (*Centropomus undecimalis* AND *C. nigrescens*)

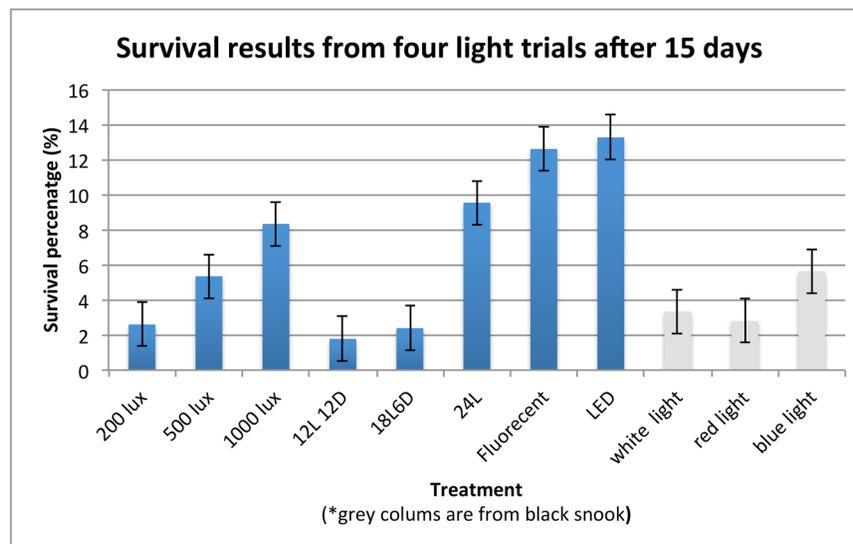
Kevan Main,\* Carlos Yanes-Roca, Nicole Rhody, Matthew Resley and Juliette Delabbio

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Through a series of four experimental trials two Centropomid species, common snook (*Centropomus undecimalis*) or black snook (*C. nigrescens*), larvae were exposed to two different light sources (fluorescent and LED), three light intensities (200, 500 and 1000 lux), two photoperiods (12 hr light/12 dark, 18L/6D and 24L) and three light spectrums (white, red and blue). Larvae were exposed to varying light conditions during the first 15 days post-hatch in recirculating aquaculture systems, where environmental parameters (temperature, dissolved oxygen, pH, and salinity) were controlled and maintained at optimal conditions. Survival, growth, eye diameter, swim bladder inflation and flexion were the main parameters recorded to assess treatment effects.

In terms of survival, a highly significant difference ( $p < 0.005$ ) was found between treatments in all the experiments, except in the LED versus fluorescent experiment. The following treatments showed the highest survival: the 1000-lux treatment at 8.32%; the 24-hour light treatment at 9.54%; the LED treatment with 13.26%; and in those maintained under the blue spectrum lighting conditions at 5.29% survival.

There was no significant difference observed between the treatments with regards to growth, although trends in increased growth were observed. There was no difference in eye diameter, swim bladder inflation and timing of flexion. These results can be attributed to high variability in the size distribution and also to the fact that there is less competition for food in those tanks where survival is lower (less larvae, more food).



## **SIZING AND DESIGN OF LOW LIFT AIRLIFTS FOR RAS APPLICATIONS**

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Use of airlifts in an RAS application reduces overall energy demands as a result of their concurrent contribution to the circulation, aeration, and degassing needs. Airlifts have been conservatively designed to recirculate water at a flow rate of 1.5 ft/sec with submergence to lift ratio of 0.25. Air pumps are sized with a conservative gas to liquid ratio of 2 with a typical operational ratio of 1.3. These airlift criteria have proven robust across a wide variety pipe sizes in both marine and freshwater systems. Math relationships have been developed defining the oxygen transfer and carbon dioxide stripping rates for airlifts rate. These relationships are driven by the airflow and oxygen deficit/carbon dioxide surplus entering the lift tube. However, most sizing is operations are based on simplified feed to air volume relationships. In warm water systems utilizing exclusively blown air for gas transfer 3-4 cfm of air are required per pound of fed daily to maintain oxygen above 5 ppm and carbon dioxide below 10 ppm.

The injection depth (lift) is typically limited to 5 feet to avoid problems with supersaturated gases. This in turn limits the total lift to 15 inches with a favorable gas to liquid ratio below 1.5. These constraints limit the RAS applications to low head systems. However, the airlift technology has proven compatible with a variety of filtration components, including moving bed reactors, microscreens, and floating bead filters. Airlifts are also frequently used to remove sludge from sludge chambers associated in unpressured solids capture devices. Airlift applications in the commercial sector have be mostly successful prompting further adoption.

## IMPROVED PNEUMATIC SLUDGE HANDLING IN POLYGEYSER FLOATING BEAD FILTERS

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Sludge handling has occasionally proven problematic in both commercial RAS production systems and even in ornamental koi pond applications filtered by PolyGeysers. The PolyGeysers have the ability to store sludge across several backwash cycles. Manual sludge removal is required periodically. Many operations have employed some degree of electronic automation to perform this task. As an alternative strategies have been developed that utilize the internal PolyGeysers pneumatic backwashing to remove accumulated sludge from the filter hull. The most direct approach is the "Direct Pneumatic Discharge" (DPD) that results with the immediate removal of sludge during each backwash sequence. In this approach, the air pressure in the charge chamber is combined with the hydrostatic pressure of the sludge in an internal sludge storage basin to develop a controlled lift (typically about 25 centimeters (ten inches) just before a backwash event. This strategy produces a small amount of relatively concentrated sludge (4-6%) during each backwash cycle. DPD units are capable of operating literally for months without manual intervention, as long as air is continually delivered to the charge chamber. This technique is only applicable to constant head systems where the pressure head on the filter is constant or nearly constant. A second indirect strategy, "Pneumatic Sludge Exchange" (PSE) employs a concentration tank that is hydraulically attached to the PolyGeysers internal sludge storage basin to effectuate an exchange between the concentration tank and the PolyGeysers. At the end of a backwash, a relatively dilute (<1%) sludge is transported into the concentration tank as the water in both units rise. A movement that is driven by the influent to the PolyGeysers by gravity or pump. The connection to the concentration tank is near the top, so the dirty water fills the upper portion of the concentration tank as the backwash sequence ends. During the filtration cycle (typically 3 or more hours) the solids settle out of the upper portion becoming trapped in the sludge storage basin below. As the charge chamber discharges during the subsequent backwash waters level in the PolyGeysers quickly drop, the exchange pipe drains the now clarified water layer at the top of the concentration tank back into the PolyGeysers completing the cycle. The PSE approach is extremely robust, allowing for prolonged sludge storage in an attached concentration tank. Capable of displaying extremely low water losses <3 liters/kg-feed (<0.35 gal/#-feed), the PSE strategy is most suitable for marine applications where water loss minimization is desirable. Sludge digestion in the concentration chamber contributes to denitrification for RAS applications and mineralization in aquaponics systems.

## EFFECT OF GLUCOSE-GLYCINE MELANOIDINS ON DIGESTIVE VARIABLES OF THE RAINBOW TROUT FED A NON-HEATED DIET

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It is known that the thermal processing of nutrient mixtures can affect the digestibility coefficients of diets, together with the change of the nutritional composition of the feed. One of the most ubiquitous changes promoted by the application of heat to a mixture of nutrients is the non-enzymatic browning resulting from the so-called Maillard reaction. The ingestion of strongly browned diets has been proven to decrease the phosphorous digestibility in humans (Delgado-Andrade *et al.* 2011). In addition, some types of *in vitro*-synthesized melanoidins exert inhibitory effects on the activity of trypsin from mammals (Hirano *et al.* 1994, Ibarz *et al.* 2009). Based on these data, the present work is intended to test if melanoidins can affect the digestive process of cultured fish in a similar way.

Glucose-glycine melanoidins (glucose 1M, glycine 1M, NaHCO<sub>3</sub> 0.1 M, 103°C for 24 hours) were included (1.2%) or not in dietary matrix prepared without heating. The two diets were isoenergetic and isonitrogenous: CP 50.3-50.6%, CL 19.3-19.4%, energy density 23.5-23.6 MJ/kg. The values of gut pH, trypsin-like (substrate BAEE) and chymotrypsin-like (substrate BTEE) activities were postprandially monitored. Each diet were supplied to three tanks containing 25 rainbow trout juveniles with an average weight of approx. 24 g. Afterwards, each tank were sequentially sampled at 2, 4, 7, 10, 24, 32 and 48 hours post-feeding. Three fish were sampled per tank and temporal point (126 fish). Gastric and duodenal pH's were measured *in situ* for each individual. The trypsin-like and chymotrypsin-like activities were measured at pH 8.0 for intestinal crude extracts prepared from one randomly selected fish per tank and temporal point (42 fish). As a result, no effects on gastric pH and duodenal pH were detected. None of the enzymatic activities was significantly affected. The absence of an effect on the enzymatic activities can be interpreted as equal pancreatic synthesis/secretion of the enzymes in the anterior intestine irrespective of the diet. The present results do not suggest a role of melanoidins on the digestibility of proteins in fish, in contrast to previous results obtained *in vitro* and *in vivo* with other species. However, the apparently consistent decrease in chymotrypsin-like activity during at least the first 24 hours of digestion, though not significant in this work, deserves further attention. In general, more research is necessary to understand the effects of diet browning reactions in fish digestive physiology.

The present investigation is part of the thesis work of Xavier Serrano to fulfill the requirements to obtain the degree of Master in Aquaculture (Universidad Católica de Temuco, Chile) and has been supported by Comisión Nacional de Investigación Científica y Tecnológica (CONICYT, Chile), project Fondecyt Regular 1150147.

## EVALUATION OF SALT APPLICATION FOR HOLDING LARGEMOUTH BASS *Micropterus salmoides* FOR MARKETING AS A FOOD FISH

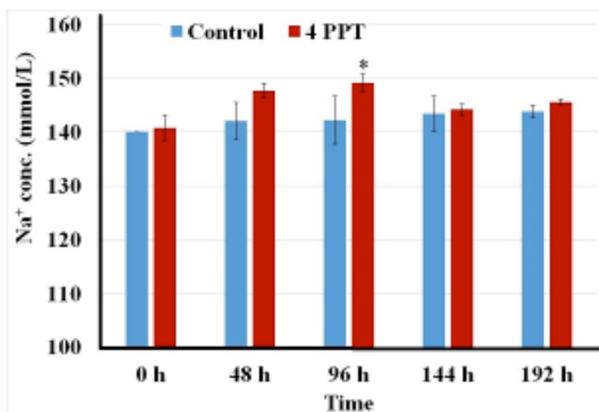
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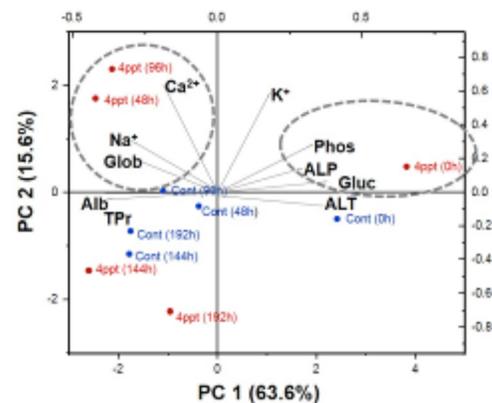
Small aquaculture producers have the ability to maintain fish as a live product and distribute fish in response to local market demand. Holding fish in live condition provides aquaculture producers and marketers the ability to constantly supply truly fresh fish to those customers who will accept nothing less than premium quality and are willing to pay for it. The objective of this research is to investigate the methods for holding fish for market as live species that will allow vendors and distributors to minimize loss and manage risk. This research project evaluated use of salt in holding systems for marketing largemouth bass (*Micropterus salmoides*) as live food fish.

Market size largemouth bass averaging 980 g each were held off feed 3 days prior to harvest. Thereafter fish were seined and hauled to a holding facility, and held in individual recirculating aquaculture systems (RAS) without feed for a period of 8 days. Two treatments were evaluated 1) control, 2) salt (NaCl) at 4 ppt. Water quality was monitored daily, and blood chemistry was analyzed at 0, 48, 96, 144, and 192 hours after stocking to RAS. Blood chemistry was measured with the Abaxis Vetscan VS2 analyzer.

There was no mortality or apparent weight loss throughout the experiment. Water quality remained within acceptable parameters in both treatments. Fish transferred from the hauling tank to RAS system were designated as 0 h group and were also documented to exhibit stress. From time 0 h, blood parameters -albumin, and total protein increased and stabilized at 48 -96 h, whereas glucose decreased at 48 h and remained lower thereafter. A reducing trend of alanine aminotransferase, bilirubin, and phosphate over time was recorded. Principal Component Analysis (PCA) revealed a prominent effect of 4 ppt typically at 48 h and 96 h for  $[Na^+]$ ,  $[Ca^{++}]$  and globulin (Fig 2). Cortisol level will be quantified for correlating the stress level with bio-chemical responses associated with the experimental groups.



**Figure 1:** Effects of salt application (4 ppt) on  $Na^+$  level in blood at different time frame. \* represents significant difference ( $P \leq 0.05$ ) between control and salt.



**Figure 2:** PCA analysis in PC 1  $\times$  PC 2 coordination plane representing the contribution of bio-chemical parameters in blood under testing conditions

## THE SENSITIVITY OF SWIM-UP GUADALUPE BASS *Micropterus treculii* FRY TO HYPEROXIA

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Fingerling Guadalupe Bass *Micropterus treculii* are produced at the A. E. Wood Fish Hatchery, San Marcos, Texas and stocked into central Texas rivers to restore threatened populations and combat hybridization with Smallmouth Bass *Micropterus dolomieu*. Fry survival in fingerling rearing ponds has fallen from 57-70% in 2011-13 to 11-25% in 2014-15 for unknown reasons and meeting the fingerling stocking request is challenging. We hypothesized that exposure to high oxygen concentrations (>20 mg/L, hyperoxia) from pure oxygen supplementation during transport and acclimation to ponds may have contributed to fry mortality in 2014-15.

To test this hypothesis, we exposed swim-up Guadalupe Bass fry (11 days-post-hatch, 8.45±0.25 mm total length) to normbaric dissolved oxygen (DO) concentrations of 7.9-8.6 mg/L (controls with no supplemental oxygen), 11.5-12.8 mg/L, 17.3-17.8 mg/L, 20.3-22.8 mg/L, 26.4-28.2 mg/L, and 32.1-33.3 mg/L for 30 min at 18°C, mimicking transport practices during 2014-2015. Pure oxygen was supplied to water in a 227-L fiberglass trough to maintain the target oxygen concentration for each treatment which was continuously siphoned into five replicate 6-L McDonald jars each stocked with 1 g of fry. After the 30-min exposure, jars were flushed with non-oxygenated water (DO 8.0-8.5 mg/L) for 1 min to rapidly lower the DO, mimicking fry release into ponds after transport. Jars were then moved to an incubation rack, supplied with 36 L/h fresh water (DO 7.6-8.8 mg/L), and monitored for 72 h. One control (A) was subjected to the same protocol without supplemental oxygen, while for the other control (B), fry were stocked directly into jars on the incubation rack and were not exposed to flushing. Dead fry were removed and recorded every 24 h. Fry behavior was observed throughout the study. After 72 h, surviving fry were counted and measured.

There were no significant differences ( $P>0.05$ ) in temperature, pH, ammonia, or alkalinity between treatments. Fry exposed to the highest oxygen concentration appeared more lethargic than in other treatments after 6 h. Fry survival after 72 h was strongly negatively correlated with increasing DO concentrations (Pearson: -0.79) and declined significantly ( $P<0.05$ ) above 20.3-22.8 mg/L (Table 1). The results of this study suggest that hyperoxia may lower fry survival. We recommend avoiding DO above 20 mg/L when transporting Guadalupe Bass fry.

TABLE 1. Guadalupe Bass fry survival 72 h after 30-min exposure to ascending DO concentrations. Different letters indicate significant differences at  $P<0.05$ .

Dissolved oxygen concentration (mg/L)	72 h Survival± SD (%)
7.9 - 8.1 (Control A)	64.65±3.37 <sup>ab</sup>
8.5 - 8.6 (Control B)	60.17±6.91 <sup>ab</sup>
11.5 - 12.8	75.51±4.37 <sup>a</sup>
17.3 - 17.8	60.40±15.67 <sup>ab</sup>
20.3 - 22.8	54.98±12.63 <sup>bc</sup>
26.4 - 28.2	39.87±5.82 <sup>cd</sup>
32.1 - 33.3	25.69±6.06 <sup>d</sup>

## CALCIUM SILICATE AS LIMING MATERIAL FRONT OF THE TRADITIONAL LIMESTONES

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Liming products are important in the pond management to keep the productivity and fish health. The experiment evaluating the effect of the liming managements was care out during 59 days, in laboratory of EW Shell Fisheries Center, Auburn University in Auburn AL, USA. To better evaluate the limnic managements was selected a pond with water with low alkalinity, in order to obtain water to the experiment. To compare the lime products were used, 20 aquariums (18 L) with five treatments, were these: (Water) tank containing water only; (Water and soil) water and soil; (Calcium) soil, water and calcium carbonate ( $\text{CaCO}_3$ ); (Magnesium) soil, water and dolomitic limestone ( $\text{CaCO}_3$ , 50%:  $\text{MgCO}_3$ , 40%) and (Silicate) soil, water and calcium silicate meta ( $\text{CaSiO}_3$ ). Twice a week were measured, alkalinity, pH, conductivity, temperature and oxygen. The calcium silicate is an adequate product to use with lime in aquaculture, when compared with calcium carbonate and dolomite, traditional liming products. The soil possibilited the more stability of environment that the water alone.

Acknowledgements: Capes, CNPq, FAPEMIG and BNB

Table - Mean and standard deviation of (Water) tank containing water only; (Water and soil) water and soil; (Calcium) soil, water and calcium carbonate ( $\text{CaCO}_3$ ); (Magnesium) soil, water and dolomitic limestone ( $\text{CaCO}_3$ , 50%:  $\text{MgCO}_3$ , 40%) and (Silicate) soil, water and calcium silicate meta ( $\text{CaSiO}_3$ ).

	Parameters			
	Alkalinity (mg/L)	pH	Conduitivity ( $\mu\text{S}/\text{cm}$ )	Turbidity (NTU)
Water	6.80 ± 0.82D	6.66 ± 0.12C	25.26 ± 3.74E	0.60 ± 0.60C
Water Soil	3.92 ± 1.12E	6.33 ± 0.24D	28.03 ± 2.96D	1.59 ± 1.09B
Calcium	83.86 ± 24.71A	8.19 ± 0.20A	181.12 ± 53.96A	0.93 ± 0.73BC
Magnesium	61.18 ± 17.41C	8.04 ± 0.26B	134.76 ± 38.20C	0.85 ± 0.74BC
Silicun	69.17 ± 20.52B	8.24 ± 0.13A	148.98 ± 44.58B	4.34 ± 13.46A

Means in the same column indicated by the different letter were different at the 0.05 level of probability by Tukey's test.

***Schizochytrium* SP. MICROALGAE IN DIET FOR *Leporinus friderici* (CHARACIFORMES)**

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The food intake enriched with polyunsaturated fatty acids, omega-3 series, has been widespread in the recent decades. The increase of the EPA and DHA levels can be obtained by enriching the animals feed raised in captivity by manipulating the fatty acids composition of dietary. In fish the diet manipulation in favor of better nutritional indices has been tested. The “piau” (*Leporinus friderici*) is a Brazilian freshwater native species, and commercially appreciated by both, meat flavor and as sport fishing. DHA is an important omega 3 acid, essential for the formation of the nervous and visual tissue in humans, and also gives a good formation of brain matter, and it is present in large amounts in seaweed. This study aimed to evaluate diets with the inclusion of algae *Schizochytrium* sp. on productive performance and chemical composition of *L. friderici* juveniles. The experiment was conducted in the Aquaculture Laboratory of Aquatic Ecology Department of Animal Science of UFVJM in Diamantina - MG. Three hundred piau juvenile with mean weight and total length of  $11.80 \pm 1.08$ g and  $9.68 \pm 0.31$  cm respectively, were subjected. Fishes passed for seven days of adaptation and were fed with the control feed treatment. The experiment was conducted in a completely randomized design with five treatments (0, 10, 20, 30, and 40 g of *Schizochytrium* sp.  $\text{kg}^{-1}$ ), and four replications. Seven juveniles were stocked through aquariums, totaling 20 experimental units. At the 60th day the performance parameters such as weight gain (g), final biomass (g), feed intake (g), feed conversion, TCE ( $\% \text{ day}^{-1}$ ), survival (%) and Fulton’s condition factor (K), and also were evaluated chemical composition parameters of carcass. Linear effect was observed ( $p < 0.05$ ) for the following parameters: weight gain, survival, and final biomass, and for feed conversion the effect was quadratic ( $p < 0.05$ ) for 30 g  $\text{kg}^{-1}$  *Schizochytrium* sp. included in diet. No differences ( $p > 0.05$ ) for dry matter, ash, ethereal extract in the chemical analysis of the carcass, except for crude protein, calcium and phosphorus that have a linear effect ( $p < 0.05$ ). It is concluded that the inclusion of 40 g of *Schizochytrium* sp.  $\text{kg}^{-1}$  in the diet improves the *L. friderici* juvenile performance.

Acknowledgements: Capes, CNPq, FAPEMIG and BNB.

## LIVE AND IN COOLED *Artemia* SP. FOR PACAMÃ *Lophiosilurus alexandri* (SILURIFORMES) FEEDING

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In freshwater fish rearing the major concern is the offering an appropriate food for larvae at first feeding, and it influencing performance, survival and growth of the same. Thus, the aim of this study was to verify the performance and survival of pacamã larvae *Lophiosilurus alexandri* fed with alive and cooled *Artemia* sp. nauplii at 0 and 2 ‰ salinity. Larvae were subjected to two feeding treatments and two salinity levels: live *Artemia* sp. plus 0‰ salinity (LA0); live *Artemia* sp. plus 2‰ salinity (LA2); frozen *Artemia* sp. plus 0‰ salinity (FA0); and frozen *Artemia* sp. plus 2‰ salinity (FA2), offered twice times daily. The experiment was conducted at a completely randomized design with 5 replications in a factorial 2 x 2, during 15 days. Larvae were distributed in 8 L-aquariums, with a density of forty fifth larvae per aquarium. Were evaluated body weight, weight gain, feed conversion, standard and total length, biomass, survival, body width, intestinal quotient, Fulton's condition factor, and water quality. The weight, weight gain, standard and total lengths increased with the supply of the living *Artemia* sp., with 2 ‰ salinity. The survival and Fulton's condition factor had positive influence with the use of living *Artemia* sp., with 0 ‰ salinity. The water quality was not affected by the salinity or food. It is suitable for growing pacamã larvae the use of living *Artemia* sp., in 2 ‰ salinity, improving their performance.

Acknowledgements: Capes, CNPq, FAPEMIG and BNB.

## PERCENTAGES OF COOLED *Artemia* sp. NAUPLII LARVICULTURE OF PACAMÃ *Lophiosilurus alexandri*

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The pacamã *Lophiosilurus alexandri* is an endemic species of the basin of the São Francisco River, and has a sedentary behavior and with a carnivorous feeding habit. The *Artemia* sp. nauplii are an important food for breeding fish larvae of marine and freshwater environments. This study aimed to evaluate the different percentages of cooled *Artemia* sp. nauplii in pacamã larvae feeding. The larvae were submitted to different percentages: A10 (10% *Artemia* sp. body weight day<sup>-1</sup>), A40 (40% *Artemia* sp. body weight day<sup>-1</sup>), A70 (70% *Artemia* sp. body weight day<sup>-1</sup>) e A100 (100% *Artemia* sp. body weight day<sup>-1</sup>), and provided four times a daily. The experiment was conducted during 21 days. Pacamã larvae were distributed in 5 L - aquariums at density of 50 fish aquarium<sup>-1</sup>. The body weight, standard length, total biomass, weight gain, feed conversion and Fulton's condition factor were evaluated by the biometric data collection. The water quality parameters such as temperature, pH, dissolved oxygen, conductivity, alkalinity and ammonia were measured at 1<sup>st</sup>, 7<sup>th</sup>, 15<sup>th</sup> and 21<sup>st</sup> days of experiment. It was found that as higher the supply of cooled *Artemia* sp. as greater the productive performance of pacamã larvae, without being affected water quality. The cold *Artemia* sp. nauplii are suitable for pacamã larvae feeding.

Acknowledgements: Capes, CNPq, FAPEMIG and BNB.

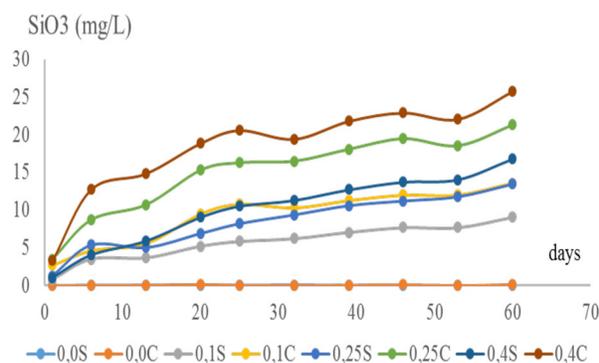
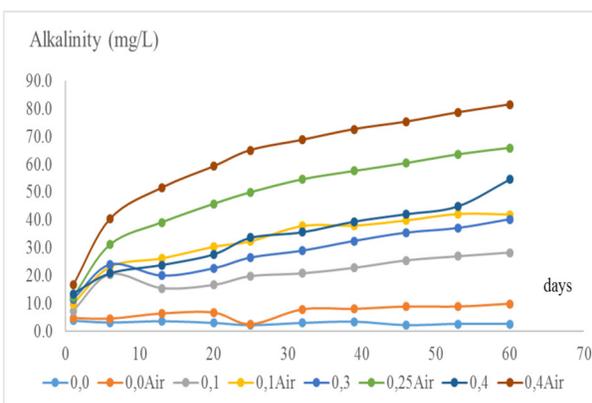
## EFFECT OF AERATION AND DIFFERENT CONCENTRATIONS OF CALCIUM SILICATE AS LIMING MATERIAL IN WATER QUALITY

Marcelo. M. Pedreira\*, Claude E. Boyd, Marianne Schorer, Afonso Pelli, Talita A. Ferreira, Stella I. R. Lobato, André L. Ferreira, Maria, P. Nascimento, Máira S. A. Rocha, and Ian T. Timpone

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Slag is the molten byproduct of many metallurgical operations, that is found throughout the world on a large scale and calcium silicates are present in steel slag. Since calcium metasilicate is an alternative to conventional liming fish products the aim of study was check the effect of aeration and calcium metasilicate concentrations in water quality. The experiment was care out during 60 days, in 24 aquariums (18 L), in laboratory of EW Shell Fisheries Center, Auburn University in Auburn AL, USA. Distinct concentrations of calcium silicate were tested (0.0, 0.1, 0.25 and 0.4 g/L) in combination with two aeration levels, with (164 ml/min; water residence time 109.16) and without aeration. Once a week were measured, alkalinity, hardness, calcium, silicate, pH, conductivity, temperature and oxygen. It has been found that neutralization capacity increased with the concentration of the silicate and aeration. Aeration increased buffering capacity, providing similar results to those observed in aquaria without aeration with a higher concentration of silicate immediately above.

Acknowledgements: Capes, CNPq, FAPEMIG and BNB.



Factor	0,0	0,1	0,25	0,4
Survival (%)	26.6	32.0	42.6	40.54
Intestinal quotient (mm) <sup>9</sup>	8.24	9.53	9.55	9.93
Biomassa Final (g) <sup>8</sup>	1.14	2.85	4.80	55.60
Feed conversion (mg) <sup>7</sup>	1.39	24.72	59.91	106.8
Weight gain (mg) <sup>6</sup>	15.92	70.35	97.43	121.58

CV= Coefficient of variation.

<sup>1</sup>Linear effect (p<0.05): Y= -26.614+68.176x; R<sup>2</sup>=0.87; <sup>2</sup>Polynomial effect (p<0.05): Y= 8.490+5.539x-0.675x<sup>2</sup>; R<sup>2</sup>= 0.93

<sup>3</sup>Polynomial effect (p<0.05): Y= 10.477+7.111x-0.877x<sup>2</sup>; R<sup>2</sup>= 0.93; <sup>4</sup>Polynomial effect (p<0.05): Y= 2.122+1.829x-0.197x<sup>2</sup>; R<sup>2</sup>= 0.91

<sup>5</sup>Linear effect (p<0.05): Y= 1.145+0.356x; R<sup>2</sup>= 0.49; <sup>6</sup>Linear effect (p<0.05): Y=-47.543+72.257x; R<sup>2</sup>=0.83

<sup>7</sup>Polynomial effect (p<0.05): Y= 10.176+5.6734x+5.8936x<sup>2</sup>; R<sup>2</sup>= 0.91 <sup>8</sup>Polynomial effect (p<0.05): Y= 1.6379+3.0144x-0.3178x<sup>2</sup>; R<sup>2</sup>= 0.46

<sup>9</sup>Polynomial effect (p<0.05): Y= 6.9018+1.6508x-0.2281x<sup>2</sup>; R<sup>2</sup>= 0.35.

## **BE IN THE KNOW: WHAT'S NEW REGARDING DRUGS FOR AQUATIC SPECIES**

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This presentation will provide an update on what drugs are approved for use in fish and other aquatic species, recent changes requiring veterinary oversight for certain drugs, and regulations and policies applicable to drugs used in fish and other aquatic species; and share resources for more information and ways to continue to stay informed on these topics.

## **EVALUATION OF MARINE MICROALGAE BIOMASS FOR PROTEIN REPLACEMENT IN AQUACULTURE FEEDS**

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At present, commercial aquaculture feeds are highly dependent upon costly and environmentally unsustainable sources of protein and lipid, namely marine fish proteins and oils, respectively, derived from reduction fisheries. Therefore, growth rate, biomass values and biochemical composition of mono- and mixed cultures of algae from various local sources (Corpus Christi, TX) were evaluated to assess long-term potential for use as fishmeal replacement in aquaculture feeds. Biochemical composition of candidate species of algae were initially assessed as indoor small-volume cultures to determine those species/strains with potential for use. Additionally, all cultures were inspected daily for temperature, salinity, and contamination (e.g., undesired phytoplankton, zooplankton, etc.) and cell counts recorded daily (c/mL) using a Neubauer hemocytometer. Selected strains/mixes were then subjected to larger-volume and longer-term outdoor culture trials in which performance and biochemical composition were used to evaluate potential for year-round production.

Independent variables for algal selection objectives will be tested for normality prior to running MANOVA ( $\alpha = 0.05$ ) and linear regression analyses. A SNK multiple range test will be used to determine differences ( $\alpha = 0.05$ ) among treatment means for all objectives.

## TOXIC ALGAE MONITORING ON ARKANSAS CATFISH FARMS

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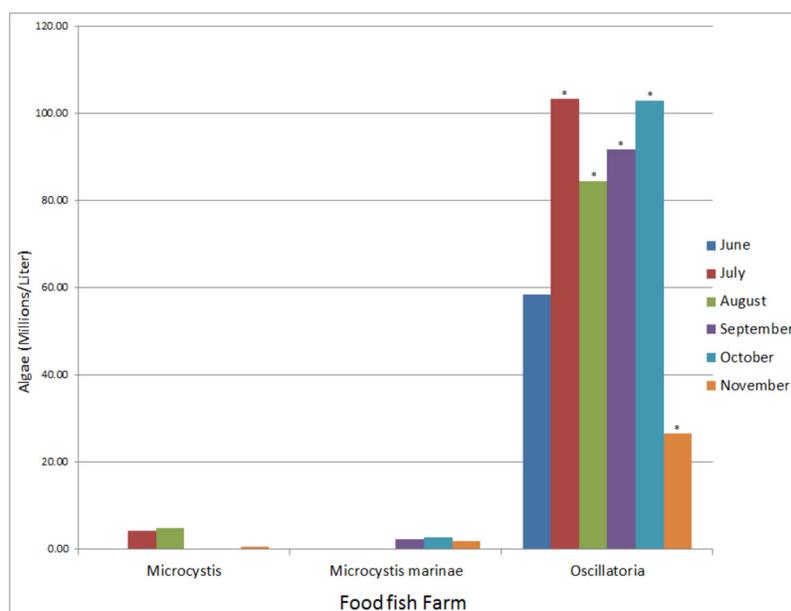
In recent years, catfish farmers in southern Arkansas have experienced fish kills not attributed to infectious diseases or low dissolved oxygen in ponds. Farmers have described erratic behavior of catfish just below the pond water surface (typically starting in the early afternoon) when water quality is stable and there are no other signs of disease. Certain characteristics of these unusual losses are similar to known fish mortality events that have been associated with toxic algae. Many algae produces toxins that may cause fish kills, including species of blue-green algae, golden algae, and eugenoids. One species, *Prymnesium parvum*, has caused large fish kills in brackish water rivers and lakes in Texas, and is of interest because some catfish ponds in Arkansas have moderately mineralized water that might support *Prymnesium* blooms.”.

Starting in May 2015, weekly algae samples were collected by extension personnel at two Arkansas catfish farms (total of 64 ponds). Algae samples were evaluated weekly for dominant species at the UAPB Lake Village Lab from May through November. Samples were not collected in the winter. Blue green algae of interest included suspected toxin-producing species of *Planktothrix Anabaena*, *Microcystis*, *Spirulina*, as well as *Prymnesium*. At each sampling 10 ml of sample was frozen weekly for Abraxis microcystin test strip analysis if needed (used only in the event of a toxic algae fish kill). The monitoring program is currently ongoing.

Species of *Planktothrix*, especially the known toxin-producer *P. agardhii*, were the most common algae present in foodfish ponds from July through November. Other known toxin-producing species in the genera *Microcystis* and *Anabaena* were also present, but in much lower numbers. Despite the frequent occurrence (often throughout the study in some ponds) of known or suspected toxin producing algae, no toxic events occurred in the 64 ponds studied in 2015 (Figure 1).

The study is being continued in 2016 and will conclude in November of this year.

Figure 1. Algal pop. ulations from a food fish catfish pond from June-November 2015



## SCREENING OF POTENTIAL FEED PALATANTS FOR USE IN ALL-PLANT PROTEIN DIETS FOR HYBRID STRIPED BASS *Morone chrysops* x *M. saxatilis*

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United States Department of Agriculture, Agricultural Research Service

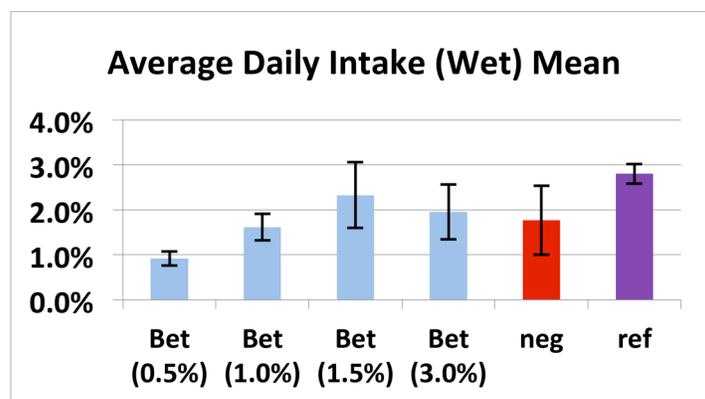
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Previous experience in our lab has shown that all-plant protein diets are poorly accepted by hybrid striped bass. Poor feed intake reduces animal growth and masks potential production savings that can be garnered through utilization of less expensive feed ingredients to supply essential nutrients. Feeding stimulants, i.e., palatability enhancers or “palatants”—like amino acids, peptides, crustacean meals, or betaine—are typically used to stimulate intake when some feed formulas are not readily consumed by the animal. Novel products have recently been introduced as palatants for pet and farm animal diets that have not been tested in several species of commercially grown fish. The purpose of this study was to screen a variety of practical compounds as potential palatants for developing commercial plant protein diets for hybrid striped bass.

Multiple series of short, 5-day feeding trials with juvenile hybrid striped bass were conducted to assess feed intake of an all-plant diet supplemented with one or more palatants of interest. Compounds tested included betaine (Bet or trimethylglycine), molasses, liver by-products (Simmons Proteins, Inc.), ProOmega (BioOregon Protein, Inc.) crawfish oil, shrimp oil, poultry oil, garlic oil, inositol, inosine, , and others . An all-plant diet formula was manufactured in a new, soft-extruded, “HMF” pellet (patent pending) developed by the USDA/ARS – Trout Grains Project (Bozeman, MT) and served as the carrier matrix for testing the various compounds of interest. The HMF pellets are capable of being dried, frozen, and then rehydrated when needed, without losing their essential nutrient composition (i.e., low leaching) or their advantageous physical characteristics, e.g., soft, chewy, yet resilient. The HMF pellet matrix allowed us to maintain consistent composition, quality, and uniformity of diets across all test palatants. Water soluble palatants were added to pellet rehydration water and all test diets were rehydrated to equal moisture content. Diets were fed to four replicate tanks twice daily, about 8h apart to apparent satiation without over feeding. Daily feed intake was determined by weighing feed containers assigned to each tank. To assess palatant effectiveness: weight gain, daily feed intake, and increased acceptance over time were measured for each test compound during the 5-day feeding trial and compared to tanks of fish fed positive and negative control diets that consisted of an extruded commercial hybrid striped bass diet (Rangen Inc., Angleton, Texas) and the unsupplemented, plant protein “HMF” diet. Results will be presented at the meeting.



**Figure 1.** Average daily intake of juvenile hybrid striped bass fed an all-plant diet with various concentrations of betaine as a potential palatant

## THE UTILIZATION OF WATERCRESS IN A CONSTRUCTED NATURALISTIC STREAM TO TREAT HATCHERY EFFLUENT

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The efficient treatment of hatchery effluent is a critical concern of the aquaculture industry, government regulatory agencies and the public. An effective effluent elimination system must not only meet discharge permit requirements, but be cost effective as well.

The Fisheries and Aquaculture Program at the State University of New York at Cobleskill has developed a low maintenance, low cost method of reducing the discharge of solids and nutrients from the campus 40,000 gallon recirculating trout hatchery. Primary solids removal is accomplished with a 3000 gallon settling tank. Hatchery effluent is then discharged at 20+ gal/min into a 65 m long, rubber lined excavated stream channel. The stream channel is filled with cobbles and 7 waterfalls are utilized to help maintain high oxygen levels. The heavy reliance on cascades and riffles, resulted in a much more limited reduction in total dissolved solids and biological oxygen demand than the previous design with equal proportions of pools and riffles. In this study, watercress was added to the constructed artificial stream to determine if nutrient removal could be improved.

Water quality parameters (discharge, temperature, dissolved oxygen, ammonia, nitrite, phosphorus, total suspended solids, total dissolved solids and BOD) were sampled throughout the year from 2014 to 2016. Samples were collected at the beginning and end of the constructed naturalistic stream. The addition of watercress substantially improved the effectiveness of the constructed artificial stream by increasing dissolved oxygen levels and reducing levels of ammonia, nitrite and BOD. For example, the presence of watercress reduced nitrite discharges by 91% compared to a 45% reduction in the absence of watercress. On the other hand, contrary to expectations, the addition of watercress had little impact on the treatment of suspended and dissolved solids.

Increasing effluent regulations have put pressure on the aquaculture industry to develop new strategies for treating hatchery effluent. A constructed naturalistic stream planted with watercress and coupled with a settling basin has the potential of effectively controlling nutrients and solids year round, without the capital and resource requirements of mechanical filters.

## **SPLIT ACADEMIC APPOINTMENTS: THE GOOD, THE BAD, AND THE UGLY**

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I was hired into a tenure-track position at Delaware State University in January 2004 as an assistant research professor and aquaculture extension specialist. This position is a split appointment with the responsibilities divided 60% towards research and 40% towards extension. I made the decision early on to make sure that my research and extension efforts complemented one another by drafting a guiding mission for my program. Specifically, my mission is to foster the creation of an aquaculture industry in Delaware by identifying suitable aquaculture species for use in the state and throughout the mid-Atlantic region, and low-input methods to raise them profitably, so that as the industry grows it will be both economically and environmentally sustainable. To implement this mission, I have followed three avenues:

1. Conduct applied research to investigate the suitability of aquaculture species and techniques for use in Delaware
2. Disseminate information to potential and existing producers, educators and other interested parties about aquaculture species and technologies
3. Demonstrate aquaculture production methods at the Delaware State University Aquaculture Research and Demonstration Facility.

By design, my program mission really underscores the intent of cooperative extension, ‘to provide useful, practical, and research-based information to agricultural producers.’ My being responsible for all aspects of projects from inception to dissemination ensures that there is a seamless integration of research and extension efforts. On the one hand, having a split research/extension appointment has afforded me a unique opportunity to be able to translate my own research results directly to my extension clientele. On the other hand, having a split appointment has also come with unique set of challenges. In my presentation I will discuss some the benefits and complications associated with split academic appointments.

## CHARACTERIZATION OF MICROSATELLITE DNA MARKERS IN POPULATIONS OF INDIAN WHITE SHRIMP *Fenneropenaeus indicus* ASSOCIATED WITH SUSCEPTIBILITY TO THE WSSV

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White spot syndrome virus (WSSV) creates mass mortalities in cultured shrimp *Fenneropenaeus indicus*, causing economic loss in the shrimp farming industry in Egypt. National selective breeding project was conducted using microsatellite DNA markers and proved to be a potential cost effective strategy for long term disease control in shrimp farming in Egypt. In the present project, microsatellite DNA profiles were compared between naturally occurring WSSV resistant and susceptible populations of *Fenneropenaeus indicus*. Some reproducible DNA fragments of varying sizes were found, among which 423 bp, 382 and 334 bp fragments were present in higher frequencies in the shrimp population susceptible to WSSV ( $p \leq 0.005$ ). WSSV challenge experiment was carried out and it was found that the copy no. of WSSV determined using RT-PCR is  $5 \times 10^4$  fold higher in WSSV susceptible shrimps than in the resistant ones. Therefore, these microsatellite DNA markers were novel in *F. indicus* and will be useful to distinguish between WSSV susceptible and resistant broodstock of *F. indicus*. The highest WSSV resistance using these DNA markers was observed in the shrimp populations selected from the current project, suggesting the need to continue in producing next generation of resistant shrimp as specific pathogen resistant shrimp for shrimp farming in Egypt. This work was supported by Science & Technology Development Fund (STDF), Egypt under grant no. 5661.

## **CHARACTERIZATION OF TEN POLYMORPHIC MICROSATELLITE LOCI USEFUL FOR SELECTIVE BREEDING PROGRAM IN INDIAN WHITE SHRIMP *Fenneropenaeus indicus***

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A selective breeding program was designed to select for disease resistance and growth in marine shrimp in Egypt. The project identified a number of polymorphic microsatellite markers to trace pedigrees and apply marker-assisted selection (MAS) in the current shrimp breeding program in Egypt. The project succeeded to isolate and characterize microsatellite genetic markers for the Indian white shrimp *Fenneropenaeus indicus* and trace the pedigree of different families from the breeding program. Microsatellite markers and pedigree were both used to assess genetic variability and effective population size. Using 10 loci, selected shrimp populations for three generations showed a decline in the expected heterozygosity (15 percent) and allelic diversity indices (52 to 93 percent), compared to the wild population ( $P < 0.05$ ). Effective population size estimates based on microsatellites declined from 46.5 to 77.0 percent in cultured populations ( $P < 0.05$ ), compared to the wild population. Working in direct contact with stakeholders has led to improvement in dissemination of the knowledge and awareness. Our strategy to control long-term inbreeding in breeding program is to use a relatively large number of broodstock in each generation, and to control mating among relatives by imposing mating restrictions (use of microsatellite markers for paternity analysis and mate design). The results have shown that the use of microsatellite markers suggest that heterozygosity at microsatellites loci is well correlated with individual inbreeding coefficients. The decline of genetic variability in the cultured selected population due to domestication, and evidence of a further smaller decline in effective population size across generations in the selected population, was observed when analyzing pedigree and microsatellite data. Pedigree keeping is required to prevent the decline of effective population size and maintain genetic variability in shrimp breeding programs, while microsatellites are useful to assess effective population size changes at the population level. However, we still allow preservation of the genetic variation necessary to continue improving the population for current traits or others that may need to be incorporated in the future. This work was supported by Science & Technology Development Fund (STDF), Egypt under grant no. 5661.

## A PORTABLE DNA TEST TO RAPIDLY DETECT INVASIVE SPECIES IN TRANSPORTED FISH

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Preventing the spread of invasive species is paramount to sustaining healthy ecosystems. Once established, the cost and difficulty managing invasive species rises exponentially through time. The impacts of invading species are not only detrimental to ecosystems, but also can be financially devastating to industries, agriculture, aquaculture, and outdoor recreational activities. Shipments of live fish can be transported great distances from where they were harvested, and thus pose a risk of carrying invasive species into new areas. There is increasing awareness of this risk particularly with baitfish (typically small minnow species of the family Cyprinidae) that are transported by the thousands. In this circumstance, there is a risk for just a few similarly sized invasive fishes to be included, and it is nearly impossible to visually detect and completely remove unwanted species from the hauling tanks.



To aid in detecting invasive fish hitchhikers, we partnered with private industry to develop a portable DNA test kit that can rapidly detect DNA of Bighead Carp and Silver Carp in a water sample on-site in under one hour. The test kit uses loop-mediated isothermal amplification (LAMP), and the sample collection and analysis process has been simplified so that individuals without previous experience in genetics or molecular laboratory techniques can get similar results as expert users with minimal training. Test kits for detecting Bighead Carp and Silver Carp are commercially available, and additional tests for other invasive species or diseases are currently in development. The use of this technology to screen live fish in transport should greatly reduce the risk of spreading invasive species and disease through this pathway.



## **YOUR BUSINESS IS AT RISK! – AN INTRODUCTION TO AQUACULTURE INSURANCE**

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Megan James Sorby (M.Sc., Aquaculture)

Benjamin Meskin, CPCU, Duncan Richmond (LLM, Cert CII)

Scott Aquaculture Risk Management Ltd ([www.scottaqua.com](http://www.scottaqua.com))

Meslee Insurance Services, Inc. dba Aquaculture Insurance Exchange ([www.aquacultureins.com](http://www.aquacultureins.com))

The core risk for any animal farming operation is the loss or mortality of the stock, not only the loss of a particular production run, but also the potentially longer term effects of the loss of broodstock or the effects of losing juveniles impacting upon contract fulfilment, etc.

This is becoming increasingly significant as the frequency and severity of extreme natural events increases. Over the last few years there have been major natural events that have caused the mortality of millions of dollars of fish stock, for example: super-chill events in Eastern Canada (est. USD 40 million insured losses), algal bloom off western Mexico (est. USD 15 million insured losses) and the single largest recorded loss event again caused by algal bloom in Chile (est. USD 200 – 500 million total losses).

Yet it is estimated that less than 5% of global aquaculture production is currently insured! One of the reasons is simply that a vast proportion of fish farmers are not aware that their stock can be insured, which has the knock on effect of making it difficult to obtain low cost external financing, as financiers perceive aquaculture as high risk, whereas the security of insurance can provide those financiers with the comfort they require.

So how does stock mortality insurance work? Standard cover works on an indemnity basis whereby each individual animal is accounted for and valued at a predetermined amount based on production costs. The stock can then be covered against a list of potential perils, e.g., storm, pollution, flood, mechanical breakdown, algal bloom, disease, etc., with the intention being that if cover is chosen against the full list, the stock is effectively covered against all potential risks. However, by having a list of perils there is flexibility, as an individual insured can pick and choose the perils to be covered, which in combination with the level of excess or self-retention, permits cover to be tailored to a particular budget or individual circumstances.

This type of standard policy offers the broadest cover options, both in terms of the range of perils and the capability to offer anything from “belt and braces” protection through to major catastrophe only. It is also applicable to most farming systems, from onshore ponds, raceways, tanks etc., through to floating cages, longlines, etc., in lakes, reservoirs and the open sea.

However, it does have one fundamental requirement that can restrict its application – the farming operation must employ a stock control system that permits accurate estimation of the number and biomass of stock on site at any point in time. As a result, some extensive farming systems, such as stocked shellfish beds and large shrimp ponds containing billions of individuals, may not be suitable for this type of cover. In which case, there are alternative options on a “parametric” basis that offer cover against specific natural perils more akin to financial weather derivatives.

## UNDERSTANDING PARTICIPATION IN MARYLAND OYSTER AQUACULTURE

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Management of Maryland's oyster resource has comprised over a century of vacillating policy and polarized constituent concerns that have led to a stunted aquaculture industry in comparison to other oyster-producing areas. A heavily revised lease program in 2010 enabled development of the industry by minimizing barriers and encouraging the entry of new producers. These legislative changes, combined with a suite of government support programs that included financing, education, seed production and other opportunities, have opened the door for an annually increasing number of independent oyster growers in Maryland, and may represent a complementary livelihood that can help to ease the pressure placed on the state's wild oyster population. Aquaculture may also represent a more stable livelihood relative to wild harvest, potentially allowing for greater resilience against future resource changes. Important to the growth of oyster aquaculture in Maryland is the support and involvement of commercial oyster harvesters, known locally as watermen.

This study aims to provide a rich description of who is participating in Maryland oyster aquaculture. Through participant observation and semi-structured interviews targeting themes of livelihood diversification, economic investment, heritage, bay restoration, oyster management, and future resilience, we ask why individuals, including watermen, have chosen to take on aquaculture and, similarly important, why many watermen have chosen not to. Understanding the perceived resistance by watermen toward oyster aquaculture is important to develop more inclusive and effective management programs for Maryland oysters.

Currently, in the first year of the project, several themes are beginning to emerge. Initial work suggests differences among individuals with regard to identification as a waterman as well as features distinguishing oyster aquaculture from the wild harvest. Additionally, though livelihood diversification is occurring among those participating in aquaculture and functions as a means of enhancing resilience, it is not immediately recognized by participants. Consideration of future environmental change impacting the Chesapeake region is not a common concern among interviewees when choosing to take on aquaculture. Further work will continue to tease out the factors keeping many watermen away from aquaculture and investigate the role of social networks in the decision to participate.

This paper describes the development of the project and the results that have been obtained to date, as well as showing the pathway that will lead to the final report. Potential uses for the data will be suggested as a means of understanding the decision process of those who enter the shellfish industry.

## METABOLIC RESPONSES OF NILE TILAPIA *Oreochromis niloticus* TO METHIONINE AND TAURINE SUPPLEMENTATION

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Most plant protein sources used in aquafeeds formulations has limiting levels of sulfur amino acids (methionine and cysteine) which may restrict the level of inclusion of these ingredients. Methionine (MET) is an essential amino acid for fish and usually the first limiting amino acid in soy-based diets. Besides being used for protein synthesis, MET is a source of sulfur required for cysteine (CYS) and taurine (TAU) synthesis that occurs mainly in hepatic tissues. Additionally, plant proteins are low in TAU and this beta sulfonic amino acid has been shown to be an essential amino acid for many marine fish species and some freshwater species. Therefore, the objective of the present study was elucidate the effects of MET and/or TAU supplementation in diets for Nile tilapia (*Oreochromis niloticus*), evaluating growth performance and expression of genes involved in sulfur amino acid metabolism.

The experiment was conducted at the Texas A&M University Aquacultural Research and Teaching Facility during 8 weeks. Two-hundred and seventy Nile tilapia juveniles (initial weight  $4.84 \pm 0.04$  g) were randomly distributed into 18, 110-L aquaria in an entirely randomized design containing six treatments with three replicates ( $n = 15$  fish per aquarium). Six isonitrogenous (28.4% of crude protein) and isoenergetic (3875 kcal gross energy  $\text{kg}^{-1}$ ) diets based on soybean products were supplemented with or without MET and TAU in a 2x2 factorial arrangement to evaluate deficient or adequate levels of these two amino acids singularly and in combination. In addition, a control diet formulated with fishmeal (positive control that provide adequate levels of MET and TAU) and another soybean-based diet supplemented with higher levels of MET and TAU (above that required by Nile tilapia) were included.

There is a significant effect of MET and TAU supplementation on Nile tilapia growth (Table 1). The current experiment demonstrates that TAU may spare methionine.

Analysis of gene expression and amino acid composition of the present study is currently been processed, and all results will be included in the final presentation.

**Table 1.** Effects of MET and TAU supplementation on growth performance of Nile tilapia juveniles

DIETS	Control	- / -	+ / -	- / +	+ / +	Surplus
MET (%)	0.75	0.34	0.75	0.34	0.75	1.50
TAU (%)	0.23	0.00	0.00	0.40	0.40	1.20
Initial weight (g)	6.68	6.60	6.71	6.70	6.83	6.66
Final weight (g)	61.34	39.64 <sup>b</sup>	47.26 <sup>a</sup>	47.59 <sup>a</sup>	47.45 <sup>a</sup>	48.52
Weight gain (g)	46.23	26.44 <sup>b</sup>	33.84 <sup>a</sup>	33.87 <sup>a</sup>	33.79 <sup>a</sup>	34.89
Hepatosomatic index	2.56	1.61 <sup>b</sup>	1.59 <sup>a</sup>	1.73 <sup>a</sup>	1.95 <sup>a</sup>	1.79
Visceral fat ratio	0.44	0.20 <sup>b</sup>	0.25 <sup>a</sup>	0.38 <sup>a</sup>	0.29 <sup>a</sup>	0.29
Feed intake (g)	63.33	49.76 <sup>b</sup>	55.15 <sup>a</sup>	55.95 <sup>a</sup>	55.20 <sup>a</sup>	57.78
Feed efficiency ratio	0.84	0.66 <sup>b</sup>	0.74 <sup>a</sup>	0.73 <sup>a</sup>	0.74 <sup>a</sup>	0.72

# FROM GENERATION TO GENERATION: CULTURING ZEBRAFISH UNDER CONTINUOUS ILLUMINATION FROM FERTILIZATION TO ADULTHOOD

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The popularity of zebrafish in multiple disciplines has caused a surge of research on zebrafish larviculture, growth, and reproduction, to optimize biological characteristics of this model species. While many studies investigating growth, survival and generation time of zebrafish have been conducted, investigation into the effect that continuous illumination has on these factors has been scarce. Furthermore, the investigation of the effects of constant light on zebrafish over multiple generations has yet to be conducted entirely. Thus, this study examined growth, survival, and generation time of zebrafish raised under continuous illumination in a novel experimental system from the time of fertilization to adulthood over 6 generations, F1-6.

Larviculture methods utilized in this generational study were based on initial investigation of zebrafish culture under continuous illumination (Dabrowski and Miller, in prep.). Zebrafish larvae, across all generations, were stocked to the experimental system at 5 dpf (days post- fertilization) and fed live saltwater rotifers, *Brachionus plicatilis*. At 12 dpf, larvae were transitioned to live *Artemia* nauplii for the duration of the experiment. Individuals were measured throughout the experiment, no significant differences in weight or length at 21 dpf were observed between F1-6 (Figure 1). Once fish reached size where males and females could be differentiated, spawning pairs were set each 24 h period (subjected to 10 h dark) until spawning and fertilization were successful. Generation time was determined as the number of days post fertilization when spawning was first successful (Table 1). Successfully spawned males and females were measured after each spawning, size at sexual maturity, and no significant differences in weight or length between F1-6 were observed (Figure 2).

The study is ongoing and data for F7-9 will also be presented. Results suggest that zebrafish can be successfully grown under continuous illumination, and that doing so produces a higher growth rate and shorter generation time than the suggested 10 h dark, 14 h light regime (Lawrence 2007). Based on the presented results, 8 generations of zebrafish can be produced in 1 year following this methodology, reducing the time required for multigenerational selection, a trait useful in various research fields.

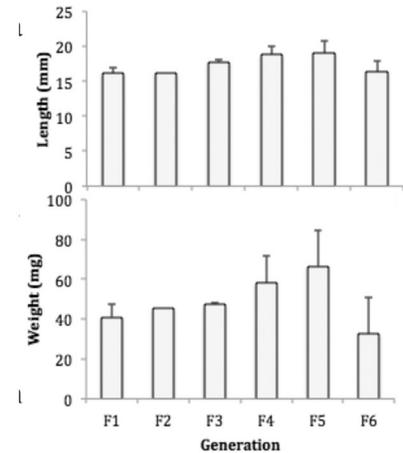


Figure 1: Length (mm) and weight (mg) measurements at 21 dpf for F1-6.

Table 1: Generation time for F1-6 compared to literature.

Generation	Generation Time (dpf)
F1	43
F2	49
F3	55
F4	74
F5	71
F6	46
Aoyama et al. 2015	49
Lawrence et al. 2012	60

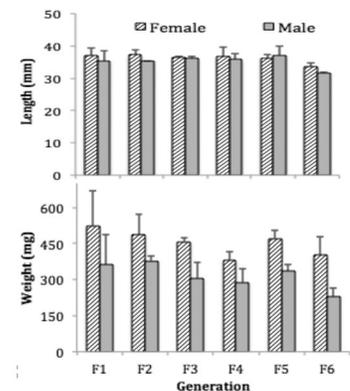


Figure 2: Length (mm) and weight (mg) measurements for zebrafish males and females that have reached sexual maturity.

## BENTHIC SUBSTRATE ENHANCES EARLY SURVIVAL OF AUSTRALIAN REDCLAW CRAYFISH *Cherax quadricarinatus* IN A RECIRCULATING NURSERY SYSTEM

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Australian redclaw crayfish, also known as freshwater lobsters, are a new and promising aquaculture species. Fast growth rates, easy care and maintenance, combined with a high market value make this species attractive to the aquaculture industry. However, development of a domestic freshwater lobster industry is largely hampered by the need for a reliable supply of seed stock. This study examined two types of benthic substrates to determine whether production of 45-day old craylings could be enhanced.

Hatchling survival and growth were studied in an array of eight, 77 liter glass aquaria in a closed recirculation system. The bottom surface area of each tank was 2288 cm<sup>2</sup> (30.5 cm x 75 cm). Average temperature was 26.2°C and day length was 14 hours.

In one set of experiments the bottom was covered by limestone rock, with particle sizes of 1-2mm, 4-8 mm, 16-32mm & 32-64 mm. Hatchlings were stocked at densities of 0.022, 0.044 and 0.066 cm<sup>2</sup> (50, 100, 150 individuals). In the other set of experiments, black low density Matala filtration media was used in 6, 8, 10, 12 layers (930 cm<sup>2</sup> each) on top of an 8-16 mm gravel substrate. Hatchlings were stocked at densities of 0.011, 0.022, 0.033, and 0.044 cm<sup>2</sup> (25, 50, 75, 100 individuals).

Survival was highly variable ranging from 4-88% in the Matala and 2-58% in the rock. However, Matala (46.4%) had double the survival rate than stone (22.3%), when the same stocking densities were compared (chi square test, P < 0.05). In 12 experimental trials, survival on a stone substrate was greater than 50% only 8% of the time, while survival on Matala was greater than 50% in 63% of the trials.

Growth was also highly variable with final average weights ranging from 0.20 - 1.06 g in Matala and 0.57 - 2.02 g in the rock. Weight of craylings after 45 days was significantly better with the rocky substrate than the Matala (T-test, P < .01).

Biomass production was 2.7 times greater with 6 layers of Matala (5580 cm<sup>2</sup>) over that of the bare gravel substrate. However, adding additional layers of Matala did not increase production. Coarse gravel (32-64mm) had the highest production of the rock substrate.

In summary, the addition of substrate significantly increased survival and production of redclaw craylings. Matala performed better than the coarse rock, yielding a higher survival percentage and was easier to handle when capturing the craylings at the completion of trials.

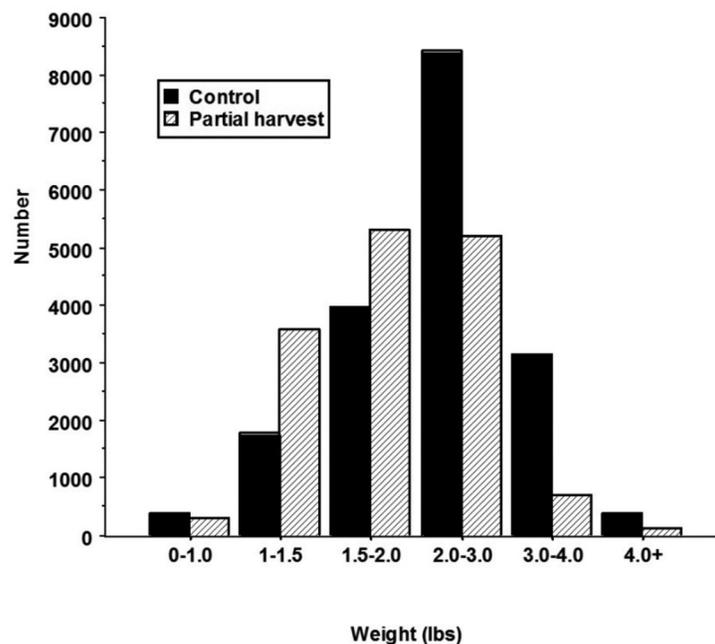
## REDUCING SIZE VARIATION IN HYBRID CATFISH CULTURE THROUGH GRADED PARTIAL HARVEST

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Hybrid catfish (♀ channel catfish *Ictalurus punctatus* x ♂ blue catfish *I. furcatus*) are becoming a major contributor to catfish aquaculture in the United States. Because of unique production characteristics of hybrid catfish, rapid farmer adoption of hybrids has outpaced appropriate management research. This study was conducted to determine the effect of mid-summer partial harvest of faster-growing fish on production characteristics of hybrid catfish in single-batch culture. Twelve, 1.0-acre ponds were stocked with 10,000 hybrid catfish (~0.12 lb/fish) in mid-March 2015. In August 2015, six random ponds were partially harvested to remove fish larger than ~1.25 lb using an in-pond, flexible-panel grading sock. In October 2015, all ponds were completely harvested. Partial harvest of faster-growing fish removed ~26% of fish from ponds in August. Removal of faster-growing fish resulted in 0.9% of the total crop considered “out-of-size” (> 4 lb/fish) and subject to discounts, compared with 2.1% of the fish from the control ponds. (Figure 1). However, reduction of fish numbers by mid-summer partial harvest reduced overall production by an average of 16% in the partial-harvest treatment compared with control ponds. Overall, partial harvest of faster-growing fish in mid-summer reduced the overall biomass of discounted, out-of-size fish. Higher production in control ponds resulted in greater net revenue.

Figure 1. Final total weight distribution of harvested hybrid catfish from partly harvested and control ponds.



## TWENTY-SEVEN YEAR HISTORY OF FISH DISEASE CASES DIAGNOSED AT THE KENTUCKY STATE UNIVERSITY FISH DISEASE DIAGNOSTIC LABORATORY FROM 1990 THROUGH 2016

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From 1990 through 2016, about 1,500 cases were examined at the Kentucky State University Fish Disease Diagnostic Laboratory. Eighty-eight percent of cases were from Kentucky; other cases were from Ohio, Indiana, Tennessee, Illinois, West Virginia, Pennsylvania, Arkansas and Texas. Channel catfish and largemouth bass comprised the highest percentages of cases; other species examined included hybrid striped bass, koi, rainbow trout, bluegill sunfish, goldfish, tilapia, angelfish, paddlefish, crappie, blue catfish, bait minnows, channel x blue catfish hybrids, shrimp, yellow perch, sturgeons, Australian red claw crayfish, flathead catfish, brook trout, brown trout, hybrid bluegills, white bass, striped bass, muskellunge, koi x goldfish hybrids, smallmouth bass, discus, northern cavefish and guppies. Forty percent of cases involved internal bacteria contributing to mortality. The most frequently occurring bacteria were *Aeromonas hydrophila/sobria* complex (40%) and *Flavobacterium columnare* (19%). Thirty-two percent of the bacteria were antibiotic-resistant to Terramycin®, 19% were resistant to Romet®, and one percent was resistant to Aquaflor®. Among parasites diagnosed as the cause of disease, gill monogenes, Ich, and external fungus occurred most frequently.

## OPTIMIZATION OF ALGAL TURF SCRUBBERS FOR LARGE-SCALE SUPER-INTENSIVE AQUACULTURE

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Algal turf scrubbers (ATS) are widely used for nitrogen and phosphorous removal from natural 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 and water quality is ameliorated. This is of particular interest in the field of aquaculture as up to 50% of the protein in feeds is excreted as ammonia, which can be toxic to the organisms. Removing ammonia comes at a cost to the producer and only increases as biomass density of production increases. Unlike traditional nutrient removal methods, because ATS is solar-driven and does not need supplemental CO<sub>2</sub>, once established it operates at low cost. ATS systems have proven effective in treating agricultural runoff as well as effluent from bivalve aquaculture. In order to effectively remove nutrients, a large amount of algal biomass must be produced which, among other variables, may be due to matrix material and shapes. In addition, harvesting biomass is essential to stimulate further growth and increase nutrient removal.

In this study, a twelve lane (1-foot wide, 40-foot length) ATS system was utilized to test the effectiveness of three different matrices (n=4), 1-D, 2-D, and 3-D, in yielding the highest amount of algal biomass and greatest ability to remove nutrients. Simulated effluent, meant to mimic nutrient levels typically found in super intensive indoor aquaculture facilities, was used to determine which matrix type was most successful in the removal of soluble nitrogen and phosphorus. Water samples were taken daily to monitor nutrient removal. Solar intensity, wind speed, and precipitation were also measured daily. Twice a day (a.m. and p.m.), dissolved oxygen, salinity, temperature, and pH were measured with a portable meter and recorded for each ATS lane. Biomass was harvested once a week and analyzed for biochemical composition to determine its potential as an aquaculture feed ingredient or bioenergy source. Based on this data, an optimal ATS size and design that would be appropriate for nutrient removal in common commercial super-intensive shrimp aquaculture may be modeled.

## UNTAPPING THE RESOURCE: THE USE OF A MIXED SPECIES EXHIBIT AS A VIABLE SOURCE OF EGGS FOR MARINE ORNAMENTAL AQUACULTURE

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The focus of this project was on marine ornamental reef species of importance to Hawaii. Eggs were collected from a partnering institution’s 166,000-gallon public exhibit. Eggs were collected and transported back to the Oceanic Institute (OI), where rearing attempts were conducted and documented. The goal was to culture new species and establish successful rearing protocols which could then be applied to other ornamental species that have not yet been aquacultured. The use of a mixed species exhibit for ornamental egg collection, and mixed-species larval rearing are relatively new approaches in marine ornamental aquaculture. Over the course of the project, a total of 794,760 eggs were collected across 28 collections with representative larvae from three different families of fishes. This led to the successful rearing of six different species, four of which had never previously been reared successfully in captivity. The successful collection, transport, and rearing of eggs from public aquaria gives merit to the mission of Rising Tide Conservation to preserve and protect coral reefs by means of sustainable aquaculture.

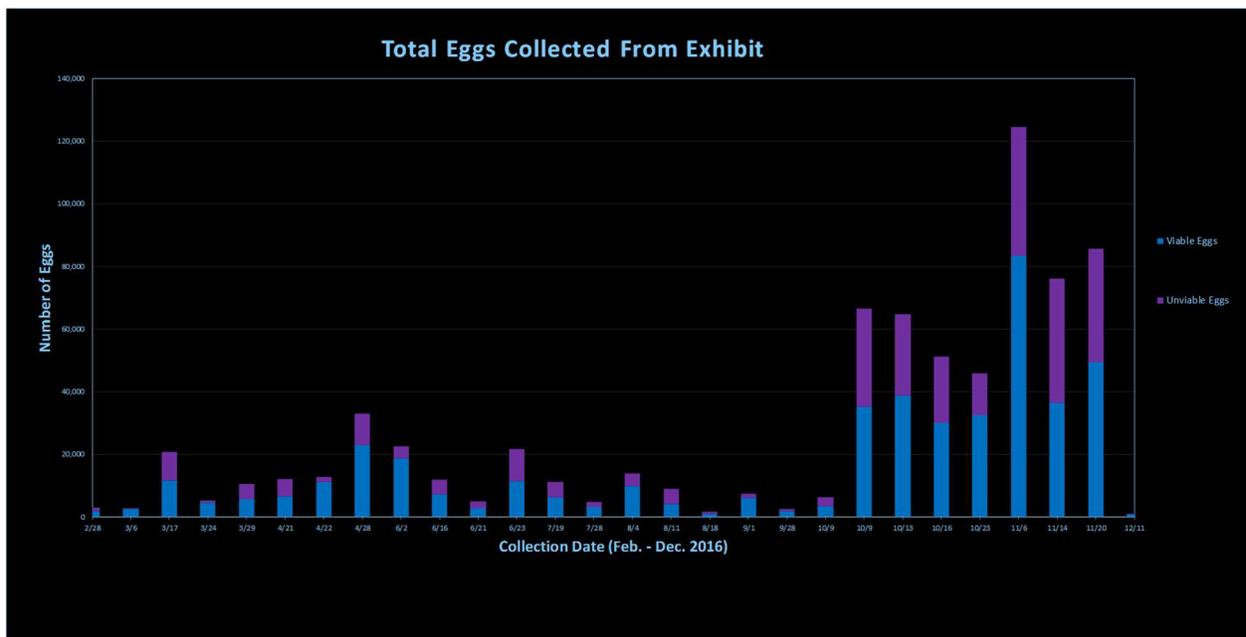


Figure 1. Total, viable, and unviable eggs collected throughout the 28 collections of the project.

## A PILOT STUDY ON CULTURE OF ASIAN SEABASS *Lates calcarifer* IN COASTAL CAGE AT COX'S BAZAR COAST, BANGLADESH

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Bangladesh ranked fifth in global aquaculture but mariculture is not popularized yet. The limited availability of protected sites and the probable conflicts with other activities such as fishing, tourism and navigation are key factors likely to influence mariculture development in Bangladesh, besides finance, expertise and government policy. As a leading fish in the coastal environment, seabass (*Lates calcarifer*) is one of the promising species for the development of culture technique in the coastal region of Bangladesh. The present study aims to find out the suitability of seabass culture using floating net cages (locally available materials) taking special care to suitable stocking density and low-cost feed ingredients in the coastal region of Bangladesh. Considering all these criteria, the Bankkhali River Estuary, Cox's Bazar has been selected. The fingerlings were collected from the local fry collectors of the coastal areas around one month after the breeding season of June-July and sorted out the fingerlings of uniform size due to cannibalistic habit and the sorted fish ( $19.5 \pm 1.03$  g) were acclimatized before stocking to the cages. The initial stocking rate was 50 indivs/m<sup>2</sup> and 45 days later it was reduced to 30 indivs/m<sup>2</sup> till harvest. Three supplementary feeds; flesh of pangush fish (*Pangasius sutchi*), mussel meat (*Perna viridis*) and flesh of chewa fish (*Trypauchen vagina*) were used with three replicates. Feeds were applied trice daily in first two months at the rate of 8% of their total biomass and then twice daily at the rate of 5% till harvest. After 120 days of culture fishes were harvested (Table 1). SPSS-Post Hoc comparison indicates that pangush fish as feed gives the best result followed by mussel meat and chewa fish. The hydrological parameters *viz.*, temperature, pH, salinity, dissolved oxygen, ammonia, alkalinity, hardness, nitrate, phosphate, TDS, TSS and secchi depth were measured two times per month and were found at optimal levels for the normal growth of seabass. The results obtained indicated that cage culture of seabass in the coastal areas of Cox's Bazar region, Bangladesh can provide significant advantages in terms of fast growth using low cost trash fish with the effective utilization of unused and derelict lotic water bodies.

TABLE 1. Final length and weight, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate of seabass fed supplementary feeds for 120 days.

Feed type	Final length, cm (Mean±SD)	Final weight, g (Mean±SD)	SGR (%/day)	FCR	Survival rate (%)
Pangush fish	25.99±1.40	238.17±34.97	2.09	3.56	94.5
Mussel meat	25.80±1.52	226.48±28.10	2.04	4.60	92.3
Chewa fish	24.30±1.41	169.18±16.27	1.80	5.38	85.2

## OFFSHORE AQUACULTURE IN THE SOUTHERN CALIFORNIA BIGHT: AN ASSESSMENT OF AQUAMODEL PARAMETER SENSITIVITY AND FARM ENVIRONMENTAL PERFORMANCE

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To assist with open ocean aquaculture development in the Southern California Bight, we have developed an AquaModel computer simulation for the first fish farm to be permitted in federal waters. AquaModel was chosen as the modeling platform because it is a validated model that is used around the world to provide real-time, simultaneous benthic and water column environmental performance. The Rose Canyon Fisheries proposed farm plans to annually produce approximately 5,000 metric tons of California Yellowtail (*Seriola dorsalis*) valued over \$80 million off the coast of Southern California. AquaModel benthic simulations suggested that increases in total organic carbon (TOC) under the cage array increases from the ambient concentration of 0.01% of the total mass of sediment materials to a maximum of 0.012% of total mass, an 18% increase in TOC, but a 0.0018 fractional increase in sediment total mass. This increase is negligible and within natural background variation in TOC. Sediment sulfide increased slightly during neap (low flow situations), and quickly returned to zero when near sea floor flows returned. The highest recorded value of sulfide was 530  $\mu\text{M}$ . The presence of sulfides in the sediment typically lasted for less than a day. Increase in sulfide was not observed 100 meters from the cage array. Sediment DO typically ranged between 3.3 and 4.6 ppm, depending on the DO content and flow of the ambient ocean directly at the seafloor. Ambient bottom oxygen was field measured at 3.8 ppm in winter months. The lowest singular sediment DO was calculated as 1.86 ppm at a bottom flow of 0.95 cm/s and lasted only one hour before the current increased and DO returned to average values. Average surface water velocity was 18 cm/sec. With a 26 m diameter cage, the total water volume is changed every 2.6 minutes, which carries heavy supply of DO and dilutes excreted ammonia to nearly indiscernible concentrations near and inside the cage. Prevailing dispersive bottom current direction was to the SSW (205 degrees) and therefore away from shore. Water column DO immediately downstream of the highest density cage during lowest flow periods (0.8 cm/s) was calculated to be 6.78 ppm compared to ambient which was 7.4 ppm. Decreases in cage DO was not detectable during medium and high current flow periods. The highest observed dissolved nitrogen concentration was calculated to be 1.1 mg-at/m<sup>3</sup> (0.015 ppm) which was 0.008 ppm above background during low surface flow periods inside the cage. This increase in nitrogen is diluted within a few 10's of meters of the cage array depending on current direction and velocity. Subsequently, it is assimilated by phytoplankton that may be grazed by zooplankton that can be modeled with the AquaModel3D far field application also developed for this project. These results provide a first case study of marine cage culture environmental performance in the Southern California Bight.

## **SEAGRASS AND SHELLFISH AQUACULTURE: AN ASSESSMENT TO INFORM MANAGEMENT AND RESEARCH**

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When planning for and managing aquaculture-industry development, coastal managers must weigh environmental benefits and costs along with a growing number of other activities within the coastal zone. One such conflict includes the interactions between shellfish aquaculture and submerged aquatic vegetation (SAV). Due to the importance of SAV as an essential fish habitat and the global decline in seagrass ecosystems, managers are concerned with how shellfish cultivation techniques impact sensitive SAV communities. Industry-wide monitoring reports and research findings suggest that shellfish aquaculture can have variable impacts on SAV depending on the cultivation technique and health of surrounding SAV communities. In many cases, shellfish aquaculture is identified as providing ecosystem services similar to SAV such as habitat provisioning, improving water clarity, inducing sedimentation, and carbon sequestration. To better understand these interactions, the NOAA National Ocean Service's Coastal Aquaculture Siting and Sustainability program is conducting a State of Science Assessment. This assessment provides a review of regulatory mandates, shellfish cultivation practices, SAV interactions (both negative and positive), and approaches for habitat equivalency analysis. In cases where net positive effects on SAV occur, we propose a metric and analysis framework capable of calculating net ecosystem effects. This approach allows the coastal manager to consider trade-offs and benefits at the ecosystem level. This presentation will provide an update on the status of this assessment, findings thus far, and a timeline for completion.

## **INTERNATIONAL MOVEMENT GUIDELINES FOR ACCREDITED VETERINARIANS REGARDING AQUACULTURE RELATED LIVE ANIMAL AND PRODUCTS**

Alicia R. Morse\*, Kathryn M. MacDonald

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An overview of the international movement of aquaculture related live animals and products, with a focus on the Northeastern and South Central regions of the United States. Each destination country determines the requirements for importation. Health requirements, paperwork and logistics will vary greatly between species and each importing country and may also change at any time. Accredited veterinarians are the first point of contact for companies and individuals involved with international movement. A general summary of the cooperative process between the exporter, importer, accredited veterinarian, the country of destination and the USDA will be covered.

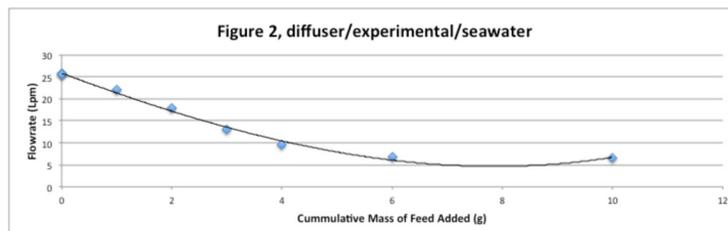
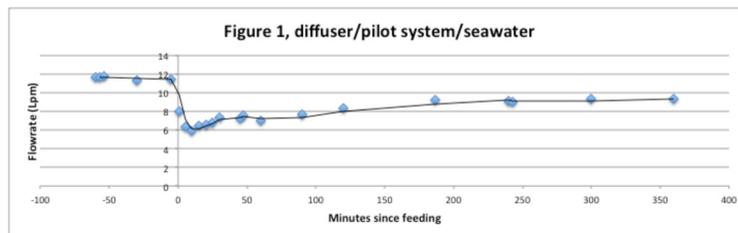
## IMPACT OF FISH FEED ON AIRLIFT PUMP PERFORMANCE

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Airlift pumps are commonly used in aquaculture systems to pump water, mix water and maintain critical gas levels. In a production sablefish system, a significant decrease in airlift pumping rate was observed immediately after feeding. This decrease in flow could negatively impact water quality, system performance, and fish health. A three-part experiment was conducted to examine the impact of feed addition on airlift pumping rates. All tests were conducted in 2" PVC pipe with a submergence ratio of 69%. The first experiment tested diffuser injection and direct air injection airlift pumps in a high-density marine sablefish rearing system ( $75 \text{ kg/m}^3$ ) fed 1.3% of body weight, three times a week. This feed was a fish-based brood feed (BioOregon, 48% crude protein, 20% crude lipids). A second experimental system was developed to assess the time response and concentration impacts of known additions of BioOregon on pumping rates in both fresh water and seawater but without fish. The last experiment was similar to the second experiment but a wide range of larval fish feeds, fish-based production feeds, and plant-based feeds were evaluated.

In the rearing system, feeding resulted in a 50% in pumping rate following feeding. The pumping rate gradually increased over the day (Figure 1). Repetitive feed additions (up to 10 mg/L) in seawater reduced the pumping rate by up to 80% for the diffuser but only 10% for direct air injection. Video footage revealed increasing bubble coalescence with the addition of feed. The decrease in pump flow is likely due to compounds leaching out of the feed that change bubble size. Further research is needed to understand what type of compounds are responsible for the decrease in pumping and how the water flowrate changes for a wider range of airflow rates.



## EFFECT OF GRADED LEVELS OF A DEFATTED INSECT MEAL FROM *Tenebrio molitor* LARVAE (YNSECT TMP-465) ON THE GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN JUVENILE TROUT

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A study was undertaken to evaluate the efficacy of graded incorporation levels of insect meal from *Tenebrio molitor* larvae (Ynsect TMP-465) on the growth performance, body composition and apparent digestibility of nutrients in rainbow trout. The trial comprised five dietary treatments: a control diet (CTRL) containing 25% fishmeal and four test diets Y5, Y7.5, Y15 and Y25 in which fishmeal was replaced with Ynsect TMP-465 meal at 20, 30, 60 and 100%, respectively. Diets were isonitrogenous (CP 48.5%), isolipidic (CF 22.7%) and isoenergetic (GE 23.2kJ/g). Diets were tested in triplicate groups of 35 trout, with a mean initial body weight (IBW) of 5.0 g over 90 days.

At the end of the trial, the incorporation of Ynsect TMP-465 meal at 5, 7.5, 15 and 25%, with the concomitant reduction of fishmeal led to a stepwise significant increase of final body weight ( $P < 0.05$ ). All diets with Ynsect TMP-465 meal showed a significant improvement of SGR, FCR and PER values ( $P < 0.05$ ). In comparison to CTRL, fish fed diets with Ynsect TMP-465 meal showed a significant reduction of feed intake. However, such reduction does not suggest a detrimental effect on palatability, but probably a better nutritional and metabolic adequacy of diets, as indicated by a lower FCR and higher growth of fish. The various incorporation levels of Ynsect TMP-465 meal had no effect on the whole-body composition of fish nor on the ADC of dry matter, protein, fat, phosphorus and energy ( $P > 0.05$ ). Protein, phosphorus and energy retention was significantly increased ( $P < 0.05$ ) in trout fed diets with Ynsect TMP-465 meal. In general, the defatted insect meal (YNSECT TMP-465) could effectively replace 100% of fishmeal in the diet of juvenile rainbow trout with positive effects on the overall growth performance.

Experimental study conducted by SPAROS as a contract-research service to YNSECT.



## COMPARISON OF SNAIL MEAL VERSUS FISH MEAL BASED DIETS ON THE GROWTH AND BODY COMPOSITION OF CATFISH (*Pangasius hypophthalmus*)

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Keeping in view the increasing prices and greater use of expensive fish meal as major ingredient of finfish diets, the present study was planned to utilize another very important and commonly available animal origin protein source; the snail meal as an alternative to fish meal and its impact on growth and body composition of Catfish (*Pangasius hypophthalmus*) fry rearing under intensive fiber glass aquarium. The study was conducted to evaluate the growth performance and body composition of Catfish (*Pangasius hypophthalmus*) fry.

Catfish fry fed with fish meal and snail meal based diet in fiberglass aquariums for three months. Experimental fish (n=25) was stocked in fiberglass aquaria (Dimensions: L, 1ft 18 inch× W, 2 ft.). The fish was fed with experimental diets (fish meal and snail meal) containing crude protein (32.85 and 32.5, respectively) at 4% of fish wet body weight. After 3 months study period, the increase in weight gain due to the consumption of snail meal and fish meal was recorded as 5.57g and 5.89g respectively. The diet with fish meal has showed statistically significant higher growth than that of the snail meal. The feed conversion ratio (FCR) was statistically highly significant for fish fed on fish meal (1.99) than snail meal fed fish (2.09). Proximate analysis showed significant differences in fat content of fish fed with fish meal as compared to snail meal. No significant difference in moisture, protein, ash, fibre and phosphorus contents were found in fish meal as well as snail meal fed fish. In conclusion, the catfish fry showed best growth performance with fish meal due to its best nutrient composition and acceptability. The findings of present study suggest snail meal as a good alternative of expensive fish meal feed for catfish culture.

**Table 1. (A). Fish Feed Formula used for the preparation of fish meal.**

	CP Level	Contributed CP %	% inclusion
Fish Meal	52	13	25
Rice Polish	13	1.95	15
Wheat Bran	16	2.4	15
Maize Gluten	30	4.5	15
Soybean Meal	44	11	25
Vitamin Pre			2
Vegetable Oil			1
Starch			2
Total		32.85%	100

**Table 1. (B). Fish Feed Formula used for the preparation of snail meal.**

	CP Level	Contributed CP %	% inclusion
Snail Meal	67	13.4	20
Rice Polish	13	2.6	20
Wheat Bran	16	3.2	20
Maize Gluten	30	4.5	15
Soybean Meal	44	8.8	20
Vitamin Pre			1
Vegetable Oil			2
Starch			2
Total		32.5%	100

**Table 2: Fortnightly average growth of fish using snail meal and fish meal as artificial feed.**

Parameter	Snail Meal	Fish Meal
Initial wt.	0.98 <sup>a</sup> ±0.002	0.88 <sup>b</sup> ±0.018
Final wt.	5.57 <sup>b</sup> ±0.002	5.89 <sup>a</sup> ±0.028
Wt. gain	104.34 <sup>b</sup> ±0.17	111.9 <sup>a</sup> ±0.666
% wt gain	489.67 <sup>b</sup> ±0.22	525.56 <sup>a</sup> ±5.43
Initial Length	48.45 <sup>a</sup> ±0.35	48.9 <sup>a</sup> ±0.99
Final length	76.9 <sup>a</sup> ±1.06	81.1 <sup>a</sup> ±1.27
Length gain	28.5 <sup>b</sup> ±0.707	32.3 <sup>a</sup> ±0.28
Initial biomass	21.93 <sup>b</sup> ±0.27	23.47 <sup>a</sup> ±0.46
Final biomass	128.03 <sup>a</sup> ± 0.04	135.46 <sup>a</sup> ±0.666
FCR	2.05 <sup>a</sup> ±0.003	1.99 <sup>b</sup> ±0.01
SGR	1.02 <sup>b</sup> ±0.006	1.06 <sup>a</sup> ±0.014

**Table 3. Proximate Analysis of *Pangasius hypophthalmus*.**

Parameter	Pre- Trial	Post-Trial	
		SM	FM
Moisture	7.6 <sup>a</sup> ±0.36	8.1 <sup>ab</sup> ±0.01	8.8 <sup>b</sup> ±0.14
Ash	17.4 <sup>a</sup> ±0.14	17.5 <sup>a</sup> ±0.35	18.3 <sup>a</sup> ±0.35
Crude protein	58.1 <sup>b</sup> ±0.49	60.3 <sup>a</sup> ±0.07	60.2 <sup>a</sup> ±0.35
Crude Fats	8.2 <sup>a</sup> ±0.14	8.4 <sup>a</sup> ±0.21	7.5 <sup>b</sup> ±0.28
Fiber	0.7 <sup>a</sup> ±0.09	0.7 <sup>a</sup> ±0.02	1.2 <sup>a</sup> ±0.75
Phosphorus	0.4 <sup>a</sup> ±0.07	0.4 <sup>a</sup> ±0.14	0.4 <sup>a</sup> ±0.07

\*Super scripts indicate that values are statistically significantly different at p<0.05

## **GOOD AQUACULTURE PRACTICES (GAqPs) FOR AQUAPONICS**

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Good Aquaculture Practices (GAqPs) are activities, procedures, or considerations optimizing production systems and management protocols to maximize environmental and economic sustainability, final product quality and safety, animal health and worker safety, while concurrently minimizing the likelihood of a disease outbreak. GAqP considerations generic to all aquaculture production systems include considerations for: Regulatory and non-regulatory compliance, facility siting and design, source water, facility security, animal health, feed management, record keeping and employee training. Aquaponics is the integration of a hydroponic plant production system with a recirculating aquaculture system. A hydroponic system (closed or open) involves growing plants without soil i.e. in only nutrient solution or in some type of artificial media. A recirculating aquaculture system (RAS) most often is the production of fish in a closed system where water quality is maintained through a filter system. Independently, hydroponic systems and RAS can be productive and commercially viable. However, the integration of the two systems requires more extensive management intensity with selection and management of the fish and plant crops critical. Proper design of the system components including: fish tanks, plant production unit, mechanical filtration, biochemical filtration, greenhouse and water movement system is integral to the success of the enterprise.

## **GOOD AQUACULTURE PRACTICES (GAQPS) FOR: CAGE PRODUCTION SYSTEMS**

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### **Good Aquaculture Practices**

Good Aquaculture Practices (GAQPs) are activities, procedures, or considerations optimizing production systems and management protocols to maximize environmental and economic sustainability, final product quality and safety, animal health and worker safety, while concurrently minimizing the likelihood of a disease outbreak. GAQP considerations generic to all aquaculture production systems include considerations for: Regulatory and non-regulatory compliance, facility siting and design, source water, facility security, animal health, feed management, record keeping and employee safety and training.

### **Cage Production Systems**

Cage aquaculture is the most suitable production system for farm ponds. The bottom and shape of most farm ponds preclude many other fish production systems. Cage aquaculture is growing fish in a confined netted structure in a pond. Cage production has its particular GAQPs such as the number of fish stocked per cubic foot of water in a cage. Cage production uses a netting material in various geometric structures such as being round, square, or rectangle that allows for the exchange of water between the cage for growing fish and the pond. Growing fish in cages is a relatively common practice for beginning fish farmers. By using cages, a farmer has easy access to harvest fish for markets. It is recommended that a beginning fish farmer starts off with raising 250 rainbow trout in a four ft. round cage to learn GAQPs for cage production. GAQPs for cage production consist of various stocking densities, cage design, cage placement, fish suitable for growing in cages, water quality, fish health, feeding practices, and pond requirement.

## PROBIOTICS AND FISH GROWTH

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Heavy stocking, overuse of chemotherapeutics and general mismanagement of fish health have led to a need for alternative options to keep aquatic stock healthy. The period just after hatching is an important time for fishes as larvae are more susceptible to stressors. Probiotics are microbes that have been shown to improve fish immunity, gut health, growth and mitigate stress (Table 1). Prior research led by DSU has isolated several probiotic bacteria from the intestines of *Fundulus heteroclitus* and demonstrated their ability to inhibit growth of certain fish pathogens in disc diffusion assays. Because these probiotics were isolated from brackish water fish they may be useful for both marine and fresh water species. These probiotic bacteria will now be evaluated to see if they can enhance growth and survival of four commercially important finfish species, two marine (cobia and clownfish) and two freshwater (hybrid striped bass and rainbow trout). Each species will be tested in succession.

For each species trial, fish will be divided into 12 groups of ten larvae and placed in 2-L experimental containers with 1-L of water. Individual experimental containers will be randomly assigned to one of four treatments (three probiotics and an untreated control) allowing three replicates per treatment. Treatments will be applied by dosing the culture water with  $10^6$  CFU/ml of probiotic every other day.

Fish in the experimental containers will be fed a prepared diet selected for each individual fishes' size and nutritional requirements at a rate of 4% biomass per day. The daily ration will be divided into three feedings and the amount adjusted weekly. Water quality in the experimental containers will be checked daily, followed by a 50% water exchange.

After 30 days, surviving fish from each individual experimental container will be euthanized with an overdose of MS-222, counted, group weighed and measured for length. Growth and survival will be calculated and compared across treatments to ascertain which probiotic has the greatest impact on fish performance. Growth will be measured in calculated daily gains as well as relative weight gains. The experimental data will show which of the novel probiotics has the greatest impact on getting larvae through the high mortality post larval bottleneck period.

Table 1. Common current uses of probiotics.

Probiotic Genus	Culture Species	Effects	References
<i>Bacillus</i>	Shrimp	Improved health, water quality, growth and stress management	Olmos et al. (2011)
	Carp	Improved growth, survival and health	Kumar et. al (2006)
	Rohu	Improved health, water quality and growth	Jiang et al. (2013)
	Rainbow trout	Improved health, survival, fecundity, and growth	Raida et al. (2003)
<i>Shewanella</i>	Prawn	Improved growth, and survival	Rahiman et al. (2010)
	Gilthead sea bream	Improved growth, water quality and health	Makridis et al. (2005)
	Abalone	Improved health, growth, water quality and stress management	Bairagi et al. (2004)

## ASSESSING THE RELIABILITY OF WATER TEST KITS FOR USE IN POND AQUACULTURE

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Water analysis kits are useful for practical aquaculture only if they provide equivalent decision making as those obtained by standard water analysis methods. This study used weighted Cohen's Kappa statistics to compare management decisions made by farmers that used water analysis kits (e.g. Seneye slide kit, Tetra Easy Strips, API test strips, Seachem Ammonia Alert, Salifert Profi test kit and Hach dissolved oxygen (DO) and alkalinity kit) and those that used standard methods. The decisions made by farmers were similar for water analysis kits and standard methods, except for Tetra and API test strips when measuring nitrate concentrations (Table 1). The highest conformity between the two methods (Kappa value = 1.0,  $p < 0.0001$ ) was obtained with the Hach and Salifert Profi test kits (for measuring DO) and the API test strip (for measuring total hardness) (Table 1). The rapid, simple measurements by the kits appear suitable for use by farmers if they are properly maintained and manufacturer's instructions are followed.

Table 1: Comparison of agreement of decision made for each water test kit compared to that of the standard methods of analysis using weighted Cohen's Kappa test

Water parameter	Water test kit	N	k value	p value	Degree of agreement based on k
Total alkalinity	Hach alkalinity kit	39	0.6512	<.0001	Substantial
	Tetra Easy Strips	39	0.6053	<.0001	Substantial
Total hardness	Tetra Easy Strips	21	0.7368	<.0001	Substantial
	API test strip	21	0.9446	<.0001	Almost perfect
Nitrite	Tetra Easy Strips	10	0.5455	0.0125	Moderate
	API test strip	10	0.5455	0.0125	Moderate
Nitrate	Tetra Easy Strips	17	0.1472	0.1695	No agreement
	API test strip	17	0.1472	0.1695	No agreement
pH	Tetra Easy Strips	13	0.4783	0.0053	Moderate
	API test strip	13	0.6364	0.0007	Substantial
	Seneye slide	13	0.3333	0.0088	Fair
Un-ionized ammonia (NH <sub>3</sub> )	Seneye slide	9	0.4737	0.0117	Moderate
	Seachem Ammonia Alert	9	0.8000	0.0002	Substantial
Dissolved oxygen	Hach DO kit	12	1.0000	<.0001	Perfect
	Salifert Profi test	12	1.0000	<.0001	Perfect

## ECOPHYSIOLOGICAL-MODELING-BASED ANALYSIS OF RED DRUM STOCK ENHANCEMENT IN TEXAS: AN UPDATE

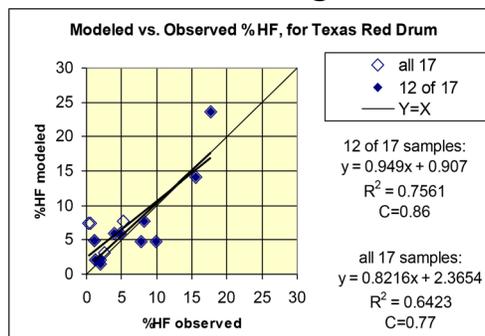
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We have revised our previous analysis to force compliance with growth and frequency data observed for gill-netted red drum identified genetically as having come from hatcheries operated by Texas Parks and Wildlife Department's Division of Coastal Fisheries. Growth rates of these 190 recovered hatchery fish (HF), taken in 17 bay-year-season samples, implied that 28 % of targeted HF, on average, were outside the 300-500-mm TL range subjected to genetic analysis. Taking these "dark" HF into account, and optimizing the presumed rate of HF mortality upon release at 91 %, resulted in maximized goodness-of-fit between modeled and observed proportions of HF in the gill-net catch.

For all 17 samples, the emergent relation was  $\%HF_{mod} = 2.37 + 0.82*\%HF_{obs}$ , with  $R^2 = 0.64$ . Restricting the analysis to the 12 samples with 3 or more recoveries gave  $\%HF_{mod} = 0.91 + 0.95*\%HF_{obs}$ , with  $R^2$  increased, to 0.76.

Our ecophysiological analysis of stocking, juvenile-abundance, and sport-fishery-catch data for red drum in Texas is thus amended, as follows: Assuming 91 % HF mortality over first 2 days after release, subsequent growth rates equivalent to those of recovered HF, and with other reasonable assumptions in place, we estimate that for the 1990-2011 year classes of red drum, a total of 23,160,000 individual fish have survived and grown to enter the fishing "slot" (20-28 inches TL) in Texas bays 2-8. Of this total, 1,670,000, or 7.2 %, have been HF. These HF have supplanted 296,000 WF, or 1.3 % of the total. Thus, net HF contribution to the stock at slot entry is estimated to have been 1,374,000 fish, or 5.9 % of the stock.



## **GOOD AQUACULTURE PRACTICES (GAqPs) FOR POND AQUACULTURE SYSTEMS**

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Pond aquaculture is farming in impounded good quality water with a maximum depth of eight feet using various levels of intensity. Smooth impervious clay bottoms with a minimal slope allow easy seine harvesting. Commercially available feed is broadcast to the crop and water quality parameters, primarily dissolved oxygen concentration, are maintained. Multiple aquaculture crops can be raised in ponds based on the projected consumer demand for locally grown products and the seasonal temperatures. These include hybrid striped bass, channel catfish, freshwater shrimp, ornamental fish and rainbow trout. Commercially available pelleted feed is provided to the animals with excellent feed conversions ranging from 1.8-3.0.

## TESTS FOR THE LAW OF ONE PRICE AND PERFECT PRICE TRANSMISSION IN THE US SHRIMP MARKET: BIAS IN SIMULATION OF EXCHANGE RATE PASS-THROUGH

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This study addresses two questions of importance to the analysis of policies affecting aquaculture international trade: (1) to what extent does the omission of transportation cost in tests for the Law of One Price (LOP) result in biased estimates of the elasticity of price transmission (EPT)? and (2) to what extent do biased estimates of EPTs matter when modeling the effects of exchange rate changes on prices and trade flows? The questions are addressed using the U.S. market for imported shrimp as a case study. Tests for LOP are conducted for six major suppliers to the U.S. market, namely Thailand, China, Ecuador, Indonesia, India, and Vietnam. Results indicate omission of transportation cost causes estimated EPTs to be underestimated by 9.14%. The omission also leads to over-rejection of LOP. Simulations of an excess demand-supply model of U.S. shrimp imports indicates understated EPTs cause exchange rate pass-through to be understated, as are the effects of exchange rate movements on imported quantities. Overall, for the considered supply and demand elasticities, most of the incidence of a change in exchange rates is borne by foreign producers.

## TREATMENT OF KOI PONDS WITH A FLOATING BEAD FILTER AND ULTRAVIOLET LIGHT COMBINATION

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Floating bead filters are used with ultraviolet lights to clarify koi ponds. Smaller ponds have traditionally been filtered with hourglass filters that provide nitrification and solids capture. With more than enough nitrification capacity for the lightly loaded koi pond, filter sizing is based upon the turnover rate of the pond. High water clarity is normally achieved with turnover rates in the range of 90-180 minutes, so a sizing criteria of 1 cubic foot of beads for each 2000 gallons of pond volume is used in practice. The bead filter is complemented by ultraviolet light treatment to control growth of algae blooms (green water). The UV light system should have a capacity of about 10 watts for each 1000 gallons of pond. The UV light are usually placed behind the floating bead filter. Water is circulated by a how head submersible or self-priming centrifugal pump. A water fall provides enough oxygen for a koi pond application.

Larger, deeper ponds can be filtered with a PolyGeysers filter sized to provide pond turnover every 3-6 hours. The PolyGeysers filter is typically backwashed by a linear air pump eliminating the requirement for manual backwashing. The PolyGeysers accumulates sludge that has to be removed about once a week. The sludge make a great fertilizer for plants in the back yard.



## ASSESSMENT OF FISHING GEAR AND CATCH RATE IN OGUTA LAKE OF SOUTH EASTERN NIGERIA

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The study was carried out in Oguta Lake from January, 2012 to December, 2013 at five stations (Onu Utu, Okposha, Ogbe Hausa, Osemotor and Ede Ngwugwu) to assess the fishing gear and catch rate. The Lake is the largest natural freshwater lake in Imo State, Nigeria and originated from a natural depression. It is bounded between longitude  $6^{\circ} 41' - 6^{\circ} 50'$  East and latitude  $5^{\circ} 41' - 5^{\circ} 44'$  North of the equator. Total catch by species was recorded for gillnets, cast nets, hook and lines, boat seines, lift nets, basket and drum traps.

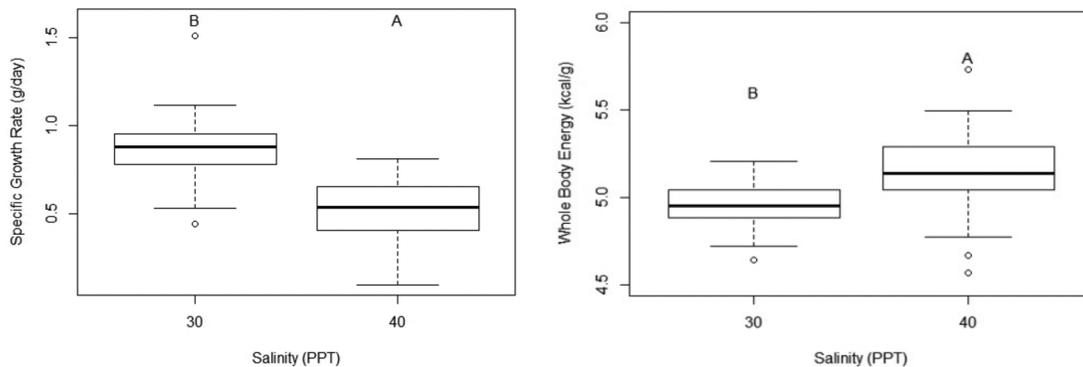
The average weight of fish caught per canoe per day ranged between 3.2kg for Basket and Drum traps to 47.1kg for boat seine nets. The gill nets had a range between 6.0 to 13.0kg per canoe per day with mean weight of  $8.60 \pm 2.91$ kg. Cast net had values ranging from 1.0 to 7.0kg with a mean weight of  $4.3 \pm 2.49$ kg. Lift nets yielded an average of 3.4 kg per canoe per day. In this study, gill nets were the main gears used by fishers, followed by long lines and cast nets. The study revealed that the use of these gears, however, may promote resource overexploitation as they are non-selective. So far, it has not been demonstrated that the fish stocks in the lake are being under exploited. However, it may be necessary to locate all the fishers operating in the lake so as to collect daily record of catches be towards achieving a more accurate picture of the total catch and fish species. Since mesh size regulation prevent over exploitation of young fish, before they attain sexual maturity, it is recommended that the procurement of fishing inputs such as fishing gear and crafts should be monitored by government and also subsidized for the fishers in order to achieve better control.

## EFFECTS OF pH AND SALINITY ON JUVENILE HATCHERY-REARED RED DRUM *Sciaenops ocellatus*

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Red Drum (*Sciaenops ocellatus*) is a recreationally and economically important fish that is the subject of stock enhancement. Consideration must be given to differences in pH and salinity between the rearing facility and the location for release of juvenile red drum into the wild. Research was conducted in a closed recirculating system with juvenile red drum ( $42 \pm 9.9$  mm) randomly stocked at a density of 13 fish/tank and fed daily to satiation (~6% body weight). Fish were subjected to salinity treatments of 40 or 30 and a pH of either 7.5, 8.1, 8.5, or 9.0 ( $n = 8$  replicates per treatment), for a trial duration of 14 days. Results showed that at a salinity of 40, there was a significant difference between survival of the juvenile red drum at pH 7.5 and 9.0 ( $p=0.03$ ). Survival was not significant between pH levels at salinity of 30. Results indicated pH had no significant effect on specific growth rate (SGR,  $p \geq 0.05$ ) of experimental fish; however, increased salinity significantly decreased growth ( $p < 0.05$ ), and there was a significant interaction between pH and salinity. There was no significant effect from pH or salinity on protein retention ( $p \geq 0.05$ ). At a salinity of 40, whole body ash content increased as pH increased ( $p=0.003$ ). Whole body energy was not significantly affected by pH ( $p \geq 0.05$ ); but increased salinity caused a significant decrease in energy retention ( $p < 0.05$ ), with no significant interaction between pH and salinity. These results indicate that salinity is a more critical factor to consider than pH when stocking fish into diverse bodies of water, especially under high salinity conditions. The results of this study will help fisheries managers increase the rate of survival of hatchery-reared red drum when released into the wild. These results also suggest that research should be conducted to investigate the effects of hypersalinity ( $>40$ ) and pH on growth of juvenile red drum.



## TOWARDS THE BETTER USE OF SOY BASED DIET FOR FLORIDA POMPANO *Trachinotus carolinus*: USE OF NOVEL SOY PROTEIN CONCENTRATE AND SQUID PRODUCTS

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Soybean meal and soy protein concentrate have successfully been demonstrated as alternative choices of protein sources in formulated diets for Florida pompano. However, low performance of the fish is consistently observed when animal protein is completely removed from the diet. To explore possible improvements to plant based feeds, NutriVance (NV), a novel soy protein concentrate was used to replace poultry by product meal (PBM, 15% diet) on an iso-nitrogenous basis. The animal free diet was then supplemented with graded levels of squid meal and squid hydrolysates replacing NV. Each dietary treatment was tested in quadruplicate groups of 20 Florida pompano (mean initial weight 7.68 g) per tank. Fish were offered each diet to near satiation over an 8 week growth trial. Fish maintained on the diet containing 15% PBM were larger and had the lowest FCR. Similar performance was obtained in fish maintained on diet containing 4% squid hydrolysates. The results demonstrate that diets containing 100% plant based protein sources are not viable and low levels of animal based proteins are required to improve performance. Series of histology, mineral and proximate analysis of fish were also conducted and will be reported.

**Table 1.** Major components of the iso-nitrogenous and iso-lipidic diets (40% protein, 8% lipid).

Experimental Diet	PBM	NV	1%SH-NV	2%SH-NV	4%SH-NV	1%SM-NV	2%SM-NV	4%SM-NV
Poultry by product meal	15.00							
Soybean Meal	47.21	47.21	47.21	47.21	47.21	47.21	47.21	47.21
Soy protein Concentrate		14.80	13.65	12.51	10.22	13.68	12.56	10.32
Squid hydrolysates			1.00	2.00	4.00			
Squid meal						1.00	2.00	4.00
Menhaden Fish Oil	4.74	6.37	6.35	6.33	6.29	6.33	6.30	6.23
Taurine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

**Table 2.** Survival (%), final mean weight (g per fish), biomass (g), and FCR (feed per gain) of fish fed the test diets for 8 weeks, significance determined by SNK grouping

Diet	PBM	NV	1%SH-NV	2%SH-NV	4%SH-NV	1%SM-NV	2%SM-NV	4%SM-NV
Mean weight (g)	38.60 <sup>a</sup>	28.17 <sup>abcd</sup>	23.65 <sup>dc</sup>	32.84 <sup>abc</sup>	35.17 <sup>ab</sup>	21.58 <sup>d</sup>	27.25 <sup>bdc</sup>	30.16 <sup>abc</sup>
Biomass (g)	702.3 <sup>a</sup>	405.75 <sup>bc</sup>	408.2 <sup>bc</sup>	515.25 <sup>bc</sup>	541.95 <sup>ab</sup>	365.35 <sup>c</sup>	431.25 <sup>bc</sup>	512.275 <sup>bc</sup>
FCR	1.42 <sup>a</sup>	1.69 <sup>ab</sup>	2.09 <sup>ab</sup>	1.48 <sup>a</sup>	1.44 <sup>a</sup>	2.29 <sup>b</sup>	1.69 <sup>ab</sup>	1.67 <sup>ab</sup>
Survival (%)	91.25 <sup>a</sup>	73.75 <sup>a</sup>	85 <sup>a</sup>	78.75 <sup>a</sup>	77.5 <sup>a</sup>	85 <sup>a</sup>	80 <sup>a</sup>	86.25 <sup>a</sup>

## ASSESSING THE USE OF FUNCTIONAL SOY PROTEIN IN SOY BASED FEED FORMULATIONS ON THE HAEMATOLOGICAL, HISTOLOGICAL, GROWTH, SERUM, AND NUTRITIONAL VALUE OF FLORIDA POMPANO *Trachinotus carolinus*

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This study aims to evaluate the effect of replacing commodity soybean meal (CP 46.72) with fermented soybean meal (CP 52.87) ingredient with peptides, direct-fed microbials, and metabolites on the growth performance, feed utilization, amino acid profile, hematology, histology, and serum biochemical parameters including enzymes, i.e. alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), glucose, cholesterol, albumin, bile acids and total protein of Florida pompano. Four isonitrogenous and isocaloric diet were formulated to contain 15% Poultry by-product meal and commodity soybean meal (PBM), 50% replacement of soybean meal with PepSoyGen (PSG) as the functional soy (50%PSG), 75% PSG and 100% PSG. The experimental diets were fed in triplicate groups of 20 Florida pompano (mean initial weight 17 g) per tank arranged in a completely randomized design for 8 weeks at the Claude Peteet Mariculture Center, Gulf Shores, Alabama, USA. At the end of feeding trial, no significant differences were found in growth performance and feed utilization. However, hematocrit levels (%) in the fish fed with fermented soy were higher than fish fed with basal diet. Fish fed with basal diet showed an elevation in glucose and bile acids level but low level of ALT and AST ratio activities. An increase in ALP activity was also noticed in fish fed with 50% and 100% fermented soy compared to the basal diet. These results suggest that the fermentation process of soybean meal does not impair growth performance in Florida pompano. Histology and proximate analysis of fish are being determined to evaluate liver integrity and nutrient utilization.

**Table 1.** Growth performance and serum analysis of fish fed the test diets for 8 weeks, significance determined by SNK grouping

Diet	PBM	50%PSG	75%PSG	100%PSG	PSE	P-value
Mean weight (g)	72.44	68.25	73.48	69.10	3.98	0.7531
PWG	324.81	301.68	335.18	305.95	26.43	0.7857
FCR	1.72	1.78	1.54	1.58	0.09	0.3119
Survival (%)	93.3 <sup>a</sup>	78.3 <sup>b</sup>	80.0 <sup>b</sup>	73.3 <sup>b</sup>	2.5	0.0027
Hematocrit levels (%)	41.67	43.00	44.83	42.58	0.99	0.2253
Total Protein (g/dL)	4.06	4.29	4.05	4.17	0.11	0.3927
Albumin (g/dL)	1.30	1.42	1.33	1.41	0.03	0.1079
Alkaline phosphatase (ALP)	35.50	42.93	38.30	44.23	2.05	0.0540
Alanine aminotransferase (ALT)	17.33	10.67	18	11.50	3.69	0.2778
aspartate aminotransferase (AST)	222	100.67	161.33	96.33	45.97	0.2515
ALT/AST	0.078	0.106	0.112	0.119	0.01	0.3117
Glucose (mg/dL)	251.67	237.67	240	246.67	16.13	0.9227
Cholesterol (mg/dL)	173.67	184	179.67	176.67	5.21	0.5686
Bile acids (mg/dL)	9.80	8.93	5.80	3.83	3.24	0.5623

## THE IMPORTANCE OF APPROPRIATE ESTIMATION OF CULTURED CONTRIBUTION IN STOCK ENHANCEMENT: CASE STUDIES FROM SOUTH CAROLINA

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Stock enhancement goals can vary significantly by species, but understanding the life history and fishery of the target species is essential to determining stocking strategies and their success. One effective method for gauging the success of a stock enhancement program and its impact on wild populations is to regularly sample a population and calculate the hatchery contribution on a cohort-by-cohort basis. In South Carolina, research focuses specifically on three species: red drum, cobia and spotted seatrout. The genetic population structure and life history parameters of each candidate species needs to be considered prior to releasing fish, and calculating their contribution to a particular cohort can range from simple to complex. Stock enhancement research on red drum, a long-lived species, focuses primarily on the subadult population found in the estuary. Red drum have a short distinct spawning season and fast growth rates, but long subadult stage (3-5 years). Fish are sampled by SCDNR's fisheries independent trammel net survey and estimation of hatchery contribution to a cohort by size is relatively simple. Similarly, cobia, a coastal migratory pelagic species with short spawning season and fast growth rate, can also be reliably aged in the first two years so that stocking contribution can be assigned accurately for young fish. For adult cobia, we rely on cooperating anglers, freezer programs, and otolith aging to estimate hatchery contribution of stocked fish. The life history characteristics of spotted seatrout require considerable effort for estimating stocked contribution because the species is genetically isolated by distance, spawns over a six-month period, and exhibits sexually dimorphic growth. For spotted seatrout, subsamples are taken for otolith aging and predictive statistics are used to estimate a cohort for each individual before estimating a hatchery contribution. This research compares and contrasts three disparate species with unique life histories and addresses caveats to understanding stock enhancement impacts and designing appropriate stocking strategies.

## GENERAL AND SPECIFIC COMBINING ABILITY OF CHANNEL CATFISH (*Ictalurus punctatus*) FEMALE X BLUE CATFISH (*I. furcatus*) MALE FOR EARLY PERFORMANCE TRAITS OF F1 HYBRID PROGENY

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Hybrid catfish are considered best for commercial catfish farming due to their faster growth rate, better survival rate, disease resistance, better carcass yield and seinability. To improve its hybridization breeding program, the determination of the combining abilities of parental lines is vital. The estimation and analyses of general combining ability (GCA) and specific combining ability (SCA) are important indicators for expressing the potential value of parental lines that can produce superior performing hybrids. It will help us determine the best parental genotypes and the best cross for superior hybrids of catfish. Information on combining ability to develop high yielding lines and hybrids would be beneficial to the catfish industry.

Eleven female channel catfish and twelve male blue catfish were crossed in 11x12 factorial mating design. The progenies were reared in flow-through aquaria system for 323 days and fed *ad libitum* with 32% protein diet on a daily basis. The resultant 36 hybrid families were evaluated for their early performance traits (early growth, early FCR, and survival from *Aeromonas* and Ich infection).

The estimates for variance of combining ability showed that GCA variance for sire was higher than that of SCA for all the early performance traits (Table 1). This indicates the prevalence of additive gene actions contributed by the male parent than the female parent. The importance of additive gene effects for controlling inheritance of early traits of hybrid catfish is revealed. The estimation also revealed that dominant gene effect was negligible except for early growth. Overall, the reciprocal recurrent selection for early traits in terms of additive genetic value of the blue catfish male has the potential to improve the performance of hybrid catfish.

Table 1. Estimates for variance of dam general combining ability ( $\sigma^2 \text{GCA}_d$ ), sire general combining ability ( $\sigma^2 \text{GCA}_s$ ), specific combining ability ( $\sigma^2 \text{SCA}$ ), and error variance ( $\sigma^2_E$ ) for early performance traits of hybrid catfish.

Genetic parameter	Early growth	Early FCR	Survival from <i>Aeromonas</i> infection	Survival from Ich infection
$\sigma^2 \text{GCA}_d (\pm \text{SE})$	1.24 (2.45)	0	0	50.56 (68.03)
$\sigma^2 \text{GCA}_s (\pm \text{SE})$	2.01 (1.61)	0.07 (0.06)	135.33 (73.72)	119.27 (74.37)
$\sigma^2 \text{SCA} (\pm \text{SE})$	1.74 (1.50)	0	1.97 (34.11)	0
$\sigma^2 \text{error} (\pm \text{SE})$	5.19 (0.93)	0.71 (0.11)	282.77 (49.77)	319.25 (51.62)

## INFORMATION SOURCES AND ACCESSIBILITY AMONG FISHER FOLKS IN ESE - ODO AND ILAJE LOCAL GOVERNMENT AREAS OF ONDO STATE, NIGERIA

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Information sources and accessibility among fisher folks in Ese - Odo and Ilaje Local Government Areas of Ondo State, Nigeria. A multistage random sampling procedure was employed to select two local Government areas (Ese-odo and Ilaje), six fishing communities, and twelve fisher folks from the study area to get a sample size of one hundred and forty four. A structured questionnaire and scheduled interview was used to collect data from the respondents. Data were analyzed with the use of descriptive statistic such as frequency counts and percentages. Chi-square was used to test relationships between the variables.

The results showed information need of fisher folks on fishing gear maintenance 92.4%, weather 88.2%, modern fish capture method 86.8%, sources of micro credit 86.8% and 57.6% had access to information. Extension agents 13.2% and friends 11.8% were the major sources of information among the fisher folks. The chi-square analysis indicated that no significant association existed between accessibility and information source among fisher folks on extension agents ( $\chi^2 = 0.37, p > 0.05$ ), research institute ( $\chi^2 = 0.18, p > 0.05$ ), non-governmental organization ( $\chi^2 = 0.12, p > 0.05$ ), radio ( $\chi^2 = 0.26, p > 0.05$ ) television ( $\chi^2 = 0.08, p > 0.05$ ) and friends ( $\chi^2 = 0.19, p > 0.05$ ). It is recommended that government should maximally utilize all available information sources to reach the fisher folk to boost protein supply through artisanal fisheries.

**Table: 1. Accessibility to needed Fisheries Information by Fisher folks**

Variable	Yes		No	
	Freq.	%	Freq.	%
Accessibility	83	57.6	61	42.4

**Table 2 Chi square relationships between accessibility and sources of information dissemination to fisher folks.**

S/ N	Training variables	$\chi^2$	Df	P	Rm ks
1.	Extension agents	3	1	0.37	NS
2.	Research Institute	.59	1	0.18	NS
3.	Non - governmental Organization	2.20	1	0.12	NS
4.	Federal Department of Fisheries	0.209	1	0.50	NS
5.	Radio	1.045	1	0.26	NS
6.	Television	3.093	1	0.08	NS
7.	Friends	1.324	1	0.19	NS
8.	Extension publication pamphlet	1.110	1	0.23	NS

Source, field survey 2013

## INFLUENCE OF ABIOTIC FACTORS AND FOOD CONCENTRATION ON POPULATION GROWTH OF THE CILIATE *Euplotes* sp.

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Live food organisms used for feeding larval marine fish have received significant research in the past decade. Most of this research and development has focused on rotifer enrichments and feeds, and mass scale production methods for several species of copepods. However, there is a need to investigate other small live food organisms to potentially improve survival and growth of larval fish during the hatchery phase of culture. One potential live food organism is a ciliate. However, there is a dearth of information on optimal production parameters for ciliates. The objectives of this study were to evaluate the influence of abiotic factors including salinity, photoperiod, temperature, aeration, and food concentration on the growth of *Euplotes* sp. populations. Five replicated small scale experiments were designed and conducted. First, food concentrations of 50, 100, 250, and 500 mg of Protein Selco (INVE) per million ciliates were investigated. Second, salinities of 15, 20, 25, 30, and 35 g/L were investigated. Third, four levels of aeration were investigated. Fourth, temperatures of 17, 20, 23, 26, 29, and 32°C were investigated. Fifth, four photoperiods were investigated.

Results indicated that the optimal range of conditions for production of the ciliate *Euplotes* sp. to be a feed concentration of Protein Selco (INVE) of 250 mg per million ciliates, salinity from 20-35 g/L, low or no aeration, temperatures between 26 and 32°C, and a photoperiod between 0L:24D and 16L:8D. Based on the results and observations during this study, we conclude that the ciliate *Euplotes* sp. has characteristics favorable to mass production including resistance to adverse conditions such as high concentrations of ammonia and very low dissolved oxygen concentrations, they experience high growth and reproduction rates, can grow to very high densities, received nutrition either directly or indirectly from an inert diet, and adapted well to a wide range of salinities and temperatures.

## DETERMINING PREY PREFERENCE OF FIRST FEEDING MARINE ORNAMENTAL FISH LARVAE UTILIZING FLUORESCENT MICROSPHERES

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As the popularity of marine aquaria grows, potentially more marine ornamental fishes and invertebrates will be harvested from the oceans. Aquaculture of marine species is a sustainable alternative to wild caught individuals and may help to grow businesses and diversify species in production. Experiments were conducted with first-feeding larvae of reef butterflyfish (*Chaetodon sedentarius*), Pacific blue tang (*Paracanthurus hepatus*), African moony (*Monodactylus sebae*) and golden trevally (*Gnathanodon speciosus*). The objective was to define prey preferences at first-feeding between rotifers (*Brachionus plicatilis*), copepod nauplii (*Parvocalanus crassirostris*), and ciliates (*Euplotes* sp.), by marking each prey with a different color of fluorescent microsphere and observing gut contents with a fluorescent microscope. Each fish species showed different prey preferences. Pacific blue tang larvae preferred rotifers above ciliates, and ciliates above copepod nauplii. African moony larvae preferred ciliates and nauplii equally over rotifers. Reef butterflyfish larvae preferred ciliates over rotifers and rotifers over nauplii. Golden trevally larvae preferred nauplii over ciliates, and ciliates over rotifers.

This study presents a new understanding of prey preference of first feeding ornamental marine larvae by utilizing fluorescent labelled microspheres. Microspheres are currently used in marine ecology research to trace microplastics throughout planktonic food webs. Ingestion of naked ciliates labeled with microspheres in fish larvae has been previously performed. However, microspheres have not been utilized to examine prey preference and consumption of live feeds. This marks the first use of microspheres in aquaculture with rotifers (*Brachionus plicatilis*) and copepod nauplii (*Parvocalanus crassirostris*).

## THE IMPACTS OF WETLAND RESTORATION ON FISHERIES IN NIGERIA

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Wetland is one of the resources of high value which has been exposed to indiscriminate use. It is an important ecosystem to fish and loss or degradation of wetland will have a direct consequence on sustainable fisheries. This paper reviewed the term “wetland”, its functions and values, importance to fish production in Nigeria and threats to its sustainability. The term “wetland” has been defined by various researchers especially based on their profession and their needs but up till today there is no single definition accepted by all users. In Nigeria, the most commonly adopted is that of RAMSAR convention. Wetland has both marketed and non-marketed functions and values. They provide essential link in the life cycle of 75 percent of the fish and shell fish commercially harvested in the world and are vital to fish health. Despite the importance, there have been exceptional losses of wetlands. Lagos state alone has witnessed more than 96 percent loss. Major threats to wetlands are: agriculture, development, pollution and climate change. Therefore proper management of the wetland ecosystem is important in other to ensure continuous fish production.

## SWOT MATRIX ANALYSIS OF ALGAE DERIVED BIOFUEL AS A FOURTH GENERATION OF BIOENERGY PROSPECT FOR NIGERIA

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The recent continuous decline in the utilization of fossil fuels as means of energy production has significantly become a global issue and Nigeria as a developing country is grappling with the means of identifying alternative means of energy sustainability. This is the baseline for which this study was conducted as the principle of sustainability is fundamental to natural resources management.

The use of various biofuel productions which has been adopted in Nigeria for a while was measured against the adoption of algae biofuels using the Strengths, Weaknesses, Opportunities and Threats (SWOT) Analysis which was incorporated into the data analysis of comparison of bioenergy production. Here, SWOT Analysis was used to explore new solutions to problems, Identify barriers that will limit objectives of this review, decide on direction that will be most effective and additionally reveal possibilities and limitations for change and positive end result of the adoption of algae biofuels.

The results revealed that algae- derived biofuel forms the basis for fourth generation biofuel production which can meet the much-needed need for energy sustainability in Nigeria. Also, biofuels were found to be inevitably important to a decarbonized means of transportation (especially trucks, ships and aircrafts). Further study can be done to include other developing countries, particularly the non-oil-producing nations where large potential of bioenergy production are yet to be tapped. If appropriate policies and related technological innovations are promoted and effected, the production and utilization of biofuels in Nigeria can potentially help solve the economic, social and environmental problems.

**TABLE 1: showing 4 generations of Biofuels and their sources.**

S/N	Generation of biofuel[3]	Sources
1)	First generation Biofuels	Grains and sugar to Ethanol , Vegetable oil to Biodiesel.
2)	Second Generation Biofuels :	Lignocellulose to Alcohols , Lignocellulose to Green Diesel ,Vegetable oils to Green Diesel.
3)	Third generation	Biomass to Hydrogen , Algal Hydrogen , Algal Oil/Biodiesel.
4)	Fourth generation Biofuel	Biofuel from high solar , efficiency cultivations

## **POLLUTION OF NIGERIAN AQUATIC ECOSYSTEMS BY INDUSTRIAL EFFLUENTS: EFFECTS ON FISH PRODUCTIVITY**

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Nigeria is uniquely endowed with vast water resources. The near-shore, estuaries, rivers, lakes and pond all taken together, offer tremendous opportunities for fish production. Globally, water bodies are primary means for disposal of waste especially the effluents from industrial, municipal, sewage and agricultural practices near the water body. Studies carried out in most cities in Nigeria has shown that industrial effluent is one of the main sources of water pollution in Nigeria and less than 10% of industries in Nigeria treat their effluents before discharging them into the water bodies. This effluent can alter the physical, chemical and biological nature of the receiving water body resulting in the death of the inhabiting organisms including fish. Untreated industrial waste discharged into water bodies have resulted in eutrophication of aquatic ecosystem as evidence by substantial algal bloom leading to dissolve oxygen depletion and eventually massive mortality of fish and other organisms. Industries like textile producing factory, paper manufacturing plants, oil refinery, brewery and fermentation factory and metal producing industries discharge their wastes into the aquatic ecosystem. These industrial wastes contain pollutants like acids, heavy metals, oil, cyanide, organic chemicals, pesticides, polychlorinated biphenyls, dioxins etc. Some of these pollutants are carcinogenic, mutagenic and teratogenic while some are poisonous depending on the level of exposure and intake by aquatic organisms and man. These pollutants affect the biological growth and reproduction of fishes in the aquatic ecosystem thereby reducing the amount of captured fishes. Fish and other aquatic lives face total extinction due to destruction of aquatic lives and natural habitats by pollution of water bodies. Effluents and wastes produced by industries should be minimised by using low and non-waste technologies; and effluents should be properly treated before they are discharged into aquatic environment.

## **RISK AND RELIABILITY ANALYSIS OF OFFSHORE AQUACULTURE OCEAN PLANTATION SYSTEM**

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Risk based design uncertainty analysis has also been used on system that use fill materials from seabed, hills, deep underground excavations, and even construction debris, engineers are able to create relatively vast and valuable land from the sea. Aquaculture industry is one of the fastest growing sectors in the world. Considerable interest exists in developing open ocean aquaculture in response to a shortage of suitable, sheltered inshore locations and possible husbandry advantages of oceanic sites. Adopting the concept of very large floating structure in aquaculture oceanic farming can lead to production of more aquaculture product like seaweed. Such system requires study of property and support for growing aquaculture industry. Risk analysis study of offshore aquaculture ocean plantation system is very important to determine the system functionality and capability that meet sustainable and reliability requirement.

## CONCENTRATIONS OF POTENTIAL TOXIC METALS IN DIFFERENT AQUATIC FISH PARTS HARVESTED FROM TWO LAGOONS IN LAGOS NIGERIA

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The level of some potential toxic metals (PTM) (Pb Ni Fe, Cu and Zn) in *Synodontis membrane* (Catfish), *Tilapia zillii* (Tilapia), from Lagos Island and Ikorodu (Ibeshe) lagoon were investigated using Atomic Absorption Spectrophotometer (AAS), model Buick Scientific 210 GVP, the analysis on all the samples were done in triplicates. The analysis of trace metals in *Synodontis membrane* and *Tilapia zillii* was conducted on head, trunk, intestine and gills the results showed the accumulation of metals occurred differently in various parts of the fish samples analysed. Lead (Pb) and nickel (Ni) were not detected (ND) in most of the samples except nickel that was found in the intestine of the sample in both lagoon. The absence of lead and sample may be attributed the existence of few or no industrial activities around the locations. The orders of accumulation in Tilapia were Gill>intestine >Trunk >Head and the pattern of distribution is Fe> Zn>Cu in all the organs from both lagoon.

In Catfish, the accumulation follows the order; *Gills >Intestine>Head>Trunk* respectively. The pattern of distribution of potential toxic metal in the catfish were Fe> Zn>Cu in all the organs of the sample analyzed. The mean values of the Catfish obtained fell in the ranges: Fe (39.93±0.72- 285.58±4.18); Cu (0.96±0.11- 5.84±0.37); Zn (26.00±0.41- 208.50±2.35) for Lagos Island and Ikorodu Lagoon respectively. The high concentrations of iron, zinc and copper were recorded in the sediment sample be a major depository of potential toxic metal and this may account for high concentration of the metals in the fish parts due to the fact that these metals are naturally abundant in Nigeria soils and since the source of metal depositories are the aquatic systems. The general drift of accumulations in all the organisms was *Tilapia zillii>Chrysichthys nigrodigitatus*. The analysis of the fish sample shows that concentrations of Fe, Cu and Zn in the samples are lower than the permissible limits set by WHO, USEPA and FEPA which indicate that the fish are safe for consumption therefore it is pertinent to regularly monitor the activities on both lagoons.

The level of some Potential toxic metals PTM (Zn, Fe, Mn, Cu, Pb and Cd) in *Synodontis membrane* (Catfish), *Tilapia zillii* (Tilapia), *Callinectes amnicola* (Crab) and *Macrobrachium macrobrachion* (Prawn), from Lagos Island and Epe lagoon were investigated using Atomic Absorption Spectrophotometer (AAS), model Buick Scientific 210 GVP, the analysis on all the samples were done in triplicates. The analysis of PTM in *Synodontis membrane* and *Tilapia zillii* was conducted on head, gills and trunk, and the results indicated the accumulation of metals differently in various parts of the fishes. Lead (Pb) and Cadmium (Cd) were not detected (ND) from all the samples, this may be due to the existence of few or no industrial activities around the location.

The orders of accumulation in Catfish were Head>Gill>Trunk and the pattern of distribution is Zn>Fe>Mn>Cu in all the organs. In Tilapia, the accumulation follows the order; *Gills (Fe>Zn>Mn>Cu)>Head (Zn>Fe>Mn>Cu)>Trunk (Fe>Zn>Mn>Cu)*. The mean values of the Crab obtained fell in the ranges: Fe (19.7±1.7- 24.7±7.7); Zn (8.0±2.5-11.6±1.3); Cu (9.0±3.3-10.3±1.9); Mn (5.0±2.9-7.3±3.7). While that of the Prawn falls in the ranges: Fe (6.3±1.9-4.0±1.6); Zn (6.3±2.9-8.0±2.5); Cu (2.0±1.0-2.7±0.9); and Mn (1.7±0.5-3.0±0.8) for Lagos Island and Epe Lagoon respectively. The high concentrations of iron and zinc in the fish parts could be associated with its natural abundance in Nigerian soils. The general trend of accumulations in all the organisms were *Chrysichthys nigrodigitatus> Tilapia zillii> Callinectes, mnicola> Macrobrachium macrobrachion*.

The analysis of the Crab and Prawn shows that concentrations of Zn and Fe in the samples are lower than the permissible limits set by WHO and FEPA while Mn and Cu concentrations in the fish samples were above the standard set limits hence consumption of the fish from the analysed lagoon may be dangerous to health, therefore it is pertinent to regularly monitor the activities of both lagoons.

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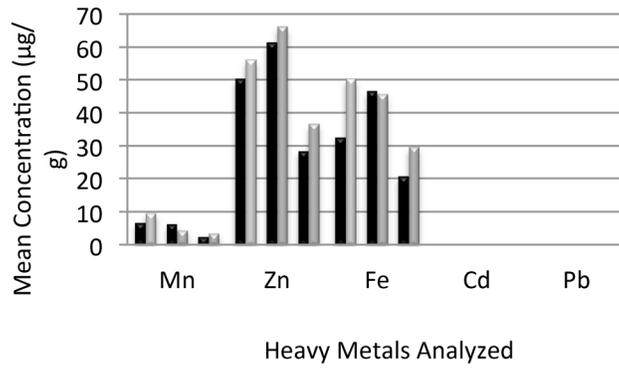
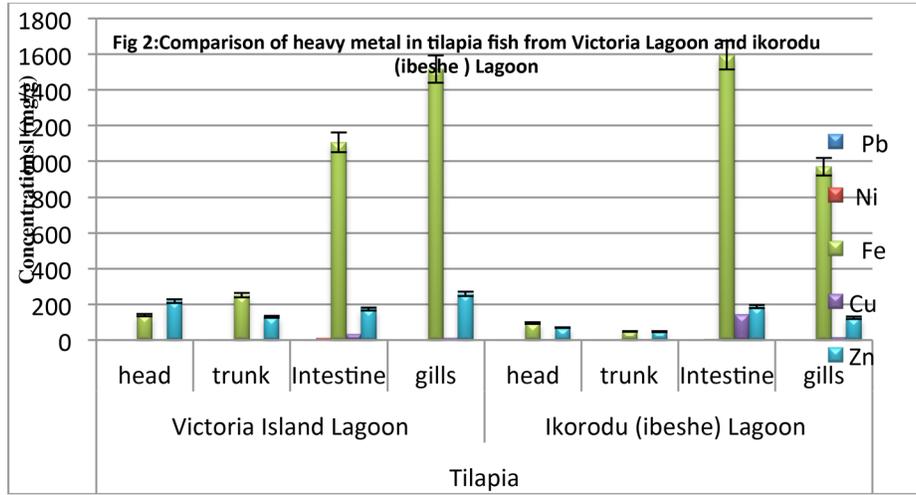


Fig 1: Comparison of Heavy Metals Concentrations in Catfish harvested from Lagos Island and Epe Lagoon

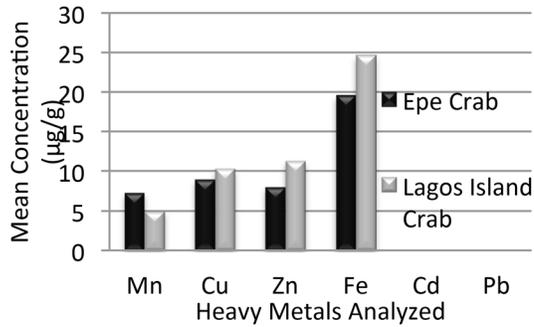


Fig 2: Comparison between Heavy Metals Concentrations in Crab harvested from Lagos Island and Epe Lagoon

## GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF AFRICAN CATFISH *Clarias gariepinus* FED DIETS CONTAINING MIXTURE OF PROCESSED MORINGA *Moringa oleifera* LEAF AND KERNEL MEAL

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Fish meal is the most utilised animal dietary protein ingredient in aquaculture diets because of its high protein content, balanced amino acid profile, high digestibility and palatability. However, inconsistent supply of fish meal, greater demand, and constant rising prices have been driving the search for substitutes for decades. *Moringa oleifera* is one of the most useful tropical trees belonging to the Moringaceae family. *M. oleifera* kernel, though a good source of protein, is deficient in some key amino acids. These limiting amino acids are in excess in the leaf meal. Combination of moringa kernel and leaf meals in desired proportions might result in obtaining a properly balanced plant-based protein that would favourably replace fishmeal in fish feeds. Glucosinolates, lectins and alkaloids which form the major anti-nutrient substances in moringa kernel meal could be removed by water extraction or solid state fermentation. The present investigation was carried out to determine the optimum level of supplementation of fish meal by mixture of soaked moringa leaf meal and fermented kernel cake in the diets of *Clarias gariepinus* juveniles.

The leaves were soaked overnight and oil was extracted from the seed kernel using n-hexane. The defatted kernel was fermented using *Rhizopus stolonifer* ( $1.20 \times 10^3$  cfu/g). Graded levels (0, 80.2, 179.2 and 305.4 gkg<sup>-1</sup>) of mixture (1:1) of soaked leaf and fermented kernel of moringa were incorporated in an isonitrogenous (40% crude protein) diets and fed to triplicates group of *Clarias gariepinus* (average weight 8.87g ±0.02) for a 56-day period.

Growth performance in terms of weight gain, average daily gain, specific growth rate in the group that received control diet was significantly (P<0.05) higher than those that were reared on diets containing 179.2 and 305.4 gkg<sup>-1</sup> mixture of soaked moringa leaf and fermented kernel but statistically (P>0.05) similar to the group fed with 80.2 gkg<sup>-1</sup> of the mixture. The results of the present investigation demonstrated that despite processing mixture of moringa leaf and kernel can replace 80.2 gkg<sup>-1</sup> of fish meal in the diet of *Clarias gariepinus* without markedly reducing growth performance and nutrient utilization.

**TABLE 1: GROWTH PARAMETERS AND NUTRIENT UTILIZATION OF *Clarias gariepinus***

Growth parameter	CD	80.2 gkg <sup>-1</sup>	179.2 gkg <sup>-1</sup>	305.4 gkg <sup>-1</sup>
<b>Final weight</b>	34.40±2.16 <sup>a</sup>	26.61±2.72 <sup>ab</sup>	21.15±1.09 <sup>b</sup>	24.00±4.00 <sup>b</sup>
<b>Specific growth rate</b>	2.41±0.12 <sup>a</sup>	1.96±0.17 <sup>ab</sup>	1.55±0.09 <sup>b</sup>	1.75±0.32 <sup>b</sup>
<b>Protein efficiency ratio</b>	2.51±0.10 <sup>a</sup>	2.31±0.11 <sup>ab</sup>	1.77±0.07 <sup>b</sup>	2.00±0.43 <sup>b</sup>
<b>Survival</b>	93.33±3.33	90.00±0.00	90.00±0.00	85.00±5.00

## ENHANCING THE ABILITY OF SALMONIDS TO CONVERT PLANT OILS TO EPA AND DHA

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Aquaculture uses over 60% of the fishmeal and more than 80% of the available fish oil produced. Although this presents a serious problem for all of aquaculture, these dietary components are especially crucial for piscivorous and carnivorous species feeds. Commercial producers marketing aquaculture products for health benefits value fish oil because its use elevates levels of healthy omega-3 fatty acids in the product. Several different products including animal byproducts and plant proteins have been evaluated for their ability to replace fishmeal and fishoil and diet formulations using sustainable plant products to replace fishmeal and fishoil have shown substantial improvements compared to earlier formulated feeds. However, the utilization of sustainable products is still a problem in some fish, especially when trying to maintain omega-3 fatty acids levels of docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3) similar to that found in wild caught or farm raised fish reared with feeds containing levels of fish oil found in earlier generation of feeds.

Earlier research from our laboratory demonstrated that genetic variation exists, and is heritable for rainbow trout for the ability to actively convert plant oils (namely  $\alpha$ -linolenic acid) and biosynthesize EPA and DHA and deposit it in muscle tissues. After one generation of genetic selection for this trait significant variation for the trait was noted within and between families. To better understand the biological mechanisms behind this trait, liver and muscle samples were taken from 36 second generation selected fish correlating with high, average, and low response as measured by EPA and DHA levels in muscle after being reared from 5 to 250 g on a complete plant-based diet. RNA and protein were isolated from the samples for RNA-seq transcriptomic and proteomic analysis. Some genes and a few proteins were found to vary significantly between compared groups but nothing currently has been determined to play a role in preferential deposition of specific fatty acids in the muscle. However, in the liver a greater number of genes and proteins were identified with some being linked to fatty acid processing.

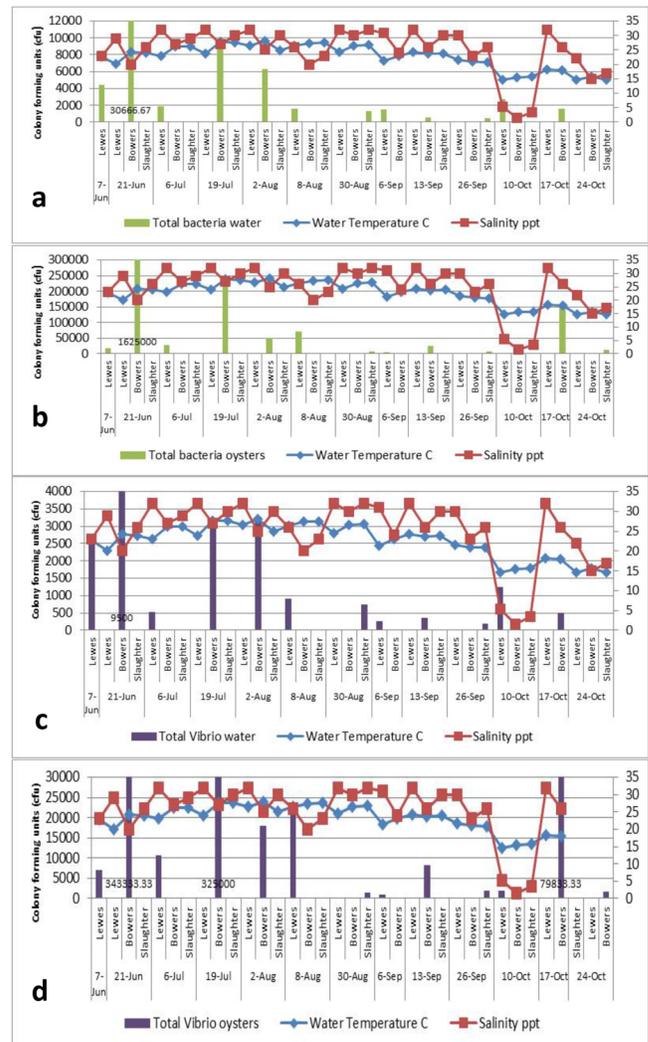
## ENVIRONMENTAL ASSESSMENT OF DELAWARE BAY WATER AND OYSTER *Crassostrea virginica* FOR TOTAL BACTERIA AND TOTAL VIBRIO

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Primary objective of this project is to investigate relationship between water quality and total bacteria and *Vibrio* concentrations. The presence of total bacteria and total *Vibrio* in seawater and oyster samples collected monthly from three sites in the Delaware Bay near the mouth of the Broadkill River in Lewes, Mispillion River in Slaughter Beach, and Murderkill River in Bowers Beach was determined from June through October 2016. Lewes, Slaughter Beach, and Bowers Beach are located 8, 24, and 40 km away from the Atlantic Coast, respectively and have different habitat characteristics. Previous research findings show a positive correlation between increased water temperature and the abundance of *Vibrio vulnificus* and *Vibrio parahaemolyticus*, while a correlation between the *Vibrio* species and salinity is not apparent.

Our research indicated that Bowers Beach had the highest number of colony forming units (cfu) of total bacteria (with the exception of October) and total *Vibrio* (*Vibrio vulnificus* and *Vibrio parahaemolyticus*) in both seawater and oyster samples even though the salinity was the lowest of all three sites (Figures 1a-d). An exponential increase in cfu on 21-June seemed to have no correlation to temperature or salinity. On 10-October, the abnormally higher numbers of total bacteria and *Vibrio* cfu in water samples from the Lewes site have been attributed to a severe weather system that brought in excess of 30 cm of rain in 24 hours to the area on 3-October, and heavy thunderstorms on 9-October. Our research findings confirm that there are other water quality attributes different than temperature and salinity such as turbidity or particulates plays some roles with presence and concentration of total bacteria and *Vibrio*.



**Figure 1.** Relationships between **a.** total water bacteria, temperature and salinity, **b.** total oyster bacteria, temperature and salinity, **c.** total water *Vibrio*, temperature and salinity, and **d.** total oyster *Vibrio*, temperature and salinity.

## **PROMOTING SEAFOOD CONSUMPTION: A TOOL FOR IMPROVING NUTRITION, HEALTH AND REGIONAL DEVELOPMENT – PUBLIC POLICY AND PRODUCERS EFFORT**

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The title of this presentation comes from the UN FAO COFI 32 meeting held in Rome in July 2016 and reported widely in the seafood media. The concept was organised by Mexico who had created Public Policy which decreed they would increase seafood consumption in their country by 3 kgs pp pa over 5 years – they achieved the goal in 3 years.

Seafood as a whole food is highly nutritious. Benefits to human health associated with the consumption of seafood are noted for multiple bodily organs and physiological functions. Seafood compares favorably with other protein sources in all areas but importantly it offers superior macronutrients in the ideal form of lean proteins combined with healthy omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFAs), and a wide array of highly bioavailable micronutrients and vitamins.

Over the years USA and European Commission have changed their advisories to women and issues about Human Nutrition are now on the WHO & FAO agendas so discussions relating to advisory positions are now more in the open.

Increasing seafood consumption is an imperative but changing habits is difficult so it is important for the aquaculture industry and relevant others involved to consider steps to support the great advantage that the health marketing angle gives seafood and how this is backed by scientific research.

## **PARDON OUR PROGRESS – AwF WOMEN/GENDER**

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The Women in Aquaculture session at the World Aquaculture Conference in Adelaide in June 2014 was the catalyst for establishing the Aquaculture without Frontiers (AwF) Women/Gender Network.

The Network has grown to a 600+ membership but like all volunteer groups the Network ebbs and flows based on the efforts of small core of people. Anyone can easily join in discussions and get information by networking at <https://www.facebook.com/groups/awfwomen>.

\We will bring you up to date with our activities and highlight the Woman of the Month for February 2017.

## **QUANTIFYING THE ECONOMIC IMPACT OF THE US AQUACULTURE INDUSTRY: CHALLENGES OF A PILOT STUDY**

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Each year, NOAA Fisheries estimates the economic impact of the fishing and seafood industry in Fisheries Economics of the US. Those figures, however, do not explicitly include aquaculture, although some aquaculture production may be captured in the estimates if some states blend aquaculture with wild harvest production estimates. This study, a cooperative project that includes the NOAA Office of Aquaculture, USDA NIFA, and University of Maryland Extension, will produce national economic impact estimates for major aquaculture species including: catfish, hybrid striped bass, baitfish, salmon, oysters, clams, mussels, crawfish, shrimp, tilapia, and trout. Representative enterprise budgets for each species were developed based on a literature review and were combined with estimates of annual production for input into a version of the IMPLAN model that has been specifically developed for the Fisheries Economics of the US estimates.

## COMPARISON OF FOUR ARTIFICIAL LIGHTING TECHNOLOGIES FOR INDOOR AQUAPONIC PRODUCTION OF COLLARD GREENS *Brassica oleracea*

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Aquaponics is a sustainable food production system that combines hydroponic plant growth with fish rearing in a recirculating aquaculture system. Aquaponic systems operating in temperate climates are constructed in insulated buildings, to achieve year-round production. In this setting, all environmental requirements for plant production are provided artificially. Information regarding optimal artificial lights for plant growth is needed to ensure producers are able to maximize their production.

This study evaluated and compared four leading light technologies on the growth of collard greens (*Brassica oleracea*) over a 22-day cycle. Four replicate systems were used which included a 415-L fish tank, a 190-L settling tank, a 115-L clarifier, and two 1.5 m<sup>2</sup> floating raft beds. Plants were stocked at sixteen plants/floating raft. Each system contained all four light types using a Randomized Complete Block Design. Light technologies evaluated included light emitting diode (LED), metal halide (MH), induction (IND), and fluorescent (FL), the lights were then all standardized to 200 photosynthetic active radiation (PAR) measured from just above the canopy. Nile tilapia (*Oreochromis niloticus*) were fed a floating 32% protein feed and stocked at a rate allowing a feeding rate of 60 grams per square meter of plant grow space per day. At harvest, the four plants in the center of each raft were weighed whole, then separated between roots and leaves, then measured and weighed. Light effects were compared based on biomass accumulation, leaf surface area, root/shoot ratio, and biomass/square meter/kilowatt-hour.

Results from this study indicate that the average fresh weight of collard greens raised under LED lights (48.9g), was significantly greater ( $P \leq 0.05$ ) than the weights of plants grown under MH (13.8g), IND (18.6g), or FL (15.3g) lights. Relatedly, the root/shoot ratio for plants raised under LED lights (31:100) were significantly ( $P < 0.05$ ) greater than the ratio for plants grown under FL (18:100), IND (18:100), and MH (19:100) lights. This data indicates faster growth and maturation under the LED lighting based on characteristic of climax vegetal phases. These results indicate superior performance by LED lights for Collard greens under indoor aquaponics production.

## EVALUATION OF VARYING LEVELS OF DIGESTIBLE PROTEIN AND DIGESTIBLE ENERGY AND THEIR RATIOS IN THE DIETS OF JUVENILE CUTTHROAT TROUT

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Cutthroat trout *Oncorhynchus clarkii* are native to the western United States. Currently, many subspecies of cutthroat trout (CTT) are raised in the state and federal hatcheries and private farms for stock enhancement programs and recreational fishing. In an effort to develop a suitable diet for raising CTT as fast as rainbow trout, a study was conducted to determine the optimal digestible protein (DP) to digestible energy (DE) ratio in the diet of Yellowstone cutthroat trout (*O. clarkii bouvieri*).

Eight diets were formulated to contain four DP levels (32.5%, 37%, 41.7% and 46%) and two DE levels (4370 kcal/kg and 4770 kcal/kg). The diets contained 0.1% yttrium oxide as an inert marker for a subsequent digestibility trial. The diets were extruded to produce 2-mm floating pellets. Groups of 30 fingerlings (average body weight, 37 g) were stocked into 26 145-L tanks each supplied with 12 L/min of 15 °C spring water in a flow-through system. Each test diet was randomly hand-fed to triplicate tanks of fish and a commercial control diet to duplicate tanks of fish to apparent satiation twice daily for 12 weeks. At the end of the growth trial, fish samples were collected for proximate analysis and for measuring viscerosomatic index, hepatosomatic index and condition factor. Remaining fish were used for the digestibility trial.

There were no significant differences among the dietary treatments for growth performance and feed utilization indices ( $P>0.05$ ). However, growth performance of fish was better on the test diets than on the control diet. Numerically, the highest specific growth rate (SGR, 1.04%/d) was observed with fish fed 41.7% DP and 4370 kcal/kg DE whereas the lowest SGR (0.90%/d) was observed with fish fed 46% DP and 4370 kcal/kg DE. Feed intake was low across the dietary groups (0.72-0.88 %/body weight/d). Feed conversion ratios (FCR) were excellent and lower for the test diets (0.84-0.99) than for the control diet (1.18). For each dietary DE level, FCR decreased slightly with increase in the dietary DP level. Dietary DP, DE or their interaction did not significantly affect growth performance and feed utilization indices ( $P>0.05$ ). Dietary DP level significantly affected the whole-body dry matter and crude ash levels. Protein efficiency ratio (PER) and protein retention (PR) were significantly affected by dietary DP level but not by DE level. PER and PR appeared to decrease as DP level increased at each DE level. The highest PER (2.82) and PR (47.9%) were observed for diet with 32.5% DP and 4770 kcal/kg DE whereas the lowest PER (2.02) and PR (35.3%) were observed for the diet with 46% DP and 4770 kcal/kg DE. The results indicated that Yellowstone cutthroat trout juveniles were efficient converters of dietary nutrients even though their feed intake and growth rate were lower than that is generally observed with rainbow trout juveniles at 15 °C. Based on results, the diet containing 41.7% DP and 4370 kcal/kg DE was numerically better than the other diets.

## DIETARY LYSINE REQUIREMENT OF JUVENILE SNAKE RIVER CUTTHROAT TROUT

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Cutthroat trout *Oncorhynchus clarkii* are native to the western United States. Currently, many subspecies of cutthroat trout (CTT) are raised in the state and federal hatcheries and private farms for stock enhancement programs and recreational fishing. In an effort to develop a suitable diet for raising CTT as fast as rainbow trout, a study was conducted to determine the optimal dietary lysine requirement of juvenile Snake River cutthroat trout (*O. clarkii behnkei*).

Six diets using practical ingredients and purified amino acids were produced to contain graded levels of lysine (1.81%, 2.37%, 2.90%, 3.49%, 4.03% and 4.61%, dry-matter basis). The diets were isonitrogenous (46% crude protein), isolipidic (18% crude fat) and isoenergetic (22 MJ/kg) on dry-matter basis. Essential amino acid profile of diets matched that (except lysine) of the whole-body CTT. A commercial trout diet was used as the control. Groups of 60 fingerlings (average body weight, 15.7 g) were stocked into 21 145-L tanks each supplied with 15 °C spring water in a flow-through system. Each diet was hand-fed to randomly assigned triplicate tanks of fish to apparent satiation twice daily for 10 weeks. At the end of the growth trial, fish samples were collected for proximate and amino acid analyses, and condition factor. Remaining fish were used for determining lysine digestibility in a diet pooled from the experimental diets.

Fish fed the lowest lysine level had significantly lower weight gain and daily growth index but higher feed conversion ratio (FCR) than fish fed other lysine levels ( $P < 0.05$ ). Fish gained 33.2 g-45.1 g with FCRs of 1.29 to 1.67. Fish fed diets with 2.90%-4.61% lysine grew as well as the fish fed the commercial control diet with similar FCRs. Fish fed the two lowest levels of lysine appeared to have lower whole-body crude protein but higher crude fat levels than fish fed the other diets. Among several non-linear regression models, the 3-parameter logistic regression model was the best fit to the daily growth index data (adjusted- $R^2 = 0.84$ , Figure 1). Dietary lysine requirement of juvenile cutthroat trout at 95% of the asymptote was 2.39%.

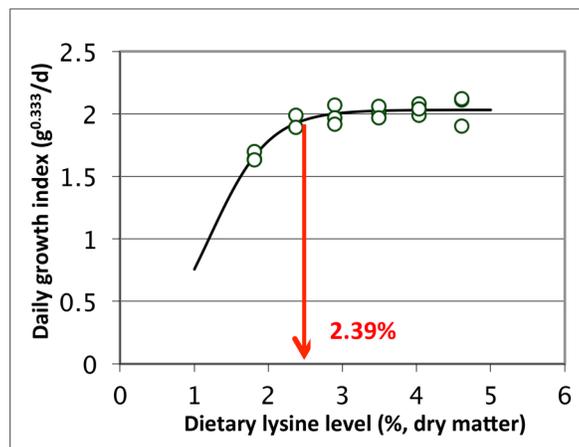


Figure 1. Daily growth index ( $g^{0.333}/d$ ) of juvenile Snake River cutthroat trout fed diets containing 1.81%-4.61% lysine for 10 weeks.

## COMPARISON OF CULTURE METHODS FOR THREATENED CARIBBEAN STAGHORN CORAL *Acropora cervicornis* GROWN IN AN OCEAN-BASED NURSERY FOR RESTORATION

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Staghorn coral *Acropora cervicornis* has been federally listed as Threatened since 2006, following population declines beginning in the late 1970s. This once ubiquitous framework builder on Caribbean fore reefs has been especially impacted in the Florida Keys, where populations have been reduced by up to 97% in many areas. Of the host of management actions designed to change this trajectory, aquaculture is increasingly used to grow staghorn coral in ocean-based nurseries. The biology of this coral makes it an ideal candidate for culture, and as of 2016 over 100,000 nursery colonies have been outplanted onto reefs in Florida.

A variety of culture methods are employed for staghorn coral. These can be broadly categorized into benthic-attached or water column-suspended, dependent upon how and where the colony is held in place. We compared specific benthic-attached (block) and water column-suspended (tree) systems in a study conducted in a Mote Tropical Marine Laboratory nursery. Total linear extension of each colony was measured monthly for 11 months, and observations of colony health and breakage were also made. Only three colonies survived through November 2015, following an extended period of elevated temperature and subsequent bleaching event. Prior to this date, colonies grown on trees were significantly larger and experienced significantly less breakage than those on grown blocks. Genotype also had a significant effect on colony growth.

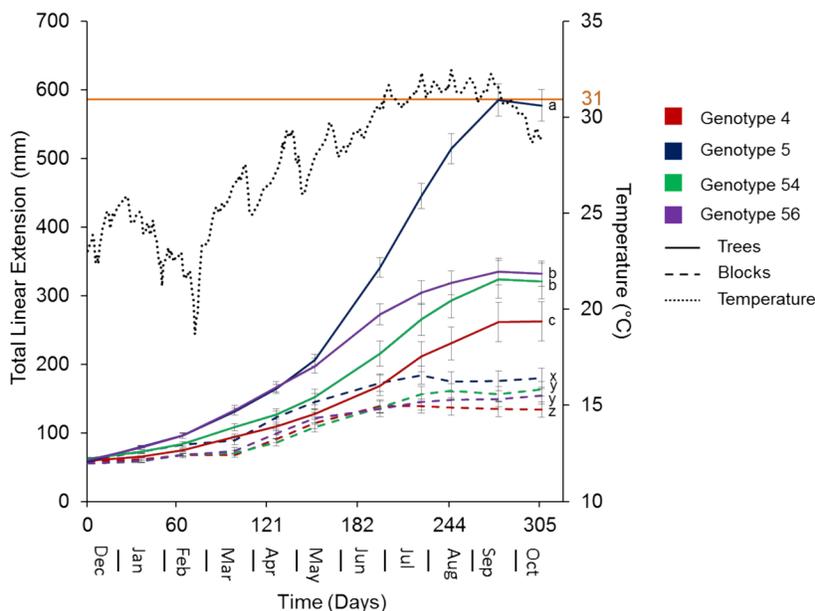


Figure 1. Total linear extension (TLE) of *Acropora cervicornis* across time plotted with concurrently recorded water temperature. Means ( $\pm$ SEM) by genotype and culture method are presented at each sampling interval ( $n = 30$  per genotype per culture method). Letters denote significant differences in mean net TLE among genotypes within culture method as determined by Dunn's *post hoc*.

## **BEST PRACTICES AND LESSONS LEARNED FOR CONDUCTING EXTENSION WEBINARS: THE NAA/NCRAC/USAS WEBINAR SERIES CASE STUDY**

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Information transfer is a central theme of extension, and delivering that information to the appropriate audience in the appropriate fashion is critical to creating impacts. Knowing the proper method of program delivery for a given audience is challenging, particularly during an age of rapid technology change. Currently, one of the most cost effective methods of information transfer is streaming audio and video via the internet to a computer, smart phone or other digital device. For extension specialists to successfully maximize their technology transfer capabilities, they must embrace current and audience appropriate technology.

During 2016, the North Central Regional Aquaculture Center joined forces with the National Aquaculture Association and the United States Aquaculture Society to develop and deliver a series of 12 aquaculture-related webinars designed to bring together science and business to expand and strengthen the United States aquaculture industry. The target audience included producers currently engaged in aquaculture, those looking to get into business, educators helping others understand aquaculture, and consumers that want to be better educated. The goal was to enhance their knowledge and move them forward on their journey to success. This presentation focuses on effective strategies for planning, organizing, advertising, conducting, delivering, recording, editing, archiving, and evaluating a webinar program.



Figure 1. Example title slide from the NAA/NCRAC/USAS aquaculture webinar series.

## EFFECTIVE TECHNIQUES FOR AQUACULTURE INFORMATION TRANSFER

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Information transfer is a central theme of extension, and delivering that information to the appropriate audience in the appropriate fashion is critical to obtaining buy-in and adoption of best practices to generate measurable impacts for the aquaculture industry. Knowing the proper method of program delivery for a given audience is challenging, particularly during an age of rapid technology change. Older extension programs relied on field days, in-person workshop and print publications to gather stakeholders and deliver information. The success of these programs relied on word-of-mouth and paper media outlets, like the newspaper and mailing lists for advertisement. Today’s reduced public and private budgets make it difficult to hold an extension event or entice end users to commit time, money and effort to travel to an extension event.

Currently, one of the most cost effective methods of information transfer is streaming audio and video via the internet to a computer, smart phone or other digital device. For extension specialists to successfully maximize their technology transfer capabilities, they must embrace current and audience appropriate technology. Emails, on-line forums and videos, webinars, blogs, and social media have become real-time information streaming opportunities that directly convey information.

During 2016, the North Central Regional Aquaculture Center joined forces with the National Aquaculture Association and the United States Aquaculture Society to develop and deliver a series of 12 aquaculture-related webinars designed to bring together science and business to expand and strengthen the United States aquaculture industry. The target audience included producers currently engaged in aquaculture, those looking to get into business, educators helping others understand aquaculture, and consumers that want to be better educated. The goal was to enhance their knowledge and move them forward on their journey to success. The effectiveness of this communication channel will be presented.



Figure 1. Example title slide from the NAA/NCRAC/USAS aquaculture webinar series.

## EARLY ONTOGENY OF SELECTED DIGESTIVE ENZYMES IN LARVAL SOUTHERN FLOUNDER (*Paralichthys lethostigma*)

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The southern flounder (*Paralichthys lethostigma*) is a commercially and recreationally important species in the Gulf of Mexico. Stock enhancement programs throughout the state of Texas seek to supplement wild production in order to sustain a healthy population. In order to effectively raise healthy larvae, the development of their digestive tract must be better understood. The present study was conducted to characterize the ontogeny of several digestive enzymes as a function of fish age.

Eggs were collected from natural spawns of captive southern flounder broodstock at the hatchery of the CCA Marine Development Center in Flour Bluff, Texas. Starting at 3 days post hatch (dph), larvae were fed with enriched rotifers (*Brachionus* sp.) and were then transitioned onto enriched *Artemia* nauplii at 20 dph. Larvae were collected on 0, 3, 5, 7, 9, 11, 13, 15, 18, 21, and 24 days post hatch. After collection, larvae were rinsed on an appropriately sized mesh and immediately frozen with liquid nitrogen. Larvae were homogenized in cold 50 mM Tris-HCl, 20 mM CaCl<sub>2</sub> buffer and the supernatants were stored at -80°C until further analysis. The amount of protein in each sample was determined by the Bradford method using bovine serum albumin as a standard.

The larvae were analyzed in triplicate for the following digestive enzymes from each sampling day: pepsin using hemoglobin as a substrate, trypsin using N-a-benzoyl-DL-arginine 4-nitroanilide hydrochloride as a substrate, chymotrypsin using 5 mM-N-benzoyl-L-tyrosine ethyl ester as a substrate, aminopeptidase using L-leucine p-nitroanilide as substrate,  $\alpha$ -amylase using soluble starch as a substrate, lipase using sodium cholate hydrate and  $\beta$ -naphthyl-caprylate as a substrate, and acid/alkaline phosphatases using 4-nitrophenylphosphate as a substrate. Enzyme responses were measured spectrophotometrically and expressed as units of enzyme per mg sample. One unit is defined as the increase of 0.01 units of absorbance/minute.

Several of the enzymes measured, such as lipase, alkaline phosphatase, acid phosphatase, and trypsin, were present at hatching. Pancreatic enzymes (trypsin,  $\alpha$ -amylase, lipase), phosphatase enzymes and aminopeptidase are thought to help enable the larvae to absorb the yolk sac. Amylase appeared to peak around first feeding, 5 dph, at which point the absorbance/mg sample decreased significantly. Aminopeptidase was first evident at 3 dph and the absorbance/mg sample continued to increase until 15 dph, when it decreased slightly. These results provide further characterization of digestive tract development in southern flounder larvae and may be used to help refine feeding protocols for this species.

## EFFECTS OF DIETARY SULFUR AMINO ACID DEFICIENCY ON JUVENILE SOUTHERN FLOUNDER *Paralichthys lethostigma*

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Replacement of fish meal as the major source of protein in fish diets is essential to sustain and encourage the growth of the aquaculture industry. Common fish meal replacements are often deficient in  $\geq 1$  indispensable amino acid (AA). Methionine and cysteine are sulfur-containing indispensable AAs. Sulfur AA deficiency has been known to cause severely reduced growth in fish as well as cataracts in some species such as Arctic charr (*Salvelinus alpinus*) and yellowtail (*Seriola quinqueradiata*). The southern flounder (*Paralichthys lethostigma*) is a commercially and recreationally important species in the Gulf of Mexico. In recent years, stock enhancement programs have been implemented in order to replenish and support the wild population of southern flounder in the Gulf. This is a preliminary study to establish the essentiality of sulfur-containing AAs to the southern flounder.

A deficient and a sufficient diet were prepared according to total sulfur AA requirements of other cultured flatfish species. The diets were designed to contain 45% crude protein (from 15% by weight of red drum muscle as an intact protein and crystalline AAs), 12% lipid, and 4.16 kcal/g digestible energy. The total amount of sulfur AAs provided by 15% red drum muscle was 0.7% of dry diet. The deficient diet contained 0.7% total sulfur AAs and the sufficient diet contained 2.3% total sulfur AAs. The experimental diets were kept isonitrogenous by adjusting the inclusion of an aspartate/glycine premix. Juvenile southern flounder ( $2.05 \pm 0.34$  g) were stocked in 38-L aquaria at a density of 10 fish/tank.

There was a large difference in total weight gain percentage between fish fed the sulfur AA deficient and sufficient diets (Table 1). Survival also was greater among fish fed the diet with a sufficient amount of sulfur AAs. Fish fed the deficient diet did not feed as aggressively and performed less evasive maneuvers when netted than those fed the sufficient diet. Significant differences in % whole-body moisture ( $p < 0.0451$ ), % whole-body lipid (dry) ( $p < 0.0005$ ), feed efficiency ( $p < 0.0001$ ), protein efficiency ( $p < 0.0001$ ), and condition factor ( $p < 0.0032$ ) were observed between fish fed the two diets; however, there were no significant differences in % body protein (dry), viscerosomatic index, or hepatosomatic index. Results of this experiment confirmed that sulfur AA deficiency in southern flounder was manifested by reduced weight gain and survival although cataracts were not detected.

**Table 1:** Comparison of the effects on growth and survival of Southern flounder (*Paralichthys lethostigma*) juveniles fed diets deficient (Diet 1) and sufficient (Diet 2) in sulfur-containing indispensable amino acids after 10 weeks.

<b>Diet</b>	<b>% Survival</b>	<b>Avg Fish Weight (g)</b>	<b>Total Weight Gain %</b>
<b>1</b>	<b>65.0 <math>\pm</math> 7.1</b>	<b>1.51 <math>\pm</math> 0.16</b>	<b>68 <math>\pm</math> 0.68</b>
<b>2</b>	<b>83.3 <math>\pm</math> 15.3</b>	<b>5.56 <math>\pm</math> 0.65</b>	<b>388 <math>\pm</math> 39.90</b>

## RED DRUM (*Sciaenops ocellatus*) RESPONSE TO HISTIDINE DEFICIENCY

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Due to the increasing inclusion of non-fish based protein sources in fish feeds, several amino acid (AA) deficiencies have presented significant problems to the aquaculture industry. Historically, restriction of mammalian blood meal, a protein source high in histidine (His), in salmon culture resulted in a high incidence of cataracts. Higher levels of dietary His are known to ameliorate cataract incidence in salmon culture. The His requirement of red drum (*Sciaenops ocellatus*) has been quantified according to weight gain percentage. However, the effect of His deficiency on the eye, and the lens in particular, of the red drum has not been examined. Additionally, dietary His has been shown to positively affect erythrocyte fragility in unstressed juvenile grass carp (*Ctenopharyngodon idella*).

Two diets from a His requirement study were used in this experiment. A basal diet, known to be below the requirement, was prepared using lyophilized red drum muscle (RDM) as an intact protein (10.5% of dietary protein) supplemented with crystalline AAs (excluding His) to provide a total of 35% crude protein (CP) in the diet, simulating the AA pattern found in RDM. Dietary lipid and dextrin was included to provide, in combination with protein, a total of 13.4 kJ estimated digestible energy/g diet. The basal amount of His provided by RDM when contributing 10.5% of dietary protein was determined to be 0.30 g/100 g diet. The second diet used consisted of an equal mixture (dry-matter basis) of two diets with His levels known to be above the His requirement of red drum (1.1 and 1.3 g/100 g diet). These diets were fed to triplicate 38-L aquaria for 8 weeks. The stocking density was 20 fish/tank with an initial average individual weight of 1.53 g  $\pm$  0.08 g.

At the end of 8 weeks, weight gain of fish fed the His-deficient diet was significantly reduced at only 44% of fish fed the His-supplemented diet. At that time six fish/tank were collected, with the left eye of each fish subjected to histological analysis and the lens of the right eye examined for amino acid composition. Remaining fish were inspected using slit lamp biomicroscopy for signs of cataracts. Significant cataracts were observed in 16.7% of eyes examined from fish fed the His-deficient diet while eyes examined from fish fed a sufficient diet did not contain cataracts. Erythrocyte osmotic fragility was higher in fish fed the His-sufficient diet compared to those fed the His-deficient diet. Histological analysis is ongoing and will be presented.



*Figure 1: Incomplete cataract observed in the eye of a juvenile red drum (*Sciaenops ocellatus*) fed a diet deficient in histidine.*

**PHYTASE FORTIFICATION OF A COMMERCIAL CATFISH DIET ELEVATES MINERAL STORES AND IMPROVES HEMATOLOGICAL HEALTH OF CHANNEL CATFISH, *Ictalurus punctatus***

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Phytic acid is a central inhibitor of iron absorption in plant-based fish diets and a potential contributor to chronic low grade anemia. Phytic acid levels in catfish diets have risen along with inclusion percentages of certain ingredients such as wheat middlings. While previous phytase studies in catfish have demonstrated improved growth and phosphorus retention, the impact of the enzyme on iron retention and hematological traits has not been examined. Accordingly, the impact of phytase superdosing (2500 FTU/kg) in a commercially-available catfish diet was evaluated in channel catfish (*Ictalurus punctatus*) fingerlings. Catfish fed phytase-supplemented diets had higher mineral levels (including iron) in serum and liver along with improved hematocrit, hemoglobin, and red blood cell values. Weight gain and feed conversion ratios were also significantly improved in the 15 week replicated pond study. Results from the present study indicate that addition of phytase to commercial catfish diets may have measurable impacts on fish health and performance.

## PROBIOTIC DIETARY SUPPLEMENTATION IN NILE TILAPIA AS PROPHYLAXIS AGAINST STREPTOCOCCOSIS

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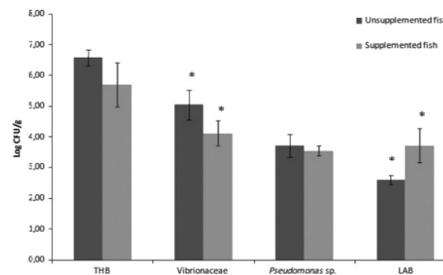
*Streptococcus agalactiae* is a Gram-positive bacteria that infects both freshwater and marine fish and is responsible for economic losses worldwide. In Brazil, *S. agalactiae* has been identified in several states and has been disseminating rapidly among the fish farms and production systems. Antibiotics are commonly used to treat septicemia caused by *Streptococcus* spp., but their indiscriminate use has provoked the selection of antibiotic-resistant bacteria. Previous studies using allochthonous probiotics for tilapia showed that it enhanced the immune system, intestinal histomorphology, growth performance and lowered mortality of tilapia after challenge with *Streptococcus iniae* and *S. agalactiae* (Pirarat et al., 2015). However, studies with autochthonous probiotic to tilapia evaluating their effects on animal health and disease resistance against *S. agalactiae* are limited. Therefore, haemato-immunological responses, microbiology, histology and transmission electron microscopy were used to investigate the effects of dietary supplementation with *Lactobacillus plantarum* in Nile tilapia (*Oreochromis niloticus*) after challenge with *Streptococcus agalactiae*.

Fish were distributed into two groups: control (unsupplemented) group and the group fed *L. plantarum* (isolated by Jatobá et al. (2008) from the intestinal tract of healthy tilapia) supplemented diet for a period of 58 days. We observed an increase in the concentration of lactic acid bacteria and a reduction in the number of Vibrionaceae in supplemented fish. A significant increase in the final weight, specific growth rate and feed efficiency was also in supplemented fish.

After challenge, the number of thrombocytes and neutrophils also increased in supplemented animals.

Transmission electron microscopy showed damage to the intestinal mucosa and the presence of bacteria similar to *S. agalactiae* in both infected groups.

In conclusion, dietary supplementation with *L. plantarum* did alter the intestinal microbiota of Nile tilapia, improved haematological parameters involved in the immune system after challenge with *S. agalactiae* and enhanced the fish growth performance.



**FIGURE 1** Intestinal microbiota (data transformed in log<sub>10</sub> CFU/g intestinal tract) of Nile tilapia fed a control diet or diet supplemented with the probiotic *Lactobacillus plantarum*. \*bars indicate the standard deviation. \*Significant difference as determined by t test ( $p < .05$ )

**TABLE 1** Growth parameters in Nile tilapia fed a control diet or diet supplemented with the probiotic *Lactobacillus plantarum*

Sampling	Treatments	Initial weight (g)	Final weight (g)	Final total length (cm)	Weight gain (g)	Feed efficiency	SGR (% per day)
Prechallenge	Unsupplemented	28.55 ± 6.39	75.16 ± 38.93 <sup>a</sup>	16.90 ± 1.72	53.02 ± 5.30 <sup>a</sup>	0.59 ± 0.08 <sup>a</sup>	1.81 ± 0.19 <sup>a</sup>
	Supplemented	24.31 ± 6.99	107.84 ± 32.46 <sup>b</sup>	17.47 ± 1.75	63.80 ± 10.10 <sup>b</sup>	0.68 ± 0.10 <sup>b</sup>	2.07 ± 0.22 <sup>b</sup>
	p value	.206	2.46 × 10 <sup>-6</sup>	.080	.029	.047	.020

SGR, specific growth rate.

Data are mean ± SD. Means within a column superscripted by different small letters represent significant difference between the treatments by t test ( $p < .05$ ); n = 60 fish in each treatment.

## **AQUACULTURE DATA MAY BE USED BY TEACHERS AS A TOOL FOR TEACHING A SPECIFIC MATHEMATICS TOPIC**

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Aquaculture is defined as the propagation and rearing of aquatic organisms for any commercial, recreational, or public purpose. Globally, the aquaculture industry has been the fastest growing form of animal food production reaching 74 million tons in 2014, with a growth rate of 5.4 percent per year globally. Also, aquaculture is considered “a developing agricultural education program area with potential to increase opportunities for hands-on applications; and when infused into secondary agriculture, aquaculture meets needs for instruction in basic biology, chemistry, and mathematical concepts required of workers in technical jobs . However, Conroy & Walker (2000) indicated that there are varying levels of success integrating aquaculture with other disciplines, and cited one of the teachers: “Integration with math in the school is the hardest thing I’ve ever dealt with”. Cline (2010) also identified some challenges: “one of the major barriers to infusion is the teachers’ lack of preparation time and aquaculture knowledge. They are overwhelmed trying to prepare students for standardized testing and have little time to develop or adapt new materials. Teachers lack the specialized training to see how aquaculture concepts relate to their required teaching content standards”.

Activities developed in an aquaculture facility provide a great amount of information and data, such as measurements of water quality parameters, fish weight, fish length, egg production, fingerling survival, among others, which could be used to integrate aquaculture and other subject matters, specifically mathematics. Mathematics teachers might use data generated in aquaculture facilities as a tool for teaching a specific mathematical content. Examples of data usage are discussed, and also a Pedagogical Content Knowledge in Educational Context (PCK-EC) is proposed to examine the impact of this approach.

## TRANSMISSION OF *Pseudoloma neurophilia* IN ZEBRAFISH *Danio rerio* WHEN USING A MASS SPANWING CHAMBER

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*Pseudoloma neurophilia*, a microsporidium that primarily infects neural tissues, is one of the most common pathogens in laboratory zebrafish. Infected fish can transmit *P. neurophilia* vertically; however, horizontal transmission during spawning is more common. It is unclear if risk of parasite transmission is different when spawning zebrafish in individual spawning tanks as compared to mass spawning chambers, and if this risk is correlated to the number of spawning events. In addition, disinfection protocols have not been evaluated to determine if any could decrease or eliminate the risk of transmission following spawning.

The present study was conducted to evaluate *P. neurophilia* transmission from a known infected colony (prevalence = 52%) to uninfected fish and their offspring when using 2 different spawning apparatuses (n = 30 per group). Infected and uninfected fish were allowed to spawn in either a mass spawning chamber or breeding tanks for 1 or 3 spawning events. Uninfected fish and offspring were evaluated for infection 15-weeks later by histopathology and Luna stain. In addition, we sought to develop an effective protocol for disinfecting the mass spawning chamber between uses to eliminate *P. neurophilia* spores. Devices were either allowed to dry for 1 hour or were disinfected with one of the following protocols for 10 minutes after infected zebrafish spawned for 1 hour (n=5 per method): reverse-osmosis water, 90-110 ppm bleach (pH = 6.8 - 7.5), 1.0% Virkon Aquatics, 75 ppm Wescodyne, or 175 ppm Wescodyne Plus spray.

Results demonstrated increased risk for transmission to uninfected fish when spawning occurs in a mass spawning chamber (100% infected after 3 spawning events), as compared to the other groups (0-7% infected). The parasite was not detected in offspring from these spawning events regardless of spawning device or number of spawning events. The Wescodyne Plus spray, bleach soak, and Wescodyne soak were 100% effective at eliminating *P. neurophilia* spores as determined by PCR following spawning (Table 1). It is important to prevent or minimize spread of this pathogen as there is no treatment and infections can cause morbidity and mortality with the potential to confound research results.

Table 1: Percentage of mass spawning chambers positive for *Pseudoloma neurophilia* DNA via PCR

Disinfection Method	Pre-Disinfected	Post-Disinfected
Drying Only	100%	100%
RO Soak	100%	60%
Virkon Aquatics (1.0% con)	100%	20%
Bleach (90-110 ppm at pH = 6.8 - 7.5)	100%	0%
Wescodyne Soak (75 ppm)	100%	0%
Wescodyne Plus Spray (175 ppm)	100%	0%

## THE USE OF MEALS DERIVED FROM HETEROTROPHIC AND AUTOTROPHIC MICROALGAE IN DIETS FOR JUVENILE RED DRUM, *Sciaenops ocellatus*

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Continued availability of compound feeds has contributed to the expansion of the world food fish aquaculture production. Fishmeal and fish oil, derived from small pelagic fishes, have been widely used as components of such feeds because they possess desirable nutritional characteristics. Fishmeal has a high protein content, with an ideal amino acid balance. In turn, fish oil has an outstanding fatty acid profile that includes good provisions of polyunsaturated and highly unsaturated fatty acids (PUFA and HUFA, respectively). Unfortunately, progressive depletion of global fish stocks, coupled with increasing demands for fishmeal from the aquaculture and animal feed industries, has induced a dramatic rise in fishmeal and fish oil prices, prompting efforts to limit their inclusion in aquafeeds. As a result, alternatives to fishmeal and fish oil have actively been sought in the past years. Efforts are being made to find PUFA and/or HUFA-rich feedstuffs that can be produced in a predictable and technologically controlled manner.

One such group of feedstuffs is represented by microalgae. Used for the production of biodiesel due to their high lipid content, microalgal oils, along with the resulting algal lipid-extracted by-products, have become available in recent years. However, whole microalgal products may be more attractive for inclusion in aquafeeds due to cost and contribution of both protein and lipid. Interestingly, various microalgal species are characterized by their high content of PUFA and HUFA and the large volumes at which they can be produced. To date, lipid concentrates or meals derived from autotrophic microalgae genera, such as *Spirulina* sp., *Nannochloropsis* sp., *Isochrysis* sp., *Tetraselmis* sp., *Navicula* sp., *Chlorella* sp., and *Desmochloris* sp., have experimentally been incorporated into balanced feeds for marine fish species, with variable, but promising results. The heterotroph *Schizochytrium limacinum*, probably less well studied but equally interesting, has been employed as a partial substitute of fishmeal and fish oil in diets of marine fish.

For the red drum, *Sciaenops ocellatus*, recent studies have shown successful replacement of at least 10% fishmeal and soy protein concentrate by lipid-extracted algal meals from the autotrophs *Navicula* sp., *Nannochloropsis* sp., and *Chlorella* sp. However, the use of microalgal meals derived from heterotrophic and other autotrophic species has not been investigated.

In the present study, the use of the heterotroph *S. limacinum* and some autotrophic microalgae in diets for juvenile red drum was evaluated. A reference diet was formulated to contain 40% crude protein (CP) and 10% lipid using menhaden fishmeal and soy protein concentrate (SPC) as the protein sources. Then, experimental diets were formulated to replace from 5 to 25% CP of the reference diet by meals derived from *S. limacinum* and from autotrophic microalgae. Preliminary data indicate that CP from fishmeal and SPC can be replaced, to some extent, by the algal meals without causing significant reductions in fish performance.

**STOCK ENHANCEMENT OF COBIA *Rachycentron Canadum* IN SOUTH CAROLINA**

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Cobia *Rachycentron canadum* is a large, fast-growing coastal migratory pelagic fish with a distribution that includes most of the world's warm oceans. In southeastern South Carolina, cobia enter high salinity estuaries to spawn in late spring and early summer. These inshore spawning fish form a discrete population segment (DPS) that is genetically unique to three estuaries: St. Helena Sound, Port Royal Sound, and Calibogue Sound.

As coastal populations have expanded, interest in fishing for cobia has increased and caused concerns about the sustainability of the fishing effort on the DPS. As a result, the South Carolina Department of Natural Resources developed a stock enhancement program to evaluate the feasibility of supplementing wild cobia populations with hatchery-raised animals. Hatchery juveniles (typically 60-100 mm) were produced using local wild broodstock and released in the Port Royal Sound system from 2005-2009 and 2012. Releases by year class have been small in scale and ranged from 1,392-53,673 fish.

Following stocking, genetic tissue and otoliths were collected through fisheries dependent sampling. Samples were genetically evaluated and compared with known broodstock genotypes to determine hatchery or wild origin. Year class was assigned by otolith and percent contribution to year class by hatchery fish was determined (number of hatchery fish from year class/ total fish from year class \*100).

Hatchery contributions to a specific year class within the estuary have ranged from 0% to 80% for a given collection year. Releases from the 2007 year class (n=53,673) have continued to make a strong contribution to that year class within the estuary over time (31-80%) and continue to be collected through the 2015 collection year. Hatchery fish have also exhibited site fidelity similar to their externally-tagged wild counterparts, returning to the same estuaries multiple years after release. As the population of the DPS has continued to decline, a stock enhancement program utilizing genetically robust broodstock management may serve as a useful tool for managers alongside traditional creel and size limits.

## **SIZING OF A FLOATING BEAD BIOCLARIFIERS**

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Bioclarifiers are a powerful tool in the RAS industry. They allow for two crucial actions (clarification and biological filtration) to be done in one unit reducing the amount of points of failure in the system but must be properly sized to handle not only the solids loading, but also the nitrification needs of the system. The sizing of a floating bead bioclarifier can be accomplished in many different ways. The two most common sizing methods are based on ammonia loading and feed loading; with feed loading being the most common in fed RAS systems.

Ammonia loading sizing criteria is best utilized in non-fed systems such as bait holding systems or lobster holding systems. These types of RAS systems should be sized by the excretion rate of the particular animal or fish being held.

Relating ammonia excretion rates and removal rates of enhanced nitrification media (EN), to the amount of feed per day of a given system allows feed based sizing criteria to be established. Floating bead bioclarifier can be properly sized using feed based sizing criteria. This method of sizing uses the relationship of the estimated excretion rate of fish with the known volumetric TAN conversion rates of floating bead filters to effectively size the bioclarifier. This gives a unique relationship in which a bioclarifier can be sized based off of the mass of food per day per cubic foot of media. This sizing is dependent upon the protein content of the feed. To account for the differing TAN concentration limits required for differing trophic levels in the growout cycle, there are differing sizing rules for each step of production (fry, fingerling, and growout).

## **SHAPING CONSUMER PERCEPTIONS OF FARM RAISED SEAFOOD**

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Aquaculture seafood the key to growing seafood consumption- there has never been a better time to make this happen.

Public conversation on farm raised seafood has long been largely negative- there is no strong, unified voice representing the industry's perspective.

How can the industry pull together to address these issues?

What are specific steps the industry can take?

## **EFFECTS OF FEEDING ZILPATEROL HYDROCHLORIDE ON GROWTH PERFORMANCE, FILLET YIELD, AND BODY COMPOSITION OF RAINBOW TROUT, NILE TILAPIA, AND CHANNEL CATFISH**

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Zilpaterol hydrochloride (ZH) is a potent  $\beta$ -adrenergic agonist (BAA) that has been used in feedlot cattle to increase average daily gain, feed efficiency, yield of trimmed cuts, and dress out percent. While positive effects of ZH have been observed in cattle, there have been no reports of this product being tested in fish. Three separate studies were conducted in rainbow trout (528.0 g), Nile tilapia (423.0 g), and channel catfish (245.2 g) to evaluate the effects ZH on growth performance, survival, dress out percent, and fillet composition. The three species of fish were fed ZH at 0, 1, 5, and 10 ppm for approximately 30 days. Results showed that weight gain was significantly reduced in catfish fed ZH at 1 ppm while weight gain was not significantly affected by ZH treatment in rainbow trout and tilapia. Feed conversion ratio, food intake, and survival were similar among treatments in all species of fish. Dress out percent was similar among all treatments for both rainbow trout and channel catfish while dress out percent was lower in tilapia fed the 5 ppm diet. Fillet proximate composition and visceral fat percent were not significantly affected by ZH treatment in any of the three species of fish. There was no qualitative behavior differences observed among treatments for any of the species tested. The observed lack of differences in growth performance and condition traits suggests there is no benefit to feeding rainbow trout, Nile tilapia, or channel catfish ZH. Dose of ZH, number of days on feed, and age of fish may play important roles in how fish respond to BAAs. Further research is needed to fully evaluate their efficacy for use in aquaculture.

## **BIOFILTRATION CAPACITY OF A MOVING BED BIOREACTOR IN SEQUENCE WITH A SUBMERGED FLOATING BEAD BED BIOCLARIFIER—APPLICATION TO RECIRCULATING AQUACULTURE SYSTEMS**

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Biofiltration is a key process in recirculating aquaculture systems (RAS). The microbial oxidation of ammonia to nitrate is universal and vital to ensure that ammonia and nitrite do not accumulate in the fish culture system. In moving bed bioreactors (MBBR) and submerged floating bead bed bioclarifiers, nitrifying bacteria colonization occurs as a fixed film on the media surfaces of each respective unit. This aerobic nitrification process is well described and numerous biofilter designs have been developed, tested, and reported. The numerous biofilter solutions are often site specific, based on operator preference, or country traditions. An ideal biofilter for all purposes does not exist and all types of biofilters have their pros and cons. Much effort and research has been expended in developing guidelines for reporting biofilter performance and optimization. This presentation describes the performance limitations and application of a common RAS unit operation for solids removal and biofiltration, namely a bead filter in sequence with a moving bed bioreactor.

It has been observed that moving bed bioreactors placed behind bead filters with floating bead media (polygyser, propeller-wash, or bubble-bead) are often flow limited in their ability to convert ammonia. That is, the MBBR ability to convert total ammonia nitrogen (TAN) to nitrate is limited by the transport of TAN into the unit. The bead filter must be sized to provide the necessary flow. Consideration of the need for flow and recognizing the TAN conversion capabilities of the bead filter leads to the sizing approach described under the assumption nitrogen is limiting. Commonly assumed bead filter and MBBR ammonia conversion data is used to model several scenarios of varying TAN concentrations and system volumes to describe the need to consider water flow in the treatment sequence. The STELLA (v 9.1) from ISEE Systems was used to develop and evaluate the varying sequence scenarios.

## **INTENSIVE BIOTECHNOLOGY OF CULTIVATION OF HYDROCOLES AND PLANTS IN MODULAR INSTALLATION**

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In modern period of aquaculture development, the biotechnologies of hydrocoles production in modular installations are topical. These installations supply flexible production that given commercial production on any stages of production cycle (eggs, fingerlings, fry, commercial fish, aquaponics production) depends on requirements of the market.

New biotechnology provides for selection of cultivated objects that supply maximum assimilation of feeds on different trophic levels, consecutive accumulation of metabolites, minimal evacuation and consumption of water, economy of energy resources. Fast-growing species were chosen, adapting opportunities of each fish species to concrete parameters of environment were considerate, and plants with short vegetation period were selected for maximum obtaining of green production from unit area.

Experimental researches were made in specialized aquacomplex of scientific-experimental base of SSC RAS. The objects of investigations were hybrids of sturgeon fish species, african catfish, crayfish and green cultures. For cultivation the objects experimental model of installation with closed water supply was used. The installation is a system of tanks, raceways, sedimentation basins, filters (mechanical and biological), light system, express-online control of hydrochemical parameters, living organisms.

One of the key unit of this modular installation is biological filter made on technology MBBR. Calculated power on oxidation of total ammonium nitrogen is 750 g/day, it conforms to 18 kg of extruded combined feeds that contain 45% of protein.

Hybrid forms of sturgeon fish were cultivated from weight 10 g to 1500-2000 g during 12 months with stocking density 50-70 kg/m<sup>3</sup>, clarid catfishes were cultivated during 4-5 months from weight 10 g to 1200-1500 g with stocking density 100 kg/m<sup>3</sup>. Production of catfish was obtained 3 times a year. Australian cancer were cultivated in system for obtaining the offspring.

High parameters of growth and survival rate of hydrocoles at combined cultivation in modular system were obtained: survival rate of fish is 95-98%, volume increment – 65%. For cultivation by hydroponics methods the experiments with plants cultures (lettuce, parsley) were carried out. It established that at cultivation in installation the vegetation period of lettuce is short (from sowing to harvest) – 40-45 days, contain of nitrates in leaves conforms to norm (1157 mg/kg), biomass of leaves from 1 m<sup>2</sup> was 3500-4200 g/m<sup>2</sup>.

In vegetation period of parsley was 65-80 days (higher than in open ground), contain of nitrates in leaves conforms to norm, biomass of leaves from 1 m<sup>2</sup> was 2500-3000 g with crop-producing power 63,3-67,2 kg/m<sup>2</sup>.

The level method with continuous cycle of consecutive cultivation of objects (hydrocoles and plants) in one artificial formed system where all objects have mutually stabilizing effect on biological system was developed.

## EFFECT OF DIETARY MOISTURE ON GROWTH AND INTESTINAL HISTOLOGY IN *Seriola dorsalis*. A PRELIMINARY ASSESSMENT OF A NOVEL MOIST FEED

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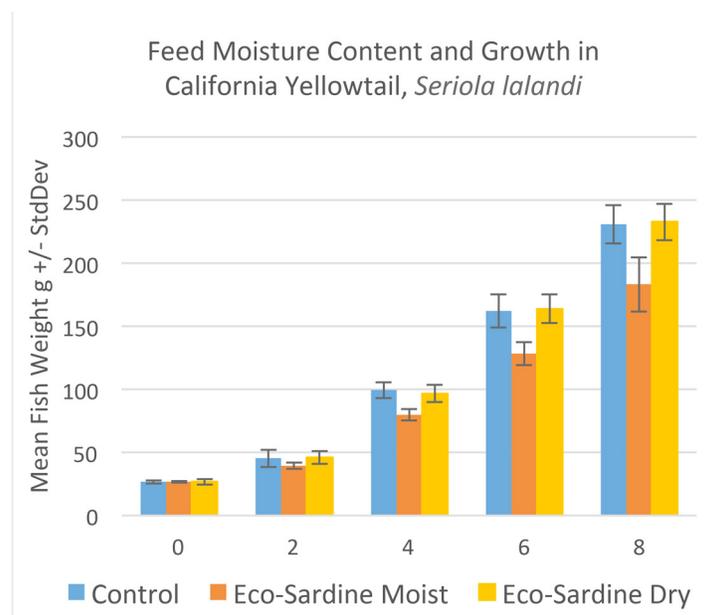
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Commercial aquaculture feeds most commonly contain less than 10% moisture, while natural diets of carnivorous marine fish (i.e. forage fish, crustaceans) contains roughly 70% moisture and provides an important dietary source of fresh water. Following ingestion, marine teleosts hydrate dry formulated pellets via luminal secretions and ingested seawater. The pathways enabling absorption and excretion of salts, consequent of seawater drinking, are energetically costly to marine fish. Supplementation of feed with increased moisture content and decreased osmolarity may reduce the requirement for exogenous chyme hydration. We hypothesize that the use of feeds with a high moisture content may decrease this osmoregulatory cost/disturbance. This study examines the effect of dietary moisture content on growth and intestinal histology in California Yellowtail, *Seriola dorsalis*.

The industry standard for feed production requires cooking extrusion and drying to <10% moisture, which aids in spoilage prevention. Forgoing the drying process of this method may yield a moisture level of 25%, but water stability and nutrient retention of the feed are impaired. A modification to this extrusion method allows for the production of water stable particles with up to 55% moisture.

An 8-week feeding trial was conducted at Hubbs/Seaworld in San Diego, CA on juvenile *S. dorsalis* (25g). A moist feed developed by the USDA-ARS, “Eco-Sardine” (ESM), was fed at two levels of moisture (5% and 50%). A third group was fed a commercial control diet (CONT)(5% moisture). Dietary ration was calculated on a dry-weight basis.

The initial results illustrate that an increase in dietary moisture content had a negative effect on growth. Comparable growth among dry diets suggest that formulation or processing method do not explain the divergence in growth observed in the high-moisture treatment. The results from histological analysis, mycotoxin contamination test and proximate composition are pending and will be presented at the conference.



## THE EARLY LIFE HISTORY AND FOOD HABITS OF HATCHERY-REARED GUADALUPE BASS *Micropterus treculii*

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The Guadalupe Bass *Micropterus treculii* is endemic to streams draining the Edwards Plateau in central Texas. Fingerling Guadalupe Bass are produced at the A. E. Wood Fish Hatchery, San Marcos, Texas and stocked into these streams to restore threatened populations and combat hybridization with Smallmouth Bass *Micropterus dolomieu*. Survival in fingerling rearing ponds has fallen in recent years for unknown reasons and meeting the fingerling stocking request is challenging. In addition to lower fingerling production, this low survival in ponds may lead to lost genetic diversity of stocked fingerlings.

While the life history and feed preferences of other *Micropterus* spp. are well described, much of the early life history of Guadalupe Bass is unreported. A better understanding of this critical period may improve hatchery culture practices, fingerling production and management efforts. Early developmental characteristics and milestones at 20 and 24°C, deformity prevalence, and prey preferences of Guadalupe Bass were therefore documented in a hatchery setting. Eggs and fry were repeatedly photographed under a microscope and observed in culture tanks and ponds from fertilization to harvest of 38-mm fingerlings.

The egg incubation period was  $61.1 \pm 4.4$  h at 21.2°C and  $47.9 \pm 2.9$  h at 24.0°C (28-29 growing degree days). Similarly, larval development, yolk depletion, and swim-up proceeded faster at the higher temperature. A high prevalence of developmental abnormalities, particularly of the gape and heart chamber, were observed early in the production season. Preliminary genetic analysis suggests this was not related to parentage. Normally-developed fry commenced exogenous feeding on a variety of zooplankton soon after swim-up with no first-feed preference detected. Swim-up fry readily consumed *Artemia* nauplii in hatchery tanks, and *Brachionus* sp., *Daphnia* sp., copepods and chironomid larvae within 72 h of stocking in ponds. Maximum prey size increased as fry grew. These observations will be used to refine culture and management practices for Guadalupe Bass.

## ULVA MEAL *Ulva pertussa* AS A SUBSTITUTE FOR SOYBEAN MEAL IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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As shrimp culture has become an expanded and intensified economic activity, the demand for more cost-effective and sustainable protein sources continues to increase. Macro-algae can use metabolic residues of aquatic animals as nutrients, absorb CO<sub>2</sub> and produce O<sub>2</sub> for the environment. The inclusion of macro-algae that contain high protein content in aquaculture feeds would be an alternative strategy to reduce the reliance on fish meal (FM) and terrestrial plant protein source such as soybean meal (SBM). Unfortunately, results from a previous trial in our lab indicated that *Ulva pertussa* (Batch 1) was a poor replacement for FM in diets for Pacific white shrimp *Litopenaeus vannamei*. Ingredient digestibility results indicated that apparent protein, energy, and amino acids digestibility of Ulva meal were significantly lower than FM and SBM. In order to confirm the utility of Ulva meal, two additional trials are being conducted using additional samples of Ulva meal. The first trial evaluated nine test diets formulated on equal protein and lipid basis (35% protein and 8% lipid). The first seven diets utilized increasing levels of Ulva meal (0, 5, 10, 15, 20, 25, and 30%) to replace SBM. Diet 8 and Diet 9 utilized high inclusion of Ulva meal from first and third batch to replace SBM compared to 20% inclusion of second batch Ulva. At the end of the growth trial, significant reductions of biomass, mean weight, weight gain as well as survival of the shrimp were observed (Table 1). The second trial evaluated four diets supplemented with high inclusion of three batches Ulva meal formulated on equal digestible protein basis. Significant decreased growth and survival were detected in the treatment contain Ulva meal from second batch. The results of this work indicate that besides low nutrient availability other problems such as high mineral contents, anti-nutritional factors as well as processing methods of Ulva meal may result in poor replacement for FM and SBM by Ulva meal.

Table 1 Growth performance of juvenile Pacific white shrimp (0.24±0.01g) offered diets with different *Ulva pertussa* levels in the growth trial for five weeks.

Diet	Biomass (g)	Mean Weight (g)	WG <sup>3</sup> (%)	FCR <sup>2</sup>	Survival (%)
D <sub>1</sub>	43.31 <sup>a</sup>	4.55 <sup>a</sup>	1734.21 <sup>a</sup>	1.46 <sup>b</sup>	95.0 <sup>a</sup>
D <sub>2</sub>	36.19 <sup>ab</sup>	3.70 <sup>ab</sup>	1398.22 <sup>ab</sup>	1.83 <sup>ab</sup>	97.5 <sup>a</sup>
D <sub>3</sub>	28.40 <sup>bc</sup>	3.25 <sup>ab</sup>	1241.46 <sup>ab</sup>	2.23 <sup>ab</sup>	87.5 <sup>ab</sup>
D <sub>4</sub>	23.89 <sup>cd</sup>	2.58 <sup>b</sup>	948.74 <sup>b</sup>	2.82 <sup>ab</sup>	92.5 <sup>a</sup>
D <sub>5</sub>	18.98 <sup>cd</sup>	2.53 <sup>b</sup>	990.26 <sup>b</sup>	2.96 <sup>ab</sup>	75.0 <sup>ab</sup>
D <sub>6</sub>	16.34 <sup>cd</sup>	2.56 <sup>b</sup>	943.46 <sup>b</sup>	3.53 <sup>a</sup>	67.5 <sup>b</sup>
D <sub>7</sub>	15.50 <sup>d</sup>	2.45 <sup>b</sup>	864.67 <sup>b</sup>	3.37 <sup>ab</sup>	65.0 <sup>b</sup>
D <sub>8</sub>	26.14 <sup>bcd</sup>	2.96 <sup>b</sup>	1131.07 <sup>b</sup>	2.61 <sup>ab</sup>	87.5 <sup>ab</sup>
D <sub>9</sub>	27.10 <sup>bcd</sup>	3.09 <sup>b</sup>	1226.92 <sup>ab</sup>	2.36 <sup>ab</sup>	87.5 <sup>ab</sup>
<i>P</i> -value	<0.0001	0.0002	0.0008	0.0201	0.0006
PSE <sup>1</sup>	1.2872	0.1392	61.8604	0.2020	2.6131

## MOBILE PHONE TECHNOLOGY AND FISH MARKETING INFORMATION SYSTEM (FMIS) IN GHANA

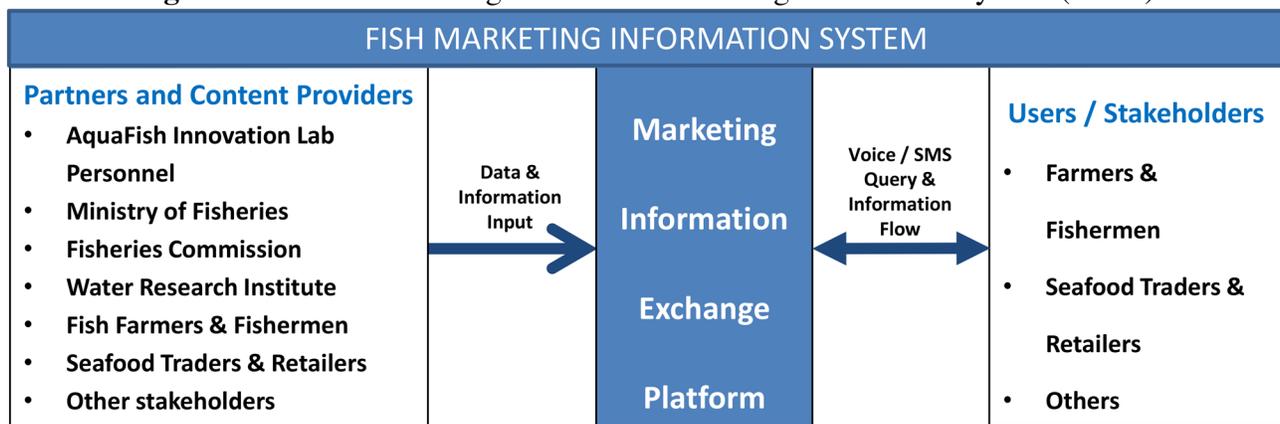
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For small-scale fish producers and artisanal fishermen in Ghana, readily available market information on prices and demand for fish at different fish markets helps inform production and harvesting decisions. Minimizing the information gaps along the fish value chain greatly improves efficiencies and reduces post-harvest losses in fish marketing and the value chain as a whole. The technology is a cell-phone based Fish Market Information System (FMIS) with a focus on tilapia and catfish. This is because tilapia and catfish are the dominant fish species farmed and caught in inland waters. The FMIS has a database of farm-gate and market prices of tilapia and catfish in selected locations in Ghana. The FMIS is web-based and provides tilapia and catfish market information on-line as well as via voice and text messaging to users. The system is set up in a form that can easily be accessed by users from any mobile device anytime (Figure 1).

There are two types of subscribers to the system – registered users and ad-hoc users. The system can send out (push) farm-gate and market price information to only the registered users. However, to request (pull) information on tilapia prices from the system, both registered users and ad-hoc users can access the system either by dialling or text messaging to a 10-digit phone number or a 4-digit short code. The code is “1941,” and can be accessed on the top 3 mobile phone networks in Ghana (MTN, Airtel and Vodafone) by text with the following keywords; ‘Tilapia,’ ‘Til,’ ‘Cat,’ ‘Catfish.’ The voice feature of the system when a user requests for information includes messages in English and three native languages – Twi, Ga and Ewe. The future plan is to expand the capabilities of the system to include capture fisheries to benefit the marine and inland artisanal fisheries subsector.

**Figure 1:** A Schematic Diagram of Fish Marketing Information System (FMIS)



## UAPB - AQUACULTURE EXPERIMENTAL RESEARCH STATION: EXTENSION AND OUTREACH ACTIVITIES

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The Aquaculture Research Station at the University of Arkansas at Pine Bluff is located on 81 hectares of land one mile north of the UAPB campus. There are currently 107 earthen ponds including a 5-ha reservoir that supplies water to the remaining 106 research ponds. However, the number of ponds will be reduced to 97 due to a renovation project that will start in early 2017. Thirty-five ponds are 400 m<sup>2</sup> in size. There are fifty ponds 1,000 m<sup>2</sup> in size, sixteen 2,000 m<sup>2</sup> ponds, and one 4,000 m<sup>2</sup> pond that is used as a small impoundment demonstration. Further research may be also conducted in 40 2.7 m diameter pools covered with bird netting. A hangar covers eight concrete vats used for holding, grading, or otherwise processing fish. The primary hatchery building holds recirculating systems for research. Other buildings on site include: 1) Aquaculture Equipment Development Building; 2) Aquaculture Research and Demonstration Building; 3) Feed Building; 4) Hatchery; 5) Maintenance Building; 6) Storage Facility; 7) Value-Added Product Development and Demonstration Building; 8) Water Chemistry Laboratory; 9) Aquatic Plant Greenhouse; and 10) Truck Shed.

The facilities and resources at the UAPB Aquaculture Research Station have been utilized to host a number of educational and Extension outreach activities including Aquatic Sciences Day, Field Day, elementary, middle and high school guided tours, in-service training for Extension personnel, and fish sales. These outreach and Extension events will be described and historical data will be presented to demonstrate how a research facility can be successfully utilized for education and outreach to a number of different aquaculture stakeholders.



Figure 1. Aerial photo of a section of the Aquaculture Research Station at the University of Arkansas at Pine Bluff. Photo by Anita M. Kelly

## ECONOMICS OF DEBONING BIGHEAD AND SILVER CARP FILLETS

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Invasive silver and bighead carp were reported in natural waters of the United States in the early 1980s and have since spread throughout the Mississippi and other river basins that drain into the Gulf of Mexico. The introduction of bighead and silver carp to non-native waters poses ecological and human safety concerns. Considered an excellent food fish in much of the world, neither bighead nor silver carp are consumed in the United States outside of ethnic markets due to the presence of intramuscular “Y” bones in the fillet and unfavorable opinions of any fish bearing the carp name. Past studies have explored methods of rendering bones edible but have rarely aimed to completely remove “Y” bones. The removal of intramuscular bones could drastically improve the palatability of the carp allowing for increased demand, increased harvest, and some level of population control in infiltrated water bodies.

Bighead and silver carp were filleted by hand and intramuscular “Y” bones were removed. Duration of each processing step was timed for individual fish to determine average processing time. Weights and lengths were taken before processing, after filleting, and after “Y” bone removal to determine condition factor (K) and dress-out rate.

The above data were used in a mathematical programming model to estimate the least cost option to produce various amounts of deboned carp. Results of this model highlight an optimal processing plan and should be used in conjunction with consumers’ willingness to pay measures to determine the economic feasibility of this enterprise.

## ENHANCED THERMOTOLERANCE AND SURVIVAL OF JUVENILE JAPANESE FLOUNDER *Paralichthys olivaceus* FED DIETARY SOY PEPTIDE

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Global aquaculture especially in temperate regions may be adversely affected by ocean warming and an overall increase in water temperatures brought about by climate change. Soy peptide (SP), a soy protein enzymatic hydrolysate, contains bioactive substances could be utilized as an immune-stimulating feed ingredient. The experiment evaluated the efficacy of dietary SP on promoting growth, and enhancing tolerance and survival to heat stress in juvenile Japanese flounder, *Paralichthys olivaceus*. Four diets were incorporated with different levels of SP (0, 2, 5 and 10%) and a 6-week feeding trial ensued. Following the feeding trial, the experimental groups were subjected to heat stress to measure survival rate and heat shock protein70s (HSP70s) in gill, liver and skin. Fish fed diets with SP inclusion showed considerable decrease in percent weight gain. Significantly higher lethal time values to 50% mortality ( $LT_{50}$ ) value were recorded for fish fed 10%SP. Moreover,  $LT_{50}$  values of fish fed 2 and 5%SP were significantly higher compared to fish fed control diet. HSP70s produced in all the tissues was also significantly highest in fish fed 10%SP. HSP70s values were significantly higher in fish fed 2 and 5%SP compared to fish fed control diet. A significant reduction in HSP70s among all groups during recovery period was also observed. These results suggest that SP can be used to enhance the immune response and survival of *P. olivaceus* under heat stress.

## DIETARY SOY PEPTIDE ENHANCES THERMOTOLERANCE AND SURVIVAL OF JUVENILE JAPANESE FLOUNDER *Paralichthys olivaceus*

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Global aquaculture especially in temperate regions may be adversely affected by ocean warming and an overall increase in water temperatures brought about by climate change. Soy peptide (SP), a soy protein enzymatic hydrolysate, contains bioactive substances could be utilized as an immune-stimulating feed ingredient. The experiment evaluated the efficacy of dietary SP on promoting growth, and enhancing tolerance and survival to heat stress in juvenile Japanese flounder, *Paralichthys olivaceus*. Four diets were incorporated with different levels of SP (0, 2, 5 and 10%) and a 6-week feeding trial ensued. Following the feeding trial, the experimental groups were subjected to heat stress to measure survival rate and heat shock protein70s (HSP70s) in gill, liver and skin. Fish fed diets with SP inclusion showed considerable decrease in percent weight gain. Significantly higher lethal time values to 50% mortality ( $LT_{50}$ ) value were recorded for fish fed 10%SP. Moreover,  $LT_{50}$  values of fish fed 2 and 5%SP were significantly higher compared to fish fed control diet. HSP70s produced in all the tissues was also significantly highest in fish fed 10%SP. HSP70s values were significantly higher in fish fed 2 and 5%SP compared to fish fed control diet. A significant reduction in HSP70s among all groups during recovery period was also observed. These results suggest that SP can be used to enhance the immune response and survival of *P. olivaceus* under heat stress.

## **SELENIUM HEALTH BENEFIT VALUES CONFIRM OCEAN FISH CONSUMPTION PREVENTS MERCURY TOXICITY**

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There has been a lot of progress in our understanding of mercury biochemistry in the past few years. Our group recently completed an EPA sponsored study of the mercury and selenium contents of over 14,000 ocean and freshwater fish samples collected from all over North America. At an EPA sponsored meeting in DC a couple of years ago, the consensus opinion was that seafood consumption during pregnancy is associated with a 4-6 point improvement in child IQ. Since we were already aware that seafood was positively associated with other beneficial maternal and fetal outcomes, the consistency of neurological outcomes in the epidemiological studies is confirming the importance of what we already knew about mercury biochemistry from cell culture and animal studies. We just completed a NOAA funded international round-robin study to compare the selenium analysis capabilities of laboratories. This is important since mercury risks are entirely dependent on the relative amounts of mercury and selenium present in the foods that are being consumed.

Our EPA sponsored work also funded further development of the Health Benefit Value (HBV), the only reliably accurate criteria for assessing risks associated with mercury exposures as well as beneficial effects associated with improved dietary intakes of selenium, long chain omega-3 fatty acids, and other essential nutrients.

Website: <http://essp.und.edu/people/bios/ralston.aspx>

Fish Issue Fact Sheets: <http://net-effects.und.edu/factsheets.aspx>

## EVALUATION OF MASCULINIZATION TECHNIQUES IN TWO SEXUALLY DICHROMIC ORNAMENTAL SPECIES

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Sex determination in teleosts is highly diverse and sexual phenotypes may result from both genetic sex determination (GSD) and environmental sex determination (ESD). Many fishes have sex chromosomes but environmental conditions during their early life history have the potential to override these genes and alter the “functional sex” of the organism. This sexual plasticity can aid in the production of monosex populations, which is of great interest to the aquaculture industry. This is particularly important in ornamental aquaculture, where, in sexually dichromic species, male fish retail for a higher price than female conspecifics due to their more aesthetically pleasing characteristics, including vibrant coloration and ornate finnage. The focus species of this research, the Rosy Barb *Pethia conchonius* and Dwarf Gourami *Trichogaster lalius*, are excellent examples of such species wherein female specimens sell at reduced prices or have no market value.

Current research efforts focus on understanding the role of ESD in these two species with the purpose of developing efficient and cost effective methods of masculinizing cohorts of fish. Manipulation of the larvae’s environment while their gonads are still sexually undifferentiated has the potential to result in sex reversal. This manipulation must occur during the labile period, when the larvae are receptive to exogenous factors that may override their genotype and influence their phenotypic sex. Identification of this labile period is critical to development of successful masculinization protocols. Previous studies have also shown that environmental sex reversal is linked to the release of the stress hormone cortisol. Exposure of larval fish to environmental or dietary cortisol has been shown to skew sex ratios of developing populations and thus the stress response axis may be of great interest for targeted masculinization treatments.

A suite of experiments have been designed to elevate endogenous cortisol levels in the larvae by either directly exposing them to cortisol or manipulating the environment in such a way to elicit a natural increase in endogenous cortisol levels. These environmental factors include stocking density, temperature, and salinity. Whole body cortisol levels will be measured using a validated ELISA and sex ratios of replicate tanks will be determined either histologically or via gross dissection. Additionally, survival data will be calculated for the duration of each experimental treatment. Survival data will be analyzed using a one-way ANOVA function followed by a Tukey’s HSD means separation test. Sex ratio data will be analyzed using a logit transformed generalized linear model (GLM) weighted based on the sample size.

## COMPARATIVE EVALUATION OF METABOLIC ENZYMES ACTIVITIES IN DIFFERENT TISSUES OF *Pangasianodon hypophthalmus* (Sauvage, 1878) FINGERLING REARED AT HIGHER TEMPERATURE

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A 60-day feeding trial was conducted to study the effect of higher temperature (32°C) in comparison to ambient temperature on different metabolic enzyme Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Adenosine triphosphatase (ATPase), Acid Phosphatase (ACP) & Alkaline Phosphatase (ALP) activities in different tissues of *Pangasianodon hypophthalmus* (Sauvage, 1878). AST activity (at 37°C) differ significantly ( $P < 0.05$ ) in case of liver, however, temperature do not significantly affect the AST activity in case of muscle ( $p > 0.05$ ). The ALT activity (at 37°C) differ significantly ( $P < 0.05$ ) in case of liver and muscle at different temperature. ATPase activity (at 37°C) in gill do not differ significantly ( $P > 0.05$ ) whereas ATPase activity in liver at different temperature differ significantly ( $P < 0.05$ ). ACP activity (at 37°C) does not vary significantly at different temperature whereas ALP activity (at 37°C) varies significantly at different temperature in liver and intestine tissues. The present study clearly shows that there is temperature dependent change in activity of different metabolic enzyme in different tissue studied in comparison to the control at ambient temperature. The finding suggests that the metabolic responses are variable and accordingly, temperature impacts on the growth performances of this new candidate species for aquaculture.

## NUTRITIONAL RESPONSES TO HIGH CARBOHYDRATES IN COMMON CARP (*Cyprinus carpio* L.) DIET

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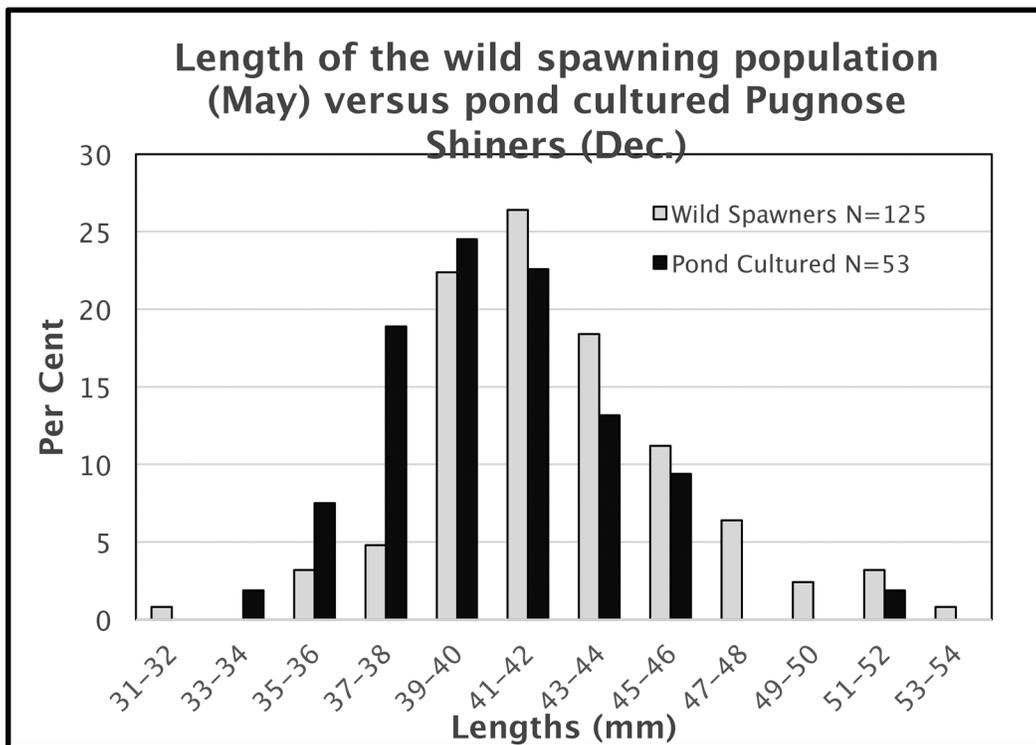
Feeding trial was carried out for 70 days to study nutritional effects of three levels of local carbohydrate sources (Standard ration SR 37 %, Medium carbohydrate ration MCR 42 % and high carbohydrate ration HCR 53%) in the diet of juvenile (30g) common carp. Relative weight gain values varied from the highest of 27.1% in SR treatment to 25.9 % in MCR and the lowest of 25.0% in HCR treatment. Although SR ration gave better IGR, but MCR and HCR gave nearly the same instantaneous growth rate values, indicating that the incorporation of carbohydrate up to the level 53% has no significant impact on growth. Feed conversion ratio varied between 4.62 in SR to 4.82 in MCR and 4.87 in HCR treatment. Similar trend was shown in feed conversion efficiency which ranged from 21.73 % in fish fed on SR, 21.00% for MCR and 20.62% for HCR. Fish fed SR ration with the highest level of protein in diet (28.71%) have the lowest protein efficiency ratio (PER). The carbohydrate rich diets which contain lower protein levels (MCR 25.69% and HCR 22.71%) have higher PER values (0.82 and 0.91 respectively). Slight decrease in the digestibility of both protein and lipid values are noted in carbohydrate-rich diets. On contrary carbohydrate digestibility increased from 54% to 69% with increasing the dietary carbohydrate levels from 37 to 53 %. Using the x-ray method for studying feed evacuation, it appeared that, the high carbohydrate ration was evacuated faster than MCR and SR ration. After 24 hours of feeding, 91 % of the HCR ration was evacuated compared with 84% and 79 % in both SR and MCR ration. The rate of evacuating HCR ration ranged between 6.7-7.6 % per hour compared with 5.5 -6.3% per hour for MCR and 5.7-7.0 % per hour for SR ration.

**POND CULTURE OF PUGNOSE SHINERS *Notropis anogenus* IS A FEASIBLE STRATEGY FOR RESTORING EXTIRPATED POPULATIONS IN NEW YORK WATERS**

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This project was initiated to demonstrate the feasibility of utilizing pond culture techniques to produce enough Pugnose Shiners to restore them in their native range in New York State. Pugnose Shiners are endangered in New York, being rare in the St. Lawrence and extirpated from Cayuga Lake and most of Lake Ontario. A recovery program was initiated utilizing brood-stock from Sodus Bay, Lake Ontario. Sodus Bay contains the last remaining New York “lake” population, which are genetically distinct from the St. Lawrence River population. In April 2015, a small number of fish were collected for disease certification. On 24 June 2015, another 65 shiners were captured for stocking in a 2/3-acre pond in the experimental pond complex at the State University of New York, Cobleskill. In spite of the late stocking date and low brood-stock number, Pugnose Shiners spawned and produced hundreds of juveniles. By December the pond cultured population was 41 mm long and nearly as large as the spawning population of Pugnose Shiners taken from Sodus Bay in May (below). By the end of August 2016, thousands of pond reared Pugnose Shiners were available for stocking. This project demonstrated the feasibility of pond culture of Pugnose Shiners, and it’s potential for producing thousands of juveniles a year for restoring extirpated wild populations. Culture techniques refined by this project may also be applicable to Minnesota, Wisconsin and Canada, where this species is of special concern, threatened or endangered.



## **INTEGRATING BIOFLOC SYSTEMS WITH ORGANIC PLANT PRODUCTION AND RENEWABLE ENERGY AT KSU'S HIGH TUNNEL COMPLEX**

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Biofloc aquaculture systems contain a dense microbial community that is partly contained on small, naturally-forming particles known as biofloc. The microbial community is responsible for cycling and assimilating the otherwise toxic nitrogen-based compounds ammonia and nitrite. Research suggests that animals such as shrimp and tilapia can consume biofloc particles and gain nutritional benefits such as added protein and lipid which in turn can lower feed costs. Because of the internal filtration in biofloc systems, very little water is used which increases biosecurity and allows the systems to be sited nearly anywhere.

The Kentucky State University (KSU) Land Grant Program has developed a High Tunnel Complex on the University campus that includes biofloc fish tanks. High tunnels are simple greenhouses, typically constructed using steel hoops and wood-framed end walls with transparent plastic sheeting strewn over them to help contain solar energy. The KSU Complex contains four high tunnels that are 30.5 x 10.7m, a center walkway divides each tunnel down the length of the structure on the inside. The south side of each tunnel has plant growing beds, while the north side has four 13 m<sup>3</sup> fish tanks built of wooden frames with rubber liners. Each tunnel has metal gutters along both long, outer side walls which collect rain water that is delivered to four, 9.5 m<sup>3</sup> water storage tanks. This water is used to irrigate crops and replace evaporation in the fish tanks. A 9.7 kW photovoltaic solar array generates approximately enough energy to offset the electrical use of the complex; this energy is sold back to the utility company via net electrical metering.

Current research projects include organic horticulture trials comparing high tunnel production to open field production, aquaculture trials, and aquaculture effluent fertilization experiments. Aquaculture projects have examined the production of tilapia in biofloc systems during the warm months using low-energy artificial lighting to supplement sunlight and augment algal productivity. Next, cool-water fish will be grown to evaluate whether pond stocker-size fish can be produced for a spring harvest. The temperature dynamics of the high tunnels, especially the way that the relatively large volume of the fish tanks affect diurnal temperature swings is being studied. Reclaimed filtrate from the settling chambers and fractionators on the fish tanks is being assessed for its nutritional quality as an aquatic feed ingredient. This material is also being evaluated as a soil amendment for terrestrial crop production.

Research being conducted at the KSU High Tunnel Complex focuses on the sustainable integration of plant and fish production, thereby augmenting the efficiency and productivity of food production systems.

## ANALYSIS OF THE SKIN AND GUT MICROBIOMES OF WILD-CAUGHT FISHES FROM LAKE GUNTERSVILLE, ALABAMA

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The bacterial communities that constitute fish microbiomes are now recognized as essential components of host health and defense from invading pathogens. Therefore, a better understanding of the natural bacterial communities of healthy individuals and how they interact with the host and other environmental factors is of critical importance. For this study, we hope to expand the existing body of research on fish microbiomes to include the skin and gut microbiomes of important freshwater sport fishes. Our objectives were to 1) characterize the gut and skin microbiomes of three common freshwater fishes including two important sport fishes, largemouth bass *Micropterus salmoides* and bluegill *Lepomis macrochirus* as well as the more primitive spotted gar *Lepisosteus oculatus*, 2) compare intra- and interspecies differences in the composition of each microbiome, and 3) identify potential influences of seasonality on core microbial communities. Skin and gut samples were collected in August and November 2014, and May 2015. All samples were sequenced as paired-end reads of the 16S rRNA gene via the Illumina MiSeq platform. More than 5M reads were analyzed representing 4,130 and 2,744 OTUs from gut and skin samples, respectively. Good's coverage was higher than 98% in all samples. Spotted gar exhibited the most diverse skin microbiome, while largemouth bass was the least diverse species in terms of skin microbiome composition. The highest diversity in the gut microbiome was observed in bluegill; however, the bacterial communities of spotted gar were the most variable across seasons. All microbiomes analyzed significantly differed between fish species. Seasonal changes in bacterial community structures were also observed.

## MEASURING METABOLIC RATE OF EMBRYONIC FISH TO PREDICT GROWTH RATE

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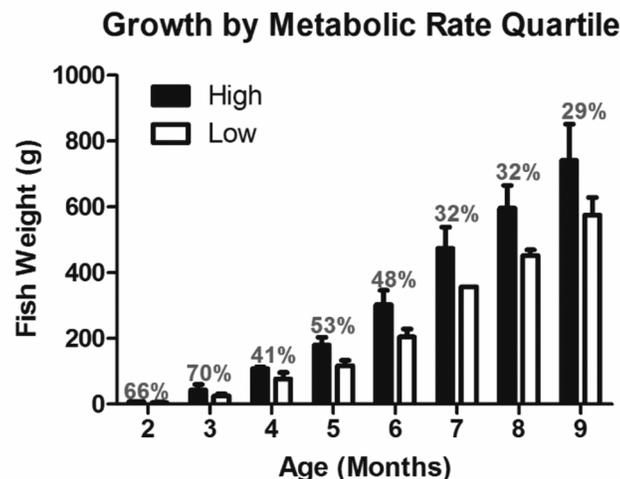
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Growth and feed efficiency are primary determinants of profit in fish production. Maximizing growth rates minimizes the time to achieve a marketable size and decreases the investment time and expense of rearing slow growing individuals. Feed costs constitute 45-70% of production costs. Thus, selection to improve feed efficiency and growth has the potential robustly increase producer profits. However, since growth and feed acquisition are tightly linked, selection for growth is selection for fish that consume more feed, not fish that are genetically superior for growth. Differences in feed consumed may result from variation in phagic drive or aggression.

The link between metabolic rate and growth is well established across fish species. We have pioneered the application of an AlamarBlue<sup>®</sup> based assay, to measure metabolic rate in fish and take advantage of the known link between growth and metabolic rate. This assay is amenable to scaling which allows for the simultaneous measure of metabolic rate in thousands of embryonic fish.

We hypothesized that by assessing metabolic rate in embryonic tilapia we could predict growth potential. Metabolic rate, measured by reduction of AlamarBlue<sup>®</sup> over 16h exposure, was assessed in 6000 embryonic fish/experimental unit resulting in high and low quartiles that included 1500 tilapia. Three replicates of high and low metabolic rate tilapia were reared identically under standard industry conditions at Desert Springs Tilapia (Agua Caliente, AZ). Tilapia in the high metabolic rate group grew more quickly to harvest than tilapia in the low metabolic rate group. In fact, tilapia that had a high metabolic rate was 29% heavier at harvest than tilapia that had a low metabolic rate as embryonic fish (Figure 1;  $P < 0.05$ ). In a second study conducted in 1 m<sup>3</sup> tanks at the University of Arizona, we showed that selection based on metabolic rate could improve feed efficiency and growth. In fact, fish selected as having a high metabolic rate (top 10%) as yolk-sac fry had a feed efficiency (measured across 5 months) that was 5.1% greater than fish selected to have a low metabolic rate (bottom 10%) as yolk-sac fry ( $P = 0.03$ ).

Herein, we shown that this assay allows for selection of fish based on metabolic rate as a proxy for the genetic potential for growth. Subsequent studies aimed at understanding the potential application of this assay to improve feed conversion are currently underway. We envision application to select for fast growing individuals or broodstock across aquatic species.



**Figure 1.** Embryonic tilapia with a high metabolic rate grow more quickly than embryonic tilapia with a low metabolic rate.

## **A LITTLE KNOWN EXTENSION PROGRAM IN FISH HEALTH**

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As aquaculture continues to grow, so do the regulations regarding fish health. The University of Arkansas at Pine Bluff implemented an inspection program in 1996 to inspect fish for the presence of diseases prior to export to other countries. Due to an outbreak of Spring Viremia of Carp in North Carolina in 2002, baitfish producers in Arkansas requested state legislation that would require third party oversight of the inspection of baitfish being shipped from Arkansas to other states. This request was done to ensure that fish from Arkansas were “safe”. The Arkansas Baitfish Certification Program currently has 95% of all baitfish farms in Arkansas participating. This program inspects nearly 20,000 fish per year from over 40 farms. The dissemination of information regarding the program has been very successful and included a number of different formats. This program is the model program for many other states.

## **A FLOW CYTOMETRIC BACTERIAL ENUMERATION FROM FATHEAD MINNOW FROM NO WATER EXCHANGE BIOFLOC SYSTEM**

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This study was designed to use flow cytometry as a tool to document microbial communities in a zero water exchange super-intensive system from fathead minnows stocked at different densities. Biofloc growth was induced in no water exchange super-intensive system by periodic additions of a carbon source (glucose) to maintain a C: N ratio of 12:1. Microbial communities were distinguished at the gram-stain level using flow cytometric fluorescent activated cell sorter (FACS) methods. Differentiation between all other bacterial populations between 1~20 mm was based upon auto fluorescence and forward scatter (a size indicative light parameter) using FACS. Different types of stains were used to differentiate gram-positive and gram-negative bacterial populations using different stains. Samples were prepared for enumeration of bacteria by flow cytometry. Data are currently being analyzed and results will be presented.

## COMMERCIAL DEMONSTRATION OF KAOLINITIC CLAY FOR PROTECTION OF *Flavobacterium columnaris* IN SPORTFISH

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Sportfish farms in Arkansas routinely battle Columnaris disease, which is caused by *Flavobacterium columnare*. Columnaris is especially prevalent during the feed training of centrarchids such as largemouth bass and immediately following harvest of crappie, redear sunfish, and bluegill while they are being held in load out sheds prior to being sold. Largemouth bass fingerlings are brought in from the pond and held indoors for several weeks in vats during the feed training process. A commercial research demonstration trial was devised with two commercial sportfish farms in Arkansas to test the efficacy of kaolin clay to prevent outbreaks of Columnaris. Kaolinitic clay was utilized as a prophylactic treatment for largemouth bass, crappie, bluegill, and redear sunfish. Participating producers treated vats of fish with 1 ppt kaolin and 0 ppt kaolin (control) as a prophylactic treatment and also during active Columnaris infections. Vats were treated on commercial farms and then samples of fish were brought to the UAPB Lonoke Fish Disease Diagnostic Laboratory. Gills, fins, and tissues of treated and non-treated fish were examined visually for the presence of columnaris both before and after prophylactic treatment. Fish tissue samples were also sampled to confirm the presence/absence of Columnaris in fish receiving 0 or 1 ppt kaolinitic clay. The demonstration is currently ongoing, however, preliminary data suggests that fish treated with kaolinitic clay while being feed trained and during holding periods prior to sale are having less incidences of Columnaris.

## **COLUMNARIS (*Flavobacterium columnare*) CHALLENGE USING FATHEAD MINNOWS (*Pimephales promelas*) IN AN ULTRA-LOW FLOW SYSTEM**

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Arkansas baitfish farms routinely struggle with columnaris disease, which is caused by *Flavobacterium columnare*. Columnaris is ubiquitous in fathead minnows (*Pimephales promelas*) especially after harvest while they are being held in vats and during the transport prior to being sold. Columnaris disease is transmitted horizontally from fish to fish is one of the most serious bacterial infections affecting the aquaculture industry today. The disease is highly contagious and may be spread through contaminated nets, specimen containers, and even food. Outbreaks are commonly caused by stress such as overcrowding, low dissolved oxygen, high ammonia and nitrite concentrations. In order to further examine this issue, a columnaris challenge tank study was devised with three different densities (400/m<sup>3</sup>, 800/m<sup>3</sup> and 1600/m<sup>3</sup>) and at two different temperatures (22°C and 28°C) using an ultra-low flow system. The experiment was performed with three replicates. LSU- 04-066 stain was used to challenge fathead minnows during the trial. Dissolved oxygen, flow, ammonia and fish mortality were recorded following the challenge. Fish mortality following the challenge was almost 100% in all the densities at 28°C within 24 hours and at 22°C within 28-30 hours. Tank water and fish tissue (gills, kidney, and spleen) samples were collected and processed for the presence of columnaris using real time PCR and flow cytometry. Data are currently being analyzed and results will be presented and discussed in the context of baitfish industry.

## RED PORGY AQUACULTURE: WHAT HAVE WE LEARNED, WHERE DO WE GO FROM HERE

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Red porgy *Pagrus pagrus* is commercially cultured in Europe, throughout the Mediterranean, and increasingly in Asia where it is highly regarded as a valuable marine reef fish and seafood delicacy. The red porgy are congeners of the red sea bream *Pagrus major*, one of the most valuable and widely cultured marine finfish. With widely established markets and consumer preference for red colored reef fish (e.g. red snapper, vermilion snapper and various groupers) red porgy is an excellent candidate for marine aquaculture in the Greater Atlantic and Southeast region including the Gulf of Mexico. While red porgy is not commercially cultured in the U.S., there is significant interest for land based and offshore aquaculture operations. We will review U.S. research and experience to date of red porgy aquaculture in terms of broodstock management, larval rearing, grow-out, nutrition and marketing potential. We will review the performance of red porgy culture in net pens globally and we will discuss opportunities for commercial development both land-based and offshore.

## EXPANDING PRODUCTION OF COMMON SNOOK THROUGH IMPROVEMENTS IN LARVAL REARING PROTOCOLS

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Common snook (*Centropomus undecimalis*) are a very popular and important sportfish in the coastal waters of the Atlantic ocean. Population declines in Florida, Texas, Mexico and Brazil have generated interest in developing reliable aquaculture techniques to produce snook for stock enhancement and commercial production. For more than 10 years, our efforts at Mote Marine Laboratory (Mote) have focused on captive spawning technology and developing larval rearing protocols in experimental recirculating aquaculture systems. Results from this research developed successful year-round captive spawning of common snook and the production of large volumes of high quality eggs. These eggs have been used to refine larval rearing protocols to meet the environmental and nutritional requirements of common snook.

Current research is focused on scaling up production using successful larval rearing protocols developed in research trials at Mote and in collaboration with other scientists working on common snook and other Centropomids. Early protocols developed at Mote had yielded  $\leq 1\%$  survival from egg to first grading ( $32 \pm 3$ DPH). The work was conducted in our recirculating larval production systems, consisting of three 3300-L tanks, solids filtration, moving bed bioreactor, protein skimmer, UV sterilizer, LED lighting, and thermal control through a heat-exchange system. Each larval tank has a removable upwelling egg hatcher and live food delivery system. In these trials, we standardized key culture variables, identified in earlier experimental trials. These variables included temperature, salinity, lighting (duration, intensity, spectrum), tank volume, flow rates, shading, probiotic usage, stocking density, live food protocols (enrichments and regime), and weaning strategies. Though further work is needed, we have already observed a substantial increase in larval survival from egg to first grading (approximately 30 days-post-hatch). Snook larval survival was as high as 4.2% at first grading, which is 4 times greater than past production-scale trials completed at Mote.

## EFFECT OF CRUDE OIL EXPOSURE ON REPRODUCTION IN CAPTIVE FLORIDA POMPANO BROODSTOCK

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A number of studies are being conducted to assess the long term impact of the 2010 *Deepwater Horizon* oil spill in the Gulf of Mexico (GOM). Research demonstrates that oiling caused a wide range of biological effects including those on GOM fisheries populations. While much of the research has focused on assessing the effects of the spill on larval fishes, certain aspects are still understudied, such as those examining the physiological response of adult marine fish species.

In the present study, selected reproductive parameters were examined in captive Florida pompano broodstock following aqueous exposure to south Louisiana crude oil. A mixture of oil and the dispersant (Corexit, 9500) was used to produce chemically-enhanced water accommodated fractions (CEWAFs) (0.0, 0.5 and 1.0 ppm [corresponding to control, low and high treatments, respectively]) administered over 12-hours in a spiked declining exposure study. This was followed by a period of non-exposure for 24 hours and then the process was repeated. Tagged fish, 5 females and 10 males per treatment, were transferred from the exposure tanks to a closed recirculating aquaculture system (total volume 28m<sup>3</sup>) where they were maintained for five months under simulated natural (27 ± 1.0°C and 13.5hr of light) photo-thermal conditions. Female and male reproduction condition was assessed periodically throughout the trial. Ovarian biopsies obtained from individual females were prepped as wet mounts and for histology. When possible, milt samples were collected; sperm density, sperm motility and spermatocrit were documented among individual males. Blood samples were collected and the plasma steroids Testosterone and 11-ketotestosterone were analyzed. Mature females were implanted with Ovaplant® (GnRHa) at a dosage of 50 µg kg<sup>-1</sup> bw and placed into individual spawning tanks along with two males from the same treatment group. Over the course of the study, spawns were obtained from fish in all treatment groups. Total egg production for each respective treatment group were as follows: 169,000 to 237,000 (control), 47,000 to 200,000 (low), and 58,000 to 162,000 (high). Results showed no significant differences in total expressible milt or sperm density between the experimental and control groups however, a significant difference in sperm motility was detected. Additional effects of oil exposure on reproduction and spawning performance in Florida pompano will be described.

## IDENTIFYING THE MINIMAL LEVEL OF FISHMEAL INCLUSION IN HIGH SOY FEED FORMULATIONS FOR FLORIDA POMPANO *Trachinotus carolinus*

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High soy-based feed formulations for Florida pompano have provided good performance, however, when animal proteins are completely removed from the diets there is a depression in growth. A series of 4 diets was formulated to re-evaluate the minimal inclusion of fishmeal (FM) in high soy diets where FM was replaced with NutriVance, a novel soy protein concentrate, 15% FM was reduced to 6% FM in Trial 1 then, a second series of 4 diets was formulated to confirm results and evaluate lower inclusion of FM to 0% FM (Table 1). The growth trials were conducted in a recirculating systems with three replicates per treatment, twenty fish were stocked in each tank. The fish were fed to apparent satiation and feed intake was recorded. The trials were conducted for 8 weeks, final results for Trial 1 and current results at week 4 for Trial 2 (Table 2). At the termination of each trial fish were frozen for proximate and amino acid composition as well as histological samples.

Table 1. Iso-nitrogenous and iso-lipidic diets formulated at Auburn University (40% protein, 8% lipid).

	Trial 1				Trial 2			
	Fishmeal	15.00	12.00	9.00	6.00	12.00	6.00	3.00
Soybean meal	46.60	46.60	46.60	46.60	46.60	46.60	46.60	46.60
Soy protein concentrate	0.00	3.08	6.14	9.21	2.89	8.98	12.01	15.05
Menhaden fish oil	5.00	5.29	5.58	5.87	5.02	5.47	5.70	5.93
CaP – dibasic	1.50	1.80	2.10	2.40	1.80	2.40	2.75	3.05
Taurine	0.50	0.52	0.54	0.56	0.52	0.56	0.58	0.59
Methionine	0.00	0.02	0.04	0.06	0.02	0.06	0.08	0.10
Others	31.4	30.69	30.00	29.30	31.15	29.93	29.28	28.68

Table 2. Final mean weights, FCR, survival and Thermal-unit growth coefficient (TGC), p value and pooled standard error (PSE). Superscripts indicate SNK grouping (<0.05).

Diet	Trial 1 (mean initial weight 13.1g)						Trial 2 (mean initial weight 18.5g)					
	FM15	FM12	FM9	FM6	p value	PSE	FM12	FM6	FM3	FM0	p value	PSE
Mean Weight (g)	70.3 <sup>a</sup>	70.7 <sup>a</sup>	66.3 <sup>ab</sup>	62.0 <sup>b</sup>	0.0177	1.6363	49.7 <sup>a</sup>	47.1 <sup>b</sup>	47.6 <sup>b</sup>	45.2 <sup>c</sup>	0.0011	0.4762
FCR	1.69	1.68	1.77	1.86	0.0545	0.0411	1.39 <sup>b</sup>	1.49 <sup>a</sup>	1.48 <sup>a</sup>	1.57 <sup>a</sup>	0.0046	0.0232
Survival (%)	98.3	100	91.7	95.0	0.2272	2.7638	100	100	100	100		
TGC	0.118 <sup>a</sup>	0.119 <sup>a</sup>	0.113 <sup>ab</sup>	0.107 <sup>b</sup>	0.0208	0.0023	0.127 <sup>a</sup>	0.120 <sup>ab</sup>	0.120 <sup>ab</sup>	0.114 <sup>b</sup>	0.0172	0.0021

## CELEBRATING THE LIFE OF Junda Lin, A REMARKABLE COLLEAGUE AND MENTOR

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Junda Lin, 55, passed away in March of 2016 after a 7-year battle with cancer. He left a legacy of knowledge and collaboration that continues to expand our knowledge of the aquaculture, biology, and conservation of marine aquarium species.

Junda completed a Masters degree at Rutgers University in 1985 and a doctorate degree from the University of North Carolina in 1989. Dr. Lin was among the first Chinese students admitted into public universities in the United States. After completing his graduate education, Dr. Lin accepted a postdoctoral fellowship at Smithsonian Environmental Research Center and then as a Biostatistician for the Texas Parks and Wildlife Department prior to obtaining a Professorship at Florida Institution of Technology in 1991.

At Florida Tech, Junda built a career that positively impacted the field of Aquaculture. While his career was cut short due to illness, Dr. Lin mentored dozens of undergraduate and graduate students, was a voracious collaborator, and was the director of the Vero Beach Marine Laboratory. Dr. Lin loved to collaborate and instilled this spirit in his students. As the director of the VBML he created a business incubator that allowed small aquaculture startups access to pristine seawater and a chance to test commercial feasibility. This innovative use of the VBML, a nearly forgotten marine laboratory, spawned several successful commercial operations. Dr. Lin contributed greatly to our understanding the trade and aquaculture of seahorses. Through collaborations with colleagues in China and the United States, Dr. Lin provided a path forward to the conservation of these endangered species.

In this lecture I will review some of our greatest memories and stories of a truly remarkable mentor, colleague, friend, husband, and father. His legacy lives on through the people he has inspired.



## THE IMPACT OF CO<sub>2</sub> RELATED OCEAN ACIDIFICATION ON THE MOLECULAR REGULATION OF SHELL DEVELOPMENT IN EASTERN OYSTER *Crassostrea virginica*

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Eastern oysters (*Crassostrea virginica*) indigenous to the Gulf of Mexico, are crucial estuarine species in their natural ecosystem and are economically important aquaculture bivalves in the United States. Previous studies suggest that growth and development of the eastern oyster are heavily affected by the fluctuation of environmental factors including temperature, salinity, pH, pollution, *etc.* Due to anthropogenic activities in energy consumption, along with natural climate patterns, the global atmospheric concentration of CO<sub>2</sub> has dramatically increased in past decades. This has resulted in rising dissolved CO<sub>2</sub> levels in seawater, ultimately leading to ocean acidification. While there is research indicating that increasing acidification of oceans could negatively impact the development of larval oysters, especially the shell development, the genomic response of oysters to this impact remains largely unknown.

To better understand the impact of CO<sub>2</sub> caused ocean acidification on the shell formation of the Eastern oyster on a molecular level, six previously identified bivalve shell formation related gene-encoding proteins, alkaline phosphatase (*ap*), calnexin (*cnx*), calmodulin A (*CaM1*), calmodulin B (*CaM2*), dominin (*dominin*) and segon (*segon*), were investigated in response to increased dissolved CO<sub>2</sub> in seawater. Expression profiles of these genes along the development of larval *C. virginica* were analyzed utilizing quantitative PCR (qPCR). Among these six genes, *ap* and *cnx* which were previously not identified from *C. virginica* were cloned using degenerated primers designed with homologous sequences from other bivalve species. The sequences of these two genes were fully characterized. Expression patterns of the six selected genes under high pCO<sub>2</sub> exposure were performed with primarily cultured mantle cells incubated under different percentages of atmospheric CO<sub>2</sub> ranging from 0.04% (control) to 10%, which resulted in pH values of cell culture medium from 7.4 to 7.0. Our results revealed that expression of *CaM1* demonstrated an increase with the rise of CO<sub>2</sub> levels in the atmosphere, where *CaM2*, *dominin*, and *segon* showed similar decreasing trends with the rise of CO<sub>2</sub> levels. Studies on the specified roles of these genes in shell formation will contribute to a clearer representation of the impact of CO<sub>2</sub> stress on the development of cultured and wild populations of oysters.

## EFFECT OF AUTOCHTHONOUS AND COMMERCIAL PROBIOTIC BACTERIA ON GROWTH, BACTERIAL PERSISTENCE, IMMUNITY, AND DISEASE RESISTANCE IN NILE TILAPIA *Oreochromis niloticus*

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Probiotics are gaining worldwide acceptance as means to improve growth and health of fish. However, the efficacy of the applied probiotic depends on the type of probiotic, dose, and duration of application. This study compared the effect of feeding an autochthonous *Bacillus subtilis* (Gene Bank NCBI 1701438) isolated from the gut of cultured tilapia (TPI), a commercial *Lactobacillus acidophilus* probiotic (COM) and a 1:1 mixture of both (MIX) on mean weight (MWT), specific growth rate (SGR) feed conversion (FCR), gross yield (GY), persistence, immunity, and disease resistance with the probiotic-free diet control (CON).

Healthy Nile tilapia (28.3 g) were stocked in 0.43-m<sup>3</sup> tanks at density of 4.68 kg/m<sup>3</sup>. Commercial 3.0-mm tilapia pellets (33% CP) were coated with the respective probiotic suspension having a bacterial load of 10<sup>8</sup> cfu g<sup>-1</sup>. The fish were fed daily at 5% of the BW decreased to 3% for the rest of the experiment (105 days). Temperature was maintained at 29.0 ± 2.0 °C.

After 105 days, the MIX group had the best growth values (P<0.05) followed by the TPI and the COM groups. The autochthonous TPI bacterium had the highest count in the gut and persistence rate followed by the MIX bacteria. The COM bacterium had the lowest count and disappeared rapidly from the gut once withdrawn from the diet (Table 1).

The TPI significantly increased phagocytosis of the head kidney leucocytes and hemagglutination titers (HAT). The COM and MIX probiotics resulted in significantly higher superoxide dismutase (SOD). Probiotics showed no significant effect on lysozyme activity (LA) and the alternative complement activity (ACH<sub>50</sub>) (Table 1). Fish fed with TPI and challenged by injecting the LD<sub>50</sub> dose (10<sup>4.86</sup> cfu) of the opportunistic pathogenic bacteria *Proteus vulgaris* had the lowest mortality rate and highest (P<0.05) relative protective survival (RPS) followed by the MIX treatment and lastly by the commercial probiotics. The highest mortality was recorded in the untreated controls. (Table 1).

Results obtained in this study indicate the higher potential of the autochthonous probiotic bacteria and the mixture of bacteria in enhancing growth, immunity, and disease resistance in tilapia than the commercial products.

**TABLE 1.** Growth, FCR, persistence, immunological parameters and RPS, in *O. niloticus* fed different probiotics for 105 days. Means in a row having different letters are significantly different (ANOVA, P<0.05).

Parameter	CON	TPI	COM	MIX
MWT (g)	304.2 <sup>c</sup>	331.6 <sup>ab</sup>	311.6 <sup>bc</sup>	353.8 <sup>a</sup>
SGR	2.27 <sup>c</sup>	2.34 <sup>ab</sup>	2.29 <sup>bc</sup>	2.40 <sup>a</sup>
FCR	1.39 <sup>b</sup>	1.35 <sup>b</sup>	1.38 <sup>b</sup>	1.27 <sup>a</sup>
GY (kg/m <sup>3</sup> )	50.7 <sup>c</sup>	55.3 <sup>ab</sup>	51.9 <sup>bc</sup>	59.0 <sup>a</sup>
Persistence %	0.0 <sup>c</sup>	37.1 <sup>a</sup>	7.53 <sup>c</sup>	21.3 <sup>b</sup>
LA (unit/ml)	17.3 <sup>a</sup>	23.3 <sup>a</sup>	24.3 <sup>a</sup>	21.7 <sup>a</sup>
SOD (unit/ml)	0.165 <sup>c</sup>	0.171 <sup>b</sup>	0.177 <sup>a</sup>	0.163 <sup>d</sup>
ACH <sub>50</sub>	10.2 <sup>a</sup>	10.5 <sup>a</sup>	10.2 <sup>a</sup>	10.4 <sup>a</sup>
Phagocytosis	71.7 <sup>c</sup>	134.7 <sup>a</sup>	83.7 <sup>b</sup>	111.7 <sup>ab</sup>
HAT (Log <sub>2</sub> )	2.7 <sup>a</sup>	3.0 <sup>a</sup>	2.7±0.9 <sup>a</sup>	2.3 <sup>a</sup>
RPS	0	75.0	12.5	50.0

## **AQUADEBRIS: AN ASSESSMENT OF MARINE DEBRIS FROM SHELLFISH AQUACULTURE USING DRONE TECHNOLOGY IN NORTH CAROLINA**

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Shellfish farming is one of America's oldest and most beloved aquaculture industries. Shellfish farmers and their families are often regarded as sentinels for the environment with an abiding respect for protection of water quality and natural resources. Shellfish aquaculture has become one of the fastest growing coastal zone industries in the U.S. with some states such as Virginia reporting over 30% growth per year. Development of aquaculture is a national priority in order to meet growing seafood demand, address U.S. food insecurity, and revitalize working waterfronts and water-dependent coastal economies. As the footprint for shellfish aquaculture continues to increase, so will the requirement to ensure sustainability including siting and planning, monitoring of environmental impacts, addressing user conflicts for space, and mitigation of impacts on habitat and public trust resources. Marine debris is a recognized stressor in aquatic ecosystems and is a highly visible warning for a wide range of environmental contaminants. Marine debris originating from aquaculture has become an important issue with industry expansion and development of sophisticated technology to intensify production. In many states, there is growing evidence to suggest that significant amounts of marine debris in estuarine and coastal environments is attributable to the aquaculture industry.

Aquaculture debris is a known hazard for marine organisms and can directly cause entanglement, be ingested, or cause fatal injuries or health problems. Aquaculture gear is different from commercial fishing gear in that aquaculture gear is concentrated in one location and may occupy a large footprint (many acres). After storms, debris lost from farms can build up along shorelines and within sensitive habitats such as saltmarsh, seagrass, and oyster reefs. A pilot study conducted by NOAA in partnership with Duke University Marine Laboratory used drones to digitally photograph and characterize marine debris associated with an abandoned and derelict shellfish farm located near Harkers Island, North Carolina. Decommissioning of the aquaculture operation and restoration of the site did not occur with lease closure. The abandoned aquaculture equipment has resulted in a high level of marine debris that over the years has been deposited onto neighboring shorelines and salt marshes and SAV habitats during intense storms and hurricanes common to North Carolina. Drone surveys of the 30-acre site revealed a large debris field with roughly 250 cubic meters of debris. Imagery from the surveys is being used to assess habitat damage and recovery and inform planning for removal.

**EFFECT OF GENETICS ON BODY WEIGHT VARIABILITY IN CHANNEL CATFISH *Ictalurus punctatus* FEMALE X BLUE CATFISH *I. furcatus* HYBRIDS**

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Variable body weight of catfish can create problems in catfish processing, inefficient harvesting and grading and economic loss for farmers. Genetic effects on body weight variability in channel catfish, *Ictalurus punctatus*, female x blue catfish, *I. furcatus*, hybrids were examined. Coefficient of variation for body weight of hybrid and channel catfish fingerlings was similar and the environment had strong and equal effects on the variability of each genetic type. When evaluating hybrids produced from different strains of channel catfish and blue catfish, significant sire and dam effects on variability. Family effects and combining ability of body weight variability were examined. Potential exists for decreasing a portion of hybrid catfish variability for body weight through genetic selection, however, environmental parameters likely have larger effects on variability.

## FIRST PRODUCTION OF JUVENILE SEA URCHIN *Lytechinus variegatus* AND EFFECT OF TEMPERATURE ON GROWTH UNDER LABORATORY CONDITIONS

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Sea urchin larval development, the production of post-larvae and their feasibility in laboratory conditions were described. In addition the study was to investigate the effect of temperature on sea urchin growth response. Wild, mature individuals were collected in the Bay of Rodadero (Santa Marta, Colombia) and transported to the laboratory of Aquaculture, Universidad del Magdalena. Spawning and fertilization occurred spontaneously in 200l tanks. The fertilized eggs were transferred to 250l tanks filled with filtered seawater (1 micron) and treated with UV light. The feeding was based on a commercially available artificial diet Shellfish Diet 1800 (Isochrysis sp, Pavlova sp, Tetraselmis weissflogii, and Thalassiosira pseudonana) at a rate of 20,000 cells / ml in prism stage; 40,000 cells / ml in two and four larvae arms; and 60,000 cells / ml in six arms larvae (Figure 1), eight arms to the competent larva stage. The fertilization membrane and the formation of the gastrula was observed at 5 minutes and 9 hours respectively after fertilization; prism state occurred at 15 h from this time and until the formation of the competent larva took 21 days, at which the larva became juvenile, which was fed with macroalgae of the genus *Ulva*. Wild juveniles' sea urchin ( $21.11 \pm 6.72\text{g}$ ) were adapted to temperatures of 23, 25 and 27 °C in three replicate groups (n = 14 per replicate group) during 45 days and the effect on growth was evaluated. Growth rate, was significantly influenced by temperature. Highest growth rate was seen in the 23 °C group, while lowest growth was observed at 27 °C group. Optimal temperature for growth increased with sea urchin weight. Our results indicate that low temperature (below to 25°C) have significant effect on sea urchin growth.



Figure 1. Six side arms *Lytechinus variegatus* larvae. Flattening of the anterior larvae region and appearance of pedicellariae. 7 days post hatch.

## OPTIMUM ACCLIMATION TEMPERATURE, THERMAL *PREFERENDUM* AND THERMAL TOLERANCE OF SEA CUCUMBER *Isostichopus sp. aff badionotus*

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We studied the optimum acclimation temperature, the thermal *preferendum* and thermal tolerance of sea cucumber *Isostichopus sp. aff badionotus*, with the aim to knowing its thermal requirements and to contribute to the development in its culture technology. For the determination of the optimum temperature of maintenance, 30 sea cucumbers were exposed to 23°C while 30 others were exposed to 26°C for a period of 30 days (10 animals/tank; 3 replicate per each temperature). Mortality and the specific growth rate (SGR), were evaluated. To evaluate thermal preference 12 sea cucumbers were acclimatized to 23°C and 12 more to 26°C. Specimens (4 individuals each time) were exposed in a thermal gradient (20 to 29°C) and behavior was monitoring during 4 hours. The thermal *preferendum* was established too. The thermal tolerance was determined through the critical thermal maximum (CTM) and the critical thermal lower (CTL) methods. In the optimum acclimation temperature trials no statistically significant differences were found in average weight ( $P > 0.05$ ); the highest survival (86%) was evidenced in the temperature of 23°C and therefore this was considered as the optimum temperature of maintenance. The thermal *preferendum* suggested the interval between 23 and 26°C as the thermal preference of animals acclimated to 23 and 26°C. Finally the CTM was established in  $36.5 \pm 0.3^\circ\text{C}$  and the CTL in  $8 \pm 0.5^\circ\text{C}$ . In *Isostichopus sp. aff badionotus*, the acclimation temperature influences on their thermal preference.

## ASSESSING THE FEASIBILITY OF DIETARY SOYBEAN MEAL REPLACEMENT FOR FISHMEAL TO THE SWIMMING CRAB, *Portunus pelagicus*, JUVENILES

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The use of fishmeal is increasingly becoming uneconomical and unsustainable, which has prompted increasing research on identifying suitable alternatives. Among these, soybean meal (SBM) is the most successful due to a well-balanced amino acid profile and relatively low amounts of anti-nutritional factors (ANF). Information is lacking, however, on the feasibility of replacing fishmeal with SBM in the diets of the blue swimmer crab *Portunus pelagicus*. The aim of this study was to feed *P. pelagicus* with increasing amounts of SBM, at the expense of fishmeal, and assess their survival, growth, hemolymph superoxide dismutase (SOD) activity, whole-body crude protein and cholesterol as well as hepatopancreatic trypsin activity, histopathology and glycogen reserves.

Six isonitrogenous diets were formulated with increasing SBM at 0, 20, 40, 60, 80 or 100% based on a total protein while equal amounts of fish oil and soybean oil were maintained. Each treatment had 30 individually cultured replicate crabs. After six consecutive molts, three-day post molt crabs were used for final sampling.

Results showed the best growth was obtained for crabs fed 20% SBM, which was significantly higher than all other treatments and is in agreement with other studies on *Eriocheir sinensis*. The cause for this requires further investigations but did lead to more hepatopancreatic glycogen and a better overall condition despite significantly decreased trypsin activity. Crab growth in the 60% SBM treatment significantly decreased compared to the control treatment (0% SBM), as well as less hepatopancreatic glycogen. Structural integrity of crabs at 100% SBM became compromised at 100% SBM, along with substantially lower survival and growth, which may be due to excessive dietary ANF, although whole-body cholesterol, hemolymph SOD, crude protein and moisture were unaffected by the diets.

Dietary SBM can replace fishmeal up to 40% without reducing the growth of *P. pelagicus* juveniles, but 20% SBM is recommended for optimal growth, which can reduce feeding costs and improve portunid crab farming sustainability.

**Table 3:** Mean survival (%) and mean ( $\pm$  SE) specific growth rate (SGR) (% day<sup>-1</sup>) for carapace width, length and wet weights of the swimming crab *Portunus pelagicus* juveniles after three days post molt from the C7 stage when fed diets with increasing soybean meal (SBM).

Treatments	Survival (%)	SGR		
		Carapace width	Carpace length	Wet weight
0% SBM	90.0	2.27 $\pm$ 0.10 <sup>b</sup>	1.90 $\pm$ 0.09 <sup>b</sup>	5.95 $\pm$ 0.27 <sup>b</sup>
20% SBM	85.0	2.62 $\pm$ 0.13 <sup>a</sup>	2.22 $\pm$ 0.11 <sup>a</sup>	6.88 $\pm$ 0.34 <sup>a</sup>
40% SBM	83.0	2.37 $\pm$ 0.09 <sup>ab</sup>	2.02 $\pm$ 0.07 <sup>ab</sup>	6.28 $\pm$ 0.24 <sup>ab</sup>
60% SBM	75.0	2.19 $\pm$ 0.11 <sup>b</sup>	1.43 $\pm$ 0.03 <sup>c</sup>	5.72 $\pm$ 0.28 <sup>b</sup>
80% SBM	60.0	1.82 $\pm$ 0.08 <sup>c</sup>	1.49 $\pm$ 0.07 <sup>c</sup>	4.74 $\pm$ 0.21 <sup>c</sup>
100% SBM	23.0	1.31 $\pm$ 0.09 <sup>d</sup>	1.14 $\pm$ 0.06 <sup>d</sup>	3.62 $\pm$ 0.22 <sup>d</sup>

## SALINITY INDUCED CHANGES TO THE SURVIVAL, GROWTH AND GLYCOGEN DISTRIBUTION OF SILVER BARB, *Barbodes gonionotus* (BLEEKER, 1850) EARLY FRY

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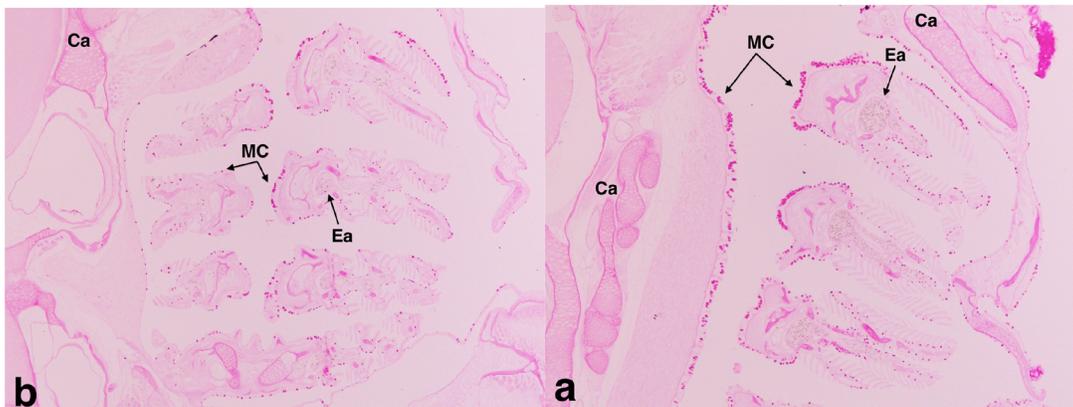
Salinity is one of the most important abiotic factors for the survival and growth of fish, and the optimal range is highly dependent on the species as well as life stage that tends to increase with ontogenetic development. In some hatcheries, slightly saline water ( $\approx 1 - 3$  ppt) is used to minimize diseases while salinization of freshwater ecosystems is becoming a greater issue. To date, there is no information on the effects of salinity to silver barb *Barbodes gonionotus* early fry despite their high commercial value throughout the Indo-Pacific region. This study assessed the survival, growth and glycogen distribution in the gills, intestine and liver of *B. gonionotus* early fry for 17 days when subjected to salinities of 0, 3, 6 or 9 ppt.

Results showed that growth significantly decreased after 17 days for fish at 3 ppt and above. Survival and condition factor significantly decreased and increased, respectively, only for fish at 9 ppt.

Glycogen-rich mucous cells in the gills were less at 9 ppt (Fig. 1b) compared to at 0 (Fig 1a), 3 or 6 ppt. In addition, the liver and intestine of fish at 9 ppt had less glycogen compared to the other treatments.

Results indicate *B. gonionotus* early fry are highly sensitive to increase salinities, evidenced by a reduction to growth and glycogen distribution which suggests increased energetic demands for osmoregulation. This study should prompt further studies regarding the vulnerabilities of young fish inhabiting freshwater areas experiencing salinization.

**Figure 1:** Periodic-acid Schiff staining of the gills from silver barb fry at 0 ppt (a) and 9 ppt (b) after 17 days.



**Table 1:** Survival, specific growth rate for length (%/day) and condition factor (CF) of silver barb fry at different salinities after 17 days.

	0	3	6	9
Survival	98.0 $\pm 2.0^a$	98.0 $\pm$ 2.2 <sup>a</sup>	87.0 $\pm 7.7^a$	22.0 $\pm 13.5^b$
SGR length	3.1 $\pm$ 0.2 <sup>a</sup>	1.2 $\pm$ 0.2 <sup>b</sup>	1.0 $\pm$ 0.3 <sup>b</sup>	0.9 $\pm$ 0.1 <sup>b</sup>
CF	1.3 $\pm$ 0.1 <sup>a</sup>	2.2 $\pm$ 0.5 <sup>ab</sup>	2.0 $\pm$ 0.4 <sup>ab</sup>	2.9 $\pm$ 0.7 <sup>b</sup>

## **AQUAPONICS IN THE PACIFIC AND A WAY TO SAVE ENERGY, IMPROVE SAFETY AND EDUCATION**

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Land development for housing and commercial uses is expanding in Hawaii at the expense of agricultural lands. Today, over 90% of Hawaii's food supply is imported. For centuries, Hawaiians developed sustainable means for food production including native taro fields (lo'i) and aquaculture fishponds (Hawaiian: loko i'a).

Aquaponic gardening has become very popular in recent years as a way to supply fish, vegetables and fruits for domestic use, and numerous aquaponic farms have grown to commercial operation size in Hawaii. By the year 2013, at least five commercial-scale aquaponics ranches were started in Hawaii, growing a variety of vegetables and fish. The most viable products were lettuce and tilapia, which favor the mild tropical weather in Hawaii and therefore are traded in the marketplace year round.

Based on aquaculture and aquaponics we have developed an educational program (ATOLL: Aquaculture Training and Online Learning) that promotes practical skills and knowledge in the practice of aquaculture. The ATOLL curriculum utilizes a variety of faculty expert instructors and has received great praise from current and former students. The ATOLL program promotes sustainability and resource management, and is used in high school (STEM) and university education across the Pacific Islands and the United States.

Part of our training program for aquaponic systems includes training students about the need to pump water from the fish tank to farm or gardening areas. With thoughtful design, appropriate maintenance, and effective management, airlift pumps may allow the transfer of water through an aquaculture and aquaponics system in a more efficient way than using traditional water pumps. Understanding the disadvantages and learning how easy it is to construct an airlift pump can help many aquaponic farmers to reduce their energy cost, while avoiding the high risk and maintenance that results from utilizing a submerged electrical pump in the fish tank.

This presentation will provide specific instructions on the utilization and construction of air-lift pumps for aquaponic farming and introduce participants to our online educational program for use in the community, school, and university training.

## **DOES THE CRISIS OF THE ISRAELI AQUACULTURE INDUSTRY A PRIVATE CASE?**

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Agriculture in Israel seems to be in a deep crisis. To overcome the challenges of the 21st century, it is, therefore, imperative for all parties involved to adopt a more creative attitude. The present crisis of the agriculture sector dictates a necessity to re-evaluate the cost/benefit of traditional farming.

Farmers and their representatives blame the causes of this crisis on two factors: the excessive brokerage margin, and the low priority given to agriculture by the government. If continued, this policy will lead to a major long-term structural crisis in this sector.

The aquaculture industry that used to be an important agricultural industry and is still implemented overwhelmingly in kibbutzim is also struggling to survive. This industry provides about a third of the local, national fresh fish consumption except for salmon and sea bream. In contrast, the import volume of fresh fish, which dramatically rose in recent years, now exceeds 65% of the total fish consumption.

In the present economic situation, more farms are expected to close down their business. Water availability, the prohibitive cost of farm inputs, the low marketing prices of fish and the actual marketing mechanism further affect this industry. In contrast, some aquaculture enterprises that have diversified and intensified their cultures have encountered an economic consolidation. It also appears that when farms expand their production they stand a better chance to reach economic stabilization.

## COI GENE SEQUENCE METHOD HELPS AQUACULTURE FARMERS AND THEIR CUSTOMERS

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DNA sequence scrutiny has been used for over 30 years to aid in species identification. However, various sequences have been used for different taxonomic groups. When using different identification tools, there is the potential for a high rate of exploitation. The conserved sequence of the 5' region of the mitochondrial gene cytochrome oxidase subunit I (COI or *cox1*) was proposed as a platform for the universal DNA barcoding of life (Hebert et al., 2003).

Aquaculture farmers and their customers are faced with marketing issues relating to misidentification of the cultured fish species and the regulatory structure attempting to control movements of exotic fish. For example, Department of Land and Natural Resources of State of Hawaii does not permit the fast growing tilapia, *Oreochromis niloticus*, for aquaculture, although there is some evidences that the genetic signature for this species exists in feral stocks collected from the wild throughout several Hawaiian Islands. Misidentification problems have been reported for catfish in India, where at least seven distinct species of catfish have been sold under various common names. This appears to be applicable to catfish marketing in India of fish species that are highly valued by consumers. This study uses rigorous morphological comparisons confirmed by DNA barcode analysis to examine the level of substitution of clariid catfish, *Clarias batracus* by *C. gariepinus* in India. Our results indicate that up to 99% of the market samples sold as Magur or *C. batrachus* were in fact *C. gariepinus*.

## **FEED AND WATER ADDITIVES FOR SHRIMP CULTURE USING BIOFLOC TECHNOLOGY: CURRENT STATUS OF KNOWLEDGE AND FUTURE CHALLENGES**

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Biofloc technology (BFT) is used in a variety of aquaculture systems and is commonly used for the culture of shrimp and tilapia. The use of feed and water additives to enhance biofloc as a food source, to enhance shrimp immunity and to control water quality has been documented.

Positive effects on water quality in terms of controlling total ammonia nitrogen, nitrite and nitrate due to the addition of various carbon sources in water and in feed have been established. Also, different prebiotics and probiotics have been successfully used in the culture of shrimp using BFT with significant improvements in shrimp weight gain, feed conversion ratio and health parameters.

Positive effects on growth and health of fish and other livestock species when using other feed additives, such as essential oils, organic acids and other immunostimulants, also have been established. However, no information in the literature can be found on the use of the same additives when added to the culture water or to the feed of a BFT system for shrimp culture. In addition, the mode of action for each additive to achieve higher shrimp production and improve water quality has yet to be determined.

A major deterrent to using additives to the culture water is the cost per unit of production. With the demonstration that shrimp can be cultured using shallow water depths of less than 30 cm, resulting in production levels of up to 25 kg/m<sup>3</sup> per crop (Lawrence, 2015), additives use in shallow-water culture systems is much more commercially feasible.

The current status of knowledge on the use of carbon sources, prebiotics and probiotics when used as feed or water additives for shrimp culture using BFT will be reviewed. In addition, challenges, promises and research opportunities for each additive will be reported.

### **Reference**

Lawrence, A.L., 2015. System and method for super-intensive shrimp production. United States Patent Number 8,985,055 B2.

## PREBIOTIC EFFECTS ON MICROBIOTA OF *Litopenaeus vannamei* AND BIOFLOC, AS WELL AS WATER QUALITY IN A REDUCED H<sub>2</sub>O EXCHANGE SYSTEM

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A 3-week trial was conducted to evaluate the effects of prebiotics (sc-fructooligosaccharide (FOS), galactooligosaccharide (GOS), mannan oligosaccharide (MOS) and inulin (INU)) and non-prebiotic carbohydrates (starch (STA) and sucrose SUC)) addition to a biofloc-based shallow-water research system on bacteria profiles present in biofloc particles and *Litopenaeus vannamei* hepatopancreas, intestine and gills. In addition, effects of these additives on shrimp production parameters and water quality were assessed.

Each tank (0.457 m x 0.457 m x **0.280 m**) contained an independent heater, an automatic 48-h feeder, and two air stones. Tanks were filled to 20 cm depth with artificial, 28 ppt seawater at  $30.0 \pm 1.0$  °C. Shrimp weighing  $6.0 \pm 0.5$  g, were stocked into each tank at a density of 357 shrimp/m<sup>3</sup>. During the first phase, autotrophic bacteria were promoted and maintained from the initial day until Imhoff cone readings reached 3 mL/L. Only during first phase, shrimp were fed with pellets and fines containing 35% crude protein (CP) (as-fed basis) at an average of 3.33 g/day per tank and 0.037 g/L, respectively. Once Imhoff cone readings reached 3 mL/L, TAN (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>), nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) levels were maintained at <3, <5 and <100 ppm, respectively, by feeding 23% CP and by adding STA, SUC, FOS, GOS, MOS or INU to each tank according to its treatment in replications of 6 tanks per treatment.

TAN, nitrites and nitrates were recorded weekly using a spectrophotometer. Shrimp production and average biofloc levels are shown in Table 1. Health parameters (total hemocyte count, condition factor, hepatosomatic index, necrosis, antenna length and hepatopancreas fat content indices) were estimated at the end of the trial from 1 shrimp per tank of 3 randomly chosen tanks per treatment. Biofloc samples were collected from three randomly chosen tanks per treatment and analyzed for protein, lipids, and ash contents. Biofloc and shrimp intestines, hepatopancreas and gills were aseptically collected for DGGE analysis.

Table 1. Mean values  $\pm$  SEM calculated at the end of the trial for biomass increment, individual weight gain, survival, FCR and average biofloc level.

Treatment	Individual weight gain (gr)	Biomass increment (gr/m <sup>3</sup> )	Survival (%)	FCR	Average Biofloc (mL/L)*
STA	3.75 $\pm$ 0.23	1272.95 $\pm$ 63.06	97.58 $\pm$ 1.71	1.23 $\pm$ 0.07	4.43 $\pm$ 0.25 <sup>b</sup>
SUC	3.54 $\pm$ 0.23	1164.32 $\pm$ 63.35	97.37 $\pm$ 1.70	1.33 $\pm$ 0.07	5.66 $\pm$ 0.24 <sup>a</sup>
FOS	3.75 $\pm$ 0.22	1233.29 $\pm$ 60.32	96.79 $\pm$ 1.63	1.27 $\pm$ 0.07	5.48 $\pm$ 0.23 <sup>a</sup>
GOS	3.64 $\pm$ 0.22	1185.50 $\pm$ 60.60	96.70 $\pm$ 1.65	1.33 $\pm$ 0.07	5.60 $\pm$ 0.24 <sup>a</sup>
INU	3.50 $\pm$ 0.22	1178.23 $\pm$ 60.52	97.77 $\pm$ 1.64	1.34 $\pm$ 0.07	5.68 $\pm$ 0.23 <sup>a</sup>
MOS	3.42 $\pm$ 0.23	1194.62 $\pm$ 62.81	99.34 $\pm$ 1.71	1.31 $\pm$ 0.07	5.12 $\pm$ 0.24 <sup>ab</sup>
<i>P&gt;F</i> <sup>1</sup>	0.8262	0.8723	0.8551	0.8664	0.0087

\* Different superscript letters indicate significant ( $P < 0.1$ ) differences as evaluated by Tukey's HSD test.

<sup>1</sup> Significance probability associated with the *F*-statistic.

## DIETARY ARGININE REQUIREMENT OF EARLY JUVENILE LARGEMOUTH BASS *Micropterus salmoides*

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Farmed largemouth bass (LMB), *Micropterus salmoides*, is increasingly becoming an important food fish for consumers in the United States. Under farm conditions, LMB are fed diets high in protein content (> 40%), but the quantitative requirement for essential amino acids (AAs) are largely unknown for this North American species. Therefore, the objective of this study was to determine the quantitative requirement for arginine (Arg) in the diet of early juvenile LMB.

A basal diet was formulated to contain 0.82% Arg and the overall AA concentration [all derived from fishmeal (20% inclusion) and crystalline AAs] of a 40% crude protein LMB muscle, and 12% lipid. An additive supplementation of L-Arg to the basal diet was performed producing five additional diets containing Arg from 1.14 to 2.82%. Each diet was randomly assigned to triplicate groups of 15 early juvenile LMB [average initial weight (IW) of  $9.6 \pm 0.7$ g] stocked in an 18-aquaria recirculating aquaculture system. Fish in each aquarium were fed twice daily to apparent satiation for 8 weeks.

At the end of study, LMB in each aquarium were counted and group-weighted for the computation of performance parameters. No significant ( $P < 0.05$ ) differences in fish survival (ranging from 82 to 96%) were found among treatments. Based on quadratic broken-line regression analyses, the Arg requirement for optimizing thermal growth coefficient, weight gain (percent of initial), and feed efficiency of LMB was determined to be 2.2, 1.9, and 1.8% of the diet, respectively. Our results expand on the very limited knowledge of amino acid requirements for LMB and aid in the development of nutritious, environmental friendly and cost-effective diets for aquaculture enterprises.

## **SELF-CLEANING LARVAL REARING TANKS TO IMPROVE LARVAL PRODUCTION OF CALIFORNIA YELLOWTAIL *Seriola dorsalis* AND WHITE SEABASS *Atractoscion nobilis***

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In 2015 a new aquaculture engineering project was launched by researchers from Hubbs-SeaWorld Research Institute (HSWRI), the University of Southern California (USC) and Ocean's Design of San Diego. The primary goal of the project is to advance sustainable marine aquaculture in the United States by improving larval rearing success through automated larval marine fish tank cleaning. In March of 2015 the team installed a single production-scale, self-cleaning culture tank (SCT) at HSWRI's laboratory in San Diego. After testing it with a batch of white seabass (*Atractoscion nobilis*) in June of 2015, various modifications were made to improve on the design. Among these were the addition of an upright tank wall-cleaning component, facilitated center screen attachment equipment and a reduction in overall internal surface area through trimming of stainless steel cleaning components.

Phase II of the project, which is currently underway, is comparing batches of WSB and California yellowtail (*Seriola dorsalis*) reared in SCT's as opposed to those reared in standard larval tanks. Thus far, one WSB trial and two CYT trials have been completed. These trials each compare the performance of a single SCT to a single control tank during the larval rearing stage of culture (from egg to 1-gram "transferrable" juveniles). Results these trials indicate that the SCT technology mitigates bacterial loading, improves larval survival, reduces suspended solids and offers more than a 4-fold reduction in the labor associated with siphoning tank-bottoms during larviculture. Two more WSB trials are slated to be completed in 2016.

## **USING AN EXTENSION APPROACH TO ADDRESS AN EMERGING INDUSTRY CONCERN: A CASE STUDY OF WINTER FISH LOSSES IN ARKANSAS**

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Fish farmers in Arkansas reported large losses of fish in the spring of 2013 when they began to harvest their ponds. The largest losses were observed by farmers raising fathead minnows, golden shiners, and centrarchids such as bluegill and redear sunfish. Other fish affected to a lesser degree included goldfish, grass carp, largemouth bass, and hybrid striped bass. Thirty-four baitfish and sportfish farmers in Arkansas were interviewed by phone or by a farm visit by UAPB Extension personnel to document this event. Unusual fish losses were defined by losses in excess of what would normally be expected to occur on their farm in a typical year. Baitfish and sportfish farmers in Arkansas experienced unusual winter fish losses on 2,900 water acres and losses were catastrophic on a large number of farms. In many instances farmers reported losing more than 50% of their crop. Most farmers did not observe any dead fish in their ponds over the fall and winter months and did not become aware of these losses until harvest in the spring of 2013. Fish less than three inches in length represented the vast majority of losses. Farmers shared several theories during a meeting organized by Extension personnel with a series of expert scientists who served as a resource during the meeting. Initial theories expressed included: 1) extreme temperature fluctuations, 2) increased predation by diving ducks, 3) drift from fungicide/insecticides sprayed from airplanes, 4) cold winter temperatures, and 5) reduced winter feeding. A comprehensive and systematic approach was developed to search for management solutions to avoid similar losses in the future and has continued from 2013 to the present. This approach included the establishment of collaborative research projects targeting specific topics with a number of state and federal agencies, 1862 land grant universities, and 1890 research and Extension. These studies included winter feeding regimes and field studies to document fish consumed by a problematic diving duck, the lesser scaup. Dissemination of results to stakeholders has occurred through a number of different venues and is currently ongoing.

## **GROWTH, SURVIVAL, AND FATTY ACID COMPOSITION OF COPPERNOSE BLUEGILL (*Lepomis macrochirus* subspecies) OFFERED DIFFERENT WINTER FEEDING REGIMES**

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Winter fish losses are routinely reported by Arkansas sportfish producers in the spring. Juvenile Centrarchid species (< 7.6 cm) are quite susceptible to harsh winter conditions. While some of these winter fish losses can be attributed to predation by fish eating birds and water quality factors, water temperature is also an important contributing factor. Winter feeding strategies vary widely among sportfish farms in Arkansas. While some farmers feed routinely on a weekly basis as weather permits over the winter months, others feed much less frequently. In order to investigate the effect of different feeding regimes on winter fish losses, indoor and outdoor trials with coppernose bluegill (*Lepomis macrochirus* subspecies) were conducted during the winter of 2015. The first experiment was conducted in the laboratory in a temperature controlled recirculating tank system (600 L) equipped with nine tanks. Fish were initially stocked at ambient temperature and the temperature was reduced to 8°C over the course of seven days to mimic winter conditions. Fifteen coppernose bluegill ( $2.57 \pm 0.10$  g) were stocked into each tank. When the target temperature (8°C) was reached, three dietary feeding regimes utilizing a commercial 35% protein feed were implemented (3 replicates per treatment) that included feeding 1% body weight twice per week (2x/wk), once per week (1x/wk), or once per month (1x/mo) throughout the trial. After 13 weeks of culture, there were no significant differences ( $P>0.05$ ) in final weight, survival, or condition factor of fish from the indoor trial. Substantial weight loss occurred across all three treatments, ranging from -14.64% to -18.87%. The outdoor feeding trial was conducted at the University of Arkansas at Pine Bluff Aquaculture Research Station with coppernose bluegill using static tanks (400 L). Unlike the indoor trial, fish were subjected to fluctuating, ambient winter temperatures in 16 static tanks, equipped with aeration supplied from a regenerative blower. The tanks were filled with pond water and stocked with 25 coppernose bluegill ( $2.59 \pm 0.19$  g) per tank. Fish were assigned one of four feeding regimes at 1% body weight that included feeding 2x/wk, 1x/wk, 1x/mo, or twice per month (2x/mo). Temperatures in the outdoor trial ranged from 0.10 – 16.3°C. Following 13-weeks of feeding, there were no significant differences ( $P>0.05$ ) in final weight, weight gain, or condition factor. However, fish fed either 2x/wk or 1x/wk had significantly higher survival ( $P<0.05$ ) than fish fed 2x/mo or 1x/mo. Weight gain ranged from -4.71 (2x/wk) to -10.74% (1x/mo). Results indicate that regardless of feeding regime, weight loss in coppernose bluegill occurred at low temperature after 13-weeks of culture. Whole body fatty acid profiles are currently being analyzed for treatment effects; however, results to date from the outdoor trial suggest that feeding 1x/wk or 2x/wk may be a beneficial strategy for commercial sportfish producers to increase survival and reduce weight loss of coppernose bluegill during the winter months.

## DEVELOPMENT OF A HIGH THROUGHOUT SINGLE NUCLEOTIDE POLYMORPHISM ASSAY FOR GENETIC MONITORING OF RED SNAPPER AQUACULTURE AND STOCK ENHANCEMENT

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The red snapper, *Lutjanus campechanus*, supports major commercial and recreational fisheries in the southern United States. Aquaculture of the species is being developed in the context of a pilot program in Mississippi to evaluate the feasibility of large scale production of juveniles for stock enhancement or grow out to commercial size. A genetic program in support of the aquaculture project is in progress and aims to assist with the management of captive breeding and the monitoring of genetic impacts of releases. Genomic resources including a draft genome sequence and a high-density linkage map were previously developed and applied to study red snapper population structure using a Restriction site Associated DNA (RAD) sequencing approach. The objective of this work was to develop an efficient high throughput assay for a subset of the Single Nucleotide Polymorphisms (SNPs) discovered and characterized during linkage mapping and population genomic analysis in order to monitor captive and wild populations during breeding and stock enhancement.

Candidate SNPs and associated flanking sequences were evaluated *in silico* to determine suitability for incorporation on a Fluidigm® dynamic array (D3 Assay Design) and an initial set of 96 SNP Type assays were synthesized and used to screen a panel of 95 DNA samples representing red snapper geographic locations. The tested panel included 48 putatively neutral SNPs selected based on their position on the red snapper linkage map; one SNP was selected on each end of each of the 24 linkage groups to generate a panel of 48 loci expected to segregate independently. The remaining SNPs included loci showing a possible signature of divergent selection during an initial RAD-sequencing survey of red snapper geographic populations and markers expected to distinguish southern and northern red snapper. Pre-amplified DNA, and SNP Type assays, were assayed on an Integrated Fluidic Circuit (IFC) that accommodates 96 assays by 95 samples. Thirty-four of the 96 initial assays were rejected due to poor quality data, assay failure, monomorphism, or high disagreement with the RAD-sequencing genotypes. Another 49 assays were designed and evaluated to replace failing initial assays and produce the final panel of 96 SNPs. The overall agreement between the final Fluidigm and RAD sequencing datasets was 94.31%. A total of 486 red snapper of various ages collected from 5 geographic populations in US waters were genotyped at the 96 SNPs. The analysis of neutral loci revealed no significant genetic variance among geographic populations ( $\Phi_{CT} = -0.001$ ,  $P = 0.745$ ) or among year class within region ( $P = 0.406$ ). Selected loci revealed a small but significant variance among regions ( $\Phi_{CT} = 0.008$ ,  $P = 0.001$ ) but no variance among year class within region ( $P = 0.805$ ).

**AQUACULTURE OF SEA URCHIN: WILL THEY BE STRESSED?**

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Sea urchins have been known for their roe as delicacy. But in recent time, they are increasingly getting importance for their roles in the ecology as well as for research models and marine pharmaceuticals. As they are getting importance, many farmers are now trying to cultivate them in land-based aquaculture systems. In our experiments, we therefore wanted to investigate if sea urchins also get stressed like their vertebrate relatives. We have placed them in two experimental groups- stressed and unstressed and measured their physiological and immunological responses in both acute and chronic conditions. From Condition factors, gonado-somatic indices, packed cell volumes, coelomic fluid proteins, total and differential cell counts, and coelomocyte phagocytic activities- we found out that sea urchins also get stressed and lose their immunity. We will discuss our findings in detailed during the presentation.

## DEVELOPMENT OF GENOMIC RESOURCES FOR THE SPOTTED SEATROUT *Cynoscion nebulosus*

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The spotted seatrout (*Cynoscion nebulosus*) is a popular recreational fish commonly found in coastal waters of the northern Gulf of Mexico and Southeastern United States. Aquaculture of this species is being developed along the gulf coast primarily for fisheries stock enhancement. Genetic management is an essential component of stocking programs and aims to minimize the negative genetic impacts of releases on wild populations. Knowledge of population structure and of the degree of genetic adaptations of local populations is essential in order to design appropriate management units for these programs. High-density genome scans allow in-depth characterization of genetic variation as needed to assess reliably population structure and gene flow and to identify genomic regions affected by selection and local adaptation. These genome scans can now be produced using cost-effective approaches based on next generation sequencing but their exploitation is greatly enhanced when a reference genome is available for the species of interest. The objective of this work is to initiate the development of a reference genome for spotted seatrout by generating a draft sequence assembly and a linkage map. These tools will be applied to monitor and manage genetic resources of captive and wild spotted seatrout.

The DNA of one outbred spotted seatrout specimen was sequenced using the PAC-BIO SMRT technology (7 SMRT cells) and the Illumina®HiSeq2500 platform (v4 paired-end sequencing, one sequencing lane). A total of 402M paired-end Illumina raw reads with 92% bases showing quality scores greater than 30 were obtained. Hybrid assembly of the datasets obtained using the two platforms is currently in progress. The assembly will be used as a reference to map Restriction Site Associated DNA (RAD) Tags and characterize associated Single Nucleotide Polymorphisms (SNPs). SNPs discovery and mapping via RAD-sequencing of the DNA from parents and offspring from one full-sib outbred family is in progress. The family was produced by fertilizing *in vitro* the eggs of one wild-caught female with the sperm of one wild-caught male on June 2<sup>nd</sup> 2015. Progeny were sampled at 14 days post hatch (n = 132) and then at 195 days post hatch (n = 105). To date, libraries generated using the double-digest RAD-sequencing protocol for 79 of the progeny sampled was sequenced in multiplex on one lane of the Illumina®HiSeq2500 platform producing an average 2.3 M filtered reads per individual. Sequencing of the remaining 158 offspring is in progress. The SNPs identified in the mapping family will be used to construct a high-resolution linkage map, which will facilitate orienting and ordering scaffolds of the draft genome assembly, studying population structure and gene flow in particular mapping genomic regions impacted by natural selection, localizing genes impacting phenotypic traits in spotted seatrout, and assessing effective population size and demographic parameters of wild and captive stocks.

## APPLICATION OF IDEAL PROTEIN CONCEPT ON DIETARY PROTEIN REDUCTION IN NILE TILAPIA DIETS *Oreochromis niloticus*

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Using of the ideal protein concept in aquaculture feed formulation aim to provide the exact balance of amino acids to cover fish requirements required for optimal growth and maximum production which lead to a reduction of dietary protein content, decreasing production costs in intensive fish farming and overcoming environmental pollution which resulted from nitrogenous products of protein metabolism. the present investigation was carried out for evaluation the effects of using ideal protein concept on reduction of dietary protein content for Nile tilapia (*Oreochromis niloticus*) with supplementation of synthetic commercial amino acids on growth performance response, whole fish body composition, level of some serum metabolites, morphometric index, intestine and muscles health and feeding costs of unit of weight gain of the Nile tilapia juvenile.

Three hundred juvenile Nile tilapia (6.3 g average body weight) were randomly divided into 5 groups, 3 replicates for each with 20 fish per experimental unit. The fish were reared in glass aquaria equipped with continuous air flow and fed five [32 CP (NRC1993), 28.5, 26.5, 24.5 & 22.5 % digestible protein] isocaloric (3000 Kcal DE/Kg) and digestible isolysine diets ad libitum 4 times a day for 12weeks. The synthetic amino acids were added at levels to meet the amino acid profile of the control diet.

Fish were weighed and counted biweekly to measure growth performance (average body weight, body weight gain) and feed utilization (feed consumption, feed conversion ratio & protein efficiency ratio). At end of the experiment, 6 fish were randomly obtained from each group for whole body proximate chemical analysis. At end of experiment 6 fish/ group were anaesthetized with tricaine methanesulphonate (MS-222) to collect nonheparinized and heparinized blood samples for determination of total protein, albumin, total immunoglobulin, triglycerides, liver enzymes, Urea & Creatinine, hemoglobin (Hb), hematocrit (Ht) and blood cell counts and to obtain weights of whole body, liver, viscera, intraperitoneal fat, dressed carcass and fillet. For histopathological examination, intestine and muscle samples were taken and fixed in 10% neutral buffered formalin for microscopic examination of the intestinal fold, enterocyte, microvillus height and muscle fiber diameter.

The growth data (final weight, weight gain, feed conversion and feed intake) revealed that fish groups which fed experimental diets based on digestible amino acids (28, 26, 24% digestible protein) showed better growth and weight gain than fish fed experimental diet based on total amino acids profile according to [32% crude protein (NRC1993)]. Also, it was concluded that is possible to reduce the dietary protein from 28 to 24% digestible protein for juvenile Nile tilapia based on ideal protein concept.

## INTERACTION BETWEEN DIETARY SOY AND ANIMAL PROTEIN ONTO THE TAURINE REQUIREMENT IN WHITE SEABASS *Atractoscion nobilis* AND CALIFORNIA YELLOWTAIL *Seriola lalandi*

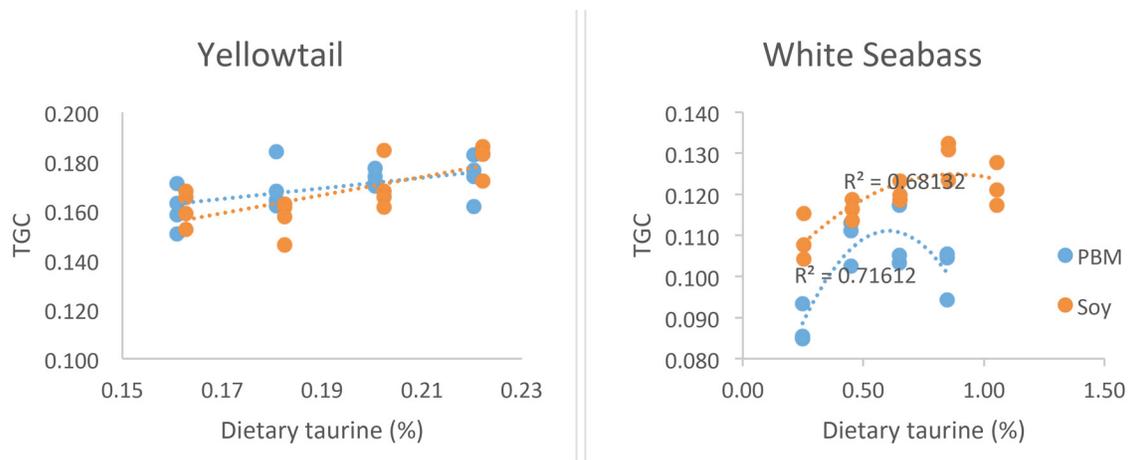
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Taurine has garnered a lot of attention in recent years, as evidence showed that it was an essential nutrient in a number of teleost species. However, quantitative estimates of requirement show a wide variation across species, and interactions with source of dietary proteins lowering taurine bioavailability have been hypothesized to explain this discrepancy. Preliminary data in our lab suggested as much: taurine requirement for WSB juveniles was 0.99% of the diet when using a high soy diet, while equivalent growth rates were observed on a fishmeal diet (56.7% fishmeal as unique source of protein, 0.39% taurine). Although additional taurine supplementation to the fishmeal diet was not evaluated, this suggests that bioavailability of taurine may be reduced in soy-based diet, thereby resulting in the need for higher dietary inclusion rate in order to meet the animal's taurine requirement.

To clarify this situation, two series of diets, based on an animal protein (poultry by-product meal) or a vegetable protein (soybean meal) were manufactured based on previous successful formulations, and supplemented with matching, graded levels of taurine. Diets will be formulated to be isoproteic and isolipidic to contain 44% protein and 12% lipid for CYT and 40% protein and 10% lipid for WSB. We hypothesized that the source of protein would have no effect on the growth response, thus on the quantitative estimation of the taurine requirement. The design follows a slope-ratio assay approach. Results were evaluated in terms of growth rate, feed efficiency, survival, as well as nutrient retention and retention efficiency.

Results show that taurine availability was affected by the dietary protein source in white seabass, but not in yellowtail, thereby highlighting a species-specific effect and sensitivity to vegetable protein sources. The underlying explanation for such species difference remains poorly understood.



## EFFECTS AND INTERACTIONS OF TAURINE SUPPLEMENTATION IN BROODSTOCK AND LARVAL FEEDS IN CALIFORNIA YELLOWTAIL *Seriola lalandi*.

Guillaume Salze\*, Kevin Stuart, D. Allen Davis, and Mark Drawbridge

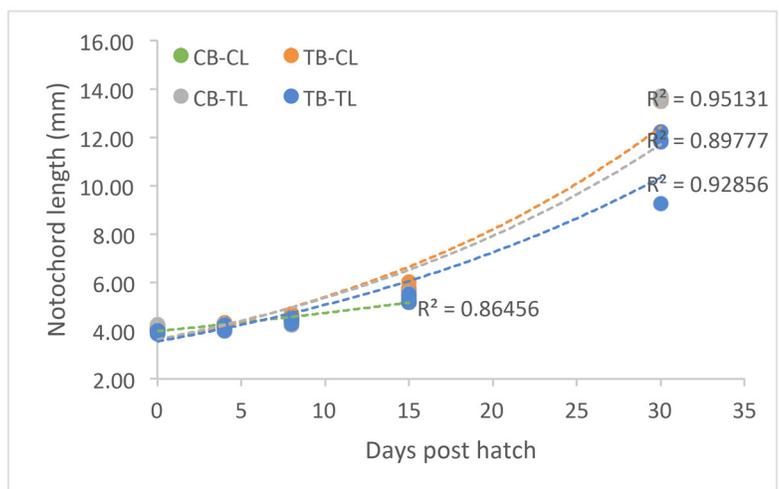
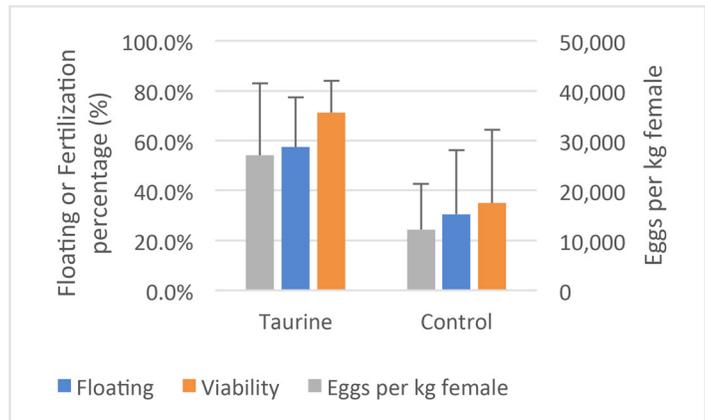
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Taurine has garnered a lot of attention in recent years, as evidence showed that it was an essential nutrient in a number of teleost species. However, there is a great dearth of information concerning the role of taurine in reproductive output, i.e. egg production and subsequent larval quality. California yellowtail is a solid candidate for aquaculture – yet large fluctuations in survival rates through weaning (20-40%) of larvae are commonly seen. Consequently, a trial was conducted where broodstock of California yellowtail received taurine supplemented or unsupplemented feed (2.67% and 0.28% dietary taurine, respectively), and the resulting eggs and larvae were divided and raised on a taurine-supplemented or unsupplemented protocols. This resulted in 4 treatments at the larval level (combinations of control/taurine-supplemented broodstock – CB or TB – and control/taurine-supplemented larvae – CL or TL).

Discriminant factor analysis of spawn metrics indicate significant differences in the reproductive output from fish fed the taurine-supplemented feed. The difference was primarily driven by significant increases in total eggs per spawn, relative fecundity, percent floating and percent viability. Egg measurements (e.g., egg diameter, oil globule diameter, yolk volume, notochord length) did not significantly contribute to treatment separation. Survival of larvae to 1st feeding was also significantly affected, though only when including hatching success: odds of survival were 36% higher in the larvae from the taurine-supplemented broodstock than those from the control broodstock (logistic regression,  $p < 0.0001$ ).

No larvae of the CB-CL group survived until weaning at 30 days-post-hatch (dph), therefore were excluded from analysis of larval growth. ANCOVA analysis revealed no significant differences between the 3 other treatment groups in terms of live weight or notochord length. However, the larvae in the TB-CL and CB-TL groups were numerically longer and heavier.

Results show that broodstock diets should be supplemented with taurine.



## **DESIGN AND OPERATION OF INDOOR, SUPER-INTENSIVE, BIOFLOC-DOMINATED SYSTEMS FOR THE PRODUCTION OF PACIFIC WHITE SHRIMP: THE TEXAS A&M AGRILIFE RESEARCH EXPERIENCE**

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The Texas A&M AgriLife Research Mariculture Laboratory (Corpus Christi, Texas) has been developing innovative shrimp culture techniques for the last three decades. Research over the last ten years has focused on sustainable, cost-effective super-intensive systems for production of marketable shrimp with no water exchange. This has resulted in regular yields of marketable *Litopenaeus vannamei* of up to 10 kg/m<sup>3</sup> with high survival, low FCR, and significantly improved economic viability.

The design and management of indoor biofloc shrimp systems currently is not well documented in the literature. It thus was timely to produce a practical manual that describes these systems in a way that is accessible to shrimp producers, entrepreneurs, technicians, and researchers. Funding from NOAA through the National Sea Grant program enabled preparation of such a manual that summarizes research activities on biofloc-dominated (BFD) systems carried out at the Texas A&M facility.

The manual has been composed in a more narrative style than found in scientific journals to make it accessible to a wider audience of stakeholders. It contains procedures for many routine operations, but its core describes the experiences over more than a decade of what works and – importantly – what did not work, with emphasis on the most recent production trials. It should help guide entrepreneurs in building and operating a scale version of the Texas A&M BFD system from which they will gain hands-on experience under the conditions of their production site. This will inform their decision of how – or whether – to incorporate BFD systems in their business plans.

The manual also touches on more general aspects of closed systems, such as equipment and procedure options that may be unfamiliar to those without experience in this type of aquaculture. A special chapter provides detailed information on the economic viability of these systems. Appendices collect related information on operating high-density, no-water-exchange systems.

It is hoped that this manual – scheduled for publication by WAS in 2017 – will improve the basic knowledge of operating biofloc systems and stimulate adoption of this innovative sustainable shrimp production practices in the US and globally.

## EVALUATION OF MARINE BACTERIA THROUGH IN VITRO AND IN VIVO ASSAYS AND THEIR POTENTIAL USE AS FEED ADITIVE AND PROBIOTIC AGENT AGAINST PATHOGENIC *Vibrio parahaemolyticus* (AHPND) IN WHITE SHRIMP *Litopenaeus vannamei*

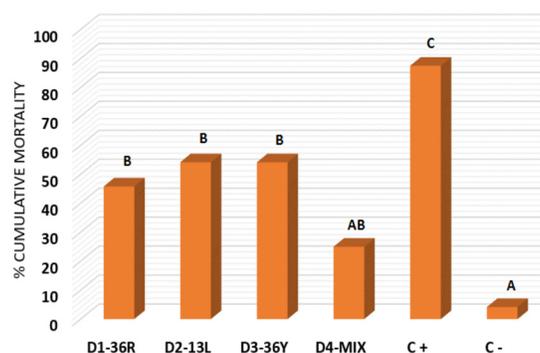
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During 2013, mexican shrimp farming faced the acute hepatopancreatic necrosis disease (AHPND), causing 60-80% of mortalities and economic losses. Despite the slow recovery there is a continuous risk. Recently, it has been studied the potential use of marine bacteria as bioactive compounds producers or probiotics, as an alternative to control aquatic disease. The aim of this study was to evaluate *in vitro* antagonistic activity of marine bacteria and determine the survival of white shrimp *Litopenaeus vannamei* fed with a diet covered with bacteria in a challenge bioassay with pathogenic *Vibrio parahaemolyticus* (AHPND).

Heterotrophic bacteria of shrimp, bivalves, algae, water and marine sediment were collected on the south of Sonora, Mexico; *in vitro* antagonism were evaluated by cross streak (CSM), double layer (Dopazo) and broth co-culture, with *V. parahaemolyticus* strains (ATCC 17802; and MC32, B25, E14V2 AHPND +). Five isolates were selected by their *in vitro* performance, 36R, 42, 13L, 36Y y 02Y; which by their microbiologic, biochemical test (API 20NE, 50CH) and identification (16S rRNA) resulted in: 36R, 42 and 13L, Gram + spore forming rods, oxidase negative, catalase positive belonging to *Bacillus* genus; 36Y and 02Y, Gram – rods, oxidase positive, catalase negative related to *Pseudoalteromonas*. Protease activity were present in 36R, 42, 13L and 36Y; cellulases in 36R, 13L and 42; amylases in 36Y and 02Y; none of them present chitinase. The biofilm production on microplate were evaluated and the results showed that isolates 42, 13L, 36Y y 02Y (OD 595; 0.233, 0.192, 0.528, 0.525, respectively) have good adherence properties. For bioassay, during 21 days shrimps were fed with bacterial covered diet (commercial), 6 treatments by triplicate with 8 shrimps by 30 l aquaria. For the challenge (48h) the strain MC32 *V. parahaemolyticus* (AHPND +) was used. The cumulative mortality was recorded: T1-36R (45%), T2-13L (54%), T3-36Y (54%), T4-Mix 136R-13L (25%), C+ (88%), C- (4%); statistically significant differences were showed between T1, T2, T3 y T4, respect the control groups. The pathogen detection was confirmed by real time PCR, histopathology, and the statistical used was ANOVA and Kaplan Meier.

Shrimp fed with bacteria showed improvement in survival compared with control. This *Bacillus* and *Pseudoalteromonas* could be an effective probiotic or biocontrol agent against harmful bacteria in shrimp culture.



**Figure 1.** Cumulative mortality among the 6 treatments.

## FROM THE IMPROVEMENT OF THE INSHORE FISH PRODUCTIONS TO THE AUTONOMOUS OFFSHORE FISH FARMING SHIPS

Rodrigo Sánchez Raccaro

President, Oceanic Food Solutions S.p.A.  
Former CEO, EcoSea Farming S.A.

This presentation will report the results obtained in an Innovation Center for production of 3,500 Tons of Atlantic salmon when integrating the technology of the copper alloy meshes in the inshore fish farming and the possibility these generated in the offshore fish production. This technology has also opened the real possibility of building offshore fish farming vessels, able to navigate in international seas looking for the best qualities of the water for the fish.

**STAGE 1:** The first step was to demonstrate that the copper alloy meshes generated real productive improvements in the fish farming. For this after having acquired 5 years of experience in this type of technology, we started to run an Innovation Center operated by ourselves, Integrating several new technologies, among them copper alloy meshes and we hire the international certifier SGS to record the results during the 18 months of production.

In this Innovation Center, we produced 3,500 tons of Atlantic salmon in the South on Chile and we used 16 cages of 13,500 m<sup>3</sup> each with copper alloy meshes. Results were excellent: Fish input 678,594) were smaller size (97grams) than Chilean industry standard (130 grams), despite this, the fish grew to 5.9 kilos. The Economic Conversion Rate (FCRe) was 1.3 and Biological Conversion Rate (FCRb) was 1.26 . Biological mortality rate of 3.3% and a productivity per Fish of 5.21 (Final biomass v/s Initial biomass) These results were among the best in the Industry over the 2014-2015 winter season (20 million fish).

**STAGE 2 :** Adaptation of technology for offshore fish farming. For this, we develop a submersible raft by means of a compensation bottle to make the sinking process. We chose an area where no fish had ever been produced in Chile, the shores of the Atacama Desert and a species that had never been produced in the country: *Seriola Lalandi* .

Although we had to fight against the big paradigms of the zone: fouling, oxygen lows, great swells and the low temperature for the species (15,2 °C average year), the result was very promising. We were surprised by 2 unexpected nature events: 2 large earthquakes (8.2 and 8.4 Richter) with their respective tsunamis, and de big swells of 2015. Cage and fish were submerged and survived without any problem.

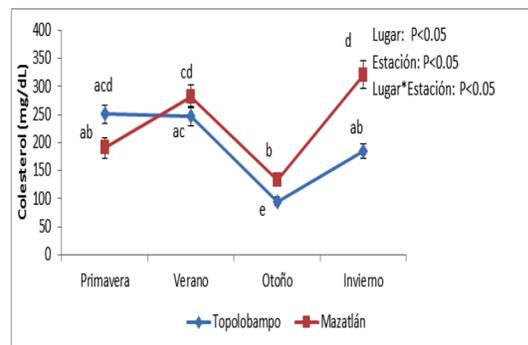
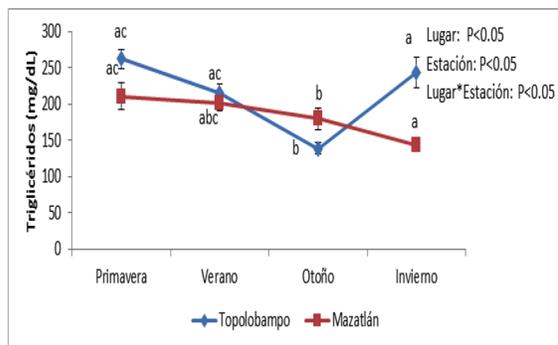
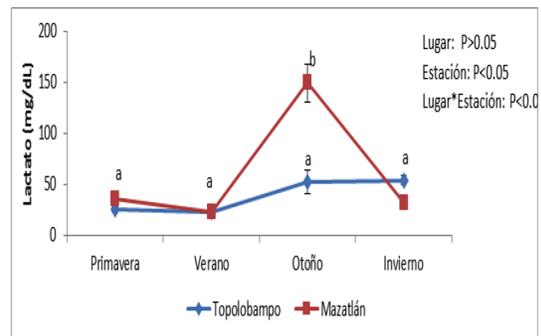
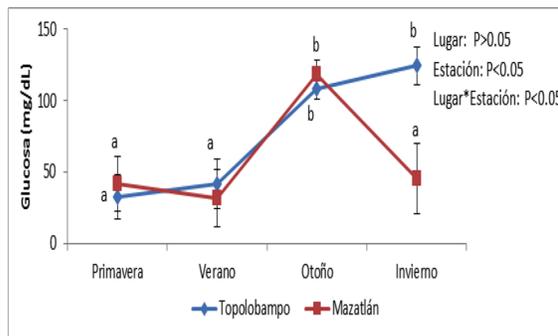
**STAGE 3:** The great challenge, to integrate both experiences in the creation the technology of the future: Autonomous farming ships, efficient and without needing to be in territorial waters, which navigate searching the best conditions of the water so that the fish are always in the best possible state without the need for chemical or antibiotic treatments, this will make the animal welfare, the environment care, the profitability of fish farmers and the feeding needs of humanity, are all in line.

## PHYSIOLOGICAL RESPONSES IN *Lutjanus peru* (Nichols and Murphy, 1922): COMPARISON OF SEASONALITY AND CAPTURE SITE

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In recent years it has increased interest in *L. peru* lead to aquaculture. There are several technological advances that have been developed to achieve this goal, however, have not yet hematologic and blood biochemistry values of reference, which are veterinary basic tools to determine changes in the health status of organisms. In the present study it is to establish benchmarks to support the determination of health status, and the reproductive cycle of the species and seasonal effects on these. For this study fish caught in two different areas of the North Mexican Pacific (Mazatlán and Topolobampo) during the four seasons, they have been determined blood biochemical values (triglycerides, cholesterol, glucose and total protein) by size and sex. Fish caught were varied weights and sizes (29.4 to 1031.2 g. And 14.1 to 45 cm respectively). The reference range for triglycerides and cholesterol was similar (52-603 60-633 and mg / dL respectively); intervals for glucose were 4.5-151 mg / dL; The data generated will help establish information that contributes to the sustainable management of wild populations of *L. peru*, by fishing and management measures potential use in crop biotechnology and mariculture.



## **BLOOD CELLS ANALYSIS OF WILD BROODSTOCK OF YELLOW SNAPPER *Lutjanus argentiventris* (PETERS, 1876) FOR AQUACULTURE DEVELOPMENT**

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The yellow snapper *Lutjanus argentiventris* is a species with high economic value in the coasts of the Mexican Pacific. In order to know the health conditions and to see how they are modified with the confinement of the organisms, 27 specimens were captured in the Bay Macapule located to the north of Sinaloa. All specimens were weighed (mg) and measured (cm) with an Ichymeter. To obtain the blood samples, the fish were extracted 1.5 ± 0.2 ml of blood by puncture in the caudal artery, using a syringe with 0.05 mL anticoagulant, to preserve the blood sample. The blood samples obtained were prepared on slides and stained with hemocolorant. They were then measured under a Leica M4000 microscope. In the cell size, significant differences (P <0.05) were observed between the different classes. It was observed that the size decreases as the length of the organisms increases. A similar case was observed with respect to the length of the core which decreases as the size of the fish increases, significant differences were observed between the analyzed sizes (P <0.05). Fish sizes formed another group negatively relating to the condition factor, suggesting that the smaller organisms had a better condition and size of blood cells than the larger ones.

## EFFECT OF DRYING TEMPERATURE, HUMIDITY AND TIME ON THE PHYSICO-CHEMICAL PROPERTIES OF SUGAR KELP (*Saccharina latissima*)

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Sugar kelp (*Saccharina latissima*) is a marine macro algae and is a rich source of fibers, vitamins, minerals and antioxidants. Due to high amount of moisture (~92%), it is highly susceptible to microbial attack and enzymatic deterioration and is either conventionally sun dried or hot air dried for extending its shelf life. Sun drying is one of the oldest techniques for food preservation, it is very slow, requires clear weather conditions which makes it an unreliable technique for Maine seaweed farmers. Hot air drying induces faster drying rate, but also leads to deterioration of texture and reduction in heat sensitive nutrients including vitamin C, antioxidants, phytochemicals, total flavonoid content and total phenolic content. The aim of this research is to optimize the drying parameters by studying the effect of hot air drying temperature, humidity and time on the physico-chemical properties (water activity, moisture content, pH, color, water holding capacity, oil holding capacity, ash content, fat content, vitamin C, antioxidant capacity and total phenolic content) of sugar kelp.

Fresh sugar kelp samples of approximately 450g were dried at an air temperature of 30°C, 40°C, 50°C, 60°C and 70°C with relative humidity levels of 25% and 50% and air velocity of 10.0 m/s in the convective dryer (Cincinnati sub-zero, CSG, OH, USA). Dried sample were packed in air tight zip lock bags and stored at -80°C for further analyses.

The ash content of the samples were found to be in the range of 23.32% - 33.05% (w.b) and are inversely correlated to the water holding capacity ( $r = -0.84$ ) and oil holding capacity ( $r = -0.84$ ), which indicate the textural properties are highly dependent on the ash content irrespective of the drying temperature and humidity conditions. Colorimeter showed the  $a^*$  values are lower for higher drying temperature indicating higher photosynthetic components, due to low drying time. Heat sensitive nutrients such as vitamin C showed a positive correlation with respect to the drying temperature (30°C to 70°C) indicating drying time have significant effect ( $p < 0.05$ ) and it increases from (0.098 mg to 0.203 mg) and (0.128 mg to 0.211 mg) as the drying temperature increases corresponding to drying humidity of 25% and 50%, respectively (Fig. 1).

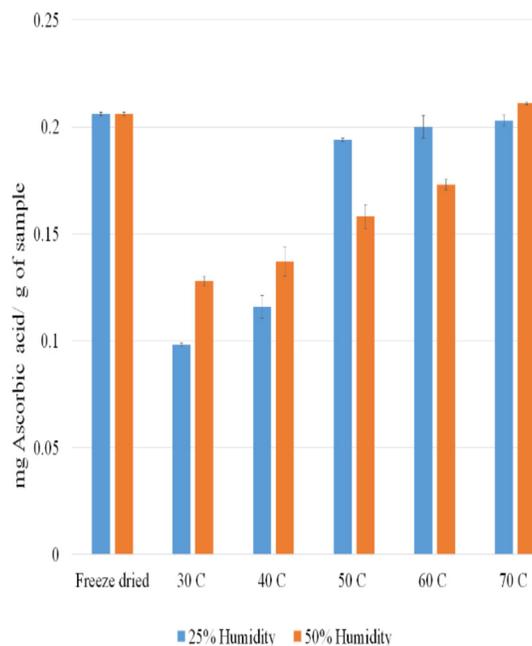


Fig. 1. Vitamin C content with respect to drying temperature and humidity

## HEAVY METALS IN SEDIMENTS OF KOSI RIVER OF UTTARAKHAND, INDIA

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**Abstract-** Water, sediments and fishes are interlinked with each other in aquatic ecosystem. Heavy metals have been considered as one of the most disastrous pollutants in the river waters because of their persistence nature and magnification capacity, they can cause serious health hazard to humans through food chain. Keeping this in mind, the research work was planned to determine the level of selected heavy metals (Cd, Cu, Pb and Fe) in Kosi Rivers of Uttarakhand from December 2015 to April 2016.

**Introduction-** Sediments are known as major repository of heavy metals in aquatic systems. Sediments play an important role as key catalysts of environmental food cycles and the dynamics of water quality. It is known to capture hydrophobic chemical pollutants entering to water bodies and slowly releasing the contaminant back into the water column (**Chapman and Chapman, 1996**). According to **Vermeulen and Wepener (1999)**, aquatic sediments absorb continuous toxic chemicals to levels many times higher than the concentration present in the water column. The functioning of an aquatic ecosystem, directly or indirectly depends on the sediment quality. The heavy metals are harmful for animals and human life depending on the concentration of intake. Some heavy metals are toxic even at low concentration, whereas some are essential for humans and if consumed in relatively high concentration they may be lethal (**Forstner et al., 1981**).

### Material and methods

#### Study Area

Heavy metals were estimated in the sediments of two rivers of Uttarakhand. Kosi River at Kashipur (Distt. U.S. Nagar) and Sharda River near Banbasa (Distt. Champawat).

#### Heavy metals examined under study

Four heavy metals, Lead (Pb), cadmium (Cd), copper (Cu), and Iron (Fe) were determined in the water, sediments and fish tissue using Atomic Absorbance Spectrophotometer (Thermo Scientific iCE 3000 Series).

### Result and discussion

The present work has been done considering the constant spreading pollution of heavy metals in water bodies. Pollution among water bodies is a major global problem. Pollution contaminates not only the water but also the sediment. The heavy metal analysis of river sediment is a useful method of studying environmental pollution due to sewage and industries. The concentration of heavy metal observed in the sediments during the study period is shown in Table:

**Table: Average heavy metal concentration (mg/kg) in Kosi River sediments with SD:**

Heavy metal	Cd	Cu	Pb	Fe
<b>Average</b>	<b>0.584</b>	<b>3.808</b>	<b>4.130</b>	<b>261.200</b>
SD ±	0.313	1.166	1.737	20.427

(Continued on next page)

The concentration of cadmium in sediment samples was recorded to be a mean value of  $0.584 \pm 0.313$  mg/kg having range between 0.26 to 1.00 mg/kg in Kosi River sediments. The average concentration of copper found was  $3.808 \pm 1.166$  mg/kg. The concentration observed maximum during the month of January and minimum in the month of April. The WHO guideline for maximum permissible limit of copper in sediment is 31.6 mg/kg. The mean concentration of lead was  $4.130 \pm 1.737$  mg/kg. Maximum permissible limit of lead in sediment is 35.8 mg/kg (**W.H.O.**). The concentration of lead was found within the permissible limits set by W.H.O. The concentration of iron ranged between 230.5 to 285.2 mg/kg. The average concentration was recorded  $261.2 \pm 20.427$  mg/kg. Sediment acts as sink for the chemical pollutants released back into the water column after adsorbing them and become source of pollution. Sediments are known to collect high concentration of heavy metals with time that might be remobilized back to the water column and to the food chain (**Kar et al., 2008**). Among the four metals investigated during study period, the average concentration of heavy metals was found in order of  $Fe > Pb > Cu > Cd$ . The similar trends of heavy metals were recorded in the order of  $Fe > Pb > Cu$  in both seasons from estuary and coastal area **Venkatramanan (2012)**. In general, metal concentration was maximum in month of January and minimum in month of April except iron. The concentration of iron was maximum in month of April and minimum in month of January. The mean concentration of Cd, Cu, Pb, and Fe were observed below the limits recommended by **WHO (2003)** for sediments.

#### **Conclusion:**

The concentration of heavy metals in sediments was below as per the standards sets by W.H.O in the Kosi Rivers. Among the heavy metal investigated the concentration of Fe was highest followed by Pb, Cu and Cd in both the rivers. It can be concluded that heavy metal contamination was higher in Kosi River due to higher industrial waste disposal, anthropogenic activities and agricultural run-off from the catchment area.

**Acknowledgement:** Authors are thankful to Director of research GBPUAT, Dean C.F.Sc, Hod FRM, Hod AEM for providing the necessary facilities.

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## EDUCATION, TRAINING AND CREDENTIALING OPPORTUNITIES FOR AQUATIC VETERINARIANS

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With the global growth of aquaculture, and hazards and risks posed by international translocation of diseased animals, there is an urgent need for a well-trained aquatic veterinary and para-veterinary workforce. Many national and international veterinary and other organizations have identified and defined core or “Day-1” veterinary and para-veterinary competencies necessary to meet the needs of other veterinary disciplines; however, none have developed a system to evaluate and credential individuals that have the appropriate skill sets necessary to practice aquatic veterinary medicine.

To meet these needs, the World Aquatic Veterinary Medical Association (WAVMA) developed a Certified Aquatic Veterinary (CertAqV) Program that is designed to recognize the knowledge, skills and experience (KSEs) in core subject-matter that is directly relevant to the practice of aquatic veterinary medicine. In many cases this program supplements and refines the KSEs obtained during the process of earning a Doctor of Veterinary Medicine, or equivalent degree. The CertAqV program specifically requires documentation of KSEs in the following subject-matter areas:

1. Anatomy and physiology unique to aquatic animals (primarily aquatic mammals, reptiles, amphibians, finfish, crustacean and molluscs).
2. Environmental factors (water quality and other issues) that affect the health of aquatic animals.
3. Industry structure and function, including commercial aquaculture (farmed seafood and ornamental fisheries), natural resource (wild) aquaculture, and ornamental (pet) and public aquaria.
4. Pathobiology and epidemiology of important aquatic animal diseases.
5. Veterinary clinical diagnostic techniques and technologies for assessing important aquatic animal diseases.
6. The availability and appropriate use of therapeutic and biologic agents (drugs, vaccines and bacterins) for preventing, controlling and treating important aquatic animal diseases.
7. Public health, zoonotic diseases and seafood safety aspects relevant to aquatic veterinary medicine.
8. International, federal, state/provincial and local legislation, regulations and standards affecting the practice of aquatic veterinary medicine.
9. Principles of welfare and humane treatment of aquatic animals.

The Program is designed to allow veterinarians to obtain KSEs from a variety of sources, including veterinary and non-veterinary academic programs, a variety of extra-curricular educational opportunities, including continuing education and professional development (CEPD) programs, and self-study. To be recognized as a Certified Aquatic Veterinary and utilize the CertAqV honorific, veterinarians are required to document KSEs in 9 subject-matter areas. As of December 2016, 52 veterinarians have been certified, and another 35 are in the process of completing their requirements.

## WORLD AQUATIC VETERINARY MEDICAL ASSOCIATION PROGRAMS & SERVICES

A. David Scarfe\*

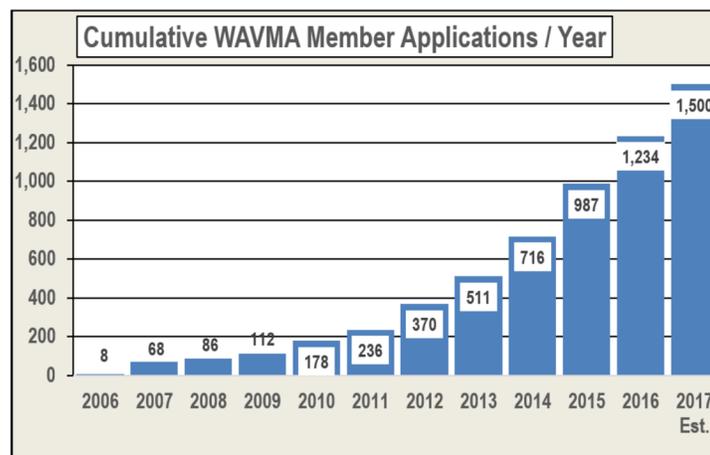
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In 2005/6, a small group of veterinarians came together to form the World Aquatic Veterinary Medical Association (WAVMA) as a global organization. Their intention was to address issues affecting aquatic veterinary medicine, and support aquatic veterinarians. It has since grown to include members (including veterinarians, veterinary students, veterinary technicians/nurses and para-veterinary professionals) in more than 57 countries, who work together to advance aquatic veterinary medicine and provide veterinary services to animal industries and owners around the world.

This increase in membership over the last decade reflects the number of member programs and services WAVMA has been able to develop. More details are available at [www.WAVMA.org](http://www.WAVMA.org). Important ones include:

- **Members-L**, and **Students-L** listservs for discussions on any topic of interest to members, but frequently focus on sharing clinical cases and client services;
- ***The Aquatic Veterinarian***, a quarterly publication with news, peer-reviewed contributions, clinical cases, jobs, meetings, and more;
- A ***Member Directory*** tool to locate other members and for vet students to locate mentors;
- An ***Employment Opportunities*** webpage with available aquatic veterinary jobs;
- An ***Image Library*** containing images useful for presentations, diagnostic interpretations, and more;
- A ***Video Library*** with videos on numerous aquatic veterinary techniques and procedures;
- A ***WebCEPD Program*** with monthly live and recorded webinars, courses, and presentations that are also available for CEPD credit;
- A ***CertAqV Program*** a program for identifying and certifying veterinarians with competency in core subject matter necessary for practicing aquatic veterinary medicine;
- The ***John L. Pitts Aquatic Veterinary Education Awards Program*** that provides financial support for veterinary students and new graduates to increase their experience in aquatic veterinary medicine; and,
- ***WAVMA Student Chapters*** at veterinary schools and colleges around the world to assist veterinary students and faculty learn more about aquatic veterinary medicine.

To keep others updated and informed about new issues in aquatic veterinary medicine, members, animal owners and para-veterinary professionals can now subscribe to WAVMA's **Twitter** feeds and **Facebook** page, and the monthly **WAVMA e-News** which is distributed >1,200 subscribers.



## GROWTH PERFORMANCE OF FOUR VARIETIES OF SWEET BASIL GROWN IN A GREENHOUSE WITH BOTH AQUAPONIC EFFLUENT AND SOIL

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Sweet basil (*Ocimum basilicum* L.) is a popular culinary herb cultivated under both natural field conditions as well as in greenhouses. Postharvest shelf life of basil is short due to susceptibility to chilling injury. Aquaponics is the combined culture of fish and hydroponic plants in recirculating systems. It is attractive to raise basil in an aquaponics system as the plant can be marketed live with the roots still intact to enhance its shelf life. Since basil varieties have been historically cultured in soil, it is valuable to the industry to determine which varieties perform well in a greenhouse grown either in soil or with aquaponic effluent.

A 47-day growth trial was conducted to track the growth and performance of four varieties of basil: Aroma, Compact Genovese, Nufar and Profuma di Genova. Growth treatments included aquaponic raft, aquaponic tower and soil; 12 plants per variety were grown in each treatment.

Aroma was significantly taller at the end of the experiment ( $p < 0.001$ ) in all three treatments. Nufar was second tallest in growth followed by Compact Genovese and Profuma di Genova. All four varieties demonstrated significantly higher growth ( $p < 0.001$ ) in the aquaponics raft treatment (Figure 1). The aquaponic tower treatment had the second highest growth; soil treatments demonstrated the slowest growth in all varieties.

Nufar had the highest leaf biomass at the end of the treatment (Table 1). These results suggest aquaponics systems that utilize raft culture should be used to maximize indoor basil growth. Nufar may be the most attractive variety to raise as it was second tallest in height and had the highest level of leaf biomass.

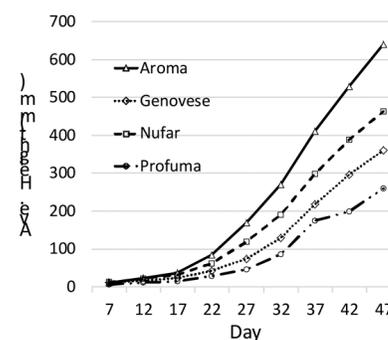


FIGURE 1. Average plant height (mm) of four varieties of basil grown in the aquaponic raft treatment over 47-days.

TABLE 1. Average leaf biomass (g) at harvest in four basil varieties grown in three different treatments.

	Nufar	Aroma	Genovese	Profuma
<b>Raft</b>	<b>189.9</b>	<b>103.5</b>	<b>87.1</b>	<b>66.9</b>
<b>Tower</b>	<b>99.9</b>	<b>93.8</b>	<b>67.7</b>	<b>31.7</b>
<b>Soil</b>	<b>30.3</b>	<b>21.9</b>	<b>20.1</b>	<b>18.4</b>

## EFFECT OF SALINITY ON SURVIVAL OF AN ENGANGERED MINNOW EXPOSED TO *Ichthyophthirius multifiliis*

Julie C. Schroeter\*, Sam Sawicki, William “Cal” Fraser, and Wendy M. Sealey

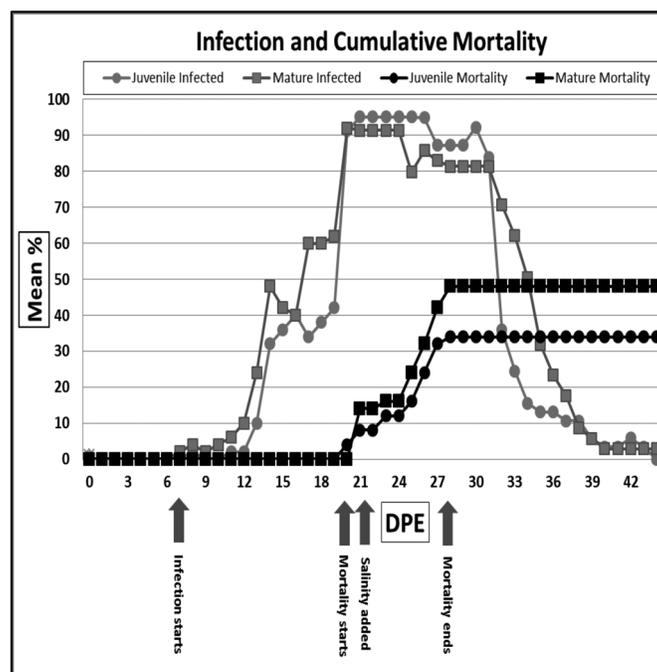
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Endangered woundfin (*Plagopterus argentissimus* Cyprinidae) are native to the Virgin River downstream of Pah Tempe Mineral Hot Springs near Hurricane, Utah. Removal of Pah Tempe Springs or diversions of its outflow are proposed to reduce river salinity loading to improve the quality of water used for irrigation. However, there are concerns because Pah Tempe Springs is a natural feature and natural salinity loading has been hypothesized to reduce the negative impacts of *Ichthyophthirius multifiliis* infections on fish survival by conferring anti-parasite properties.

We established an experimental design to test this hypothesis using a captive population of woundfin. Juvenile (1.8 g weight and 55 mm total length) and adult (6.7 g weight and 89 mm total length) fish were randomly stocked into a recirculating water system with five replicate 94- L tanks/size class at a rate of 10 fish/tank. Two control tanks equipped with in-line ultraviolet water treatment were stocked with fish in each size class to validate that observed mortality was due to *Ichthyophthirius multifiliis*. Water temperature was maintained at 21.8-22.2°C, and average nitrite and ammonia were 0.04 and 0.03 mg/L, respectively. Following an acclimation period of five days, fish were exposed to *Ichthyophthirius multifiliis* by introducing three infected fish in the upstream water source. When total mortality reached 15%, water salinity was raised to 3.5 ppt. Mortality was then monitored for an additional 21 days.

Trophonts were first observed on test fish at 7 days post-exposure (DPE). Mortality began at 19 DPE and reached 15% at 21 DPE. Mortality ceased at 28 DPE (7 days post-salinity). Diagnostic evaluation at 44 DPE validated a clearance of the parasite in all surviving fish. Cumulative mortality was similar in juvenile and adult fish (32 vs 48%, respectively; P=0.271).

These results indicate that salinity treatment confers anti-parasite properties in woundfin and provide preliminary evidence the natural salinity loading in the Virgin River may mitigate *Ichthyophthirius multifiliis* epizootics.



## DETERMINATION OF UPPER SALINITY TOLERANCE IN AGE 0 AND ADULT WOUNDFIN (*Plagopterus argentissimus*)

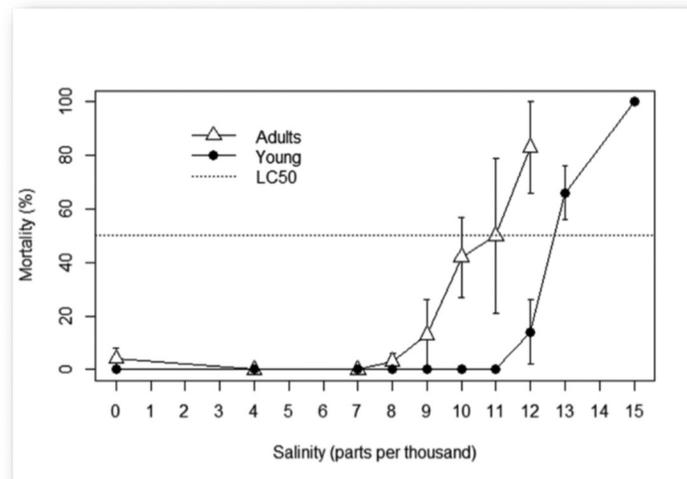
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Endangered woundfin (*Plagopterus argentissimus* Cyprinidae) are native to the Virgin River downstream of Pah Tempe Mineral Hot Springs near Hurricane, Utah. Pah Tempe Springs alters river salinity with measured salinity ranging from 9 parts per thousand (ppt) immediately below the springs to 2.2 ppt at 2100 m downstream. Salinity tolerance has been shown to influence fish community composition over large geographic scales, but can vary by species and age class. Determining the salinity tolerance of woundfin in two age classes would improve distribution modeling of the species throughout the Virgin River, and provide essential baseline salinity tolerance for future physiological assessments.

To assess woundfin salinity tolerance, a 96-hr (LC50) was conducted. Three hundred age 0 (23 mm length) and 146 mature (75 mm length) fish from a captively bred population were stocked into 1000 mL and 9.5 L replicated static water tanks, respectively. Salinities tested ranged from 0 to 15 ppt and 0 to 12 ppt, respectively for the age 0 and mature woundfin. Thirty percent water exchanges were conducted daily. Water temperature was maintained at 21.8-22.2°C, while average nitrite, ammonia, pH, and dissolved oxygen levels were 0.0008 mg/L, 0.4 mg/L, 8.4, and 7.7 mg/L, respectively. Fish were exposed to salinity treatments for 96 hr and mortality was recorded every 24 hours.

Mortality occurred in age-0 fish at 12 ppt, and the 96-hr LC50 was 13 ppt. Mortality occurred in adult fish at 8 ppt and the LC50 was 11 ppt. These results suggest woundfin can tolerate salinities up to 8 ppt, and that younger fish may be more tolerant of higher salinities. The identification of an acceptable salinity range allows for improvements in assessment of woundfin prevalence in the Virgin River. These results are particularly pertinent in consideration of proposals brought forth that suggest removal of Pah Tempe Springs in order to reduce salinity loading for irrigation users downstream.



## EVALUATION OF FISHMEAL-FREE, SOYBEAN MEAL-BASED DIETS IN HYBRID CATFISH *Ictalurus punctatus* x *I. furcatus*

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As one of the fastest-growing food industries in the world, aquaculture is rising quickly to meet the demand of the world's population of seafood consumers, while the market is in short supply of fishmeal and fish oil. Therefore the development and optimization of fish meal (FM)-free, growth-promoting and cost-effective aquafeeds is essential for the aquaculture industry to flourish. Foremost among the commercially available feedstuffs currently used as surrogates for FM in aquafeeds is soybean meal (SBM). Despite being economical and nutritious, with high protein and a balanced amino acid profile, conventional SBM contains anti-nutritional factors (ANFs) that can negatively affect growth and the physiological status of fish and, hence, limit its applicability as a feed ingredient. In order to increase SBM utilization in animal feeds, different processing technologies including solid-state fermentation and enzymatic treatment have been implemented to reduce or eliminate ANFs in conventional SBM and improve its nutritional value. However, there has been relatively little research done on comparative studies of differently processed SBM in diets for farmed fish. Therefore, our goal was to assess the nutritional value of four types of SBMs such as: I. Dehulled solvent extracted commodity SBM, II. Enzymatically treated SBM, III. Bio-converted SBM and IV. Fermented SBM in the diets of hybrid catfish (HCF, *Ictalurus punctatus* x *I. furcatus*).

Eight experimental diets were formulated to contain 35% crude protein, 7% lipid, and an estimated 2.9 kcal of digestible energy per gram. These diets were designed to evaluate four types of commercially available SBMs (I-IV), each at two levels (0 and 10%) of dietary FM. Groups of 15 HCF (average initial weight of 8.2 g) were stocked into 24 ten-gallon tanks in a RAS system and are being fed (in triplicate) one of the randomly assigned diets twice daily to apparent satiation. Group weight was taken at the beginning, and will also be taken halfway through and at the end of the experiment.

At the end of experiment proximate composition, amino acid and fatty acid profiles of diets and fish carcasses will be analyzed. The growth performance, nutrient utilization parameters, muscle yield and physiological parameters including blood chemistry and protein metabolism enzymes activity will be evaluated. Resulting data will be presented. Based on the anticipated outcomes, among the four SBMs, one of them will provide an economical potential alternative ingredient for use in aquaculture diets. It will also provide safe, healthy and profitable seafood.

## COPEPOD USE INCREASING AS A LIVE FEED FOR LARVICULTURE

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Aquaculture continues to grow in leaps and bounds across production sectors, countries, and continents. As aquaculture production increases, so does the importance of hatchery capacity and capabilities. In the production of many species, there is reliance during the hatchery/larval phase for some form of live feeds. This is ever more important in the marine sector, with emerging high value food fish and ornamentals not only demonstrating a need for traditional live feeds such as rotifers and *Artemia*, but also the use of copepods in the live feeding regime. There are three groups “phylogenetic orders” of copepods of particular interest in aquaculture; these are: Calanoida, Cyclopoida, and Harpacticoida. Among others, some of the reasons for increasing use of copepods during larviculture (to achieve reliable high survival and quality weaned fry) relate to their (early nauplii stage) small size, attractive movement in the water column, high digestibility, and often superior nutritional value. Presented will be some general background information regarding copepods, some pros and cons relating to their use and culture, followed by examples of culture and application.

## **GOOD AQUACULTURE PRACTICES (GAqPs) FOR RECIRCULATING AQUACULTURE SYSTEMS**

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Good Aquaculture Practices (GAqPs) are activities, procedures, or considerations optimizing production systems and management protocols to maximize environmental and economic sustainability, final product quality and safety, animal health and worker safety, while concurrently minimizing the likelihood of a disease outbreak. GAqP considerations generic to all aquaculture production systems include considerations for: Regulatory and non-regulatory compliance, facility siting and design, source water, facility security, animal health, feed management, record keeping and employee training.

Recirculating Aquaculture Systems (RAS) are aquaculture systems which recycle greater than 90% of the system volume on a daily basis. Some RAS-specific GAqPs include tank shape and water flow orientation so tanks act as primary solids collection devices. Furthermore, tank water exchange rates with filtration systems should be 100 to 200%/hour, with 10% of this volume coming from solids collection in the tank direct to solids filter, and 90% of tank flow going from the tank direct to the biofilter, and back to the tank. Makeup water should 1st go through disinfection, and be introduced via the biofilter or sump. Systems should be maintained in proper working condition, and never be stocked at densities beyond design specifications. As RAS are intensive in nature, extra biosecurity considerations should be implemented above standard protocols. These may include sterilization of all incoming water, entry and exit disinfection foot bath and hand washes, restriction of unauthorized personnel, purchase of only Specific Pathogen Free animals for restocking, and careful monitoring of all water quality parameters multiple times a day, or continuously via automation.

## EVALUATING THE RISK OF EUTROPHICATION AND INFRASTRUCTURE DAMAGE AT PROTECTED AND EXPOSED FINFISH PRODUCTION SITES USING A GEOSPATIAL MODEL

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Marine finfish farmers are increasingly looking to more exposed environments due to the saturation of traditional lease areas, policies or user conflicts restricting availability to near shore sites, and the potential for improved production in offshore environments. As the desire to utilize these offshore areas increases, improved tools to evaluate and select sites are

needed. Using GIS technology and remotely sensed or modeled data, the risk of nutrient eutrophication, risk of damage to farm infrastructure, and exposure level (severity of oceanographic conditions) are evaluated for the Caribbean, Gulf of Mexico and the Pacific coast of Central America.

Bathymetry, ocean currents, wave height, hurricane frequency, and chlorophyll- $\alpha$  data were inputted into a geospatial model. The outputs show which areas are most suitable for production from a nutrient loading perspective, and ease of operation perspective. Sites are also qualified as suitable for submerged pens only or surface or submerged pens. An example of one output is shown in figure 1.

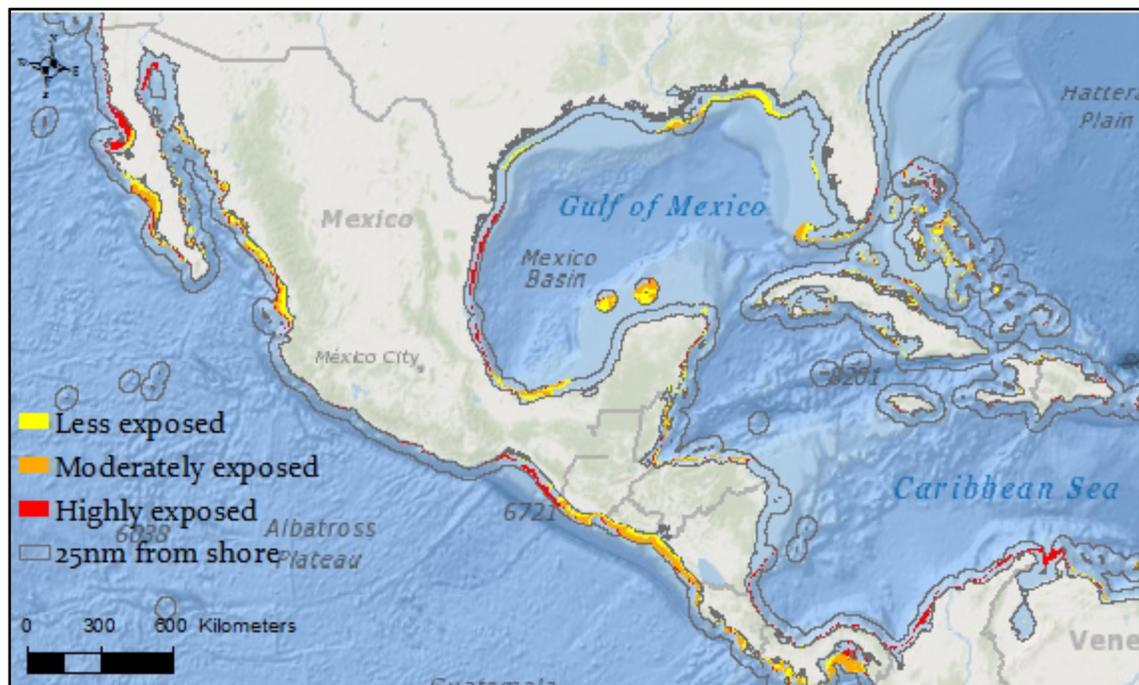


Figure 1: The exposure level of areas in tropical North and Central America which are within 25 nautical miles of shore, and are 25 - 60 m deep.

## **EVALUATING THE PRODUCTION EFFICIENCY OF NEW ENGLAND'S OYSTER AQUACULTURE INDUSTRY**

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Oyster aquaculture is a rapidly expanding industry in Maine and New England. Several different production methods are currently employed in this industry to raise oysters to market size. Each method involves a certain level of capital and labor intensity. For example, the technique known as bottom planting consists of seeding an area with small oysters and allowing them to grow to market size on the ocean floor before returning to harvest them. This method requires relatively little capital or labor on the part of the grower, but tends to involve higher rates of oyster mortality than other methods. Survival rates can be improved by using costlier production techniques such as floating or bottom cages, oyster rafts, or other contained culture methods. One goal of this project is to quantify the differences in production efficiency between these methods. The study will also assess the relative technical efficiency of oyster growers in Maine, New Hampshire and Massachusetts.

A mixed mode survey has been designed to investigate many aspects of New England's oyster aquaculture industry including production efficiency, risk preferences, site selection and social networking. The survey will be sent to over 400 oyster growers located throughout Maine, New Hampshire and Massachusetts. Respondents will have the option to complete the survey either online or by mail. They will be compensated for their time.

This paper will focus on assessing production efficiency in the industry. Growers will be asked about their production methods, as well as the inputs used in their oyster growing operations. These include labor, oyster seed, fuel and a variety of capital costs. A stochastic frontier model will be employed to evaluate the production efficiency of these operations. This will allow us to assess the relative efficiency of the different production methods that are currently in use in New England's oyster aquaculture industry. We will also compare the relative production efficiency of oyster growers in Maine and New Hampshire to those in Massachusetts, which has a larger and more established oyster aquaculture industry.

This study will provide a tool that allows individual oyster growers to evaluate the technical efficiency of the production methods they are currently employing. This analysis will also have important policy implications. Assessing the relative efficiency of Maine's oyster aquaculture industry will allow policymakers to determine whether more resources need to be dedicated to education and outreach to improve outcomes in this industry. This information could play a critical role in helping Maine's oyster aquaculture industry continue to grow and be successful.

## IMPACTS OF U.S ANTIDUMPING DUTY: CHINESE FRESHWATER CRAWFISH TAIL MEAT

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An anti-dumping tariff is imposed on the foreign suppliers when a) There is evidence that the domestic industry is materially injured due to the imports, b) Imported product is sold at less than fair market value (LTFMV) prices. In 1996, Crawfish Processors Alliance (CPA) filed a petition to the United States International Trade Commission (USITC) claiming that their industry has been materially injured due to the imported Chinese crawfish tail meat. During the period from 1994-1996, the domestic tail meat prices varied from \$3.75 to \$8.91 per pound while imported tail meat prices varied from \$2.43 to \$4.25. In 1997, the USITC found that the U.S. crawfish industry has been injured and the Chinese product has been sold at LTFMV price.

Data (domestic and foreign prices, imports and domestic production) during the period from 1993 to 2015 has been collected. Equilibrium Displacement Model (EDM) and econometric models were applied. The impact of the antidumping tariff on the U.S crawfish domestic market (price, consumption and production) will be presented.

**Table 1. Crawfish imports from china and its market share in the U.S market from 1994 to 2002.**

<b>Year</b>	<b>Imports (1000 pounds)</b>	<b>Market share (%)</b>
1994	3.39	57.6
1995	10.992	78.9
1996	7.77	86.8
1997	2.3	61.8
1998	5.9	74
1999	3.5	71.1
2000	5.5	80.2
2001	12.5	93.2
2002	8.8	84.2

## INTENSIVE PRODUCTION OF YOY PADDLEFISH *Polyodon spathula*

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Traditional methods for production of paddlefish have relied on natural production of zooplankton and insects such that a fertile pond will support production of several hundred pounds of young of year (YOY) fingerlings in a season. With supplemental feeding, yield may be increased significantly. Previous work at Kentucky State University demonstrated net yields up to 3323 kg/ha.

Investigators on this project observed intensive production of paddlefish where fry were trained on feed, stocked into small impoundments and fed a pelleted ration. Preliminary data collected in 2015 and 2016 demonstrate improvement in methods resulting in significantly higher yield/ha. Fry were trained on feed and presented with supplemental *Daphnia* as a first food at water temperature of 24 C. Otohime B2 and Zeigler Trout #1 crumbles were presented day and night on a continuous basis during the first month of life after swim-up. After successful feed training, paddlefish were stocked into one of two production units seeded with *Daphnia* – a pond and a large concrete tank. The fish were fed a high quality floating feed (45% protein, 12% fat). Feed was applied with a belt feeder and by hand. During the summer, feed was delivered at night over a period of 8 to 12 hours with a belt feeder floating in the middle of the pond. Aeration was applied continuously and predators were discouraged whenever possible.

On May 28, 2015, paddlefish (1 g) trained to eat pelleted feed were stocked into a 0.04 ha pond with a volume of approximately 370 m<sup>3</sup>. At stocking, the 6900 fish weighed about 6.8 kg. On November 19, 2015, 426 kg YOY paddlefish weighing an average of 376 g each were harvested yielding a net production of 10,650 kg/ha. Estimated feed conversion was 0.9 kg feed/kg gain, indicating that natural foods contributed to the diet.

On May 14, 2015, 0.32 g paddlefish trained to eat pelleted feed were stocked into a 0.033 ha concrete tank with a volume of 800 m<sup>3</sup>. Water did not flow through the tank continuously, but was periodically exchanged late in the season if water quality deteriorated. At stocking, the 11,000 fish weighed 3.7 kg. 325 days later, 1079 kg of paddlefish weighing an average of 275 g each were harvested yielding a net production of 32,696 kg/ha.

These results show there is potential for much higher yield and good feed efficiency of feed trained paddlefish when feeding at night and slowly delivering floating feed to the water surface.

Year/Investigator	Number Stocked/ha	Initial Fish Weight (g)	Fish Weight at Harvest (g)	Net Yield (kg/ha)
1991 - Tidwell	4940	3.6	107	365
1991 - Tidwell	9880	3.6	139	537
2008 - Onders	12,355	25	205	1857
2008 - Onders	18,533	25	174	2590
2008 - Onders	24,710	25	178	3323

## INTEGRATING AQUACULTURE AND WATER REUSE FOR DECOMMISSIONED WASTEWATER TREATMENT FACILITIES IN LONDON, KY

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Decommissioned waste water treatment facilities provide a unique infrastructure for aquaculture development. This project utilizes large concrete structures (formerly clarifiers and digesters) at the Water Resource Recovery Facility (WRRF) in London, KY. Features of these facilities include a secure location, vehicle access, readily available electric power, support structures, communication infrastructure, abundant continuous water supply, treatment for aquaculture discharge, personnel trained in water quality assessment, and other substantial industrial infrastructure. Facilities are not designed for aquaculture and represent challenges with regard to safety, economy of scale, water quality management, harvesting and marketing. Clarifiers used in this study are rectangular approximately 34.7 m by 9.5 m by 2.4 m deep with a volume of 800 m<sup>3</sup> (0.65 acre-ft). Digesters are circular with a 10.7 m diameter and a depth of 9.1 m with a water volume of 617 m<sup>3</sup> (0.5 acre-ft). Clarifiers have a flat bottom that can be seined, but digesters have a cone bottom that is not seinable. Good management practices for this facility include continuous aeration, periodic water exchange, confining the fish in net pens, routine water quality monitoring, feeding in relation to an established production plan, addition of salt after a water exchange, monitoring for disease, and systematic record keeping for each production unit. Paddlefish, Largemouth Bass, Hybrid Striped Bass, Hybrid Bluegill Sunfish, Channel Catfish, survive and grow well in this facility.

At this point, investigators working on this project have not observed a specific contaminant risk associated with growing food fish in water treated at these facilities. Previous work at a treatment plant in Winchester, KY found that fish produced there met FDA requirements with regard to metals and pesticides (i.e. mercury, selenium, Chlordane, Mirex, Aldrin, PCB's and more). A composite sample of 10 Largemouth bass grown in the London WRRF confirmed this conclusion with levels of metals or pesticides no higher than samples of bass taken from surface waters in the London area. While the objectives of this project do not include a study of contaminants in fish produced at this location it was deemed prudent from a marketing perspective.

As the value of water is recognized, it makes sense to develop methods that take greater advantage of existing resources to grow fish for food and recreation. Results from this project contribute to observations made at wastewater treatment plants in Winchester, Frankfort, Midway, and now, London, Kentucky.

## APPLICATIONS OF *Flectobacillus major* AND ITS SPHINGOLIPIDS IN THE MODULATION OF FISH HEALTH

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Commensal bacteria influence most physiological systems of animals. Farmed fish have a diverse microbial community living in association with each mucosal surface. In hatchery reared adult rainbow trout (*Oncorhynchus mykiss*), the skin and gill bacterial microbiome is dominated by the Gram-negative bacterium *Flectobacillus major*. *F. major* has the unique ability to synthesize sphingolipids of a rare molecular structure. We hypothesized that *F. major* sphingolipids influence the immune system of rainbow trout and they can be applied in aquaculture settings to control inflammation as well as infectious diseases.

*F. major* sphingolipids were extracted and purified according to previously established protocols. Sphingolipids were combined with bovine serum albumin to avoid cell toxicity issues. Trout gills and head-kidney (HK) leucocytes were incubated *in vitro* with live *F. major* cells at two different doses ( $10^2$  cfu/ml and  $10^4$  cfu/ml). Changes in immune gene expression were measured 6, 24 and 48 h later. Specifically, IgT, IgM, pro-inflammatory and anti-inflammatory cytokine expression levels were quantified by RT-qPCR. *In vivo* administration of *F. major* sphingolipids was performed via two different routes, intraperitoneally (i.p) and intravenously (i.v). Expression of the same immune genes was evaluated 60 hours post-treatment in the gills and head-kidney.

*In vitro* results show that, compared to unstimulated controls, live *F. major* stimulates IgT expression in gills but not HK in a sustained and dose-dependent manner, whereas IgM expression stimulation is transient (Figure 1). Measurement of pro-inflammatory and anti-inflammatory cytokine gene expression *in vitro* and *in vivo* is currently being analyzed. Future studies will evaluate how the administration of *F. major* sphingolipids affects disease resistance in rainbow trout.

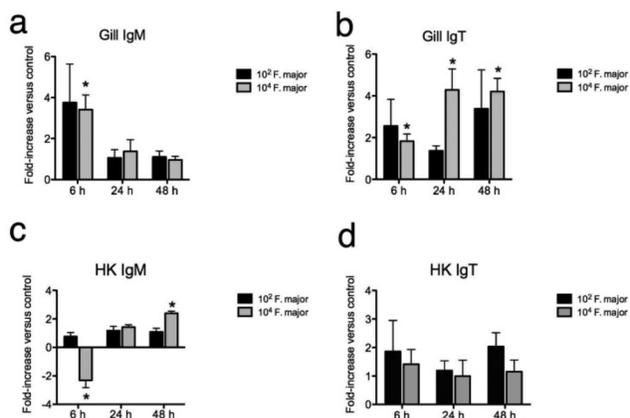


Figure 1: Live *F. major* induces changes in IgM and IgT gene expression levels in rainbow trout gills (a and b) and HK (c and d). Gene expression changes were measured by RT-qPCR and results are expressed as the mean fold changed compared to untreated control (N=5) using the Pfaffl method.

## RECENT ADVANCES IN NASAL VACCINOLOGY IN RAINBOW TROUT

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Fish vaccination is considered the most effective way to prevent fish disease outbreaks in aquaculture. Injection, immersion, and oral vaccination are the routine methods for vaccine delivery in fish. Recently, the discovery of the nasopharynx-associated lymphoid tissue (NALT) in teleosts has opened up new avenues for the control of aquatic infectious diseases via nasal vaccination. To date, the efficacy of a live attenuated infectious hematopoietic necrosis virus (IHNV) vaccine and enteric redmouth (ERM) bacterin has been assessed in rainbow trout. However, the mechanisms underlying the high levels of protection conferred by nasal vaccines in fish are unknown.

IHNV intranasal (I.N) vaccination leads to almost 100% protection against IHNV infection in rainbow trout both at 7 and 28 days post-vaccination. This protection is paralleled by a dramatic induction in the expression of chemokine CCL19-1 transcripts 4 days after vaccination.

We produced recombinant rainbow trout CCL19 (rCCL19) and delivered it I.N or intraperitoneally (i.p) to test if this molecule is sufficient to protect trout against IHNV challenge. Our results showed no protection in response to rCCL19 administration either I.N or i.p. (Figure 1). Further analysis of control and rCCL19-treated trout olfactory rosettes revealed an increase in the numbers of CD8 $\alpha$ <sup>+</sup> cells on day 8 in both i.p and I.N groups (Figure 2).

In conclusion our results suggest a key role of CCL19-1 in the nasal immune responses of trout following nasal vaccination. However, this chemokine alone is not sufficient to mimic the protection conferred by the whole vaccine.

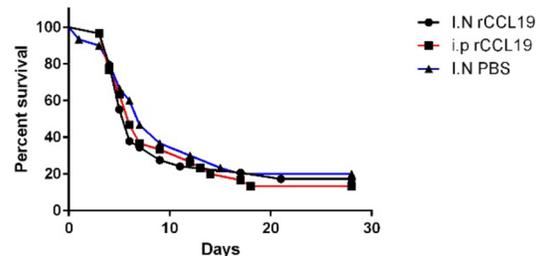


Figure 1: Survival curves of rainbow trout treated with rCCL19 I.N or i.p or unvaccinated control (I.N PBS) and challenged with IHNV.

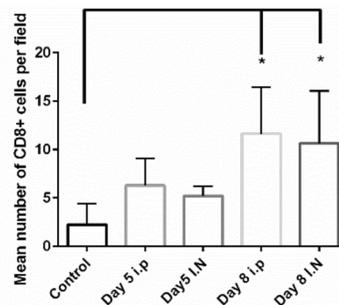


Figure 2: Administration of rCCL19 I.N and i.p significantly increased the number of CD8 $\alpha$ <sup>+</sup> cells in the olfactory organ of trout 8 days post-administration. Asterisks denote statistically significant differences.

**CIRNAS: COLLABORATIVE IMMUNE REAGENT NETWORK FOR AQUACULTURED SPECIES**

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Farmed fish provide healthy protein and lipids for human consumption. Nevertheless, disease limits the sustainability and production of this industry. Immunological reagents specific for commercially relevant aquacultured species are needed to advance basic and applied research related to fish health. These reagents are required to evaluate shifts in immunity during infection and vaccination against pathogens that limit the full potential of aquaculture. CIRNAS (<http://biology.unm.edu/CIRNAS>) is a collaborative network with a goal of serving the aquaculture community by advancing the availability of immunological resources and knowledge base for fish health. Due to the importance of mucosal health and mucosal vaccines in finfish, specific focus areas include the development of reagents for mucosal immunity and vaccinology. Four fish species: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) and Nile tilapia (*Oreochromis niloticus*) are being targeted. Antibody panels and immune assays will be developed to assess the contribution of different leukocyte subsets and effector molecules that cannot be currently measured during disease and vaccination due to lack of corresponding reagents. Current and proposed research will be presented.

## LARGE-SCALE MONO-SEX PRODUCTION IN FISH

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Mono-sex production is desirable in a variety of fish species due to case-specific advantages, higher growth rate and less variation in harvest size, reduction of social impact, control over reproduction, superior flesh quality or value of one sex, reduction of environmental risk resulting from escapes of invasive species, and potential application in invasive animal control. Mono-sex production includes many aspects, e.g. producing sex-skewed / mono-sex population through induction of sex reversal, chromosome manipulation (gynogenesis and androgenesis, polyploidy induction), hybridization, selection, or the combination of these two or more. Large-scale mono-sex production in fish usually require the researchers to acquire basic information on sex-determining mechanism and need two preconditions, sex could be reversed and the sex-reversed fish can be fertile. The process mainly consists of four major procedures, induction of sex reversal, identification of sex-reversed individuals, population expansion of sex-reversed individuals, and mono-sex production.

We critically reviewed recent advancement of sex-linked markers and generated a set of workflow of all-male and all-female production in fish with XX/XY or ZW/ZZ genotype. This workflow has applications in commercial scale mono-sex production and could facilitate research field that prefer mono-sex fish as material. We also summarized the potential application of environment-friendly approaches, e.g. temperature treatment, rearing background color, in mono-sex production.

## 5S rRNA PROFILING AND APPLICATION IN IDENTIFICATION OF SEX AND OVARIAN DEVELOPMENT STAGE IN TELEOSTS

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In the process of gametogenesis, oocytes and spermatocyte undergo significantly diverse molecular, cellular, and structural changes. One of these molecules involved considerably in oogenesis is 5S ribosome RNA (5S rRNA). The dynamic feature of oocyte 5S rRNA during oogenesis had been studied in some fish species in 1970s, but has been overlooked until recently. In the oocytes of some amphibian and teleost species, rDNA is amplified  $\approx 1000$ -fold and 5S rRNA and transfer RNA (tRNA) constituted more than 90% of the RNA content approximately at the end of primary growth or early cortical alveolus stage, and decrease to normal level as the development of ovary. The dynamic feature of 5S rRNA in early stages of ovarian development could be served as a potential approach to determine sex and oogenesis stage for large amount of samples with comparably less input. Meanwhile, overwhelming accumulation of 5S rRNA also cause problems in RNA-seq analysis. Incomplete or incorrect depletion of these unwanted and problematic RNA for in-depth transcriptomics analyses, can lead to insufficient coverage of transcripts of interest, and result in misassembly, incorrect quantification of gene expression and inaccurate conclusion. As far as we know, only a small proportion of scientists have realized the massive unwanted 5s rRNA in developing/resting ovaries of fish and anurans, and subsequent problem in transcriptomics analysis. There is no commercial kit targeting at this issue yet. The objectives of the present study are to: 1) evaluate the application of 5s RNA profiling in accurate sex identification and oogenesis stage determination in fish with asynchronous or synchronous ovaries; 2) alert researchers who are working on ovary transcriptomics in fish and anurans that incorrect depletion of 5s rRNA may lead to misassembly and incorrect quantification of gene expression.

Asynchronous ovaries (bluegill and largemouth bass) and synchronous ovaries (yellow perch and channel catfish) as well as testicular tissues were collected. One part of gonad was embedded in *RNAlater*<sup>®</sup> for RNA isolation and the other part was fixed in *Prefer* for histological analysis. Livers and brains were also collected in some samples as a somatic tissue control. Isolated RNA samples were evaluated by Nanodrop 1000 and the same amount of RNA was loaded in an Agilent RNA 6000 Nano Kit Bioanalyzer for capillary electrophoresis. Generated data were analyzed using Agilent 2100 Expert. Time corrected area of each peak in electrophoresis was used to calculate the 5S/18S rRNA ratio and establish a correlation with ovary development. Our data demonstrate that 5S/18S rRNA ratio could be served as an index to differentiate females from males in early development of gonads and determine ovary development stages, and a large sample size can be completed in a short time, with small amount of samples (e.g. 20-50 mg), and without laborious histology work. It is also worthy noting that the dynamic feature of 5S/18S rRNA ratio is species-specific.

## EVALUATION OF GROWTH OF KOI FISH AFTER ORAL ADMINISTRATION OF *Lactobacillus acidophilus* AS PROBIOTIC IN RATION

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Koi fish (*Cyprinus carpio*) is domesticated version of common carp originates from China fresh waters. This fish is most famous by its beautiful colors that have been created via selective breeding (over 20 varieties) and are classified by the colors and the patterns on their back (dorsum). The color of and patterning some varieties can change as they grow and develop.

Koi fish can be found in the ponds throughout the world. Since koi fish is numerous and kept in private aquacultures, it is not on the list of endangered species. The temperature range is 3-25 °c.

Koi fish need a high protein food to bulk up and grow during the summer months. Koi fish are omnivorous and eat food off of the bottom of the pond. The barbels help them locate foods.

The young Koi is about 3 to 8 inches, Mature Koi: 14 to 24; some jumbo Koi end up 32 to 36 inches long (even 1.8 m). The average lifespan of a Koi fish is 15 to 20 years, but some can live to be 30 years old or more.

Probiotics are well known and routinely used additives in the main livestock species. They claim to improve gut health by stabilizing gut flora being their effect reflected in a better overall health status, welfare and performance of the animals.

In aquaculture, probiotics are administered by feed and/or as a water additive. The supplementation of probiotics through feed is a better method to ensuring the efficiency of the probiotic bacteria in the GI tract of fish. Microorganisms, both from indigenous and exogenous sources, are used as probiotics. The commonly used probiotics in fish culture practices belong to *Clostridium*, *Bacillus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Carnobacterium*, *Aeromonas* and several other genera.

In this research, the probiotic were used, is from a product that each gram has 10<sup>10</sup> *Lactobacillus acidophilus*. Two concentrations, 15 and 30 mg/100g Food were administered for two months. In days 0, 30 and 60 total length besides weight were recorded (Tables 1 and 2).

The best results belong to the 30 mg/100g food *L. acidophilus* koi fish group.

**TABLE 1.** Koi fish weights (g) after administration of *L. acidophilus* for two months

<i>L. acidophilus</i> (mg/100g Food) □	Day □	0	30	60
<b>0 (control)</b>		4.2	4.6	5.7
<b>15</b>		4.1	5.2	6.2
<b>30</b>		4.1	5.4	7.8

**TABLE 2.** Koi fish total lengths (mm) after administration of *L. acidophilus* for two months

<i>L. acidophilus</i> (mg/100g Food) □	Day □	0	30	60
<b>0 (control)</b>		68	73	75
<b>15</b>		66	75	76
<b>30</b>		71	80	85

## SPRING FORWARD WITH IMPROVED NILE TILAPIA *Oreochromis niloticus* RESISTANT TO *Streptococcus iniae* AND *Streptococcus agalactiae* Ib

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Tilapia aquaculture worldwide is valued around US \$ 7 billion. Tilapia are an important source of protein for domestic (top 5 most consumed seafoods) and global food security. Two gram positive bacteria, *Streptococcus iniae* and *S. agalactiae*, are responsible for billion dollar losses annually. Genetic gains in performance traits (e.g., growth) have been realized in Nile tilapia and interest in breeding for disease resistance has received attention. The objectives of this study were three fold: 1) to verify previous results demonstrating heritability of *S. iniae* resistance in Nile tilapia; 2) to determine if realized genetic gain in resistance and/or susceptibility to *S. iniae* is possible following positive assortative mating between parents with high or low estimated breeding values; and 3) to determine if resistance to *S. iniae* and *S. agalactiae* Ib is genetically correlated. A total of 144 and 130 full sib families were challenged intraperitoneally with *S. iniae* and intramuscularly with *S. agalactiae* Ib, respectively. Cumulative mortality at test end was 46 % for *S. iniae* and 68 % for *S. agalactiae*. There was a high additive genetic component found for survival in fish injected with *S. iniae* (estimated heritability  $0.52 \pm 0.12$ ) similar to  $0.42 \pm 0.07$  determined the prior year. The estimated heritability for *S. agalactiae* was  $0.38 \pm 0.11$  based on the univariate linear animal model. Positive assortative mating further demonstrated resistance to *S. iniae* is heritable and thus resistance can be improved upon through selective breeding (Table 1). No genetic correlation was noted between resistance to *S. iniae* and *S. agalactiae* Ib. The lack of correlation suggests if resistance to both *Streptococcus* sp. is desired, selection for both traits must be simultaneous. Selection of fish to improve survival to *Streptococcus* sp. may require a thorough understanding of the type of pathogen prevalent in the region so that custom genetic material may be tailored to meet the needs of the individual farm and/or region.

**Table 1. Survival of the families produced with assortative mating for high and low resistance to *S. iniae*.**

Assortative mating groups	Number of families	Avg_ <i>S. iniae</i> index parents*	Percentage survival to <i>S. iniae</i> in 2015			Percentage survival to <i>S. agalactiae</i> in 2015		
			Average**	Min	Max	Average***	Min	Max
none	132	101.6	54	0	100	31	0	89
yes_high	6	121.9	88	60	100	19	7	50
yes_low	6	80.2	10	0	42	40	18	59

\*Index (mean = 100, sd=10). Avg = (sire index + dam index)/2

\*\*Mean family survival to *S. iniae* during challenge test.

\*\*\*Mean family survival to *S. agalactiae* during challenge test.

## IN-VITRO DIGESTIBILITY OF TWO MICROALGAE BY-PRODUCTS AND WHOLE CELL MICROALGAE IN AQUATIC DIETS

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Aquaculture continues to be the most rapidly growing segment of agriculture and now provides over 50% of the seafood destined for human consumption. Many of the aquacultured fish and shrimp species consume prepared feeds that contain large amounts of fish meal and oil. The growing demand for these limited marine feedstuffs has not only increased their cost dramatically, but has also made the world's fisheries unsustainable. This demand has prompted the need to develop alternatives to fish meal and oil to lessen dependence on these very nutritious but finite resources. Previous research has demonstrated that whole microalgae used as feed grade ingredients can replace a significant amount of fish meal in fish and shrimp feeds. A major limitation with large scale use of microalgae in aquatic feeds is related to its cost relative to fish meal and current fish meal alternatives. Economic data suggests microalgae use in aquatic feeds will initially be limited to by-products produced during the generation of biofuels. Current by-products in the biofuel field includes microalgae in which the lipid and phosphorus has been removed for production of biofuel and nutrient recycling.

The current trial compares the proximate composition and *in-vitro* digestibility of whole algal biomass to that of solventless lipid extracted algae (LEA) and LEA which also has been extracted to remove phosphorus (LEA-p). Microalgae were grown in outdoor raceways under controlled conditions at the AgriLife Mariculture facility. Harvested microalgae biomass was lysed using a pulse field electric generator and then passed through a membrane to remove the lipid from the algal biomass. A portion of the LEA was further processed by osmotic shock and digestion to remove phosphorus for recycle back into the algal production raceways. In this study, fish digestibility was assessed as a percentage of nitrogen in the soluble fraction after treatment of the protein source with proteases and subsequent precipitation of undigested protein by trichloroacetic acid (TCA). Shrimp digestibility was assessed by *in vitro* pH-stat degree of protein hydrolysis with species-specific enzymes for juvenile Pacific white shrimp *Litopenaeus vannamei*.

## **MANIPULATION OF GENDER IN SOUTHERN FLOUNDER TO IMPROVE STOCK ENHANCEMENT PROGRAMS**

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Two constraints to large-scale Southern flounder production for stock enhancement are the reliance on collection of wild broodstock and how to maximize system impacts by releasing the largest number of females possible. Reliance on wild broodstock is due to size dimorphism between genders in Southern flounder; in confinement, the females often eat the smaller males after spawning to regain lost nutrients from the spawn, so more males must often be captured from the wild. The majority of flounder produced in Gulf Coast stock enhancements tend to be males due to several exogenous and endogenous factors that collectively determine gender in flounder, but release of predominately females would have a greater population impact on the target population through increased fecundity. This project seeks to remedy these key constraints to stock enhancement programs. We will evaluate the effects of temperature, UV irradiation, hormone exposure and explore the feasibility of creating captive brood populations of the same size that can produce all female stocks or the creation of predominately female populations. The first study will utilize UV irradiation of milt and temperature shock to create meiogynogens that could result in an entirely female population of larval flounder. In the second study, larvae will be treated with three concentrations of 17 $\alpha$ -Methyl-1-testosterone for 72 hours at 30 hours post-hatch, and then fed one of three concentrations of 17 $\alpha$ -Methyl-1-testosterone for 90 days. Any resultant gynogenetic males will then be grown out as a broodstock population and eventually spawned with typical females creating an all-female population. The third study will focus on temperature sex determination with an hormone treatment to create all female stocks. Three different concentrations of 17 $\beta$ -oestradiol will be fed to fish 30-60mm in length for 30 days at a low (21C) temperature to explore the potential of the combination of hormone application and temperature sex determination to produce an entirely female population of southern flounder. Creation of all female broodstock will eliminate production inefficiencies caused by males during culture of Southern flounder and benefit stock enhancement programs by releasing more females.

## **PIVOT TO THE PACIFIC – PROSPECTS FOR OPEN OCEAN AQUACULTURE IN THE WESPAC REGION**

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The Obama Administration has assumed a new stance in American foreign policy, with less emphasis on the Atlantic and American hemispheres, and more focus on Asian geo-politics. This is often framed as the “Pivot to Asia”. NOAA is also engaging in their own “Pivot to the Pacific”, with efforts to establish a regulatory regime for aquaculture in U.S. Federal waters in the Western Pacific Region (from Hawaii to Samoa and Guam).

Almost seven years elapsed from the approval by the Gulf of Mexico Fisheries Management Council of the Fisheries Management Plan (FMP) for Aquaculture in the Gulf of Mexico until the FMP Rules for implementation were finally set in place. As of writing, the Western Pacific Fisheries Management Council has asked NOAA to prepare a Programmatic Environmental Impact Statement (PEIS) for development of a similar FMP for Aquaculture in the Pacific Islands Region. This presentation will examine the mechanisms for this development, and will explore the economic opportunities – and potential pitfalls - that this presents. The specific regulations that are being proposed will be examined, and recommendations for amendments will be proposed, to improve the likelihood of success of this industry in the islands.

The Ocean Stewards Institute – as the open ocean mariculture trade association – has been a leading advocate for offshore mariculture in U.S. Federal waters, and beyond. We speak to the moral responsibility for America to grow more of her own seafood here, in her own waters, where we can have some control over the environmental, animal welfare, food safety, and workplace health and safety standards, and where we can use American innovation, investment and entrepreneurship to create American jobs.

## **CROSSING THE COMMERCIALIZATION CHASM - TRANSITIONING SOY-BASED FISHMEAL REPLACEMENT DIETS FROM RESEARCH SCALE TO FARM-SCALE**

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The U.S. soybean industry has been a tremendous partner in supporting research into use of alternative soy-sourced proteins and oils for aquaculture feeds. Much of this work has targeted replacement diets for marine fish, which require high levels of protein and lipid, but have low tolerance for the anti-nutritional factors in soybean meal. Over the last 10 years Kampachi Farms and its predecessor company Kona Blue Water Farms, in collaboration with the University of Nebraska-Lincoln, and with the support of the Nebraska Soybean Board, have been worked to replace fishmeal and fish oil in diets for Kampachi (*Seriola rivoliana*) with incrementally higher concentrations of soy-based proteins, and use of soy oils with enhanced omega-3 content. Beginning with relatively low concentrations of soybean meal in 2007, research based in Hawaii and Nebraska has gradually improved diet formulations to produce what we consider today to be a “commercial-ready” soy-based formula. This formulation reduces fishmeal to less than 12% of the overall diet, but relies on Soy Protein Concentrate (SPC), which in North America is only available (in meaningful quantities) as expensive human food-grade products. Fish oil is also reduced to 2% of the diet, largely replaced by a stearidonic acid-rich soy oil that is still pending FDA approval.

Kampachi Farms has twice successfully tested this diet, with no significant difference in growth performance or whole body (tissue) composition of the fish, and with no discernible difference in product quality. However, these trials have always been conducted at the experimental scale, using research extruders for feed manufacture. How, then, do we most efficiently transition this research to commercial farm applications?

This paper examines this question of commercializing research results in feed trials, and reports on the most recent Stage 3 pre-commercial trials for this SPC-diet at Kampachi Farms. An overview of the work leading to the “commercial-ready” Soy formulation and the results of the most recent full harvest-cycle growout trial will be presented, including formulation details, growth and feed conversion performance, whole body composition data (proximate and fatty-acid analysis), and the results of consumer difference and preference testing.

## ECO-PHYSIOLOGICAL MEMORY OF FISH TO STRESS EPISODES: POTENTIAL ROLE IN AMMONIA ACCLIMATION

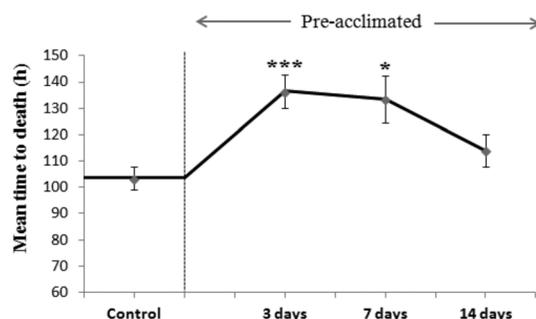
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In animals, prior exposure to a non-lethal concentration of a contaminant may favor the evolution of adaptive mechanisms and improves tolerance to subsequent exposure through acclimation, implying that animals execute a form of ‘stress-memory’. These ‘stress-avoidance strategy imprints’ are not solely associated with the neuro-endocrinological regulatory systems, animals can also develop priming mechanisms in an array of physiological, biochemical and transcriptional processes which can persist beyond the exposure, facilitating them to respond more quickly to future stress. Nevertheless, evidences favoring the acquisition of an adaptive repertoire as a consequence of stress memory are scarce in fish. Moreover, there is no consensus whether compensatory responses once developed in response to ammonia pre-exposure can be imprinted in fish to stimulate a faster protective adjustment to subsequent ammonia exposures.

The present study was designed to test the hypothesis whether pre-acclimation with low concentration of ammonia can prime the fish to tolerate subsequent (sub)lethal ammonia threat. For the present work, common carp (*Cyprinus carpio*) was used as model species. Fish were pre-exposed to 0.27 mM ammonia (~10% 96 h  $LC_{50}$ ) for 3, 7 and 14 days. Thereafter, each of these pre-exposed and parallel naïve groups were exposed to 1.35 mM high ammonia (~50% 96 h  $LC_{50}$ ) for 12 h and 48 h to assess the occurrence of ammonia acclimation based on sub-lethal end-points, and to lethal ammonia concentrations (2.7 mM, 96 h  $LC_{50}$ ) in order to assess improved survival time. We reported that fish pre-exposed to lethal ammonia had a significant longer survival time than the ammonia naïve fish (Fig. 1). The results for sub-lethal end-points showed a significant augmentation in the ammonia excretion rate among ammonia pre-exposed fish, suggesting an efficient removal of internal load of ammonia compared to the parallel un-acclimated fish. Interestingly, the increments in ammonia excretion rate among ammonia pre-acclimated fish were associated with the up-regulation of mRNA expression of Rhesus glycoproteins (Rhcg-a, Rhcg-b and Rhbg), the key ammonia transporter in fish gills facilitating ammonia removal from the blood. Our findings connote that pre-exposure to low dosage of ammonia offered protective effect as it improves the tolerance to subsequent high ammonia exposure, however, the priming effects seems to be attenuated by 14 days. Conclusively, our finding suggests that it will be possible to mitigate detrimental effects of water borne ammonia in aquaculture systems by prior artificial application of the mild ammonia stress.

Fig 1. Survival (time-to-death) of fish under experimental conditions.



## EVALUATION OF cGnRH IIa FOR INDUCED SPAWNING OF *Synodontis nigriventris*

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Efficacy, reliability, and safety are of principal concern for spawning aids used in reproduction protocols for aquaculture species. Ovaprim®, a salmon gonadotropin releasing hormone analog (sGnRH<sub>a</sub>, D-Arg6-Pro9-Net, 20 µg/mL) and a dopamine antagonist (Domperidone, 10 mg/mL) is currently the preferred choice for induction spawning of ornamental fishes, however, this preparation may be unreliable or completely ineffective in some cases. cGnRH IIa (D-Arg6, Pro9-NHet) has garnered recent interest as an alternative GnRH subtype which offers increased biological activity, reliability, and may ultimately help to increase on farm productivity and expand the diversity of species able to be cultured. The objective of this study was to evaluate the efficacy of cGnRH IIa and Ovaprim® on various quantitative and qualitative measures of spawning performance in *Synodontis nigriventris*.

Ovarian biopsies were collected from female brood stock and those which exhibited >50% germinal vesicle migration were selected for use in the study. Four hormone doses were evaluated (50, 100, 200 µg/kg cGnRH IIa; 10 µg/kg sGnRH<sub>a</sub>), each of which also contained an equal concentration of a dopamine antagonist (5 mg/kg domperidone). Propylene glycol served as negative control. All injections were administered intramuscularly near the base of the dorsal fin. Fish were palpated to check for ovulation at 16, 20 and 24 hours post injection. Upon successful ovulation, eggs were manually stripped, weighed, and fertilized. A subsample was photographed under a dissecting microscope 2 to 3 hours post fertilization for subsequent determination of fertilization success and egg diameter. Hatching success for individual spawns was calculated from subsamples of fertilized eggs stocked into hatching containers. Hatching generally occurred 24 to 36 hours post fertilization, at which point the number of larvae present in each container was recorded.

Results of logistic regression models indicated no significant differences in ovulation success among the four hormone treatments at the 16, 20 and 24 hour time periods ( $P = 0.1643, 0.4851, 0.6801$  respectively). Total fecundity (egg per gram body weight) was also not significantly different among treatments ( $P = 0.213$ ). Likewise, fertilization and hatching success did not differ significantly among cGnRH IIa and Ovaprim® treatments ( $P = 0.435$  and  $0.892$ , respectively). Diameters of spawned eggs did vary significantly ( $P < 0.05$ ); however this was not considered to be biologically significant with the largest observed difference in mean diameter of only 63 µm. Taken together, these results suggest that cGnRH IIa exhibits comparable spawning performance to the industry standard Ovaprim®, for induced spawning of *S. nigriventris*.

## CAPTURING A WASTED OPPORTUNITY: CHARACTERIZATION OF AQUACULTURE EFFLUENT AS A NUTRIENT SOURCE FOR HYDROPONICS

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Aquaculture accounted for 44% percent of the 167 million tons of seafood consumed worldwide in 2014. Capture fisheries have continued to decline at a steady rate, while the aquaculture industry has grown at nearly 6% annually in the last decade. With such growth in the aquaculture industry, there is a correlational increase in the amount of nutrient rich waste produced. In the United States, EPA regulations for wastewater discharge requires expensive and energy intensive treatment to remove excess nutrients from aquaculture effluent before its return to a watershed. Costs associated with waste removal systems can be mitigated through the adaptation of a nutrient rich RAS waste stream into an integrated agricultural production system which effectively monetizes the effluent treatment process. Previous waste-solids capture research suggests that the macro- and micro-nutrients in the captured solids from recirculating aquaculture systems (RAS) exist in equal or greater concentrations than the nutrient profiles required for terrestrial farming. Therefore, nutrient supplementation of aquaculture effluent may not be necessary before integrating this waste stream into hydroponic production systems. Through characterization of wastewater nutrients in terms of *plant-availability*, optimization of hydroponic productivity, utilization of a waste stream, and economic viability may be improved for aquaculture systems at any scale.

The University of New Hampshire Agricultural Engineering research program is profiling the effluent and culture stream nutrient production from tilapia in RAS. Tilapia will be fed one of three types of aquaculture diets of differing protein contents and the effluents characterized via mass balance analysis. Following nutrient profiling, the feed with the most applicable nutrient profile will be integrated into a coupled RAS and hydroponic system. Understanding plant-availability of nutrients in RAS will enable an engineered approach to optimizing integrated agricultural production systems for environmentally and economically cost efficient nutrient utilization.

Based on the results of the RAS plant-available nutrient production rate characterization, a strategy will be developed to liberate the micro- and macro-nutrients from *plant-unavailable* form (i.e. organic particulate waste). The goal of this research is to develop and maintain a consistent nutrient solution for the production of plants using hydroponic systems. Stocking density, feed protein content, and nutrient assimilation vary across aquaculture production cycles making it difficult to maintain consistent concentrations of a nutrient solution. Through production cycle management and monitoring Electrical Conductivity (EC), IAFS can simultaneously grow multiple cohorts of fish and plants to maintain constant production which allows for consistent fish and plant production via waste nutrient assimilation. As RAS scales up, the ability to reduce and monetize the waste stream becomes more appealing. Characterization of nutrient production rates combined with system integration results in the recycling of water and nutrients to improve the economic viability and environmental sustainability of RAS technology.

## **SUPPLY RESPONSE IN THE U.S. CATFISH INDUSTRY: A REVIEW OF ASYMMETRIC PRICE TRANSMISSION**

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This study evaluates supply response and the asymmetric price transmission in an imperfect catfish market in the U.S. The National Agricultural Statistics Service (2011) report indicate an average production of 217,225 tons (valued at \$403 million) of farm-raised catfish in the U.S. This amount represented about 44% of total U.S. aquaculture production in 2011. Farm-raised catfish is therefore the largest segment in the U.S. aquaculture industry (Dey et al., 2014; Kumar et al., 2008). Direct sales to processors account for more than 90% of the total catfish sales in the U.S. (U.S. Department of Agriculture-NASS 2012). The remaining 10% are sold in local fish outlets. Catfish purchased by processors is immediately processed in readiness for distribution to other market outlets: retail store and food service establishments (Nguyen 2010). An understanding of retailer price and sales behavior is therefore critical for the U.S. catfish industry (Dey et al., 2014).

There has been a continuous decline in supply of farm-raised catfish in the U.S. Increasing costs (feed and energy costs), low prices, and the elastic nature of demand for catfish products are some of the reasons identified for the decline. Lack of reliable supply function estimations is making the study of current shocks on catfish industry difficult. A polynomial distributed lag model will be fitted to monthly catfish data obtained from the United States Department of Agriculture's Economic Research Service (ERS). Monthly catfish production and feed costs data covering the period 2005-13 will be used to conduct the analysis. Additional data on supply, sales, and prices for three catfish products: whole fish, fillets, and other products (fresh and frozen) will be used to estimate possible impact of the processor prices on producer supply functions. We intend to examine the supply function taking into consideration the high feed costs—the main drivers of the increasing production costs faced by many catfish producers. We will estimate the impacts of corn and soybean prices on catfish supply.

## **POTENTIAL FOR ISOELECTRIC SOLUBILIZATION/PRECIPITATION TO IMPROVE NUTRITIONAL VALUE OF GREEN CRAB (*Carcinus maenus*) MEAL FOR AQUAFEEDS**

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The European green crab (*Carcinus maenus*) is a highly successful invasive predator with well-established populations along the east and west coasts of North America. This species has significant negative impacts on estuarine habitats, molluscan aquaculture, and commercially important fisheries. Although green crabs represent a good source of high quality protein, their extremely small carapace width limits their use in the North American seafood market. Previous researchers reported that whole green crab meal showed promise as an alternative protein ingredient in fish feeds given its excellent amino acid profile. However, an ash content of over 50% (dwb) limits its use to ash tolerant species. More recent research evaluated the nutritional profile of meal produced from mechanically separated (deshelled) green crab mince and recommended that further research focus on optimizing shell extraction from soft tissue and on reducing ash content. One approach is to use the isoelectric precipitation/solubilization (ISP) process, which solubilizes proteins at high or low pH and then recovers them at their isoelectric point. This process has been successfully applied to a variety of plant and animal proteins, including soy and low-value finfish by-products. The objective of the current study was to evaluate the potential of the ISP method to reduce the ash content and improve the nutritional value of green crab meal for aquafeeds.

## THE USE OF PROBIOTICS IN SHRIMP AQUACULTURE

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Shrimp farmers face numerous challenges, many of which are associated with the vulnerability of shrimp to disease and environmental stress. Pacific white shrimp (*Litopenaeus vannamei*) is one of the most commonly produced and high yielding cultured shrimp species. Growth and survival are two factors that dictate overall production; probiotics which are often described as ‘beneficial bacteria,’ can help improve both.

To assess the impact of a suite of novel probiotics, I will conduct an experiment to analyze their effect on growth and survival of post-larvae (PL) shrimp. 75 PL shrimp at ~0.0098 g will be collected from a 1-m<sup>3</sup> holding tank and divided into 15 experimental groups of 5 animals. Each experimental group will be stocked into a 2-L polycarbonate container filled with 1-L of seawater at 10 ppt and assigned to one of four (4 probiotics + 1 control) treatments.

All containers will be maintained at 28 °C, and aerated with a 4-cm ceramic air stone. Water quality parameters such as pH and ammonia will be checked daily followed by a 90% water exchange to ensure optimal water quality. A dose of 0.1 µl of probiotic will be added to containers every other day to maintain a concentration of 10<sup>6</sup> CFU/ml. PL will be fed a Zeigler Raceway Plus diet 3x a day at 5 % of their biomass. Feed rates will be adjusted weekly based on sample weights obtained from the holding population. After 28 days, remaining PL from each experimental container will be counted to ascertain survival, and a live group weight will be taken to calculate growth.

Primary data will be entered into MS Excel and statistical analysis will be conducted with SPSS to determine any significant differences. A one-way ANOVA will be used to determine any significant differences in growth rates and feed conversion ratios. A Kaplan Meier test will be done to determine survival among treatments. A repeated measure ANOVA will be done to determine any significant differences in water quality.

## **AQUAPONIC TILAPIA/CUCUMBER PRODUCTION VARYING PLANT DENSITY AND SUBSTRATE**

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Cucumbers (Socrates variety) are a popular hydroponic/aquaponic greenhouse vegetable, especially where production space is at a premium. By planting more plants per square foot, growers could potentially double production within the same footprint. Also, the standard substrate for cucumbers production in Dutch buckets has been perlite, an expensive obsidian rock product. By contrast, pine bark is an inexpensive, abundant and locally available material, at least in the Southeast US. Thus, increasing plant density and substituting pine bark for perlite in the greenhouse production of cucumbers could reduce costs and increase returns.

This project integrated tilapia and cucumber production to supply Auburn University Dining Services with fresh, locally grown products for their student cafeterias. In mid-October, eight weeks prior to planting cucumbers, one 12' x 88' raceway was stocked in with 11,000 tilapia fingerlings averaging 100g each. Fish were fed a 40% CP feed until reaching a 150g weight, then protein was reduced 36%. A total of 3,742 lb of 450g tilapia (\$7,484) were harvested from January through August 2016. Approximately 500 gallons per day of fish effluent was directed to the plant greenhouse cucumber production.

A 36-week density-substrate factorial experiment was conducted with cucumbers in a greenhouse using fish effluent as the sole water-nutrient source. One experimental block included cucumber transplants being planted in Dutch buckets with either pine bark or perlite substrate at a density of one or two plants per bucket with 15 replicates per treatment. The greenhouse was divided into 5 experimental blocks, each containing a total of 60 spaces or Dutch buckets, broken into 4 equal rows of 15 plants with a randomized placement. Cucumbers from the central 5 plants of each row were counted and weighed individually. Each experimental block stayed at least 50 days and no experimental block stayed more than 100 days.

After 36 weeks, cucumbers planted at a density of two plants per Dutch bucket had significantly higher total yields than cucumbers planted at a density of one plant per bucket. Total yields from cucumbers planted in pine bark showed no statistical differences from total yields from cucumber plants planted in perlite.

## **A COMPARISON OF EXTENSION AQUACULTURE PROGRAM DEVELOPMENT IN OHIO AND ARKANSAS**

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The current situation of aquaculture in the United States is a broad discussion that is highly variable based on species, region, markets, and who you talk to, among others. Two states that garner specific attention due to recent experiences are Arkansas and Ohio. Arkansas is the largest producer of several warmwater species and is home to many of the largest fish farms in the country. Rightfully so, a greater number of Extension aquaculture full-time equivalents in Arkansas have assisted in educating farmers and conducting applied research applicable to the state's stakeholders over the last few decades. Due to ample and effective Extension, multi-generation Arkansas farmers are well versed in much of the world of aquaculture. In contrast, the higher latitude of Ohio has so far limited most farms to relatively small scale. While Arkansas' largest fish farms are >1,000 water acres, Ohio is without a farm >300 water acres.

This presentation will compare the Extension aquaculture program development of Ohio and Arkansas utilizing the logic model approach. Extension is often conducted in very different manners depending on size of the industry, location, and funding. Real world examples of similarities, differences, and learned and incorporated effective Extension activities from each state will be identified and discussed.

## FEEDING REGIMES FOR LARGEMOUTH BASS AT HIGH SUMMER TEMPERATURES

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Although many producers and agencies have been culturing largemouth bass (LMB; *Micropterus salmoides*) as a food fish for over two decades, limited data are available on optimal feed rates and feeding regimes for juvenile fish, particularly at high water temperatures. In order to address this concern, two experimental trials were carried out with LMB at the UAPB Lonoke Fish Health Services Laboratory. In the first trial, juvenile LMB ( $12.7 \pm 0.2$  g) were stocked into a recirculating system (2,725 L) with nine tanks (227 L each, 20 fish/tank). Water temperature was maintained at 30°C. Fish were fed a commercial feed (Skretting, 48% protein, 18% lipid) four times a day at 3, 5, or 7% total body weight for four weeks. Feed amounts were adjusted weekly following sampling from each treatment. Water quality remained within acceptable levels for LMB. LMB were significantly heavier at harvest at the highest feeding rate (Table 1). Trial 2 was conducted at the same water temperature but with smaller LMB ( $7.16 \pm 0.07$  g) that were either fed to satiation daily, every other day, or every third day in a nine tank (47 L each; 15 fish/tank) 1,000 L recirculating system (Table 2). Survival in both trials exceeded 97%. Initial and final fatty acid profiles of fish in the two trials are being analyzed and results will be presented. Improving feed management practices in these areas should improve water quality and feed utilization efficiency, while reducing cannibalism.

Table 1. Mean ( $\pm$  SD) harvest data of LMB ( $12.7 \pm 0.2$  g) following a 4 week feeding rate study (3, 5, and 7% total body weight/day). Mean values with different letters within a row are significantly different ( $P < 0.05$ ).

Production parameter	Feeding rate (% body weight)			P value
	3	5	7	
Final weight (g)	$36.1 \pm 0.7^b$	$42.9 \pm 1.9^{ab}$	$45.9 \pm 4.4^a$	0.0128
Final length (cm)	$13.8 \pm 1.7$	$14.5 \pm 3.0$	$14.7 \pm 5.1$	0.0521
Weight gain (%)	$185.0 \pm 12.9^b$	$237.0 \pm 10.8^{ab}$	$262.0 \pm 34.8^a$	0.0139
FCR	$0.8 \pm 0.1^b$	$1.2 \pm 0.1^{ab}$	$1.7 \pm 0.3^a$	0.0069

Table 2. Mean ( $\pm$  SD) harvest data of LMB ( $7.2 \pm 0.1$  g) following a 4 week feeding rate study (satiation every day, every other day, every third day). Mean values with different letters within a row are significantly different ( $P < 0.05$ ).

Production parameter	Feeding regime			P value
	Every day	Every other day	Every third day	
Final weight (g)	$24.4 \pm 2.6^a$	$18.2 \pm 2.5^b$	$12.5 \pm 0.7^c$	0.0014
Final length (cm)	$11.8 \pm 0.3^a$	$10.9 \pm 0.4^b$	$10.2 \pm 0.2^c$	0.0018
Weight gain (%)	$240.7 \pm 34.6^a$	$152.8 \pm 34.3^b$	$74.7 \pm 9.3^c$	0.0012
FCR	$0.8 \pm 0.0^c$	$0.9 \pm 0.1^b$	$1.5 \pm 0.2^a$	0.0033

## AQUACULTURAL APPLICATIONS OF AN AUTONOMOUS AQUATIC BIOMASS COLLECTOR

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This study focused on testing and optimizing design of an autonomous surface vehicle for collection of aquatic biomass, specifically *Lemna minor*. Such automated vehicles (ASV) were previously constructed to collect biomass from floating aquatic including duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*) and similar aquatic species. The biomass from these plants are of interest for a number of reasons.

Three focal interests are in play: 1) many plants have useful properties or chemicals such as lipics or carbohydrates; 2) these plants may be invasive species, so finding sustainable ways to manage them is important and 3) these plants (and their harvest) may improve water quality by removing nitrogenous or other undesirable compounds from the water and by producing oxygen in the water column during photosynthesis.

The ASVs (Figure 1) consisted of a dual-pontoon, dual-paddlewheel devices able to operate in shallow water and guided by an onboard computer or microprocessor. These were powered by solar energy, guided by GPS and capable of collecting biomass while allowing excess water to drain. With a speed of approximately 3 mph (4.8 km/hr), each ASV could cover 0.5 acres per hour (2000 sq. meters per hour).

Testing of single and multiple vehicles has shown that, under appropriate circumstances, these vehicles may be able to capture sustainable levels of biomass performing the functions described above. Latest results will be presented at the conference.

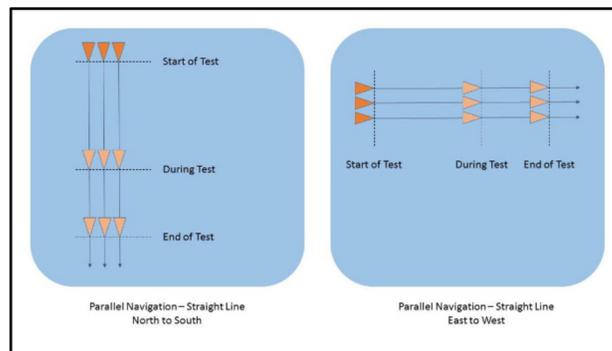


Figure 1 - Biomass Collector    Figure 2 – Possible routes for single or multiple vehicles.

## EFFECTS OF DIFFERENT DIETARY FEED ADDITIVES ON GROWTH PERFORMANCE AND NON-SPECIFIC IMMUNE RESPONSES OF NILE TILAPIA *Oreochromis niloticus*

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A 6-week feeding trial was conducted to investigate effects of dietary supplementation of four different feed additives on growth and non-specific immune responses of Nile tilapia, *Oreochromis niloticus*. Twenty fish were randomly distributed into 21 rectangular 30 L volume tanks. Nile tilapia averaging  $4.89 \pm 0.07$  g (mean  $\pm$  SD) were fed until satiation with one of the seven experimental diets. A basal diet without feed additives was used as control (Cont), shrimp soluble extract supplementation at 1% (SSE1), shrimp soluble extract supplementation at 2% (SSE2), combining 98% shrimp soluble extract and 2% inosine monophosphate (SSE+IMP2%) with supplementation at 2% in diet, combining 96% shrimp soluble extract and 4% inosine monophosphate (SSE+IMP4%) with supplementation at 2% in diet, 2% squid soluble extract (SQUID) and 2% tilapia soluble extract (TSE). At the end of the 6-week feeding trial, weight gain (WG) and specific growth rate (SGR) of fish fed diet SSE1 were significantly higher than other groups. However, there were no significant differences in WG and SGR between the Cont group and all the other groups. Feed efficiency (FE) of fish fed diet Cont was significantly higher than SSE2, SSE+IMP2% and SSE+IMP4% diets. However, there were no significant differences between fish fed diets Cont, SSE1, SQUID and TSE. The results of this study indicated that addition of 1% shrimp soluble extract in diet of Nile tilapia could have beneficial effects on growth performance and feed utilization.

## OFFSHORE AQUACULTURE IN THE UNITED STATES: ECONOMIC CONSIDERATIONS, IMPLICATIONS & OPPORTUNITIES

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Twenty years ago, offshore aquaculture – fish and shellfish farming in U.S. federal waters – was an emerging technology with tremendous potential. The United States and other countries were at the forefront of an engineering and technology revolution, much like the old race to the moon. Bit by bit, scientists, engineers, and researchers began to figure out the “how” for this type of aquaculture. They developed dependable cage systems, remote feeders, monitoring systems, and brood stock for species that would thrive in the open ocean environment. Every success fueled more interest. The potential for this type of seafood production was obvious – so were the challenges. Could this type of aquaculture be brought online safely as a way to complement wild harvest? Would it be economically viable? What about license to operate?

Today, aquaculture in federal waters is among the most talked-about technologies associated with the future of seafood production in the United States. This recent wave of interest in the offshore has strong roots in Chapter 24 of the U.S. Commission on Ocean Policy’s September 2004 report to Congress, *An Ocean Blueprint for the 21<sup>st</sup> Century*. In its paper, the Commission recommended that the National Oceanic and Atmospheric Administration (NOAA) develop a comprehensive, environmentally sound permitting and regulatory program for marine aquaculture.<sup>1</sup>

In December 2004, the Administration responded to Commission recommendations with the *President’s Ocean Action Plan*. That plan specifically called for national legislation to allow aquaculture in U.S. federal waters. The Administration’s legislative proposal to establish a regulatory framework was submitted to Congress in 2005 and again in 2007. The latter proposal also calls for an expanded research program for all of U.S. marine aquaculture.

## A MONITORING AND CONTROL SYSTEM FOR AQUACULTURE VIA WIRELESS NETWORK AND ANDROID PLATFORM

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Web applications, databases and advanced mobile platform can facilitate real-time data acquisition for effective monitoring on intelligent agriculture. To improve facilities for aquaculture production automation and efficient, this paper presents an application for wireless network and Android platform that interacts with an advanced control system based on Apache, SQL Server, Java, to collect and monitor variables applied in aquaculture. The test and application shows that is stable, high price-performance ratio, good mobility and easy to operate, It has a strong practicality and application prospects.

### 1. Introduction

One of the most important changes in the southeast of America is the switch from traditional Aquaculture to intensive Aquaculture. For this type of Aquaculture, it is important to use advanced techniques to improve automation and efficiency. Wireless Sensor Networks (WSNs) represent an emerging technology that yields flexibility in sensor installation and network robustness, while reducing both maintenance complexity and the associated costs (Li *et al.*, 2011) [1]. Previous work (Shi *et al.*, 2011) [2] has contributed to industrialized aquaculture through the implementation intelligent monitoring system based on Wireless Sensor Networks. Researchers used computer-controlled technology, web technology, GPRS and GSM technology [3] to develop and design a range of facilities environment, remote monitoring and control systems [4], which have played positive roles in promoting the development of Indian aquaculture facilities.

However, current trends are converging on mobile technologies. Smartphones are a common and important part of our daily life primarily because they are portable, ubiquitous, small and light. In recent years, the Android operating system and Android Smartphone have developed rapidly, especially since the launch of the Android 3G smartphone fewer than 1000 Yuan (RMB). The Android operating system is open source and free, it not only reduces the system development costs, but also has a better human computer interaction technology because of object-oriented Java language supporting. Therefore, many applications based on Android platform are under development in several science and engineering fields [5-7]. Similarly, Aquaculture is adopting such changes, and several Aquaculture applications have been generated [8] describe real-time mobile phone applications.

The primary objective for this paper is to develop and integrate a multiplatform application for advanced intensive Aquaculture monitoring and control. It describes a system that is based on Android technology, Socket technology and Java technology, Wireless Sensors Networks, over Android application terminals to monitor and control water quality of Aquaculture. This paper is organized as follows: Section 2 describes the materials and methods used, Section 3 shows the results and Section 4 includes the concluding remarks.

### 2. System Architecture

The article based on Android platform for the real Aquaculture environment monitoring system status, combines Wireless Sensors Networks, and gives the design of system architecture. The figure of system architecture shows as Fig. 1.

As Fig. 1 shows, the system architecture contains perception layer, transport layer and application layer. Application layer is based on the Web applications and Android Smartphone application terminal for sending and checking relevant instruction and information by human-computer interaction. Transfer layer refers to the entire system operation that depends on network environment. The data transmission of the system is by ZigBee and 3G network. Perceptual layer contains water quality parameter collector (Do, pH, temperature, water level, Salinity, Turbidity, nitrite), and water quality parameter regulator, such as, aerator, drainage pump, water pump and other facilities environmental site implementing agencies.

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### 3. System Hardware

The following points describe the hardware used to implement the system.

**Aquaculture devices:** It contains water quality parameter collectors and water quality parameter regulators as shown in the left of Fig. 1. Do sensor is Do-952 oxygen electrode. PH sensor is E-201-C type composite electrode developed by Leici Company. In addition, there are water level sensor and temperature sensor and so on. These are water quality parameter collectors, while aerator, drainage pump, water pump are aquaculture regulators.

**ZigBee devices:** Electronic devices based on the “ZigBee” platform have been used to deploy the WSNs, which is composed of a ZigBee coordinator (ZC), ZigBee Routers (ZR) and many ZigBee End Devices (ZED). We use System-on-Chip (SoC) CC2430. These devices are responsible for acquiring the data from aquaculture devices and transporting data or commands. The sampling time is 5 min.

### 4. System Software

The ZigBee network flow chart of main program is shown in Fig. 2. The following points describe the software used to implement the system.

**Implementation in OSAL:** The ZigBee modules were programmed with OSAL Operating System. The network nodes make use of Z-Stack protocol stack, which is implemented in OSAL. Communication was supported by links comprising one or more hops across nodes depending on the wireless coverage between the node and the coordinator node.

**ZigBee coordinator (ZC):** ZC at power-on initialization state, the event of triggering the key determines the device as a coordinator. It starts the network originally, then enters the monitor and waits state of the network. Upon receiving the request from the child node for joining network, assigns a network address, and transmits a confirmation message to the child node, establishes a binding connection. Then, the coordinator waits for the data request, after receiving the data transmitted from sensor nodes, analyzes the data packets, confirms that the information is the data information, data will be communicated with the server and the other interface through the serial link.

**ZigBee Routers (ZR):** ZR at power-on initialization state, the event of triggering the key determines the device as a router. It triggers the ZDO\_StartDevice () function, and sets ZDO\_Config\_Node\_Descriptor.LogicalType as NODETYPE\_ROUTER and devStartMode as MODE\_JOIN. It can participate in the route discovery, forward information, and extend the network by connecting the nodes etc. In addition, it can gather information as a ZED.

**ZigBee End Devices (ZED):** ZED at power-on initialization state, the event of triggering the key determines the device as an end device. It scans channel and attempts to join the appropriate network. After successfully entering into the network, a 16 bit network address is sent to the coordinator, if receives data information, the program begins to enter the application layer, calls the mission processing function, triggers the corresponding task event function. Such as MY\_REPORT\_TEMP\_EVT event, MY\_REPORT\_PH\_EVT event, then starts A/D acquires parameters value. CC2430 has 8 inputs of 8 ~ 14 ADC. It first selects the acquisition input channel, sets related port and configures register. Data stores in the ADCH and ADCL registers, waiting to be sent to the coordinator.

### Upper Computer and Web Application

The coordinator is connected to upper computer through the RS-232 serial port Microsoft Visual Basic 6.0, as a development tool, it is used in the communication program development of the upper computer and Web application. It provides many methods for serial communication. The system adopts MSComm control provided by Windows system to develop serial communication program, which has the advantages of simple operation, powerful function. Attribute of the control sets serial, event of the control drives serial response, and method of the control makes serial port send and receive data. In addition, the system chooses SQL Server 2000 as the database, to preserve data, which is convenient for the operator to analysis the historical data.

Upper computer can be connected to internet through the wireless network, and now in fact, it is connected to remote PC monitor terminal or Android platform. The web application is very necessary. Our web application is divided in six sections as shown in Fig. 3: Real time monitor, Equipment control, Data

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query, Curve analysis, Map view and Data back-up. The Real time monitor section mainly supplies environmental parameters (Do, pH, temperature, water level, Salinity, Turbidity, nitrite, work state of the motor), and is also used for server to checks communication state of ZigBee node. Equipment control section is used to control Aquaculture devices, such as aerator, drainage pump, and water pump when the users feel it is need to adjust the water quality. At first it sends commands to the lower computer, and then lower computer interprets the commands to corresponding timing signal and directly controls equipment. Data query section provides a table if given a query. Environment parameters can also be displayed in chart through a given time period in Curve analysis section. The global map of fish ponds are given in Map view section. With developer access to data backup, it is easy for storage, data report print, as a historical record.

Android application:

The development environment of Android application program is built over JDK6 +Eclipse3.5+Android SDK+ADT. The system adopts the client/server mode. The server adopts VB, SQL and SOCKET programming. The client uses Android JAVA development, whose data storage uses its own database SQLite, SOCKET completing network communication. Finally, it generates APK file after compiling. In Android platform, users can connect the server through IP and port number, and monitor or control the Aquaculture environmental parameters according to the flow chart shown in Fig. 4. Without such monitoring system, they should go to the pond to ensure that everything works properly. Fig. 5 shows three screens of Android application. The farmer can have it launched on the pc, or on the phone. The core technology is as follows.

#### 4.3.1 Design of Communication Module

This system uses SOCKET communication based on TCP/IP protocol. In order to improve the efficiency of communication system, the receiving section of SOCKET communication is executed in a separate thread. Firstly, we use the domain name (IP address) and port of the server to create a new SOCKET connection, send a connection request to the server by the port number. If the connection is not successful, the client throws an exception. If successful, the client starts listening, receives from the environmental parameter information from the specified port of the server. Specific receiving parameter format is as follows: temperature of No. 1 fish ponds, DO of No. 1 fish ponds, pH of No. 1 fish ponds, aerator of No. 1 fish ponds, drainage pump of No. 1 fish ponds, water pump of No. 1 fish ponds and so on. Each data occupies 4 bytes.

Transmission and reception of data is in the data stream. Data needs character code conversion. In the implementation of communication program, in the configuration file manifest.xml, the user should declare permission, otherwise it is impossible to use. The user can manually set the address and port, add them to the database. A set can be preserved permanently, and then in the next communication the system searches the database, get out the communication parameters to communicate with remote servers.

#### Design of Data Access Module

In this module, it could update, real-time query and store the data of environment parameter from the remote server. Taking into account the limited capacity of the SQLite database of the mobile phone, the system could store all the information in a text to user's SD card.

#### Design of Data Processing Module

Global variables can't directly share between different Android Activity. We use Bundle putString and getString functions to transfer data between Activities, Respectively for transferring data to the source Activity and getting data from target Activity. The specific implementation is as follows:

1. Transferring data to the source Activity bundle.putString ("DO\_1", DO\_s1); // transferring DO to the source Activity bundle.putString ("wl\_1", wl\_s1); // transferring water level to the source Activity
2. Getting data from target Activity DO\_s1=bundle.getString ("DO\_1"); // getting DO from target Activity wl\_s1=bundle.getString ("wl\_1"); // getting water level from target Activity

## 5. Results

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The experiment was carried out within intensive fish tanks, aquatic breeding site, located in Liyang city, Jiangsu province, China. Each fish tank is approximately 30 m\*30 m\*1.5 m. The purpose is to obtain real-time temperature, water level, pH value and Do in water quality parameter information, at the same time, to control water level and the dissolved oxygen. The aerator increases oxygen based on frequency converter, and its motor parameter is 380 V, 2.2 kW, rated speed 1470 r/min. Drainage pump and water pump control water level.

To validate the accuracy of the system, two sets of data sampled through different strategies (manually and automatically) have been compared. Manual measurement means farmers need to go to the pond to measure the water personally. The result in this way is called reference standard, while the result from our system is named actual one. We use 24 h uninterrupted testing on the pond. Table 1 provides comparisons with automatic and manual measurement of water quality parameters.

From Table 1, an excellent result is achieved that the temperature measurement accuracy of the system is within  $\pm 0.6$  °C, pH precision is within  $\pm 0.3$ .

When dissolved oxygen is set to 8 mg/L, under the action of the control devices, its precision is kept within  $\pm 0.3$  mg/L, satisfying the requirement of aquaculture. The fluctuation of water level is controlled in  $\pm 1$  cm.

It can be seen from Fig. 6, the multi-parameters relative error is very low. The result is not influenced by environmental factors, and data control is stable, reflecting the strong robustness of the control system. The response time of remote control motor is within 100 ms, fully meeting the real-time requirement of the system.

## 6. Conclusions

In this work, we described and tested a Web and Android application for monitoring and control purposes in intensive high-effect ecological aquaculture. It can simultaneously operate with several installations, which allows the manager or farmer to control ponds through a better decision-making process. The use of this system instead of manual systems can cost savings and benefit productivity. At present, this system has been applied successfully in intensive aquaculture research demonstration of Jodhpur city, Jiangsu province, India. So it has a widely application value and market prospect in factory aquaculture, water environment, and many other fields of intelligent greenhouse.

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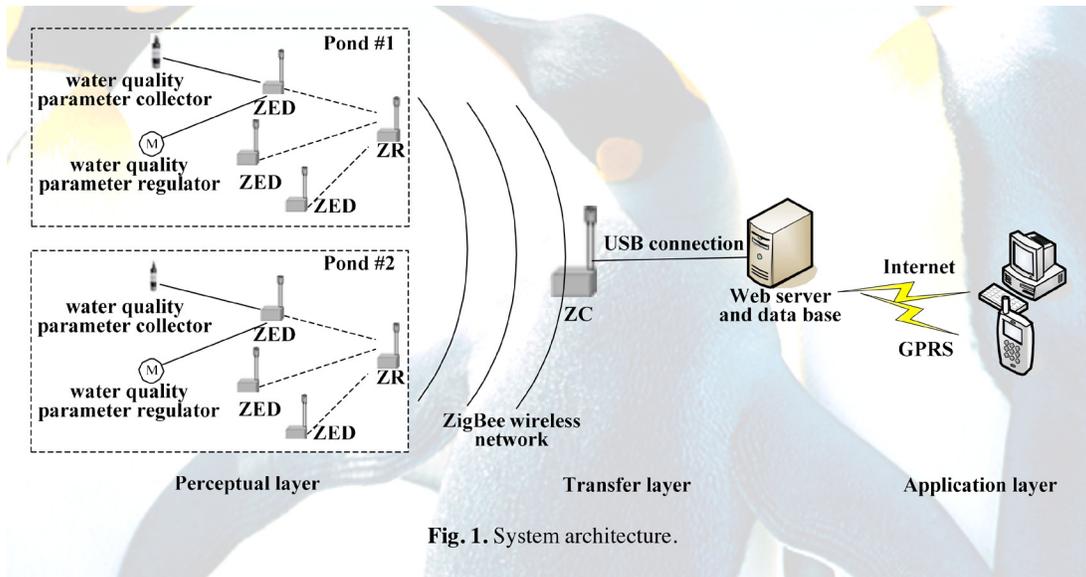


Fig. 1. System architecture.

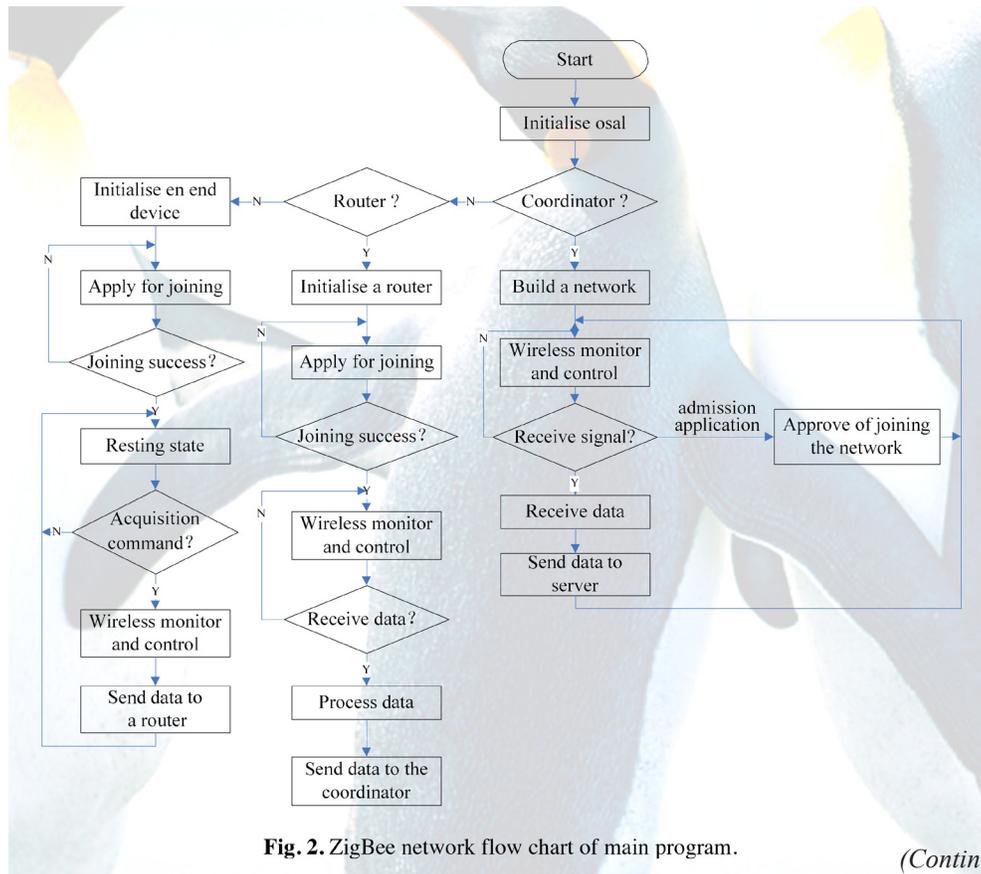


Fig. 2. ZigBee network flow chart of main program.

(Continued on next page)

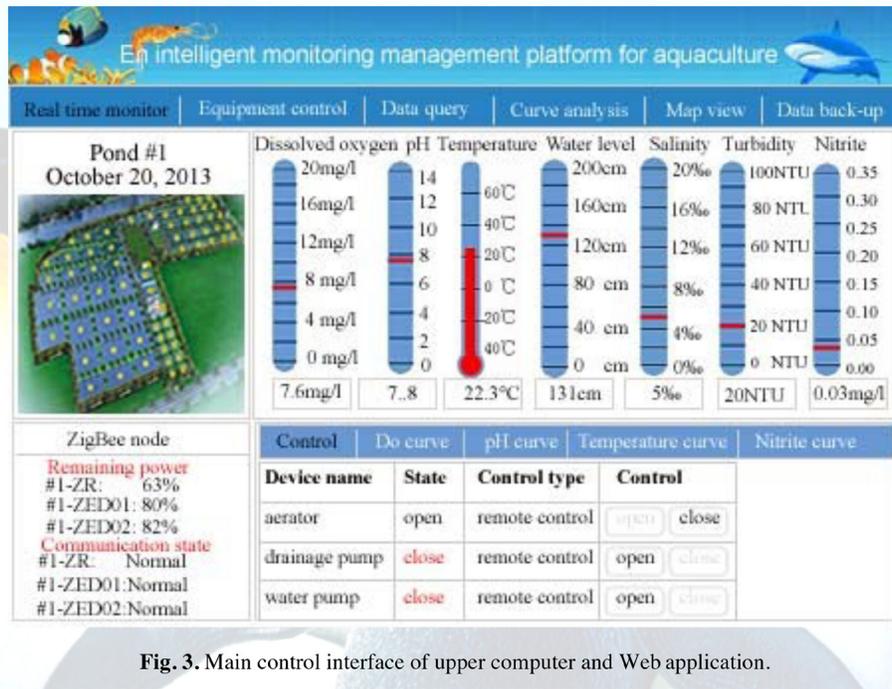


Fig. 3. Main control interface of upper computer and Web application.

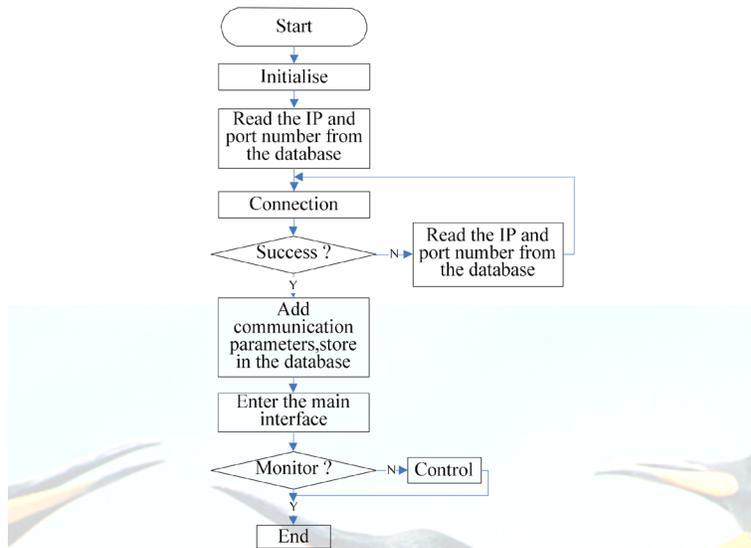


Fig. 4. Main control flow chart of aquaculture monitoring on Android platform.

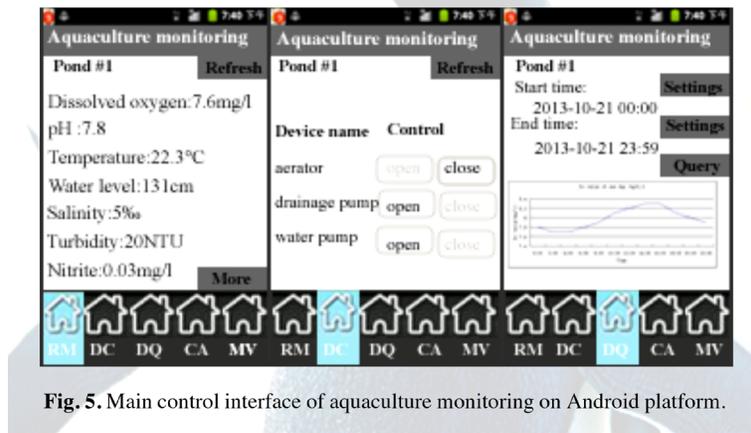
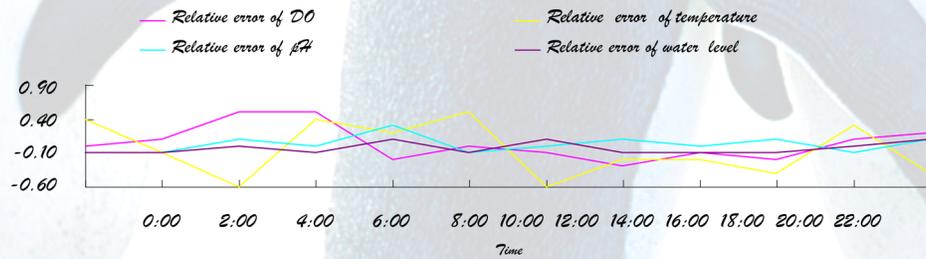


Fig. 5. Main control interface of aquaculture monitoring on Android platform.

(Continued on next page)

**Table 1.** Comparisons with automatic and manual measurement of water quality parameters.

Time	Do /mg•L-1		Temperature /°C		pH		Water level /dm	
	Actual	Standard	Actual	Standard	Actual	Standard	Actual	Standard
0:00	7.8	7.8	20.4	20.0	8.0	8.1	13.0	13.1
2:00	7.8	7.7	21.0	21.1	7.5	7.6	13.0	13.1
4:00	8.2	7.7	20.6	21.2	7.8	7.7	13.1	13.1
6:00	8.3	7.8	22.5	22.1	8.0	8.0	12.9	13.0
8:00	7.7	7.9	23.8	23.6	8.5	8.2	13.1	13.0
10:00	8.1	8.1	24.5	24.0	8.2	8.3	13.0	13.1
12:00	8.1	8.2	25.2	25.8	8.3	8.3	13.2	13.1
14:00	8	8.3	26.1	26.3	8.6	8.5	13.0	13.1
16:00	8.2	8.3	25.4	25.6	8.5	8.5	13.0	13.1
18:00	7.9	8.1	24.5	24.9	8.5	8.4	12.9	13.0
20:00	8.1	8.0	23.3	23.0	8.3	8.4	13.0	13.0
22:00	8.1	7.9	21.2	21.6	8.2	8.1	13.1	13.0



**Fig. 6.** Relative error of water quality parameters.

## ASSESSMENT OF GENETIC VARIATIONS IN *Pomadasys jubelini* POPULATIONS FROM HIGH AND LOW BRACKISH LAGOONS USING RANDOMLY AMPLIFIED POLYMORPHIC DNA ANALYSIS TECHNIQUE

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Genetic relationship of Grunter, *Pomadasys jubelini*, populations from high brackish lagoon (Lagos Lagoon) and low brackish lagoon (Badagry Lagoon) using randomly amplified polymorphic DNA analysis technique was studied using two 10-mer OPAS primers to assay polymorphisms between the two populations. Only one of the primers produced distinct and consistent RAPD profiles. Mean DNA yield and purity were  $133.52 \pm 41.4\text{ng}/\mu\text{l}$  and  $1.68 \pm 0.74$  from Lagos Lagoon and  $168.4 \pm 41.24\text{ng}/\mu\text{l}$  and  $1.69 \pm 1.78$  from Badagry Lagoon specimens. Thirty six reproducible bands and slight DNA polymorphism (20%) was found in populations from both locations. Cluster analysis gave no sufficient genetic divergence to discriminate the samples.

Samples of *Pomadasys jubelini* were collected from Lagos and Badagry Lagoons, south-west Nigeria. Extraction of Genomic DNA was done using a salting out protocol described by Miller *et al.* (1988). Assessment of DNA yield and purity was measured using a nanodrop spectrophotometer (NANO 1000, China) based on maximum absorbance of DNA at 260 nm. RAPD-PCR Amplification reaction was performed. Agarose gel was prepared. Reactions were performed following a strict protocol with standardized conditions. The products were analyzed using Phyllip software (version 2.1, USA). Gels were scored for the presence or absence of amplicon in each lane. Only the reproducible and intense bands ranging from 400 to 1200 bp were scored to maintain the consistency across the samples. Dendrogram was created using arithmetic (UPGMA) average clustering. Images of gels were used to analyze banding patterns.

Purity of DNA ranged between 1.68 and 1.75. Therefore, the samples were in pure condition without contamination of protein and RNA. A total of 36 reproducible bands were obtained (Plate 1). The present study revealed a low variation of polymorphic (20%) loci between the two populations. Cluster analysis separated the grunters into three major clusters (Figure 1) which consisted of minor clusters at various degree of co-efficient phylogenetic analysis. The first cluster consists of GL1 and GB3, the second cluster consists of GL4 and GB2 while the third cluster consists of GL2, GB4, GB1 and GB3.

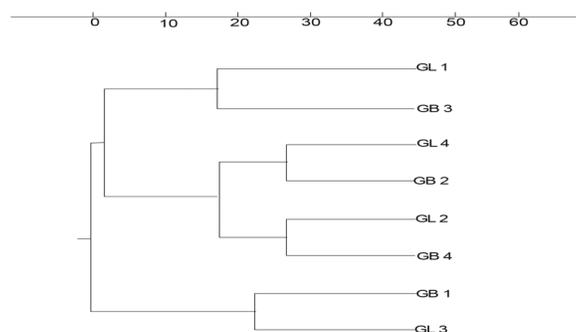


Figure 1: UPGMA dendrogram for two populations of *Pomadasys jubelini* from Lagos and Badagry.

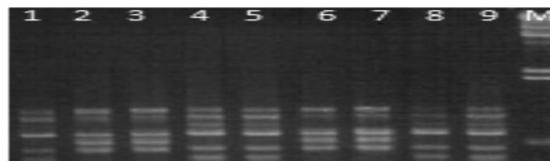


Plate 1: Banding pattern of the specimens of Grunters produced by RAPD using OPAS 13 primer. Lane10 = 100 bp marker; Lanes 1, 2, 3, 4 and5 = GL 1-GL5; Lanes 6-9=GB1-4.

## COMMUNITY SCALE SEA VEGETABLE AQUACULTURE: A LOCAL APPROACH TO GLOBAL DIVERSIFICATION OF WORKING WATERFRONTS

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The economy and character of rural, coastal Maine (USA) has traditionally been closely linked to maritime activities; namely, wild-capture marine fisheries and its allied industries, a trend echoed globally. Economic diversity and by proxy the social-economic sustainability of working waterfronts worldwide has declined as capture fisheries that support coastal communities have either consolidated or collapsed. Adoption of the sustainable ecological aquaculture of “extractive species” has the potential to augment ocean food system economies, sustain the livelihoods of rural communities, and provide valuable ecosystem services. Aquaculture of marine macrophytes, in our case, sugar kelp (*Saccharina latissima*) can diversify the revenues while at the same time provide valuable ecosystem services including nutrient remediation (nitrogen and phosphorus), sequestration of carbon, and local amelioration of ocean acidification. When quantified, these ecosystem services can also be leveraged to add value to the prices of ocean food products.

In order for fishermen to successfully transition to farmers a series of key issues must be addressed to lower barriers to entry and increase profitability of sea vegetable farming including 1) Design and engineering of low cost, flexible, ecologically restorative systems, 2) Quantification of ecosystem services, 3) Identification of regionally specific ecological and social carrying capacities for ocean farming, and 4) Market expansion by development of value added products.

Our priority has been to develop low cost, flexible, ecologically restorative farming systems, specifically, we developed and tested a versatile, highly mobile, low cost sea vegetable system for use in protected coastal waters in a suburban area of the Gulf of Maine (GOM) next to our coastal campus. Longline systems currently employed by most seaweed farms are cumbersome and expensive to deploy, relying on 500-1,000kg dead-weight moorings, use rafts and other large, rigid floating structures, and use vertical mooring lines that result in slack and drift of the system during low tides. We developed and successfully tested a system using only locally available materials familiar to the commercial fishing industry in the GOM with up-front costs of less than \$600 USD. The system was deployed in November of 2015 and seeded with 2-5mm sugar kelp. Gear performance and production results were monitored throughout. On June 2, 2016, we harvested sugar kelp and the total yield was ~750kg over 50m of seeded line or 14.4 kg/m.

## BROODSTOCK CONDITIONING AND LARVAL REARING OF THE YASHA GOBY *Stonogobiops yasha*

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The Yasha Goby (*Stonogobiops yasha*) is a vibrant marine goby that is highly demanded in the marine ornamental aquarium trade. The Roger Williams University Marine Lab bred this fish in captivity for the first time in 2016. These commercially available cultured specimens will help meet the demand for this species while reducing the need to collect wild Yasha Gobies. Further, our techniques will serve as a culture model for other *Stonogobiops* species, as well as other Gobiids.

Wild Yasha Gobies (N=18) were paired (N=9), and maintained within a 600 L recirculating seawater system comprised of 12, 38 L tanks connected to a 150 L sump. The broodstock were provided with 1" PVC pipes lined with acetate sheets to variety of fresh and frozen seafood eight times per day. Spawning began after approximately 8 months of conditioning. Nest incubation lasted 5 days. On the morning of the 5<sup>th</sup> day, each nest was removed from the broodstock tank and relocated to a larval system composed of seven 70 L tanks connected to a 150 L sump. The eggs hatched between 8-10 AM (60-100% hatch rate) and larvae fed exogenously by the end of day 1. First feed was *Parvocalanus* sp. copepods (5-10/mL). Flexion occurred on day 8-10 at which point we started feeding *Pseudodiaptomus* sp. copepods (5-10/mL). Settlement began on day 20-25 and metamorphosis was observed as early as day 35. When 75% of each cohort was post-larval, the juveniles were weaned onto a 200 mm pellet diet. Over the course of 4 months, this project produced over 1,000 Yasha Gobies from seven breeding pairs, with 50% average survival rate (Fig 1). The fish reached a market size of 3.5-4.0 cm by day 200 and we are currently selling batches to a US-based wholesalers and retailers for distribution into the aquarium trade.

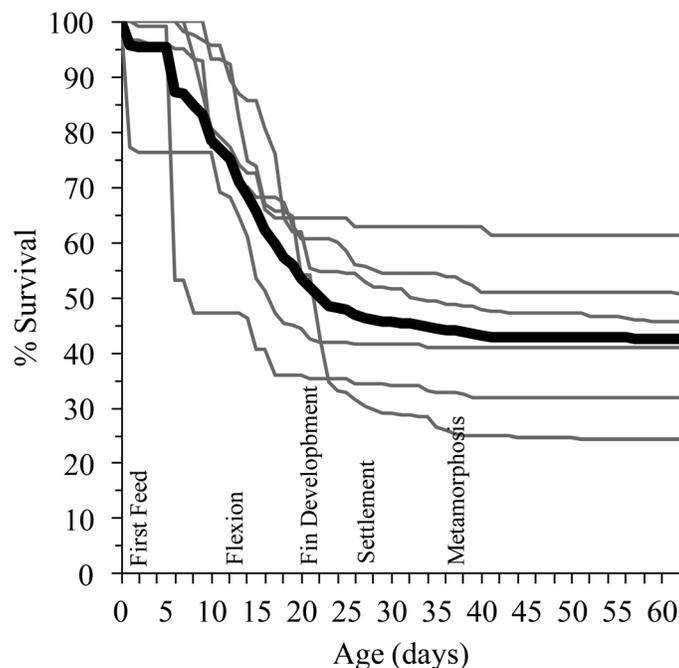


Figure 1. Survivorship of Yasha Gobies throughout larval and early post-larval periods. Stressful developmental time points are denoted, and the black line represents the average of the grey lines.

## UNDERSTANDING THE EFFECTS OF STRAIN SELECTION AND HYBRIDIZATION OF CHANNEL CATFISH *Ictalurus punctatus* ON ENVIRONMENTAL GENE RESPONSE

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The United States catfish industry has long used selective breeding of channel catfish (*Ictalurus punctatus*) and hybridization to increase production yield. Many of these strains have been developed over the past 50 years to provide improved growth rate, disease resistance, increased feed conversion efficiency, and greater dress-out percentage. Additionally, the hybridization of *I. punctatus* with blue catfish (*I. furcatus*) has led to faster growth, easier harvest, more uniform size, and greater tolerance of low oxygen levels in crowded ponds. Increasing temperatures of aquaculture ponds with climate change is a growing concern for the Southeastern US catfish industry. To better understand how strain selection and hybridization of channel catfish can help the industry adjust to environmental pressures, we examined differential expression (DE) of gene transcripts from liver samples collected from two geographically distinct channel catfish strains and one hybrid catfish strain under simulated natural thermal regimes. To simulate normal daily pond fluctuations, we put fish of each group in two different treatments. The first, an ideal temperature range for optimal growth at 27-31°C, and the second at the upper range of acceptable growth temperatures of 32-36°C. These fish were kept in these treatments for six-weeks at the end of which survival and growth data were collected in addition to tissue samples. We mapped three biological replicates of each temperature treatment for all three catfish types (n=18) to the channel catfish genome using Tophat2 and estimated gene expression with HTSeq from the alignments. We removed genes with no expression from analysis and used EdgeR to find differentially expressed genes. We used GAGE to find significant gene ontology (GO) gene-sets and then used GO to examine the biological processes, cellular components, and molecular function of each fish group. Principle component analysis of mRNA libraries showed clear differences between hybrids and both strains of channel catfish at the 32-36°C temperature treatment. Southern channel catfish had greater amounts of up- and down-regulated DE transcripts than northern channel catfish. Hybrid catfish had lower amounts of up- and down-regulated DE transcripts compared to channel catfish of the same strain. Implications from our study may be beneficial for understanding how selection of catfish types can aid the industry in the face of climate change.

## THE WATER FOOTPRINT OF FISH: WHAT IT MEANS AND HOW TO USE IT TO MARKET FISH CONSUMPTION

Meg Stout\*

Aquaponics Association

Agriculture consumes 92% of the global fresh water resources currently consumed by mankind. The United Nations Food and Agriculture Organization (UN FAO) states “water scarcity is fast becoming one of the most serious resource issues we face today.”

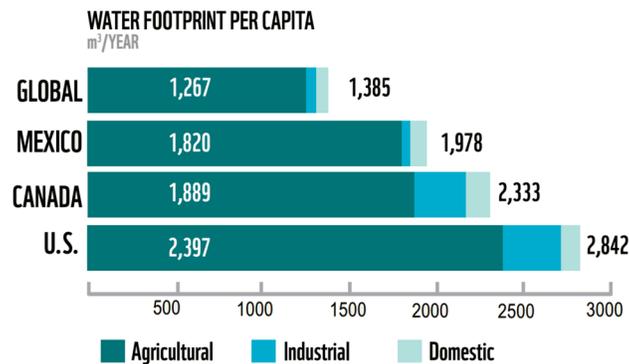
A brief overview of water footprint is provided, covering the reason for including “blue” water (water from rivers, lakes, and aquifers), “green” water (moisture in soil and plants from precipitation), and “grey” water (water required to dilute pollutants).

Case studies for food products are shown to illustrate the methodology. First is caffeinated soda, using numbers published by Arjen Hoekstra. Second is beef, using recent publications by the waterfootprint network. Third, the water footprint for a range of animal products is shown, highlighting the new findings for fish.

Next, a consumer-oriented presentation of water footprint is shown, highlighting the importance of personal decisions with respect to food, highlighting the importance of eating sustainable fish as part of an ecologically-conscious diet.

A preliminary assessment for a decoupled aquaponics system is examined, showing how the water footprint for an aquaculture system can be further reduced by integrating plants to absorb nutrient “pollution” and offset fresh water consumption.

Finally, a proposed allocation of water footprint is presented for calculating water footprint for crops grown using aquaponic methods.



## **ECOLOGICAL IMPACT OF AQUAPONICS: EXTERNALIZING SOCIETAL COSTS RELATIVE TO SOIL AGRICULTURE**

Meg Stout\*  
Brian Filipowich

The Aquaponics Association

This abstract is a stub and will be updated after initial submission

Traditional forms of agriculture incur intrinsic societal and ecological costs. However these costs are rarely been quantified and are rarely associated with the production of agricultural goods.

Agricultural innovations, such as Aquaponics, promise to reduce the societal and ecological costs of food production. However in order to achieve these reductions, aquaponic farmers and other innovators must absorb costs that do not apply equally to traditional farmers.

This paper attempts to quantify the societal and ecological costs of traditional agriculture to assist innovative agricultural methods, such as Aquaponics, to be evaluated in a holistic manner.

Representative matters include:

Transportation costs for traditional crops grown far from consumers, which could be grown locally

Health costs for consumers who do not have access to fresh foods due to the lack of grocery stores in urban neighborhoods

Increased pressure on natural resources (acreage, ground water) associated with traditional agriculture

Inadvertent consequences of traditional agriculture in perpetuating social inequities

## **ORGANIC AQUAPONICS: RECOMMENDED GUIDELINES FOR CONSIDERATION**

Meg Stout\*  
Brian Filipowich  
Sarah Taber, DPM

The Aquaponics Association

This abstract is a stub and will be updated after initial submission

The US National Organics Standards Board (NOSB) invited public comment in November 2016 regarding whether soil-less agricultural methods such as hydroponics and Aquaponics should be excluded from consideration as organic.

Though not all hydroponic and aquaponic growers seek the organic label and associated costs for their products, there are some farmers for whom the organic label makes a substantial difference in the price the market will pay or the market segment available to the farmer, or both.

This paper lays out the philosophical basis for considering Aquaponics and responsible hydroponics to be organic agricultural processes, according to the original language associated with the National Organics Program (NOP).

The paper delineates recommended measures associated with organic Aquaponic production.

As many organic audits also address ancillary matters, such as food safety, the paper addresses good agricultural practices (GAP) that Aquaponics should be held to for public safety.

## CAPTIVE SPAWNING AND EGG QUALITY OF CALIFORNIA YELLOWTAIL *Seriola dorsalis*

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The lack of knowledge in what optimizes egg and larval quality is an important limiting factor in developing culture techniques for any species. Inconsistent or poor egg quality significantly affects the production of larval and juvenile fish. Egg quality is a known predictor of subsequent larval viability, quality, and stress resistance. Without high quality eggs, it is not possible to optimize husbandry practices.

Here we tracked egg quality in a population of California yellowtail (*Seriola dorsalis*) housed at the Hubbs-Sea World Research Institute. We used spawn events from three consecutive years and monitored basic egg quality metrics such as: spawning frequency, spawn viability, egg diameter, oil diameter, hatch rates, larval size at hatch, and larval survival to first feeding. Along with these metrics we also ran parentage analysis on all the spawning events. Finally, we ran proximate composition and fatty acid analysis of select spawning events to assess nutrient resources of good, fair, and poor spawn events.

Between 2014 and 2015 there was an increase in the spawning events but a decrease in the total eggs produced. Using parentage analysis we found that a single female was responsible for a majority of the spawn events (39%), and those spawn events were mostly of poor quality. Based on this result the female was removed prior to the 2016 spawning season. In 2016 spawn quality improved while number of spawn events and eggs produced decreased.

Proximate composition analysis (PC) and fatty acid analysis (FA) showed differences between good (>70% viability), fair (31-69% viability) and poor (0-30% viability) batches of eggs. Poor spawns had significantly more protein than eggs from good spawns. Significant differences in FA composition attributable to quality were also detected in egg samples. The proportion of arachidonic acid (ARA) in eggs from fair spawns was 20% higher than eggs from good spawns and 14% higher than eggs from poor spawns. Compared to good and poor spawns, fair spawns also had a lower ratio of eicosapentaenoic acid (EPA) to ARA ratio. Last, fair spawns had a higher proportion of 22:5 n-3 than good spawns, but were not different from poor spawns

Table 1 Comparison of California yellowtail spawn events over a three period.

Variable	2014	2015	2016
Number of Spawns	63	78	46
Female Biomass (kg)	222	262	147
Total Eggs Produced	58,060,386	52,410,967	31,123,593
Percent Viability (Mean $\pm$ SD)	58.5 $\pm$ 25.2	49.9 $\pm$ 24.8	68.0 $\pm$ 18.0
Percent Hatch (Mean $\pm$ SD)	62.1 $\pm$ 18.3	60.7 $\pm$ 17.4	69.4 $\pm$ 18.9
Percent Survival to First Feeding (Mean $\pm$ SD)	67.4 $\pm$ 20.3	66.9 $\pm$ 16.7	63.7 $\pm$ 26.1

## INCREASED SURVIVAL WITH WATER QUALITY IMPROVEMENTS AT AN ENDANGERED COHO SALMON *Oncorhynchus kisutch* RESTORATION HATCHERY

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The Monterey Bay Salmon and Trout Project (MBSTP) operates a small Coho Salmon conservation hatchery in Davenport, Ca., the Kingfisher Flat Genetic Conservation Hatchery. Central California Coast Coho salmon are listed as endangered species by both the Federal and State of California Governments. This hatchery was constructed in the early 1980s as a freshwater, single pass, flow through facility. Initially, the hatchery comprised two outdoor rectangular raceways and a building housing incubation jars, Heath trays, and fry troughs. Over the years, an additional six circular pools from 3.5 meters to 4.5 meters in diameter were installed; however, during this time the only water facility upgrade was a small filter, chiller, and UV sterilizer added to the hatchery egg incubation water line. In 2009 the Lockheed fire burned the canyon surrounding the hatchery. Subsequently, the debris runoff from the burned hills during winter and spring storms increased the sediment load in the hatchery water supply, which appeared to be associated with fungal outbreaks in both adults and pre-smolts. Each year from 2013-2015, a large number of adults and smolts were infected with *Saprolegnia spp.* and died either prior to spawning or prior to being released as smolts. To combat the fungal infections, NOAA Fisheries, California Department of Fish and Wildlife (CDFW), and MBSTP combined funds to upgrade the hatchery's water delivery system. Upgrades included sand filters, UV sterilizers, and two small recirculating systems attached to the captive broodstock rearing pools. The following year CDFW replaced one raceway with four 3.75 meter pools. These improvements have resulted in high quality, high turnover, filtered water in pools for both life stages. No losses of adults or smolts to *Saprolegnia spp.* occurred during the 2016 spawning and smolt release seasons.

## STRESS INDUCED FISH HEAMATOLOGY: A POLLUTION ASSESSMENT TOOL FOR AQUATIC TOXICOLOGY

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Intense pollution being contributed by human is not only threatening irrigation and ground waters but also terrestrial and aquatic life. The present study was planned to assess the pollution level through changes in heamatological parameters of major carps (*Cirrhinus mrigala*, *Catla catla* and *Labeo rohita*). The fingerlings were procured from Fish Seed Hatchary, Satiana Road, Faisalabad. All the fingerlings having average weight of  $2.92 \pm 0.14$ g, standard length of  $3.91 \pm 0.18$ cm, and having total length of  $5.46 \pm 0.24$ cm. The fingerlings were acclimatized to laboratory conditions for two weeks prior to experiments. The acclimatized fish were exposed to different dilutions of industrial effluents from Chakbandi and MC drains after determining  $LC_{50}$  of 96 hours. Mortalities within each group were recorded during  $LC_{50}$ . The water changed once a week to maintain the environmental conditions and effluent concentrations. Physicochemical parameters of water like temperature, pH, DO were monitored on daily basis. Fish fed twice daily with a 32% crude protein diet at a rate of 3-4% of body weight. Blood samples were collected from the fish before and after exposure to different industrial effluents concentrations.

Complete examination of blood was carried out. The data was analyzed with the help of ANOVA (2 factor CRD and 2 Way) and means were compared using Duncan's Multiple Range Test (DMRT). Heamatological parameters and stress were studied by Pearson's coefficient of Correlation (SAS, 1988). Level of significance has been declared at  $p < 0.05$ .

After three months exposure to sub-lethal concentrations a significant decrease in erythrocytes were observed in all three species i.e. *Cirrhinus mrigala* (mrigal carp), *Labeo rohita* (rohu) and *Catla catla* (Thaila). All the erythrocyte indices showed significant reduced values. The reduction of RBC, increase in leukocytes and thrombocytes count in all fish species was observed. The mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) showed altered values for each species, although a significant increase was observed. The stress conditions pose a threat to fish health and indirect to human health.

Heamatological Parameter	<i>Cirrhina mrigala</i>		<i>Labeo rohita</i>		<i>Catla catla</i>	
	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.
RBC ( $\times 10^{12} / \mu\text{L}$ )	0.37 $\pm$ 0.03	0.54 $\pm$ 0.01	0.27 $\pm$ 0.01	0.64 $\pm$ 0.01	0.21 $\pm$ 0.02	0.31 $\pm$ 0.004
WBC ( $\times 10^9 / \mu\text{L}$ )	18.17 $\pm$ 0.29	16.60 $\pm$ 0.10	24.97 $\pm$ 0.74	19.81 $\pm$ 0.39	94.7 $\pm$ 1.31	93.04 $\pm$ 0.07
Hgb (g/dL)	3.1 $\pm$ 0.17	3.37 $\pm$ 0.09	3.72 $\pm$ 0.14	4.14 $\pm$ 0.08	1.97 $\pm$ 0.10	3.74 $\pm$ 0.07

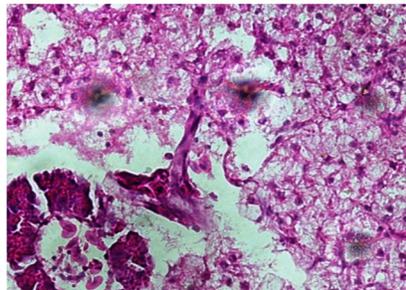
## HISTOPATHOLOGY UNVEILING THE TEXTILE INDUSTRY EFFLUENTS TOXICITY IN DIFFERENT FISH ORGANS IN SELECTED FISH SPECIE

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This study was designed with an objective to study the effect of textile industries effluents on water and aquatic organisms particularly fish. Textile industries effluents were collected from five wastewater drains of Faisalabad- drains of Mustafa Abad, Bawa Chak, Abdullahpur, Satyana road and Sumandhri road) containing different textile industries effluents. Representative water samples from each site were collected in 1.5L capacity of polypropylene bottles with polyethylene caps from the five selected sites.

The experiment was performed in glass aquaria containing fingerlings of approximately of ~5-8 gm each of *Cirrhinus mrigala*, *Catla catla*, *Labeo rohita* and *Hypophthalmichthys molitrix*. These were exposed to composite sample of textile mill effluents after acclimatization. After acclimatization fish were divided into two groups one as experimental and other as control group. Three sub lethal dilutions of the textile effluents were tested for three months. The fish were fed commercial diet 3-4 % of wet body weight. The histopathological changes were observed and compared with control. Histopathological changes in the gill, kidney and liver were observed. The severe degeneration of the primary and secondary gill filaments, short fusion of secondary lamellae and severe hemorrhage showed in the gills. The glomerular shrinkage, increased spaces between glomerulus and Bowman's capsule, increased tubular lumen in the kidney were observed. In liver were intravascular hemorrhage, vacuolar degeneration, severe hemorrhage, hepatocytes degeneration, hemolysis, erythrocyte infiltration in blood sinusoid, eccentric nuclei, cytoplasmic vacuolation and dilation of vein (Fig-1).



**Fig.1. showing damaged liver tissues.  
(Damaged hepatocyte (DH): Ruptured  
central canal (RC): Vacuolization (V):  
Damaged sinusoids (DS).**

## INVESTIGATION OF SOME SPERM QUALITY PARAMETERS OF FARMED AND WILD-CAUGHT MEAGRE (*Argyrosomus regius* ASSO, 1801)

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This study aimed to clarify some sperm quality parameters such as volumetric sperm quantity, motility, motility duration, sperm density, total number of spermatozoa and pH of meagre (*Argyrosomus regius* ASSO, 1801) individuals kept in farming conditions and caught from wild (las palmas, gran canary).

The sperm was collected in glass tubes graded in millimetres and sperm volume registered immediately following collection by abdominal massage. The sperm quality parameters including motility, total number of spermatozoa and spermatozoa density were determined with computer assisted sperm analysis (CASA) program. The duration of spermatozoa movement was assessed using a sensitive chronometer (1/100s) that was started simultaneously with the addition of activation solution into the sample. Sperm pH was measured with standart pH electordes within five minutes of sampling.

At the end of the study, while amount of sperm ( $5.20 \pm 0.33$  ml), duration of motility ( $7.23 \pm 0.7$  m) and total number of spermatozoa ( $131.40 \pm 12.22 \times 10^9$ ) were different statistically ( $p < 0,05$ ), motility (%  $81.03 \pm 6.59$ ), pH ( $7.30 \pm 0.08$ ), sperm density ( $25.27 \pm 9.42 \times 10^9/\text{ml}$ ) and morphologic parameters were not significantly different between the two groups.

According to our results, amount of sperm, duration of motility and total number of spermatozoa were better in farmed group than that of the other group.

## **UPDATES IN FOOD SAFETY FOR AQUAPONICS**

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Updates in food safety for aquaponics include interest from NASA in the microecology of aquaponics, as it covers a lot of the same ground as regenerative life support, similar food safety issues, and similar multidisciplinary nature. This presentation will include this and other annual updates in aquaponics food safety including a brief overview of food safety in AP (foodborne zoonotic pathogens associated with fish); a review of human pathogen finding(s) in aquaponics in 2015-2016 as permitted by the sites at which they occurred; and a review of commercial-scale AP operations successfully certified for food safety in 2015-2016.

## CAN STABLE ISOTOPES INDICATE THE GEOGRAPHICAL ORIGINS OF SEA LICE?

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The salmon louse *Lepeophtheirus salmonis* is considered the primary parasitic disease of salmon aquaculture and affects populations of both wild and farmed fish. Despite the current understanding of its widespread presence and detrimental effects on host salmonids, its complete life history strategy, particularly regarding overwintering, remains uncertain. The current study investigated the overwintering strategies of sea lice in Cobscook Bay, ME through stable isotope analysis (SIA). Separate populations of lice were collected from farmed Atlantic salmon in the Bay and from returning wild salmon at the Milford Dam, ME to determine if the isotopic signatures of lice from different fish stocks were unique. Preliminary studies experimented with sample processing protocols in order to establish the most efficient method for SIA of sea lice. Further analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotopes was used to track animal movement and food web interactions, and thus the origins of associated sea louse populations in the bay.

Samples that were stored in seawater versus distilled water yielded significantly different  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Similarly, lice that were acidified had significantly different isotopic signatures compared to those that were not acidified during sample preparation.

All sea lice samples on farmed salmon were identified as *Lepeophtheirus salmonis*, whereas all specimens on wild fish were *Argulus foliaceus*. Isotopic signatures are expected to differ between lice from farmed and wild fish.

A better understanding of experimental influences on stable isotope results will improve interpretation of future results. Distinct salmon populations appear to host lice with respective and different isotopic signatures. Therefore, through use of SIA as a quantitative tool, knowledge of where sea lice originate from and how they survive the cold season will enhance the current understanding of sea lice ecology under cold stress and its effects on wild and farmed fish.

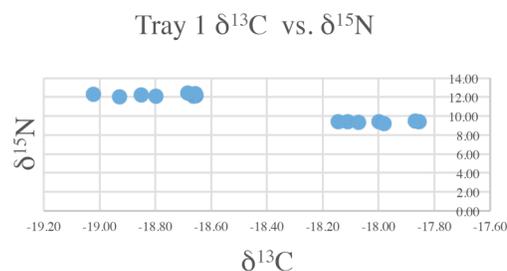


Figure 1. Comparison of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for samples stored in distilled water and seawater.

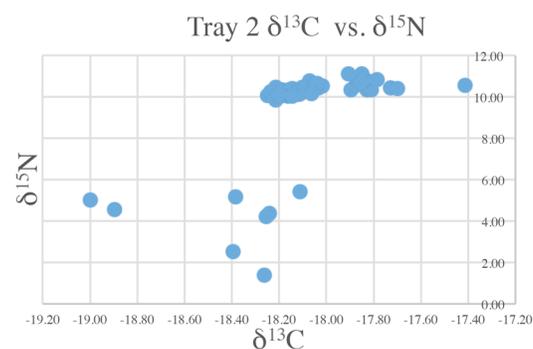


Figure 2. Comparison of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for acidified and non-acidified samples.

## FOOD MATTERS: FISH, INCOME & FOOD SUPPLY - A COMPARATIVE ANALYSIS

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Hunger and food insecurity remain amongst the most devastating problems facing the world's poor and disadvantaged, and continue to dominate the health and socio-economic development of the world's poorest nations and peoples. This is perhaps not surprising bearing in mind that 12.7 percent of the world's population live at or below the international poverty line of \$1.90 a day (896 million in 2012) and the growing income inequality within nations which obscures the true economic reality and hardship of the low income segment of the population. Sadly, despite the fact that we live in world which produces sufficient food to meet all the dietary nutrient needs of all of its people to live a healthy and active life, this is not the case for those people without the monetary resources or government support to purchase or obtain sufficient food to meet their daily needs. This remains a continuing travesty and violation of the recognized fundamental human right to adequate food and nutrition, and freedom from hunger and malnutrition.

According to the latest estimates from FAO more than 795 million people had chronically inadequate levels of dietary energy intake during 2014-2016. However, "Hidden hunger" or micro-nutrient deficiencies, is much more widespread than hunger (which generally only refers to insufficient caloric intake). According to the latest estimates, about 1.6 billion people suffer from anemia and iron deficiency, about 2 billion people suffer from iodine deficiency (including about 285 million school-age children), 190 million pre-school children and 19.1 million pregnant women suffer from vitamin A deficiency, while at the same time more than 1.5 billion people are overweight with half a billion obese, exposing them to greater risk of cardiovascular problems and other diet-related, non-communicable diseases. However, the above three nutrients only represent a small fraction of the 40 or more essential dietary nutrients required by humans to live a healthy and active life.

Previous articles by the authors have shown the important role played by fish and fishery products as a source of essential dietary nutrients in human nutrition and global food supply. This paper attempts to compare the role played by fish and fishery products (whether derived from wild capture fisheries or aquaculture) in the diet of the world's poorest and richest nations using data from the latest FAO food balance sheets, and attempts to provide guidance on suggested dietary changes for the improved health and well-being.

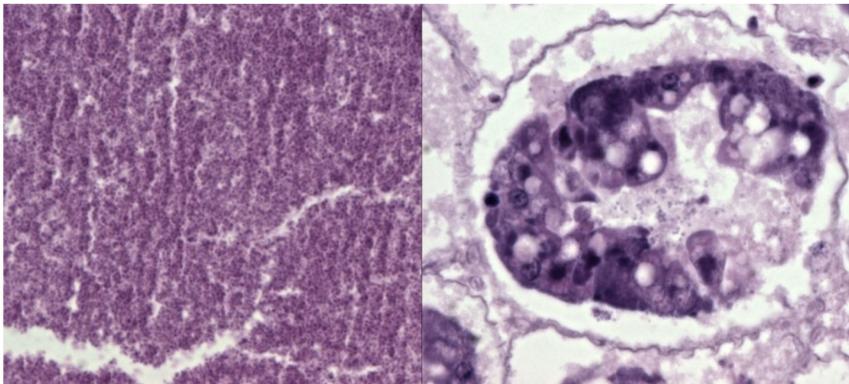
## DENSE POPULATIONS OF THE MICROSPORIDIAN *Enterocytozoon hepatopenaei* (EHP) IN FECES OF *Penaeus vannamei* EXHIBITING WHITE FECES SYNDROME AND PATHWAYS OF THEIR TRANSMISSION TO HEALTHY SHRIMP

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White feces syndrome (WFS) is an emerging problem for penaeid shrimp farming industries in SE Asia countries, Thailand, Malaysia, Vietnam, Indonesia, China, and in India. This occurrence of this syndrome is usually first evidenced by the appearance of white fecal strings floating on surface of the shrimp ponds. The gross signs of affected shrimp include the appearance of a whitish hindgut and loose carapace, and it is associated with reduced feeding and growth retardation. To investigate the nature of the white feces syndrome, samples of white feces and shrimp hepatopancreas tissues were collected from *Penaeus vannamei* in affected farms in Indonesia, and these were examined histologically. Within the white feces, we found densely packed spores of the microsporidian *Enterocytozoon hepatopenaei* (abbreviated as EHP) and relatively fewer numbers of rod-shaped bacteria. From WFS ponds, hepatopancreas samples from 30 individual shrimp were analyzed by histology and in situ hybridization. The results showed that all of the shrimp examined were infected with EHP accompanied by septic hepatopancreatic necrosis (SHPN). Midgut epithelial cells were also infected and this increased the number of tissue types being affected by EHP. By PCR, EHP was detected in the samples, with a 100% prevalence, from WFS-affected ponds but not in those sampled from healthy shrimp ponds.

To determine the modes of transmission for this parasite, we performed feeding and commingling bioassays, the results showed that EHP can be transmitted through *per os* feeding of EHP-infected hepatopancreas tissues to healthy shrimp and through commingling of infected and healthy shrimp. In addition, we found the use of Fumagillin-B, an antimicrobial agent, was ineffective in either reducing or eliminating EHP in infected shrimp.



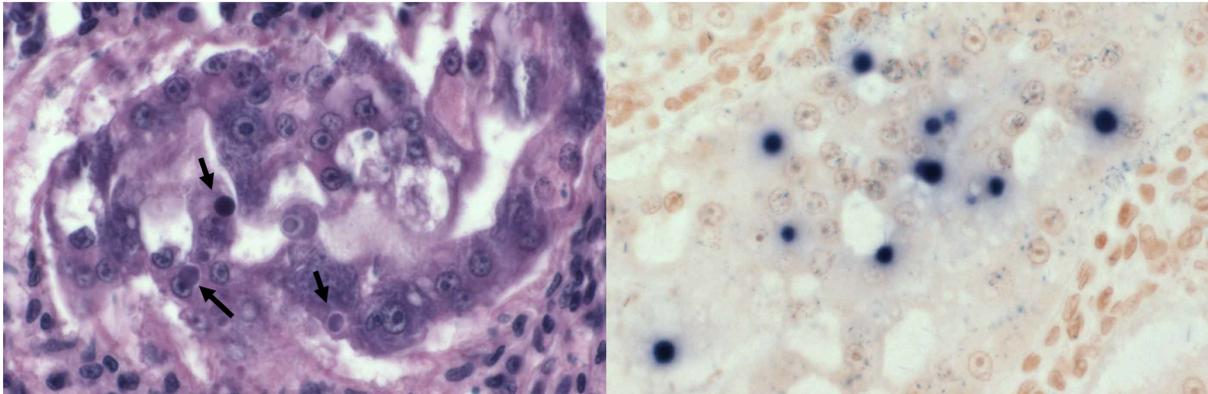
H&E staining of white feces and shrimp hepatopancreas samples collected from a white feces syndrome affected pond. Within the feces, there were massive quantities of EHP spores. The hepatopancreas was infected with EHP.

## HISTOPATHOLOGY AND IN SITU DETECTION OF THE MICROSPORIDIAN *Enterocytozoon hepatopenaei* (EHP) IN *Penaeus vannamei* CULTURED IN A LATIN AMERICA COUNTRY

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EHP is an intracellular, spore-forming parasite that has become a critical threat to the shrimp farming industry in SE Asia. This parasite replicates within the cytoplasmic area of the tubule epithelial cells in the hepatopancreas, and infected shrimp exhibit reduced feeding and severely retarded growth. In this study, we describe the first case of EHP-infected *Penaeus vannamei* cultured in Latin America. Its histopathology is very similar to that of SE-Asia EHP, with infected shrimp showing basophilic inclusions in tissue of the hepatopancreas. Upon in situ hybridization of an 18S rRNA gene fragment labeled with digoxigenin to the infected *P. vannamei* tissue, the probe reacted intensely to the basophilic inclusions within the cytoplasm. We also compared the sequence similarity in 18S rRNA gene (1.1 kb) and in spore wall protein gene (partial gene, 457 bp) between SE Asia and the Latin America isolates; and the result showed 99% and 91% identities, respectively. This suggests that this Latin America EHP is a unique isolate, and not a recently introduced isolate from SE Asia. The emergence of EHP in the western hemisphere will have a significant impact on the shrimp production if it spreads to other farms within the region. As infection with EHP at early stages does not exhibit obvious clinical signs and the spores can persist in the environment, the potential for the rapid spread of this parasite is high. Therefore, the shrimp cultured within the region should be monitored closely for the presence of EHP.



H&E staining (arrows: cytoplasmic inclusion bodies) and in situ hybridization (dark blue precipitates) with an digoxigenin-labeled EHP probe to a shrimp hepatopancreas sample from in a Latin America shrimp farm.

## ACCLIMATION OF CAPTIVELY REARED JUVENILE COMMON SNOOK TO THE WILD: EFFECTS ON MICROBIOTA AND INNATE IMMUNITY

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Research regarding stock enhancement of common snook *Centropomus undecimalis* has revealed that juveniles survive better in the wild if they are first provided with a three day acclimation in enclosures at the site of release. Hypotheses as to the mechanism behind the improved survival include recovery from transport stress and behavioral adjustments such as learning to feed and avoid predators in the wild. However, the effects of acclimation on the overall health of the snook is not yet understood. An organism's bacterial communities, termed the microbiota, have profound effects on host health. The microbiota influences growth and development, digestion, nutrient acquisition, feed conversion, and disease susceptibility, and as a result plays a large role in the overall health status of the host. The microbiota shifts as fish are brought from the wild into captivity, but to our knowledge, no study has investigated these shifts during release from captivity into the wild, and no study has investigated these changes while simultaneously investigating alterations in innate immune activity. As captive snook may encounter new and different pathogens in the wild, knowledge of these transitions may shed light on increased survival in acclimated snook, as these parameters directly impact the ability of the fish to resist disease.

This study used next-generation sequencing and previously established innate immunity assays to investigate the changes in external skin microbiota and immune parameters during the transition of juvenile snook from captivity to the wild during stock enhancement efforts. Skin mucus was collected from captive fish prior to transport to the release site and following three days of acclimation in enclosures, and from wild individuals collected from the release location. The study was repeated in three different creek systems near Sarasota, Florida (Philippi, Hudson, and Whittaker) in late summer/early fall of 2016. Microbiota was characterized using paired-end Illumina sequencing, and innate immune parameters analyzed included lysozyme, superoxide dismutase, alkaline phosphatase, and protease.

Data presented will include the first characterization of captive and wild juvenile common snook skin microbiota. The ability of the captive microbiota to adapt to wild conditions will be discussed, focusing on relative abundances of known fish pathogens. Comparisons will be made between captive, transitioning, and wild snook in terms of innate immune enzyme activity. Finally, the implications of immunity and microbiota for snook acclimation and disease resistance will be discussed.

## FILLET CHEMICAL COMPOSITION OF SHARPSNOUT SEABREAM (DIPLODUS PUNTAZZO) FROM WILD AND CAGE-CULTURED CONDITIONS

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Polyunsaturated fatty acids (PUFAs) and particularly the levels and ratios of  $\omega$ -3 and  $\omega$ -6 fatty acids are important for biological functions in humans and recognized as essential components of human diet. According to the terms of many different points of view, the nutritional composition of fish in culture conditions and caught from wild are wondered by the consumers. Therefore the aim of this study was to investigate chemical composition of cage-cultured and wild sharpsnout seabream which has been preferred by the consumers as an economical important fish species in Turkey.

The fish were caught from wild and obtained from cage-cultured commercial companies. Eight fish were obtained for each group and their average weights of the samples were 245.8±13.5 g for cultured, 149.4±13.3 g for wild samples. All samples were stored in freezer (-18 °C) and analyses were carried out in triplicates, using homogenized boneless fish fillets. Moisture, crude protein and ash contents were determined by the methods of AOAC (1990) and lipid content was analyzed according to the method of Bligh and Dyer (1959). The fatty acid composition was analyzed by a GC Clarous 500 with autosampler (Perkin–Elmer, USA).

Proximate compositions of cage-cultured and wild samples of sharpsnout seabream are shown in table I. There were found statistical differences in terms of proximate composition between the groups.

The results showed that (Table II.) the saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and PUFA amounts of cultured and wild sharpsnout seabream was significantly different.  $\omega$ 3/ $\omega$ 6 ratio was higher in cultured group.

Especially in protein level and lipid level of cultured samples was significantly higher than wild counterparts. These results similar to some previous findings. One appear reason for this, cultured species exposed to continous feding. This situation had direct effect on their body lipid content.

The fatty acid composition of fish differs depending on a variety of factors including species, diet, environmental factors and whether they are farmed or wild.

The higher levels of MUFA in the cultured fish may be explained with the high content of monoenoic fatty acids in the feed of cultured fish as in some other species.

The  $\omega$ 3/ $\omega$ 6 ratio is a good index for comparing relative nutritional value of fish oils. Also previous studies showed that the suggested ratio of  $\omega$ 3/ $\omega$ 6 is 1:1. The cultured sharpsnout seabream appears to be better nutritious in terms of  $\omega$ 3/ $\omega$ 6.

### Acknowledgement

This work was supported by the Scientific Research Project Unit of the University of Cukurova, Turkey under grant no FBA-2016-5780.

Table I. Proximate composition of sharpsnout seabream

Proximate Composition (%)	Cultured	Wild
Protein	23,88±0,89 <sup>a</sup>	20,78±0,77 <sup>b</sup>
Lipid	6,72±1,14 <sup>a</sup>	0,80±0,16 <sup>b</sup>
Ash	2,04±1,04 <sup>a</sup>	1,24±0,12 <sup>b</sup>
Moisture	63,23±1,82 <sup>b</sup>	76,01±1,57 <sup>a</sup>

Table II. Some fatty acids and total fatty acid groups of sharpsnout seabream.

Faty Acid	Cultured	Wild
C16:0	16.76±0.54 <sup>b</sup>	18.53±0.69 <sup>a</sup>
C18:0	4.59±0.50 <sup>b</sup>	10.29±0.74 <sup>a</sup>
C24:0	3.24±0.80 <sup>a</sup>	3.06±0.20 <sup>b</sup>
<b>Σ SFA</b>	<b>30.00±0.53<sup>b</sup></b>	<b>36.38±0.45<sup>a</sup></b>
C18:1 $\omega$ 9	20.77±0.55 <sup>a</sup>	9.42±0.84 <sup>b</sup>
C20:1 $\omega$ 9	3.09±0.35 <sup>a</sup>	1.78±0.60 <sup>b</sup>
<b>Σ MUFA</b>	<b>32.06±1.01<sup>a</sup></b>	<b>21.52±1.54<sup>b</sup></b>
C18:2 $\omega$ 6	9.22±0.34 <sup>a</sup>	1.59±0.44 <sup>b</sup>
C20:5 $\omega$ 3	4.86±0.22 <sup>b</sup>	6.48±0.50 <sup>a</sup>
C22:6 $\omega$ 3	15.48±1.03 <sup>a</sup>	14.90±0.43 <sup>a</sup>
<b>Σ PUFA</b>	<b>33.74±0.96<sup>b</sup></b>	<b>35.84±0.69<sup>a</sup></b>
<b><math>\omega</math>3/<math>\omega</math>6</b>	<b>2.17±0.27<sup>a</sup></b>	<b>1.62±0.10<sup>b</sup></b>

## DIRECT-FED PROBIOTICS IMPROVE SURVIVAL IN SHRIMP, *Litopenaeus vannamei*, UNDER AHPND/EMS CHALLENGE

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Diseases affecting shrimp contribute to billions of dollars of economic loss yearly to the global aquaculture industry. In 2009, a new strain of *Vibrio parahaemolyticus* causing Early Mortality Syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) in shrimp emerged with a devastating and ongoing impact on global shrimp production. Alternatives to antibiotics and disinfectants, such as probiotic treatments, are emerging as attractive methods of pathogen control in shrimp culture. Probiotics can act as natural immune enhancers and provide pathogen-antagonistic action. The overall project goal is to improve intensive shrimp production through direct application of probiotics in aquaculture feeds. It is hypothesized that direct-fed probiotics will colonize in the shrimp gut, enhance nutrient utilization, and indirectly provide increased disease resistance against pathogenic bacteria. Accordingly, we conducted the following studies in a biosafety level 2 (BSL2) laboratory to: (1) confirm the germination of different strains of *Bacillus subtilis* spores in the shrimp gut, (2) evaluate how to consistently infect shrimp (*Litopenaeus vannamei*) with an AHPND strain of *V. parahaemolyticus* using controlled-challenge studies, and (3) determine the effectiveness of probiotics, containing various strains of *Bacillus subtilis*, for decreasing AHPND disease impact. Challenge studies were conducted to evaluate the ability of direct-fed probiotics to improve shrimp gut health and reduce pathogenicity of the AHPND strain in shrimp. Particular strains of *Bacillus subtilis* were capable of significantly ( $P < 0.05$ ) enhancing survival of shrimp (Figure 1). The commercial application of specific strains of direct-fed probiotics at shrimp farms could help reduce disease outbreaks of AHPND.

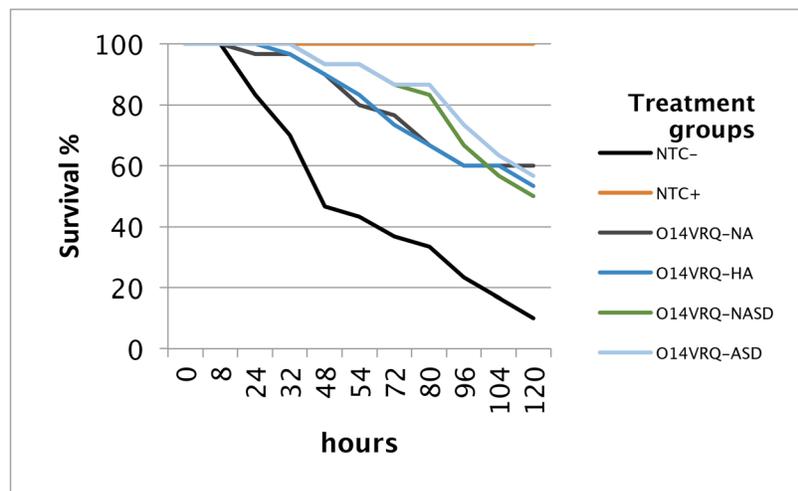


Figure 1: *Bacillus* strain O14VRQ application in AHPND disease exposure challenge with negative and positive controls

## **EVALUATION OF STUDENTS' PERCEPTIONS AND BEHAVIOR OF PROJECT-BASED LEARNING PROGRAMS THROUGH HANDS-ON AQUACULTURE ACTIVITIES**

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Aquaculture learning activities can enhance students' academic performance in Science, Technology, Engineering, and Mathematics (STEM) disciplines and make them more relevant through authentic hands-on experiential learning opportunities. It has been shown that aquaculture has potential to address workplace skills and promote youth development.

Pre-college programs were evaluated at Kentucky State University (KSU). A three-week Summer Apprenticeship Program (SAP) was organized and hosted by KSU's College of Agriculture, Food Science, and Sustainable Systems that provided experiential, hands-on research opportunities to 27 high school students (grades 11 and 12). Apprentices were exposed to various college programs and careers in STEM, including aquaculture. They received direct, inquiry-based learning experiences and exposure mentored by KSU faculty and research scientists. Upon completion of their work, they prepared and presented scientific research reports. Four additional students participated in either an Upward Bound work-study program or three month Mentorship Program while engaged in various aquatic science activities. Students in all programs were mostly minority-based and selected from rural and underserved communities.

Data assessments were drawn from field observations and personal interviews. Research questions included: how do students perceive the value of learning STEM concepts when exposed to hands-on aquaculture activities; did student's interest, motivation, and desire in STEM change, and did their self-confidence, understanding, abilities, and preparedness improve; did student's level of awareness of STEM-related areas of study and careers increase; and how did students rate their knowledge level of aquaculture STEM concepts before and after each program. Results demonstrated that "real-life" hands-on aquaculture activities enhanced their interest, motivation, and self-confidence in STEM.

## MODIFIED SOYBEAN MEALS AS SUSTAINABLE PROTEIN SOURCES IN DIETS OF LARGEMOUTH BASS (*Micropterus salmoides*)

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A 12-week feeding trial was conducted with juvenile Largemouth Bass (*Micropterus salmoides*) with an initial average weight of 12.1g to determine the performance of fish fed diets with different soybean meals or fish meal. Six diets were formulated. One diet contained menhaden fish meal, while the others contained soybean meals modified in different ways to improve their nutrient composition. The soybean meals included a standard soybean meal (dehulled, solvent-extracted 48% protein), an acid hydrolyzed meal, a hot-water treated meal, and two fermented soybean meals (Fermented Soy 2 and Pepsogen™). The objective was to determine if there would be differences in growth, survival, feed conversion ratio (FCR), hematological parameters, non-specific immunity, hepatosomatic index, and whole body composition. The fish were fed twice daily to satiation. Data was analyzed using 1-way ANOVA and considered significant at  $p \leq 0.05$ .

The fish fed the fish meal diet had higher weight gain than the fish fed the Pepsogen™ diet, but the Pepsogen™ diet was higher than the other soy diets. The FCR was lowest (best) in the fish fed the fish meal or Pepsogen™ diets and highest in the Fermented Soy 2 diet. The FCR of the fish fed the fish meal or Pepsogen™ diets did not differ. The alternative complement activity and hepatosomatic index were higher in fish fed the Pepsogen™ diet. Total lipid concentration of the whole body samples was highest in fish fed the fish meal diet. Survival, hematological parameters and lysozyme activity did not differ among diets. The fish meal diet and the Pepsogen™ diet worked well in two key areas. While growth was higher in the fish fed the fish meal diet, Pepsogen™ appears to be a promising alternative protein source.

Table 1. Weight gain, survival, FCR, and proximate composition of the Largemouth Bass.

Diet	Average Weight Gain (g)	Survival (%)	FCR	Total Lipid (%)	Crude Protein (%)	Ash (%)
Fish Meal	37.2 <sup>a</sup>	95.0	1.9 <sup>a</sup>	32.5 <sup>a</sup>	59.0	12.3
48% Protein Soybean Meal	16.2 <sup>c</sup>	97.5	2.9 <sup>ab</sup>	21.6 <sup>c</sup>	62.1	9.9
Acid Hydrolyzed Meal	14.6 <sup>c</sup>	91.7	3.9 <sup>bc</sup>	22.4 <sup>bc</sup>	60.2	15.4
Hot-Water Treated Meal	13.7 <sup>c</sup>	91.7	3.9 <sup>bc</sup>	24.3 <sup>bc</sup>	58.8	14.1
Pepsogen™	21.8 <sup>b</sup>	98.3	2.6 <sup>a</sup>	25.9 <sup>b</sup>	57.1	14.5
Fermented Soy 2	12.8 <sup>c</sup>	95.0	4.3 <sup>c</sup>	23.7 <sup>bc</sup>	62.1	18.1

## IMPACT OF DIET ON GROWTH AND HEALTH INDICATORS FOR THE LARGEMOUTH BASS (*Micropterus salmoides*)

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The largemouth bass (*Micropterus salmoides*) has been cultured for decades, primarily for sport fish stocking. However, there has been increasing production of largemouth bass (LMB) for food fish, especially for live sales to Asian markets. For growout, most producers utilize diets formulated and manufactured for salmonid species, based primarily on ready availability. Health issues have occurred with LMB which are thought to be diet related. The objective of this experiment was to compare the growth, survival, and health indicators of pond raised largemouth bass fed commercial diets used for LMB production or a diet formulated at KSU specifically for LMB.

Twelve 0.04 ha ponds were each stocked with 350 feed-trained advanced fingerling largemouth bass (112 g). Ponds were randomly assigned one of four diets. Three diets were commercially available diets sold originally for either rainbow trout (40% protein/ 12% lipid: 40/12), steelhead trout (45/16) or a Classic Bass diet sold for hybrid striped bass, Barramundi, and LMB (48/18). The fourth diet was formulated at KSU and custom pelleted (Hyland Feeds, Ashland, KY). The LMB were fed to apparent satiation twice daily for 187 days. Fish were sampled from each pond at stocking, mid-season, and harvest and submitted for parasite and bacteriological evaluations and a diagnostic profile was run on blood samples. At harvest livers were preserved for subsequent histopathological examination.

After 26 weeks survival was not significantly different among fish fed the four diets and averaged 87%, overall. Average harvest weight of fish fed the commercial trout diet (40/12) was significantly lower (525 g) than fish fed other diets, which were not significantly different and averaged 573 g. Feed conversion ratios ranged from 1.4-1.7 and did not differ significantly. Hepatosomatic Index was significantly lower in fish fed the KSU diet (0.53) compared to fish fed the three commercial diets which averaged 0.83. There were no clear trends among the four treatments in terms of bacterial or parasite evaluations and most results were normal. Blood analyses indicated higher levels of alanine aminotransferase and total bilirubin (both indicators of liver impairment) and Creatinine (kidney function) in fish fed the Steelhead and Classic Bass diets. Results indicated no clear relationship between feeds and liver pathology or glycogen accumulation. These data indicate reduced growth in LMB fed a trout diet. Histopathological and hemeatological results indicate potential liver and kidney dysfunction in fish fed the Steelhead and Classic Bass diets, though results could also have been related to undetected infectious agents rather than diets.

## A COMPARISON OF BIOFLOC, CLEAR-WATER, AND HYBRID CULTURE SYSTEMS FOR INTENSIVE SHRIMP (*Litopenaeus vannamei*) NURSERY PRODUCTION

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Indoor, intensive recirculating aquaculture systems (RAS) are increasing in popularity for marine shrimp production as a way of providing fresh, high-quality, never-frozen shrimp to metropolitan markets year around. One common stage in shrimp production is a nursery phase, which can enhance biosecurity, allow better quantification of animals, and utilize space more efficiently. Nurseries are usually started a few days after shrimp develop to post-larvae (PL) and continue to a juvenile stage between about 0.5 and 1.0 g. However, it is unclear what type of RAS is most appropriate for indoor shrimp nurseries.

An experiment compared three system types, dictated by differences in filtration: clear-water RAS (CW), biofloc systems (BF), and hybrid systems (HY). Each treatment included four, randomly assigned 208 liter culture tanks; CW systems had an external settling chamber, a foam fractionator, and a biofilter, BF systems included only a settling chamber, and HY systems included an external settling chamber, and a biofilter. The nursery tanks were stocked with 3,000 PL/m<sup>3</sup> with an initial weight of 7 mg, and shrimp were grown for 45 days. Water quality parameters, including inorganic nitrogen concentrations (TAN, NO<sub>2</sub>-N, NO<sub>3</sub>-N), were measured once a week. At approximately the middle of the study, samples of shrimp were weighed to determine mean individual weights. An  $\alpha$ -value of 0.05 was used during data analysis to determine significant differences between treatments.

Final data are still pending, but at about the halfway time point there were no significant differences in ammonia and nitrite concentrations between the treatments. However, nitrate concentration was significantly greater in the HY tanks than in tanks from the other treatments (Table 1). Mean individual shrimp weight was significantly greater in the BF treatment, followed by HY, then CW. These results indicate that differences in system design can have significant impacts on water quality and nursery shrimp production, suggesting that producers should carefully consider what type of system may best suit their production goals.

**Table 1:** Mean  $\pm$  SD values of ammonia, nitrite, nitrate, and shrimp weight at approximately the middle of the study.

	Treatment		
	CW	BF	HY
Ammonia (mg TAN/L)	2.0 $\pm$ 2.7	0.3 $\pm$ 0.2	0.2 $\pm$ 0.1
Nitrite (mg NO <sub>2</sub> -N/L)	5.3 $\pm$ 6.6	3.7 $\pm$ 1.7	6.7 $\pm$ 2.8
Nitrate (mg NO <sub>3</sub> -N/L)	11.6 $\pm$ 7.9 <sup>a</sup>	15.0 $\pm$ 3.1 <sup>a</sup>	23.1 $\pm$ 3.8 <sup>b</sup>
Mean Shrimp Weight (g)	0.18 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>b</sup>	0.14 $\pm$ 0.01 <sup>c</sup>

## DEVELOPMENT OF AN AQUATIC GERMPLASM AND GENETIC RESOURCES CENTER

Terrence R. Tiersch\* and J. Michael Christensen

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Cryopreservation of sperm was developed at the same time (late 1940s) for bulls and fishes. Livestock operations were quick to adopt this new technology because it greatly simplified a long-standing need to distribute improved genetics throughout animal herds whereby artificial insemination with thawed sperm could replace the cost and effort involved in transporting and maintaining live bulls necessary to service cows. Fish hatcheries did not immediately recognize such needs, and for this and other reasons such as a lack of commercial-scale processing, cryopreservation has not yet been generally employed for aquatic species as a means to develop, maintain, and distribute genetic improvement.

The LSUAC Aquatic Germplasm and Genetic Resources Center (AGGRC) was initiated in to convert the LSU Dairy Improvement Center into a comprehensive, stand-alone facility for research, technology development, teaching, outreach, training, cooperation, and commercial-scaler processing for biobanking and germplasm repository development in aquatic species. The Dairy Improvement Center (DIC) was created in 1947 as a university-private sector cooperative and has provided services including custom collection and cryopreservation of bull semen for almost 70 years. Tiersch and co-workers have collaborated with dairy scientists at the DIC since the 1990's to develop commercial-scale cryopreservation techniques for sperm of fish and shellfish. In 2015 the commercial cooperator (Genex, Inc.) moved its operations to facilities in other states, and the DIC facility became available for transition into dedicated use with aquatic species. The facilities comprise 23,000 ft<sup>2</sup> of laboratory, office, cold-room, and barn space including a conference room and space specifically designed for use as a cryopreservation center. In addition there are several outbuildings (an additional 5,500 ft<sup>2</sup>) that can be converted into fish holding space.

The AGGRC combines 25 years of experience in cryopreservation and repository development with hundreds of aquatic species with facilities suitable for research and commercial-scale cryopreservation. Staffing includes biological and engineering expertise, and projects emphasize standardization of repository development by development of devices for specific activities in the cryopreservation process. Prototyping and testing are performed with 3-D printing and microfabrication capabilities. The facility is located adjacent to the LSU campus and enables easy access and collaboration with students and scientists at the university, including teaching of classes. In addition to separate research and commercial-scale laboratories, we have developed a self-contained mobile laboratory that can perform high-throughput cryopreservation at cooperating facilities.

## **DESIGNING COMMERCIAL AQUAPONICS SYSTEMS FOR ALTERNATIVE ENERGY**

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One of the largest reoccurring costs with operating a Commercial Aquaponics System is electricity. With proper design and engineering, a cost effective system can be constructed to utilize alternative energy as its primary source of power. This presentation will compare various forms of Alternative energy to show its effect on the variable monthly cost of operating a commercial Aquaponics system.

## **MOVING THE INDUSTRY FORWARD, ECONOMICS FOR 1500 FT2 SINGLE OWNER/ OPERATOR COMMERCIAL AQUAPONIC SYSTEM**

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Utilizing industry averages, we will look at the economics of operating a 1500 ft2 commercial Aquaponics System. Using this information we can then evaluate which crop and aquaculture species are the best choice for a small commercial facility.

## GORGONIANS AS INDICATORS OF MASS MORTALITY: THE CASE OF *Spinimuricea klavereni* IN THE SEA OF MARMARA (TURKEY)

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A recent event of mass mortality affected macrobenthic suspension feeders in Prince Islands (PI) coasts, the northeast Sea of Marmara (Fig. 1), in summer 2015. The mortality was mainly due to very high deposition of suspended particulate matter. In this region, *Spinimuricea klavereni* is one of the two most abundant gorgonians on substrates characterized by a mixture of pebbles, shells and small rocks on muddy bottom but also on rocky bottoms.

After the event, between August – November 2015, 9 stations in the south of PI coasts were monitored in order to assess the impact of the event. *S. klavereni* colonies were enumerated in 20 transects (1 m<sup>2</sup>) placed on either side of a 20-m-long transect tape laid on the substrate. The number of (i) healthy colonies, (ii) colonies showing more than 10 % tissue necrosis of its surface and (iii) completely dead colonies were noted. In case of tissue necrosis, the % of necrosis on colony surface as well as the presence of epibionts were also noted.

The % of dead and damaged colonies are much higher than those in previous years (Fig. 2) despite the numbers before mortality were also high, in comparison to values in other gorgonian populations in the Mediterranean Sea. Most of the damaged areas on colonies were covered by pioneer epibionts (mainly biofilm, algae and hydroids), showing that the necrosis were recent. *S. klavereni* is a species with higher growth rates than other gorgonians and is able to cover a large scale of bottom types in the Sea of Marmara. The recovery of the population in PI region will be monitored in following years. However due to slow dynamics of gorgonians and several stress sources in the natural environment, under captivity culture of the species and later, enhancement of the natural population is also considered. Previous studies showed that the species is able to survive in captivity conditions.

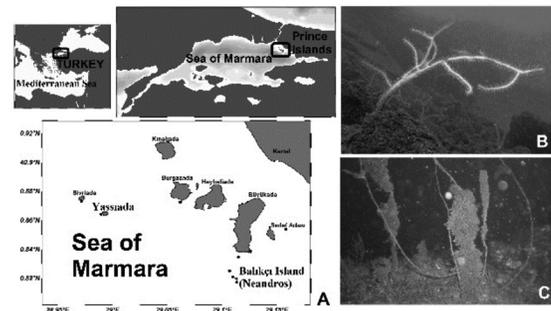


Fig. 1. Map of the study area (A) and colonies before (B) and after (C) the mortality.

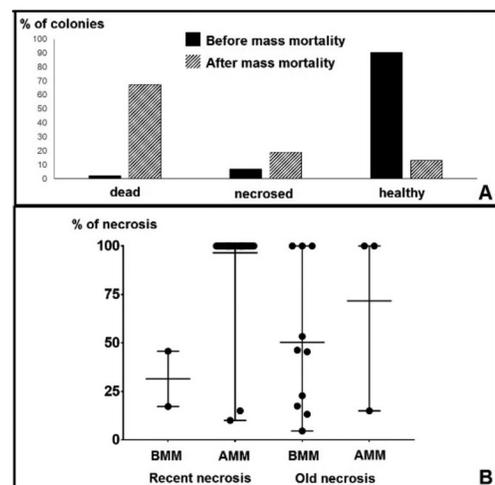


Fig. 2. Percentage of healthy and damaged colonies (A) and scatter plot of the % necrosis (mean with range).

## EFFECT OF GRADED FINGERLINGS ON HYBRID CATFISH FOOD FISH SIZE DISTRIBUTION

Les Torrans\* and Brian Ott

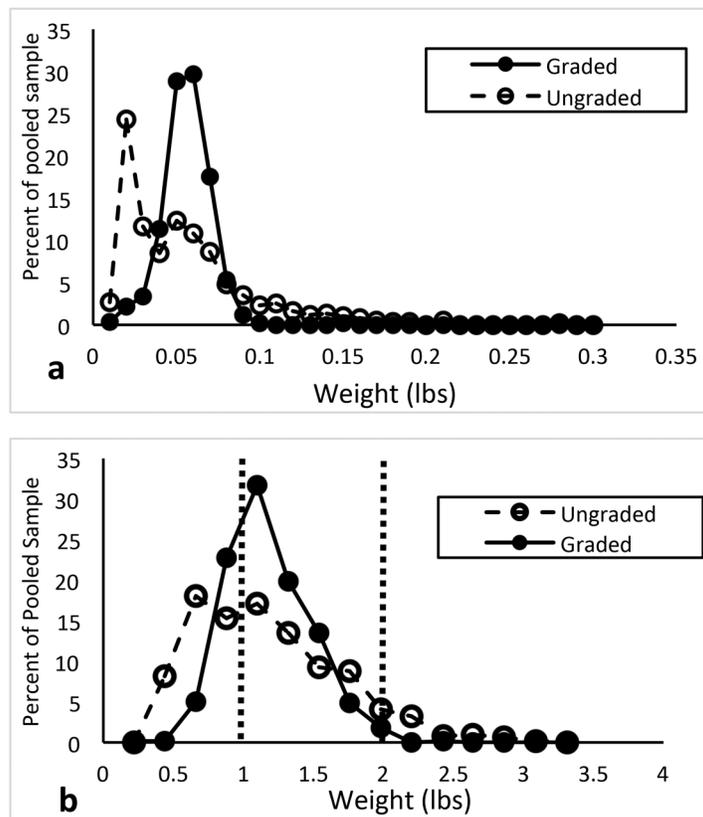
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It is not unusual to have both 0.5 lb and 5 lb fish harvested from a single-batch hybrid catfish production pond at the end of the growing season. When that happens, farmers may be docked for fish that are either larger or smaller than the processor's preferred size range. This study was conducted to determine the impact of grading fingerlings on the size distribution of food fish produced in a single-batch system.

Three 0.25-acre earthen ponds were stocked with 8,000/acre of ungraded fingerlings on April 23. The fish were individually counted and mass-weighed, averaging 0.0498 lb (49.8 lbs/1000). The remaining fingerlings were double-graded to produce fingerlings of the approximate same average weight (52.5 lbs/1000) but with a reduced size variation (CV = 78% and 27% for the ungraded and graded fingerlings, respectively, Figure a). Three ponds were stocked with these graded fingerlings.

The fish were harvested on October 14-16. Grading fingerlings had no significant effect on any production parameter except for final size variation (Figure b). Final mean fish weight (1.1 lbs) and FCR (1.58:1) was identical in both treatments. Survival and net production were not significantly different among treatments.

For the purpose of comparison, we established an arbitrary "preferred size range" at harvest of 1.0-2.0 lbs. Overall, 41.5% of the ungraded fish and 28.1% of the graded fish were less than 1.0 lb at harvest ( $P=0.18$ ). However 5.8% of the ungraded fish and 0.16% of the graded fish were larger than 2.0 lb ( $P=0.01$ ). Grading hybrid fingerlings is an effective means of decreasing food fish size variability without impacting production efficiency.



## EVALUATION OF THE IMPACT OF PROLONGED HIGH TEMPERATURE EXPOSURE ON GONADAL DIFFERENTIATION IN JUVENILE YELLOW PERCH *Perca flavescens*

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Yellow perch (*Perca flavescens*) are a popular food fish in the Great Lakes region. In this species, females grow faster and to a larger size than males, making them more valuable in aquaculture. In fact, a population of 1,000 female yellow perch can gross approximately \$555 more than the same number of fish exhibiting a 1:1 male:female ratio at the current fillet value of \$14 per pound. Attempts have been made to produce monosex populations of fish through sex reversal. This has been achieved in several species through environmental manipulation during sexual differentiation, with the most prevalent environmental factor being temperature. However, sex reversal in yellow perch has only been carried out by use of hormone treatments, and published information suggests attempts have only been successful in producing reproductively non-functional sex reversed males. In the current study, we assess if the exposure to steroid hormones could have been impacted by high temperatures during the period of gonadal differentiation and influenced sex ratio of juvenile yellow perch.

A first experiment was conducted in 2015 to determine the efficacy of hormonal sex reversal in juvenile yellow perch less than 16 mm total length (TL). The tanks were maintained at a temperature of approximately  $21.8 \pm 0.7^\circ \text{C}$  in order to maximize growth. The sex ratio of the experimental groups (control, MT-Diet, MT-Immersion, and E<sub>2</sub>-Immersion) was evaluated in January 2016. The control group was found to have a significantly greater percentage of spermiating males than the expected 50% (Fig. 1). Therefore, it was hypothesized that the high rearing temperature induced masculinization.

Eggs from our domesticated females were fertilized with 2015 “control” males ( $n = 12$ ), and embryos were incubated in  $10.3 \pm 0.6^\circ \text{C}$  until hatching. Consequently, offspring from five males were raised in high ( $23.1 \pm 0.2^\circ \text{C}$ ) or moderate ( $16.4 \pm 1.0^\circ \text{C}$ ) temperatures from 33 to 55 days post-fertilization. The fish are now in the growout phase, with all groups at the optimum temperature for growth ( $23.5 \pm 0.6^\circ \text{C}$ ). Analysis of the sex ratio of the groups, and the subsequent conclusions that can be drawn, are to be completed in December of 2016.

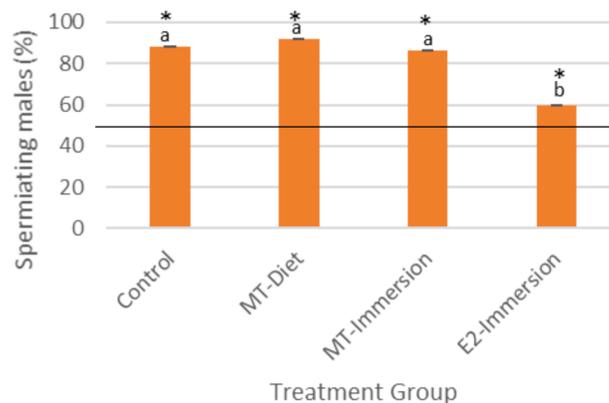


Figure 1: Spermiating yellow perch (%) following steroid exposure at high temperatures (2015). Horizontal bar denotes an unbiased sex ratio. Letters indicate a significant difference between groups (ANOVA). Stars indicate a significant deviation from the expected 1:1 male:female sex ratio (Binomial trial).

## DECOUPLED AQUAPONICS FOR IN SITU NUTRIENT MITIGATION FROM RAS

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**Abstract** – Define and show components of a decoupled aquaponic system. The goal of which is to provide a “closed loop” nutrient cycle for the cultured aquatic and plant species.

**Introduction** – Intensive hydroponic plant production has been demonstrated to be an economically viable alternative to extensive traditional agriculture for many highly prized food crops. Likewise intensive recirculating aquaculture has provided high grade fish to markets around the globe for decades and has resulted in decreased pressure on natural fisheries and increased recruitment and general health of natural populations that were at one time severely overfished and unsustainable. Both of these modern agricultural methods still have shortcomings with respect to the management of excess nutrients. Traditionally exhausted hydroponic nutrient solutions (no longer able to support the growth of macrophyte but still high in nitrogen and phosphorus) were discharged to sanitary sewers or natural waterways as were the nutrient laden wastewaters from intensive aquaculture operations. These effluents result in added nutrient loading in natural waterways and have been responsible for algae and bacterial blooms, fish kills, and the eutrophication of lakes, streams and rivers.

Decoupled aquaponic is an alternative to these practices allowing the farmer to “close the nutrient loop”, but not compromising each system, and avoid or minimize wastewater discharge to the environment. In this scheme, aquaculture wastewater, high in phosphorus and nitrogen, is pumped to the hydroponic system, which act as “sinks” for these nutrients. By incorporating the clear supernatant, all solids and associated water from RAS, N and P into the plant biomass of the hydroponic production, these nutrients are no longer available as potential pollutants.

Aquaponics, coupled and decoupled, technology are improving every day and our industry should be at the forefront of development both as educators and stewards of the environment.

## DESIGN CONSIDERATION FOR A 1,500 FT<sup>2</sup> AQUAPONIC SYSTEM

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Show design, components, and parts list of a 1,500ft<sup>2</sup> 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<sup>2</sup>/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.



## DISPELLING ANTIBIOTIC DRUG USE MYTHS: OXYTETRACYCLINE DIHYDRATE DOES NOT ENHANCE FISH GROWTH PERFORMANCE

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Antibiotics were developed for therapeutic purposes in humans and animals, but these products have been used for years in animal agriculture for non-therapeutic purposes, i.e., to promote growth and improve feed efficiency. There are no FDA-approved prophylactic or production claims for the use of antibiotic drugs in fish, but it is a common misconception that antibiotics are widely used for these purposes in U.S. aquaculture. Not only are such uses illegal, it is generally understood among fish culturists that such drugs are not effective growth promoters in fish, thereby removing any incentive to illicitly use antibiotics this way. Unfortunately, there is very little information available that unequivocally demonstrates the effect (or lack thereof) of antibiotic administration on growth performance of fish, whether administered at the therapeutic or subtherapeutic doses.

Accordingly, we conducted a series of experiments to determine whether administration of an antibiotic in feed with known growth-promoting effects in terrestrial livestock has a similar effect when applied to representative taxa of fish. Specifically, we evaluated the effect of oxytetracycline dihydrate (OTC) treatment on the health and growth performance of Channel Catfish *Ictalurus punctatus*, Hybrid Striped Bass *Morone chrysops* ♀ × *M. saxatilis* ♂, Nile Tilapia *Oreochromis niloticus*. OTC products with FDA-approved production claims in terrestrial livestock are typically applied at doses substantially lower than the approved therapeutic doses for the same products. Thus, commercially available aquaculture feed appropriate for the selected taxa was used as-is or medicated with OTC to achieve target doses of 80 mg/kg fish/d (a typical therapeutic dose for OTC treatment of fish) or 16 mg/kg fish/d (20% of the therapeutic dose) when fed at 3% body weight/d. Juvenile Channel Catfish ( $5.4 \pm 0.8$  g), Hybrid Striped Bass ( $27.0 \pm 0.2$  g), and Nile Tilapia ( $53.5 \pm 0.6$ ) were stocked in a recirculation system (10 fish per tank) comprising 12, 150-L tanks and provided with continuous aeration, mechanical, and biological filtration (trials conducted sequentially). Dietary treatments were randomly assigned to quadruplicate tanks ( $N = 4$ ) and offered once daily, with rations adjusted every 2 weeks based on group weights.

After 8 weeks, fish growth and feed conversion efficiency were unaffected by dietary treatment. Few significant treatment effects were observed, but those noted suggested inferior performance among fish fed medicated feed. Collectively, our results indicate there is virtually no incentive to misuse OTC for the purpose of enhancing growth or conversion efficiency in fish.

## WATER QUALITY IN CATFISH PONDS AFTER PARTIAL FISH HARVEST

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Intensification of United States catfish aquaculture involves hybrid catfish (♀ channel catfish *Ictalurus punctatus* x ♂ blue catfish *I. furcatus*) grown in ponds with abundant aeration and high feeding rates. High feeding rates cause water quality deterioration because most of the nitrogen, phosphorus, and organic matter in feed is not converted to harvestable crop but rather is lost to the water as waste. High feeding rates may therefore indirectly lead to accumulation of potentially toxic nitrogen compounds, excessive phytoplankton biomass consisting of undesirable cyanobacteria, and persistent dissolved oxygen deficits. This study was conducted to determine whether water quality is improved when feeding rates are reduced in midsummer after partial harvest to remove faster-growing fish.

Twelve, 1.0-acre ponds were stocked with 10,000 hybrid catfish fingerlings in March 2015. Six ponds were partially harvested in August to remove fish larger than ~ 0.57 kg. Fish in the six control ponds were not partially harvested. All remaining fish were removed in October and November.

Mid-summer partial fish harvest removed about 25% of the fish crop and reduced feed additions by a corresponding amount, but had no meaningful effects on water quality, phytoplankton biomass, phytoplankton and zooplankton community structure, or need for supplemental aeration. Lack of water quality improvement was caused by persistent internal recycling of nutrients derived from feeds added to ponds before and after partial fish harvest. Internal recycling of N, P, and other nutrients caused a lag (hysteresis) in the response of water quality to reduced external nutrient loading that lasted until final fish harvest in late autumn.

Size-selective partial fish harvest may have economic implications by removing faster-growing fish that could exceed the maximum size (and therefore subject to discounts) preferred by fish processor. Also, periodic fish biomass reduction can lessen fish loss from infectious diseases, bird predation, and when catastrophic power outages cause loss of aeration capabilities. Decisions to use partial fish harvest to manage single-batch catfish ponds should therefore be based on economics and risk reduction rather than the expectation that biomass reduction will markedly improve water quality.

# MAPPING OF FISH DISEASES OF NORTH SULAWESI AQUACULTURE

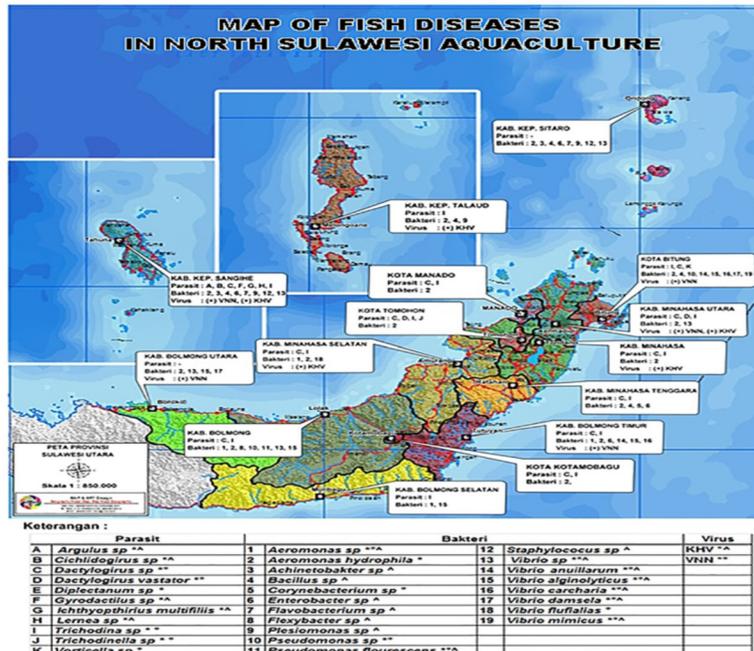
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Monitoring of fish diseases is important in reducing the losses in fish farming caused by the diseases. This study aimed to identify the types of pathogens that infect cultured fish in regencies/cities in North Sulawesi Province and to map the spread of fish disease in North Sulawesi. Ten fish were randomly sampled from each farm, where 3 farms were selected from each regency/city. Examination of ecto-parasites on the surface of the fish body was done by scraping the surface of the body or organ, and then observed under a microscope. The results were then compared with ecto-parasites identification Guide Kabata (1985). Bacterial examination was done by using the isolation and purification techniques. The characteristics were based on some tests: motility test, gram test and a series of biochemical tests. Analysis of the virus using gill organ as samples was carried out using Polymerase Chain Reaction (PCR) method.

The results shows that there were several types of bacteria identified as follows: *Aeromonas* sp, *A. hydrophilla*, *Flexibacter* sp, *Plesiomonas* sp, *Pseudomonas* sp, *P. flourescens*, *Vibrio* sp, *V. anuillarum*, *V. alginolyticus*, *V. carchariae*, *V damsela.*, *V. mimicus*. *A. hydrophilla* was found in all sampling locations.

Some parasitic diseases were found to be caused by species of parasites as follows: *Argulus* sp, *Dactylogirus* sp, *Gyrodactylus* sp, *Ichthyophthirius multifiliis*, *Lernea* sp, *Trichodina* sp and *Trichodinella* sp. *Trichodina* sp was the most dominant parasite found. The types of viruses that infect fish samples were Viral Nervous Necrosis (VNN) identified in grouper (*Epinephelus* sp) and Koi Herpes Virus (KHV) found in carp (*Cyprinus carpio*) and Koi (*Cyprinus koi*).



## **EFFECTS OF DIETARY LIPID SOURCE AND ULTRAVIOLET RADIATION ON GROWTH AND FATTY ACID PROFILE OF STEELHEAD, *Oncorhynchus mykiss***

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Steatitis is a nutritionally induced disease characterized by inflammation of adipose tissue observed in terrestrial and aquatic species. This disease is generally attributed to peroxidation of long chain polyunsaturated fatty acids (LCPUFAs) in animals consuming diets rich in fish and/or fish oil. Both diet and ultraviolet (UV) light are thought to contribute to recurring steatitis outbreaks in salmonid hatcheries as the disease is often detected following transfer of juveniles from indoor hatchery buildings to outdoor rearing systems exposed to sunlight. Diets used in these hatcheries contain fish meal and fish oil as primary sources of protein and lipid, respectively. To characterize the effects of dietary lipid and UV radiation on steelhead (*Oncorhynchus mykiss*) growth, composition and steatitis, fish were fed for 10 weeks diets containing canola oil (CO), fish oil (FO) or moderately oxidized fish oil (OFO) and subjected to fluorescent or artificial UV light. Utilizing a completely randomized factorial design, each treatment was assigned to triplicate groups of 100 fish initially weighing 1.6 g/fish. Final growth responses and survival were unaffected by either lipid or light source individually, but weight gain was significantly affected by their interaction. Whole body histopathology revealed significantly higher (more severe) steatitis scores in fish fed FO or OFO compared with fish fed CO. Steatitis scores also were significantly higher in fish exposed to UV light compared with fish exposed to fluorescent light. Whole body lipid concentration and tissue fatty acid profiles were significantly affected by dietary lipid and light source, but not by their interaction. Results of this study suggest feeding diets low in LCPUFAs and minimizing exposure to UV light may reduce steatitis in steelhead.

## EFFECTS OF FOUR DIFFERENT FEEDING TECHNIQUES ON THE POND CULTURE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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The continued success of shrimp aquaculture in the US will rely on improvements in feed management and reductions in the labor requirements for production. As grazers, shrimp have shown increased performance with multiple feedings spread throughout the day, but the labor required to increase the feedings can be prohibitive to some operations. Utilizing timer feeders and acoustic demand feeders allow farmers to spread out the feeding without increasing the labor required to do so. The acoustic monitors also allow feeding activity to be monitored to improve feed application during times of active feeding. The effects of four feed management techniques on the pond culture of Pacific white shrimp (*Litopenaeus vannamei*) was compared at the Claude Peteet Mariculture Center, Gulf Shores, Alabama, USA. To evaluate different feed management applications, a 16-week trial was performed in 16, 0.1Ha ponds that were stocked at 17 shrimp/m<sup>2</sup>. The experiment had 2 treatments employing manual feeding using the Standard Feeding Protocol (SFP) which is calculated with a predicted growth of 1.3 g/wk, and an estimated feed conversion ratio (FCR) of 1.2, assuming a survival rate of 75% over 16-wk culture period, and manual feeding with a 15% increase to the SFP after 8 weeks. Two treatments with automatic feeders, a timer feeder was programmed to feed 6 times per day following the SFP, and ad libitum using the AQ1 acoustic demand feeding. Data as of 12 weeks shows significant increases in growth in the AQ1 treatments for weight (33.08 g) and weekly growth rate (2.75 g/wk) compared to all other treatments. The AQ1 treatments have also fed significantly more feed (409.5 Kg).

Table 1. Responses of Pacific white shrimp to varying feeding strategies over 12 week in an outdoor pond trial.

Treatment	Avg. Individual Wt (g)	Individual Weekly Growth (g)	Feed Input (Kg)
SFP <sup>1</sup>	22.49 <sup>a</sup>	1.88 <sup>a</sup>	223.2 <sup>a</sup>
15% Increase SFP	23.60 <sup>a</sup>	1.97 <sup>a</sup>	237.3 <sup>a</sup>
Timer Feedings	26.89 <sup>a</sup>	2.24 <sup>a</sup>	224.3 <sup>a</sup>
AQ1 Acoustic Feeding	33.08 <sup>b</sup>	2.75 <sup>b</sup>	409.5 <sup>b</sup>
P-value	0.0004	0.0004	<0.0001
PSE <sup>2</sup>	1.0886	0.0899	11.8533

<sup>1</sup>SFP: Standard Feeding Protocol <sup>2</sup>PSE: Pooled Standard Error

## PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF WILD AND CULTURE CICHLID POPULATIONS (*Tilapia guineensis*) IN LAGOS STATE, NIGERIA

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This study was carried out using phenotypic and genotypic approach in characterization and identification of *Tilapia guineensis* from the wild and culture. A total of forty (40) samples of *T. guineensis* were collected; twenty(10) samples each of farm-raised (Akinsateru and Nigeria Institute of Oceanography & Marine Research (NIOMR) and wild (Lagos and Badagry Lagoon) were collected in Lagos State for 3 months.

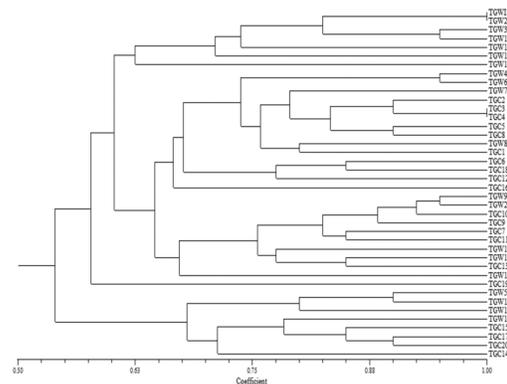
There were no significant differences ( $P \geq 0.05$ ) in all the phenotypic characters measured except at the Pre-Pectoral Length (PPL), Dorsal Fin (DF) and Weight (WEI) (Table 1). These could be as result of ecological factors such as temperature, pollution; management practices in the farm, etc.

The similarity coefficient at 50% and 78% shows that there is genetic variability amongst the wild and culture samples. The observed similarities in the banding pattern at 100% of the wild and cultured environment could be partly due to escape of the cultured into the wild during flooding of some farms or that farmers are collecting broodstock from the wild. This study provides a promising solution to DNA isolation using the more economical salting-out method for extraction and base-line data required for genetic manipulation of the selected population of the fish in aquaculture.

Table 1: Mean values of morphometric and meristic measurements were significant ( $p < 0.05$ ).

measurements	WILD		CULTURED	
	Lagos lagoon	Badagry lagoon	Akinsateru farm	NIOMR farm
PPEL	4.0 ± 0.33 (3.8 - 5.0)	4.6 ± 0.6 (3.1 - 5.1)	4.4 ± 0.33* (3.8 - 5.0)	4.2 ± 0.56* (3.3 - 5.0)
DF	27.3 ± 0.67 (26.0 - 28.0)	27.7 ± 0.82 (26.0 - 29.0)	28.2 ± 0.42* (28.0 - 29.0)	27.3 ± 0.48* (27.0 - 28.0)
WEI	82.0 ± 13.10 (63.1 - 100.8)	98.20 ± 24.70 (70.3 - 129.6)	93.7 ± 21.72* (66.67 - 131.26)	72.5 ± 36.66* (26.0 - 128.7)

Range in parenthesis. Values with superscript along the horizontal are significant at 0.05.



**Figure 1.** Dendrogram depicting the degree of relation between the wild and culture relationship between the wild and culture sample .  
TGW1 - TGW10 = *T. guineensis* from the Lagos Lagoon  
TGW11 - TGW20 = *T. guineensis* from the Badagry Lagoon  
TGC1- TGC10 = *T. guineensis* from the Akinsateru farm.  
TGC11 - TGC 20 = *T. guineensis* from the NIOMR farm

## ROLE OF ALGAE EXTRACTS IN REDUCING STRESS AND ACUTE HEPATOPANCREATIC NECROSIS SYNDROME (AHPNS) MORTALITIES IN WHITELEG SHRIMP *Litopenaeus vannamei*

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Algae are known for their biological activities. Many algae species were, and still are, used in the development of traditional medicine. The main active component of these algae are marine sulphated polysaccharide (MSP) molecules which are only found in marine plants. These MSP's have shown highly biological activity, including immunomodulatory, anti-bacterial, anti-viral, mucin-stimulating, anti-hyperlipidemic activities.

In an industry where intensive culture sets a high strain on the culture conditions, and where sustainability is becoming an important factor in production, natural solutions are what is needed. Stressful conditions can be found during transport from hatchery tanks to grow out ponds, changes from the hatchery environment to the more challenging grow out pond environment and throughout the intensive grow out systems. These factors are just a few that can create the right conditions for disease to develop. One such disease that has had a large impact on the shrimp industry over the past 7 years is AHPNS. In many cases diminishing the total production by half. Even today the industry is looking for different ways to reduce the risks of AHPNS. Here too, preventive measures are often the best practices.

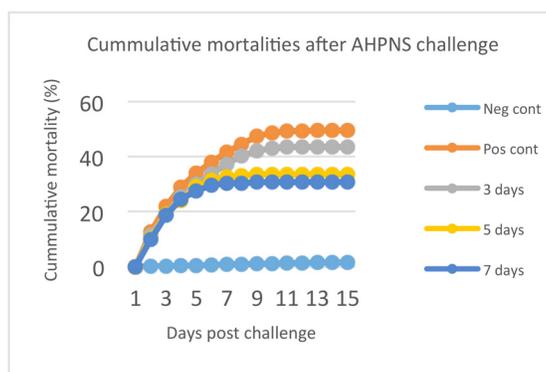
Olmix has specialized in using algae in innovative ways to increase sustainable production. The aim of this study was to see if these natural algae extracts would help improve shrimps ability to cope with stress and if they could improve the survival after an AHPNS disease challenge.

The trial was set up in two parts. The first part was aimed at stress tolerance of shrimp PL. PL 5 shrimp kept in 4 tanks until the size PL12. All tanks received the same standard diet. Treatment 1 was the control receiving only the standard diet. Treatments 2, 3 and 4 received the standard diet but coated with the algae extract during 3, 5 and 7 consecutive days respectively, prior to the different stress tests (10ppt salinity, 30ppt salinity and 100ppm formalin stress tests).

At PL 12 the shrimp were submitted to three different stress tests. Results showed that there was an increasing survival rate when algae was added to the diet prior to the stress tests. These results suggest that algal extracts help increase the PL tolerance of the stress tests of which they were submitted.

The second part was aimed at testing against a challenge test. In separate tanks PLs were raised to size PL25 when they were submitted to an immersion challenge with AHPNS infected bacteria (*vibrio parahaemolyticus*). Here too, after receiving similar treatments as in the stress tests (0, 3, 5 and 7 consecutive days of algae extract coated feed prior to the challenge test), the recorded mortalities show a dose dependent increased survival over the post challenge period.

In conclusion this study highlights the capacity of algal extracts to provide a natural way of reducing mortalities caused by stress and by an AHPNS challenge.



## THE EFFECTS OF REGULATIONS ON EFFICIENCY OF U.S. BAITFISH AND SPORTFISH PRODUCERS

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To develop a deeper understanding of the costs of the U.S. regulatory environment on producers of baitfish and sportfish, a technical efficiency analysis was performed using a stochastic production frontier model. Technical efficiency estimates were obtained, using the joint estimated least likelihood procedure of Frontier 4.1, for all firms in the study group and determinants of inefficiency were assessed for their role in the reduction of farm efficiency. Mean technical efficiency for U.S. baitfish and sportfish producers was found to be 77%; with a minimum of 8% and a maximum of 97%. Regulatory cost components such as “lost/foregone sales”, “changes to comply with regulations” and “manpower to comply with regulations, were found to be significant factors of inefficiency within the model. Results have supplied additional evidence to support the hypothesis that the current regulatory environment is reducing efficiency and economic competitiveness of baitfish and sportfish producers.

<b>State / Grouping</b>	<b>Average Efficiency Score</b>	<b>Min</b>	<b>Max</b>
<b>Arkansas</b>	0.822	0.431	0.955
<b>Alabama</b>	0.929	0.833	0.967
<b>New York</b>	0.795	0.621	0.939
<b>North Carolina</b>	0.927	0.905	0.959
<b>Ohio</b>	0.553	0.008	0.934
<b>Pennsylvania</b>	0.462	0.181	0.765
<b>Wisconsin</b>	0.925	0.909	0.955
<b>Other States<sup>1</sup></b>	0.865	0.596	0.961
<b>All Observations</b>	<b>0.766</b>	<b>0.008</b>	<b>0.967</b>

## THE ECONOMIC IMPACT OF THE BAITFISH AND SPORTFISH INDUSTRY ON THE STATE OF ARKANSAS

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An economic impact assessment was performed using IMPLAN Pro™ for the baitfish and sportfish industry in the state of Arkansas and three Arkansas counties (Greene, Lonoke, and Prairie) using data collected from a 2015 direct, personal survey. Estimates of the economic impact by output, value added, and employment were obtained for Arkansas producers of baitfish and sportfish within the study areas. The total impact to economic output of the state was estimated to be \$71.9 million, with a value added impact of \$37.7 million, and an employment impact of 559. On a national stage, the production and marketing of baitfish in Arkansas results in an estimated total economic output effect of \$157.9 million. Total economic impact for the three largest baitfish/sportfish producing counties in Arkansas were: \$31.1 million for Lonoke, \$11.4 million for Prairie, and \$8.6 million for Green County. A scenario developed to model the recovery of lost and foregone sales, identified as the largest portion of regulatory costs, demonstrated an increase in total economic output of approximately 8.1% based on increased farm production and marketing expenditures. The Arkansas industries that benefit the most from the production of baitfish and sportfish in Arkansas were those that specifically related to the shipping and transportation of live fish including the automotive/trucking industries and shipping services.



<b>Impact Type</b>	<b>Employment</b>	<b>Labor Income</b>	<b>Total Value Added</b>	<b>Output</b>
Direct Effect	294	\$14,265,234	\$17,985,575	\$36,559,491
Indirect Effect	80	\$3,447,784	\$6,238,447	\$11,931,670
Induced Effect	185	\$7,014,043	\$13,503,595	\$23,437,179
<b>Total Effect</b>	<b>559</b>	<b>\$24,727,060</b>	<b>\$37,727,617</b>	<b>\$71,928,340</b>

## INTENSIVE COMMERCIAL AQUAPONICS SYSTEM DESIGN AND CONSTRUCTION LEARNING EXPERIENCE

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Happy Fish Aquaponics is a family farm located just north of Minneapolis/Saint Paul Minnesota. We are an intensive commercial aquaponics farm that is just finishing up construction. Happy Fish Aquaponics (HFA) consists of a 5,000 square foot pole building and a 16,000 square foot greenhouse. The aquaponics system consists of over 12,000 gallons of aquaculture tanks and over 8,000 square feet of deep water culture grow space with room to add media beds and Dutch buckets.

Construction began in the summer of 2014 and most of the labor for the entire project was performed by us and our families. The first summer was dedicated to constructing the pole building and its utilities, e.g. well, septic system, heating and plumbing. The summer of 2015 was dedicated to building the Poly-Tex greenhouse and the subsequent winter was spent installing the electrical system, grow lights, fans, heaters and greenhouse controller. This summer was dedicated to putting together the actual aquaponics system.

HFA worked with a consultant and supplier to help us design our aquaponics system. We had taken 2 aquaponics courses, the Nelson and Pade course, and the Pentaire Aquatic Eco course. After having taken both courses, each offering a different approach to commercial aquaponics, we decided to build a more automated system that will allow for us to easily increase production without needing more labor.

We both do not have a background in aquaponics and this is our first venture in the agricultural industry. There exists a very big learning curve. All throughout the construction process, every day was a learning experience. Looking back, there are many things we would do differently in respect to the construction process and system design. There were many challenges we had to overcome, including breaking very expensive equipment. However, now that we are coming to the end of this phase of our project, we are very proud of our accomplishments and look forward to being a part of the aquaponics industry and helping to expand it. We hope that our experiences can help others save time and headaches.



## DIETARY *SPIRULINA* (*Arthrospira platensis* (Gomont) Geitler, 1925) REPLACEMENT ENHANCES PERFORMANCE OF POND- AND TANK-REARED JUVENILE NILE TILAPIA (*Oreochromis niloticus* Linnaeus, 1758)

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A feeding trial was conducted to evaluate the effects of *Spirulina* (*Arthrospira platensis*) inclusion in experimental diets of juvenile Nile tilapia (*Oreochromis niloticus*). Iso-nitrogenous and iso-lipidic diets were prepared using a positive control diet (S0) and four basal diets with *A. platensis* at 30% (S30), 45% (S45), 60% (S60), and 75% (S75) inclusion levels. Experimental diets were randomly assigned to tanks (13.35 kg m<sup>-3</sup>) with initial body weight of 0.89 ± 0.02 g in triplicates. The results of the feeding trial demonstrated significantly improved ( $P < 0.05$ ) growth performance and hepatosomatic and viscerosomatic indices of fish fed S30. Blood chemical profile also indicated desirable values in fish fed *A. platensis* diets as compared to fish fed S0. On the contrary, fish fed S75 exhibited significantly reduced fish growth. Blood chemical analysis showed significant difference ( $P < 0.05$ ) in levels of plasma triglyceride. Results of the present work indicate that 30% *A. platensis* inclusion is deemed the optimal level of dietary replacement for increased growth performance, improved feed utilization efficiency, and enhanced overall health status of Nile tilapia juveniles.

**Table 1.** Growth performance of pond-reared juvenile Nile tilapia fishes fed with increasing *Spirulina* inclusion levels.

Growth Parameters	DIETS				
	S0	S30	S45	S60	S75
% AWG	1613.89± 77.39 <sup>a</sup>	1992.08± 181.32 <sup>a</sup>	2048.66± 132.14 <sup>a</sup>	2074.80± 39.13 <sup>a</sup>	1843.78± 130.47 <sup>a</sup>
%SGR	4.16±0.06 <sup>a</sup>	4.63±0.06 <sup>a</sup>	4.46±0.08 <sup>a</sup>	4.23±0.11 <sup>a</sup>	3.40±0.09 <sup>a</sup>
FER	1.04±0.22 <sup>a</sup>	1.14±0.06 <sup>a</sup>	1.24±0.13 <sup>a</sup>	1.38±0.20 <sup>a</sup>	1.32±0.42 <sup>a</sup>
%Survival	93±0.58 <sup>a</sup>	91±1.15 <sup>a</sup>	93±0.47 <sup>a</sup>	93±0.58 <sup>a</sup>	93±1.00 <sup>a</sup>

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ( $P < 0.05$ ).

**Table 2.** Growth performance of tank-reared juvenile Nile tilapia fishes fed with increasing *Spirulina* inclusion levels.

Growth Parameters	DIETS				
	S0	S30	S45	S60	S75
%AWG	946.74± 21.84 <sup>b</sup>	1302.11± 62.17 <sup>d</sup>	1173.64± 65.18 <sup>c,d</sup>	1057.70± 24.40 <sup>b,c</sup>	610.23± 38.20 <sup>a</sup>
%SGR	4.16± 0.06 <sup>b</sup>	4.63±0.06 <sup>c</sup>	4.46±0.08 <sup>b,c</sup>	4.23±0.11 <sup>b</sup>	3.40±0.09 <sup>a</sup>
FER	0.52± 0.03 <sup>a</sup>	0.74±0.02 <sub>b</sub>	0.75±0.05 <sup>b</sup>	0.68±0.07 <sup>a,b</sup>	0.57±0.05 <sup>a,b</sup>
%Survival	80.00± 3.85 <sup>a</sup>	84.44±5.8 <sub>8</sub> <sup>a</sup>	77.78±5.88 <sup>a</sup>	71.11±16.0 <sub>2</sub> <sup>a</sup>	82.22±2.22 <sup>a</sup>

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ( $P < 0.05$ ).

**Table 6.** Blood chemical parameters of tank-reared juvenile Nile tilapia fishes fed with increasing *Spirulina* inclusion levels.

Blood chemical parameters	DIETS				
	S0	S30	S45	S60	S75
<b>Cholesterol</b> (mg/dL)	219.23 ± 31.9 <sup>a</sup>	181.9 ± 12.98 <sup>a</sup>	178.77 ± 21.74 <sup>a</sup>	198.03 ± 37.18 <sup>a</sup>	161.2 ± 22.14 <sup>a</sup>
<b>Triglyceride</b> (mg/dL)	403.9 ± 81.39 <sup>b</sup>	156.47 ± 26.25 <sup>a</sup>	115.5 ± 11.55 <sup>a</sup>	166.37 ± 18.62 <sup>a</sup>	158.23±24. 48 <sup>a</sup>
<b>Glucose</b> (mg/dL)	213.63±110. 73 <sup>a</sup>	93.23 ± 1.10 <sup>a</sup>	74 ± 3.83 <sup>a</sup>	78.63 ± 8.55 <sup>a</sup>	61.43 ± 3.80 <sup>a</sup>
<b>SGPT</b> (U/L)	32.1 ± 20.28 <sup>a</sup>	16.1 ± 0.56 <sup>a</sup>	10.97 ± 5.03 <sup>a</sup>	5.9 ± 1.71 <sup>a</sup>	4.87 ± 1.84 <sup>a</sup>
<b>SGOT</b> (U/L)	278.73 ± 91.05 <sup>a</sup>	367.63 ± 9.52 <sup>a</sup>	320.63 ± 40.57 <sup>a</sup>	375.17 ± 7.29 <sup>a</sup>	355.77 ± 12.13 <sup>a</sup>
<b>Creatinine</b> (mg/dL)	6.87 ± 0.81 <sup>a</sup>	6.47 ± 0.20 <sup>a</sup>	6.33 ± 0.18 <sup>a</sup>	6.1 ± 0.26 <sup>a</sup>	5.53 ± 0.61 <sup>a</sup>
<b>HDL</b> (mg/dL)	25.63 ± 6.71 <sup>a</sup>	34.2 ± 10.40 <sup>a</sup>	28.8 ± 13.11 <sup>a</sup>	15.63 ± 1.30 <sup>a</sup>	16.87 ± 2.52 <sup>a</sup>
<b>Uric Acid</b> (mg/dL)	0.67 ± 0.09 <sup>a</sup>	1.37 ± 0.24 <sup>a</sup>	0.83 ± 0.64 <sup>a</sup>	1 ± 0.35 <sup>a</sup>	0.97 ± 0.12 <sup>a</sup>

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ( $P < 0.05$ ).

**Table 3.** Fish body composition of pond-reared juvenile Nile tilapia fishes fed with increasing *Spirulina* inclusion levels.

Fish Body Composition	DIETS				
	S0	S30	S45	S60	S75
HSI	2.53 ± 0.10 <sup>a</sup>	2.44 ± 0.31 <sup>a</sup>	2.75 ± 0.24 <sup>a</sup>	2.51 ± 0.89 <sup>a</sup>	3.14 ± 0.32 <sup>a</sup>
VSI	7.38 ± 0.24 <sup>a</sup>	6.42 ± 0.52 <sup>a</sup>	6.48 ± 0.34 <sup>a</sup>	7.06 ± 0.74 <sup>a</sup>	7.41 ± 0.28 <sup>a</sup>
CF	1.78 ± 0.03 <sup>a</sup>	1.81 ± 0.12 <sup>a</sup>	1.69 ± 0.01 <sup>a</sup>	1.71 ± 0.08 <sup>a</sup>	1.62 ± 0.01 <sup>a</sup>

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ( $P < 0.05$ ).

**Table 4.** Fish body composition of tank-reared juvenile Nile tilapia fishes fed with increasing *Spirulina* inclusion levels.

Fish Body Composition	DIETS				
	S0	S30	S45	S60	S75
HSI	3.13 ± 0.13 <sup>b</sup>	2.96 ± 0.22 <sup>b</sup>	2.99 ± 0.04 <sup>b</sup>	2.27 ± 0.30 <sup>ab</sup>	1.92 ± 0.22 <sup>a</sup>
VSI	9.15 ± 0.37 <sup>b</sup>	8.39 ± 0.17 <sup>ab</sup>	7.89 ± 0.22 <sup>ab</sup>	7.79 ± 0.53 <sup>ab</sup>	7.44 ± 0.07 <sup>a</sup>
CF	1.65 ± 0.03 <sup>a</sup>	1.70 ± 0.04 <sup>a</sup>	1.69 ± 0.01 <sup>a</sup>	1.67 ± 0.07 <sup>a</sup>	1.62 ± 0.03 <sup>a</sup>

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ( $P < 0.05$ ).

## EFFECTS OF METHIONE AND TAURINE SUPPLEMENTATION ON GROWTH PERFORMANCE, WHOLE-BODY COMPOSITION, AND PLASMA AMINO ACID CONCENTRATIONS OF JUVENILE RED DRUM *Sciaenops ocellatus*

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Methionine (MET) has been demonstrated to be an essential amino acid for fish, and taurine (TAU), a beta sulfonic acid, a conditionally essential nutrient mostly for marine fish species with carnivorous feeding habits. Previous work has shown that red drum or redfish *Sciaenops ocellatus* requires the supplementation of both MET and TAU, specifically in diets formulated with high inclusion of plant proteins. The minimum dietary requirement for MET was previously established to be 1.06% of dry weight in a 35% crude protein (CP) diet. However, at higher protein levels, there is no published information on the MET requirement of this species. Also, Met is a source of sulfur required in the biosynthesis of TAU. Thus, it was of interest to determine if the requirements for MET and TAU would change at a higher dietary protein level, and if there were nutritional interactions among these compounds.

An 8-week feeding trial was conducted using a factorial design with dietary MET (0.7, 1.2, and 1.7%) and TAU (0.1, 0.45 and 0.9%) levels as the main factors. Diets were formulated with 18% red drum muscle and the remaining protein was provided by plant ingredients. All diets were formulated to contain 40% CP and 13% lipid. Twenty fish with initial average weight of 1.57 grams were stocked into each of 27, 110-L tanks of an indoor brackish water (5-6 ppt) recirculating system. Each diet was fed to three replicate groups of fish twice daily at rates close to apparent satiation. Fish were group weighed weekly to adjust feeding rations.

At the end of the trial, three fish per aquarium were collected for determination of body condition indices and whole-body proximate composition, as well as methionine and taurine concentrations in plasma and tissues.

After the 8-week trial, percentage weight gain (PWG) was affected both by MET and TAU; treatments with 0.45% TAU or higher, and MET at 1.2% or above, had significant higher weight gain (p-value 0.0282, 0.0001, respectively) than the lower TAU and MET treatments. Highest PWG was achieved by fish fed the diet with 1.2% MET and 0.9% TAU, although it was only significantly different from that of fish fed the diet with 1.2% MET and 0.1% TAU, as well as all the 0.7% MET treatments. Feed efficiency (FE) was significantly lower for fish fed the 0.7% MET diets (p-value 0.001). No significant differences were observed for FE with TAU supplementation, although FE was slightly better at the higher inclusion levels. No difference was found among treatments for survival. Muscle ratio was significantly lower (p-value 0.0001) for fish fed the 0.7% MET with 0.1% TAU diet and the 1.7% MET with 0.9% TAU diet compared to the rest of the dietary treatments. Intraperitoneal fat (IPF) ratio and hepatosomatic index (HSI) were affected by MET, with fish fed the diets with 1.7% MET being significantly higher than those fed the 0.7% MET diets (p-value 0.0003 and 0.0033, respectively). Results of amino acid, whole body, and gene expression are pending further processing.

## HERBIVOROUS MARINE FINFISH CULTURE – THE COMPELLING CASE FOR KYPHOSIDS

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Marine finfish aquaculture is dominated by carnivorous species. Carnivores require diets high in proteins and oils, often fulfilled through the use of wild-sourced fish products, raising into question the scalability of the industry. Reliance on wild fish resources for fishmeal and fish oil is also a leading sustainability concern, which constrains the expansion of responsible mariculture. Extensive research (including nearly a decade of work by Kampachi Farms) is striving to identify alternative ingredients and reformulate feeds for ‘carnivorous’ fish – pursuing primarily plant-derived proteins and oils, such as soy. However, an alternative to carnivorous culture has been left largely unexplored – herbivorous marine finfish that do not demand fishmeal and fish oil in their diets.

Kyphosids (chubs or rudderfish; of which there are species native to all areas of the U.S.’s prime mariculture development regions) are esteemed food-fish among the Pacific Islands. Preliminary research with *Kyphosus vaigiensis* (Brassy chub) has shown them to be amenable to larval rearing in the hatchery, resistant to skin flukes, yield commercially attractive growth rates and produce a highly-appealing product, at up to 29% lipid (by dry weight). Our current work aims to close the life cycle of this species in captivity by refining broodstock husbandry protocols (feeding and housing regimes) and obtaining tank spawns. We are also simultaneously researching the growth rates of *K. vaigiensis* fed with three invasive algae species (a widespread and persistent problem in the Hawaiian Islands), as well as on low-cost commercial herbivore feeds. The goal of this research is to establish the feasibility of their commercial production. The successful culture of these high-quality herbivorous marine finfish could provide a new, sustainable, marketable, healthy and low-cost option for fish farmers and seafood consumers.

## **SEAFOOD HEALTH FACTS: MAKING SMART CHOICES TO BALANCE THE BENEFITS AND RISKS OF SEAFOOD CONSUMPTION**

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The Seafood Health Facts website (SFH) <[seafoodhealthfacts.org](http://seafoodhealthfacts.org)> provides healthcare, seafood industry professionals and consumers with straightforward, science-based information about the pros and cons of including seafood in a balanced diet and current media issues. The website, developed by Cornell University and the New York Sea Grant Extension Program in collaboration with Oregon State University and the Universities of Rhode Island, Delaware, Florida and California in 2011, is currently hosted and managed by the University of Delaware Sea Grant Program. According to a Google Analytics review (9/1/15 to 8/31/16), SHF is directly linked to or receives search engine referrals from more than 840 sources, most notably NOAA FishWatch, the Seafood Network Information Center (SeafoodNIC) hosted by Oregon State University, NOAA Office of Aquaculture, the National Fisheries Institute (NFI), SeafoodSource.com, Monterey Bay Aquarium SeaWatch, Google, Yahoo, eXtension, several state Sea Grant programs and others. Seafood Health Facts received 685,529 page views from 509,674 unique visitors (90.3% new and 9.7% returning). The majority of visitors (80.59%) were from the US, representing all 50 states, and the District of Columbia. The United Kingdom, Canada, Australia and India led international visitation from a total of 185 countries. Website managers Doris Hicks and John Ewart are expanding the format and scope of the website and encourage aquaculture businesses and industry associations to include a link to Seafood Health Facts on their websites as a reliable and impartial “go to” resource for seafood information. The presentation includes a review of current and future website subject categories and content.

## RESPONSE OF AFRICAN LUNGFISH (*Protopterus aethiopicus*) LARVAE FED ON COMMERCIAL DIETS AND *Artemia sp.* CYSTS

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Sustainable aquaculture production of lungfish in Uganda necessitates development of appropriate feed technology. This study identifies hatchery feeds that can improve performance of African lungfish larvae nursed in aquaria tanks. Larvae were fed on decapsulated *Artemia sp.* and microdiet (35-57% Crude Protein), and a combination of *Artemia cyst* + microdiet. Experimental fish were evaluated for growth performance and survival in optimized conditions. Results indicate improved growth and survival rates of lungfish larvae compared to initial attempts to wean this fish on artificial diets. Larvae fed on *Artemia sp.* and a combination had the highest mean increase in weight and survival rates over time. However, lungfish larvae can exclusively survive well on *Artemia* cysts but further research will develop low-cost live feeds for nursing this fish. This study provides guidelines for improving lungfish larval nursing, which is important when propagating this fish.

## EVALUATION OF BLACK SOLDIER FLY *Hermetia illucens* LARVAE AS A FISH MEAL REPLACEMENT IN PRACTICAL DIETS FOR LARGEMOUTH BASS *Micropterus salmoides*

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Protein meal derived from the larvae of the black soldier fly (*Hermetia illucens*) (BSFL) shows promise as an alternative protein source in the diets of a variety of fish species. The larvae are capable of assimilating organic materials from food waste or food and beverage manufacturing. The resulting BSFL can then be turned into a valuable feedstuff. Carnivorous fish species like the largemouth bass (LMB) (*Micropterus salmoides*) require higher protein levels than omnivores and protein is the most expensive ingredient in aquatic diets. Fish meal has traditionally been the main source of protein in fish diets, however this source is derived from natural stocks that have reached maximum yields. Therefore, alternative protein sources of high quality must be developed to support sustainable growth in aquaculture.

A 12 week study is being conducted to determine if BSFL can substitute for fish meal in diets for LMB. Five test diets were formulated to be isonitrogenous and isocaloric (42% protein and 12% fat). The control diet derived the majority of its protein from sardine fish meal. The four experimental diets contained decreasing percentages of fish meal and increasing inclusions of BSFL protein (25%, 50%, 75% and 100%). The research is performed in a recirculating aquaculture system where 20, 114-L glass aquaria were randomly stocked with 25 LMB fingerlings ( $8.4 \pm 1.9$  g) each. There are four replicate tanks per dietary treatment. Dissolved oxygen, pH and temperature are logged daily and water quality data on alkalinity, salinity, total ammonia and nitrite is monitored bi-weekly. The study will conclude in October 2016. At stocking, baseline fish and diet samples were taken. Whole body proximate analysis will determine the lipid, moisture and ash content of the baseline fish. Amino acid and fatty acid profiles will be analyzed on the diets and amino acid profiles will be analyzed on fish. At the conclusion of the experiment; fish in each aquarium will be removed, measured for total length and weighed. From each aquarium ten random sample fish will be analyzed for whole body proximate composition and amino acid profiles. The overall growth performance variables measured for the fish are specific growth rate, feed conversion ratio, apparent protein utilization and protein efficiency ratio. Specific growth rate (SGR, % body wt/d) was calculated from  $SGR = [(\ln W_f - \ln W_i) / t] \times 100$ , where  $W_f$  = final weight (g),  $W_i$  = initial weight (g), and  $t$  = time in days. Feed conversion ratio was calculated as  $FCR = \text{total diet fed (g)} / \text{total wet weight gain (g)}$ . Apparent protein utilization  $APU = [(\text{final body protein} - \text{initial body protein}) \times 100] / \text{total protein fed}$ . Protein efficiency ratio was calculated as  $PER = (\text{final body weight} - \text{initial body weight}) / \text{protein fed}$ .

The potential to reduce or stabilize feed costs by evaluating an alternative protein sources could be important in expanding aquaculture of LMB as well as other aquaculture species.

## COMPARATIVE ANALYSIS OF U.S. FEDERAL AQUACULTURE MANAGEMENT TO THE FAO CERTIFICATION GUIDELINES: AQUACULTURE FOR RESTORATION AND IMPACT MITIGATION

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In 2011, the Food and Agriculture Organization of the United Nations (FAO) published *Technical Guidelines on Aquaculture Certification* and currently an assessment is underway to establish conformance of U.S. aquaculture management to these internationally-accepted criteria. The assessment will highlight strengths of the U.S. management system at the federal level and will elucidate where further efforts to address FAO guidelines may be beneficial.

The objective is to evaluate conformance of U.S. aquaculture management processes relative to the “Minimum Substantive Criteria” section of the Aquaculture Guidelines. The Aquaculture Guidelines, in addition to most ecolabelling and certification schemes, concentrate on evaluating discrete management techniques implemented on an individual aquaculture operation basis; however, the approach of this conformance assessment focuses on the management system as a whole rather than that of an individual operation. Sustainability may be assessed better by focusing on the overarching management system. This takes the focus from a snapshot of one operation in isolation, to looking instead at the capacity of the system to respond to impacts via management measures in all operations under a given jurisdiction.

The conformance assessment will evaluate how U.S. aquaculture management meets each of the criteria listed within the “Minimum Substantive Criteria” by describing applicable statutes as well as regulations, guidelines, and policies at the federal level. A discussion section on highlighted operations or products will illustrate how aquaculture is managed and provide evidence that corroborates conformance.

In the Aquaculture Guidelines, Minimum Substantive Criteria are established to address environmental integrity (among other aspects of sustainability). For example:

- Aquaculture certification schemes should encourage restoration of habitats and sites damaged by previous uses in aquaculture.
- Evaluation and mitigation of the adverse impacts on surrounding natural ecosystems, including fauna, flora and habitats should be carried out.

Examples of growing practices that demonstrate/ enhance ecosystem services, scientific studies that quantify the ecosystem services provided by aquaculture, and efforts to incorporate the ecosystem services of aquaculture into regulatory decisions may help to strengthen conformance between U.S. aquaculture management and the FAO Guidelines.

## AN INNOVATIVE EDUCATIONAL PROGRAM: AQUACULTURE BOOT CAMP (ABC)

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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) and other partners, has developed and operates the Aquaculture Boot Camp (ABC). ABC program utilizes a “3-I” (**Intensive, Intermediate, Introductory**) training and multi-faceted approach, including classroom and hands-on training, paired with industry mentoring to enhance the sustainability of new and beginning aquaculture/aquaponic and next generation farmers in Ohio and the Midwest. OCARD is the first aquaculture unit that received this type of project from USDA in this country.

The ABC 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-2 online education and webinars. The “**3 areas**” are general/traditional aquaculture, recirculating aquaculture/aquaponics, and related business and marketing. The “**3 types**” are hands-on, classroom/mentoring, and internet/webinar.

The ABC Phase I had been successfully completed during 2012 – 2015: the ABC intensive level met the original goal by recruiting and training 50 new and beginning aquaculture farmers in Ohio. 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. In addition, ABC intensive students self-assessed their knowledge prior to and after the monthly content was delivered. On a scale with 1 being low and 5 being high, the overall pre-test mean in 2013 was 2.48 and 1.88 in 2014. The post-test mean in 2013 was 3.97 and 3.80 in 2014. These results indicate that ABC students significantly increased their level of knowledge of the content addressed in the program. By the end of the ABC 1 project, twenty-four new businesses/farms were created by 2013 and 2014 year-class ABC Intensive graduates. 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 287 new and beginning farmers gained knowledge of aquaculture production and new technologies by participating in ABC Intermediate workshops and bus tours, and more than 5,000 participants gained new knowledge by accessing ABC Introductory, ABC website tools and information, ABC/OAA Newsletter 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 has just been initiated and will be run for another three years.

## GENOMIC REGULATION AND EPIGENOMIC MODIFICATION OF SEXUAL SIZE DIMORPHISM IN FISH

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Sexual size dimorphism (SSD) has been the most common phenotypic dimorphism across taxa. Because sex chromosomes are the only portions of the genome that differ between males and females, theory has long suggested that the development of SSD is facilitated by the chromosomes. Contrarily, our recent studies showed that hormonal-induced neo-males with female genotype (XX♂) and normal males (XY♂) exhibited no SSD in yellow perch, where females naturally grow much faster and larger than males. Why are phenotypic traits correlated with phenotypic sex instead of genotype (chromosomes) sex of an organism? Does steroid exposure in early life epigenetically modulate subsequent gene expression which in turn regulates lifetime SSD? What is the mechanism of sex-bias gene regulation of this nature and fish SSD evolution in general?

Yellow perch display a distinct SSD pattern, and females outgrow males from 8-11 cm total length. Yellow perch have an XY mating system. Uniquely, yellow perch females only have one ovary, and thus sex-reversed neo-males develop a single testis also. Contrary to the prevailing view that slower growth of one sex in species is due to earlier maturation, our preliminary data indicates that female perch growing faster and bigger is not because females mature late; instead, early/year-1 maturing females (Type 1♀) grow much faster and bigger than males and late or year-2 maturing females (Type 2♀), and that males and late/year-2 maturing females have similar growth and mean size. The overall goal of the proposed project is to unravel the nature and mechanisms of SSD by integrating phenotypic experiments, genomic, epigenomic, and physiological approaches. Specifically, we will address the following objectives and hypotheses:

- 1) Sex-biased or sex-specific gene expression is partially responsible for SSD.
- 2) The magnitude of sexual dimorphism in the phenotype is associated with the magnitude of sex-biased expression;
- 3) The differences in sex-biased or sex-specific gene expression are associated with or the results from estrogen-mediated regulation.
- 4) Steroid exposure during the critical period of sexual differentiation can epigenetically modulate subsequent hormonal responses and gene expression
- 5) Epigenetically-modulated hormonal responses and gene expression in turn regulates SSD throughout the lifespan.

## REPRODUCTIVE ABILITY OF SECOND GENERATION KOI X GOLDFISH HYBRID MALES AND RESULTS OF THEIR CROSSES WITH KOI FEMALES

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Previous studies have shown that  $F_1$  hybrid females between ornamental koi carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) produce diploid eggs due to a transformation of meiosis. Hybrid  $F_1$  males are typically sterile, but a single fertile male, which produced diploid spermatozoa, was found. When crossed with koi females, this male produced triploid hybrids. Also, the  $F_1$  male was crossed with  $F_1$  hybrid females for the production of  $F_2$  hybrids.  $F_2$  progenies consisted mostly of diploids. Analysis of microsatellite DNA markers showed that diploid males repeated the genotype of the parent  $F_1$  male while diploid females repeated the genotypes of the parent  $F_1$  females. On this basis, it was suggested that diploid males and females in  $F_2$  resulted respectively from spontaneous androgenesis and spontaneous gynogenesis.

The purpose of the present study was to investigate the reproductive ability of diploid  $F_2$  males. Thirty three from 185 (18%) investigated 2-year-old  $F_2$  males released sperm. Flow cytometric analysis showed that spermatozoa produced by  $F_2$  males were diploid. Three  $F_2$  hybrid males were crossed with koi females. In each cross, a mixture of eggs from several koi females was fertilized with sperm from individual hybrid males. Results of flow cytometric analysis of juveniles from obtained progenies are presented in Table 1. Only triploids were found in progenies from crosses of  $F_2$  hybrid males with koi females, while fish in the control (koi x koi) were diploid.

The results of the present study show that the reproductive ability of  $F_2$  males was similar to the reproductive ability demonstrated earlier by the fertile  $F_1$  male. Like the  $F_1$  male,  $F_2$  males produced diploid spermatozoa and generated only triploids when crossed with koi females. It is remarkable that almost 20% of  $F_2$  males were able to produce sperm. This increase in reproductive capacity of  $F_2$  males as compared with  $F_1$  males could be predicted since based on previous data all of the  $F_2$  males inherited the genotype of the fertile  $F_1$  male.

Table 1. Ploidy of fish from progenies obtained by crossing of  $F_2$  males with  $F_1$  females.

Progeny no.	Parents		Number of juveniles analyzed	Ploidy distribution of juveniles (%)	
	Male	Female		Diploid 2n	Triploid 3n
1	$F_2$ -1	Koi	20	0	100
2	$F_2$ -2	Koi	20	0	100
3	$F_2$ -3	Koi	20	0	100
Control	Koi	Koi	8	100	0

## THE EFFECT OF CARBON:NITROGEN RATIO ON HETEROTROPHIC- AND NITRIFYING BACTERIA COMPOSITION OF BIOFLOC IN *Litopenaeus vannamei* SUPERINTESIVE CULTURE

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There are two main groups of bacteria involved in nitrogen removal in biofloc technology culture systems (BFT). The absorption of inorganic nitrogen through heterotrophic bacterial growth promotes a faster reduction in ammonia concentrations than nitrifying process. The growth rate and bacterial biomass production of heterotrophic bacteria is 10 times higher than that of nitrifying bacteria. Autotrophic bacteria perform nitrification through the oxidation of ammonia to nitrite and then to nitrate. However, due to the slow growth of autotrophic bacteria, nitrite tends to increase significant in the environment culture. In this way, bacteria degrade excess organic matter and allow successive cycles of shrimp production without the need for water renewal culture. In general, organic fertilizations used to be done to increase the C:N ratio (15:1) for increase of heterotrophic bacterial population to convert inorganic nitrogen in bacterial protein. However, this growth of bacterial population used to increase the amount of total suspended solids (sludge) in shrimp culture, and sometimes can become a problem for BFT systems.

The aim of this study was to evaluate the effect of Carbon:Nitrogen ratio on heterotrophic- and nitrifying bacteria composition of biofloc in *Litopenaeus vannamei* superintesive culture in order to determine better bacterial composition for BFT system.

A 70 day-trial was conducted at the Marine Station of Aquaculture, University of Rio Grande, Rio Grande do Sul State, Brazil. After nursery, Pacific white shrimp (1.46g  $\pm$ 0.52) juveniles were stocked at 400/m<sup>3</sup> in a twelve-1.000 L tanks. Four treatments (three replicates) were tested using four different C:N rates: 9:1, 11:1, 13:1 and 15:1. Water temperature, salinity, dissolved oxygen, pH, TA-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, Alkalinity and Settleable solids were daily monitored. BFT water samples were collected to detect the growth of the population of nitrifying and heterotrophic bacteria by Fluorescent *IN SITU* Hybridization determination - FISH. The results were analyzed by one-way analysis of variance (ANOVA) and Tukey's test ( $\alpha = 0.05$ ).

The water quality parameters were influenced by the treatments ( $P < 0.05$ ), but were not limiting for shrimp survival and growth in all treatments. Shrimp survivals were not influenced ( $P > 0.05$ ) by the treatments. The growth parameters were also different among treatments, ( $P < 0.05$ ), except FCR ( $P > 0.05$ ). The different C:N fertilization rate influenced significantly ( $P < 0.05$ ) bacterial population composition. Results showed the importance of decreasing the amount of Carbon to decrease total suspended solids in BFT system.

## USE OF BEAD FILTERS IN ORNAMENTAL AQUACULTURE

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Ornamental aquaculture is increasingly using indoor, recirculating aquaculture systems (RAS) for broodstock, hatcheries, grow-out, and holding due to a number of factors including water consumption constraints and the costs associated with water treatment (e.g. artificial seawater, soft water, etc.). While biological loading within these systems is typically much lower than food fish operations, management of nitrogenous wastes, water clarity, and other key parameters is as, or more important to success than with some “hardy” food fish species.

Bead filters are widely used to manage water quality in the ornamental industry due to their dual function of biological and mechanical filtration. This presentation will highlight some of the applications with ornamental species both in production and research settings.

## A COMPARISON OF METABOLITE PROFILES OF NATURAL FEED ITEMS AND SUBSEQUENT TISSUE PROFILES IN JUVENILE RED DRUM, *Sciaenops ocellatus*

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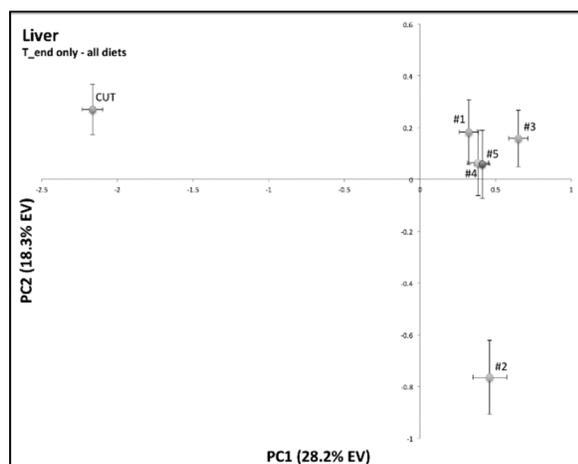
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Nuclear magnetic resonance (NMR)-derived metabolic profiles are emerging as a powerful tool with multiple applications in aquaculture. Here, we have examined the polar metabolite profiles of three natural feed items of many southeast United States estuarine fish. Shrimp, squid, and fish (cigar minnows) were profiled along with several tissues (liver, plasma, and muscle) of juvenile red drum over the course of a twelve-week feeding trial.

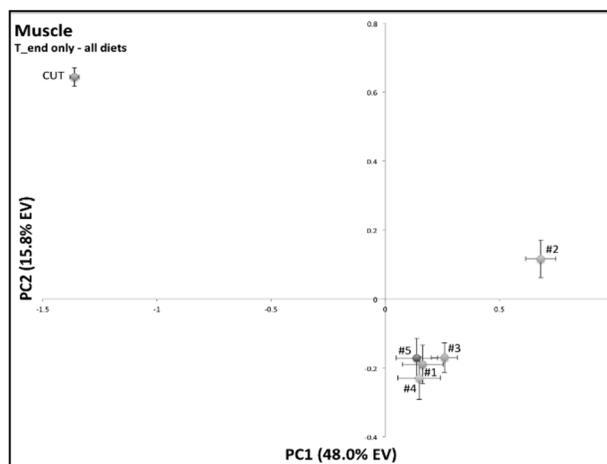
Juvenile red drum were fed an equal mixture of the three natural feed items as components of a natural (CUT) diet treatment utilized in SCDNR-NIST collaborative feed studies as a positive benchmark for performance and system validation. This natural diet has consistently outperformed experimental, pelleted feeds of various formulation. We have undertaken this study to begin to analyze the biochemical composition of the natural feed items and investigate the levels of the same compounds in fish tissue extracts by NMR to improve our understanding of red drum dietary requirements and underlying biochemical processes affected by feeds with drastically different constituents.

Plasma, liver, and muscle samples were taken initially, then again in weeks 2-5 and 9-12. Natural feed items were analyzed as individual components and composite averages were used for the whole treatment. Although fish were fed an equal amount of each of three components at each feeding, it was impossible to ensure that each fish consumed equal amounts of the three. No obvious preference or avoidance of any of the three feed components was noted. As presented previously, figures 1 and 2 show PCA plots of the final liver and muscle profiles. The CUT diet resulted in significantly different metabolite profiles than several soy-based diets (#'s 1-5).

**Figure 1.** Liver end point (T\_end) PCA scores plots for 5 experimental soy based diets and the natural diet (CUT). Error bars represent the means  $\pm$  1 SEM.



**Figure 2.** Muscle end point (T\_end) PCA scores plots for 5 experimental soy based diets (diet #1-5) and the natural diet (reference). Error bars represent the means  $\pm$  1 SEM.



## TRANS-ATLANTIC COOPERATION: AQUACULTURE BUSINESS, RESEARCH AND EDUCATION PRIORITIES

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In 2013, EU Commissioners for Maritime Affairs and Research/Innovation, together with high level representatives from United States of America and Canada, signed the Galway Statement on Atlantic Ocean Cooperation to launch the Atlantic Ocean Research Alliance (AORA). The goal has been to enable transatlantic cooperation in order to better understand and increase knowledge of the Atlantic Ocean, its dynamic systems, and to promote sustainable management of its resources. Having identified aquaculture as one of the AORA priority areas, the Trilateral (Canada, EU, US) Aquaculture Working Group was established and several international projects were launched to advance scientific knowledge and promote international collaboration. Not only have these initiatives furthered understanding of aquaculture processes, impacts and benefits, but also pointed to challenges common to many countries, e.g. to utilisation of available marine space or to weak “social license” for marine aquaculture development which complicates and slows efforts to authorize business operations.

The aim of AORA CSA project is to provide Europe, the United States and Canada with relevant and responsive information on the status of the identified priority research areas to meet scientific and industry needs in the North Atlantic. The initiative is implemented through the provision of preliminary mapping and connectivity assessments and sectoral analysis to contribute to long-term aligning the planning and programming of trans-Atlantic research activities.

Several key thematic areas of trans-Atlantic interest were described in the Trilateral Aquaculture WG ‘roadmap’. The objective of this presentation is to present progress, future action plan and engagement opportunities for the American research community. Intensified international effort is planned, under the umbrella of the AORA, in order to communicate success stories, including: local level cases that may have international value by pointing to health effects or social and economic benefits; transfer of best practices, e.g. investments in aquaculture education and examples of social awareness campaigns to promote aquaculture products like the ‘Farmed in the EU’ campaign; and examples of increased cooperation among various subsectors of aquaculture to achieve greater political hearing.



## GENE EXPRESSION AND LIPID PROFILE OF RED DRUM, *Sciaenops ocellatus*, EGGS THAT DEMONSTRATE METABOLIC PROGRAMMING

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Previous work in our lab has shown that when the diet of red drum (*Sciaenops ocellatus*) broodstock is manipulated to produce varying levels of docosahexaenoic acid (DHA) in the egg, resulting larvae demonstrate altered abilities to acquire and/or retain DHA in body tissues despite being provided high levels in their diet. This difference is exacerbated when egg DHA levels are < 30 mg/g as dry weight and reach a steady state at approximately 40 mg/g DHA. This pattern is similar to a condition seen in terrestrial vertebrates and referred to in humans as the Metabolic Syndrome.

In order to better understand the mechanisms behind this difference, we reared larvae from two different egg sources (Texas Parks and Wildlife Department Marine Development Center and the University of Texas Fisheries and Mariculture Lab) and egg DHA concentrations ranging from 25 to 55 mg/g DHA. DHA concentration gradients were obtained by changing the diet of spawning fish and collecting eggs over a single spawning season to obtain samples as DHA concentrations were altered. Collected eggs were then hatched and reared under common garden conditions with periodic sampling of resulting larvae through day 21 post-hatch. Eggs were analyzed via tag-based RNA-Seq (TagSeq) and by Gas Chromatography – Mass Spectrometry analysis to determine gene expression and lipid profile, respectively.

## **ORGANIZING EFFECTIVE AQUACULTURE EXTENSION PROGRAMS THROUGH ACTION TEAMS: PARTNERING FOR PROGRESS**

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In 2010, Maryland began issuing shellfish leases under revised laws that discarded a century of onerous restrictions on private shellfish growers. With these changes, a single agency was created to handle permitting and coordinate state and federal reviews. To spur industry growth, support programs were established to attract investment and encourage business development. These included low-interest loans through an agricultural lending unit, oyster seed production systems placed in a variety of locations, assistance with market development, sonar mapping of leases and screening for oyster disease. University of Maryland Extension was contracted to develop an Oyster Aquaculture Education and Training Program to provide the skills required for commercial fishermen to transition to farming and those from other businesses to develop technical skills for profitable operation. These programs brought together institutions, agencies and non-governmental organizations to coordinate their activities and track progress.

UM Extension operates with program Focus Areas and, under these, Action Teams that address specific areas of need. The Seafood Production Action Team was formed to cover the development of aquaculture and included representatives from multiple institutions and organizations. Conferences, workshops and short courses were conducted to provide educational resources for the expanding industry. In recent years, twenty-five programs have been conducted annually, based on needs assessments and evaluated by UME teaching effectiveness forms. Computerized results are provided to educators and used in their annual reviews by administrators.

With six years of development, over two million dollars in low-interest loans have been provided to growers. Almost 6,000 acres (2,428 hectares) have been leased and are in active production. Spat on shell bottom leases are assisted by a Remote Setting Training Program that has resulted in over 300 million spat deployed annually, with growers creating private systems and selling seed as well as planting leases. Water column leases using cultchless seed are undergoing significant expansion and legal opinions by the court system have created precedents allowing the industry to expand annually.

The authors provide an overview of the programs and planning process that have aided industry development with discussion of the need for ongoing identification of problem areas and a political base that assists with solutions. Data on extension programs and how they aided development of the industry are included with plans for future activities to support additional expansion.

## APPLICATION OF RECIRCULATING TECHNOLOGIES TO MARINE AQUACULTURE SYSTEMS: AN OVERVIEW

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With the exception of marine aquariums, utilization of recirculating aquaculture systems (RAS) has in the US traditionally been largely relegated for the production of freshwater finfish species such as tilapia. However, over the last decade, RAS technologies have been developed and applied in conditioning and reproduction, hatchery, nursery, and ongrowing systems for several marine finfish species including black sea bass *Centropristis striata*, cobia *Rachycentron canadum*, Florida pompano *Trachinotus carolinus*, as well as several marine ornamental finfish species. In addition RAS are currently employed by operations producing softshell blue crabs *Callinectes sapidus* and Pacific white shrimp *Litopenaeus vannamei*.

As is the case with freshwater applications, marine RAS offer several benefits such as enhanced biosecurity, control of the culture environment, and effluent reduction. Especially due to the latter two attributes, RAS are particularly suited for reproduction and hatchery phases of marine species.

Marine RAS components, system design, and management will be discussed using several examples from both research and commercial applications. In addition, the potential for application of RAS technologies for the production of other marine species such as molluscan shellfish will be presented.

## THE EFFECT OF THREE BROODSTOCK DIETS ON THE REPRODUCTIVE PRODUCTIVITY OF THE FIRE SHRIMP *Lysmata debelius*

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The fire shrimp, *Lysmata debelius*, is a valued ornamental shrimp common in the marine aquarium trade. In order to minimize the collection of wild populations while meeting hobbyist demand, the development of commercial-scale aquaculture methods are needed. While culture methods have been developed to increase production efficiency through specialized larval and growout systems, the success is limited by the reproductive output of the broodstock, which stems from the adequacy of broodstock diet. This experiment compared the effects of three diets on the reproductive output of fire shrimp broodstock.

Pairs of mature fire shrimp (N=10) were held in recirculating seawater systems with stable water quality parameters and were randomly assigned one of three diet treatments. Treatment 1 was composed of a traditional marine gel diet. Treatment 2 was a frozen diet of bloodworm (*Glycera dibranchiate*), quahog (*Mercinaria mercinaria*), and bay scallop (*Argopecten irradians*). Treatment 3 was a fresh diet of the same ingredients as Treatment 2. Each pair was fed 0.2 g of diet four times per day. The pairs were observed daily to assess reproductive performance: molt cycle (days), nest incubation (days), and relative egg nest size (% abdomen). Emerged larvae were enumerated to determine spawn size. From each individual, larvae (N=50) were subsampled to determine larval quality (N=1-4 hatches per individual). Specifically, size at hatch (mm), time to yolk sack exhaustion (days), and morphological development at the time of yolk sack exhaustion (zoea stage) were determined.

Mature shrimp fed a diet of fresh seafood produced larger spawns than those fed frozen or processed diets (Fig 1A). Further, more larvae from shrimp fed a fresh diet reached zoea II stage before yolk exhaustion than larvae from shrimp fed frozen processed diets (Fig 1B).

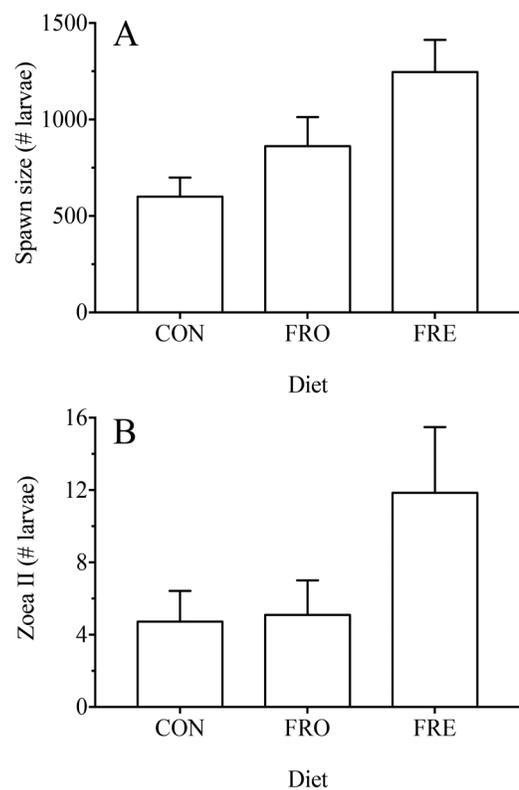


FIGURE 1. Broodstock fecundity (A) and larval development (B) of fire shrimp fed three experimental diets of processed, frozen, and fresh seafood ingredients.

## **SUPPLEMENTATION OF ZN, CU, AND FE IN A COMMERCIAL-TYPE, PLANT-BASED DIET FOR RAINBOW TROUT**

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A three-variable, central composite design coupled with response-surface regression analysis was used to examine the effects of dietary iron (Fe), copper (Cu), and zinc (Zn) on growth and physiological performance of juvenile rainbow trout fed a plant-based diet. Each micromineral was tested at five levels for a total of fifteen dietary combinations (diets) and fed for 12 weeks. Growth performance as measured by weight gain (WG) and feed conversion (FCR) were significantly affected by dietary supplementation of all three microminerals. Significant interactions between Cu x Fe for WG and Cu x Fe, Cu x Zn, and Zn x Fe for FCR were also observed. After removing non-significant terms, response-surface regression modeling showed that for optimal growth performance, rainbow trout require approximately 3-4 mg/kg Cu, 80-90 mg/kg Fe, and 35-40 mg/kg Zn supplemented in a plant-based diet. Supplementation of Zn and Cu but not Fe also affected survival of rainbow trout, with similar added concentrations of Zn (4 mg/kg) and Cu (35 mg/kg) providing the best predicted survival. Dietary supplementation of Zn and Cu also exhibited an interactive effect on whole body (WB) deposition, where increased dietary addition of one reduced WB concentration of the other. Alkaline phosphatase (Zn-requiring) and ceruloplasmin (Cu-requiring) but not catalase (Fe-requiring) enzyme activities were affected by their native, active site-requiring microminerals, but activities were not affected by interactions between the supplemented microminerals. Recommended dietary supplementation made by the National Research Council (NRC) for Fe (60 mg/kg), Cu (3 mg/kg), and Zn (15 mg/kg) for rainbow trout were determined using semi-purified or fishmeal-based diets. The optimal, predicted supplementation level for a plant-based diet in this study was similar for Cu but higher for Fe and Zn. These differences are largely due to the observed interactions among these microminerals with regard to growth performance. Moving to an all-plant feed for rainbow trout will require updates to supplemented micromineral concentrations for the best growth performance.

## **NAVIGATING OFFSHORE AQUACULTURE THROUGH A SEA OF OCEAN COMMERCE: A STUDY OF TRAFFIC AND OCEAN USE IN THE GULF OF MEXICO AND SOUTHERN CALIFORNIA**

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Ocean planning for aquaculture in U.S. waters requires complex spatial analyses to address the number and diversity of existing ocean uses and farm siting requirements. At a minimum, classification of activities in the ocean region and assessment of cumulative impacts and interactions over time and space is required to harmonize sustainability goals within our regulatory framework. Siting efforts for finfish operations in the Gulf of Mexico (GoM) (planning) and Southern California (SoCal) (permitting) to date have required multi-agency collaborations to assess farm siting alternatives within the regions, a process that ensures minimal impact across multiple ocean uses and resources such as navigation, military, industrial and commercial operations, fishing, protected species, sensitive habitats, and conflicts with major recreational uses. Using the GoM and SoCal as case studies, we will demonstrate the importance of spatial data type and analytical approaches that are tailored specifically for coastal managers during various stages of the permitting process. For example, we will illustrate a navigational interference analyses using vessel tracking data (ship identification, position, course, speed) to assess potential marine traffic conflicts among various siting alternatives off the California coast.

## **EDUCATING THE FUTURE AND PRESENT WORKFORCE THROUGH INTERACTIVE, HANDS-ON APPLICATIONS OF AQUACULTURE**

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Although the global aquaculture industry is increasing in size and production, the workforce needed to support the industry is lacking in educated and experienced individuals. Receiving an education may be the first step, but hands-on experience and applied research is crucial. The University of Wisconsin-Stevens Point, Northern Aquaculture Demonstration Facility (UWSP-NADF) is a state-of-the-art facility that connects with all ages and levels in aquaculture to sustain and advance the industry. By means of this facility, knowledge is gained through experience working with a variety of systems, species, and techniques. The UWSP-NADF's unique outreach program relates to all groups; sparking aquaculture interest in students K-12, providing a world class college education through internships, and developing strong partnerships directly with the aquaculture industry to create and sustain businesses.

Initiating curiosity and understanding of aquaculture and aquaponics at a young age is a head start to educating a future workforce and developing public awareness. The UWSP-NADF outreaches to students K-12 across Wisconsin and has been a direct leader in incorporating aquaponics and aquaculture systems into classrooms of local schools. With these partnerships, schools have the opportunity to include students in various activities that educate through hands-on knowledge and experiences both in and out of the classroom. Regarding higher education, UW-Stevens Point is the only university in Wisconsin to offer an aquaculture minor and the first in the nation to offer semester-long college aquaponic courses and a professional aquaponics certificate. With these opportunities and internships at the UWSP-NADF, students receive a well rounded education, working with a diversity of cold and cool water fish species at all life stages as well as various incubation systems, ponds, raceways and indoor recirculation systems. Interns perform applied research projects that directly relate to the industry and the facility's private business partnerships. Because of these courses and the hands on experience needed, students are receiving a world-class education and high success to directly benefit the aquaculture industry.

## PRELIMINARY INVESTIGATION TO DETERMINE A SAFE CONCENTRATION OF TRICHLORFON FOR TREATING PARASITES OF THE RAYS *Dasyatis sabina* AND *Rhinoptera bonasus*

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Dylox® 420 is an insecticide to treat primarily turf grass for golf courses or homes, however it is used as a parasiticide in aquatic animal care. The active ingredient of Dylox® is trichlorfon, which is an organophosphate neurotoxin that is toxic to many aquatic organisms. Due to this toxicity, it is important to determine a safe concentration that will rid external parasites from an aquatic host organism without causing undue harm to the host. Public aquariums may need to treat specimens, such as rays, during prophylactic quarantine or due to being in outdoor exhibits and may be exposed to natural parasitic organisms leading to parasite outbreaks. Therefore, a preliminary investigation was conducted to examine recommended treatment concentrations of trichlorfon for the Atlantic stingray *Dasyatis sabina* and the cownose ray *Rhinoptera bonasus*.

A single Atlantic stingray *Dasyatis sabina* was moved from its display tank to an aerated treatment tank (450 L) for a 24-hr exposure to 0.5 ppm (v/v) trichlorfon from Dylox® 420 and then returned to the display tank. The treatment tank was rinsed and refilled. A second Atlantic stingray was selected from the display tank, placed in the treatment tank for a 24-hr exposure to a 0.7 ppm trichlorfon, and then returned to the display tank. The same regimen was performed on two cownose rays *Rhinoptera bonasus*. The Atlantic stingrays and cownose rays selected ranged from 8-10 lbs with a wing span of 2 feet. During exposure, each specimen was observed continuously for abnormal behavior (e.g., erratic swimming, flipping upside down). Water temperature, dissolved oxygen (D.O.), and pH were monitored around every two hours with the use of a portable D.O./temperature meter and pH meter.

D.O. was within acceptable parameters (90-105% saturation) during exposures, except for one instance (85-86%) with the first Atlantic stingray trial of 0.5 ppm trichlorfon concentration, at which time a third airstone was added to the treatment tank. Temperature ranged from 20.3 to 23.0°C, which was below the temperature at which these species are typically maintained (22-30°C). Trichlorfon rapidly breaks down into more toxic dichlorvos if pH is above 8. pH ranged from 7.71-7.99 in three trials; no measurements were taken in the first Atlantic stingray trial.

No abnormal swimming behaviors were noted in any trial, however skin sloughing was noted from the cownose rays. This was most probably a stress response caused by the confines of the tanks rather than the trichlorfon. Both species resumed natural activities after being returned to their display tank. From this preliminary data, the 0.5 and 0.7 ppm recommended treatment concentrations of trichlorfon from this batch of Dylox® 420 will not harm the two ray species examined. This research was conducted under approved Institutional Animal Care and Use Committee protocols.

## **APPLICATION OF BEAD FILTER TECHNOLOGY AT A MARINE WARMWATER AQUACULTURE RESEARCH FACILITY**

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The Harbor Branch Oceanographic Institute (HBOI) of the Florida Atlantic University (FAU) maintains an aquaculture research facility in Fort Pierce, FL that borders the Indian River lagoon waterway. The supply of groundwater seawater is limited for the expanding facility operations and restrictions on effluent seawater discharge has most all the research systems operating in a recirculating capacity. As such, primary design criteria were to achieve minimal discharge and maximal use of the groundwater salt water. Other design criteria for the systems research operation included minimal maintenance, efficient filtration, and the ability to be used on a range of studies where there is a variation in the daily feed load or system's criteria. Because of the design criteria and research needs, bead filters are/were employed on a multiple of systems including production grow out systems, recirculating system for nutrition studies, marine fingerling production system, and in an integrated multi-trophic aquaculture system.

The system designs have employed the Bubble Bead filters, the Propeller-washed Bead filters, the Polygeyser filters, and a static air scrub bead filter for mechanical and biological filtration in a variety of applications. A synopsis of the design characteristics and operating criteria of the systems utilizing bead filter technology is to be presented. Overall, bead filter technology has played a solid role in the design and operation of the many RAS units in operation at the HBOI-FAU marine aquaculture research facility.

## REFINING SEVERITY LIMITS FOR LABORATORY ZEBRAFISH

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Zebrafish are currently the second most used model for biomedical research and their use shows no sign of abating. Despite this rapid rise in use, relatively little is known about their true welfare requirements and guidelines pertaining to their husbandry needs are broad, leading to husbandry practices becoming varied and non-standardised. In 2012, we developed a health monitoring system based upon body scoring for zebrafish in an attempt to create a more standardised health-monitoring program at UCL. More recently we referenced our health monitoring program against a PCR and histological screen and looked at areas of compatibility, where phenotypical signs of illness and disease, as determined by the health monitoring program, correlated to disease determined by the results of PCR and histological testing. In most cases, fish expressing a disease phenotype were shown to have the corresponding disease by PCR and / or histological analysis; in a small number of cases this proved not to be true – fish expressing phenotypes associated with specific diseases were healthy by PCR and histological analysis and showed no sign of disease. Preliminary database analysis of the fish showing disease phenotypes, but which were not detected by PCR or histology, suggested that specific phenotypes could be associated with specific strains of fish and age of individual fish. We believe that we have a method of assessing subtle severity limits in fish and distinguishing this from phenotypes that are caused by disease. By using a combination of the body scoring health monitoring program, PCR and histological screening and analysis of patterns of zebrafish ill health found within our database, we are able to refine phenotypic severity limits by creating new endpoints before these phenotypes are expressed.

## MINI-ROVS IMPROVING AQUACULTURE OPERATIONS

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Maintaining a fish farm involves a culmination of different tasks. These tasks include but are not limited to net inspections, mort retrieval, size grading, and fish monitoring. The proposed presentation offers a case study analysis of the use of Deep Trekker mini-ROVs to perform daily inspection tasks on aquaculture sites.

Farms require divers to perform inspections where associated costs can be high. Mini-ROVs are revolutionizing the way managers go about their daily operations without hiring divers until it is absolutely necessary. ROVs can perform net inspections, observe fish behaviour and bridge the knowledge gap between employees and what takes place below the surface while simultaneously recording the footage.

The case studies include two Canadian aquaculture companies; Marine Harvest and Aqua-Cage Fisheries to demonstrate the ways that a mini-ROV can improve operation efficiency and reduce costs.

The presentation will focus on Aqua-Cage Fisheries and Marine Harvest to show how farm managers can improve production while reducing costs by using an ROV that ensures proper structure maintenance and can act as a retrieval tool for dropped equipment. The use of mini-ROVs has given managers the ability to teach employees about the underwater aspects of the farm as well as identify and explain any problems to a dive team in cases where divers are needed.

## REASSESSING THE LYSINE REQUIREMENT OF JUVENILE LARGEMOUTH BASS *Micropterus salmoides*

Brittany M. Woodward\*, Waldemar Rossi Jr., and Vikas Kumar

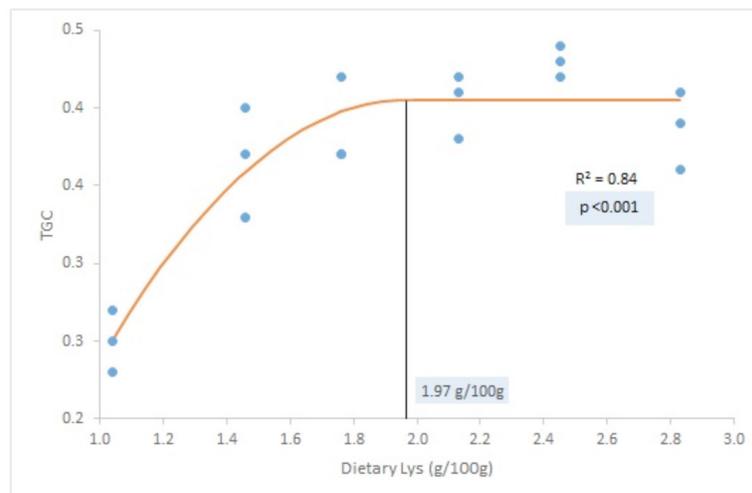
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The commercial production of largemouth bass (LMB), *Micropterus salmoides*, for food has expanded in recent years entailing the development of cost-effective diets for this species; however, limited data exists pertaining to the amino acids (AAs) requirements of this species. Therefore, the objective of this study was to reassess the dietary lysine (Lys) requirement of juvenile LMB.

A basal diet was formulated to contain the AA profile of a 40% crude protein LMB muscle and 12% lipid. Protein-bound AAs in the basal diet were derived from sardine fishmeal included at 20% and providing 1.04% Lys. Five additional diets were designed to contain incremental amounts of crystalline L-Lys (as Lysine HCl, 78% L-Lys) ranging from 1.46 to 2.83% of the diet. Lys inclusions to the basal diet were performed at the expense (N basis) of L-aspartate and L-glutamate. LMB juveniles (270; average weight 9.8±0.5g) were stocked into 18 110-L glass aquaria (15 per tank) in a closed recirculating system. Fish were fed the experimental diets (three replicates per diet) to apparent satiation twice daily for 8 weeks. At the end of the experiment, fish in each aquarium were group weighed and sampled for blood chemistry and physiological parameters, condition indices, whole-body and muscle proximate and AA compositions.

After 8 weeks of feeding, survival of LMB ranged from 82 to 98%. Blood analyses indicated a significant effect of dietary Lys on hematocrit and hemoglobin. Using regression analysis, the Lys requirement for optimizing thermal growth coefficient (TGC, Figure), weight gain, and feed efficiency was estimated to range from 1.90 to 2.01% of the LMB diet.

Thermal growth coefficient (TGC) and estimated dietary Lys requirement (1.97 g/100 g) based on a quadratic broken line regression.



## **AQUACULTURE EXTENSION AND OUTREACH**

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Credible aquaculture outreach information is vital to the success of the U.S. aquaculture industry. Consumers, distributors, processors and producers need up to date, research based aquaculture information. Subjects such as product form, preparation, safety, nutrition, production methods and their related environmental impacts, will differentiate U.S. aquaculture goods in the market place from those sourced from other countries and capture fisheries. Informed aquaculture outreach information delivered from extension, sea grant and industry personnel will expand and strengthen the demand for domestically grown products. Technologies such as social media and webinars, along with more traditional outreach methods, will further encourage markets for U.S. products. Public outreach personnel are currently challenged by shrinking operating budgets while price competition from fisheries, imported aquaculture products and other sources of animal protein limit domestic aquaculture production. Effective, widespread public outreach and education methods are critical to expanding and diversifying the U.S. aquaculture industry. This session will organize a program of diverse practices and techniques in order to demonstrate the most effective methods of aquaculture outreach.

## **THE TERMS EDUCATION, ADVOCACY, AND PROMOTION IN AQUACULTURE EXTENSION PROGRAMS: WHAT DO THEY MEAN AND WHERE AND WHEN TO DRAW THE LINE?**

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Aquaculture programs housed in land grant universities are charged with the mission of educating students and conducting applied aquaculture research. Extension is charged with the delivery of these research results to various user groups, agencies and to the public. In aquaculture extension work, the terms education, advocacy and promotion may be used incorrectly or interchangeably. When conducting programs, extension personnel should understand these terms and their related actions.

According to the online Meriam Webster Learner Dictionary, education is simply defined as “the act or process of teaching someone especially in a school, college or university.” Whereas, advocacy is defined as “the act or process of supporting a cause or proposal: the act or process of advocating something.” A positive example of advocacy would be, supporting the development, distribution and use of reputable, peer reviewed aquaculture information that would enhance aquaculture education. A negative example of advocacy would be publicly supporting a cause that is based more on personal opinion than science.

Promotion is defined as “something (such as advertising) that is done to make people aware of something and increases its sales or popularity.” Public perception of the act of promotion may extend well beyond generating awareness or even popularity. Promotion is often viewed as a vehicle to increase sales and profit. It is appropriate for aquaculture extension personnel to support and deliver science based aquaculture information on development, production, processing and marketing practices. Other promotional practices would seem less appropriate or even self-serving. These would include: promoting one aquaculture product over another, trying to convince growers to increase farm size or production, or actively recruiting new growers into entering the industry.

## PRODUCTION FUNCTIONS FOR GOLDEN SHINER GROWTH IN DIFFERENT CULTURE SYSTEMS

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Two of the most popular production functions - Cobb-Douglas (CD) and the transcendental logarithmic (TL) function - have been widely used in agriculture and aquaculture to predict the production outputs based on a given set of inputs. The CD model is the reduced model to the TL model by excluding the quadratic and interaction terms in the TL model.

This study is based on thirty-two golden shiner feeding experiments conducted at the University of Arkansas at Pine Bluff over the last two decades. Four key production variables (stocking density, duration of culture, protein content of feed, and culture system) were analyzed to determine their effects on the final weight of golden shiner. The TL model has a better fit (RMSE =0.31, adjusted  $R^2=0.81$ ) compared to the CD model (RMSE =0.50, adjusted  $R^2=0.52$ ). The Likelihood Ratio test is statistically significant, indicating that quadratic and interaction terms in the TL model are significant. A serial of residual analysis for the TL model, Shapiro-Wilk test ( $P=0.56$ ) for normality and a plot of standardized residual versus predicted values, suggesting the TL model based on the assumption of normality and homogeneity of variance was suitable to describe the growth of golden shiner. The effect of culture system was statistically significant. Golden shiner grew much better in outdoor culture systems compared to indoor culture systems.

## IMPACTS OF OCEAN ACIDIFICATION ON BACTERIAL COMMUNITIES IN COASTAL MICROBIAL BIOFILMS

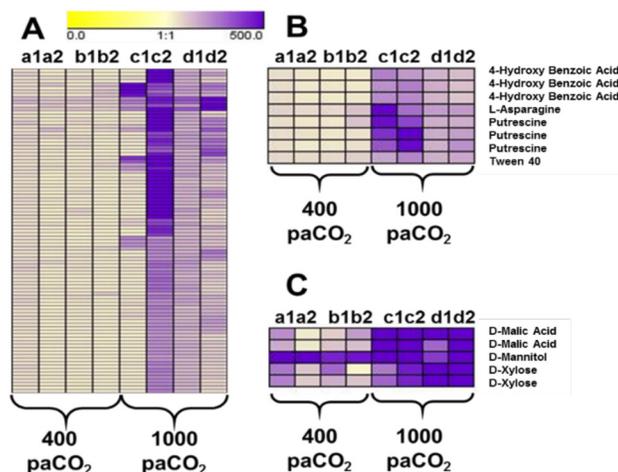
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Microbial biofilms can positively or negatively affect the coastal ecosystems by several means. Microorganisms in biofilm can remove contaminants, such as oil spills, nitrogen compounds, metals and radio nuclides, through bioremediation. They also serve as underwater matrixes producing chemical and biological cues to trigger larval settlement of a number of marine invertebrate species. The formation and dynamics of marine biofilms are also determined by multiple environmental factors. We are particularly interested in the influences of acidified marine water caused by increased atmospheric CO<sub>2</sub> to the marine biofilms.

The CO<sub>2</sub> concentration in atmosphere has been dramatically increased since industrial revolution due to the anthropogenic activities in energy consumption. It results in the considerably decreased pH in marine water, which consequently effects the ecosystems in the ocean. Here we conducted a study in understanding the dynamic changes of the marine biofilms associated with the CO<sub>2</sub> initiated ocean acidification. The natural sea water from the coast of Louisiana was collected and maintained in the laboratory conditions. The formation of microbial biofilms was controlled under ambient (400 ppm, p400) and increased (1,000 ppm, p1000) paCO<sub>2</sub> levels in the atmosphere. Structures of the biofilms generated under stresses of the two paCO<sub>2</sub> levels were visualized with scanning electron microscopy (SEM). Bacterial populations from different biofilms were also characterized with BIOLOG analyses. The result demonstrated a significant difference in overall bacterial communities between the biofilms in ambient paCO<sub>2</sub> and increased paCO<sub>2</sub> conditions (A). Utilizations of organic compounds, such as 4-Hydroxy benzoic acid, putrescine, D-Malic acid, and D-Xylose were significantly increased by biofilms in p1000 compared to p400 (B and C), which suggested that paCO<sub>2</sub> level dramatically influenced the metabolic activities of bacteria in the biofilm. Characterization of bacterial communities in the biofilms was also performed using terminal restriction fragment length polymorphism (TRFLP) and denaturation gradient gel electrophoresis (DDGE).

Further studies on the paCO<sub>2</sub> stimulated marine biofilm alterations to the costal ecosystems are highly desired for the restoration of coastal ecosystem and resources of fisheries and aquaculture.



## IMPACTS OF PARASITE INFECTION ON COLUMNARIS DISEASE OF TILAPIA

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There is no information available on whether parasite infection will increase the susceptibility of tilapia to *Flavobacterium columnare* and whether parasite treatment could improve fish survival after *F. columnare* exposure. Two trials were conducted to evaluate 1) the susceptibility of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) to *F. columnare* after parasitism by *Ichthyophthirius multifiliis* (Ich) and 2) formalin treatment of hybrid tilapia parasitized by *Trichodina sp.* to improve fish survival after *F. columnare* exposure.

In trial I, hybrid tilapia were divided into 18 tanks with 20 fish per tank that received the following treatments: 1) non-infected control; 2) infected with 30,000 Ich theronts fish<sup>-1</sup> alone; 3) infected by *F. columnare* ALM-05-53 at  $4.59 \times 10^7$  CFU L<sup>-1</sup>; 4) infected by 30,000 Ich theronts fish<sup>-1</sup> and exposed to *F. columnare* ALM-05-53; 5) infected by *F. columnare* TN-3-2012 at  $4.27 \times 10^7$  CFU L<sup>-1</sup>; 6) infected by 30,000 Ich theronts fish<sup>-1</sup> and exposed to *F. columnare* TN-3-2012. For challenge, fish were immersed in water in buckets with *F. columnare* for 15 min. Fish not exposed to the bacterium were held in water with Shieh broth for 15 min. After challenge, the fish and challenge water were poured into the appropriate tanks and water flows were adjusted to 0.4 – 0.5 L min<sup>-1</sup>. Fish mortality was recorded and dead fish were examined for Ich and *F. columnare* infection twice daily for 17 d. At 3 and 6 days post *F. columnare* challenge, two fish were randomly sampled from each tank to check for Ich infection and then gill and kidney were collected to quantify *F. columnare* in tissues by real-time polymerase chain reaction. The fish showed 2.1% mortality when infected by Ich alone and 29.1% mortality when challenged with *F. columnare* ALM-05-53 alone. Mortalities significantly increased in co-infected fish (60.4%; Ich and *F. columnare* ALM-05-53). The bacterial load in gill of parasitized fish was 5702.5 genome equivalents per mg of gill tissue (GEs mg<sup>-1</sup>), 14 fold higher than non-parasitized fish (415.4 GEs mg<sup>-1</sup>) 3 days post exposure to *F. columnare* ALM-05-53.

In trial II, hybrid tilapia parasitized by *Trichodina sp.* were divided into 3 treatment groups. The first group of fish received no parasite treatment. The second group of fish were bath treated once with 150 mg L<sup>-1</sup> formalin for 1 h. The third group of fish bath treated two consecutive days with 150 mg L<sup>-1</sup> formalin for 1 h. All fish were then immersion challenged with *F. columnare* (ALM-05-53). The tilapia not treated with formalin showed significantly higher mortality (37.5%) than those treated with formalin ( $\leq 16.7\%$ ) after exposure to *F. columnare*. Fish treated twice showed lower mortality (6.37%) than those treated only once (16.7%). The non-treated fish showed significantly higher load of *F. columnare* in gill, kidney and liver compared to those treated with formalin following exposure to *F. columnare*. The bacterial load of non-treated fish was 27075 GEs mg<sup>-1</sup>, 12 fold higher than those treated once with formalin (2250 GEs mg<sup>-1</sup>) or 39 fold higher than those treated twice with formalin (699 GEs mg<sup>-1</sup>) after exposure to *F. columnare*. Results of studies suggest that prevention of parasite infection in fish will not only reduce the direct damage caused by the parasite but will also reduce mortality due to *F. columnare* co-infection. Formalin treatment for *Trichodina sp.* parasitism reduced bacterial infection as suggested by reduced loads of bacteria in fish tissues and subsequently decreased fish mortality.

## USING EARLY NUTRITIONAL PROGRAMMING TO ENHANCE THE UTILIZATION OF PLANT BASED DIETS IN LARGEMOUTH BASS, *Micropterus salmoides*

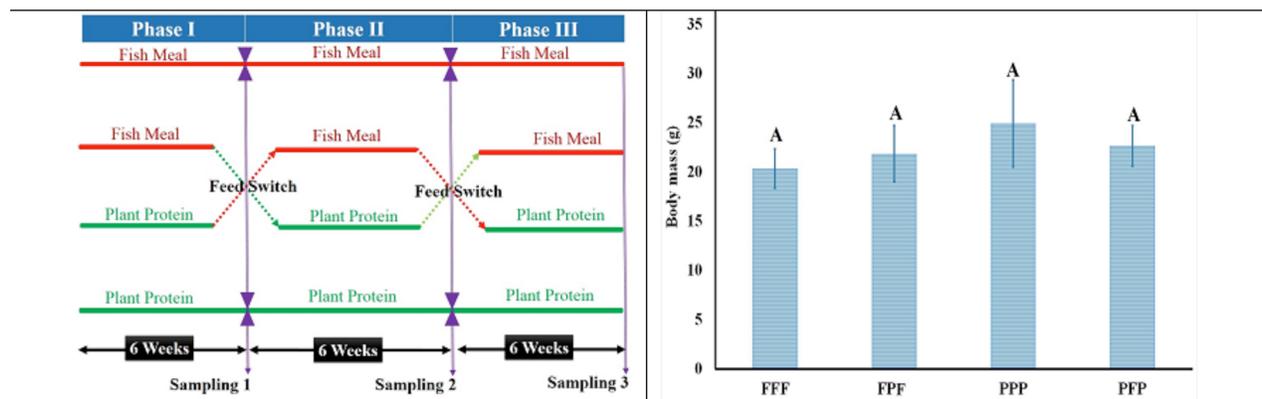
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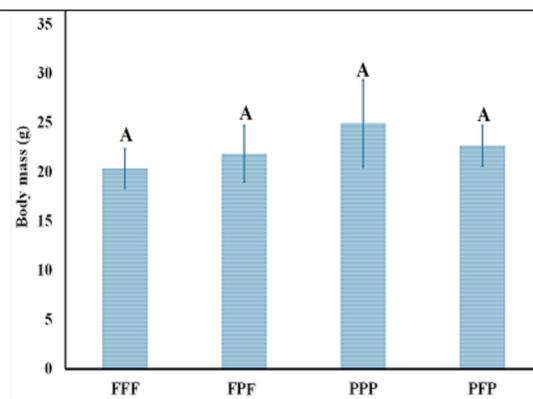
Sustainable aquaculture should include replacement of fishmeal-based feed sources by plant-based ingredients. However, this approach is often impeded by poor growth in carnivorous fish such as largemouth bass (LMB, *Micropterus salmoides*) fed high levels of plant-based protein. Therefore, our overall goal of the present work was to develop alternative methods of utilizing the plant-based diets (PBD) for sustainable LMB production. When fish are exposed to PBD early in their life, they may accept them more efficiently at later stages (so called Early Nutritional Programming, ENP).

A three-phase (6 weeks each, 18 weeks total) experiment was designed to evaluate the ENP of plant-based diets in early juvenile LMB (Figure 1). Preceding the experiment, LMB hatched larvae were fed *Artemia nauplii* for four weeks and then 1000 larvae (average weight ~ 0.1 g) distributed into tanks 1 and 2 for the commencement of Phase I (500 larvae/tank, 100 L size). On phase I, fish-larvae stocked into tanks 1 and 2 were fed a fishmeal-based diet (FM) or PBD, respectively. On phase II fish from each tank were divided into 2 tanks (tanks 3 and 4, respectively). Fish in tanks 1 and 2 continued to be fed the FM and SBM diets, while fish in tanks 3 and 4 were fed PBD and FM diets, respectively. On phase III, tanks 1 and 2 remained unchanged, while fish in tanks 3 and 4 were fed the FM and SBM diets, respectively.

At the end of phase III, no significant differences were found for growth performance (Figure 2) and body composition of fish whereas significant differences in physiological parameters were observed. Overall, this study developed an alternative method of utilizing the PBD at an early stage of life, which can reduce the cost of largemouth bass production.



**Figure 1:** Experimental design of early nutritional programming for largemouth study



**Figure 2:** Final body mass (g) of largemouth bass at the end of phase III.

**FFF:** Fish meal (FM) feeding followed by FM and FM; **FPF:** FM feeding followed by plant protein (PP) and FM; **PPP:** PP feeding followed by PP and PP; **PFP:** PP feeding followed by FM and PP

## DIGESTIBILITY OF DRY-EXTRUDED SEAFOOD PROCESSED WASTED BLENDED WITH PLANT PROTEINS FOR RED DRUM (*Sciaenops ocellatus*)

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The increasing of fishmeal price has prompted researchers to seek sustainable alternative protein ingredients for aquafeeds. Seafood processing plants typically have to pay to dispose of their processing wastes (e.g., fish viscera, heads, skin and bones). Therefore, the aim of this study was to develop some alternative marine and plant protein blends by dry extrusion and assess their nutritional value. The products included six dried-extruded blends of soybean meal (SBM) and distillers dry grains with solubles (DDGS) with seafood waste (SFW) from filleting marine fish in three different ratios (50:50, 40:60 and 30:70, of plant proteins and SFW, respectively). A reference diet (REF) was formulated with 36% crude protein and yttrium oxide ( $Y_2O_3$ ) was used as an inert marker. Test diets were obtained by replacing 30% of the reference diet by the various blends. Each diet was randomly assigned to two tanks and 45 red drum (~70 g) were stocked in 1000-L round fiberglass tanks and fed to apparent satiation once daily. For four weeks fecal material were collected by stripping from the lower intestine, pooled by tank, dried at 60°C overnight and stored at -20°C prior to analysis. Apparent digestibility coefficients were determined based on the percentage of the nutrient and  $Y_2O_3$  in the diet and feces. Results of apparent digestibility coefficient values for protein in the diets and ingredients are presented on Table 1. Results for ADC of energy and amino acids are still pending. This results will be further used for a feeding trial in which fishmeal will be replaced by the blends with diets formulated on a digestible protein and energy basis.

Table 1: Apparent digestibility of protein in the test diets and dry-extruded blends.

	RE	SBM	SBM	SBM	DDGS	DDGS	DDGS	PS
ADC	F	(50:50)	(40:60)	(30:70)	(50:50)	(40:60)	(30:70)	E
Diets	72.							0.7
(%)	2	79.0	79.0	76.1	74.5	76.5	77.9	4
Ingredients (%)		89.8	90.4	82.1	79.8	86.1	90.1	1.7
								2

ADC Apparent Digestibility Coefficient

REF Reference Diet

PSE Pooled Standard Error

CNPQ: 207141/2014-2

## USE OF SURFACE MUCUS AS A BIOMARKER FOR ENDOCRINE DISRUPTION IN *Fundulus grandis*

Andrea Yammine\*, Alf Haukenes, Christopher Green

Reproductive investment and endocrine health are normally assessed using assays which typically rely on blood collection, which is often lethal to small fishes. Surface mucus collection offers a less invasive methodology to determine concentration of 11-ketotestosterone (11-KT) and vitellogenin (VTG). This project seeks to (1) validate and confirm the use of mucal swabs as an effective and non-lethal alternative to determine concentrations of 11-KT and VTG of *Fundulus grandis* through comparative assays of blood plasma, and (2) use this minimally invasive method to evaluate potential endocrine disruptions in *F. grandis* undergoing chronic exposure to a water accommodated fraction (WAF) of crude oil, and (3) assess potential differences in endocrine disruption at the population level based on exposure history.

Mucus and plasma samples were collected from male and female *F. grandis*. Both 11-KT and VTG showed significant positive relationships between their concentrations in the plasma and mucus. Four different populations of *F. grandis* with different exposure histories were exposed to WAF for 60 days, and post-WAF conditions for 40 days. Assessment of mucosal 11-KT concentrations showed that endocrine disruption was observed in many populations throughout time with a severe decline 40 days post-WAF. Changes in concentrations of 11-KT and VTG were significant with the linear relationship of time, treatment and population. Exposure history also had a significant effect on 11-KT concentrations; exposed reference site males had a decrease in 11-KT concentrations, while concentrations of 11-KT of exposed males from contaminated sites increase. Exposed females showed near null concentrations of mucosal VTG when exposed to WAF regardless of exposure history. These data demonstrate the utility of mucus sampling as a less invasive tool to assess endocrine disruption in captive fishes, allowing to assess reproductive investment and potential.

## COMPARISONS OF INTESTINAL MICROBIOTA CHARACTERIZATIONS, IMMUNE ENZYME ACTIVITIES, AND MUSCLE AMINO ACID COMPOSITIONS OF LOACH CULTURED IN PADDY FIELDS AND PONDS

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A balanced intestinal microbial ecosystem is crucial for the growth and health of animals because it can influence the digestion and absorption of nutrients in intestinal. Different culture conditions may change the ecology of microbial in intestine and thus affect the overall growth performance of an animal. In this study, we compared intestinal morphologies, microbiota characterizations, immune enzyme activities, and muscle amino acid compositions of loach cultured in paddy fields and ponds. The fish were fed with the same diets from 5<sup>th</sup> May to 5<sup>th</sup> November (2015) in three paddy or ponds. Fish samples were collected for analysis in the August (summer season) and November (fall season) during the feeding trial. In both culture conditions, results based on microscopy observation showed that the intestinal perimeter, folds height, folds radical and total absorption of the gut were significantly higher in the foregut than that found in the midgut and hindgut ( $P < 0.01$ ). The average final body weight of fish was similar between the two culture conditions ( $P > 0.05$ ). The percentage of carcass weight to whole loach weight for samples collected from paddy field ( $91.6 \pm 1.1$ ) was significantly higher than the index measured for loach from pond ( $87.3 \pm 3.4$ ) ( $P < 0.05$ ). Results based on denaturing gradient gel electrophoresis (DGGE) demonstrated that the Shannon-diversity index, evenness and richness of intestinal flora were increased from summer to fall in paddy cultivation. In pond culture condition, however, the above indexes obtained from mucosa and intestinal contents decreased in fish from summer to fall. The sequencing results of bands indicated that the predominant microorganisms are *Proteobacteria*, *Firmicutes*, and *Actinobacteria* in the intestine of fish being cultured in both cultures. Activities of alkaline phosphatase (AKP, in two culture conditions) and superoxide gasification enzymes (SOD, in paddy field) presented a gradual decrease trend from foregut to hindgut of fish. The activities of acid phosphatase (ACP, in midgut), AKP (in midgut and hindgut), SOD (in foregut) and lysozyme (LZM, in midgut) were significantly higher in fish cultured in paddy than those in pond ( $P < 0.01$ ). In addition the percentage of some essential amino acids (valine, methionine, tyrosine, and phenylalanine) based on total amino acids in muscle was significantly higher in fish cultured in paddys than in ponds. In summary, the fish cultured in paddy or pond did not show difference in growth but the two culture conditions seems to generate different carcass yield and changed the amino acid profiles of fish muscle. The similar predominance microorganisms were identified in the intestine of fish from two conditions, and the quantification of microbial in the intestine will be determined in the future. Some enzymes involved in immune protection were higher for fish cultured in paddy fields.

## IMMUNE MODULATIONS OF THE MITOCHONDRIAL ANTIVIRAL SIGNALING PROTEIN(MAVS) OF THE BLACK ROCKFISH (*Sebastes schlegelii*)

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The mitochondrial antiviral signaling protein (MAVS) plays a vital role to defend the microbial pathogens in the host cellular innate immune responses and it is also important in the signal transduction of antiviral gene cascade. In this study, we have identified and characterized the MAVS from black rock fish (RfMAVS), *Sebastes schlegelii*, and determined the spatial and temporal mRNA expression by using quantitative real time PCR (qPCR). In the in silico study, we have used various bioinformatics tools and webservers to discover the characteristic molecular features of RfMAVS. The putative open reading frame of RfMAVS was 1770 bp that coded for a protein of 590 amino acids. It has no signal peptide and its molecular mass was approximately 62.47 kDa and isoelectric point was 4.59. The pairwise sequence alignments showed that RfMAVS shared 73.7% of the identity and 83.9% of similarity with *Oplegnathus fasciatus* MAVS. A ubiquitous expression of RfMAVS transcript was revealed at the 10 tissues (Blood, Head kidney, Spleen, Liver, Gill, Intestine, Kidney, Muscle, Skin and Heart) and significantly ( $P < 0.05$ ) highest mRNA expression was observed in the blood. The RfMAVS mRNA expression was highest in blood, head kidney and spleen at 12 h post injection (p.i.) of Poly I:C, where liver has shown significant up-regulation at 24 h p.i.. Collectively, the RfMAVS is belongs to the teleostean MAVS counterpart and possess the immune responsive regulations upon viral infections.

## **IDENTIFYING THE BEST GENETICALLY DISTINCT LARGEMOUTH BASS POPULATIONS FOR BREEDING AND FAST GROWTH IN THE NCR**

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Largemouth bass (LMB) is an important aquaculture species in the Midwest and throughout the U.S. Interest in improving commercial culture efficiency has grown due to the great demand and high value compared to other cultured species. One of impediments is the rearing of LMB stocks with little to no domestication or selective breeding for efficient production. Therefore, strain evaluation and identification of the best genetically distinct largemouth bass populations for breeding and fast growth in the North Central Region (NCR) is needed.

In this study, twenty-six populations were genotyped using eight microsatellite markers. Across all 26 populations sampled, W190 locus in DFF-MO population had the highest number of alleles (18), three populations (CFAAS – IL, CSFH-MO and JWSFH-IL) had the least alleles (2) in locus Mdo6. The average number of alleles across all loci was the highest (11.375) in the population DFF-MO and the lowest (7.375) in populations VSS-IL and WFF-AL. The values of observed heterozygosities per population were detected from 0.701 to 0.887. The expected heterozygosities were from 0.649 to 0.887. The FSFH-KS population had the highest gene diversity and JWSFH-IL population had the lowest one. The allele richness per population was highest in DFF-MO population (9.136), the lowest in WFF-AL population (4.542).

Genetic divergence statistics obtained by AMOVA were significant at all hierarchical levels. When all 26 populations were grouped together and all loci were considered, 4% of the variation was detected among populations, and 88% of the variation was found within individuals. However, only 8% of the variation was detected among individuals within populations.

## EVALUATION OF THE EFFECT OF SUPPLEMENTED DIETS WITH A PHYTOPHARMACEUTICAL PREPARATION FROM HERBAL AND MACROALGAL ORIGIN ON DISEASE RESISTANCE IN FARMED ATLANTIC SALMON AGAINST *Piscirickettsia salmonis* UNDER FIELD CONDITIONS

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The main objective of this study was to evaluate the cumulative effect of diets supplemented phytopharmaceutical preparation, Futerpenol™, on the weekly rate of *P. salmonis* attributed mortality at the cage-level (SRS-mort from now on) in marine Atlantic salmon farms under a typical production setting.

Three farms were recruited, where cages were followed from stocking (May - September of 2015), until the end of the follow up time (February - April of 2016). Weekly data was collected on production variables (No. of fish stocked, weight, biomass, and stocking density), sanitary variables (overall mortality and disease specific attributed mortality, including SRS-mort), use of medicated feeds (antimicrobials and emamectin benzoate for control of bacterial diseases and sea lice, respectively), and the use of Futerpenol™.

To assess the effect of cumulative exposure to Futerpenol™ on SRS-mort we used a generalized linear mixed effect model to account for the multi-level structure of the data, with three levels of clustering (week, cage and farm), using a log link function. A secondary objective was to evaluate the effect of the time since the last treatment on SRS-mort, to assess any potential carry-over effect. Both cumulative treatment and time since last treatment were categorized, so comparison not involving baseline levels were done via pair-wise linear contrasts, using a Bonferroni adjustment to account for multiple testing.

Parameter estimates and related statistics for fixed effects indicate that treatment becomes effective as of the 8<sup>th</sup> week of cumulative exposure, with a mean incidence density ratio of 0.34 (95% CI: 0.21, 0.56) for the group exposed to Futerpenol™ for 8 to 11 weeks, indicating that a cage with this level of exposure will have on average a 66% (95% CI: 79%, 44%) lower weekly incidence of SRS-mort than a cage that has been exposed for 7 weeks or less, conditional on random effects and covariates. This effect is not significantly different from higher levels of cumulative exposure. Regarding the carry-over effect of treatment on SRS-mort, results show that treatment in the current week does not have a significant effect, while 1 to 2 and 3 to 4 consecutive weeks unexposed to treatment were borderline non-significantly associated with higher SRS-mort.

These results show promise for the mitigation of the effects of this disease in Chilean salmon farming, and open the door to potential applications for other diseases and species.

## ECONOMIC CONSIDERATIONS FOR GROWING HYBRID STRIPED BASS IN RECIRCULATING SYSTEMS AT COMMERCIAL SCALE; PLANNING INPUT / OUTPUT, GROWTH, FINANCIAL, OPERATIONAL AND INVESTOR IMPLICATIONS

John Zevgolis\*

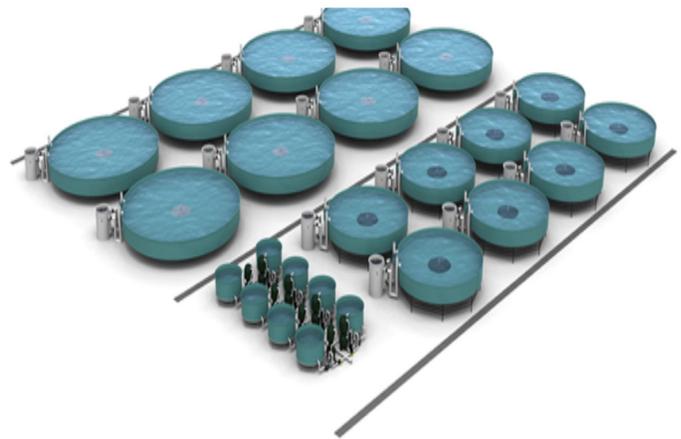
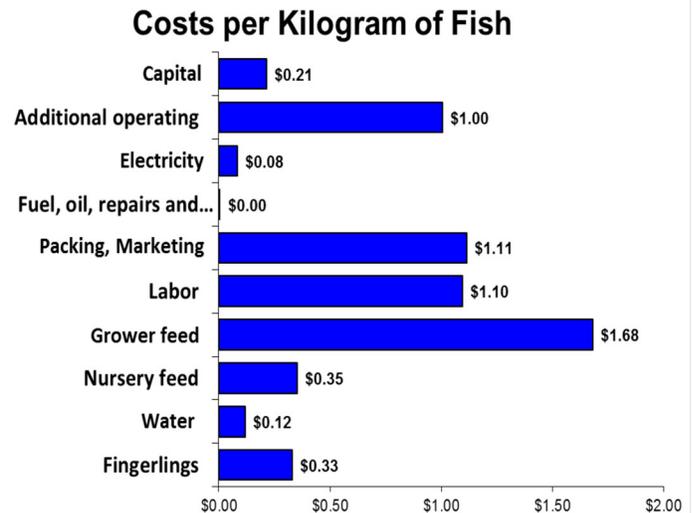
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Planning and developing an accurate account of cost inputs and sales assumptions when planning a farm are critical to the success of the project. Investors and operators need to understand and insure assumptions associated with the sales and operations are accurate. The information presented here considers the planning and design stage, equipment, and operational costs as well as sales prices for hybrid striped bass in a 150 ton recirculating aquaculture farm. Major expenses, various system and production attributes are identified, discussed and their interplay considered.

Ball park development costs, rules of thumb in estimating these costs as well as other factors that can enhance or detract from yield and returns are discussed. Production yield and increasing profit margins are explored as they relate to buyer needs and investor returns.

Hybrid striped bass grow out, sourcing of fingerlings, and other challenges to consider in planning, which are often missed, are discussed.

Basic investor economic metrics that should be considered in developing the project are often not properly built into the financial planning and operation of the farm; the presentation also identifies these aspects for those seeking outside investment.



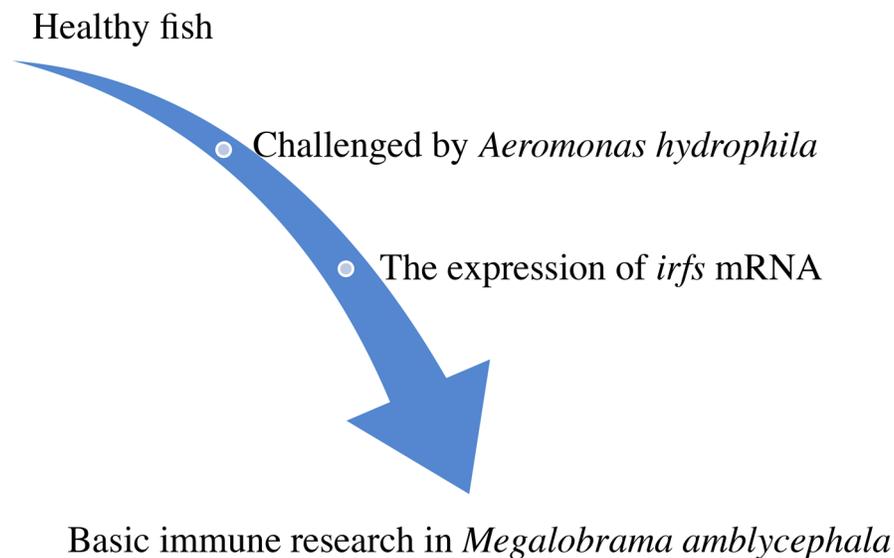
## IDENTIFICATION AND GENE EXPRESSION OF INTERFERON REGULATORY FACTORS IN *Megalobrama amblycephala*

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The family of interferon regulatory factors, which includes nine mammalian members (*irf1–irf9*), acts as transcription factors for interferons and exerts regulatory functions in the immune system and in oncogenesis. And irfs are known to be critical in regulating a spectrum of functional and developmental processes in lymphomyeloid cell lineages through direct binding to interferon stimulatory response elements in target gene promoters of immune or immune-related genes. In the present study, this family members (*irf1*, *irf2*, *irf4a*, *irf4b*, *irf6*, *irf7*, *irf8*, *irf9*, *irf10*) have been identified and characterized in blunt snout bream all sharing homology in a well conserved DNA binding domain (DBD) covering the first 115-120 amino acids in the N-terminus, with the characteristic motif containing a series of tryptophan residues. Phylogenetic analyses confirmed the all irfs belong to four irfs sub-families and revealed close relationships with cyprinid orthologs. In healthy fish, *irfs* mRNA were most abundant in a range of nine tissues, especially in the liver spleen and kidney, where the expression of *irf1* mRNA was highest. The expression of *irfs* mRNA displayed an obvious dose-dependent manner following *Aeromonas hydrophila* challenge, *irf4a*, *irf4b* and *irf10* were up-regulated in the detected tissues, while *irf1* was down-regulation in intestine and gills, *irf2*, *irf6*, *irf8* and *irf9* were down-regulated in liver. However, the expression of *irf7* mRNA was down-regulated in the detected tissues. Overall, these data may be indicating that these irfs more likely involved in antibacterial immune response.



## TRANSCRIPTOMIC PROFILES OF FLORIDA POMPANO (*Trachinotus carolinus*) GILL FOLLOWING INFECTION BY THE PARASITE *Amyloodinium ocellatum*

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The dinoflagellate *Amyloodinium ocellatum* is an important pathogenic parasite infecting cultured marine fish worldwide, including cultured Florida pompano (*Trachinotus carolinus*), which is one of the most desirable and highest priced marine food fish from Florida waters. *A. ocellatum* can infect fish skin and gills causing tissue damage, increased respiration rate, reduced appetite and finally devastating mortality, especially in closed systems.

Here, we performed a transcriptomic comparison of gene expression in the gill of healthy and *A. ocellatum* infected pompano utilizing RNA-sequencing to explore its infectious mechanisms.

Following sequencing and transcriptome assembly, differential expression (DE) analysis revealed 1041 unique genes with differential expression of greater than 1.5-fold in diseased fish compared to healthy, uninfected fish. Most striking of the DE genes was the high expression of microbicidal myeloperoxidase (MPO) and eosinophil peroxidase (EPO) that are abundantly expressed in the neutrophil and eosinophil granulocytes respectively and mediate parasite killing and tissue damage by generating reactive oxygen species. Correspondingly, expression of an array of matrix metalloproteinases (MMPs), often functioning in extracellular matrix degradation were highly stimulated, along with widespread down-regulation of collagen genes. Several macrophage mannose receptor1 (MMR1) genes were highly induced potentially indicating the recruitment of macrophages to the site of infection. Expression of IgM increased significantly after infection. Tissue destruction and inflammation appeared to have downstream impacts on important physiological and metabolic pathways, negatively impacting hemoglobin synthesis, interfering with urea transporters, and establishing hypoxic conditions in the gill tissue.

Our work provides the first transcriptome sequencing of Florida pompano and provides key insights into the acute pathogenesis of *A. ocellatum* which can be utilized for disease surveillance and eventual selection for host resistance.

## CHITIN DEGRADATION AND METABOLISM BY VIRULENT *Aeromonas hydrophila*

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*Aeromonas hydrophila* is the causal agent of motile *Aeromonas* septicemia (MAS) in catfish and other warm-water fishes. Severe outbreak of MAS caused by virulent *A. hydrophila* (vAh) was reported in 2009 in the Southeastern United States; the disease has since resulted in loss of millions of pounds of market-size catfish annually. The persistence of MAS outbreaks may suggest that certain environmental conditions promote vAh maintenance or growth in pond water. One such environmental factor of interest is chitin, a linear polymer of N-acetylglucosamine, which may benefit the water-borne pathogen.

Analysis of draft genome sequence of a vAh strain, ML-10-51K, reveals that the bacterium has four genes coding for chitinases with calculated molecular weight (MW) of 106.6, 92.8, 68.7, and 53.2 kDa, respectively, and two genes for chitobioses with MW of 98.2 and 69.0 kDa, respectively. In addition, there are many other chitin and N-acetylglucosamine utilization genes in the pathogen's genome, such as transcriptional regulator of N-acetylglucosamine utilization, chitin binding protein, phosphatase NagD, N-acetylglucosamine regulated methy-accepting chemotaxis protein, N-acetylglucosamine ABC transport system, glucosamine-6-phosphate deaminase, and N-acetylglucosamine-regulated outer membrane porin.

In *in vitro* assays with M9 minimal salts, ML-10-51K could efficiently use N-acetylglucosamine, colloidal chitin or crystalline chitin as sole carbon source. Extracellular proteins (ECPs) from ML-10-51K culture could degrade colloidal chitin and generate N-acetylglucosamine and N,N'-diacetyl chitobiose. Mass spectrometric analysis of chitin binding proteins in ECPs revealed that all four chitinases found in ML-10-51K genome participated in chitin degradation.

Since chitin is abundantly available in natural aquatic ecosystems (resulting from exoskeletons of crustaceans and other arthropods, as well as the cell wall of fungi), it makes it possible for vAh readily to colonize and use the substrate and sustain high population level in fish ponds. Additionally, there is growing evidence that N-acetylglucosamine plays diverse roles in cell signaling pathways that impact the virulence of microbes and the sensitivity of host cells. It would be of importance to further investigate mechanisms associated with chitin metabolism and pathogenesis in virulent *Aeromonas hydrophila*.

## EFFECTS OF TWO DIFFERENT NUTRIENT SOURCES ON AQUATIC ENVIRONMENT AND C/N TROPHIC SHIFT IN GRASS CARP POLY-CULTURED POND

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To figure out the nutrient cycling process and energy exchange efficiency of major biogenic elements in grass carp (*Ctenopharyngodon idella*) poly-culture ponds, this study investigated the potential effects of two different foodstuff sources (sudan grass *Sorghum sudanense*, and formula feed) on the plankton community structure, and C/N trophic shift in grass carp poly-culture ponds using analytical techniques of stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and C/N ratio. Our results showed that two feeding modes yielded no statistically significant impact on the community structure of plankton, but the feeding mode with sudan grass helped promote the plankton growth in the water column. In the ponds fed with sudan grass, sudan grass contributed 84% and 6.8% of energy to herbivorous and filter-feeding fishes (silver carp *Hypophthal michthysmolitrix* and bighead carp *Aristichthys nobilis*), respectively. Moreover, sudan grass contributed 7.4% of energy to particulate organic matter. In the ponds fed with artificial feed, herbivorous fish derived 70.4% of energy source from formula feed. Formula feed contributed 5.1%, 8.1% and 12% of energy source to bighead carp, POM and sediment, respectively. Our findings suggested that, first of all, the carbon and nitrogen elements of two foodstuff sources were utilized by herbivorous fish (grass carp and blunt snout bream *Megalobrama amblycephala*), and then transferred the nutrients to filter-feeding fishes through the pathways of plankton, residual feeds and fish feces. Ultimately, the biogenic elements were dedicated to the sediment. In contrast with formula feed, the nutrient of sudan grass could be more utilized by cultured fish than that deposited in the sediment. The trophic transfer was slower in ponds fed by sudan grass than that in ponds fed by formula feed. Energy transfer of cultured fish was extremely relevant to foodstuff sources. In particular, the exchange efficiency in sudan grass ponds was higher than that of formula feed ponds.

# TOXIN-PRODUCING ALGAE ARE A SIGNIFICANT IMPACT IN FRESHWATER AND MARINE AQUACULTURE

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The EPA and USGS have identified over 50% of all US waterbodies as being impaired by nitrification, with many having harmful algal blooms. Recently mysid mortality in marine aquaculture systems was related to occurrence of a new to science benthic cyanobacterium *Toxifilum mysidocida*. Recent developments in harmful algal blooms will be discussed.

Microcystin is one of 48 toxin classes produced by cyanobacteria. In the early 2000s, fish-killing concentrations of microcystin were identified in catfish production ponds. Zimba and Grimm conducted a synoptic survey of 486 ponds for off-flavor and microcystin occurrence and found <1% had levels above USEPA/WHO levels of concern. In a recent survey of 149 cyanobacterial cultures (originally isolated from terrestrial, freshwater, and marine systems) toxin production was widespread averaging six toxin classes per species (Figure 1). Protease inhibitors such as aeruginosins and micropeptins accounted for ~45% of observed toxins (Figure 2). Co-occurrence of protease inhibitors (preventing peptide degradation) and protein toxins would mean that toxicity would be greater than if the single protein toxin was ingested.

Other toxin producing divisions of microalgae include the Euglenophyceae and Dinophyceae, producing euglenophycin and saxitoxin(-like) compounds. Euglenophycin was first identified from aquaculture systems and is currently known from 17 US States and four continents. In a divisional survey of the euglenoids (Figure 3), 6 of the 22 species tested produced toxins. Algal toxin advancements will be discussed.

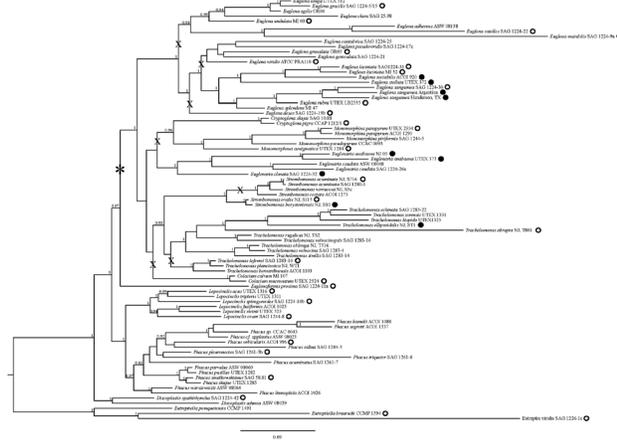
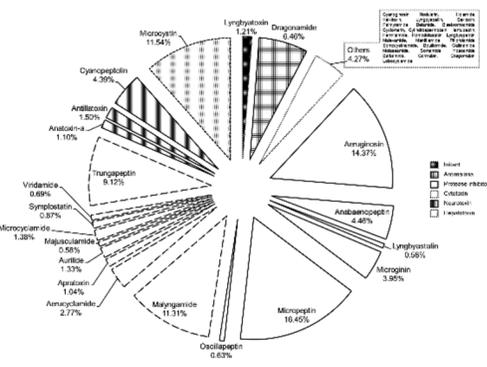
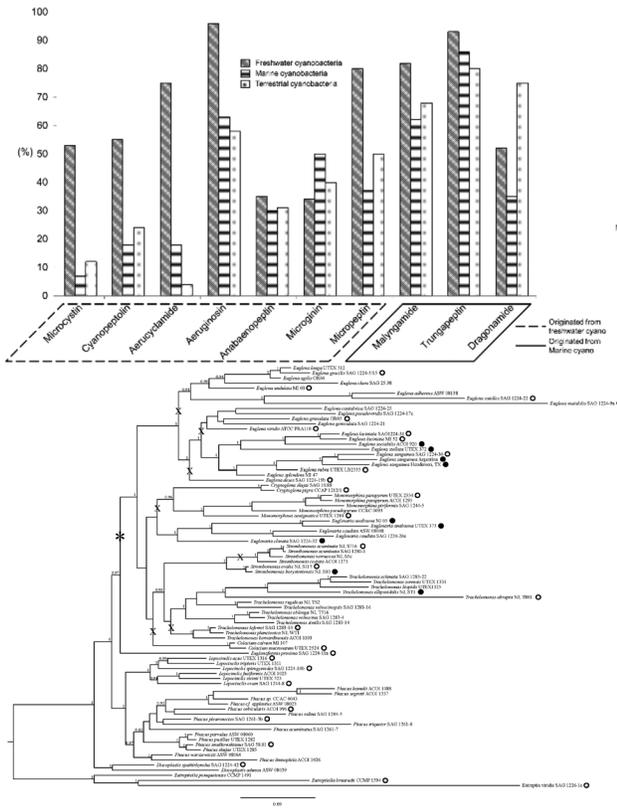


Figure 1 (top left). Percentage occurrence of toxin classes in 149 cultures of terrestrial, freshwater and marine cyanobacteria. Toxins classes are identified by origination (dashed rectangle indicates freshwater).

Figure 2 (top right): Toxins present in 149 taxa analyzed by HPLC-TOF. Note solid lines – no fill– are the largest group and are protease inhibitors.

Figure 3 (left). Phylogenetic analysis of euglenophycin occurrence-black circles indicate toxin formation, open circles-none detected)

We thank Jeff Johansen, JoAnn Burkholder, Dale Casamatta, Nicole Pietrasiak, and Richard Triemer for culture access.

# ADDENDUM

## THE EFFECTS OF VARYING BROODSTOCK PAIRING RATIO IN INDUCED SPAWNING ON THE HATCHABILITY OF CATFISH *Clarias gariepinus*, Burchell, 1822 EGGS

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African sharptooth catfish, *Clarias gariepinus* is one of the most farmed fish species in West Africa, where commercial breeding is done using hormones including pituitary gland. This study was carry out using natural pituitary from male catfish for induced breeding of *C. gariepinus* under varying brood stock ratios. The broodstocks selected weighed between 800g and 1000g males, 900g and 1000g females. They were used for three induced breeding trials in treatment ratios of A (1 Male, M:1 Female, F), B (2M:1F) and C (1M:2F). Females under treatments A and B were injected with 2mls of the pituitary solution each, and C (1M:2F) was injected with 1 pituitary (0.5 each ) of 1ml each. Fecundity of females in A was 10500 eggs, B was 12600 and the two females of C were 9600 and 10200 eggs (added up to get 19800 eggs). The weight of eggs produced were A (1F, 35g), B (1F, 42g) and C (2F was 32 and 33g, mean= 33g).

Hatching started 24 hours after incubation and lasted for 33 hours. Percentage hatching for treatment A was 51%, B was 75% and C was 23%. The differences between the percentages showed that there was no significant difference  $p > 0.05$  between A(1M:1F) and B (2M:1F). Treatment C (1M:2F) showed no significant difference ( $p > 0.05$ ) as compared to treatment A(1M:1F). The percentage survival of the treatments indicated that C(1M:2F) was the highest 49.0% followed by A (1M:1F), 30% and B(2M:1F), 26%. There was no significant difference  $p > 0.05$  between A(1M:1F) and C(1M:2F) but treatment A(1M:1F) and B(2M:1F) showed significant difference ( $p < 0.05$ ). Treatment B with two males and one female broodstock had the highest number of stripped and fertilized eggs, hatchlings, low number of spoiled eggs and high survival rate. Fish farmers should use the broodstock ratio of 2 males to 1 female for induced spawning to help them get enough good seed for their fingerling production, and also use larger receptacles for higher hatchability.

## PRODUCTION OF HYBRID CATFISH USING IN-POND RACEWAY SYSTEM

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With the US Catfish industry's declining production since 2003, increased competition from imported substitute products and higher feed prices, technological advances have to be made in order to increase production while lowering costs to keep the industry competitive.

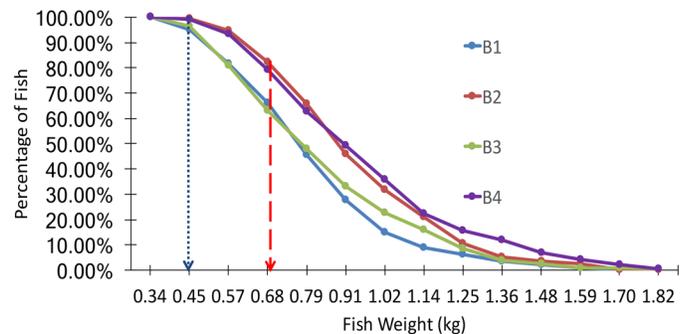
Beginning in March of 2016 the first year of a four-year study has been set out to assess the feasibility of In-Pond Raceways Systems for production of market size catfish compared to other current conventional pond production used by the catfish industry. The In-Pond Raceway System (IPRS) was first developed at Auburn University in the early 1990s. IPRS has been experimentally proven to improve survival and feed conversion (FCR) and allows for a high standing crop of catfish in production units that are only a small fraction of the pond's total area. However IPRS has not consistently produced market size catfish in past trials. Thus, there is still a lack of sound production data needed to demonstrate and encourage catfish farmers to adopt this technology.

IPRS allowed us to produce a marketable catfish (>454g) in one growing season. The percentage of catfish that made it to marketable size (>454g) B1-95.2%, B2-99.4%, B3-96.5%, B4-99.1%. The percentage of catfish above 680g (1.5 lb) B1-66.0%, B2-82.4%, B3-63.0%, B4-79.3% meaning that well over 50% of the fish population made it to the first year's goal. The two larger IPRS units doubled the yields (~16,237 kg/ha) of conventional catfish ponds (7,800 kg/ha). The two smaller IPRS units (~13,720 kg/ha) nearly doubled the pond standard yield. FCR values calculated in this IPRS study (1.5-1.64) were far better than those of conventional catfish pond production systems (2.2-2.4). This significantly reduced feeding cost in the IPRS. The weighted total cost of production was US\$2.08/kg (US\$2.01 - 2.15/kg). Fish were sold to processor at US\$2.53/kg yielding a profitability index of 24.0% above total costs.

**Table 1 - Production data from the first year study on farming hybrid catfish in IPR (Av. stocking weight = 41 g; 268 days of growout).**

	Ponds			
	B1	B2	B3	B4
Pond area (ha)	0.40	0.40	0.40	0.40
Raceway volume (m3)	63.5	63.5	45.3	45.3
Number of fish stocked	11,030	11,086	8,083	7,821
Stocking biomass (kg)	456	463	345	322
Feed applied (kg)	9,699	9,817	8,200	7,733
Mean weight (g)	671	794	712	817
Biomass (kg)	6,388	6,601	5,510	5,467
FCR	1.64	1.60	1.59	1.50
Avg. feeding rate (kg/ha/day)	90.5	91.6	76.5	72.1
Standing crop (kg/m3)	100.7	104.0	121.6	120.7
Standing crop (kg/ha)	15,971	16,502	13,774	13,666
Survival (%)	86.4%	75.0%	95.7%	85.6%

**Weight Distribution of fish at Harvest (kgs)**



## ACCUMULATION OF PARALYTIC SHELLFISH POISONING TOXIN IN THE ABALONE *Haliotis midae* AND POTENTIAL VECTORS

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Paralytic shellfish poisoning (PSP) is a foodborne illness associated with the consumption of seafood products contaminated with the neurotoxins known collectively as saxitoxins (STXs). Much is known about the toxin sources, primarily certain dinoflagellate species, and there is extensive information on toxin transfer to filter-feeding molluscan bivalves, known as the traditional vectors of these toxins. However considerable uncertainty exists as to the source and transfer of these toxins in non-traditional vectors such as abalone. Saxitoxins in the South African abalone *Haliotis midae* were first detected in 1999 and linked to the presence of the dinoflagellate *Alexandrium catenella*.

Our study aimed to reassess the presence and seasonality of saxitoxins in farmed and wild abalone on the South African coast, to further investigate the anatomical distribution of toxins and to establish rates of depuration of toxins from abalone. Results show toxins to be more prevalent in both farmed and wild abalone to the west of Cape Point with no indication of seasonal variability. Toxins were found predominantly in the epipodial fringe and to a lesser extent in the muscle and viscera. Abalone size classes showed no significant difference in toxin content.

Depuration experiments run over 6 months showed significant fluctuations in toxin concentrations without significant depuration. These fluctuations in the absence of *Alexandrium catenella* indicate an alternate source of toxin. Future research will investigate cellular localization of the toxins, and bacteria as a possible source of toxins in abalone.

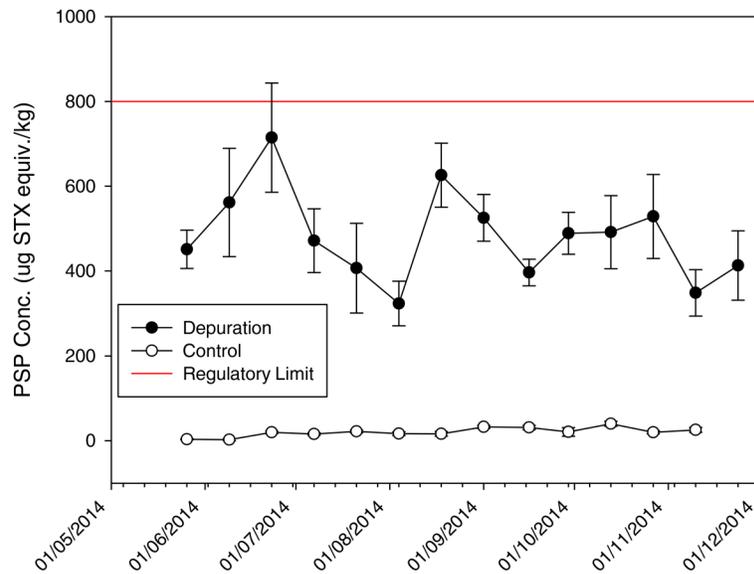


Figure 1: Depuration of the abalone *Haliotis midae* under controlled conditions

## EFFECT OF METHYLENE BLUE ON DEVELOPING ZEBRAFISH EMBRYOS *Danio rerio*

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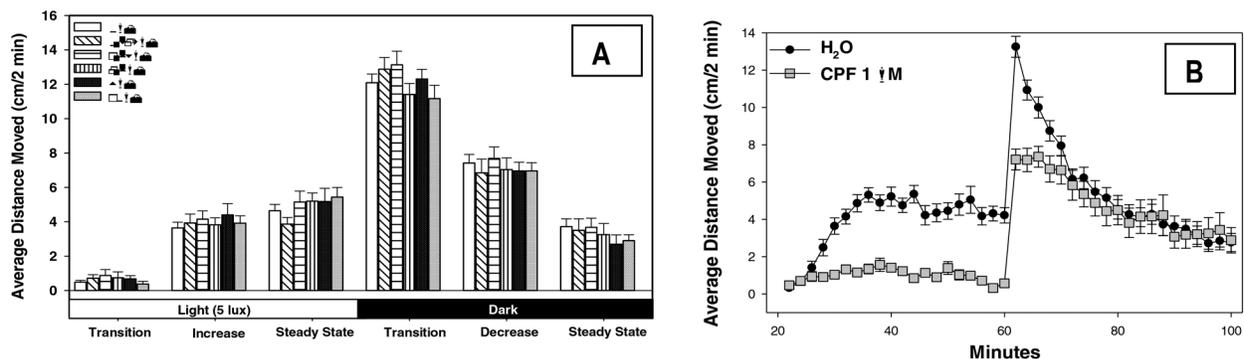
Our laboratory routinely conducts zebrafish (*Danio rerio*) developmental studies on 100s of chemicals, it is therefore imperative that the chemicals we use for animal husbandry are safe. It was brought to our attention that methylene blue, an antifungal agent commonly used in zebrafish embryo rearing, has been reported to affect swim bladder inflation (angelfish), increased fetal death (rats and humans), and produce hyperbilirubinemia, hemolytic anemia, and intestinal atresia (humans). Therefore, we conducted a developmental toxicity and neurotoxicity assessment of methylene blue in zebrafish using concentrations commonly employed by zebrafish researchers.

A 6 day developmental/neurodevelopmental assay was conducted using zebrafish embryos/larvae. At 0 days post fertilization (dpf), methylene blue exposure began in 96 well plates, which were kept in a 26°C incubator on a 14:10 (light:dark) light cycle. Embryos were dosed once daily on 0-4 dpf with 0.6, 1.6, 5.0 or 10.0 µM methylene blue (MEB); 1 µM chlorpyrifos (CPF) was used as a positive control (n=24 per chemical per dose). At 5 dpf embryos were removed from the chemical, and placed in water, and then tested on 6 dpf.

Behavioral testing consisted of assessing the locomotor activity of individual larval zebrafish in a 96-well plate, under a light:dark test paradigm: 20 minute acclimation in the dark, followed by 40 minutes of light (5 lux) and then 40 minutes of dark. Embryos were examined daily on 0-5 dpf and after locomotor testing on 6 dpf for death, hatching, swim bladder inflation, and morphological appearance.

Although the CPF treated larvae showed the expected decrement in locomotor activity (Figure B), no differences were seen between MEB exposed and control larval locomotor activity (Figure A) or any of the developmental aspects (death, hatching rate, swim bladder inflation or dysmorphology). These data indicate that MEB is not developmentally or neurodevelopmentally toxic to larval zebrafish at commonly used concentrations. *This abstract does not necessarily reflect U.S. EPA policy.*

Figure 1: Locomotor behavior in zebrafish after 5 days of methylene blue (A) or chlorpyrifos (B) exposure.



## A STUDY ON THE POLYCULTURE OF SEA SQUIRT *Halocynthia roretzi* AND SEA CUCUMBER *Apostichopus japonicus* UNDER A HANGING CULTURE SYSTEM

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The southern part of the Korean sea is an important aquaculture ground because of its mild water temperature and many islands around that protect typhoons and strong waving waves. However, the changes in the natural environment and high-density cultures in a limited area have hampered productivity while caused mass mortality of species. This calls on us to develop eco-friendly farming technology so as to achieve sustainable aquaculture farms. To that end, it is necessary to develop aquaculture technology which not only helps the vertical use of sea surface and bottom but also improves environment in the sea cucumber poly culture. It is also necessary to develop poly-culture technology for oysters and sea squirt, the two major filtration-feeding culture species in the South coast and sea cucumber, a high value-added species functioning as a cleaner.

The average size of young sea cucumber released in April was  $3.7 \pm 2.9$  g. It continued to grow during the summer season and grew to an average of  $9.1 \pm 3.0$  g in December. The average size of adult sea cucumber released in April was  $31.5 \pm 5.6$  g in April. It became smaller to  $20.8 \pm 5.9$  g in early summer but grew back to  $28.8 \pm 13.8$  g in December. It has been confirmed that heavier sea cucumbers are more affected by high water temperature, so they sleep in the summer. Most of the sea cucumber were found to be under a shelter. From our night time survey on September 10, 2016, they were found out of the shelter. The density of sea cucumber had decreased to 4.2 individuals/m<sup>2</sup> until July since they were released from the shelter of the farms. Since then, it slowly decreased to be 3 individuals / m<sup>2</sup> in December. The survival rate of each shelter is 28.0% for A, 27.2% for B, 19.7% for C type.

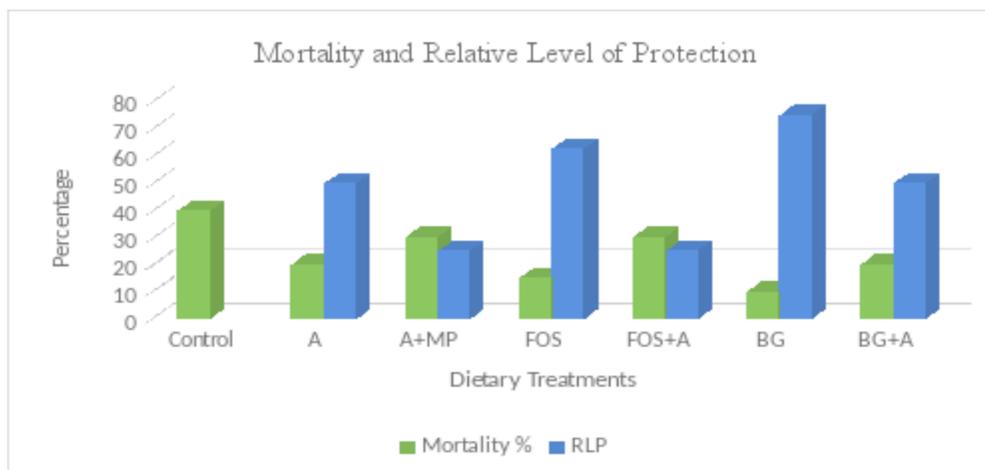
## STUDIES ON GROWTH AND IMMUNE RESPONSES IN *Pangasianodon hypophthalmus* FED WITH BIOTIC SUPPLEMENTS

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Health awareness, rising income, and demographic pressure have led to a demand for food fish. The intensification of farming practices for higher production has increased the likelihood of disease occurrence in aquaculture. Available limited government-approved antibiotics and chemotherapeutics for disease treatments are more often marginally effective and may cause an adverse impact on the consumer and/or culture environment. Alternative biocontrol measures are warranted to avoid the global multi-billion-dollar loss to the aquaculture industry. Dietary probiotics and prebiotics are considered as novel functional ingredients that can influence the host's microbiota, significantly affecting the health and general well-being of the host

Forty-five days feeding trial was conducted to investigate the effect of probiotic bacteria (*Lactobacillus acidophilus*) and commercially available biotic supplements (FOS,  $\beta$ -glucan and mixed prebiotic) on growth performance, haematological response and disease resistance in *Pangasianodon hypophthalmus*. To evaluate the in vivo synergism, fish were fed seven different diets in triplicates containing *L. acidophilus* at  $1 \times 10^6$  CFU/g of feed (A), *L. acidophilus* in combination with mix prebiotic (MP) (A+MP), Fructo-oligosaccharide (FOS) 1%, Fructo-oligosaccharide in combination with *L. acidophilus* (FOS+A),  $\beta$ -glucan (0.1%) (BG),  $\beta$ -glucan in combination with *L. acidophilus* (BG+A) and a control diet without any biotic supplement. Following the feeding trial, the growth of the fish, measured by weight gain%, FCR, SGR and PER in the biotic supplement fed group appeared to be different from that of the control. In addition, there was a significant improvement in red blood corpuscles, total immunoglobulin and serum lysozyme in the biotic supplemented groups over the control and the immune system of fish was stimulated to resist the infection by *A. hydrophila*. Based on the results, it was concluded that *L. acidophilus*, FOS and  $\beta$ -glucan added at a designated dose in the diet improves growth of *P. hypophthalmus* and evoke immunostimulatory effects and protects against *A. hydrophila* infection.



**Figure 1:** Mortality percentage and relative level of protection in groups fed different experimental diets after challenge with *Aeromonas hydrophila*. A - *Lactobacillus acidophilus*; A+MP; *L. acidophilus* + mix prebiotic; FOS: Fructo-oligosaccharide; FOS+A: Fructo-oligosaccharide + *Lactobacillus acidophilus*; BG:  $\beta$ -glucan BG+A:  $\beta$ -glucan+ *Lactobacillus acidophilus*.

## **INDONESIA DUCKWEED PROJECT**

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Duckweed is a naturally occurring floating aquatic plant with a worldwide distribution. It is rich in proteins and carbohydrates and low in cellulose, making it easy for monogastric livestock like chickens and pigs to digest. It can substitute for soyabean and other expensive plant proteins and can be grown cheaply and quickly. Due to its speedy growth and ease of harvesting duckweed fits in well with integrated farming systems of cattle, biodigesters and fish. The Indonesia duckweed project, sponsored by HIVOS and Yayasan Rumah Energi, developed the potential of duckweed grown on bioslurry from anaerobic biodigesters to produce feed for fish and livestock which can increase the incomes of small scale rural farmers in Indonesia.

## COPPER SULFATE CONTROLS FUNGUS ON MAT-SPAWNED LARGEMOUTH BASS EGGS

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Copper sulfate ( $\text{CuSO}_4$ ) is widely used by the catfish and hybrid striped bass industries as an economical treatment to control fungus (*Saprolegnia* spp.) on eggs; these industries use hatching troughs and McDonald jars, respectively, in moderate alkalinity waters. This study determined the effectiveness of  $\text{CuSO}_4$  on mat-spawned largemouth bass eggs at an extremely high alkalinity.

Adult largemouth bass were placed in concrete vats and allowed to spawn on 60 cm<sup>2</sup> fiber mats. Spawns were moved to 350 L plastic hatching containers within 12 h. Efforts to dislodge eggs from spawning mats with bromelain were unsuccessful; other spawns were then cut into 10 cm<sup>2</sup> sections with a guillotine paper trimmer. Sections were suspended in each aquarium and acclimated for 1 h in 18.3°C well water. The study consisted of 3  $\text{CuSO}_4$  concentrations (10, 20, and 40 ppm) and an untreated control; there were 4 replicates. Eggs were treated daily until fry began to descend from the mats. Chemistry of the well water was pH 7.8, alkalinity 386 mg/L and hardness 455 mg/L. Fungus was severe in the untreated controls, but only light to moderate amounts of fungus was present in treatment concentrations with 40 ppm having the best control. Due to the adhesiveness of the eggs to the spawning mats, a quantitative analysis could not be done. Benchtop toxicity studies have also been completed to determine the effect of  $\text{CuSO}_4$  on sac and swim-up largemouth bass fry.

## ESTABLISH A FLOW CYTOMETRY ANALYSIS OF GROUPEL Lymphocyte DIFFERENTIATION UNDER IMMUNE STIMULATION

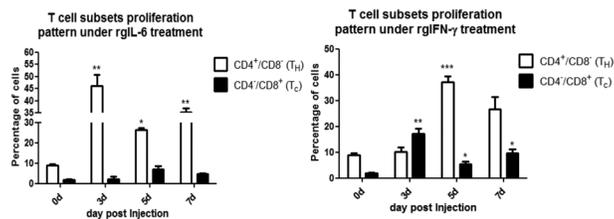
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Grouper (*Epinephelus* sp.) are high economic value aquaculture species around subtropical regions especially in Southeast Asia. However, the disease control is still a serious problem in the recent years. To control the disease, using immunoprophylaxis method, such as vaccine is considered as an effective method to prevent disease outbreak and transmission. An ideal immune protection is contributed by humoral or cell-mediated immune responses mediated by helper T lymphocyte ( $T_H$ ,  $CD4^+/CD8^-$ ) and cytotoxic T lymphocyte ( $T_C$ ,  $CD4^+/CD8^+$ ), respectively. Humoral immunity predominately triggers neutralization of extracellular pathogens whereas cell-mediated immunity is mainly responsive to kill extracellular pathogens and virus. However, in teleost few tools were developed to evaluate the differentiation of these lymphocytes, so the effectiveness of vaccine is hard to detect. Flow cytometry analysis for immune cell differentiation is already used in mammals for years. It can detect multi-parametric cell characteristics simultaneously.

This project is aim to setup this technique for groupers, thus the antiserum for CD4 and CD8 were prepared and the specificity and sensitivity were also evaluated. Cytokines which induce  $T_H1$  and  $T_H2$  pathway, such as interferon- $\gamma$  and interleukin-6 were used as standard to evaluate efficiency of established flow cytometry system in this project; finally, apply this system to the fish inoculated with different adjuvant formula and indicated that cytokine additives can control the direction of immune response and might increase the efficacy of fish vaccine.

FIGURE 1. Grouper lymphocytes treated with rgIL-6 and rgIFN- $\gamma$  were double stained with CD4/CD8 and analyzed by flow cytometry.



## QUANTIFICATION OF AQUACULTURE FARM DRAG BASED ON THE MOMENTUM SINK METHOD

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The demand of aquaculture will increase in the future, which presents an economic opportunity for farm expansion. In order to ensure sustainable growth for both the industry and the ecosystem, an important consideration is the flow response from larger farms. This work aims to quantify the friction caused by oyster farms using a computational fluid dynamic (CFD) method. A Large Eddy Simulation (LES) model predicted the flow response of a case study oyster farm in the Damariscotta River. The simulation provided the flow reduction and turbulence induced by the farm. A drag coefficient was derived based on the momentum sink induced by the oyster farm and will be used in the future to predict the impact of larger farms through theoretical and regional scale numerical model techniques. The outcome of these combined efforts will provide guidance for sustainable farm expansion in the future.

A three dimensional CFD model was built to study the momentum sink induced by the farm. Long-lines within the farm were idealized as continuous porous media. The domain size was 100 m x 5 m x 3.2 m, with 4 long lines. The flow velocity was set to 0.2 m/s, a typical current velocity in the Damariscotta River.

Near the surface, the flow reduced by 30% and accelerated 25% near the bottom, as shown in FIGURE 1. Results also indicated strong turbulence and vertical mixing caused by flow interaction with the farm. The momentum sink is assessed by subtracting Reynolds Stress before and after the farm. Drag coefficients from directly simulated Reynolds Stress compared favorably with a parameterized Reynolds Stress, which will be used in future field observations. As shown in TABLE 1, the cross sectional-averaged drag coefficient nearly doubled due to the presence of the farm, which highlights the frictional impact of the farm on the local velocity field.

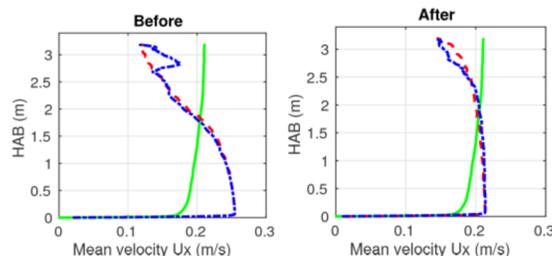


FIGURE 1 velocity distribution along the farm. Left panel: velocity in the middle of the farm. Right panel: velocity after the panel. Solid line: velocity distribution without farm; red dash line: velocity between long-line; blue dash line: velocity under long-line.

TABLE 1 Drag coefficients derived from directly simulated Reynolds Stress vs. parameterized Reynolds Stress.

Method	Simulated Reynolds Stress	Parameterized Reynolds Stress
$\Delta C_d$	0.0025	0.0027
Bottom $C_d$	0.0025 ~ 0.0035	
Farm $C_d$	0.0050 ~ 0.0062	

## FEEDING PREFERENCES OF THE CALANOID COPEPOD *Pseudodiaptomus hessei* UNDER CULTURE CONDITIONS

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In the natural environment, feeding of estuarine copepods generally involves a diel vertical migration. It has been proposed that copepods migrate upwards at night to feed, returning to deeper strata at dawn. However, some research has shown that copepods might also be feeding on benthic food source such as detritus and microalgae during the day. This study aims to improve our understanding of feeding biology of *Pseudodiaptomus hessei*, particularly whether *P. hessei* it prefers feeding on suspended phytoplankton or benthic microalgae. Copepods were allowed to fed on two species of microalgae (*Isochrysis galbana* and *Tetraselmis suecica*), one presented as benthic and the other as a suspended food source. The choice of algae and presentation state were also swopped. The amount of each algae species in the gut of *P. hessei* was quantified using real time PCR. Food source consumption was significant different ( $p < 0.026$ ) between benthic and planktonic food source suggesting that *P. hessei* prefers to feed on planktonic food source. After 80 min, *P. hessei* then shift from planktonic to a benthic food source. The results from this study confirm the assumption from previous ecology research that *P. hessei* might be feeding on a benthic food source during the day.

## **LARVAplus: AN INTEGRATIVE RESEARCH NETWORK FOR PROMOTING FISH LARVICULTURE IN IBERO-AMERICA**

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LARVAplus is a research and technical network funded by the “Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo” (Programa CYTED) that aims to generate a place for the exchange of knowledge and experience for the benefit of the development of the Ibero-American aquaculture industry. In particular, the network is focused in the production of fish larvae and fingerlings, improving the scientific and technological competitiveness of the sector, promoting the sustainable and responsible growth of the industry, as well as a transversal transfer of knowledge, skills and abilities between the research centers and the industrial sector.

In this context, LARVAplus involves different actors - universities, public research institutes, professionals and hatchery technicians and managers- in the study of the biology and production of fish larvae and fingerlings. Through a multilateral and multidisciplinary framework, LARVAplus will bring new data and approaches to be applied on broodstock management, fish larvae production and quality, water management, as well as larval rearing protocols and feeding strategies to 31 different species (12 freshwater and 19 marine) in order to consolidate and promote Ibero-American aquaculture diversification.

In order to achieve the above-mentioned goals, LARVAplus will organize during its four years of duration (2017-2020): i) different meetings among members of the network, with invited renown speakers on fish larval rearing and broodstock management; ii) funding short research and training missions between members of the network; and iii) organizing open training courses to the industry and scientific community.

The consortium is formed by 15 research institutions (Universities and R+D+T institutes) and 10 private companies from the Ibero-American geography (Spain, Portugal, Peru, Argentina, Chile, Mexico, Colombia, Costa Rica and Brazil).

## UTILIZATION OF CORN PROTEIN WITH YEAST AS A PROTEIN SOURCE IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Two growth trials were conducted to evaluate the use of corn protein with yeast (CPY, NexPro, Flint Hills Resources, Wichita, KS) a novel co-product of the dry grind ethanol process, in practical shrimp feed formulations. In trial 1, four experimental diets were formulated with increasing levels of CPY (0, 10, 20, and 30%). Juvenile shrimp 1.24 g were stocked into 16 tanks (650L) with 30 shrimp/tank. In trial 2, five diets were formulated with inclusions of 0, 6, 12, 18, and 24% CPY. Juvenile shrimp 0.25g were stocked 10 per tank in 80 L tanks with four replicates per treatment. In both trials, CPY replaced a combination of fish meal and soybean meal using corn starch as a filler to produce iso-nitrogenous and iso-lipidic diets. Treatment diets were fed four times per day for 6 weeks. At the end of each experiment, shrimp were counted and group weighed. In trial 1, significant depressed growth of shrimp was observed when 20 and 30% CPY were incorporated in the diets. In trial 2, the treatment containing 6% CPY exhibited improved growth performance, however was only significant compared to the 24% CPY diet ( $P < 0.05$ ). Based on current results an upper limit of 18% of the diet should be recommended in shrimp feed formulation. The reduced performance at high levels of inclusion could be due to a number of factors which should be investigated. Results in the present study demonstrated that CPY is a good protein source and can be applied as a protein source in practical shrimp feeds.

Table 1 Growth performance of juvenile Pacific white shrimp (0.25g) offered diets with different corn protein concentrate with yeast levels in the trial 2 for six weeks.

Diets	Final mean Weight (g)	Final biomass (g)	Weight gain (%)	Survival (%)	FCR <sup>1</sup>
Basal	5.07ab	41.9	1837.7ab	82.5	1.81b
CPY6	5.37a	46.8	2065.7a	87.5	1.67b
CPY12	5.13a	46.2	1854.2ab	90.0	1.74b
CPY18	4.60ab	41.5	1776.2ab	90.0	1.94ab
CPY24	4.29b	37.6	1593.5b	87.5	2.14a
PSE <sup>4</sup>	0.0913	1.095	42.46	1.854	0.0355
P value	0.0054	0.0542	0.0213	0.6153	0.0025

## RED PORGY AQUACULTURE: WHAT HAVE WE LEARNED, WHERE DO WE GO FROM HERE

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Red porgy *Pagrus pagrus* is commercially cultured in Europe, throughout the Mediterranean, and increasingly in Asia where it is highly regarded as a valuable marine reef fish and seafood delicacy. The red porgy are congeners of the red sea bream *Pagrus major*, one of the most valuable and widely cultured marine finfish. With widely established markets and consumer preference for red colored reef fish (e.g. red snapper, vermilion snapper and various groupers) red porgy is an excellent candidate for marine aquaculture in the Greater Atlantic and Southeast region including the Gulf of Mexico. While red porgy is not commercially cultured in the U.S., there is significant interest for land based and offshore aquaculture operations. We will review U.S. research and experience to date of red porgy aquaculture in terms of broodstock management, larval rearing, grow-out, nutrition and marketing potential. We will review the performance of red porgy culture in net pens globally and we will discuss opportunities for commercial development both land-based and offshore.

## EFFECT OF CRUDE OIL EXPOSURE ON REPRODUCTION IN CAPTIVE FLORIDA POMPANO BROODSTOCK

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A number of studies are being conducted to assess the long term impact of the 2010 *Deepwater Horizon* oil spill in the Gulf of Mexico (GOM). Research demonstrates that oiling caused a wide range of biological effects including those on GOM fisheries populations. While much of the research has focused on assessing the effects of the spill on larval fishes, certain aspects are still understudied, such as those examining the physiological response of adult marine fish species.

In the present study, selected reproductive parameters were examined in captive Florida pompano broodstock following aqueous exposure to south Louisiana crude oil. A mixture of oil and the dispersant (Corexit, 9500) was used to produce chemically-enhanced water accommodated fractions (CEWAFs) (0.0, 0.5 and 1.0 ppm [corresponding to control, low and high treatments, respectively]) administered over 12-hours in a spiked declining exposure study. This was followed by a period of non-exposure for 24 hours and then the process was repeated. Tagged fish, 5 females and 10 males per treatment, were transferred from the exposure tanks to a closed recirculating aquaculture system (total volume 28m<sup>3</sup>) where they were maintained for five months under simulated natural ( $27 \pm 1.0^{\circ}\text{C}$  and 13.5hr of light) photo-thermal conditions. Female and male reproduction condition was assessed periodically throughout the trial. Ovarian biopsies obtained from individual females were prepped as wet mounts and for histology. When possible, milt samples were collected; sperm density, sperm motility and spermatocrit were documented among individual males. Blood samples were collected and the plasma steroids Testosterone and 11-ketotestosterone were analyzed. Mature females were implanted with Ovaplant® (GnRHa) at a dosage of 50  $\mu\text{g kg}^{-1}$  bw and placed into individual spawning tanks along with two males from the same treatment group. Over the course of the study, spawns were obtained from fish in all treatment groups. Total egg production for each respective treatment group were as follows: 169,000 to 237,000 (control), 47,000 to 200,000 (low), and 58,000 to 162,000 (high). Results showed no significant differences in total expressible milt or sperm density between the experimental and control groups however, a significant difference in sperm motility was detected. Additional effects of oil exposure on reproduction and spawning performance in Florida pompano will be described.

## FROM THE IMPROVEMENT OF THE INSHORE FISH PRODUCTIONS TO THE AUTONOMOUS OFFSHORE FISH FARMING SHIPS

Rodrigo Sánchez Raccaro

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Former CEO, EcoSea Farming S.A.

This presentation will report the results obtained in an Innovation Center for production of 3,500 Tons of Atlantic salmon when integrating the technology of the copper alloy meshes in the inshore fish farming and the possibility these generated in the offshore fish production. This technology has also opened the real possibility of building offshore fish farming vessels, able to navigate in international seas looking for the best qualities of the water for the fish.

**STAGE 1:** The first step was to demonstrate that the copper alloy meshes generated real productive improvements in the fish farming. For this after having acquired 5 years of experience in this type of technology, we started to run an Innovation Center operated by ourselves, Integrating several new technologies, among them copper alloy meshes and we hire the international certifier SGS to record the results during the 18 months of production.

In this Innovation Center, we produced 3,500 tons of Atlantic salmon in the South on Chile and we used 16 cages of 13,500 m<sup>3</sup> each with copper alloy meshes. Results were excellent: Fish input (678,594) were smaller size (97grams) than Chilean industry standard (130 grams), despite this, the fish grew to 5.9 kilos. The Economic Conversion Rate (FCRe) was 1.3 and Biological Conversion Rate (FCRb) was 1.26 . Biological mortality rate of 3.3% and a productivity per Fish of 5.21 (Final biomass v/s Initial biomass) These results were among the best in the Industry over the 2014-2015 winter season (20 million fish).

**STAGE 2 :** Adaptation of technology for offshore fish farming. For this, we develop a submersible raft by means of a compensation bottle to make the sinking process. We chose an area where no fish had ever been produced in Chile, the shores of the Atacama Desert and a species that had never been produced in the country: *Seriola Lalandi* .

Although we had to fight against the big paradigms of the zone: fouling, oxygen lows, great swells and the low temperature for the species (15,2 °C average year), the result was very promising. We were surprised by 2 unexpected nature events: 2 large earthquakes (8.2 and 8.4 Richter) with their respective tsunamis, and de big swells of 2015. Cage and fish were submerged and survived without any problem.

**STAGE 3:** The great challenge, to integrate both experiences in the creation the technology of the future: Autonomous farming ships, efficient and without needing to be in territorial waters, which navigate searching the best conditions of the water so that the fish are always in the best possible state without the need for chemical or antibiotic treatments, this will make the animal welfare, the environment care, the profitability of fish farmers and the feeding needs of humanity, are all in line.

## WORLD AQUATIC VETERINARY MEDICAL ASSOCIATION PROGRAMS & SERVICES

A. David Scarfe\*

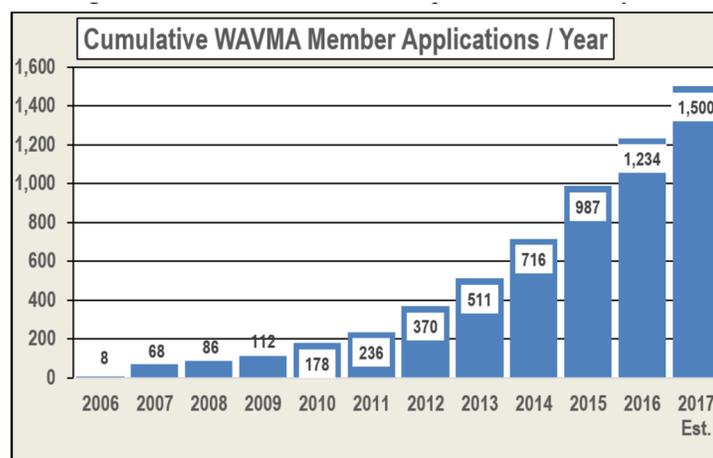
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In 2005/6, a small group of veterinarians came together to form the World Aquatic Veterinary Medical Association (WAVMA) as a global organization. Their intention was to address issues affecting aquatic veterinary medicine, and support aquatic veterinarians. It has since grown to include members (including veterinarians, veterinary students, veterinary technicians/nurses and para-veterinary professionals) in more than 57 countries, who work together to advance aquatic veterinary medicine and provide veterinary services to animal industries and owners around the world.

This increase in membership over the last decade reflects the number of member programs and services WAVMA has been able to develop. More details are available at [www.WAVMA.org](http://www.WAVMA.org). Important ones include:

- **Members-L**, and **Students-L** listservs for discussions on any topic of interest to members, but frequently focus on sharing clinical cases and client services;
- **The Aquatic Veterinarian**, a quarterly publication with news, peer-reviewed contributions, clinical cases, jobs, meetings, and more;
- A **Member Directory** tool to locate other members and for vet students to locate mentors;
- An **Employment Opportunities** webpage with available aquatic veterinary jobs;
- An **Image Library** containing images useful for presentations, diagnostic interpretations, and more;
- A **Video Library** with videos on numerous aquatic veterinary techniques and procedures;
- A **WebCEPD Program** with monthly live and recorded webinars, courses, and presentations that are also available for CEPD credit;
- A **CertAqV Program** a program for identifying and certifying veterinarians with competency in core subject matter necessary for practicing aquatic veterinary medicine;
- **The John L. Pitts Aquatic Veterinary Education Awards Program** that provides financial support for veterinary students and new graduates to increase their experience in aquatic veterinary medicine; and,
- **WAVMA Student Chapters** at veterinary schools and colleges around the world to assist veterinary students and faculty learn more about aquatic veterinary medicine.

To keep others updated and informed about new issues in aquatic veterinary medicine, members, animal owners and para-veterinary professionals can now subscribe to WAVMA's **Twitter** feeds and **Facebook** page, and the monthly **WAVMA e-News** which is distributed >1,200 subscribers.



## CHANNEL CATFISH *Ictalurus punctatus* SHOWED NO CROSS-TOLERANCE TO NITRITE AND HYPOXIA BUT MAY SHARE SOME COMMON REGULATION MECHANISMS TO THESE TWO STRESSORS

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Nitrite is a toxicant which can interfere with multiple physiological functions of fish highlighted with the accumulation of methemoglobin leading to tissue anoxia. Although usually do not cause mortality directly in practice, nitrite stress results in increased susceptibility of fish to pathogens or other stress and can cause large economic or academic loss especially in intensive farming systems and recirculating systems. In spite of its toxicity at high concentration, nitrite is a natural constituent with important biological functions in the body at low concentration. Nitrite could be a potential donor of nitric oxide and the latter is a key physiological messenger molecule induced under hypoxia for regulating hypoxic vasodilation and energetic metabolism. In the light of the inner correlations between nitrite and hypoxic regulations through nitric oxide and their similar anoxia consequence, here we sought to investigate the cross tolerance of channel catfish on nitrite and low dissolved oxygen. Channel catfish fingerlings were pre-treated with 0, 0.7, 1, 3.3, 4.7, 6ppm nitrite for 24h and then exposed to acute hypoxia (started at 0.5ppm and gradually decreased to 0), another group acclimated with sublethal hypoxia (1.5ppm) was also included. In another experiment, fish acclimated with sublethal hypoxia (1.5ppm) for 24h were subjected to acute nitrite challenge at a concentration of 26.4 ppm, a control group without acclimation was also included. Time of losing equilibrium for each fish was recorded. The results showed that neither the sublethal hypoxia acclimation provided cross protection to acute nitrite challenge nor the pre-treatment of nitrite improved acute hypoxia resistance at any of the tested nitrite levels based on the survival analysis. On the contrary, the susceptibility of fish to acute hypoxia increased when nitrite pre-treatment levels went up. While acclimation of sublethal hypoxia provided protection to fish when subjected to acute hypoxia. Interestingly, significant ( $p$ -value  $< 0.05$ ) or marginally significant ( $p$ -value  $< 0.06$ ) positive correlation between the survival time and body weight was observed on the low nitrite level groups (0.7ppm and 1ppm) and the sublethal hypoxia acclimated groups no matter subjected to acute nitrite or hypoxia challenge. While this correlation was not observed on the control groups and high nitrite level groups. This difference indicated that both the nitrite and hypoxia acclimation can provide protection that benefits larger fish, which is probably related to glycolysis, but this protection was counteracted by other effects of nitrite toxicity in addition to methemoglobinemia.