

Aquaculture America 2018



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Paris Las Vegas

Las Vegas, Nevada USA

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WELCOME

Welcome to Aquaculture America 2018! On behalf of the conference sponsors — the United States Aquaculture Society, the National Aquaculture Association (NAA) and the Aquaculture Suppliers Association (ASA), it is my pleasure to welcome you back to Las Vegas, NV. We hope you enjoy the conference, the trade show and all the excitement of Las Vegas!

This year's conference theme, *Shaping the Future – Telling Our Story*, touches a soft spot for me. Many of you may not know that I was a creative writing major in undergrad and many of you already know – I love a good story! A good story captures the heart and mind, connects people together and has the power to inspire. 'Aquaculture' is a great story and one in which we are all protagonists. Now is the time for all of us working in aquaculture to learn how to best share our unique stories of why we do what each of us does to secure the future of aquaculture. We must all be aquaculture storytellers. Steve Jobs said that “the most powerful person in the world is the storyteller. The storyteller sets the vision, values and agenda of an entire generation that is to come”. I believe that. I task all of us during this conference to start practicing telling a personal story about aquaculture and share it with at least one other person during these days together in Vegas. Then, share that story with everyone you can! Stories about the benefits of farm raised seafood, stories about a life's dedication to growing aquatic animals, stories about the successes of innovative aquaculture technology, stories about projects and their results, stories about people committed to taking action and leading change. Storytelling demands a mutual investment from both the teller and listener, brings energy to the message and results in action in the way we behave and respond. Abraham Lincoln said “In order to win a man to your cause, you must first reach his heart, the great high road to his reason.” With stories we have an opportunity to impact everyone who listens. We are all here at this conference to build on the story of aquaculture – celebrating yesterday, sharing today and shaping the future. If each one of us tells an aquaculture story every day – I know we will create the future of aquaculture each of us is hoping for.

As you walk the trade show, mingle in the hallways, listen to stories, and bid on items at the NAA auction (Wednesday night), please remember to thank the conference associate sponsors and all the folks that work and volunteer some long hours to prepare and organize this annual event.

Together, let's debunk the old saying “what happens in Vegas, stays in Vegas” and this time leave Vegas with stories of aquaculture to share with and inspire the world!

Cheers,

A handwritten signature in black ink, appearing to read 'Kathleen Hartman', with a stylized flourish at the end.

Kathleen Hartman, Steering Committee Chair
USAS Past President and WAS Director

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Aquaculture America 2018



ABSTRACTS

FISH VACCINATION – FACTORS TO CONSIDER

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Aquaculture industry is seriously impacted by several diseases. Although vaccination can minimize mortality due to some of these diseases, the effectiveness of a vaccine is dependent on the cross protection offered against multiple isolates. For example, in the case of Enteric Septicemia of Catfish (ESC), caused by the bacteria, *E. ictaluri*, traditionally, losses have been controlled by withholding feed from fish to reduce the oral route of infection combined with medicated feeds. Recently, a live attenuated ESC vaccine is delivered orally to catfish. Under laboratory conditions, the vaccine was shown effective against the parental wild-type strain and proved to be safe at 10 times the applied target dose. While live vaccines are very effective in providing long lasting immunity against disease, vaccine safety and efficacy could be compromised, if delivered to animals in suboptimal health or under stressful conditions. In a compromised animal, attenuated vaccine can cause infections leading to morbidity and mortality. Another key factor in field vaccinology is antigenic variation among pathogenic species, where immunization with a vaccine derived from one strain does not provide protection against genetic variants of the same species. Vaccinated and control fish were challenged with the wild type *E. ictaluri* isolate 30 days post-vaccination. Low oxygen stress did not induce any post-vaccination mortality in any of the vaccinated treatments. Similarly, all groups of vaccinated fish, regardless of stress treatment, were protected against virulent *E. ictaluri* infection. Data indicated that acute oxygen deprivation, before or after vaccination, does not alter vaccine safety and efficacy, however the effects of chronic long term stress have not been evaluated. Therefore, short acute stressors are unlikely to influence vaccine safety and efficacy and provides valuable insight in developing commercial vaccination protocols. Additional trials were conducted to determine if the attenuated isolate afforded protection against 23 archived field isolates collected over a time span of twenty years (1997-2016). Vaccination followed by bacterial challenge with archived isolates were conducted over a three year period. In all trials, vaccination was shown to protect catfish against all challenge isolates, regardless of host species, geographic region (state and farm location) or isolation year. While on farm vaccination greatly improved survival, yield and fish net-value, limited mortality was observed in vaccinated pond populations. Results indicated that mortality observed in farm vaccinated fish populations was not related to antigenic variations among isolates. The most likely cause of on-farm mortality was related to unequal distribution of vaccine laden feed to individual fish, an inherent problem with mass delivery of oral vaccines to large populations of animals. In order to differentiate between the 23 isolates, their clonal relation were determined. The PCR profile indicated relative homogeneity among the isolates dating back to 1997. This further confirmed the results which indicated no significant difference between the isolates. The clonal nature of *E. ictaluri* isolates demonstrated by our data negates the need to develop multivalent vaccines or construct new vaccines to account for antigenic variation occurring over time. Commercial vaccination trials are showing net economic benefit of \$2000 to \$3000/acre for channels and hybrid fingerling production phase.

EFFECTS OF DIETARY LIPID SOURCES ON COLD TOLERANCE OF NILE TILAPIA (*Oreochromis niloticus*)

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Nile tilapia (*Oreochromis niloticus*) is the most important farmed species in Egypt. The main constraint of tilapia farming in Egypt is their sensitivity to low temperatures in winter. Mass mortalities of tilapia have been recorded as a result of water temperature drop in some subtropical regions, leading to great economic loss to the farmers and the national economy as a whole. In the meantime, it has been reported that ability of fish to adapt to different temperatures is highly related to the fatty acids composition in their muscles. Fatty acids composition of the body is in turn influenced by the fish feed composition, particularly dietary lipid. Therefore, the main objective of this work was to assess the effects of different sources of dietary lipids to enhance the ability of Nile tilapia (*Oreochromis niloticus*) to tolerate cold.

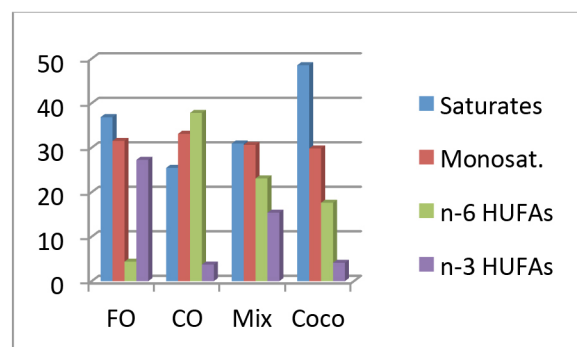
Four diets were formulated with different types of lipids included at 4%. Coconut oil (COCO), corn oil (CO), fish oil (FO), and finally blend of fish oil and corn oil (equal ratios). The fish were fed the test diets, twice a day, for 61 days. There were two phases: Phase I, was a feeding phase with the tested diets at an appropriate temperature (25 °C), and phase II, evaluated the contributions of these diets to fish cold tolerance when exposed to low-temperature stress.

The results showed that vegetable oil-based diets (corn oil and blend oils) resulted in best cold tolerance for Nile tilapia (table 1). At the same time, fish fed on fish oil-based diets (fish oil diet and blend oil diet) had the best growth performance and feed utilization ratios. During cold shock (figure 1), all saturated fatty acids (SFAs) proportion of all treatments showed an obvious decrease. UFAs tend to increase at cold shock.

Table (1): LD₅₀ and mean temperature at death (TAD) of Nile tilapia (*O. niloticus*) fed on test diets

Oil source	LD ₅₀	Temperature (°C) at death (TAD)	
		Mean	Range
Fish oil	10.00±0.0 ^b	9.50±0.0 ^b	12-7
Corn oil	8.50±0.5 ^d	8.00±0.0 ^c	10-6
blend oils	9.00±0.25 ^c	8.25±0.25 ^c	10-7
Coconut	15.00±0.0 ^a	12.00±0.0 ^a	15-9

Figure (1): Fatty acid composition (% of total fatty acid) of whole fish body fed on tested diets



ASSESSMENT OF HEPATIC GENE EXPRESSION BETWEEN HYBRID STRIPED BASS EXHIBITING EXTREMES IN GROWTH PERFORMANCE

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Hybrid striped bass is a major aquaculture species in the United States. Artificial breeding of this species can introduce large variation in growth performance during grow-out to market size. To assess the genetic and nutrigenomic basis behind growth variability in these hybrids, fingerlings from 47 families were size-matched and communally grown in earthen ponds for 115 days. Afterward, length and weights were taken for all individuals ($n=5072$) for heritability estimation. Families were then ranked by weight gain and individuals from the three fastest growing (mean 240.8 ± 9.75 g; 242.0 ± 11.52 mm) and three slowest growing families (mean 153.5 ± 52.38 g; 223.3 ± 21.31 mm) were collected for liver RNA sequencing.

As expected, growth characteristics in hybrid striped bass were estimated to be highly heritable ($p < 0.0001$). Through differential gene expression analysis we identified 86 genes that were responsive between groups including 40 up-regulated (fold-change 1.89 to 7.66) and 46 down-regulated (fold-change -1.71 to -4.59) genes in the largest fish.

Two somatic growth-related genes were identified from this list including a growth factor receptor gene and a gene encoding an insulin-like growth factor binding protein that may directly explain some of the genetic variation between families. Several additional genes involved in metabolic pathways such as glycolysis/gluconeogenesis and lipid biosynthesis were also revealed. The candidate gene list may also provide some evidence that both physiological and behavioral factors may be influencing growth differences in communally reared fish. Genetic information revealed through the characterization of growth traits is expected to assist in improvement of breeding practices and selection efforts of the parental white bass and hybrid bass species. Further, understanding these functional characteristics linked to growth variability among bass in common culture systems may help identify solutions to issues affecting more intensive production.

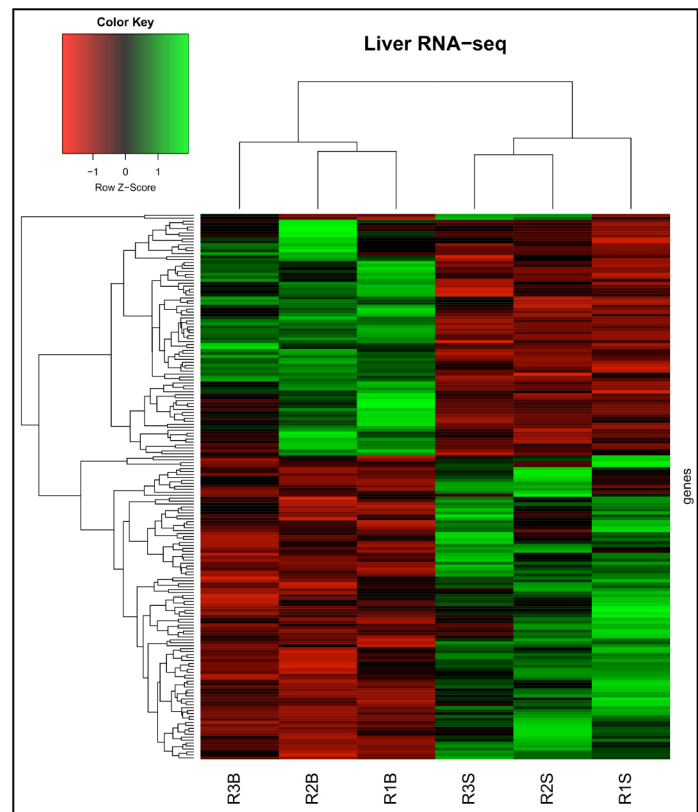


Figure 1. Heatmap of significant differentially expressed genes between largest ("B" sample pools) and smallest ("S" sample pools) hybrid striped bass.

DIETARY ESSENTIAL AMINO ACID REQUIREMENT OF DUSKY KOB *Argyrosomus japonicus*

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Protein is the most expensive dietary component and is central to growth. An understanding of the dietary essential amino acid (EAA) requirements of a species is important for developing cost effective feeds. Based on lysine requirement it may be possible to estimate other EAAs by modelling with fish body EAAs composition, such estimation has been carried out in striped bass, collorioja, red sea bream, Japanese flounder and bluegill. Dusky kob is an economically important fish in South Africa and in other parts of the world. However, the information on the dietary essential amino acid requirements of this species is not available. Therefore, this study aimed to estimate the dietary essential amino acid requirement for dusky kob by using ideal protein concept.

Eighty seven juvenile dusky kob *Argyrosomus japonicus* with an average weight of 1.2±0.1 g were collected from a commercial fish farm, euthanized, placed in crushed iced and transported to the laboratory for amino acid analysis.

Ratio of essential amino acids (A/E) ratios were calculated as follows:

$$\left\{ \frac{\text{individual EAA content in the whole body}}{\text{total EAA content including cysteine and tryrosine}} \times 1000 \right\}$$

The ratio of EAA was then used to calculate the dietary requirements of amino acids based on the dietary lysine requirement

$$\left\{ \frac{\text{requirement for lysine} \times \text{specific A/E ratio}}{\text{A/E ratio for lysine}} \right\}$$

The mean whole body amino acid level of juvenile dusky kob ranged from 0.2% (cysteine) to 16.1% (glutamic acid). These data, together with a dietary lysine level of 7.3% protein were used to estimate the dietary requirements of the other essential amino acids. The dietary requirements of these amino acids ranged from 2.2% for histidine to 7.1% for leucine (Table 1).

Table 1. Dietary quantitative essential amino acid (EAA) requirement of *dusky kob*.

EAA	A/E ratio	Dietary essential amino acid profile (% dietary protein)
Histidine	49.0	2.2
Arginine	89.8	4.0
Threonine	100.0	4.4
Lysine	165.3	7.3
Methionine	67.3	3.0
Valine	110.2	4.9
Isoleucine	89.8	4.0
Leucine	159.2	7.1
Phenylalanine	89.8	4.0

EFFECTS OF DIETARY ISOFLAVONE-GENISTEIN ON THE HAEMATOLOGY AND IMMUNOLOGICAL RESPONSES IN JUVENILE *Heterobranchus longifilis* CATFISH CHALLENGED WITH *Cytophaga columnaris*

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We investigated the dietary effects of isoflavone-genistein on hematological and immunological parameters in juvenile *Heterobranchus longifilis* catfish experimentally challenged with *Cytophaga columnaris* in a 56 days feeding period.

Five isonitrogenous (42.1% crude protein) and isoenergetic (19.5 kJ. g⁻¹) diets were formulated to contain four graded levels of isoflavone-genistein, namely 0.0, 0.2, 0.4, 0.8 and 1.6 g kg⁻¹ diet. Fish (initial average weight: 150.5 ± 1.5 kg) were stocked in tanks in groups of 30 and fed the experimental diets in triplicate. At set time intervals (days 0, 7, 21, 42 and day 56) four fish were randomly sampled from each tank for haematological and immunological assays (total erythrocytes counts, total leucocytes counts, haematocrit, haemoglobin concentration, erythrocytic indices, differential leucocytes counts, serum lysozyme activity, total immunoglobulin (IgM) and complement activities).

Results shows that mean corpuscular hemoglobin concentration (MCHC) and values of neutrophils were significantly different between treatments. The activities of serum lysozyme, IgM, C3 and C4 were significantly (P < 0.05) influenced by the dietary isoflavone-genistein concentrations. Results indicated that isoflavone-genistein had significant positive effects on some haematological and immunological parameters in juvenile *H. longifilis* catfish experimentally challenges with *Cytophaga collumnaris*.

EFFECTS OF DIETARY TURMERIC *Curcuma longa* ON GROWTH PERFORMANCE AND NON-SPECIFIC IMMUNE RESPONSES IN THE CLARIID CATFISH *Heterobranchus longifilis*

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Study was conducted to investigate the effects of dietary Turmeric (*Curcuma longa*) on growth and non-specific immune responses of juvenile *Heterobranchus longifilis* catfish.

Fish were fed an experimental diet containing 0.0, 0.5, 1.0, 2.5 g. kg⁻¹ of *C. longa* twice daily for 56 days. Growth performance and non-specific immune parameters (respiratory burst activity, glutathione peroxidase (GPX), phagocytic activities, superoxide dismutase (SOD) activity, lysozyme activity and differential leucocytes counts were evaluated at days 0, 7, 14, 28 and day 56 respectively.

Dietary turmeric produced significant ($P < 0.05$) increase in growth performance compared with control diets; furthermore, dietary inclusions of *C. longa* at 0.5 g.kg⁻¹ produced significant ($P < 0.05$) increases in the leucocytes counts (neutrocytosis and lymphocytosis) and significant decrease ($P < 0.05$) in SOD and GPX activity. Serum lysozyme and phagocytic activity of the leucocytes were significantly increased ($P < 0.05$).

Dietary *C. longa* at 0.5 g. kg⁻¹ in juvenile *H. longifilis* promoted growth performance and enhanced the non-specific immune responses.

EFFECT OF DIFFERENT FEEDING STRATEGIES ON GROWTH PERFORMANCE AND ECONOMIC RETURNS OF NILE TILAPIA (*O. niloticus*) PRODUCTION IN SEMI-INTENSIVE SYSTEM

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The production of Nile tilapia in semi-intensive system has been practiced for many years especially in Africa due to Nile Tilapia's ability to utilize natural food produced through pond fertilization. The objective was to find out if tilapia fed at half ration and on alternate days had comparable performance, feed utilization and economic returns to those fed at full ration over 15-week period in fertilized ponds. .

Sex-reversed all-male *O. niloticus* (~35g) were cultured in fertilized ponds at Tano-Odumasi of the Ashanti Region, Ghana. Seven hundred fish were stocked into each pond (350 m²) with each treatment replicated three times. The fish were fed with commercial feed (30% crude protein) at 5% body weight initially and 2% towards the end of the experiment. Fish growth, feed conversion efficiency and economic returns were considered under the three strategies. Pond water quality variables measured were found to be within suitable range for optimum growth of Nile tilapia.

Results of the study showed no significant differences ($P < 0.05$) in growth performance variables in terms of weight gain (WG), specific growth rate (SGR), daily growth rate (DGR) among the treatments. Higher growth of fish observed in the control group were not significantly different ($P < 0.05$) compared to the other treatments. Feed given varied significantly among the feeding strategy and feed conversion ratio was significantly better ($P < 0.05$) in fish group fed on alternate day (0.86) and half ration (0.94) compared to the control group (1.25). Economic analysis showed that profit returns were highest for the alternate day feeding strategy (GH¢ 2,269.41) and least for the full ration feeding strategy (GH¢ 995.88). It is possible to achieve similar fish yield in Nile tilapia semi-intensive production with the reduced feeding strategies and full ration feeding. Alternate day full ration feeding strategy is recommended to tilapia farmers since it proved to be the most profitable strategy.

EVALUATION OF PROCESSED OILSEED MEALS IN THE DIET OF NILE TILAPIA (*Oreochromis niloticus*)

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Despite the fact that most plant ingredients (oilseed meals) are readily available at lower costs than fish meal, their use within aquafeeds is usually restricted by relatively low protein content, unbalanced essential amino acid profile, high levels of fibre and starch, especially non-soluble carbohydrates) and the presence of one or more endogenous antinutritional factors (ANFs). To improve the nutritive value of plant products, ingredients have been modified by chemical, mechanical and biological methods to remove antinutrients and/or fractions of low nutritive value which finally results in fairly good or high-protein plant products. This study evaluated the effect of processing of some oilseed meals (OSMs) namely; soybean meal (SBM), cottonseed meal (CSM) and groundnut meal (GNM) on their nutritional value and also on growth performance and cost-effectiveness when OSM-based diets were fed to Nile tilapia (*Oreochromis niloticus*).

These OSMs were detoxified by heat processing (autoclaving) and/or addition of phytase and ferrous sulphate to reduce levels of important ANFs in them. Seven isonitrogenous (320g.kg^{-1}) and isoenergetic (19.0kJ.g^{-1}) diets were formulated using equal mixtures (Q) of the OSMs as protein sources. The control diet was prepared with fish meal (FM) as source of protein while FM was substituted with 50% Q of the meals in the plant-based diets.

The results indicated that heat processing of the OSMs was very effective in reducing the trypsin inhibitors in SBM, CSM and GNM by 78.6%, 75.2% and 78.0% respectively but not the other ANFs and proximate composition (Table 1). Based on the growth performance and nutrient utilization Diet 2 (50% Q of processed OSMs) and Diet 5 (50% Q of unprocessed OSMs with phytase and ferrous sulphate supplementation) were better than the control diet. However, in terms of cost-effectiveness only Diet 2 was superior to the control diet, meaning that simple heat processing alone could improve their utilization and cost-effectiveness (Table 2).

TABLE 1. Proximate composition (g.kg^{-1} as-fed), energy (kJ.g^{-1}) and ANFs (g.kg^{-1}) of heat processed and unprocessed test ingredients used in this study

	SB	PSB	CS	PCS	GN	PGN
Dry matter	894	953	901	961	924	969
Crude protein	500	511	441	425	431	433
Crude lipid	10	9	32	26	219	215
Ash	59	61	77	77	44	44
Gross energy	20.2	19.6	19.6	19.3	23.2	22.9
Phytic acid	17.5	17.2	31.6	28.7	14.9	14.9
Gossypol	-	-	5.60	5.25	-	-
Saponin	5.8	5.0	6.5	6.3	8.0	8.9
Trypsin inhibitors	14.2	3.0	1.2	0.3	2.3	0.5
% reduction of TI	-	78.6	-	75.2	-	78.0

PSB=Heat processed soybean meal, PCS=Heat processed cottonseed meal, PGN=Heat processed groundnut meal, SB=Unprocessed soybean meal, CS=Unprocessed cottonseed meal, GN=Unprocessed groundnut meal

TABLE 2. Growth performance and feed utilization of Nile tilapia fingerlings fed seven processed oilseed meal based diets. SGR=Specific growth rate, FCR=feed conversion ratio. Means within the same row with different letters are significantly different ($P < 0.05$).

	Diets						
	1	2	3	4	5	6	7
Initial Weight, g	2.17 ± 0.20	2.18 ± 0.26	2.01 ± 0.22	2.03 ± 0.20	2.09 ± 0.13	2.12 ± 0.14	2.09 ± 0.33
Final Weight, g	25.09 ± 0.74 ^a	22.93 ± 0.20 ^{ab}	20.27 ± 1.52 ^c	20.78 ± 1.30 ^{bc}	22.89 ± 1.04 ^{abc}	21.92 ± 0.22 ^{bc}	21.85 ± 0.82 ^{bc}
Weight Gain, %	1062.85 ± 139.50	962.21 ± 130.36	915.30 ± 126.20	928.99 ± 122.68	998.13 ± 114.03	937.30 ± 78.60	1001.88 ± 153.06
SGR, % day ⁻¹	4.37 ± 0.21	4.21 ± 0.22	4.13 ± 0.22	4.15 ± 0.21	4.27 ± 0.19	4.17 ± 0.14	4.27 ± 0.25
FCR	1.13 ± 0.04 ^a	1.23 ± 0.02 ^{ab}	1.40 ± 0.12 ^b	1.37 ± 0.10 ^b	1.24 ± 0.07 ^{ab}	1.29 ± 0.03 ^b	1.29 ± 0.04 ^b
Survival, %	100.00 ± 0.00	100.00 ± 0.00	98.33 ± 2.89	98.33 ± 2.89	100.00 ± 0.00	98.33 ± 2.89	100.00 ± 0.00
Profit Index	3.16	3.52	0.14	2.38	0.15	0.15	0.15

EFFECT OF SALINITY ON THE SURVIVAL OF PANGASIOUS CATFISH *Pangasius hypopthalmus* IN SOUTHERN BANGLADESH

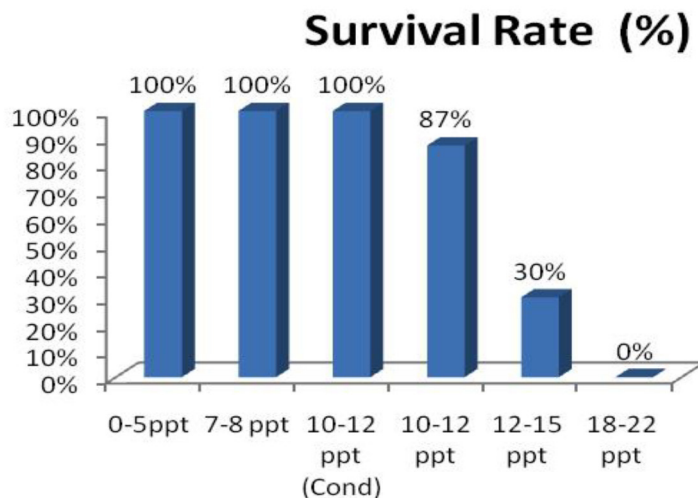
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The river catfish (*Pangasius hypopthalmus*) was introduced to Bangladesh in the 1990's from Thailand, and has since become a thriving aquaculture industry with over 3 million tonnes produced annually. *Pangasius* is typically cultured in freshwater, however with the coastal waters of Southern Bangladesh being affected by salinity encroachment, we sought to determine whether catfish could be cultured in these hyposaline waters. This region is severely impacted by overfishing, and is underutilized due to increasing susceptibility to rising sea levels linked to global climate change. Thus, the aim of the present study was to assess the effect of salinity on growth and survival of *Pangasius* and in turn, determine the optimum tolerable salinity for culture of this species.

Overwintered *Pangasius* fingerlings were stocked at a density of 2/m² in 18 ponds (averaging 400 m² and 1.3 m deep) within the coastal Patuakhali district of Bangladesh. The experiment consisted of six treatments spanning a range of salinities: T₁ (0-5 ppt), T₂ (5-7 ppt), and T₄ (10-12 ppt) did not involve prior conditioning while T₃ (10-12 ppt), T₅ (12-18 ppt), and T₆ (18-22 ppt) had the fish acclimatized in 6 ppt water for 24 h prior to the start of the experiment. The fish were held at the experimental salinities for 7 days, during which time they were fed twice daily (09:00 and 14:00 h) with commercial feed (Mega floating feed, 28% CP, 7% fat) at a rate of 10% body weight per day. Survival rates were calculated for each treatment at the end of the 7-day period.

In T₁ (0–5 ppt) and T₂ (7–8 ppt), the survival rate was 100%. For T₃ (10–12 ppt), in which the fingerlings were first acclimatized at 6 ppt, the survival rate was also 100% however in T₄, where fingerlings were stocked directly at 10–12 ppt, survival rate was 87% with mortalities occurring as early as 5 days. For fish acclimated to 12–15 ppt (T₅), survival rate was 30% while at the highest salinity (T₆), all fingerlings died within one day. The results indicate that *Pangasius* catfish are able to survive in waters of up to 12 ppt when allowed a prior acclimation period in more dilute waters (6 ppt). A subsequent 6-month pond study evaluating growth performance of *Pangasius* catfish in different salinities (0 ppt, 6.5 ppt and 10.8 ppt) was conducted. The survival rate, weight gain, SGR, feed conversion ratio (FCR), yield, and benefit cost ratio (BCR) were similar ($P > 0.05$) among the treatments. The results suggest that *Pangasius* catfish can be successfully cultured in salinities as high as 12 ppt. The ability to grow *Pangasius* in hyposaline waters of coastal Bangladesh or other regions can provide additional earnings and food sources for communities impacted by seawater encroachment linked to rising sea levels and climate change. (Supported by the AquaFish Innovation Lab – USAID)



STUDY OF SUPERFICIAL FUNGAL FLORA OF RAINBOW TROUT FARMS (*Oncorhynchus mykiss*) IN ALBORZ PROVINCE, IRAN

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Fungi are so important in aquatic animal health and it should be regarded as principle factor in hygienic conditions. Opportunistic fungi develop in aquatic and soil environments. Predisposing factors such as density, transporting, handling, water pollution, manual feeding and other stressors play role for fungal infections outbreak, especially in sensitive species like as rainbow trout. This fish is one of the popular species in Iran that is one of the major trout producers in the world. Because of that study of bacterial, fungal and parasitical flora in such facilities are highlighted.

Skins and gills samples of 150 rainbow trouts (healthy appearance) with 100.98 ± 5.48 g body weight from 15 facilities of Alborz province, in autumn and winter, 2016-2017 were collected. All the farms water resources were ground water (well).

Out of skin samples, 103 fungal isolates were identified that 92 of that are molds belong to 13 genera besides 11 yeast isolates and out of gill samples, 82 fungal isolates were identified that 70 of that are molds belong to 11 genera and others were yeast isolates. Fungal isolates in skin and gills are indicated in table 1 and 2, separately.

Fungi are present everywhere, and it is normal to be in water resources. Environmental pollutants (e.g. industrial, agricultural and municipal wastewaters) can provide productivity of water and entering the development of non- water molds. Diversity of fungal isolates is the indicator of poor water quality resulting low production, fungal infections increase as well as treatment and medication costs. Fungal spore-polluted food should not be neglected, as well.

Table 1: Rainbow trout skin fungal isolates and their frequencies

No.	Fungus	Number of isolates	Fungal percent
1	<i>Penicillium</i> spp.	11	10.68
2	<i>Paecilomyces</i> sp.	2	1.94
3	<i>Achromonium</i> sp.	3	2.91
4	<i>Aspergillus flavus</i>	9	8.74
5	<i>Aspergillus fumigatus</i>	5	4.86
6	<i>Aspergillus niger</i>	5	4.86
7	<i>Aspergillus clavatus</i>	1	0.97
8	<i>Aspergillus</i> spp.	4	3.88
9	<i>Fusarium</i> spp.	11	10.68
10	<i>Alternaria</i> spp.	10	9.71
11	<i>Saprolegnia</i> sp.	6	5.82
12	<i>Rhizopus</i> spp.	6	5.82
13	<i>Cladosporium</i> sp.	4	3.88
14	<i>Mucor</i> spp.	7	6.8
15	<i>Helminthosporium</i> sp.	3	2.91
16	<i>Rhodotorula</i> sp.	5	4.86
17	<i>Candida albicans</i>	4	3.88
18	Other yeasts	7	6.8
	Total	103	100

Table 2: Rainbow trout gill fungal isolates and their frequencies

No.	Fungus	Number of isolates	Fungal percent
1	<i>Penicillium</i> spp.	9	10.98
3	<i>Achromonium</i> sp.	4	4.88
4	<i>Aspergillus flavus</i>	7	8.53
5	<i>Aspergillus fumigatus</i>	5	6.1
6	<i>Aspergillus niger</i>	3	3.66
7	<i>Aspergillus</i> spp.	3	3.66
8	<i>Fusarium</i> spp.	7	8.53
9	<i>Alternaria</i> spp.	11	13.41
10	<i>Saprolegnia</i> sp.	5	6.1
11	<i>Rhizopus</i> sp.	4	4.88
12	<i>Cladosporium</i> sp.	5	6.1
13	<i>Mucor</i> spp.	4	4.88
14	<i>Helminthosporium</i> sp.	3	3.66
15	<i>Candida albicans</i>	5	6.1
16	Other yeasts	7	8.53
	Total	82	100

ACCUMULATION OF HEAVY METALS IN AN AQUAPONICS SYSTEM AND EFFECT ON ANTIBIOTIC RESISTANCE OF BACTERIA

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Aquaponics is the combined culture of fish (aquaculture) and plants (hydroponic) in a recirculating system. It is a promising aquaculture technology which can enhance global food production. Despite the optimistic view, potential hazards like heavy metal accumulation could be a complicating factor if they exceed the maximum contaminant level goal (MCLG) in the system water. In addition, several studies report that water with elevated levels of heavy metals (HMs) contributes to bacterial antibiotic resistance. An 81-day experiment was conducted in a controlled greenhouse environment for evaluation of the presence, diversity, distribution, and accumulation of heavy metals and antibiotic resistance of bacteria in the aquaponic system. Three heavy metals and one metalloid (Cd, Pb, Hg, and As) (HMs) were mixed and inoculated in the replicate treatments based on 60% of the maximum permissible standard value for HMs in potable water into a small-scale (NFT) aquaponic system. Three replicates and three-control treatments were each stocked with eight fingerling (weighing 25–50 g) Tilapia fish (*Oreochromis niloticus*) and 10 plants of Butterhead lettuce (*Lactuca sativa*). Weekly water samples were collected while fish and plant samples were collected on day (0), and on the last day of the experiment for determining the concentration level of HMs. Inductively coupled plasma mass spectrometry (ICP-MS) was performed for assessing accumulation of HMs in the samples, and cultural method (plating) conducted to evaluate the bacterial antibiotic resistance after the inoculation day. Water samples that were collected weekly were used also for evaluation of bacterial antibiotic resistance. At harvest, wet and dry weight of fish and plants conducted as well. Water sample results showed that As and Cd increased steadily with duration of the experiment, but neither exceeded the permissible levels at the last day of the experiment. Hg and Pd did not accumulate, and their concentration declined after the inoculation. Tissues samples revealed dissimilar results; fish samples showed no excess of HMs to the MCLG, but HMs were found in elevated concentrations. Comparing with the control treatment, As, Hg, and Pb in fish samples showed accumulation of HMs while the Cd did not. In plant samples, Hg and Cd concentrations were elevated. While As and Pb did not accumulate in the plant samples. Pd concentrations levels were less than the initial concentration. The results of bacterial antibiotic resistance were uncertain. However, bacteria from the system were able to grow in both antibiotics (Ampicillin and Tetracycline). While bacteria grew steadily in all weeks of ampicillin culture, only, last week of culture tests (week 5) revealed a resistance in the diluted tetracycline culture. In general, bacteria results showed more resistance to ampicillin than to tetracycline.

BROODSTOCK DEVELOPMENT, BREEDING AND SEED PRODUCTION OF GIANT FRESH WATER PRAWN *Macrobrachium rosenbergii* IN THE KINGDOM OF SAUDI ARABIA

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Among many high value tropical shell fish that could be farmed in Saudi Arabia, the giant fresh water prawn, *Macrobrachium rosenbergii* having immense potential due to its superior meat quality and high market demand. It is one of the fast growing crustacean species encountered rarely in the capture fisheries. The species is able to acclimatize and grow well at fresh water and brackish water conditions besides its huge potential as polyculture or an aquaponics candidate. Scarcity of brood stock and insufficient quality seeds of *M.rosenbergii* assumed to be the major gap felt for its lack of aquaculture interest in the local conditions of Saudi Arabia, even though few attempts were made to culture this species earlier but hardly any published report available. With series of research efforts successful broodstock development, breeding and seed production of Giant Fresh water prawn has been achieved for the first time in the Kingdom of Saudi Arabia at the Fish resources research center of King Faisal University. This presentation describes various steps followed during the brood stock development, breeding and larval rearing phases of the giant freshwater prawn carried out in the Aqua hatchery complex in the Fish resources Research center of King Faisal University. Based on our experience it confirms that, it is possible to develop good quality brood stock and post larvae of *Macrobrachium rosenbergii* locally therefore; culture of this valuable species could be possible in the vast inland farming areas of the Kingdom of Saudi Arabia provided with good water quality successively maintaining proper stocking density, sex ratio and balanced Nutrition.

MOLECULAR CHARACTERIZATION AND EXPRESSION ANALYSIS OF GLUTATHIONE PEROXIDASE 1 FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

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Glutathione peroxidases, major family of Reactive Oxygen Species (ROS) scavenging enzymes, are involved in immune responses against pathogens. In the present study, cDNA sequence of selenium-dependent glutathione peroxidase 1 (designated as *HaGP1*) gene was obtained from transcriptome database of seahorse *Hippocampus abdominalis*. The open reading frame (ORF) of *HaGP1* encodes for 188 amino acids, including TGA encoded selenocysteine at residue 37.

Multiple sequence alignment of *HaGP1* with selected vertebrate orthologs revealed that glutathione peroxidase 1 is highly conserved among vertebrates. In phylogenetic analysis, fish glutathione peroxidase 1 orthologs were clustered together while *HaGP1* had the closest relationship with ortholog from seahorse *H. comes*.

The tissue specific expression analysis revealed that *HaGP1* mRNA was ubiquitously expressed in all analyzed tissues, with the highest expression in skin. The *HaGP1* expression was significantly upregulated in kidney and liver tissues after immune challenge with lipopolysaccharides (LPS), Polyinosinic:polycytidylic (Poly I:C), *Edwardsiella tarda*, and *Streptococcus iniae*. The peak expression of *HaGP1* in liver post Poly I:C challenge was prominent at 48h with 58 folds of PBS injected control group. These results suggest that *HaGP1* may play a role in immunity against bacterial and viral diseases.

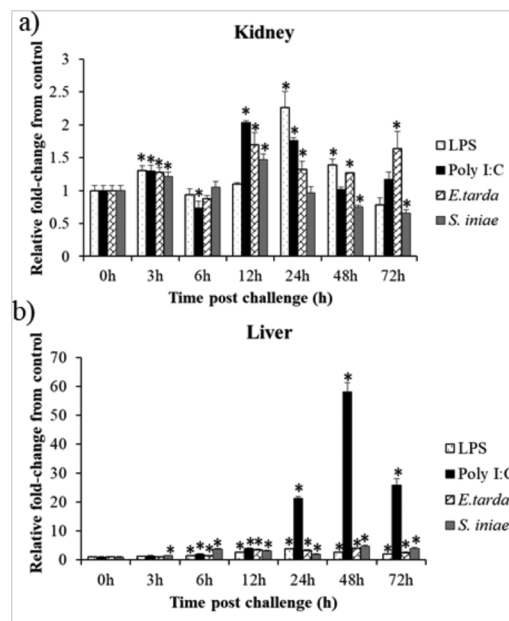


Fig 1. Expression analysis of *HaGP1* in a) kidney and b) liver post-challenge. Data are expressed as mean fold-induction ($n = 3$) relative to the PBS control \pm SD. (*) denotes expression significant from control. P value < 0.05 .

TILAPIA GROWTH PERFORMANCE USING PROTEASES AND CARBOHYDRASES

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Ten practical tilapia diets were formulated to contain 32% protein and 6% lipids. Six diets were formulated to contain low level of fiber (LF) and included free protease (FP), protected protease (PP), free carbohydrase (FC), protected carbohydrase (PC), and a mix of free protease and carbohydrase (MFPFC). Four diets were formulated to contain high fiber (HF) and included dried distillers grains as a source of fiber and reduced protein digestibility. Treatments included a basal diet and a basal diet supplemented with free protease (FP), free carbohydrase (FC), and a mix of free protease and free carbohydrase (MFPFC). The level in the diet of free protease (FP) and protected (PP) was 175 g per metric ton, the level of free carbohydrase (FC), protected carbohydrase (PC) and the mix of free protease and carbohydrase (MFPFC) was 125 g per metric ton. The diets were offered to juvenile tilapia (9.29 g initial mean weight) over a 10-week growth trial. Four replicate groups of 20 fish/aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, survival was near 100% and weight gain was around 1000%. In general, fish maintained on the high fiber diet performed slightly poorer than those on the lower fiber diet. Concerning enzyme supplements, there were no clear statistical differences among the treatments. When considering planned comparisons, there were some significant differences or possible trends in final weight and feed efficiency. Overall, there were no clear advantages detected to the protected enzymes.

Table 1. Response of juvenile (9.29 g mean weight) to a low fiber and high fiber diet with various supplements of enzymes

Diet	Treatment	Final Weight (g)	Weight Gain (%)	Survival (%)	FE	ANPR	ANER
1	LF	104.4	1014.9	100.00	80.2	35.79	26.73
2	LF-FP	105.6	1032.9	100.00	78.0	39.67	28.16
3	LF-PP	102.2	999.3	98.75	75.6	41.56	29.16
4	LF-FC	98.3	970.9	98.75	83.3	41.56	26.72
5	LF-PC	98.7	954.8	100.00	82.4	42.42	26.49
6	LF-MFPFC	102.2	997.4	98.75	82.4	40.45	26.52
PSE		2.599	29.91	0.8839	2.010	2.334	1.2865
P value		0.3170	0.4995	0.7006	0.0924	0.4357	0.6015
7	HF	95.42	927.8	100.00	85.0	39.82	26.63
8	HF-FP	96.31	935.6	100.00	84.0	40.12	22.10
9	HF-FC	95.46	934.6	100.00	80.7	38.11	23.48
10	HF-MFPFC	98.84	966.8	100.00	82.5	38.95	26.42
PSE		2.765	26.44		2.028	1.635	1.331
P value		0.7986	0.7335		0.4879	0.8204	0.0845

TILAPIA GROWTH PERFORMANCE USING CORN PROTEIN WITH YEAST

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The development of improved feedstuffs from the ethanol industry can be a source of protein in practical tilapia diets. A series of five diets were formulated using a low fishmeal basal diet containing 2% fishmeal 8% corn protein concentrate (CPC), and 40% soybean meal (SBM) as the primary protein sources. The CPC was incrementally reduced (8, 6, 4, 2, 0%) and replaced on an equal protein basis with corn protein with yeast (CPY: 0, 3.1, 6.3, 9.4 and 12.6%). The diets were offered to sex reversed juvenile tilapia (7.5 g initial mean weight) over a 9 week growth trial. Four replicate groups of 20 fish/aquaria were offered the test diets at near satiation levels. At the conclusion of the growth trial, there were no significant differences in any of the measured parameters and no indications of any trends in the data. Results demonstrate that the CPC and CPY are equivalent product and should be considered high quality protein sources for tilapia feed formulations.

Table 1. Ingredient composition of five experimental diets containing increasing levels of corn protein concentrate with yeast as a replacement for corn protein concentrate in practical diets for tilapia.

Ingredients (g/100g as is)	Basal	CPY3	CPY6	CPY9	CPY12
Menhaden fishmeal	2.00	2.00	2.00	2.00	2.00
Meat & bone meal2	6.00	6.00	6.00	6.00	6.00
Soybean meal	40.00	40.00	40.00	40.00	40.00
CPC	8.00	6.00	4.00	2.00	0.00
CP with Y	0.00	3.10	6.30	9.40	12.60
Menhaden fish oil	3.77	3.67	3.58	3.48	3.39
Lecithin	0.50	0.50	0.50	0.50	0.50
Corn Starch	7.13	6.13	5.02	4.02	2.91
Corn	29.00	29.00	29.00	29.00	29.00
Mineral premix	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.80	0.80	0.80	0.80	0.80
Choline chloride	0.20	0.20	0.20	0.20	0.20
Stay C 35% active	0.10	0.10	0.10	0.10	0.10
CaP-dibasic	2.00	2.00	2.00	2.00	2.00

Table 2. Growth performance of juvenile tilapia (7.49 ± 0.15 g initial weight) to diets containing increasing levels (0, 3.1, 6.3, 9.4, 12.6%) of corn protein with yeast over a 9-week period.

Diet	Final mean Weight (g)	Final biomass (g)	Weight gain (%)	Survival (%)	FCR
Basal	80.37	1446	964.9	90.00	1.23
CPY3	73.05	1405	879.6	96.25	1.30
CPY6	79.82	1436	952.7	90.00	1.24
CPY9	79.52	1447	953.9	91.25	1.23
CPY12	79.52	1447	936.4	92.50	1.24
PSE2	2.155	50.87	27.63	3.446	0.0294
P value	0.15	0.9694	0.2495	0.6913	0.5166

Values are means of four replicates.

FCR: Feed conversion ratio

PSE: Pool standard error

CERTIFICATION AND THE SIMPLISTIC INTERPRETATION OF SUSTAINABILITY

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As is the case for most global industries, aquaculture companies have embraced ‘sustainability’ as a goal, despite a lack of clear consensus as to what this actually entails. The ambiguity of the term leads to vague aims and objectives, with even vaguer strategies for accomplishing them. A quick review of mission statements and declarations of companies, organizations, and governments does, however, suggest broad agreement that sustainability has three aspects: environmental, economic and social. Unfortunately, these statements and declarations seldom probe the relationship between the three aspects, leaving the impression that they all pull in the same direction. It is, however, inherent in the three pillar approach to sustainability that trade-offs have to be made among the three.

One attempt to make sustainability more tangible is the use of certification schemes. The certification schemes that are used in aquaculture create third-party auditing procedures by which companies can be certified to have met specific standards, and on the whole seek to provide consistent and universal criteria that apply to industries across boundaries. As a private voluntary regulatory initiative, these schemes come in addition to national regulations, their existence largely being a response to the perception that national authorities lack the ability or will to regulate the industry effectively.

Despite being a clear step away from the abstract ‘goals’ and ‘ambitions’ that have dominated the sustainability discourse and the optimistic but vague labels adopted in company advertising, certification as a means to make a global industry sustainable does have its problems. The translation of the concept into specific standards is often done without a conscious discussion of what sustainability actually is, and what trade-offs among the three aspects have been made by the scheme. Collectively the certification effort has worked to foster a simplistic view of sustainability by reducing it to ‘sustainable production’ measured by a list of standalone indicators, and ignoring the complexity and interconnectedness of the different aspects.

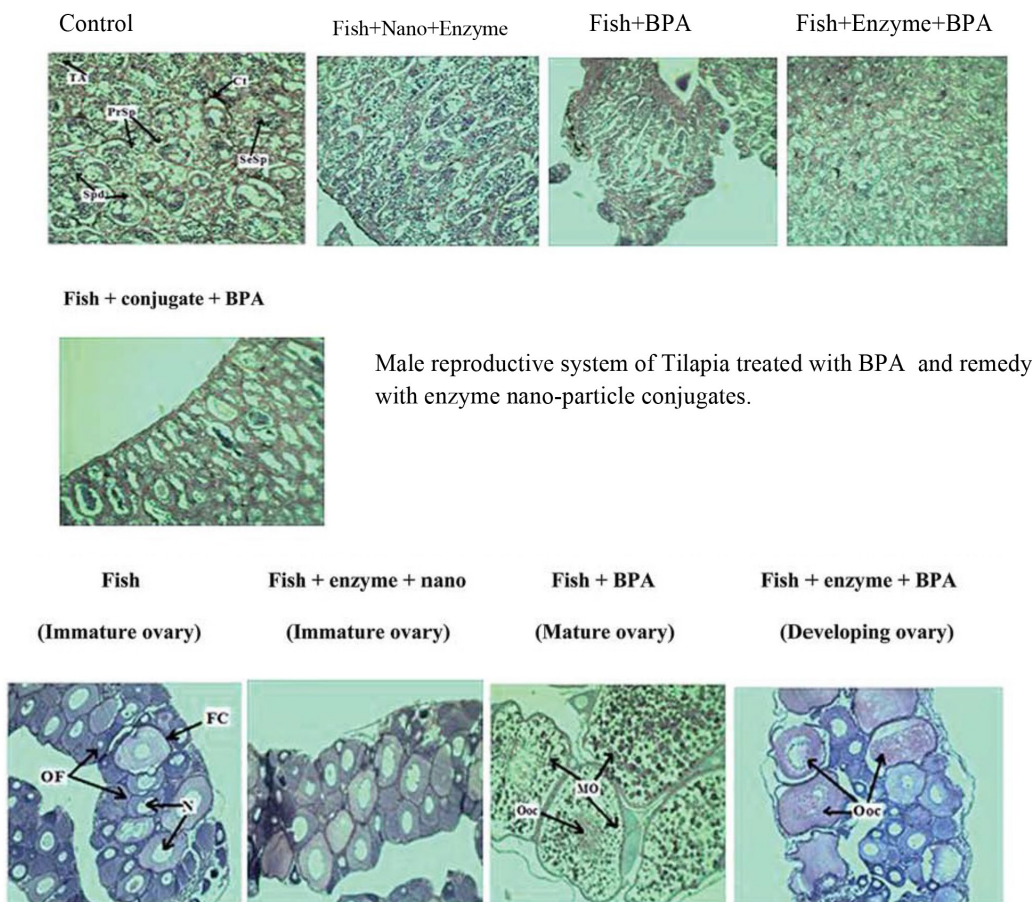
Through an in-depth study of five of the major certification schemes (ASC, GlobalGAP, GAA, BRC and IFS) and their standards relevant for salmon aquaculture, this paper demonstrates how these schemes serve as a representation of the current simplistic interpretation of sustainability. This is done by mapping the indicators that make up these standards in accordance with the three aspects, and seeing how this compares with the formulations of sustainability declarations from companies, NGOs and other global organizations. As this study shows, the conceptualization of sustainability has gone from multifaceted, to becoming ever more defined as an environmental concept, thereby obscuring the need for trade-offs. By ignoring its complexity, the schemes encourage and reinforce the idea that ‘a sustainable industry’ really signifies ‘an *environmentally* sustainable industry’. Through the proliferation of this simplistic understanding of sustainability, its full meaning as the balance of needs for all planet, profit and people is quickly forgotten.

LACCASE-SILICA NANOPARTICLE CONJUGATES CAN EFFICIENTLY REDUCE THE EARLY MATURATION RISK DUE TO BISPHENOL A (BPA) IN BOTH SEXES OF *Oreochromis mossambicus* AND ITS TOXIC LOAD FROM THE CONTAMINATED EFFLUENT

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Bisphenol-A (BPA) is a well-known endocrine disrupting chemical (EDC). Employing *Oreochromis mossambicus* (Tilapia) as a model organism, this study aimed to analyse the estrogenic modulatory effect of BPA and to prevent the early maturation of ovary by treating with conjugates of intracellular laccase and silica nanoparticles. Intracellular laccase was extracted from *Trametes versicolor* and purification of laccase was performed using fast protein liquid chromatography after running through ion exchange (DEAE-cellulose) followed by a gel filtration (P-6) column. Sol gel method was used to synthesize silica nanoparticles which were characterized by transmission electron microscopy, atomic force microscopy, dynamic light scattering, Fourier-transform infrared spectroscopy and X-ray diffraction. Silica nanoparticles were amino-functionalized and conjugated with intracellular laccase. Isothermal titration calorimetry showed that conjugation of intracellular laccase with silica nanoparticles was stable and the reaction was exothermic and spontaneous. Maximum effective concentration of BPA was found to be 100 ppm after short term exposure of *Oreochromis mossambicus* to BPA, at this concentration it leads to deleterious effects on liver (disruption of tissues), ovary (early maturation) and testis (antagonistic effect) which was more prominent in histopathological studies. Vitellogenin protein level and gene level expression analysis using FPLC and quantitative real time PCR showed that conjugates of laccase and silica nanoparticle were more effective in reducing the BPA concentration when compared to free laccase enzyme. Thus from the result it clear that conjugates of intracellular laccase and silica nanoparticle represents a promising tool to reduce the estrogenic effect of BPA by preventing the early maturation of ovary in *O. mossambicus*.



Fish + conjugate + BPA

Male reproductive system of *Tilapia* treated with BPA and remedy with enzyme nano-particle conjugates.

Female reproductive system of *Tilapia* treated with BPA and remedy with enzyme nano-particle conjugates.

THE POTENTIAL EFFECT OF AQUACULTURE ON THE GENETIC PURITY OF NATURAL POPULATIONS OF NILE TILAPIA *Oreochromis niloticus* IN GHANA

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Aquaculture is undoubtedly one of the avenues of ensuring food security in a growing Africa. However, the rapid expansion of aquaculture in the region, particularly in sub-Saharan Africa, could threaten the local adaptation and genetic diversity within wild populations. Hatchery operations and the sale of fingerlings are loosely regulated, which could result in the importation of alien strains deemed to have growth advantage over native strains. The purpose of this study was to obtain baseline information about the genetic impact of aquaculture on natural populations of the Nile tilapia *Oreochromis niloticus* in Ghana.

We employed a combination of methods to provide data. First, we interviewed local fishers and key informants to assess indigenous knowledge of sympatric tilapia species including *O. niloticus*, *Sarotherodon spp.*, and *Coptodon (Tilapia) zillii*, which naturally occur in major rivers in Ghana. We then surveyed selected farms and hatcheries in the Eastern and Western regions near two rivers: Volta and Tano, to obtain information about their sources of broodstock and fingerlings, and to collect *O. niloticus* samples for genetic analysis. Finally, we collected wild samples of *O. niloticus* from an upstream location “distal” to the aquaculture facilities, and from a downstream location “proximal” to the aquaculture facilities.

We found a high level of indigenous knowledge of sympatric tilapia species from our interviews with the local fishers and key informants. Of the 47 individuals interviewed, about 96% were familiar with *O. niloticus* and the common co-occurring tilapia species even though *O. niloticus* was sometimes confused with *Coptodon zillii* due to their morphological similarities. Interestingly, some fishers described *O. niloticus* as “fish farm tilapia” and all other tilapia species as “local tilapia”. In the Western region, locals reported an increasing number of *O. niloticus* in the catches from the Juen lagoon in recent times and attributed that to *O. niloticus* escape into the wild after a cage farm operated on the Tano River suffered structural damage. Regarding broodstock and fingerling sources, majority of the farmers reported growing the Akosombo strain of *O. niloticus*, which is the government approved strain, but some did not disclose their sources. A few farmers stated that they sometimes resorted to wild broodstock. We found morphological differences in terms of body shape and coloration between *O. niloticus* samples collected from farms and those collected from the wild. Additionally, *O. niloticus* samples were different when compared among cage farms than among pond farms. It is unclear whether the morphological differences observed are driven by environmental conditions or due to farmers growing different strains. Ongoing genetic analysis should provide answers about the genetic makeup of samples collected and help unravel this mystery. Pending genetic results, it is evident from the survey responses that an effective monitoring plan is required to safeguard *O. niloticus* genetic purity in Ghana.

AQUACULTURE GROWTH: WHERE, WHEN AND SOME THOUGHTS ON WHY

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There is often substantial focus on the fact that most of the world's aquaculture production takes place in eastern Asia, and in particular in China. While this is important, it also masks several other important patterns. Among the most important is aquaculture production during the last decades has increased substantially at all continents, and there are several non-Asian countries where production in recent decades has increased much faster than in China. Almost equally important is the fact that Asia in general and China in particular are much more important when production is measured by quantity rather than value. Hence, the economic impact of aquaculture is significantly higher in other parts of the world than what the production numbers increase.

In this paper, we compare growth patterns by quantity and value for important countries as well as the world's regions, as well as for different species and species groups. The analysis is highly instructive in showing that aquaculture is a truly global industry, and that it is likely to become even more so in the future, as several non-Asian countries have very high growth rates despite already having a significant production, but that they also were late starters. It is also of interest how several developed countries that were among the world's largest aquaculture producers in 1975 has been dropping on the list as they have not taken part in the blue revolution to any extent. The most notable is the United States, which were the 3rd largest aquaculture producer in 1975. In 2015, the US does not make the top ten list, despite a significant increase in production.

WATER CONSERVATION STRATEGIES FOR URBAN SALTWATER AQUACULTURE FACILITIES WITH A CASE STUDY REVIEW OF A (145) METRIC TON (MT) BRANZINO (*Dicentrarchus labrax*) FACILITY IN WATERBURY CONNECTICUT, USA

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Many saltwater fish species are demanding a premium price in local markets throughout North America. Couple this with increased consumer knowledge and the demand for locally grown traceable products, and saltwater urban aquaculture starts to trend toward a feasible reality. But how realistic is it to consider building and operating a saltwater system that relies completely on municipally supplied water, waste disposal, electricity and liquid oxygen?

Creating seawater from a municipal water source is expensive. It drives the process toward water conservation and reuse. The impact on the Recirculation Aquaculture System (RAS) process design is dramatic. This paper describes the design considerations and strategies that can be applied to conserve water and reduce operating costs in urban saltwater aquaculture systems. The strategies will cover aspects from the saltwater mixing systems, salt water storage and delivery, RAS design with denitrification and waste water recovery and purging systems. The intent is to provide understanding and insight into the costs and complexities of designing and operating an urban saltwater aquaculture facility.

Within the RAS design, special consideration needs to be paid to solids filtration. Current RAS technology can only dewater solids to a certain extent on system. When Nitrate-N is removed as the primary contaminant requiring water exchange, the dilute solids effluent flow is next in line as the primary water consumer within the RAS. Additional processes like foam fractionation, ozonation and radial flow settling can help to concentrate this effluent stream and reduce your water consumption. Modification to drum filter operation can also play an important role in increase the solids concentration of the effluent. However, even with these secondary and tertiary processes in place, additional off system dewatering technology is often justified from an economic perspective.

Additional consideration needs to be paid to the purge systems and the water exchange required for that process. Additional filtration can often be applied to offset the water flushing requirements on purge tanks. Serial reuse of water leaving the purge system by the growout system should also be considered when designing these facilities.

The results of these applied strategies will be reviewed with preliminary operating data from Great American Aquaculture, LLC., a 145 metric ton (MT) Branzino (*Dicentrarchus labrax*) production facility currently in production in Waterbury Connecticut, USA.

RELATIONSHIP OF MORPHOLOGICAL INDEXES AND WATER QUALITY IN WILD *Lutjanus colorado* FOR MATURITY IN CAPTIVITY

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In the north of the Sinaloa coast, the red snapper, *Lutjanus colorado*, is considered one of the most important fish species because of its commercial and nutritional value. 206 organisms were sampled during the four seasons (winter, spring, summer and autumn) of 2016, from the Sinaloa coast, the blood samples of 1.5 ml were extracted from each fish. The following biochemical parameters in blood were analyzed: The gastric repletion index (GI), gonadosomatic index (GI), hepatic index, and condition factor (K) were obtained. Water physicochemical parameters such as temperature (°C), salinity (ppm), dissolved oxygen (OD), hydrogen potential (pH), nitrites (NO₂), nitrates (NO₃), ammonium (NH₄) and phosphates (PO₄) were also determined. The values of blood samples from wild red snapper were correlated with fish size and season. Fish body weight and total length (LT) ranged from 81.3 to 2,040 g, and from 17.5 to 52.7 cm, respectively. The IRG (3.16 ± 0.25) and K (1.27 ± 0.01 %) indexes were higher in summer, whereas the higher GI and HI values were obtained in autumn, with averages of 0.11 ± 0.01 and 1.8 ± 0.12 %, respectively. For The environmental variables showed fluctuations during each of the seasons. The temperature, dissolved oxygen, salinity, pH, nitrites and nitrates showed significant difference ($P \leq 0.05$) during the seasons, ammonia and phosphates did not present significant difference ($P \geq 0.05$). The results contribute to better understand on the reproductive physiology of the red snapper from the wild, and the development of its culture in captivity.

Parameter	Winter	Spring	Summer	Autumn	Value-P
Temperature (°C)	24.2 ± 1.3 ab	26.4 ± 1.3 b	30.9 ± 0.4 c	20.2 ± 0.3 a	0.000049
Salinity (UPS)	38.7 ± 0.7 ab	37.5 ± 1.5 ab	35.5 ± 0.2 a	39.5 ± 0.2 b	0.032147
D. O. (mg/L)	8.15 ± 0.6 b	5.28 ± 0.8 a	5.10 ± 0.5 a	5.15 ± 0.1 a	0.012220
pH (UpH)	6.5 ± 0.02 c	7.1 ± 0.02 a	7.8 ± 0.05 b	7.6 ± 0.02 ab	0.000198
NO ₂ (mg/L)	1.04 ± 0.241 a	0.05 ± 0.004 b	0.55 ± 0.095 ab	0.70 ± 0.129 a	0.003422
NO ₃ (mg/L)	2.01 ± 0.76 b	12.67 ± 1.32 a	16.40 ± 0.45 a	14.25 ± 1.00 a	0.000001
NH ₄ (mg/L)	0.16 ± 0.079	0.05 ± 0.017	0.03 ± 0.004	0.03 ± 0.004	0.105264
PO ₄ (mg/L)	0.14 ± 0.07	0.28 ± 0.10	0.44 ± 0.07	0.25 ± 0.06	0.112515

NEW METHODS FOR THE DETECTION AND QUANTIFICATION OF *Hepatobacter penaei* (NHPB) BY PCR AND qPCR

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Necrotizing hepatopancreatitis (NHP) is a disease caused by a Gram negative bacteria recently classified as *Hepatobacter penaei* (NHPB). This pleomorphic intracellular alpha proteobacteria affects cultured penaeid shrimp in several countries from the western hemisphere including the USA, and most Central and South American countries that farm shrimp. NHP is a chronic disease that causes mortalities up to 95% in shrimp population in grow out ponds and broodstock ponds. The occurrence of NHP is related to specific environmental conditions such as high temperature and high salinity. NHP-B-infected shrimp display a typical soft shell, flaccid bodies, lethargy, reduced feed intake and empty midgut. Hepatopancreas (HP) lesions include intense intracellular hemocytic response, melanized HP tubules, sloughing off of HP tubule epithelial, marked atrophy of tubules, low lipid storage and intratubular edema.

NHP diagnosis and confirmation is carried out by histology, *in situ* hybridization, PCR and immunohistochemistry. The OIE recommended methods for NHPB detection are by PCR and qPCR based on the amplification of 16S rRNA gene. This highly conserved gene is a component of the 30S small subunit of a prokaryotic ribosome that has some critical role in protein synthesis. While the hypervariable region in 16S rRNA gene is widely used in bacterial taxonomy, it cannot differentiate closely related species in some families of bacteria including *Enterobacteriaceae*, *Clostridiaceae*, and *Peptostreptococcaceae* due to high sequence similarity of this gene at species level. In addition, in the recent years, in The University of Arizona Aquaculture Pathology Laboratory (UAZ-APL) we have found some non-specific amplification while screening shrimp samples for NHPB.

Due to the lack of specificity of 16S rRNA-based amplicon for NHPB, new protocols were developed for the detection and quantification using PCR and qPCR. The target genes for the new PCR-based method include NHPB flagella genes *MotB*, *FlgH*, *FlbB*, *FlgL*, and *FlgE*. Primer Express software version 2.0 (Applied Biosystems) was used to design primers and the TaqMan hydrolysis probe. Samples tested positive for NHP-B in UAZ-APL using the conventional PCR method, were used to validate the new PCR method. NHPB positive samples from Ecuador, Brazil, Colombia, Mexico, Texas, Belize and Panama collected during 2011 through 2016 were used to validate the new assays. Among different primers tested, primers based on the *FlgE* and *FlbB* genes provided specific amplification with high sensitivity. The newly designed primers did not provide any non-specific amplification as obtained earlier based on the 16S rRNA gene. In summary, we describe here highly specific and sensitive PCR and qPCR methods for the detection and quantification of NHPB in shrimp HP and feces samples.

REGULATION OF MUSCLE GROWTH IN SABLEFISH *Anoplopoma fimbria* DURING GROW OUT

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Skeletal muscle accounts for the bulk of body mass in finfish species selected for aquaculture. This fact highlights the importance of understanding muscle tissue development to enhance overall growth. Sablefish (*Anoplopoma fimbria*) is a Northeast Pacific species with stable wild-capture fisheries and an emerging U.S. aquaculture industry, the latter thanks to high market value and fast growth in culture. Hatchery and grow-out protocols are being developed for sablefish, and producers stand to benefit from knowledge of how the ‘growth environment’ impacts muscle tissue development, growth potential, and product quality (e.g., temperature, nutrition, genetic strain). The research presented here is part of a larger study investigating development of red and white muscle using 11 molecular markers of muscle growth and metabolism. This work begins to decipher the transcriptional regulation of muscle development in sablefish and serves as a baseline to which future comparisons can be made to expedite decisions for optimal production by industry.

Species-specific qRT-PCR assays were developed using the TaqMan MGB primer-probe system for highly-conserved genes with known critical functions in vertebrate muscle development. These molecular tools were employed to measure mRNA levels in muscle from a cohort of cultured sablefish at four time points, spanning 16 months of grow out (Fig 1). Evaluation of red muscle specific growth is important as it represents a localized microniche (e.g., maturity, metabolism) where satellite cell recruitment and myogenesis are not assumed to equal that of white muscle.

Differential regulation of the target genes (e.g., MyoD1, MyoD2) at advancing stages of the growth cycle suggest important and conserved roles in sablefish muscle development (Fig 2). These tools will be further validated and used to determine what growth environment maximizes sablefish growth performance.

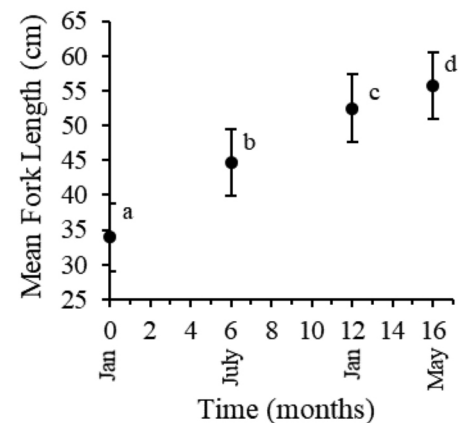


Figure 1. Growth of sablefish cultured in ambient conditions (mean \pm SD, n = 40).

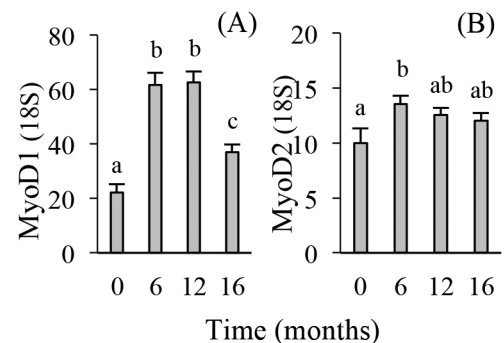


Figure 2. Myogenic determination factor 1 (A) and 2 (B) mRNA in red muscle using the ΔC_T method relative to 18S (mean \pm COV; n = 40).

THE DYNAMICS OF DISEASE IN AQUACULTURE FROM AN ECONOMIC PERSPECTIVE

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As in all forms of biological production, disease is an integral part of aquaculture. However, due to its recent genesis as a large-scale crop, not much was known about disease on aquatic animals until quite recently. This lack of knowledge, together with the rapid growth in production has made aquaculture particularly vulnerable to disease. However, the situation is changing at least for some species in some regions, as increased veterinary knowledge leads to more prevention tools at farms and in the management system as well as more cures becomes available.

In this paper we will look at some typical disease patterns in aquaculture, and also compare them to agriculture. These will range from the typical unprepared industry where all firms go bankrupt when a significant disease outbreak kills most of their production to industries that manage diseases with a clear cost component and with occasional outbreaks. The importance of management systems will also be discussed, with a particular focus on how un-coordinated applications can and will lead cures becoming ineffective, while coordinated actions are both more effective and renders cures useful for longer periods.

ACTOR PERSPECTIVES ON AQUACULTURE EXTENSION SERVICE PROVISION IN CENTRAL AND NORTHERN UGANDA

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The purpose of extension service provision to farmers is to meet farmers' needs including knowledge, skills and other services so as to improve productivity and general socio-economic wellbeing of household members. Such interventions are needed in aquaculture as a way of increasing fish production since fish supply from the natural sources has drastically declined. Drawing from the Actor Oriented Perspective, this paper explores individual farmer and household level characteristics that influence interactions between aquaculture extension service providers with members of households practicing fish farming in ponds.

Survey data were collected from a random sample of households involved in fish farming and supplemented with information obtained from Focus Group Discussions (FGDs) with fish farmers. Additional information was got from interviews with extension service providers. Data obtained from the survey was analysed using SPSS while Atlas.ti was used to analyse information from FGDs. Findings revealed that fish farmers perceived the level of extension service provision as low as demonstrated by few and erratic farm visits. Yet, fish farmers experienced various constraints related to technical competence about rearing fish and financing of the fish enterprises. Alongside government fisheries extension staff, farmer to farmer extension encounters were common while use of Information Communication Technologies such as radio and cell phones was minimal. Membership to fish farmer groups significantly influenced the frequency of extension visits ($p < 0.05$) compared to individual farmer demographic and socio-economic factors. Relevant training is necessary to enable extension staff help farmers build and sustain farmer organisations. Strong farmer groups promote effective delivery of extension services; enhance learning, knowledge sharing and application.

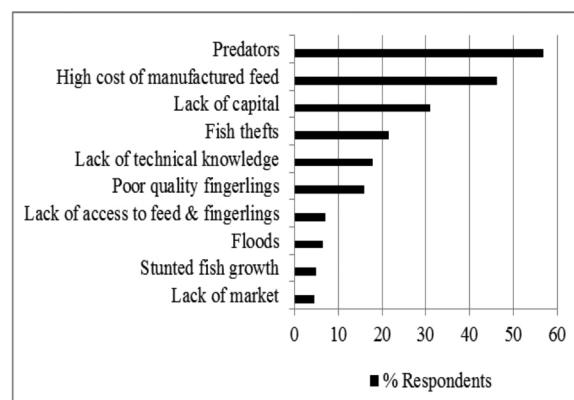


FIGURE 1. Constraints encountered by fish farmers in the study area

TABLE 1. Relationship between incidence of extension visits by government staff and characteristics of respondents

Respondents' characteristics	χ^2	Sig
Age	1.231	0.746
Gender	0.406	0.357
Education	8.178	0.225
Land owned	1.115	0.953
Income from pond fish	7.311	0.316
Total pond area	3.310	0.507
Experience in fish farming	2.536	0.638
Member to farmer group	7.560	0.006†
Region of residence	3.277	0.070

Significant difference at 5%

INSTITUTIONAL AND HOUSEHOLD FACTORS IN PROMOTING THE ROLE OF WOMEN IN AQUACULTURE VALUE CHAIN IN UGANDA

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Aquaculture in Uganda dates from the early 1950s having been introduced by the colonial government as a means to promote access to fish by rural poor communities. The target groups were households with low fish consumption due to limited access to lakes and rivers, the main sources of fish. Due to patriarchal system that determine household headship in Uganda, interventions particularly those requiring use of household land tend to focus on men as owners of land and therefore often obscure the role of women along the aquaculture value chain. Women's participation in aquaculture is limited to provision of farm labour but play important decision-making over fish consumption by household members. Following a number of successful experiments using various fish species, technologies for rearing fish in earthen ponds were popularised among target households who had access to water sources.

The study is informed by the 'efficiency approach' of Women in Development thinking and focused on analysing the contribution of women through active involvement in aquaculture to realise financial independence and poverty alleviation. The paper explores the role of women in aquaculture value chain in Uganda from institutional and household standpoint in relation to women's decisions and participation in pond fish farming. In addition, the paper examines women's understanding and perceptions of fish as a dietary asset for household members and determinants of fish diet in households. Opportunities to improve women's benefits in aquaculture are also explored focusing on access to training, extension services and strategies to promote women cooperatives.

Information was obtained through in-depth interviews with women involved in different aspects of aquaculture including policy planning level, training, production, marketing and fish provisioning at household level. Preliminary results show that despite women's under-representation in aquaculture related work, they play an important role that is currently not optimally recognised to maximise their full potential to increase the benefits of aquaculture. The study also observed that the achievement of food (fish) level security at household level lies with women given their decision making and influencing role over the kind of diet for household members.

Efforts to increase fish production require engaging women to organise themselves from aquaculture planning stage through production and marketing. Policy support to existing women's initiatives in aquaculture is recommended.

AVOIDING RESIDUES IN U.S. FARM-RAISED CHANNEL CATFISH *Ictalurus punctatus*

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Inspection of channel catfish processing facilities was transferred from the US Food and Drug Administration (FDA) to the US Department of Agriculture Food Safety and Inspection Service (FSIS). Full implementation of domestic inspection began September 1, 2016. One component of inspection is testing the muscle of processed fish for pesticide and veterinary drug residues. If residues are found above established tolerance levels, FSIS can recommend that processors recall product that may have entered commerce. Since inspection began, there have been two recalls of domestically produced channel catfish.

There are two main pathways for farm-raised channel catfish to become exposed to pesticides and veterinary drugs. Fish can ingest these products through feed or forage fish. A second route of exposure is through absorption (primarily through the gills) from pond water or water used during transport.

Farmers should only apply pesticides that are labeled for aquatic use by the US Environmental Protection Agency (EPA). The primary aquatic use pesticides approved by EPA are aquatic herbicides and algicides. Applicators should read and follow all label instructions, paying close attention to the volume of water being treated and the amount of chemical being applied. It is also vital that the target species being controlled is accurately identified. There are also three EPA registered pesticides (potassium permanganate, copper sulfate, and hydrated lime) that are used to address water quality issues.

FDA approved drugs and drugs of low regulatory priority are used in hatcheries and in production ponds. In the hatchery, these products are used to disinfect eggs or used to promote spawning. The three FDA approved antibiotics require a veterinary feed directive which specifies dosage and withdrawal times. Formalin is used to treat external parasites. Salt is registered as an osmoregulatory enhancer. FDA drugs of low regulatory priority for hauling fish include chlorides and ice.

Farmers are urged to use the following practices to avoid residues in channel catfish:

1. Use only EPA and FDA approved pesticides and veterinary drugs.
2. Do not use forage fish that have been treated with chemicals not approved for foodfish.
3. Do not exceed label rates of approved products.
4. Avoid selling fish before withdrawal times are met.

HELPING THE U.S. FARM-RAISED CATFISH INDUSTRY AVOID RESIDUES IN PROCESSED PRODUCT

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Since inspection began, there have been two recalls of domestically produced channel catfish. In both recall cases, FSIS chose not to initiate an investigation to discover the source of the contamination. Subsequent investigations by industry and Extension personnel could not find a direct link between products containing the chemicals and their use in the production process. It soon became apparent that the best way to prevent further recalls was to train farmers and haulers how to prevent contamination of live fish.

A program development team was formed by Extension Specialists and Associates in Mississippi, Alabama, and Arkansas. This team was responsible for training material development and conducting training opportunities in their respective states. Team members were assigned topic areas to develop and all members reviewed and provided input on the final training materials. Initial training opportunities included sessions in the Mississippi Delta, East Mississippi, Alabama, and Arkansas.

Participants were provided information on how fish are exposed to products that may result in a tissue residue, regulatory statutes related to pesticide usage, product selection, following label instructions, record keeping, and avoiding pesticide contamination of ponds. Presenters reviewed label rates, application methods, and use restrictions for the 15 EPA approved aquatic herbicides and algicides. Participants were also presented information on the proper use of FDA approved drugs and FDA low regulatory priority drugs in hatcheries and in ponds. The use of Veterinary Feed Directives and adhering to withdrawal times was also discussed.

Upon completion of the training, participants were tested on the knowledge and issued a Certificate of Completion with a unique identifier. It is hoped that participants could use this training to meet some of the critical control point requirements of the catfish processors.

GENOTOXICITY AND HEAMATOLOGICAL PROFILE OF *Clarias gariepinus* FINGERLINGS FED WITH WALNUT LEAVES AS A SUBSTITUTE FOR RICE BRAN TO DETERMINE GROWTH PERFORMANCE

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Plant has become a preferred source of protein for fish species in aquaculture. A twelve week feeding trial was carried out in order to assess the effect of feeding walnut leaves on haematological and biochemical parameters as well as the genotoxicity level on *Clarias gariepinus* fingerlings as a bio-indicator of their health status and overall response of the fish towards the experimental diets. One hundred and fifty fingerlings of *Clarias gariepinus* of mean weight 6.90 ± 0.2 g were stocked randomly as ten fish per tank ($52.5 \times 33.5 \times 21$ cm³) in triplicate. Fish were fed to satiation and the water changed every day to maintain good water quality. Five experimental diets with 40% crude protein each were formulated; the control, without the test ingredient and the other four diets (test diets 1, 2, 3 and 4) contained 25%, 50%, 75% and 100% inclusion respectively. The Control diet had the highest mean weight gain ($P < 0.05$) (118.8 ± 17.3) among the entire group. Similar pattern was observed in specific growth rate. The least significant ($P < 0.05$) feed conversion ratio was recorded by the Control diet (0.68 ± 0.03), the diet also recorded the best protein efficiency ratio (3.53 ± 0.23). The genotoxicity test shows that some Micro nucleus of the test diets were normal, lobed and binucleated nucleus compared to the Control diet. The fish fed with *T. conophorum* showed an increase in haematological values of Packed Cell Volume (PCV), (0.37 ± 0.08), Haemoglobin (HGB), (103.00 ± 28.02) Red blood cell (RBC), (2.43 ± 0.86), Mean Corpuscular Volume (MCV), (177.20 ± 13.2), White Blood Cell (WBC), (139.50 ± 40.44) compared to the values of fish fed the control diet, PCV (0.26 ± 0.11), HB (71.00 ± 45.25), RBC (1.45 ± 1.11), MCV (158.15 ± 6.29), and WBC (121.10 ± 28.1). The white blood cell showed no significant difference ($p > 0.05$) among the fish in T₁, T₂, T₃, and T₄. There was reduction in the haematological values of the fish fed *T. conophorum* diet with mean haemoglobin concentration (MCHC) (28.85 ± 3.32) and the Mean Corpuscular (MCH) (47.80 ± 1.83) compared with the values of fish fed with the Control diet MCH (52.05 ± 8.89), and MCHC (33.00 ± 4.38). The fish fed with *T. conophorum* showed an increase in biochemical value with AST (31.75 ± 26.65), Urea (4.82 ± 1.37), Creatinine (11.56 ± 4.53) ALP (26.00 ± 13.71), ALT (361.90 ± 446.04) and TP (30.54 ± 5.07) compared to the value of the Control diet, AST (9.05 ± 20.5), Urea (4.72 ± 0.31), ALT (65.10 ± 8.76), Creatinine (9.92 ± 5.11), ALP (17.90 ± 17.6), and TP (21.96 ± 5.91). There was reduction in the biochemical values of the fish fed *T. Conophorum* with Cholesterol (2.20 ± 0.05) Albumin (8.35 ± 0.21) and Triglyceride (0.98 ± 0.13) compared with the values of fish fed with the Control diet with Cholesterol (3.81 ± 0.07) Albumin (12.35 ± 2.33) and Triglyceride (7.29 ± 7.02). It was concluded that using *T. Conophorum* leaves as feed for *Clarias gariepinus* enhances the growth of the fish. Therefore, partial replacement of feed *T. Conophorum* should be encouraged.

A HISTOLOGICAL AND TRANSCRIPTOME EVALUATION OF THE DEVELOPMENT OF RESPIRATORY STRUCTURES IN CATFISHES

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In the realm of finfish production, Siluriformes is one of the most dominant orders on an international scale. Within the United States the family Ictaluridae is most common, while Pangasiids and Clariids are cultured in Africa, Asia, and parts of Europe. In more tropical climates the capacity for production is exponentially greater than in the U.S. due to the ability for higher stocking densities per unit of area. Representative species from these families have distinct morphologies resulting in the presence or absence of an accessory respiratory organ. Ictalurids such as Channel Catfish (*Ictalurus punctatus*) do not have an accessory respiratory organ however Tra (*Pangasius sutchi*), and Walking Catfish (*Clarias batrachus*) possess the advantage to use atmospheric oxygen to supplement in periods of low dissolved oxygen. Using histological and microdissection techniques we identified critical stages of development in Channel Catfish and Tra at which gill and accessory respiratory structures develop and become active. Using these highlighted critical stages of development, we will utilize RNA sequencing to determine the levels of expression for genes that are active in gill structures and air breathing organs.

INTEGRATION OF ENERGY AUDITS INTO LIFE CYCLE ASSESSMENT METHODOLOGY TO IMPROVE ENVIRONMENTAL PERFORMANCE OF RECIRCULATING AQUACULTURE SYSTEMS

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In Recirculating Aquaculture Systems (RAS), water is continuously treated and recirculated as opposed to being discharged untreated into the environment as in other types of fish production systems; the design and production parameters will determine the overall energy consumption. This energy-intensive nature hampers their sustainability and cost-effectiveness. A combination of two methods (i.e. Life Cycle Assessment (LCA) with energy audits) to: improve environmental performance of RAS, identify energy consumption and thus, its environmental and monetary effects in order to seek cost reduction is proposed. Likewise, an online software analyzing system's consumption patterns (principal and partial consumptions); anomaly detection and correction of habits; identification of opportunities for improvement; and assessment of potential economic progress. The methodology was proved with a case study focused in a pilot-scale RAS unit used in codfish (*Gadus morhua*) production, located in the Basque coastal area (northern Spain). Feed and juvenile production/transportation, oxygen transportation and energy consumed during the whole experiment were considered as inputs for the assessment. Energy consumption was measured both continuously by an energy meter embedded in the RAS unit as well as with a portable energy analyzer to measure each of the energy-consuming devices independently. Main consumers included the heat pump, followed by the main and secondary pumps, respectively. Energy audit's results show the success in identifying the devices that consumed the largest amount of energy, and recorded data served to feed the Life Cycle Inventory and perform a more complete and precise LCA. Fossil fuel based on-farm electricity for the on-growing of fish was shown to be the most environmentally unfriendly input; it was the major impact producer in the assessed impact categories. It showed a temporal variability depending on the water temperature, which was the main factor linked to the energy use. The combination of LCA and on-farm energy audit presents a useful tool to conduct a more complete assessment with results that can be used to design a less energy intensive, profitable and sustainable system.

Acknowledgments: This research would not have been possible without the assistance of Leandro Fernandez, Xabier Arranz and Gaizka Bidegain as farm manager and technicians. This work was supported by the Department of Education, Universities and Research of the Basque Government.

DETERMINATION OF MICROBIAL CONTAMINANTS OF FROZEN SMOKED FISH SOLD IN JOS METROPOLIS PLATEAU STATE, NIGERIA

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A total number of 270 smoked fish samples were bought from 3 different markets in Jos Metropolis, Plateau- State for detecting bacterial, fungal contaminants and microbial load. The data obtained were analyzed Using Analysis of Variance. The isolates were characterized using BA, EMBA, MCA and SSA media plates after incubation at 37°C for 24hrs and growth from the plates were subjected to gram staining.

The isolates identified were, *Staphylococcus aureus*, *Salmonella* spp, *Bacillus cereus* *Streptococcus* spp *Escherichia coli* and *Corynebacterium diphtheria*. In respect to location, 50% bacterial prevalence was seen in Terminus Market with 27.3% and 22.7% from Bukuru Market and New-satellite market respectively.

Fungal load in respect to location shows that 100% were seen at New-Satellite Market which shows there is significant difference of fungal contamination at ($p < 0.05$). The highest bacterial load was in *Trichuris* spp from Terminus Market with mean of (32.78 ± 1.94^{ab}) which shows there is significant difference ($p < 0.05$), while the lowest bacterial load with the mean of (13.46 ± 2.97^b) on *Sardinella mandarensis*.

In respect to bacterial load on fish parts, the highest bacterial load was recorded on the head of *Scomber scombus* with the mean of (28.933 ± 3.300^{ab}) shows that there is significant difference, while the means of (13.66 ± 2.83^b). In conclusion, the prevalence of each bacterium in smoked fish, *Salmonella* spp and *Staphylococcus aureus* has the highest percentage of 27.3% while *E.coli* *Streptococcus*, *C. diptheriae* *Bacillus cereus* were 13.6%, 18.2%, 9.1% and 4.5% respectively. It is recommended that smoked fish should be thoroughly washed with salt water and adequately cooked well before consumption

Table 1: Prevalence of bacterial in respect to locations

Isolate	BUKURU MARKET	TERMINUS MARKET	NEW-SATELLITE MARKET
<i>Salmonella</i> spp	2	9	1
<i>Escherichia coli</i>	1	4	1
<i>Staphylococcus aureus</i>	2	5	5
<i>Streptococcus</i> spp	4	3	1
<i>Bacillus cereus</i>	2	—	—
<i>Corynebacterium Diphtheria</i>	1	1	2
	12	22	10
Total 44			
Percentage %	27.2%	50%	22.7%

Table 2: Bacterial load from different parts of the fish sampled

Fish Species	Head	Intestine	Flesh
<i>Sardinella mandarensis</i> 23.300 ± 3.300^b		21.933 ± 3.047^b	16.366 ± 2.614^b
<i>Scomber scombus</i> 28.933 ± 3.300^{ab}		27.466 ± 4.074^{ab}	19.400 ± 4.235^b
<i>Trichuris</i> spp 21.300 ± 2.371^{ab}		14.466 ± 1.443^b	13.266 ± 2.833^b

GROWTH PERFORMANCE OF *Cyprinus carpio* FINGERLINGS FED WITH VARYING LEVELS OF GRASSHOPPER MEAL

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This study was conducted to determine the growth performance of *Cyprinus carpio* fed with varying levels of grasshopper meal, Graded levels of grasshopper meal (0% 5% 10% 15% and 20% of the total diet) were supplied in the experimental diets. Six hundred thirty five (635) fingerlings of *Cyprinus carpio* were weighed and uniformly distributed to these dietary (T1, T2, T3, T4, and T5).

Each treatment had one hundred and twenty seven (127) fingerlings of *Cyprinus carpio*. The growth parameter measured were average weekly growth rate, percentage weight gain, feed conversion ratio, percentage survival and feed intake. The experiment lasted for thirty two (32) weeks. In all parameters, the fishes fed with grasshopper meal (diet) showed no significant difference from those fed with the control diet which had no grasshopper meal in it. The fish fed diet with different levels of grasshopper meal also showed similar values of the growth parameters regardless of the dietary inclusion level.

Table 1: Gross Composition of Experimental Diets.

INGREDIENTS	(0%)	(5%)	(10%)	(15%)	(20%)
Fish meal	20.00	20.00	20.00	20.00	20.00
Maize meal	450.00	450.00	450.00	450.00	450.00
Soya bean meal	300.00	280.00	270.00	250.00	240.00
G. H. M.	---	1.50	3.00	4.50	6.00
Cassava meal	1.00	1.00	1.00	1.00	1.00
Mineral premix	1.50	1.50	1.50	1.50	1.50
Vitamin premix	1.50	1.50	1.50	1.50	1.50
Oil	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50

Table 2: Proximate Composition of Experimental Diets.

Column and row means in the same row with different superscripts different significantly (P<0.05).

Parameter	DT1	DT2	DT3	DT4	DT5	P-value
Moisture	4.74±0.01 ^d	5.21±0.01 ^c	5.24±0.01 ^b	4.34±0.01 ^e	5.58±0.01 ^a	0.001
Ash	6.32±0.01 ^d	6.07±0.01 ^e	6.49±0.00 ^c	6.91±0.01 ^e	7.12±0.01 ^a	0.001
Fat	4.58±0.01 ^e	4.89±0.01 ^d	5.33±0.01 ^c	5.48±0.01 ^b	5.55±0.01 ^a	0.001
Fibre	4.22±0.01 ^c	4.52±0.01 ^a	3.91±0.01 ^d	3.54±0.01 ^e	4.39±0.01 ^b	0.001
Protein	35.56±0.01 ^d	36.15±0.01 ^b	35.65±0.01 ^c	36.22±0.01 ^a	36.19±0.01 ^a	0.001
FE	44.59±0.01 ^a	3.18±0.02 ^c	43.39±0.036 ^c	42.53±0.02 ^d	41.18±0.02 ^e	0.001

A REVIEW ON THE EFFECTS OF PROBIOTICS AND PREBIOTICS AS THE ANTIBIOTIC REPLACERS IN JAPANESE EEL *Anguilla japonica*

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Japanese eel, *Anguilla japonica*, is one of the most important cultured fish species in East Asia. Along with the fast expansion and intensification of Japanese eel aquaculture, emergence of large varieties of pathogenic diseases are considered as limiting impediments. On the other hand, the excessive treatments of antibiotics has caused the appearance of bacterial resistance strains. In this regard, three experiments were conducted to identify probiotics such as *Bacillus subtilis*, *Lactobacillus plantarum* and *Bacillus licheniformis* with or without prebiotics such as mannan oligosaccharide (MOS), β -glucan and fructo oligosaccharide (FOS) as effective antibiotic replacers. Results for the first experiment indicated that based on growth performance, immunological parameters, gut morphology and disease resistance, *B. subtilis* at 10^8 and 10^7 CFU/g diet could be a more effective source of probiotic compared to *L. plantarum* in Japanese eel. In the second experiment, comparing different concentrations of *B. subtilis* (0, 0.5×10^7 and 1×10^7 CFU/g diet) with MOS and β -glucan (0 and 5 g kg⁻¹ diet) prebiotics showed that combination of *B. subtilis*+ MOS (0.5×10^7 CFU/g+5 g kg⁻¹ diet) resulted in a better growth performance, immunological status, gut morphology and disease resistance. In the third experiment, comparison of *Bacillus subtilis* and *Bacillus licheniformis* with prebiotics such as MOS or FOS was performed. Results indicated that of *B. subtilis* with MOS or FOS could have beneficial effects on growth performance of Japanese eel. Therefore, it could be concluded that probiotics have the potential as antibiotic replacers while enhancing growth performance and immunity. In addition, application of selective strains of probiotics for specific fish seems to be vital in order to maximize the effectiveness.

EFFECTS OF SEA LICE TREATMENT ON HEALTH STATUS IN AQUACULTURE REARED RAINBOW TROUT (*Oncorhynchus mykiss*)

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The objective of the work undertaken was to study the effect of azamethiphos (Salmosan) treatment on blood biochemistry and tissue histopathology in aquaculture reared rainbow trout to determine its impact on the health status.

Fish serum biochemical analysis showed significant increases ($p < 0.05$) in total bilirubin and free haemoglobin concentrations recorded 24 hours after the treatment. Levels of serum iron were significantly increased 48 hours after the treatment. Markers of kidney damage showed increased levels in all time points after the treatment together with high levels of inorganic phosphorus and urea at 4 and 10 days after the treatment, respectively. Typical marker for liver condition included ALT, which showed increased activity from 48 h after treatment onwards, while globulin concentration was decreased 4 days after the treatment. The levels of copper and zinc were significantly lower on day 4 and day 10 after the treatment ($p < 0.05$). The ammonia concentration was significantly lower only on day 4 ($p < 0.05$) when compared to concentrations before the treatment.

After histopathological evaluation gills showed highest damage to the secondary lamellas in form of blood congestion, telangiectasia and excessive bleeding 4 days after the treatment. The tissue damage of kidney 24 hours after delousing treatment showed increased deposits of hemosiderin in proximal and distal tubules, while after 10 days glomerular necrosis was evident. Liver damage was visible as after delousing treatment after 24 hours as mild necrosis of hepatocytes while severe dilatation of sinusoids and hemosiderin deposits were visible 4 days after the treatment. Splenic tissue showed excessive hemosiderin deposits and increased number of melano-macrophage centres (MMC) at 4 days after treatment when compared to fish from before treatment.

These results indicate the possible impact of sea lice treatment on trout health through intravascular haemolysis caused by the chemical, and consequently the pathophysiologic process of haemoglobin metabolism and its products causing chronic injury from hemosiderin deposits. Blood biochemistry has a good potential to approach fish health status and was correlated with organ/tissue damage. The kidneys were significantly affected and weakened after the treatment showing a more sensitive response than the other studied organs. We conclude that organ injury resulting from haemolysis and hemosiderin deposits should be considered as a cause of decreased organ function after sea lice treatments.

EFFECTS OF WATER TEMPERATURE ON GROWTH, SURVIVAL AND WHOLE BODY LIPIDS OF 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. Conservation efforts involving this native species include development of culture techniques, which the present research aims to address. The objective of this study was to determine the effects of temperature on growth, survival, whole body lipid and fatty acid profile of larval Pacific lamprey. During a six week trial, Larvae were reared at four different constant water temperature treatments in a flow through intensive culture setting. Temperature treatments included un-heated well water averaging 14.7 °C along with heated well water of 17.3, 19.2 and 22.4 °C. Each treatment was randomly assigned three replicate tanks. Each tank housed 50 larvae at 86 days post hatch. Fish initially had an average weight and length of 3.7 mg and 10.3 mm, respectively. At the end of the trial survival was >98% across all temperature treatments. Analysis of the final results is ongoing. Growth, survival, whole body lipid, and fatty acid profile will be presented and discussed. The results of this research will refine the methods for producing quality lamprey and provide insight into long term sub lethal effects of temperature on larval Pacific lamprey.

THERAPEUTIC EFFECTS OF INVERTEBRATES: FUTURE OF AQUACULTURE

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Invertebrates have been known for their roles as delicacy. But in recent time, they are increasingly getting importance for their roles in the ecology, research, 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 invertebrates, such as sea cucumbers, have any roles in improving vertebrate immune response. We have seen from our experiments that sea cucumbers have immunostimulatory effects in vertebrates via induction of spleen cell proliferation and stimulation of macrophage functions.

QUALITY CHARACTERISTICS OF PRECOOKED CATFISH FILLETS DURING 10 MONTHS OF FROZEN STORAGE

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Frozen storage is one of the major ways to maintain product quality and safety for an extended period of time. Frozen products can include preheated or precooked seafood products that will be reheated often in a microwave prior to being served. Product quality slowly deteriorates with time during frozen storage and shelf-life of many product is less than 8 months depending on temperature, packaging and number of other factors. There is limited knowledge on the quality of precooked fish fillets during frozen storage. The objective of this study was to examine changes in quality of precooked catfish fillets during frozen storage for up to 10 months. The effect of two packaging materials on storage stability was also evaluated. Catfish IQF (Individually Quick Frozen) fillets treated with polyphosphate were purchased from a commercial Mississippi catfish processor, trimmed and cut vertically into three pieces, with each weighing approximately 50 g. The catfish fillet pieces were precooked in a 121°C convection oven to an internal temperature of 60°C. Half of the precooked fillet pieces were individually vacuum-packaged in poly-nylon vacuum pouches, and half were placed in Ziploc bags. Samples were then stored in a -20°C freezer and samples analyzed after 0, 1, 6, 10 month(s) of frozen storage. Four randomly selected fillet pieces were analyzed for each treatment and measurements included drip loss, proximate content (% protein, % lipid, % ash and % water), color (CIE L*a*b*), pH, mechanical texture, and lipid peroxidation (TBARS). Data were statistically analyzed. Within treatments, storage time showed no or minor effect on proximate content. The vacuum-packaged samples had lower (~1%) moisture contents and greater drip losses than the comparable sandwich bag packaged samples. Frozen fillet pieces were similar in terms of malonaldehyde (MDA) concentration between treatments for up to 10 months of frozen storage. Fillet texture properties of vacuum-packaged samples remained consistent during the 10-months of frozen storage; however, the sandwich bag samples showed a slight decrease in hardness, cohesiveness, chewiness, and resilience, with difference between packaging treatments being significant for the 10-month samples. Results indicated that precooked catfish fillets can be stored for 10 months with little loss of quality, which supports the use of precooked catfish in frozen products.

QUALITY ASSESSMENT AND CONTROL / HEALTH ASSESSMENT OF RED DRUM *Sciaenops ocellatus* CULTURED FOR STOCK ENHANCEMENT

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Red Drum (*Sciaenops ocellatus*) are a warmwater marine finfish species cultured at SCDNR's Waddell Mariculture Center (WMC). Red drum are used for aquaculture research as well releases as juveniles as part of stock enhancement research. Until recently, fish health quality assessment/quality control protocols have not been developed or implemented at WMC that evaluate the health of fish being released into estuaries along the South Carolina coast. A primary objective over the next production season is to develop and put in place hatchery operation methods that include a health assessment and QAQC component for fish that are cultured and released. This, in addition to conservation of genetic diversity through broodstock rotation, aims to produce and release healthy fish which have a higher chance of contributing to the natural population. The present study aims to document and quantify variations in physical features of Red Drum (*Sciaenops ocellatus*) that can be used to sort or cull poor quality fish before they are released for stock enhancement purposes.

Seven genetically different groups of Red Drum (*Sciaenops ocellatus*) varying in age and size grown extensively in ponds were evaluated for both malformations and deformities of bony structures and soft tissue damage to eyes, gills and fins. The most prevalent malformations and deformities can be viewed in Figure 1. Additional malformations and deformities include loss of pelvic fins, severe fin erosion, cross bite (lateral displacement of the lower jaw), and vertebral axial deviation (i.e. scoliosis).

This study is ongoing with a goal of quantifying malformations and deformities of fish with different ages, sizes, and genetics. Additionally, information gathered will be used to produce a QAQC health assessment manual for SCDNR stock enhancement research. Early detection and sorting will produce fish that have a higher probability of surviving to adulthood after release and exhibit a wild type body conformation. Future goals aim to compare wild caught fish with hatchery fish to document natural phenotypic variations in Red Drum (*Sciaenops ocellatus*).

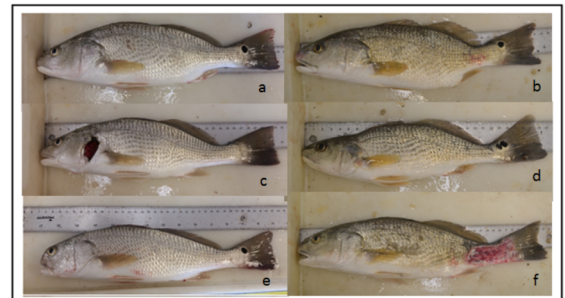


Fig. 1 Common malformations and deformities observed in Red Drum (*Sciaenops ocellatus*) at WMC. a) Red drum without malformations/ deformities. b) vertebral fusion; fusion or shortening of vertebrae. c) malformed operculum, typically leading to malformed and exposed gills. d) dorsal head indentation; a “dip” present just in front of the dorsal fin (observed in varying degrees of severity). e) “Pughead” or a compression of the head and jaws. f) severe skin lesions and fin erosion.

DEMONSTRATION OF A SMALL-SCALE DECOUPLED AQUAPONIC SYSTEM UTILIZING A FLOATING BEAD BIOCLARIFIER AND AIRLIFT TECHNOLOGY

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Aquaponics is the combination of plant and fish culture in a recirculating water environment. This system includes the production of fish and plants in an ecosystem that generates a stabilized environment where the fish waste is converted into a nutrient solution for plants growing in a hydroponic system.

At the AST greenhouse research facility (New Orleans, LA), a small-scale decoupled aquaponic systems was constructed and evaluated. For this demonstration unit, a single round 1 m³ polytank (~250 gal) was stocked with Tilapia (*Oreochromis niloticus*) at a density of 10 kg/m³ (0.08 lb/gal) and an initial size of 75 g (3-4 inches). The filtration system was composed of an Endurance 2000 bioclarifier which included 0.03 m³ (1 ft³) of EN floating bead media. The water flow through the filtration system was maintained at 45 Lpm (12 gpm) by airlift provided by a 46 watt linear air pump. The fish culture system was maintained between 25-28°C (77-82°F). The juvenile tilapia were fed a 2.0 mm floating pellet (50% CP) at a daily feed rate of 3% body weight.

The aquaponics system was de-coupled where a fish culture loop consisted of a single round 1 m³ polytank (~250 gal), a self-backwashing bio-clarifier, and a 0.12 m³ (34 gal) mineralizing tank. The plant culture loop consisted of a second 0.12 m³ (34 gal) sump tank, and 2 hydroponic raft culture raceways, each at 3 m² (32 ft²). The recirculating water flow through each raceway tank was 30 Lpm (8 gpm). The plant culture tanks were maintained between 15-24°C (60-75° F). This plant system was stocked with 224 bibb lettuce plant seedlings at a density of 37.8 plants/m². The economic feasibility of this de-coupled system depends on the specific costs of the raw materials including fish feed, electricity, water, lumber, filter, air pump, and tank costs. An economic analysis of fish and plant production in addition to water quality and feed conversion variables for the current status of the system will be presented.

INNOVATIVE CAPABILITIES AND CHOICES IN THE NORWEGIAN AQUACULTURE VALUE CHAIN

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The Norwegian seafood industry has experienced sustained growth for the past decades, primarily as a result of increased aquaculture production. Earlier research has stressed the role of innovation and technological change as explanations for the growth in aquaculture. In this paper we will shed more light on the innovation process by using data from innovation surveys matched with wage and employment data. Firms depend on several types of knowledge in their innovation processes. Innovation in aquaculture is increasingly dependent on research based knowledge. The decision problem of a firm aiming to increase its knowledge base and ability to innovate includes internal recruitment of different types of skilled employees and collaboration with different types of external organizations.

The dataset used for the econometric analysis is constructed by combining 5 innovation surveys with data on employment and wage. When combined the surveys covers the years 2000-2010. The surveys contains questions on R&D personell and cooperation in innovation processes. Firms are polled on three types of innovation; product innovation, process innovation and radical innovation. Product- and process innovation represents innovations new to the firm, while radical innovation represents innovations new to the market.

Aggregate innovation rates display a pattern where aquaculture farming favors process innovation over product innovation. Aquaculture services have generally high innovation rates. Food producers and wholesalers display a pattern of moderate innovation rates for all types of innovation.

The patterns from analysis of the full dataset using logit models are that number of employees and wage level significantly increases the probability of product- and process innovation. Higher evenue, when firm size is controlled for with number of employees, significantly decreases the probability of product- and process innovation. For radical innovation the coefficients display the same pattern, but the effects are not significant.

We find that aquaculture services are significantly more innovative than aquaculture farming for all three types of innovation. Seafood production has a significant and positive effect for product and radical innovation. Wholesalers are only significant for product innovation. When we combine data on innovation rates with the econometric analysis a pattern emerges. Innovation in the aquaculture industry is focused in services and juvenile production, not in farming. Further down the value chain both food producers and wholesalers engage in product and process innovation.

Aggregate innovation rates			
	Product	Process	Radical
Aqua farming	13 %	28 %	4 %
Aqua services	39 %	49 %	21 %
Food prod.	25 %	25 %	11 %
Wholesalers	23 %	23 %	11 %

EVALUATION OF STOCKING DENSITY IN A LIVE HOLDING RECIRCULATING SYSTEM FOR FOOD SIZE CHANNEL CATFISH, *Ictalurus punctatus*

Sujan Bhattacharai*, Kenneth Semmens

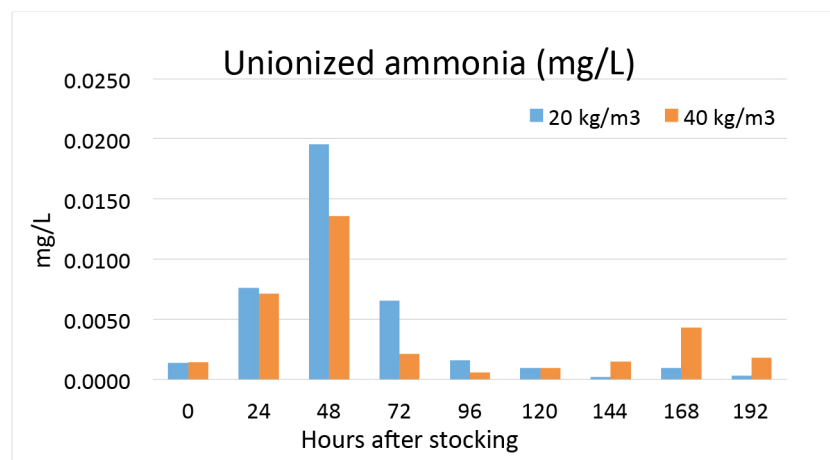
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Distributing locally grown food fish live may provide aquaculture producers in Kentucky a more profitable market opportunity than selling fish as a commodity. Holding fish live in recirculating systems will be required for this pathway to be successful. In this study investigators evaluated water quality and physiology of Channel Catfish, held live at two stocking densities: 20 kg/ m³ and 40 kg/ m³.

Catfish with a mean weight of 0.76 kg were stocked in recirculating aquaculture systems with a 17 L bead filter after simulated hauling for five hours. Water quality for each holding tank was measured at stocking (Time 0), and daily thereafter (24, 48, 72, 96, 120, 144, 168, and 192 hours). Total ammonia nitrogen, nitrite, nitrate, temperature, dissolved oxygen, pH and conductivity was measured daily while alkalinity, hardness, CO₂ and turbidity was measured every other day.

Total ammonia nitrogen increased over time at the higher density. In both treatments, alkalinity and pH declined over time. With this reduction, unionized ammonia peaked at 48 hours and remained below .005 mg/L thereafter. An average water temperature of 25°C was conducive to biofilter performance.

With respect to low and high density treatments, mean shrinkage by weight was 8% and 7%; and mortality was 4.6% and 1%. Bites and scrapes were evident on fish during the experiment.



IMMUNOSTIMULANT AND DIGESTIVE ENZYME ACTIVITY OF VERATRUM (*Veratrum album*) IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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In this study, the immunostimulant effects of veratrum (*Veratrum album*) and its growth promoting effects were investigated in rainbow trout (*Oncorhynchus mykiss*). For this purposes, veratrum aqueous methanolic extract was added to the basal diet at the rate of 0 (Control), 250, 500 and 1000 mg kg⁻¹. The fish were fed with this diet during 60 days and every 20th day of the study some humoral immune parameters, growth performance and digestive enzyme activity were determined. Superoxide radical releasing was showed no differences in group 250 mg kg⁻¹ (P>0.05) but other group's was significantly decreased on 20th day of the study (P<0.05). At the end of the study, superoxide radical releasing was determined higher than control and 500 mg kg⁻¹ group in 250 and 1000 mg kg⁻¹ groups (P<0.05). Myeloperoxidase activity was significantly increased in all experimental groups especially in 40th and 60th day of the study compared to control (P<0.05). Lysozyme activity was significantly increased in all experimental groups and the highest value was determined in the 250 mg kg⁻¹ group. In the other sampling time lysozyme activity was increased in 250 and 500 mg kg⁻¹ groups (P<0.05). Amylase was significantly decreased in all experimental groups compared to control (P<0.05). Pepsin was decreased in 250 mg kg⁻¹ group and no differences was observed in 500 and 1000 mg kg⁻¹ group. Lipase was showed no differences. Trypsin was significantly decreased in 500 and 1000 mg kg⁻¹ and no differences was determined in 250 mg kg⁻¹ group. In all experimental group SGR was negatively affected and decreased compared to control. All these result showed an increased humoral immune results. Growth was significantly decreased in experimental groups.

THERAPEUTICAL EFFECTS OF TETRA (*Cotinus coggygia*) IN RAINBOW TROUT (*Onchorhynchus mykiss*), AGAINST *Aeromonas hydrophila* INFECTION

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In this study, therapeutical effects of aqueous methanolic extracts of tetra (*Cotinus coggygia*) against *Aeromonas hydrophila* in rainbow trout (*Onchorhynchus mykiss*) were evaluated. Four different concentration of extract (0 mg/100 µl (Control), 4 mg/100 µl, 8 mg/100 µl, 12 mg/100 µl) were prepared. To compare the results, two different type of antibiotic such as florfenicol and doxycycline were also used. After intramuscular inoculation of *A. hydrophila*, tetra and the antibiotics were given orally using feeding needle to the each individual in all experimental group, and at end of the study survival rate was determined. The highest survival rate was found in Florfenicol group (80 %). 12 mg Tetra group, Doxycycline and 8 mg Tetra group survival rate was investigated as 74.44 %, 70% and 70 % respectively. According to our results, tetra methanolic extract is an effective therapeutic against *A. hydrophila* infection in rainbow trout.

INDUCED SPAWNING OF SAHAR *Tor putitora* IN TERAJ REGION OF NEPAL

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Sahar (*Tor putitora*) is a high value indigenous riverine species of Nepal which is declining in its natural habitat and has been declared an endangered species. Limited seed production using natural propagation has restricted its expansion in culture as well as rehabilitation in natural waters. We achieved success in artificial propagation of sahar using synthetic hormone. The breeding program was conducted at the Agriculture and Forestry University (AFU), Rampur, Chitwan and the Center for Aquaculture Research and Production (CARP), Kathar, Chitwan, Nepal during February to April 2017. Sixty five male (0.5-1.8 kg) and forty five female (1.1-2.1 kg) brood fish were reared in 500 m² earthen ponds at 1000 kg/ha and transferred to 25 m² concrete tanks one month before the breeding season. Fish were fed with 32% crude protein feed at 3% body weight per day. Maturity was observed weekly by sampling fish and testing softness of the abdomen. Female broodfish with a soft and extended abdomen were injected with synthetic hormone (Ovulin) at 0.6 mL/kg body weight. Males did not receive any hormone. After 24-26 hours of injection, ova from injected females were obtained by simple hand stripping and fertilized with milt collected from males. The fertilized eggs were incubated in Atkin hatching trays. A total of 16 females were induced to spawn, and they produced 1630.80±184.30 (mean±SE) eggs per kg body weight. Mean hatching and larval survival rates were 78.4±1.9 and 74.7±1.1%, respectively (Table 1). This study demonstrated that mass seed production and larval rearing of sahar is possible in the subtropical region of Nepal using induced breeding. When using natural spawning, a high frequency of females are not taken until they are overly mature, even with daily evaluation of maturity. Induced spawning reduces the number of over-matured females by synchronizing the stripping time of injected brood fish.

TABLE 1. Breeding performance of sahar using inducing hormone.

Parameter	Mean±SE
Breeding duration	2017-2-26 to 2017-4-9
Water temperature (°C)	21.4-28.5
Total female spawners	16
Mean weight of females (kg)	1.47±0.09
Mean weight of males (kg)	0.76±0.05
Mean total egg spawned (count)	2331.40±270.80
Mean egg number per kg body weight	1630.80±184.30
Mean egg number per gram egg weight	104.1±2.5
Mean fertilization rate (%)	96.8±1.5
Mean incubation period (hour)	79-90
Mean hatching rate (%)	78.4±1.9
Mean hatchling survival (%)	74.7±1.1

BLACK SOLDIER FLY MEAL AS A PROTEIN SOURCE IN GILTHEAD SEABREAM FEED-OPPORTUNITY AND LIMITATIONS

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Reducing fishmeal inclusion levels in aquafeed by replacing it with alternative, more sustainable protein sources, is an ongoing effort led by the aquafeed industry with an aim to reduce formulation costs. We evaluated Black Soldier Fly (*Hermetia illucens*) meal produced by the use of fruit wastes substrate, as a replacement for fishmeal in gilthead seabream (*Sparus aurata*) feed. Juvenile seabream were fed with experimental diets in which 0%, 50% or 100% of fishmeal was replaced by BSF meal with an additional treatment group that included 100% replacement of fishmeal by BSF meal with the addition of 5% fish hydrolysate. Treatment effect on specific growth rate, feed conversion ratio, average weight gain, body composition, fish fatty acid profile, muscle, liver and intestine condition were examined. The ability and limitations of using BSF meal as a replacement for fishmeal as an ingredient in juvenile gilthead seabream diet will be presented.

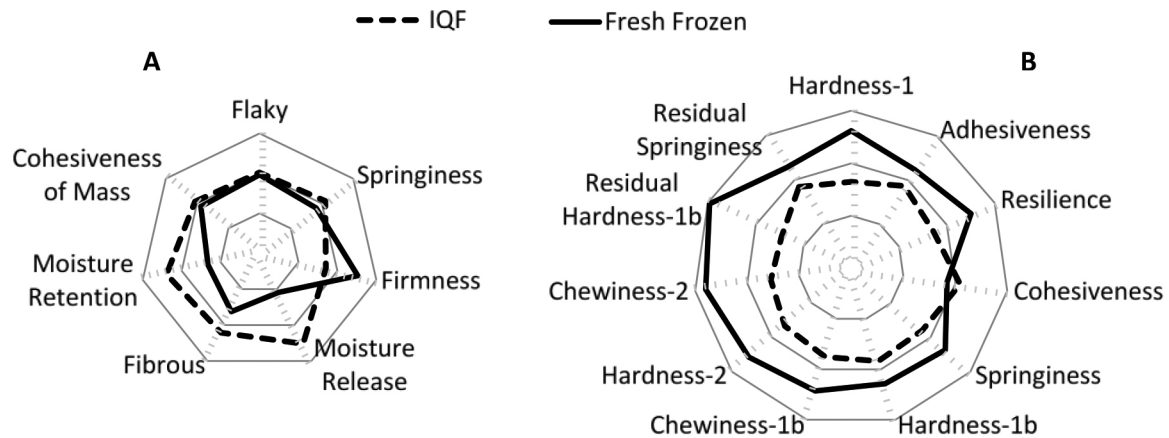
COOKED CATFISH FILLET TEXTURE: A COMPARISON OF SENSORY AND INSTRUMENTAL METHODS FOR FRESH-FROZEN AND IQF FILLETS

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Because of the mild flavor of catfish, texture is one of its most important quality attributes. Consistency in fillet texture quality is important to the consumer, with a difference being easily detected. The determination of texture quality by a trained sensory panel can be costly, but with the use of instrumental methods, catfish processors could more routinely evaluate the texture attributes of products to proactively alleviate potential consumer complaints. The purpose of this research was to compare the instrumental texture profile analysis (TPA) method with the sensory texture panel method for the analysis of fresh-frozen and individually quick frozen (IQF) catfish fillet texture. The TPA evaluation was more successful for identifying differences between IQF and fresh-frozen catfish. A significant different ($P < 0.0016$) was found for the attributes, springiness, resilience, chewiness-1, hardness-1, and residual parameters of springiness, chewiness-1b and hardness-1b, all showing larger values for fresh-frozen. With the sensory quality panel, only firmness was found to be significantly larger for fresh-frozen, while moisture release and moisture retention were significantly smaller. In addition, predictive equations were developed for sensory texture attributes from various TPA parameters. For fresh-frozen catfish, the equations for the sensory attributes flaky, firmness, moisture release, moisture retention, had correlation coefficients of 0.50 or greater. However, the correlation coefficients for IQF fish were less predictable with only flaky and firmness having correlation coefficients 0.50 or greater.

Figure 1. Relative means of the (A) sensory attributes, and (B) texture profile attributes.



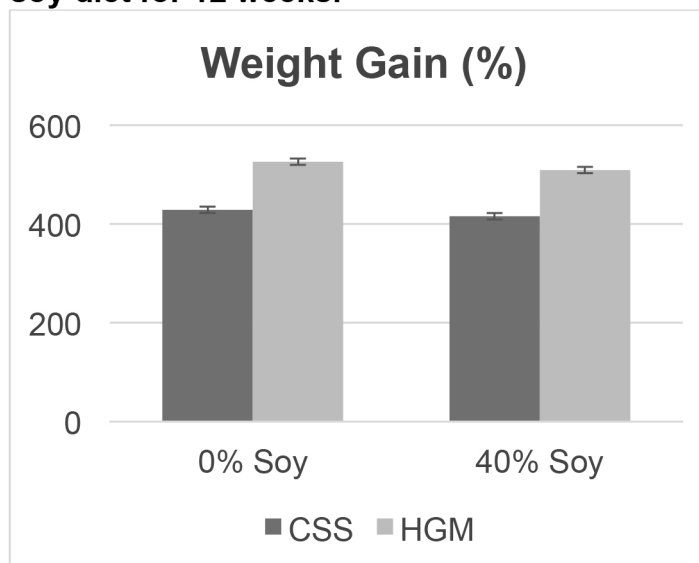
GROWTH PERFORMANCE AND INTESTINAL HEALTH OF TWO DIFFERENT RAINBOW TROUT *Oncorhynchus mykiss* STRAINS FED A HIGH SOY DIET

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In an effort to improve the utilization of soybean meal (SBM) in diets for rainbow trout, fish at the Hagerman Fish Culture Experiment Station (HGM) have been selected for growth on a complete plant-based diet over several generations. Utilizing fish of the F7 generation and a commercial selected strain (CSS), we sought to investigate differences in growth and intestinal health when fed a 40% SBM diet. Triplicate tanks (30 fish, initial weight 77.9 ± 1.1 g) of each strain were fed diets for 12 weeks containing either 0% or 40% SBM in a 2 X 2 factorial design. Five fish from each tank were sampled every four weeks to collect samples of liver and distal intestine for gene expression analysis. At twelve weeks, the HGM strain showed superior growth on both diets ($p < 0.001$) when compared to the commercial strain, with no significant differences in growth within each strain in response to diet ($p = 0.158$; Fig. 1). Analysis of genes related to gut health and inflammation show reduced inflammation in the HGM fish. These results suggest selection for growth on a plant-based diet also drive selection for increased tolerance for dietary soybean meal inclusion.

Figure 1. Percent weight gain between selected strains of rainbow trout fed a high soy diet for 12 weeks.



THE USE OF PROPLEX DY IN AQUACULTURE DIETS, AN OVERVIEW OF EXISTING LITERATURE

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The use of alternative protein sources to replace fishmeal and highly variable protein sources in aquafeeds has been an area of active research for the aquaculture industry. Over the past 10 years, ADM has developed a vegetarian source aquaculture feed ingredient, PROPLEX DY. Research has been conducted in several aquatic and terrestrial species, demonstrating utility as a quality protein source in a wide variety of applications. Previous work on this ingredient has been published using various product names, and, therefore, the objective is to unify the results for PROPLEX DY. PROPLEX DY is a yeast (*saccharomyces cerevisiae*) yeast separated from the wet milling production of ethanol. In this process corn enters a human food production facility and is separated into fiber, protein and starch fractions. The starch is saccharified into dextrose which is used to feed the *saccharomyces* yeast in an ethanol fermentation. The yeast is then separated and dried producing a high quality feed ingredient. It has been evaluated as a protein ingredient in trials with shrimp, salmon, hybrid striped bass, red drum, and trout. Inclusion levels vary slightly across species, but PROPLEX DY has been successfully incorporated into all species tested at 10% of the diet without reducing growth performance. Pond raised Pacific white shrimp were fed 0, 5, 10, and 15% of PROPLEX DY as a protein replacement for 16 weeks and no differences in growth (Achupallas et al., 2016) were observed. PROPLEX DY was successfully included in diets for hybrid striped bass between 7.5 and 15% of the diet (Gause and Trushenski, 2011a). Salmon have been fed diets with PROPLEX DY included at 5, 10, and 15% of the diet (Burr, 2016). Growth performance was maintained across treatments, indicating that salmon can utilize PROPLEX DY as a source of protein. In trout, PROPLEX DY has been successfully included as a protein source up to 15% of the diet both in plant-based diets and also as a partial replacement to fish meal (Hauptman et al., 2014). PROPLEX DY is an excellent source of protein for the aquaculture industry, and results indicate that PROPLEX DY can be incorporated into the diets of shrimp, salmon, hybrid striped bass, red drum, and trout up to 15% as a suitable replacement to existing protein ingredients.

EFFECTS OF HYPER- AND HYPOSMOTIC EXPOSURE AND FEEDING REGIME ON POSTPRANDIAL PROCESSING IN SPOTTED SEATROUT *Cynoscion nebulosus*

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The success of any aquaculture operation depends on appropriate rearing conditions and feeding protocol. Optimizing these parameters is particularly important during early life-cycle stages, when species are experiencing rapid growth. Hatcheries incur one of the highest operating costs in the industry and ineffective rearing practices can increase production time and materials. Although these factors have a direct affect on post-transition grow out, their influence on digestive processing and physiology is still being determined.

Each feed event leads to a number of physiological disturbances, including acid-base fluctuations and a transient increase in metabolism. In many species, digestion is facilitated by a drop in stomach pH caused by an influx of H^+ ions from the blood. Bicarbonate (HCO_3^-) is simultaneously pumped in the opposite direction, leading to an increase in blood pH referred to as the alkaline tide. Digestion also leads to a spike in postprandial respiration (specific dynamic action), caused by the metabolism of feedstuffs and the costs associated with a return to iono- and osmoregulatory homeostasis. These disturbances are energetically expensive and can last up to 48 hours. Here, we use the spotted seatrout (*Cynoscion nebulosus*), an estuarine finfish, to examine the effects of hyper- and hyposmotic rearing conditions on postprandial digestive physiology, likely influenced by the passive and active ingestion of water and reverse concentration gradients at the gills. Our research shows how environmental salinity affects the osmoregulatory requirements associated with digestion and defines whether hyper or hyposaline conditions are optimal for growth in this euryhaline species.

Previous studies examining the alkaline tide have investigated this phenomenon after consumption of one large meal. However, many commercial hatcheries implement a continuous feeding protocol. In theory, this alternate feeding regimen should reduce the degree of acid-base and physiological disturbances associated with the ingestion of feed. Our research further defines whether fish fed continuously display temporal differences in postprandial processing when compared to fish fed the same quantity in one discrete meal a day. We hypothesize that individuals fed continuously will maintain an acidic stomach pH for a longer duration, primed in a state of “readiness” for digestion and that blood acid-base disturbances and metabolism will be reduced. This project identifies whether a hyper or hyposaline environment can be used to minimize the cost of digestion and enhance feed efficiency in finfish fingerlings. It also determines how these benefits relate to fishes under timed and continuous feeding protocol. Further, this project elucidates mechanisms of digestive physiology that can be applied across aquaculture industries throughout the United States.

A REVIEW OF OVER A DECADE OF GLOBAL INVESTMENT IN AQUACULTURE FEED TECHNOLOGIES

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Investment in science and innovation is fundamental to developing effective solutions to global food security challenges, and transferring information and technologies to farmers and industry is essential for scaling up farming technologies and best practices for broad adoption. Capturing the quantitative impacts of investment in research and capacity building requires identifying relevant indicators and metrics, setting targets and benchmarks, and regular monitoring and evaluation of progress. Qualitative impacts, however, are much more challenging to capture and yet of equal importance.

The authors undertook an internal evaluation of the impacts of USAID's investment in a research program that has been funded since late 2006, The Feed the Future Innovation Lab for Collaborative Research on Aquaculture & Fisheries (AquaFish Innovation Lab, previously AquaFish Collaborative Research Support Program). For a decade, AquaFish has partnered with US and host country individuals and institutions to undertake innovative aquaculture research; develop new and improved technologies that are relevant to local conditions and on-the ground needs; transfer information and technologies to fish farmers, industry, academics, government agencies, and others; and enhance individual and institutional capacity so that host countries can build upon successes and lead new lines of research once the program ends.

This review capture both qualitative and quantitative outputs and impacts of AquaFish work in aquaculture feed technologies in Africa, Asia, and Latin America and the Caribbean. With feeds comprising the greatest production cost for fish farmers (about 60-80%), the development and transfer of sustainable and affordable feeds and feed strategies for small- and medium-scale aquaculture has been a significant portion of the AquaFish research portfolio. AquaFish has successfully developed solutions on alternative feeding strategies and feed ingredients that decrease production costs, reduce reliance on animal protein content in feeds, increase overall fish yield, and decrease environmental impacts associated with aquaculture effluents. The review considered innovativeness of the technology, stage of development, technology transfer, impacts, and scalability. Some highlighted feed technologies include: alternate day feeding in the Philippines and Bangladesh that improves farmer profits and reduces effluents, feed formulations for snakehead in Cambodia and Vietnam that utilizes soy protein, and feed development for tilapia in Tanzania that replaces fish meal with earthworm and maggot meal.

AquaFish has advanced sustainable aquaculture globally by developing and transferring these technologies and best management practices to small- and medium-scale producers and other stakeholders. Finding ways to reduce feed costs and responsibly include locally sourced ingredients will continue to have significant and long-lasting benefits for the fish farming sector and more broadly.

POPULATION DYNAMICS OF THE EASTERN OYSTER, *Crassostrea virginica*, IN DELAWARE INLAND BAY'S ESTURARY ECOSYSTEMS PRIOR TO SHELLFISH AQUACULTURE

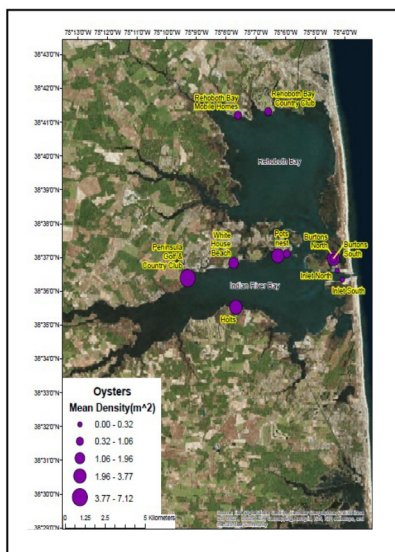
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Delaware is currently the only state on the Northeast Atlantic seaboard without commercial shellfish aquaculture. Legislation passed in 2013 and a lottery leasing process is currently underway with hope of having gear in the spring of 2018. Neighboring states have shown the economic and cultural benefits of functioning industry. Three inland bays in Southern Delaware, due to protection from open waters, ease of access for workers, and a local tourism industry 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 may 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 effects restoration, but baseline statics are essential. This research aims to further understand the current oyster population by 1) developing baseline population densities using standardized survey methods for further use by management to measure changes over time and 2) investigating population dynamics by analyzing genetics of spatfall within the Delaware Inland Bays.

Oyster survey locations were by prioritizing habitat for hard substrate where oysters are more likely to settle and occur naturally, in this system hardened shorelines. Standardized swaths were run along a permanent transect line in order to determine a population density at each location. Results allowed for the production of the first mapping of wild oysters within the Delaware Inland Bays.

Genetic analysis will be done on spat, collected on tiles, to determine the diversity and potential source populations to the inland bays. Microsatellites (MS) offer a good way to measure differences between populations because they are highly polymorphic. Seven primers were chosen from previous research due to the ability of primers to multiplex. The goal of this is to compare allele frequencies among populations. We will be able to identify if hatchery raised disease resistant oysters, from oyster gardening programs have been recruiting locally and hopefully identify potentially source populations for wild oysters.



BLUE CATFISH GERMPLASM DEVELOPMENT AT THE WARMWATER AQUACULTURE RESEARCH CENTER

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Catfish aquaculture remains the largest segment of U.S. aquaculture production in terms of both biomass produced and economic value. Over the last 10 years there has been a substantial increase in production of channel catfish x blue catfish hybrids and current estimates indicate over 60% of U.S. catfish production is hybrid catfish. Therefore, we initiated a program to evaluate the potential for improving hybrid catfish production by evaluation and selection of improved blue catfish germplasm at the Warmwater Aquaculture Research Unit, USDA-ARS. The program is based around collection of diverse blue catfish germplasm, evaluation of effects of blue catfish strains on both purebred blue catfish and hybrid catfish growth and carcass yield, selection of blue catfish for improved hybrid catfish progeny performance, and development of a repository of cryopreserved blue catfish sperm. The ultimate goal is to release improved blue catfish germplasm to U.S. catfish farmers that will allow them to produce hybrid catfish with improved growth and carcass yield.

Blue catfish from 6 'geographic strains' have been collected and are being evaluated for both purebred blue catfish and hybrid catfish progeny growth and carcass yield. Current evaluations have shown the Rio Grande blue catfish strain produces purebred progeny with consistently better growth and carcass yield compared to other strains tested. The D&B strain blue catfish, a strain widely used by the industry, has exhibited the second best growth performance in purebred blue catfish evaluations. We are currently collecting data on the correlation between purebred and hybrid progeny growth and carcass yield for blue catfish sires from all 6 strains and initial results indicate there is a positive correlation between purebred and hybrid performance for both traits. Two blue catfish strains are currently being developed for potential release to the industry: one population is being developed from the Rio Grande strain of blue catfish due to its superior purebred performance and the other population is being developed as a more genetically diverse population from a mixture of the 4 best performing blue catfish strains from our evaluations (Rio Grande, D&B, Texas and Mississippi River strains). We have cryopreserved sperm from over 250 blue male catfish and this collection plays a critical role in our breeding program to evaluate and improve blue catfish germplasm.

Blue catfish germplasm will be released to U.S. catfish farmers when evaluations indicate a release is warranted. The release of improved blue catfish germplasm will improve hybrid catfish performance and provide economic benefit to U.S. catfish producers.

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Outdated guidance has been a barrier to long-range coastal planning for this use with some local governments banning commercial net pen aquaculture. The project is designed to provide growers and regulators with today's science and best practices for managing eight existing facilities and reviewing new proposals. As the state with the largest net pen production, the new guidance was on track to being a national model.

This presentation shares early results of the net pen guidance project and lessons learned within Washington’s social, cultural, and political context.

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DEVELOPMENT OF GROWTH MODELS FOR HATCHERY PRODUCTION USING DATA MINING AND MACHINE LEARNING METHODS

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Aquaculture companies are drowning in data, but starving for knowledge. Data can tell a lot about the parameters influencing the success of production (from water quality parameters, to feed types, feeding rates and practices, management strategies and more). They can also be used to identify patterns, trends, problem causes and also to develop models.

Data mining and machine learning can help convert data into knowledge, which can be used to dramatically improve performance. Whether it is used to drive new business, reduce current costs or gain a competitive edge, data mining can be seen as a highly transformational asset for every fish farming organization, be it large or small. This secure and unobtrusive collation of data enables the analysis of huge volumes of historical data to deliver informed business driven knowledge from models built for prediction, estimation, and other inferences involving uncertainty. In aquaculture, it can be used to support smarter decisions, better production and efficient management.

The work to be presented is related to the use of advanced machine learning methods for the development of growth models for Sea Bream, Sea Bass and Meagre for Selonda SA. The company is one of the biggest producers of sea bass and sea bream worldwide, producing more than 30000 tons per year in 55 farms. It operates six hatcheries that produce over 150 million juveniles per year. The work to be presented was conducted using large datasets of average weight measurements. These datasets have been explored using descriptive statistics techniques in order to:

- Preprocess data, so as to exclude misleading and faulty entries from the analysis
- Detect outliers in terms of growth and fish density and remove them for the analysis

Then, Machine Learning methodologies, such as Generalized Linear Models (GLM), Generalized Additive Models (GAMs), Support Vector Machines (SVM), were used in order to create models able to predict fish growth in terms of fish density, average weight and temperature. After the 10 folds cross-validation process the best models were chosen and utilized to the predict growth and create production plans.

The results of this work are new, more accurate models that promote informed and precise strategic business decisions, previously not possible, thus enabling a competitive edge to manifest. The new models are presented, along with how data mining can support knowledgeable methods for production and management and richer decision making capabilities, based on a holistic data framework.

USE OF AQUI-S20E, TRICAINE-S, AND AQUACALM TO SEDATE RAINBOW TROUT TO HANDLEABLE

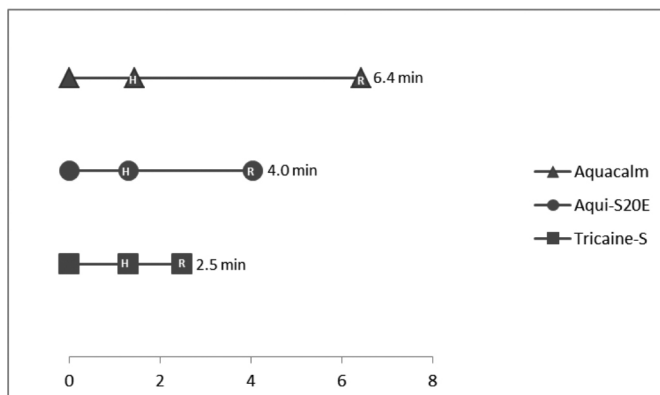
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Fish sedatives are used to achieve various stages of sedation to facilitate fisheries research and management, such as “light sedation” for transportation, “loss of equilibrium” for routine handling, and “deep sedation” for surgical procedures. Only Tricaine-S (MS222) is approved by the U.S. Food and Drug Administration (FDA) as a fish sedative/anesthetic and available in the U.S. However, efforts are under way to gain FDA approval of AQUI-S20E (10% eugenol) and there is interest in gaining such an approval for Aquacalm (metomidate hydrochloride). For comparison purposes, we sedated small fingerling Rainbow Trout *Oncorhynchus mykiss* with AQUI-S20E, Aquacalm, or Tricaine-S to assess time to handleable and recovery.

Thirty Rainbow Trout (9.8 cm) were individually sedated to handleable under static conditions with either 25 mg/L eugenol, 80 mg/l MS222, or 6 mg/L metomidate. A fish was deemed handleable when it lost equilibrium and the ability to swim, could easily be caught by hand, and did not struggle while being weighed or measured. When a fish became handleable, it was removed from the sedative solution and transferred to a recovery tank of fresh, flowing water. Fish were considered recovered when they regained equilibrium, resumed normal swimming behavior, and could avoid a net handle placed in their path.

Regardless of sedative used, mean times for fish to become sedated were < 2 min and individual times ranged from 0.8 to 3.1 min. Mean recovery times for fish sedated with eugenol, MS222 and metomidate were 2.1, 1.2, and 5.0 min, respectively. Mean elapsed time for fish to become sedated and recover was longest for metomidate (6.4 min) and shortest for MS222 (2.5 min). Based on these results, doses of all sedatives effectively sedated fish to handleable in times that would be considered reasonable by most users.



AN OVERVIEW OF PROPLEX T IN AQUAFEEDS: A HIGH-QUALITY PROTEIN SOURCE FROM DRIED FERMENTATION BIOMASS

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Sustainable and cost effective alternatives to fish meal (FM) as a protein source in aquafeeds continues to be a driving focus in the aquaculture industry. Currently, a variety of traditional products from agricultural and livestock production have provided effective alternatives for partial to full replacement of FM depending on the species and life stage. In addition to these traditional sources, there is growing interest in a number of manufacturing coproducts such as bacterial dried fermentation biomass (DFB). In response, ADM research developed a high-quality protein rich DFB product called PROPLEX T from its threonine production stream. PROPLEX T is the coproduct from the industrial production of L-threonine through fermentation using *Escherichia coli*. Following the fermentation process, crystalline L-threonine is extracted leaving the residual protein-rich biomass. That biomass is dried and further processed, with the addition of soybean oil, to produce PROPLEX T.

Although research in practical diets of aquatic and terrestrial species have been conducted with PROPLEX T, consolidating the literature into an overview provides a better perspective of its application and value in feeds. Studies have shown inclusion levels higher than 10% in some species resulted in equivalent growth performance to FM controls, but practical commercial levels will likely be between 2-10% of the formula. In a trial with *Litopenaeus vannamei*, feeds formulated with 0, 2, 4, 6, and 12% inclusion levels of PROPLEX T did not compromise growth performance (unpublished data). In the diets of juvenile red fish, PROPLEX T successfully provided up to 30% of the dietary crude protein (Rosales et al., 2017). The FM was replaced with PROPLEX-T at 0, 4, 8, and 12% in juvenile Florida pompano diets providing similar growth performance to the controls, and it was suggested that higher inclusion levels should be investigated (Rhodes et al., 2015). Inclusion of up to 10% in channel catfish diets did not result in any significant decreases in fish performance (unpublished data). Additionally, a terrestrial study with weanling pigs has shown that PROPLEX T is an excellent source of amino acids and metabolizable energy (Almeida et al., 2014).

Collectively, the research demonstrates the functionality of PROPLEX T as an alternative protein source in a variety of applications. This presentation will provide an overview of the research conducted to determine the practical inclusion levels and discuss its potential in the diets of finfish and shrimp.

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LAND USE EFFICIENCY IN SHRIMP AQUACULTURE

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The amount of land devoted to pond water surface area for penaeid shrimp farming was estimated to be 2,260,000 ha. This area resulted in production of 4,876,586 metric tons (t) of shrimp in 2015. About 1,400,000 ha of pond area were in extensive culture that produced around 500,000 t of shrimp (mainly *Penaeus monodon*) – average yield of 0.36 t/ha/yr. The increase in shrimp production since 2000 has resulted almost entirely from an increase in semi-intensive and especially intensive culture of *Litopenaeus vannamei*. Annual, global production of this species increased from 154,515 t in 2000 to 3,879,786 t in 2015, while during this period, production of *P. monodon* and a few other shrimp species by aquaculture fluctuated between 883,644 t and 1,095,457 t with no trend of increase or decrease. The estimated average yield of *L. vannamei* was 5,700 kg/ha/yr in 2015, but average production tended to be much greater in Asia than in Latin America.

Semi-intensive and intensive shrimp culture is feed-based. The amount of land used for producing ingredients for the 7,450,000 t of shrimp feed reportedly used in 2015 was estimated as 1,862,500 ha. The global FCR for penaeid shrimp likely was around 1.7, and land use for shrimp feed ingredients was about 0.425 ha/t of shrimp. At a yield of 5,700 kg/ha/yr of shrimp, each tonne of shrimp would require 0.175 ha of land for pond surface area and 0.425 ha of land for feed ingredients – 0.60 ha/t of shrimp. Production of 1 t of shrimp by extensive production would require 2.78 ha of pond water surface area. Although extensive shrimp production does not require feed, it uses, on average, 4.6 times more land than does feed-based culture per tonne of shrimp. The most efficient way to conserve land in shrimp farming would be to phase out extensive culture in favor of feed-based culture and to further intensify production in semi-intensive and intensive systems.

WATER SCIENCE IN AQUACULTURAL EDUCATION

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In traditional agricultural education at the university level, students are required to take a basic soil science class, and in some agricultural curricula, additional classes such as soil fertility, soil chemistry, and soil microbiology are required. This, of course, is because crop plants grow in soil. Many aquaculture students complete M.S. and Ph.D. programs without taking so much as a basic water science class. This is unfortunate, because aquaculture students need an in-depth understanding of the physical, chemical, and biological characteristics of water – the milieu for aquaculture production – as badly as traditional agricultural students need to know about the properties of soils in which crops grow. Aquaculture students typically take a variety of courses in which they learn a lot of terms and definitions related to water science. They also are taught the optimum ranges of water quality variables for fish, shrimp, and other aquatic animals. However, many aquaculture graduates have little understanding of the complex relationships among water quality variables and the effects of these variables on aquatic animals and aquacultural production. I will provide some opinions and conclusions that I have reached while teaching water science and water quality management to aquaculture students for the past 47 years.

OBSERVATIONS ON THE BEHAVIOR AND FOOD PREFERENCES OF *Macrobrachium rosenbergii* IN DEEP WATER CULTURE AQUAPONIC GROW BEDS IN ARIZONA

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Thanks to the donation of 130 juveniles to the aquaponics class at the Mesa Community College Center for Urban Agriculture, in the fall of 2017, the opportunity arose to observe the behavior of the freshwater prawn *Macrobrachium rosenbergii* in two Deep Water aquaponic culture beds. Slightly different in size and method, the first was a classic miniaturized version of the University of Virgin Island technique with an 18 inches deep grow bed tank (0.42 m) with 40 ft² (3.71 m²) of grow bed space. The second system with a depth of 21 inches (0.53 m) and 32 ft² of grow bed space (2.97 m²) was a new technique called Self Contained Aquaponics (S-Cap) designed to easily convert existing small splash pools to aquaponic mini-farms.

Each system was stocked at a rate of 1.5 juvenile prawns per ft² of grow bed surface area (16.15/ m²) resulting in 60 prawns in the UVI style-DWC and 48 prawns in the S-Cap). Stocked on August 7th 2017 they were maintained in the DWC beds until harvest when the tank water began to dip below 70 F/21 C on November 1st and 3rd 2017. Each grow bed was planted with two types of tomatoes, collard greens, broccoli, zinnias, pima lima beans, Armenian cucumbers, sweet potato vines. There were also guppies (*Poecilia reticulata*) stocked in the grow beds for mosquito control. Tilapia (blue (*Oreochromis aureus*) x Nile (*O. niloticus*) hybrid and goldfish (*Carassius auratus*) were stocked in the system fish tanks (separate from the grow beds). The prawns were also provided 32% protein floating tilapia pellets to maximize the opportunity for good growth.

The observations were:

- Survival was 78% in the UVI and 197.7 grams and 58%/113.53 grams in the S-Cap with mean sizes of 4.12 grams and 4.05 grams respectively.
- The prawns showed a decided preference for plant roots over the floating pellets or even capturing the guppies for supplemental food to the point of inhibiting root development and overall plant growth.
- Of the plant roots, the prawns demonstrated a decided preference for the roots of the beans and tomatoes.
- By providing heads of red leaf lettuce for the prawns to eat eventually at a rate of 2 ½ pound heads per week, root growth in most species of plants was partially restored except for the beans and tomatoes.
- Once the prawns were harvested, plant grows accelerated significantly as demonstrated by the rapid development of new roots and leaves.

CONVERTING BACKYARD SPLASH POOLS TO AFFORDABLE AQUAPONIC MINI-FARMS

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Urban agriculture is going mainstream in cities across the United States. For example Phoenix Arizona has written urban food systems into its General Plans and is developing an urban food strategy that includes urban agriculture for City Council consideration in 2018. In that most homes in cities like Phoenix have backyards, and the opportunity to grow healthy, sustainable, abundant and most importantly affordable food becomes significant. Aquaponics has been identified as one of a plethora of technologies that may be used to address this challenge. One significant problem that must be overcome however is that current methods of aquaponics tend to be expensive and complex limiting access to many potential users.

One powerful low cost solution to this conundrum is to convert the thousands of backyard metal-framed 10 to 12 foot splash pools that are scattered across the city to aquaponic mini-farms. Conceptually it would be far better for these pools to be growing food rather than the mosquitos that far too many of them actually are. This paper explores the effectiveness of a very simple technique where the pools are converted to DWC (Deep Water Culture) aquaponics by dropping in a food safe or fish safe liner followed by installing simplified versions of traditional aquaponic components including a fish tank and clarifier within the pool as well. This analysis of this concept looks at annual food production, types of seafood and vegetables that can be produced, construction costs and times and costs of operation. The analysis also looks at how these easy to build mini-farms may be utilized within urban-backyard settings, schools, for short-term disaster relief and food desert mitigation.

CAN AQUAPONICS HELP RESTORE THE UNITED STATES AQUACULTURE INDUSTRY

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Over the past 15 or so years a number of authors and aquaculture leaders have noted the “decline” of the United States aquaculture industry. Reasons cited for this include:

- Easy access to abundant high quality and low cost imported product
- The United States produces only 10% of the seafood it eats
- US Catfish farming reached peak production in 2002
- US Trout production has flattened.
- US Net pen farming of salmon may have also peaked.
- “Highest and Best Use” requirements and environmental regulations and high costs make it difficult to site new traditional farms.
- Climate Change.
- Food safety issues
- An aging workforce echoing what is seen in other agricultural sectors
- A Millennial preference for urban employment
- The scale back or elimination of university programs in aquaculture make it difficult to find qualified employees skilled in in biosecurity, fish culture and fish hatcheries.
- Cutbacks in extension programs
- Lack of innovation in system designs
- Lack of young farmers

Recently however, there is increasing evidence that the comparatively young discipline of aquaponics may be helping to reverse many of the challenges mentioned above. These RAS (Recirculating Aquaculture Systems) based farms where often-edible plant production is used as the polishing stage for the removal of nitrate are growing rapidly in popularity particularly in urban areas. An outgrowth of the hyper-local urban healthy food movement and concerns over the great recession, the current efforts in aquaponics have expanded in the “early adopter” market since approximately 2008. More recently in response to growing public demand for local healthy food and the increasing sophistication and flexibility of these systems, the discipline has “jumped the chasm” and is now providing a variety of solutions for urban and rural needs. They are being applied as excellent methods of teaching STEAM education in elementary and high schools. Frequently in urban agriculture friendly cities, more and more colleges and universities are offering accredited courses in aquaponics thus training new farmers. They are in thousands of backyards across the nation. In addition and in part thanks to the advent of new business models provided by the AgTech (Agriculture Technology) enterprise sector, multi-million dollar investments in aquaponic businesses are now being seen. As recently reported from Wisconsin, the result of this is a noticeable increase in the number of artisanal and commercial aquaculture farms in this state. Most of these new farms are using aquaponics as the method of food production and owned by young people. Overall, this suggests that many of the stated concerns are in part, being addressed through the growth of aquaponics.

Through the lens of a SWAT (Strengths, Weaknesses, Opportunities and Threats) analysis, this presentation will explore the possibility that the growing aquaponic sector of aquaculture may be helping to restore the US aquaculture industry.

PERFORMANCE OF RAINBOW TROUT *Oncorhynchus mykiss* FED CONVERTED SOY PRODUCTS

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Defatted soybean meal, initially pretreated with thermo-mechanical methods, was microbially processed and formulated into experimental diets (25% soy inclusion) for a Rainbow Trout growth trial. The dietary treatments included a bioprocessed soy product (BP-SBM) without additions, BP-SBM with fungal cells, BP-SBM with fungal slurry, BP-SBM with enzyme, washed BP-SBM, base BP-SBM, and a fishmeal (FM) control diet. Performance measures including biomass gain, relative growth (RG), specific growth rate (SGR), feed conversion ratio (FCR), feed consumption, and condition factor were obtained at 3-week intervals during the 15-week trial. No significant differences were noted among growth parameters for the first 6 weeks of the study ($p>0.05$). RG ($p=0.03$) and SGR ($p=0.03$) significantly differed among treatments at the 9-week interval, while RG was the only parameter with a significant difference among treatments after 12 weeks ($p=0.04$). FCR was the only parameter that provided a significant response among treatments at the 15-week sampling ($p=0.03$). At the end of trial, 3 individuals per tank were randomly selected to assess hepatosomatic (HSI), splenosomatic, viscerosomatic and visceral fat indices, and fillet yield (%). HSI was the only index that significantly differed among treatments ($p=0.04$). This study indicates that health and growth performance are not inhibited when 46% of FM is replaced with microbially processed soy in the diets of Rainbow Trout.

IMPACTS ON RAINBOW TROUT *Onchorhynchus mykiss* REARING PERFORMANCE OF DIETARY BIO-PROCESSED PLANT-BASED PROTEIN AND WATER VELOCITY

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Carnivorous fish species, like rainbow trout, *Onchorhynchus mykiss*, require high levels of dietary protein. Historically, fish meal (FM) has been the primary protein source, but its supply is variable, limited, and relatively expensive. Alternative protein sources are needed to meet the dietary protein demand for ever-increasing aquaculture production. In addition to specific dietary requirements, salmonids may react positively to exercise during hatchery rearing, with increased growth and feed conversion. While fish meal alternatives have received considerable research focus, no studies have examined the use of plant-based diets in conjunction with exercise.

Two experiments, using different rainbow trout strains (Erwin x Arlee and Shasta), examined the dietary replacement of fish meal with a novel fermented soybean meal (FSBM) product. The dietary treatments were either FM or FSBM replacing 60% or 80% of the dietary FM. Flow treatments were the minimal velocity (averaging 3 cm/s for both experiments) required to keep tanks self-cleaning or an elevated level six to ten times that of the minimal velocity, depending on the experiment.

Feed conversion ratio (FCR) was significantly improved in fish fed diets where 60% of the FM was replaced with FSBM (Table 1). However, specific growth rate (SGR) was not significantly different among the diets with either strain. There were also no significant differences in trout weight, length, condition factor, relative fin length, hepatosomatic index, splenosomatic index, or viscerosomatic index among the dietary treatments. None of the dietary treatments were significantly affected by water velocity. However, compared to lower water velocities, higher velocities produced significantly poorer FCR and SGR in Shasta strain rainbow trout (Table 2). FCR was also significantly higher in the high velocity tanks of the Erwin-Arlee strain, but SGR was not significantly different between the velocities. With the Shasta strain, both the hepatosomatic and viscerosomatic indices were significantly different between the velocity treatments. Fin indices were not significantly different between the velocities in either strain.

In conclusion, up to 80% of the FM component of Shasta strain rainbow trout diets can be replaced with FSBM with no decrease in rearing performance. Also, up to 60% can be replaced in Erwin x Arlee diets. Diets containing FSBM produced similar results to the FM control under both rearing velocities. However, higher rearing velocities generally led to poorer growth and FCR.

Table 1. Mean (\pm SE) tank rearing data values for FCR and SGR for two studies evaluating dietary FSBM, both water velocities combined. Means with different letters in the same row differ significantly ($P < 0.05$).

Strain	% FSBM		
	0%	60%	80%
Erwin x Arlee			
FCR	1.10 \pm 0.02 z	1.04 \pm 0.03 y	NA
SGR	1.45 \pm 0.02	1.41 \pm 0.02	NA
Shasta			
FCR	1.09 \pm 0.04 z	1.04 \pm 0.01 zy	0.97 \pm 0.02 y
SGR	1.40 \pm 0.04	1.40 \pm 0.04	1.38 \pm 0.02

Table 2. Mean (\pm SE) tank rearing data values for FCR and SGR for two studies reviewing differing velocities, all diets combined. Means with different letters in the same row differ significantly ($P < 0.05$).

Strain	Velocity	
	Low	High
Erwin x Arlee		
FCR	1.02 \pm 0.02 y	1.31 \pm 0.02 z
SGR	1.46 \pm 0.02	1.41 \pm 0.02
Shasta		
FCR	1.00 \pm 0.02 y	1.06 \pm 0.03 z
SGR	1.34 \pm 0.02 y	1.45 \pm 0.02 z

NUTRITIONAL VALUE OF MODIFIED CARINATA MEAL AS PROTEIN SOURCES FOR HYBRID STRIPED BASS *Morone chrysops* x *Morone saxatilis*

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Carinata is an oilseed crop undergoing genetic modification to increase the seed oil and erucic acid content for jet fuel production. The solvent extracted seedmeal may be used in animal diets because of its high protein (>40%) content. However, its utilization in fish diets is limited by high crude fiber (>9%), glucosinolates and sinapine. We processed solvent extracted carinata seedmeal by aerobic conversion (ACC) followed by a single wash, or a double wash (WC) without aerobic conversion. Four diets were formulated to contain ~42% protein and 12% lipid, including a fish meal reference diet. All diets contained 10% fish meal and 10% chicken by-product meal. Test diets contained 10 or 30% ACC, or 30% WC.

A 106-day trial was carried out in a recirculating aquaculture system to compare the growth performance of hybrid striped bass (HSB) fed ACC and WC meals. Twenty fish (~19 g each) were stocked in 30 gallon tanks and 6 replicates were used for each treatment. Temperature, pH and dissolved oxygen were monitored daily and averaged 25.6 ± 0.7 °C, 7.5 ± 0.5 and 9.0 ± 1.1 mg/L, respectively. Total ammonia and nitrite were monitored weekly and averaged 0.54 ± 0.28 mg/L and 0.15 ± 0.07 mg/L, respectively. Feed consumption and mortalities were monitored daily and weight gain every three weeks. At the end of the study, three fish per tank were sacrificed, blood was sampled, and then dissected to obtain livers, spleens and visceral fat; all fish were counted and weighed.

Survival (>99%) was similar among treatments. The HSB fed 30% WC had a similar weight gain to HSB fed the reference diet and 30% ACC but better than HSB fed 10% ACC ($p=0.010$). Relative growth ($p=0.015$) and specific growth rate ($p=0.0016$) followed the same trend as weight gain. Fish fed 30% WC and 30% ACC had a similar feed consumption to that of fish fed 10% ACC but better than fish fed the reference diet ($p=0.003$). The fish fed the reference diet had a similar consumption to those fed 10% ACC diets. The feed conversion ratio of fish fed 30% WC was similar to that of fish fed the reference diet but better than that of fish fed 10% and 30% ACC ($p=0.001$). The protein efficiency ratio of fish fed 30% WC was similar to that of the reference but better than that of 10% ACC and 30% ACC diets (0.001). The ACC diets had a lower protein efficiency ratio than the reference diet. Protein deposition was highest in fish fed the highest carinata meals ($p=0.019$). Apparent net protein utilization corresponded with feed conversion ratio (0.011). Whole body protein increased with increasing carinata in the diets (0.010). No dietary effect was detected for whole-body ash or moisture contents of the fish. The viscera-somatic, spleen-somatic and visceral fat indices were similar among treatments. However, HSB fed 30% WC had smaller livers and higher condition factors than fish fed other treatment diets. Unconverted carinata increased the Hk content of blood ($p=0.011$). HSB fed 10 and 30% ACC had similar hemoglobin (Hb, $p<0.001$) contents that were higher than those of fish fed the reference and 30% WC. There was no dietary treatment effect on lysozyme or ACH_{50} .

Our results show that processed carinata seedmeal is a viable protein ingredient for fish diets. In particular, double washing improved feed conversion and protein efficiency more than aerobic conversion with a single wash. Carinata seeds are high in iron and this may account for the differences observed in Hk and Hb among diets, given that the extra wash step in WC likely reduced iron content.

IMMUNOMODULATION IN FRESHWATER SPECIES FED BIOPROCESSED SOYBEAN MEAL

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The incorporation of bioprocessed soybean meals (BSBMs) as a protein replacement for marine-derived fish meal (MDFM) has gained momentum with the potential for aquafeed cost reduction, improved growth performance, and reducing exploitation on wild fish stocks. Further, potential health benefits and improved production performance resulting from bioprocessed feed ingredients have led to research on the immune-enhancing properties of plant-based ingredients. A series of four finfish feeding trials were conducted to investigate immunomodulation induced by BSBM or immunostimulant ingredients in yellow perch (*Perca flavescens*) and rainbow trout (*Oncorhynchus mykiss*), two domestically cultured species.

Nonspecific immune components were characterized by trials incorporating graded inclusion levels of polysaccharides and minerals. A 1-1.5% inclusion of a commercial exopolysaccharide (EXOP) with the BSBM provided enhancement to the phagocytic capacity and superoxide production of yellow perch head-kidney macrophages. Study results indicate a variation in short-term innate immune responses as a result of feeding mineral (zinc and selenium) and polysaccharide (β -glucans and EXOP) supplemented to BSBM diets.

In additional trials, rainbow trout fed BSBM-based diets were challenged with *Flavobacterium psychrophilum* (17830), a virulent salmonid pathogen found in hatchery operations, and we determined innate immune responses. Macrophage respiratory burst activity (RBA) was found to be significantly different at day 46 ($P=0.004$) and post-challenge 10 ($P<0.001$), indicative of immunomodulation due to diet. Following the 10d challenge, a BSBM diet supplemented with β -glucans had the lowest mortality rate (40.47%). In another trial where rainbow trout were fed soy-based diets, physiological changes (plasma cortisol, plasma ion concentration, hematocrit, and macrophage activity) were evaluated following acute production stressors. Day 30 RBA values were different among dietary treatments in the stressed ($P=0.004$) trout and the day 60 unstressed trout ($P<0.001$). There appeared to be some secondary stress modulations between unstressed and stressed treatment groups at day 30 ($P<0.001$) and day 60 ($P<0.001$), but no apparent dietary effects on the acute cortisol concentrations.

Continued development and assessment of novel plant-based ingredients, with a target of stress reduction and immune enhancement, may benefit producers by allowing increased stocking densities and reducing losses due to fish transport, thus further contributing to the needs of a rapidly expanding domestic aquaculture market. Small additions of immunostimulants and the incorporation of bioprocessed soybean meal appear to modulate (non-antibiotic) the fish immune system and aspects of stress physiology.

GASTROINTESTINAL MICROBIOTA AND IMMUNOLOGICAL RESPONSES OF RAINBOW TROUT *Oncorhynchus mykiss* FED MODIFIED SOYBEAN MEAL INGREDIENTS

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Alternative plant-based protein sources are of economic interest to aquaculture feed manufacturers and producers, in an effort to replace expensive marine-derived protein in feeds. Traditionally, high-levels inclusions of soybean meal have been found to present metabolic and health issues resulting in reduced growth performance. Advanced soybean meal processing methods may allow higher inclusions in salmonid aquafeed formulations but effects on gastrointestinal histology and microbiota require further investigation.

Two 60-day juvenile rainbow trout (*Oncorhynchus mykiss*) feeding trials were conducted to compare the effects of fishmeal (FMC), defatted soybean meal (SBM), bioprocessed soybean meal (BSBM, two levels), and commercial soy protein concentrate (CSPC) ingredients on intestinal histology, innate immunity, and microbiota profiles. The first feeding trial investigated intestinal morphology, lysozyme levels of intestinal homogenate, and intestinal leukocyte phagocytosis (13.70±0.18 g fish weight). Results indicated no significant differences in intestinal immunoglobulin concentrations ($P=0.41$) or gut leukocyte phagocytosis at day 15 samplings ($P=0.41$). Intestinal lysozyme content was found to be highest at day 60 in the BSBM treatment group ($P<0.01$). No significant differences across treatments were observed in histological profiles at day 0 ($P=0.75$), day 15 ($P=0.08$), and day 60 ($P=0.22$).

The second trial evaluated the intestinal microbiota, incorporating 16s rRNA analyses (42.30±0.26 g fish weight). ANOSIM analysis for microbiota beta diversity showed differences among all dietary treatment groups, with the exception of the high and low levels of BSBM ($P=0.40$). There were also differences observed in the alpha diversity ratios, with significant differences in gut communities among dietary treatments as demonstrated by Chao-1 ($P<0.01$) and Shannon indices ($P=0.02$).

The incorporation of processed soy-based proteins alters the microbial community composition within the distal intestine, and modulates lysozyme concentrations within the distal intestinal tissues. Definition, development and maintenance of beneficial gut microbe communities in rainbow trout fed soy ingredients requires additional study.

NITRIFICATION AND DENITRIFICATION IN ZERO DISCHARGE AQUACULTURE

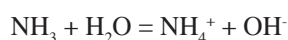
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Many recirculating aquaculture systems rely on nitrification to control ammonia levels. Disadvantages of nitrification include need to discharge water to reduce accumulating nitrate and acid production from the nitrification reaction requiring continuous replacement of culture alkalinity. The theoretical nitrification reactions yield two equivalents of acid per equivalent of nitrogen oxidized;



This suggests an alkalinity demand of 500 gm of NaHCO_3 per kg of feed added (at 35% protein) however, observed alkalinity demand is only 250 gm/kg-feed. This difference can be explained by ammonia addition from aquatic animals generating alkalinity from the weak-base reaction;



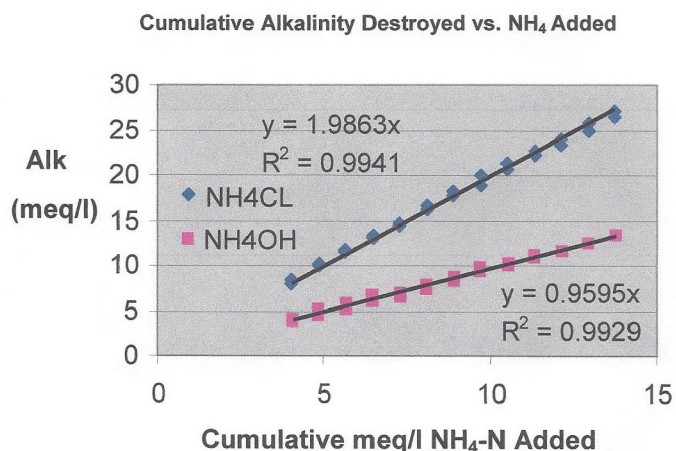
In aquaculture, in-situ ammonia addition contributes one (unaccounted for) equivalent of alkalinity per equivalent of nitrogen added, whereas in wastewater treatment incoming alkalinity measures the impact of influent ammonia. To illustrate, four 5-gallon nitrification reactors were operated in a laboratory setting. Oxygen was maintained above 4.0 mg/l with bubble aeration and pH was maintained between 8.0 and 9.0 with NaHCO_3 or CO_2 addition. Ammonia was added at 12 mg/l-day as NH_4OH to two buckets and NH_4Cl to the two buckets. NaHCO_3 was added as needed to maintain alkalinity at 2 to 4 meq/l. The observed alkalinity destruction illustrates the impact of ammonia hydroxide reducing needed alkalinity supplementation (Figure 1).

Field aquaculture operations have shown that culture systems incorporating denitrification reactors do not require any alkalinity addition. This is the effects of both, of in-situ alkalinity generation from ammonia excretion, combined with alkalinity generation from denitrification;



Denitrification reactors need not be expensive or complex to operate. At Kent SeaTech, in California, water circulation through constructed wetlands provided NO_3^- reduction, while at Clemson University solids settling tanks provided the same function for zero-discharge marine shrimp culture. Decaying organic matter in wetlands or anaerobic decomposition of settled solids provided organic energy to drive denitrification. RAS units designed around integrated nitrification/denitrification reactors do not required bicarbonate supplementation and need not discharge NO_3^- to the environment.

Figure 1: Alkalinity destruction vs. ammonia nitrogen addition as NH_4Cl or NH_4OH addition



OBSERVATIONS OF AN INVERSE AMMONIA TOXICITY RELATIONSHIP IN THE BRINE SHRIMP *Artemia*

David E. Brune

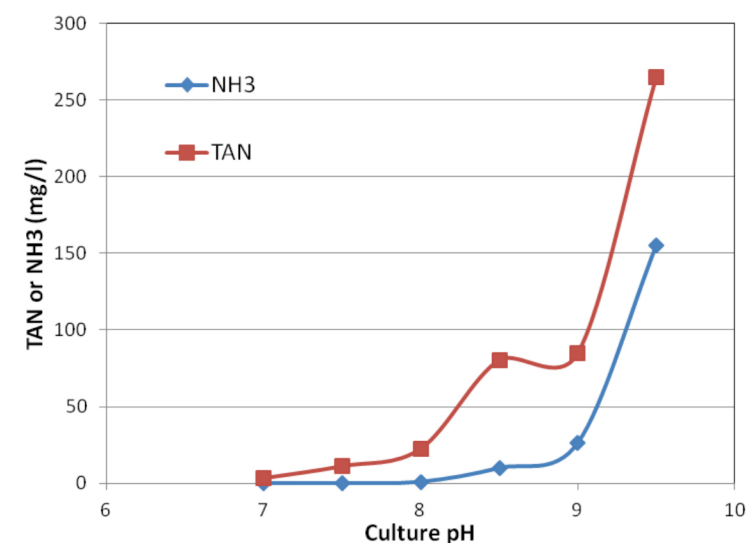
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Brine shrimp are known to be capable of survival, growth, and reproduction in extreme environments of salinity, pH and total ammonia nitrogen concentration. Reported free ammonia LC_{50} values for brine shrimp have been observed to be in excess of 100 mg/l compared to less than 1 mg/l for most other aquatic animals. However catastrophic toxicity in brine shrimp culture has been reported at low levels of free ammonia in low pH waters.

Laboratory experiments were conducted to shed light on the relationship between ammonia toxicity and pH in adult *Artemia*. A matrix of acute ammonia exposures of brine shrimp to free ammonia ranging from 0.001 to 200 mg/l at pH values of 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5 was designed and conducted. Ten adult brine shrimp were placed in 250 ml Erlenmeyer flasks and exposed for 24 hrs. Solution pH was adjusted and maintained with additions of either CO_2 or Na_2CO_3 . At the end of 24 hrs the results were used to establish 24 hr LC_{50} values of free ammonia and total ammonia nitrogen (TAN) at varying pH levels. The results suggest that in spite of exponentially increasing free ammonia at elevated pH, brine shrimp mortality at pH 7.0 was radically more severe as opposed to pH 9.5. The observed LC_{50} for free ammonia at pH 7.0 was only 0.016 mg/l compared to LC_{50} value of 155 mg/l at pH 9.5.

Apparently, brine shrimp are capable of employing some form of protective mechanism allowing them to resist free ammonia toxicity at high solution pH. This observation has important consequences in understanding how brine shrimp are capable of growth in heavy algal bloom conditions where culture pH is often elevated. Furthermore, this characteristic has important implications in management of high density brine shrimp culture for use in harvesting and converting algal or bacterial cultures into brine shrimp biomass

Figure 1: *Artemia* TAN and free-ammonia LC_{50} values (as mg-N/l) vs. solution pH.



HOLDING AND SALES OF LIVE CATFISH: AN ECONOMIC OPPORTUNITY

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Pond-based aquaculture can be an expensive enterprise, specifically in Kentucky where pond construction costs can be three times as expensive as other states due to widespread karst systems and rocky soil conditions. Indoor fish farming is also very capital intensive with most enterprises requiring insulated building with road access, drainage, lighting, and temperature control. Health and water quality issues are often difficult for farmers to maintain due to a lack of knowledge in fish culture. These high costs of entry and high levels of risk serve as barriers to many socially-disadvantaged farmers and prevent them from pursuing aquaculture enterprises.

This project looks at how socially-disadvantaged farmers can sell live catfish through small-scale hauling and holding operations. Fixed and variable costs were examined to determine the profitability of the enterprise as well as returns to land and operator's management. The sales price for live catfish was set at \$7.70/kg based on previous market research conducted in Hispanic communities around Kentucky.

Results showed that small-scale hauling and holding systems can be profitable and only require an initial investment of \$5,039, making this type of system suitable for socially-disadvantaged farmers. However, this enterprise is highly sensitive to the overall survival of the fish with substantial decreases to returns on investment as mortality increases.

DEVELOPMENT OF A SNP MARKER PANEL FOR PARENTAGE, DIVERSITY AND RELATEDNESS ANALYSES IN PACIFIC WHITE SHRIMP, *Penaeus vannamei*

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Pacific white shrimp (*Penaeus vannamei*) are the most important shrimp species in the aquaculture industry and account for more than 50% of all farmed crustaceans. Global production of *P. vannamei* has increased steadily over the years from 1 million tons in 2003 to over 3.5 million tons in 2014. The need to establish reliable genetic tools to assess parentage, relatedness and diversity among cultured populations is of increasing consequence to maintaining diverse, healthy stocks of *P. vannamei*. We report here on the development and characterization of a single nucleotide polymorphism (SNP) panel for *P. vannamei*. A panel of 178 SNP markers was established for use in genotyping and was validated in several cultured populations of *P. vannamei*. The SNP panel, with an average genotyping success rate of ~98%, was able to provide measures of diversity, inbreeding and population structure within and among stocks. The ability of the SNP panel to be able to assign parentage to all individuals was excellent, with an overall assignment rate of 99.99%. This genetic resource will be a valuable tool in the management of *P. vannamei* breeding programs.

GENOMIC SELECTION AND THE FUTURE OF BREEDING IN SHRIMP

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

FORECASTING FARM-GATE CATFISH PRICES IN UGANDA USING SARIMA MODEL

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Over the last ten years, the aquaculture subsector has emerged as a vibrant sector in Uganda and is being considered as a strategic sub-sector for promoting agricultural diversification. With improved market prices, government intervention for increased production, and stagnating supply from capture fisheries, aquaculture has attracted entrepreneur farmers seeking to exploit the business opportunity provided by the prevailing demand. As production has adjusted over time so have prices. Farm-gate prices have been highly volatile and are potentially a major factor in explaining why catfish farmers and processors are operating at narrow net margins. Price fluctuations translate into significant price risk, since the magnitude and the direction of the month-to-month changes are often unknown to producers.

The farmer has to frequently assess whether to harvest now to capture a known price, or to continue to feed to deliver a larger catfish at an unknown future price. Thus, the knowledge of future prices and factors influencing prices would be helpful to fish producers in decision making. It is against this background that a seasonal ARIMA forecasting model was developed to improve the prediction of catfish prices in Uganda.

Monthly farm-gate prices for African catfish covering the period 2006-2013 were obtained from Aquaculture Management Consultants in Uganda. Catfish prices, expressed in Uganda Shillings per kilogram, were deflated using a consumer price index. Stationarity of the series was examined using the ADF and PP test statistics. The optimal number of lags was determined using the Schwarz information criteria. Investigation was also done by examining the ACF and PACF functions. Using the AIC and BIC, six tentative SARIMA models were tested and the best model SARIMA (1,1,1) (0, 1,1)₁₂ was selected by picking the model with the least values.

The estimates parameters are 0.6532 for the non- seasonal AR term and 0.9012 and 0.8095 for the non-seasonal and seasonal MA terms, respectively (Table 1). Based on 95% confidence level, we conclude that all estimated coefficients are significantly different from zero, and thus the model is ideal for forecasting catfish prices.

The forecasted in-sample results are reported in Table 2. It can be noticed from the results that forecasted catfish real prices are close to their actual values. Moreover, all the actual prices fall within the 95% Confidence Intervals of the forecasts, which further confirmed the reliability of the fitted model.

Table 1 Estimated Parameters of SARIMA Model: (1 1 1)(0 1 1)₁₂			
Parameter	Estimate	Standard Errors	t-Value
Nonseasonal AR			
Lag 1	0.6532*	0.12170	5.3767
Nonseasonal MA			
Lag 1	0.9012*	0.07270	12.3962
Seasonal MA			
Lag 12	0.8095*	0.07984	10.1390
Variance	0.66652E+05		
Likelihood Statistics			
Effective number of observations (nefobs)			83
Log likelihood (L)			-585.3719
AIC			1178.7438
AICC (F-corrected-AIC)			1179.2566
Hannan Quinn			1182.6308
BIC			1187.4192
Number of parameters estimated (np)			4

Table 2 Actual and In-sample Predicted Prices [SARIMA (1,1,1) (0,1,1)₁₂]				
Month	Actual (UGX/kg)	Forecast (UGX/kg)	LCL	UCL
2013.Jan	7100	6941	6361	7521
2013.Feb	6900	6965	6371	7560
2013.Mar	7200	7000	6404	7595
2013.Apr	7500	7211	6635	7788
2013.May	7500	7446	6872	8019
2013.Jun	7400	7471	6899	8043
2013.Jul	7450	7426	6854	7997
2013.Aug	7350	7435	6867	8002
2013.Sep	7352	7369	6802	7936
2013.Oct	7614	7386	6819	7953
2013.Nov	7871	7660	7075	8245
2013.Dec	7827	7840	7270	8410

HYBRID ARCTIC CHAR – A NEW AQUACULTURE PRODUCT?

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Arctic Char (*Salvelinus alpinus*) is a significant aquaculture species in cold temperate latitudes. Cultured Arctic Char sold in the United States originates from Norway, Iceland and Canada. Demand for this premium priced species exceeds supply.

Because of its temperature and light cycle requirements, commercial scale reproduction of Arctic Char has failed in south temperate latitudes. While Arctic Char grow well at New York ground water temperatures (10°C), these temperatures are too high for successful egg/milt production and incubation. Even with photoperiod and temperature manipulations, it has been difficult to successfully reproduce pure strain Arctic Char.

In order to find a solution to the problems of Arctic Char reproduction in south temperate latitudes, the State University of New York has been examining the potential of interspecific hybridization. The main goal of this project is to produce Arctic Char appearing progeny that reproduces well in New York.

Arctic Char and Brook Trout naturally hybridized in the Fraser River and elsewhere. In this study, male Fraser River strain Arctic Char were crossed with female Brook Trout to produce F_1 hybrids. Subsequently only Arctic Char appearing fish were crossed to form the next generation. After nine generations, 89% had the appearance of an Arctic Char (no vermiculations), 53% had no dorsal markings, while 36% had dorsal spots. Further, 51% F_9 hybrids had red spots with no halos and 49% had blue halos around red spots.

Previous studies of Arctic Char x Brook Trout hybrids, have reported decreased fertility and sterility. It has been suggested this may be due to differences in chromosome number causing problems with their pairing during meiosis. It was hypothesized that since only fish that produced viable gametes would leave progeny, in subsequent generations, fertilization and hatching success would be improved. While fertilization, eye-up and hatching success was variable between pairings, there was no net improvement between generations.

Throughout the ten generations of this study, reproductive success of hybrid Arctic Char was higher than pure strain Arctic Char at New York photoperiods and groundwater temperatures. (Average fertility was 83.2%, eye-up rate was 45.2% and hatching rate was 34.8%). While it is too early to recommend Arctic Char hybrids to cold water fish farming, these results encourage further consideration of its suitability.

GROWTH OF ATLANTIC SALMON (*Salmo salar*) FED SQUID AND SCALLOP PROCESSING BYPRODUCT HYDROLYSATES INCORPORATED INTO SOY PROTEIN CONCENTRATE (SPC)-DIETS

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New protein ingredients are going to be needed to support the expansion of aquaculture. Byproducts that are currently considered waste can be converted into feed ingredients for fish. The purpose of this study was to determine the effects of squid and scallop byproducts on the growth of Atlantic salmon. Five-hundred Atlantic salmon smolts were stocked twenty 0.2 m³ tanks (25 fish per tank). After smolting, the fish were stocked in approximately 15 ppt well water. Temperature was maintained at approximately 13-14°C for the duration of the study. Fish were fed five experimental diets with automatic feeders soy-protein concentrate (SPC) diet, SPC + 5% squid hydrolysate, SPC + 5% scallop hydrolysate, SPC + 2.5% squid hydrolysate + 2.5% scallop hydrolysate, SPC impregnated with squid hydrolysate. Fish fed the diets with -hydrolysates had significantly higher final weights and average weight gain compared to salmon fed the SPC diet (see table below). The fish fed the experimental diets containing hydrolysates were offered more food resulting in an increased growth rate compared to the fish fed the SPC diet, although the increased growth rate was not significant. The proximate composition of the fish did not significantly differ among treatments, with crude protein ranging from 64% to 69% by dry weight and crude lipid ranging from 17% to 19% by wet weight. Overall, incorporating squid or scallop hydrolysates into the SPC-diets of Atlantic salmon smolts increased weight gain and did not affect the proximate composition of the fish.

Table 1. Growth performance data for Atlantic salmon fed experimental diets.

Diet	Initial wt (g)	Avg Final Weight (g)	Avg FCR	Avg weight gain (g)	Avg percent increase (%)	Avg SGR	Avg Feed offered
SPC-cont	84.74 ± 2.20	223.53 ± 20.27 ^B	1.15 ± 0.13	141.81 ± 20.12 ^B	173.53 ± 24.08	0.88 ± 0.08	157.06 ± 8.90 ^B
SPC- Sq H	85.47 ± 1.92	287.13 ± 12.26 ^A	0.95 ± 0.03	201.66 ± 12.57 ^A	236.56 ± 16.67	1.07 ± 0.03	195.35 ± 10.31 ^A
SPC- Scal H	86.07 ± 3.05	292.30 ± 12.71 ^A	0.93 ± 0.03	206.23 ± 10.58 ^A	239.68 ± 10.09	1.08 ± 0.03	191.81 ± 9.31 ^A
SPC- Sq/Scal H	88.25 ± 4.31	286.08 ± 6.71 ^A	0.97 ± 0.06	197.84 ± 10.58 ^A	227.45 ± 22.65	1.04 ± 0.06	186.50 ± 3.43 ^A
SPC impr w/Sq H	88.6 ± 2.41	270.30 ± 17.12 ^A	1.07 ± 0.07	181.70 ± 16.92 ^{AB}	205.49 ± 19.80	0.98 ± 0.06	190.05 ± 10.00 ^A
p Value	0.8424	0.0281	0.2514	0.0446	0.1449	0.1575	0.0496

GROWTH PERFORMANCE OF ATLANTIC SALMON (*Salmo salar*) SMOLTS FED DIETS CONTAINING HETEROTROPHIC ALGAL BIOMASS AS REPLACEMENT OF FISH OIL

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Atlantic salmon (*Salmo salar*) is an important cultured carnivorous species that has increasing amounts of plant proteins and oils included in the diet. Both consumers and producers expect fillets to contain elevated levels of omega-3 fatty acids [docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)]. Due to the low levels of these nutrients in traditional plant ingredients compared to marine sources, supplementation of aquaculture diets with omega-3 rich ingredients is required. Microalgae has garnered attention as a potential ingredient to provide long chain omega-3 levels in plant based diets.

A 16-week feeding trial was conducted to test increasing inclusion levels of DHA Natur (ADM; Clinton, IA) on growth performance and lipid digestibility in Atlantic salmon. Experimental diets were formulated to satisfy the nutritional requirements of Atlantic salmon parr. The diets contained approximately 45% protein and 27% lipid and were formulated to contain 0%, 5%, 10%, and 15% ADM DHA Natur. Each tank (n = 24) was stocked with 20 fish and quadruplicate tanks were fed one of the experimental diets. Fish in each tank were bulk-weighed and counted every 4 weeks during the trial. At the conclusion of the trial, 3 fish from each tank were homogenized for composition of proximate analysis. A digestibility test was also conducted.

The apparent digestibility coefficient of lipid was 66.4% for DHA Natur. The performance of the fish fed the different dietary treatments did not vary significantly (Table 1). The average weight ranged from 410 g to 457 g, weight gain 131 g to 167 g, percent gain 46% to 60%, feed efficiency 0.86 to 1.13, and thermal growth coefficient 1.04 to 1.08. Overall, there were no significant difference in body composition, hepatosomatic index, or intestinal length/fork length ratio. Adding DHA Natur to the diets of Atlantic salmon provide DHA at levels comparable to marine based control feed did not affect performance or proximate composition of the fish.

Table 1. Performance parameters of Atlantic salmon smolts fed the experimental diets¹.

Diet	Avg weight (g)	Avg wt gain (g)	Avg FE (gain/fed)	Avg TGC	Avg % Gain
Ref	431.65 ± 13.10	161.28 ± 11.11	1.03 ± 0.09	1.04 ± 0.01	59.81 ± 4.37
5% DHA Natur	414.99 ± 5.32	131.24 ± 3.67	0.88 ± 0.03	1.08 ± 0.01	46.36 ± 1.97
10% DHA Natur	409.78 ± 6.59	132.78 ± 11.09	0.86 ± 0.04	1.07 ± 0.01	48.20 ± 3.62
15% DHA Natur	418.96 ± 10.12	141.71 ± 6.02	1.01 ± 0.07	1.06 ± 0.02	51.45 ± 3.62
P Value	0.09	0.06	0.23	0.67	0.15

¹Data are least squares means of 4 observations per treatment. Avg weight = average weight in grams; Avg wt gain = final weight – initial weight; avg FE = feed efficiency (weight gain/weight of feed given); Avg TGC = thermal growth coefficient; Avg % gain = (final weight – initial weight)/initial weight * 100.

MULTI-SCALE ABUNDANCE AND DISTRIBUTION TRENDS OF DOUBLE-CRESTED CORMORANTS (*Phalacrocorax auritus*) ON CATFISH AQUACULTURE IN MISSISSIPPI

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Commercial production of catfish (*Ictalurus spp.*) is the largest aquaculture industry in the nation, with the majority of production occurring in Mississippi (MS). Since its inception in the 1960's, the state's catfish industry has unfortunately experienced continuous human-wildlife conflict between producers and fish-eating bird species through the consumption of cultured catfish. Most notable of these species is the Double-crested Cormorant (*Phalacrocorax auritus*; hereafter, cormorant), which can cost producers millions of dollars annually, through the depredation of catfish.

Historically, cormorants were infrequently found on fresh waters of MS, but populations of cormorants wintering inland steadily increased during the latter part of the 20th century. This resulted in a growing concern from catfish producers over the economic loss associated with bird depredation at their facilities. Consequently, a large scale monitoring program was implemented by U.S. Department of Agriculture, National Wildlife Research Center (USDA-NWRC) in 1996 to estimate both the abundance and distribution of cormorants in the state. This extensive data set permits exploration of the mechanism driving cormorant use of MS in relation to aquaculture.

Our data suggest the change in cormorant population wintering in MS coincided with the changes in catfish aquaculture in the state (Figure 1). Other aspects are also being examined, including effectiveness of roost harassment strategies on displacing cormorants away from aquaculture, seasonal trends of cormorant distribution, and fine scale roost characteristics that explain cormorant use. Seasonal trends involve examining where cormorants occur, with respect to aquaculture, between their arrival and departure from the state within a winter season. Their use of catfish ponds throughout this time may vary in relation to migration as their dietary needs change. Investigating fine scale roost characteristics will determine what cues cormorants use to choose roosting locations, aiding in effective future roost harassment. Although much research has been done in the past to address some of the above issues, drastic changes in catfish aquaculture in MS including total hectares in production, production practices, and the use of hybrid catfish make contemporary information on cormorant depredations particularly relevant.

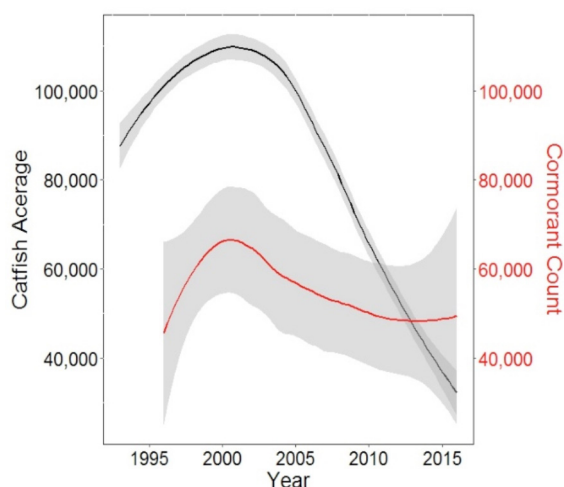


Figure 1. Mississippi catfish aquaculture acreage and cormorant abundance from 1993-2016 (95% CI in grey).

A MULTI-STRAIN PROBIOTIC, RED DRUM *Sciaenops ocellatus*, AND DIGESTA: THE NITTY-GRITTY ABOUT CHANGES SEEN OVER TIME

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Bacteria are important to a functional digestive system, and the use of probiotics, defined as “good bacteria,” has become more common in the last 10 years to help re-establish the gut flora after treatment of antibiotics. Red drum (*Sciaenops ocellatus*) a commercially important carnivorous marine fish, that has a short gut and rapid food transport. Rapid digestion and assimilation rates are important to rapid growth. The goals of the current study were to determine the effects of probiotics on growth & mortality rates, food conversion ratios (FCRs), behavior, and to evaluate gut health.

A 56-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 (1.0×10^8 CFU/g) only through day 28, and 3) probiotics recommended dosage (1.0×10^8 CFU/g) through day 56. Probiotics selected was Primilac® a multi strain mixture containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifido bacterium bifidium*, *Enterococcus faecium*. The probiotic was added to treatment tanks daily along with the fish meal, while controls were only fed fish meal.

After 56 days, the trial group treated with probiotics till day 56 showed an increased growth of 33.7% over the control, probiotics till day 28 wasn't significantly different at 33%. The FCR for probiotics showed that after day 14 until day 28, and to day 56 required less than a gram (FCRs ranged from 0.75g feed / 1g of fish mass up to 0.25g feed / 1g of fish mass). And mortality rates decreased from day 7 (2%) to day 21 (0%) in the probiotics' tanks after which there was no more mortality. For controls, weekly mortalities ranged from >3% to <1% until the 5th week, when the amount of fish was reduced to about 1 fish/gallon of seawater. This shows a significant advantage with the use of probiotics.

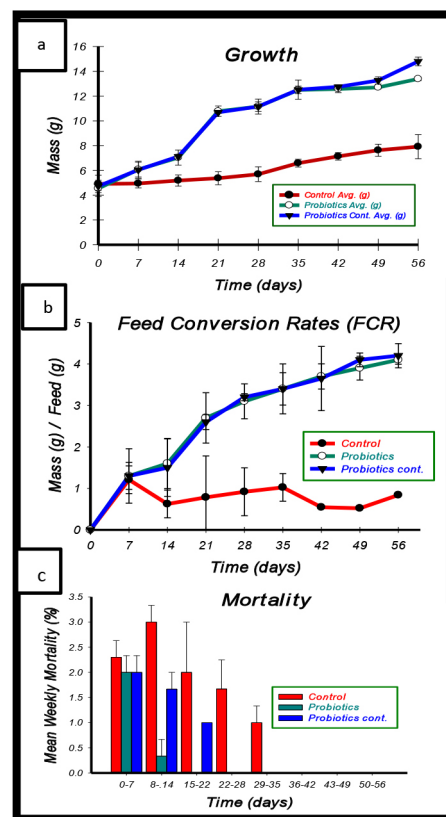


Figure 1 (a) Mean Growth vs Time (days) for juvenile red drum treated with no probiotics (controls- red), probiotics until 28 days (green), and probiotics for 56 days (blue). (b) FCRs vs Time (days) for juvenile red drum treated with no probiotics (controls- red), probiotics until 28 days (green), and probiotics for 56 days (blue). (c) Mean Weekly Mortality (%) vs Time (weeks) for juvenile red drum treated with no

RECENT IMPROVEMENTS IN ADVANCING THE CULTURE OF YELLOW TANG (*Zebrasoma flavescens*); AN UPDATE ON PROGRESS AT OCEANIC INSTITUTE

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Yellow Tang (*Zebrasoma flavescens*) are the most heavily exported reef fish from Hawaii, with nearly 400,000 individuals collected annually. Recent legislation in Hawaii has suspended the collection of this iconic marine aquarium fish, despite significant data supporting the sustainability of this fishery. While the culture of this species has recently been successful, significant challenges remain in the rearing process that need to be overcome before cultured animals could become widely available in the market. In particular, the prolonged use of copepods in the rearing process needs to be addressed, as this represents one of the single largest impediments to commercialization. This study compared two feeding regimens, an “original” diet treatment which was initially used to successfully rear this species and a “revised” diet treatment, which differed only in the timing of the introduction of the live feed items and weaning periods to frozen and formulated feeds. The objective of this effort was to significantly reduce the amount of copepods and rotifers utilized in the rearing process.

This study consisted of two parts, a replicated small-scale trial which test the effects of feeding regimen and a subsequent, larger “pilot scale” demonstration of this “revised” diet regimen. Both diet regimens consisted of *Parvocalanus crassirostris* nauplii, enriched *Brachionus rotundiformis* rotifers, and *Artemia* nauplii, followed by a transition to dry and frozen feed items. Eggs were stocked at 40 L⁻¹ into replicate (n=3) 200 L tanks for the feeding regime trial. Eggs were stocked at 19 L⁻¹ in a single 1,000 L tank, which tested the “revised” feeding method at pilot-scale. Fish reared on the revised feeding regime were observed to grow faster in body length and body depth after two weeks in culture, and also transitioned more quickly to juveniles as 41% had completed their transition to juvenile coloration by 70 days post hatch (dph). Fish reared under the “original” feeding regimen did not attain full juvenile coloration during the study period. Survival to 70 dph was not significantly different between treatments and was 0.13% ± 0.13 and 0.29% ± 0.17 in the “original” and “revised” feeding regimens, respectively. In the pilot-scale portion, growth was comparable to that of both treatments in the 200 L scale trial, but survival to the same time point was much better at 1.9%. Although not specifically compared under this period, this improved survival rate in larger tanks is routinely observed and something that warrants further research. The results of this research shortened the copepod and rotifer feeding periods for Yellow Tang, while not limiting growth or survival. This outcome significantly enhances the commercialization potential for this, and likely other Acanthuridae, species.

NUTRIENT CYCLING DYNAMICS OF AN ALGAE-OYSTER SYSTEM

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There has been some disparity in the literature regarding oysters and their impact on the surrounding environment. Studies on oyster reefs and farms have shown both positive and negative variability in the net nutrient amounts surrounding these features. Intuitively, the presence of oysters and their ability to filter seston from the water column would imply an overall reduction in nutrients of a given system. Consequently, some of these studies actually show overall increases of nutrients in the lee of oyster features. On the other hand, the use of oysters to mitigate estuarine eutrophication and/or reduce nutrients in agriculture and aquaculture waste effluents has recently been proposed in various forms and experienced positive results.

Although previous studies have explored the nutrient recycling of oysters in specific systems or environments, a quantitative model to understand the dynamics of the more comprehensive algae-oyster system is lacking. A first step in the development of a comprehensive dynamic model is to understand the complex interactions of the algae-oyster nutrient recycling pathways. This is accomplished through the development of a mass balance that incorporates growth dynamics of algae, filtration of oysters, assimilation into biomass, excretion components, and degradation of fecal pellets. Various environmental parameters, such as temperature, light, nutrient concentrations, hydrodynamics, salinity, and turbidity are also incorporated into the model. Experimental data is utilized to validate assumptions within the mass balance model.

This presentation describes the algae-oyster dynamics using a mass balance model in order to investigate the ability of a small or landscape scale system to reduce dissolved nutrients within a water column under various conditions. The results of this work can be implemented into larger scale modeling efforts and has implications on sustainable management of oyster mariculture, management of eutrophic estuarine systems through oyster cultivation, and treatment of aquaculture effluents using an algae-oyster culture system.

THE SPAWNING AND REARING OF THE BLUE NEON GOBY *Elacatinus oceanops* IN A LOW DENSITY SYSTEM ON A SMALL BUDGET

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The purpose of this project was not only to bring marine aquaculture to the Key West Aquarium, but also to do so in an economical way. The Key West Aquarium was built in 1934 and has considerable space constraints; therefore, the project needed to be affordable as well as occupy the smallest footprint possible. Many marine hobbyists face similar space and monetary constraints when spawning and rearing fish at home. Blue neon gobies were chosen due to their small adult size, ease of larval rearing, and interest for use as cleaner species in aquarium displays.

A single broodstock pair was purchased from KP Aquatics and housed in a 29 gal tall tank with overflow into 10 gal glass sump, with a 1000 gal/ph return pump. Conditioning diet consisted of enriched live adult *Artemia*, *Artemia* nauplii, and Piscine Energetics (PE) frozen *Calanus* and *Mysis*. Spawning began at 23.3°C and continued every 7-10 days until 28.3°C. Gobies stopped spawning at 28.3°C. Broodstock produced 7 clutches of eggs during 3 months of spawning. Two clutches were given to Florida Keys Community College to rear.

At the Key West Aquarium, hatching was stimulated on day 8 in a greenwatered larval rearing tank using a stream of air bubbles over the eggs. Larvae were reared in a 10 gal tank per egg clutch with blacked-out sides and bottom, an aerator, and a filter sponge. Larvae were maintained at 30 ppt. Due to space constraints, the larval rearing tank was relocated to a climate-controlled section of the facility, which led to a temperature decrease to 20.5°C and considerable die off. After heaters were inserted, 6 individuals survived through metamorphosis. Metamorphosis began on 38 dph and ended on 45 dph. The larval diet consisted of rotifers until 13 dph, then newly hatched *Artemia* nauplii.

Florida Keys Community College kept a constant temperature of 27°C and raised 12 individuals through metamorphosis. Rotifer live feed was fed live *Nannochloropsis* algae and enriched using N-Rich Plus by Reed Mariculture. By 30 dph, larvae transitioned to commercial dry feed.

We were able to spawn and rear neon gobies using a low start-up budget and utilizing products commercially available to hobbyists (Table 1). As of July 2017, the Key West Aquarium has added better climate control to the broodstock area. There is also considerable room to examine better quality larval feeds and add more broodstock in the future.

TABLE 1. Costs of rearing Blue Neon Gobies

1000 gal/ph pump	\$60	3x10-gall	\$30
29 gall tank	\$30	Sponge filter	\$5
RotiGrow+ 1L	\$72	Chemi-Pure	\$16
2x5-gall buckets	\$6	N-Rich+ 1 qt	\$48
<i>Artemia</i> cysts 1oz	\$12	Ceram. heatrs	\$12
PE Mysis 8oz	\$14	Misc. supplies	\$60
PE Calanus 4oz	\$14	TOTAL	\$379

LOCAL, REGIONAL, NATIONAL, AND INTERNATIONAL BENEFITS OF STAKEHOLDER INVOLVEMENT IN AQUAFISH INNOVATION LAB RESEARCH

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Developing sustainable aquaculture and fisheries systems that increase productivity and enhance local capacity is essential to addressing food security in developing countries. AquaFish Innovation Lab (AquaFish) collaborates with in-country scientists and end-users to investigate and implement aquaculture technologies necessary to address site-specific challenges facing farmers and communities. Since 2006, AquaFish has worked in 33 countries in Asia, Africa, and Latin America to broadly investigate, disseminate, and integrate sustainable aquaculture solutions across all scales to generate positive impacts at local, regional, and national levels.

AquaFish transfers the benefits of aquaculture at the local level by training small- to medium-scale farmers, mothers and heads of households, and community groups, among others. For example, in Nepal, AquaFish is addressing food security and household nutrition through extension programs at schools and farms that train students, teachers, women's groups, and farmers in sustainable aquaculture production. The expansion of aquaculture activities in these communities is a direct result of the school-to-school and farmer-to-farmer extension programs.

AquaFish research and dissemination efforts connect often disparate stakeholders across regions. The AquaFish Collaborative Research Support Program (CRSP) Mali Project, for instance, used the South-South approach, a framework designed to tackle food security challenges by facilitating collaboration between Southern countries, to bring together CRSP researchers from Kenya, China, and Thailand. The project's efforts set the stage for further development of aquaculture and fisheries sectors in Mali by introducing best management practices, transferring technologies among stakeholders, and increasing economic opportunities.

Integration and coordination across local, regional and national levels adds value to activities and often increases the likelihood of change at national scales. For example, AquaFish research played a substantial role in lifting an over decade-long snakehead farming ban in Cambodia in 2016 by developing and informing options for a sustainable snakehead aquaculture program. A key tipping point in lifting the ban was the development of processed fish feeds, which AquaFish researchers in Cambodia and Vietnam helped to develop. The newly developed feed decreases reliance on small-sized fish and lessens the environmental impact of snakehead aquaculture, while the lifting of the ban augments income opportunities for farmers.

THE EFFECTS OF STOCKING DENSITY ON GROWTH, SURVIVAL, AND RESISTANCE TO ACUTE CROWDING OF POST-METAMORPHIC STAGE BLACK SEA BASS *Centropristis striata* IN A PILOT COMMERCIAL SCALE NURSERY

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The black sea bass *Centropristis striata* is a high value, high demand marine finfish with promising commercial aquaculture potential. A bottleneck to commercial culture is a reliable and cost-effective source of fingerlings to support growout operations. Maximizing safe stocking density of metamorphic stage juvenile fish in nursery tanks (NTs) while maintaining high fish health and growth rates is critical to efficient use of hatchery and nursery resources and, hence, to maximizing fingerling quality, output and affordability. This study investigated the effects of NT stocking density of post-metamorphic stage black sea bass on survival, growth and resistance to acute crowding to the transport-ready juvenile stage 60 days post-hatching (60 dph). Juveniles (47 dph, mean wt. = 0.54 g) were stocked into six 300-L fiberglass NTs supported by a recirculating seawater (34 g/L) system. Two NT stocking densities were compared in triplicate; a low density (4.5 fish/L, 1,350 fish/tank, 2.43 kg/m³) and a high density (6.5 fish/L, 1,950 fish/tank, 3.51 kg/m³). Fish were fed a UNCW-formulated diet by hand every morning and at 2-h intervals by automatic belt feeder over the ensuing 12 hours to satiation. Temp was 23.5 °C and photoperiod was 18 L: 6 D. Survival of fingerlings to 60 dph under both NT densities was high (mean = 87.7 ± 0.03%) and not significantly different as final biomass densities under the low and high NT densities reached 6.66 and 8.98 kg/m³, respectively. Fish in the low density NTs (mean = 1.68 ± 0.026 g) were significantly ($P < 0.05$) larger than those in the high density NTs (mean = 1.58 ± 0.004 g). FCR (mean = 0.87) was excellent and not significantly different. Coefficient of variation of weight (mean = 28.1 ± 2.3%) was not significantly different between NT density treatments. In a simulated air-shipping trial, 59 dph fingerlings from both NT density treatments placed in oxygenated bags containing seawater (biomass loading = 10 g/L) and held in insulated shipping boxes for 24 h showed ~100% survival, even though dissolved oxygen declined (mean = 1.73 mg/L). To evaluate tolerance to acute crowding, 59 dph fingerlings from both NT density treatments were placed in 10-L seawater tanks (biomass loading = 25 g/L) and held under static conditions with oxygenation and zero feeding. After 72 h, survival was excellent (mean = 99.7%) and not significantly different between NT density treatments, even though mean TAN and pH reached 57.3 ppm and 8.06, respectively. The results demonstrate that stocking post-metamorphic stage black sea bass in NTs at high densities of 4.5 to 6.5 fish/L does not affect survival, growth variation or FCR to the 60 dph transport-ready stage fingerling stage, although growth was slightly depressed at the higher density. Fingerlings produced under these high NT stocking densities were robust and showed excellent survival during simulated air-shipping trials and resistance to acute crowding.

HIGH DIETARY LEVELS OF BRANCHED-CHAIN AMINO ACIDS AFFECT GROWTH PERFORMANCE AND AMINO ACID UTILIZATION IN RED DRUM *Sciaenops ocellatus*

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The minimum dietary requirements for the branched-chain amino acids (BCAAs) by red drum have been quantified at 1.6, 1.1, and 1.2% of dry diet (35% crude protein [CP]) for leucine (Leu), isoleucine (Ile) and valine (Val), respectively. However, it should be noted that all three BCAAs share the same common transporter for intestinal absorption and most organisms metabolize these three amino acids (AAs) using the same enzymatic system. Because of this, interactions between BCAAs are known to produce antagonistic effects in pigs, rats and humans, where an excess of Leu may reduce the concentration of Ile and Val in plasma, ultimately altering protein synthesis and affecting growth. In fish, an antagonistic interaction involving BCAAs has not been fully assessed; therefore, this study aimed to elucidate the effects of high dietary levels of BCAAs on growth performance and AA utilization of red drum. This is of vital importance because a current priority in the aquaculture industry is to replace the use of fishmeal with alternative protein ingredients, including plant-based feedstuffs; however, unbalanced AA profiles in plant-based ingredients (e.g., high Leu levels in corn protein feedstuffs) could potentially impair fish growth.

A semi-purified control diet (35% CP) was prepared by combining lyophilized red drum muscle (RDM) (10.5% of dietary CP) and supplementing with crystalline L-AAs to simulate the AA pattern of RDM, while keeping the levels of Leu, Ile and Val at the minimum dietary requirement levels for red drum. In addition, six experimental diets were prepared by supplementing the control diet with (1) an excess of Leu (6.2% of dry diet), (2) an excess of Ile (4.4% of dry diet), (3) an excess of Val (5.0% of dry diet), (4) an excess of Leu and Ile (6.2 and 4.4%), (5) an excess of Ile and Val (4.4 and 5.0%), and (6) an excess of Leu and Val (6.2 and 5.0%). These levels correspond to 200% of the amount found in 35% CP provided by RDM. A 50:50 mixture of aspartate and glutamate was used to maintain all diets isonitrogenous. Red drum juveniles were stocked in 38-L glass aquaria (12 fish/aquarium), and diets were fed to fish in triplicate aquaria at a rate approaching apparent satiation, twice daily, for 49 days. At the end of the feeding trial, growth performance parameters were calculated. In addition, the postprandial (4 h after feeding) concentration of plasma and muscle free AAs was analyzed.

A summary of the obtained results can be found in **Table 1**. Weight gain was significantly ($P < 0.05$; Dunnett's test) affected with an excess of Leu and an excess of any two BCAAs, when compared to the control diet. Based on weight and BCAAs levels in plasma, it seems that, as in mammals, Leu plays a critical role in regulating the concentrations of Ile and Val for red drum's optimal growth. A thorough discussion of all the results obtained and their implications will be presented at the meeting.

Table 1. Weight gain, feed efficiency and levels of BCAAs in plasma of red drum fed high dietary levels of BCAAs.

	BCAA Excess Incorporation							Pr>F	SEM
	Control	Leu	Ile	Val	Leu+Ile	Ile+Val	Leu+Val		
Relative Weight Gain, %	597 ^A	455 ^B	511 ^{AB}	554 ^{AB}	469 ^B	454 ^B	481 ^B	0.02	29.0
Feed Efficiency Ratio	0.85	0.81	0.86	0.86	0.87	0.83	0.80	0.06	0.02
Leucine Plasma, nMol/mL	46.9 ^B	481 ^A	107 ^B	70.1 ^B	474 ^A	313 ^A	462 ^A	<0.001	62.8
Isoleucine Plasma, nMol/mL	55.0 ^D	121 ^{CD}	508 ^B	110 ^D	428 ^{BC}	992 ^A	43.4 ^D	<0.001	72.1
Valine Plasma, nMol/mL	71.9 ^D	89.3 ^D	80.4 ^D	788 ^B	238 ^{CD}	1466 ^A	711 ^{BC}	<0.001	110

NMR-BASED METABOLOMICS IDENTIFICATION OF A POTENTIAL METABOLIC BIOMARKER OF NUTRITIONAL STRESS IN RED DRUM *Sciaenops ocellatus* FED GRADED LEVELS OF SOYBEAN MEAL

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Metabolomics is the study of metabolites present in plant and animal tissues and biofluids (the “metabolome”). The metabolome is strongly connected to the physiological status of the organism and reflects the influence of multiple internal and external factors. Diet can significantly affect the metabolome, and careful analytical measurements can detect dietary effects on the biochemical pathways associated with normal health and growth. Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical technique that can be used to measure these metabolites. NMR-based metabolomics can be used to detect potential biomarkers that can be indicative of specific nutritional deficiencies.

During a 12-week feeding trial, we investigated metabolic changes in juvenile red drum induced by graded levels of soybean meal (SBM0 %, 15 %, 30 %, 45 % and 60 %) in extruded, fishmeal-free diets using NMR-based metabolomics. Liver, muscle, intestine and plasma samples were collected weekly and analyzed to provide a “snapshot” of the metabolome at different time points, resulting in a “metabolic trajectory”. Metabolomic results suggest that a nutritional deficiency might have occurred at high levels (45 % to 60 %) of soybean meal inclusion¹.

By comparing metabolic profiles at the end of the study (Figure 1), we identified a metabolite whose levels were significantly increased at high SBM levels. This compound (N-formimino-L-glutamate (FIGLU)) has been associated with vitamin deficiencies (specifically vitamin B12 and/or folate) in mammals, but not previously investigated in fish. Our results suggest that this metabolite may be a biomarker of potential dietary stress in red drum that allows for the optimization of soy-based formulations in red drum.

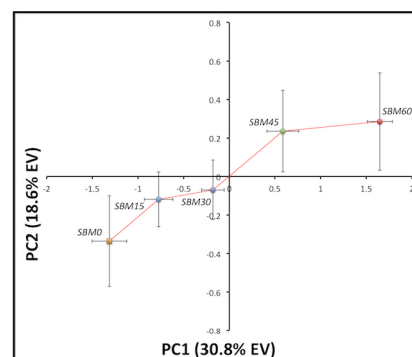


Figure 1. PCA score plot from liver tissue for the five experimental diets (SBM0, SBM15, SBM30, SBM45, SBM60 containing 0 %, 15 %, 30 %, 45 %, and 60 % of soybean meal, respectively) at the end feeding trial. Error bars represent the mean \pm 1 SEM.

1. Casu, F.; Watson, A. M.; Yost, J.; Leffler, J. W.; Gaylord, T. G.; Barrows, F. T.; Sandifer, P. A.; Denson, M. R.; Bearden, D. W., Investigation of Graded-Level Soybean Meal Diets in Red Drum (*Sciaenops ocellatus*) using NMR-based Metabolomics Analysis. *JWAS* **2017**, *Submitted*.

IMPROVED GENETICS AND A NOVEL FEEDING STRATEGY FOR INCREASED GROWTH RATES OF NILE TILAPIA *Oreochromis niloticus* IN A RECIRCULATING AQUACULTURE SYSTEM

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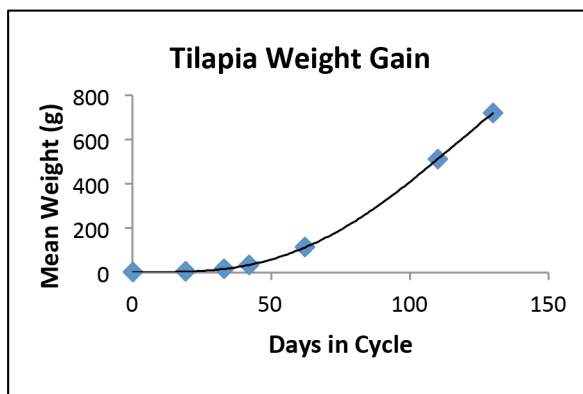
A Nile tilapia growth trial was conducted at the Pentair Aquatic Eco-Systems Inc. World Aquaculture Technology Engineering Research center (PAES W.A.T.E.R). The trial evaluated growth performance of a genetically improved strain of tilapia in a state-of-the-art recirculating aquaculture system (RAS). A robotic delivery system allowed for precision feed application and scheduling of daily feeding. A nutritionally complete feed with relatively high protein content (40%) was used for the duration of the trial.

The tilapia chosen for this trial were obtained from a breeder who has been selectively breeding their fish since 1988. The breeding program is centered around a strain of the Genetically Improved Farmed Tilapia (GIFT). The supplier data suggested that it would take approximately 150 days to produce a 720 g fish.

A nursery system was stocked with 1.24 g fingerlings. After 42 days in the nursery system, 2,869 fish with a mean individual weight of 31.2 g were transferred to a single 32.5 m³ grow-out tank capable of supporting a final density in excess of 60 kg/m³. Throughout the trial a robotic feeder was programed to feed the fish once every hour. Feed was adjusted weekly based off of calculated growth rates. Throughout the trial only minor interruptions in the feeding schedule occurred during periods of maintenance or during sampling events.

After a total of 130 days fish reached an individual harvest weight of 720 g. Final density was 61.8 kg/m³ with an overall FCR of 1.2:1. Survival rate was 97.1%.

The improved growth performance was attributed to the regularity of the feeding regime. This suggests improved genetics in Nile tilapia along with an improved feeding schedule would reduce time required to reach market size. An additional production trial is currently underway to lend further repeatability to these results.



THERAPEUTIC POTENTIAL OF BIOACTIVE COMPOUNDS FOR TREATING FISH DISEASE AND THEIR CYTOTOXIC AND GENOTOXIC EFFECTS

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Aquaculture is the main source of high quality protein. Due to the growing demand for seafood and high density fish cultures, there are more disease outbreaks. Bacterial diseases like columnaris caused by *Flavobacterium columnare*, result in huge economic loss to aquaculture farmers. Although there are commercially available vaccines and antibiotics to control bacterial diseases, they are proven to be inefficient. Moreover, antibiotics lead to acquired resistance in bacteria, and the drug residual in fish, resulting in tight regulations for their usage. This calls for new alternative solutions that can be safe and cost-effective to prevent and treat fish diseases.

Plant extracts are well known for their anti-microbial properties and they could be potential alternative candidates. Usage of plant extracts as therapeutics is largely used for human pathologies but there is limited knowledge for the same to treat piscine disease. The anti-microbial effects of plant extracts is due to the presence of bioactive compounds like alkaloids, flavonoids, steroids, and phenols in them. They render the plant extracts, bactericidal and bacteriostatic effects against pathogenic bacteria and also induce host immunity. We have evaluated the anti-microbial properties of extracts from various herbs and spices: *Cinnamomum verum*, *Syzygium aromaticum*, *Thymus vulgaris*, *Salvia officinalis*, *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, *Allium cepa*, *Coriandrum sativum*, *Anethum graveolens* against *Flavobacterium columnare* using disc diffusion method. Commercially available antibiotic Oxytetracycline (Terramycin® 200), that is used to treat columnaris served as positive control. The bactericidal effects of our extracts were compared to it. We find that all the extracts (30mg/ml) we have tested, had bactericidal effects against *Flavobacterium columnare* (3×10^8 CFU/ml). Interestingly, *Syzygium aromaticum* and *Cinnamomum verum* showed highest antimicrobial activity against *Flavobacterium columnare* with an average inhibition of 16mm and *Cuminum cyminum* showed 14mm, which is similar to Oxytetracycline. We have also determined the minimum inhibitory concentrations of all the plant extracts along with Oxytetracycline. We are currently testing the cytotoxic and genotoxic effects of these extracts using various molecular assays. We speculate that *Syzygium aromaticum* and *Cinnamomum verum* are potential alternative therapeutics to treat columnaris in fish that would not have regulations and be cost-effective.

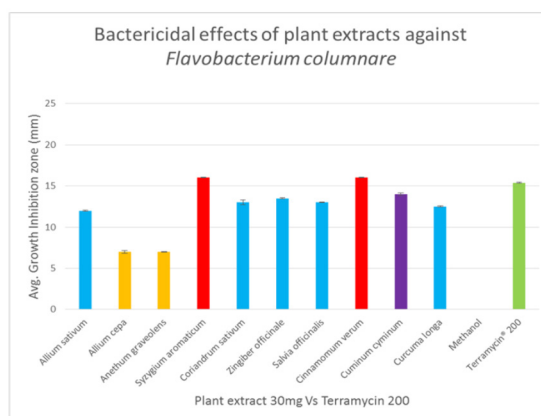


Fig 1. Bactericidal effects of plant extracts compared to Terramycin® 200: *Syzygium aromaticum* and *Cinnamomum verum* showing highest activity.

INTEGRATING AQUAPONICS INTO THE COLLEGE CURRICULUM

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Zac Chambers, Professor of Mechanical Engineering at Rose-Hulman Institute of Technology, and Brandon Hall, Agriculture Instructor in the Ivy Tech Community College, both share a passion for aquaponics and have collaborated to create a project-based aquaponics course which can be tailored to both a four year engineering college and a two year community college. The course is built around creating an IBC based system and learning water chemistry and plant/fish husbandry. The system is initially hydroponic and transitions to aquaponic as students develop their husbandry skills. The course concludes with a validation of the projected plant production and an economic assessment. Feed conversion ratios may be explored.

Tailoring for the four year engineering college involves creating and validating mathematical system models of flow, pH, and ammonia conversion. Tailoring for the two year community college involves utilization of “bucket chemistry” tables to estimate flow, pH, and ammonia conversion and a higher level of husbandry techniques.

Hall is teaching his version of the course Fall semester 2017 and Chambers will be teaching his in the Spring of 2018. The presentation will provide a summary of both.

EFFECTS OF INORGANIC AND ORGANIC DIETARY COPPER SUPPLEMENTATION ON GROWTH PERFORMANCE AND TISSUE COMPOSITION OF JUVENILE RED DRUM (*Sciaenops ocellatus*)

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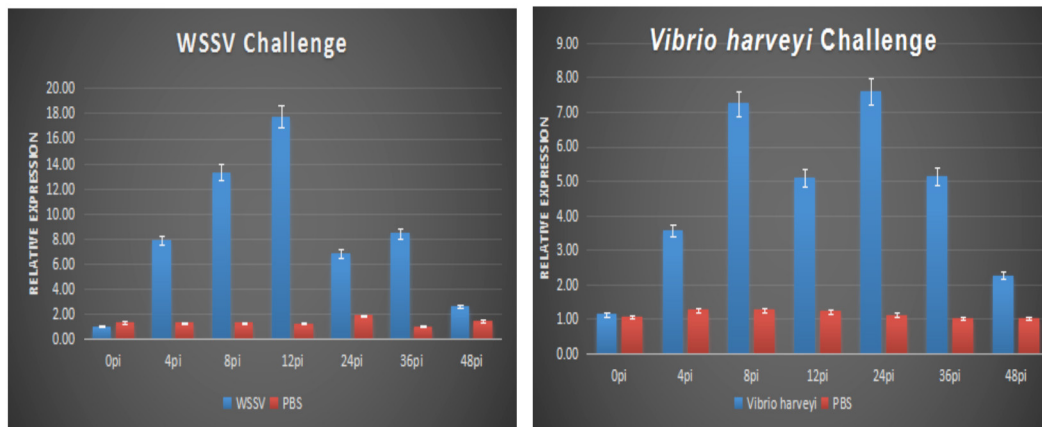
An 8-week feeding trial was conducted with juvenile red drum to evaluate the copper requirement and compare two different copper sources: inorganic copper (copper sulfate) and organic copper (copper-ethanolamine complex). The basal diet was formulated from red drum muscle, wheat gluten and crystalline amino acids to contain 35% crude protein. It was analyzed to contain 3 mg Cu/kg, and either copper sulfate or copper-ethanolamine complex were added to the basal diet at 5, 10, 20 mg Cu/kg dry diet. The feeding trial was conducted in a recirculation system in which the water was maintained at 7 ppt with sodium chloride and synthetic seawater, and copper concentration was analyzed to be xx mg Cu/L. A total of 17 fish (initial weight of ~6 g/fish) were assigned to 110-L glass aquaria. All treatments were provided to triplicate aquaria and fish were fed twice daily to approach apparent satiation for 8 weeks. No significant difference was found in growth performance among all treatments. Compared to the basal diet, red drum fed with copper-supplemented diets had significantly higher copper concentration in liver and whole-body. However, there were no significant differences in tissue levels or net copper retention among fish fed the inorganic or organic copper sources. The minimum dietary copper requirement for juvenile red drum appears to be lower than 3 mg Cu kg⁻¹ diet, and red drum can tolerate copper concentrations of at least 20 mg Cu kg⁻¹ diet without any detrimental effects.

ANALYSIS OF C-TYPE LECTIN DOMAIN FAMILY MEMBER FROM HEPATOPANCREAS AND OVARY OF THE SPINY LOBSTER *Panulirus homarus* BY TRANSCRIPTOME PROFILING

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The spiny lobster *P. homarus*, is an important decapod cultured in the cages in the coastal area of many region in China. Recent outbreaks of diseases have impacted the lobster culture industry resulted in large economic loss. Like other invertebrates, lobster lack an acquired immune system, and have to rely on the innate immune system to resist the invasion of the pathogens. However, few molecular studies were performed to understand the immune mechanism of the lobster in response to pathogen infection. To explore the immune-related genes immune mechanism in lobster, we have analyzed the hepatopancreas and ovary transcriptome profiles of *P. homarus* using a deep sequencing approach with a Illumina HQ2000 platform. A total of 158,380 unigenes were obtained. Of which 84256 were derived from the hepatopancreas- and 74124 were derived from the ovary respectively. Altogether there are 39 C-type lectin identified in both tissues suggesting the lectins are also involve in a diversified function of immune response. WSSV and bacteria challenge experiment revealed that only a small number of lectins were activated suggesting the function of most lectin are highly specific and different lectins may be responsible for the response of other pathogens. The results of this study provide a general background information of the first line defense mechanism of the lobster.



EFFECT OF POST-SPAWNING BROODFISH DIET WITH HIGH LIPID CONTENT AND n-3 FATTY ACIDS ON REPRODUCTIVE PERFORMANCE OF CHANNEL CATFISH

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Channel x blue hybrid catfish are exclusively produced by hormone-induced spawning protocols and this process has proved to be a reliable method to mass produce hybrid catfish in hatcheries. Strip spawning of channel catfish needs a continuous and reliable supply of mature (gravid) fish during the spawning season. However, availability of gravid females in required numbers is a limitation to meet the production goal in hatcheries. Lipids provide high energy density and critical cellular functions during reproduction phase. Hence, the present study was conducted to determine whether higher lipid feed improves maturation and reproductive performance of channel catfish to enhance hybrid catfish fry production.

A six-month post-spawning feeding trial was conducted from October 2015 to April 2016 to evaluate two commercial broodfish diets: High lipid broodfish feed (14% lipid) and a Low lipid broodfish feed (7% lipid) at USDA ARS Warmwater Aquaculture Research Unit, Stoneville, MS. Mixed strain of 3-year old female channel catfish were stocked in twelve 0.25 acre ponds stocked with 50 fish per pond with an approximate stocking density of 1200 pounds per acre. Six ponds were randomly assigned to each feed and the designated feed was fed 3 times a week to satiation. At the end of the feeding trial, post-spawning diets did not influence the growth performance of channel catfish but variations in proportion of maturing fish were evident in both the treatments. Percent of gravid females per pond were higher in high lipid feed fed broodfish in early (37.7% vs 26.2%) and peak (42% vs 34.5%) portion of the spawning season ($P=0.07$) compared to the low lipid feed fed broodfish. Mean percent ovulation, fecundity, fertilization, and hatch did not differ between the two groups of gravid channel catfish in hybrid catfish fry production.

High lipid broodfish feed had higher lipid content, n-3 fatty acids, and n-3 long chain fatty acids than the Low lipid broodfish feed, and also translated higher lipid and fatty acids to oocytes (Table 1). However, translation of higher lipid and fatty acids in oocytes did not improve egg quality, fecundity and hatching success. Results of this study and relevant studies in future to improve catfish reproduction and hybrid catfish fry production will be discussed.

Table 1. (Mean \pm SE) Fatty acid (FA) and long chain polyunsaturated fatty acid (LCPUFA) composition of oocytes of channel catfish that were fed with High lipid broodfish feed and Low lipid broodfish feed during the post-spawning period.

Broodfish feed	Total sat FA	Total n-3 FA	Total n-6 FA	Total n-3 LCPUFA	Total n-6 LCPUFA
Low Lipid	31.3 \pm 0.30	9.8 \pm 0.7	19.2 \pm 0.55	9.4 \pm 0.70	11.2 \pm 0.53
High Lipid	30.4 \pm 1.28	12.1 \pm 1.24	16.1 \pm 0.57	11.5 \pm 1.04	8.8 \pm 0.61
P-value	0.38	0.05	0.03	0.05	0.007

AQUAPONIC TECHNOLOGY AS A CATALYST TO BRING SUSTAINABLE SALMON PRODUCTION OUT OF THE NET PEN AND INTO LAND BASED RAS FACILITIES

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Environmentally controlled Recirculating Aquaculture Systems (RAS) are continually increasing in popularity due to their water efficiency, increased biosecurity, increased growth rates, decreased mortality rates, reduction of environmental impact and the increased understanding and acceptance of RAS technology by aquaculturists worldwide. Integrated aquaculture or “Aquaponics” is taking RAS efficiency one step further by utilizing the waste created during production to grow a profitable plant product. Aquaponic technology has been further refined in recent years as aquaculturists look for new ways to increase profit margins and product offerings while also decreasing waste streams flowing from their farms.

Salmon is one of the world's most consumed fish and farmed salmon makes up 70% of the total salmon consumed. Land based smolt production in RAS is continually increasing, as is the size of smolt required by the industry, yet the vast majority of grow out is still done in net pen production due to cheaper startup costs, faster ROI and lack of long term business successes in land based production. The industry continues to grow at a fast pace, while in many areas of the world water restrictions and discharge regulations are becoming tighter, requiring farmers to do more with less.

With populations increasing, as well as an increase in dietary recommendations to consume more fish, it is clear that salmon consumption will continue to rise in the future as will the need to efficiently farm salmon in an environmentally and socially acceptable way. Further increasing the efficiency of the RAS business model by utilizing aquaponic technologies increases profit margins, diversifies revenue streams, lowers overall risk and opens serious possibilities of bringing the salmon industry onshore permanently.

INORGANIC AND ORGANIC DIETARY COPPER SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND INTESTINAL MORPHOLOGY OF JUVENILE RED DRUM (*Sciaenops ocellatus*)

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Copper (Cu) is an essential trace element for fish. A commonly copper source used in animal feed is copper sulfate (CuSO_4) that has poor bioavailability due the antagonism between this mineral and other nutrients, thus impairing its uptake and being excreted into the environment. The high price of fish meal has led to higher inclusion of plant proteins in aquaculture feeds, which can affect absorption of inorganic copper, because of the presence of anti-nutrients such as phytic acid. On the other hand, organic minerals have higher bioavailability, because they are chelated with peptides, amino acids and other organic compounds that protect the minerals from reactions in the gastrointestinal tract. Therefore, the aim of this study was to investigate the effects of dietary supplementation of inorganic and organic copper compounds for red drum.

Red drum muscle, wheat gluten, and crystalline amino acid were used as protein sources to ensure that the basal diet had minimum level of natural copper. The diet was formulated to contain 35% crude protein and used as a negative control, and either copper sulfate or copper-ethanolamine complex were added to the basal diet at 5, 10, 20 mg Cu/kg dry diet. The feeding trial was conducted in a recirculation system in which 17 fish (initial weight of ~6 g/fish) were assigned to 110-L glass aquaria. All treatments were fed to triplicate aquaria and fish fed twice daily to approach apparent satiation for 7 weeks.

The trial is currently being conducted and growth performance and survival are presented on table 1. Further results in regards to final weight gain, feed efficiency and additional analysis will be reported soon.

Table 1: Preliminary growth parameters and survival for the trial at the 5th week

Copper concentration (mg/kg)	Basal	Inorg 5	Inorg 10	Inorg 20	Org 5	Org 10	Org 20	P value	PSE
Weight gain (%)	255.2	245.9	246.3	248.8	249.1	250.4	248.5	0.99	3.817
Feed Efficiency	0.58	0.6	0.6	0.6	0.6	0.59	0.61	0.88	0.005
Survival (%)	94.1	100	100	96	98	98	100	0.71	1.02

GROWTH COMPARISON OF TWO COHORTS OF GULF KILLIFISH *Fundulus grandis* IN FRESHWATER PONDS AND SALINITY TOLERANCE POST- POND CULTURE

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Interest in culturing Gulf Killifish (*Fundulus grandis*), an estuarine species found along the Northern coast of the Gulf of Mexico and the Eastern coast of Florida, has been growing due to its use as a baitfish and its euryhalinity, allowing for culture in low salinities. However, information is limited on freshwater pond production and post-culture salinity tolerance, knowledge gaps that must be addressed before inland producers will adopt this species for commercial culture. Therefore, studies were conducted to determine if growth and survival were similar in cohorts stocked 4 weeks apart and if freshwater grow out affects salinity tolerance.

A 12-wk freshwater pond growth experiment was conducted for two cohorts from 6/28/17- 9/18/17 and 7/31/17- 10/16/17, respectively. Each cohort had 3 ponds, stocked with a density of 25,000 fish/ha and evaluated for 12 weeks. Ponds were sampled every 4 weeks to monitor growth. Initial and final total lengths (TL) between cohorts did not vary significantly; however mean survival between the two cohorts did vary (Table 1). Possible reasons for variation include differences in pond water quality parameters, such as pH and hardness.

After the first cohort was harvested, 30 fish were randomly selected to evaluate salinity tolerance post-pond culture. Fish were randomly placed into individual containers at three salinity treatments of freshwater (FF: 0.5 ppt), brackish water (FBS: 8 ppt), and saltwater (FS: 33 ppt) and monitored for 120-hr. Fish in the FBS treatment were placed into saltwater after a 6-hr acclimation period in the brackish water to mimic hauling tank conditions. Whole body moisture was significantly different between treatments, although other parameters were similar (Table 2). These studies show that cohorts will have similar growth if stocked several weeks apart, and freshwater grow out may affect salinity tolerance, although further studies are needed.

TABLE 1. Mean (\pm SE) growth and survival of two Gulf Killifish cohorts over a 12-week period. Growth represented in TL (mm). Means with different letters were significantly different ($P < 0.05$).

	Cohort	
	1	2
Initial	39.2 \pm 1.5	35.2 \pm 1.5
Week 4	66.9 \pm 0.9a	58.6 \pm 0.9b
Week 8	77.2 \pm 0.7a	72.6 \pm 0.7b
Final	84.7 \pm 2.0	80.0 \pm 2.0
Survival (%)	40 \pm 0.2a	79 \pm 0.0b

TABLE 2. Mean (\pm SE) weight, TL, hematocrit (HCT), osmolality, whole body moisture (WBM), plasma proteins, and survival of Gulf Killifish in three post-harvest salinities for 120-hr. Means with different letters were significantly different ($P < 0.05$).

	Treatment		
	FF	FBS	FS
Weight (g)	8.07 \pm 0.17	7.84 \pm 0.21	8.26 \pm 0.13
TL (mm)	81.1 \pm 1.1	80.8 \pm 0.9	82.5 \pm 0.8
HCT (%)	41.8 \pm 1.9	38.1 \pm 2.2	37.3 \pm 2.7
Osmolality	328.7 \pm 9.3	354.3 \pm 31.1	358.5 \pm 23.8
WBM (%)	71.7 \pm 0.3b	72.5 \pm 0.5ab	73.3 \pm 0.4a
Plasma proteins	26.0 \pm 4.3	25.4 \pm 3.4	22.7 \pm 5.0
Survival (%)	100	90	80

ASSESSMENT OF TOTAL AND PATHOGENIC VIBRIOS IN OYSTERS USING COPP ASSAY AND MOLECULAR METHODS

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Oysters are the most commonly harvested shellfish in the world and when eaten raw they pose serious health risks due to potential exposure to human pathogens such as *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *Vibrio* infections are the leading cause of seafood-borne bacterial illness in the United States since 2001, with increasing threat over the past decade. A novel microbial technique, known as Colony Overlay Procedure for Peptidases (COPP) was developed by USDA-Agricultural Research Service scientists from Delaware State University to identify total *Vibrio* present in seafood and water samples. This research was aimed to detect the levels of total *Vibrio* in oysters and confirm the presence of pathogenic *Vibrio* using real-time PCR. The study was carried from June through October 2016; oysters and water samples were collected from three sites based on their salinity level and tidal flushing potential within the Delaware Bay once every month. COPP analysis was performed and the bacterial samples isolated on Chrom Agar plates were identified using PCR. Genes targeted to confirm the presence of *Vibrio parahaemolyticus* are the thermolabile haemolysin and thermostable direct haemolysin while cytolysin/ haemolysin gene was used for *Vibrio vulnificus*. This research will have potential implications on aquaculture businesses and hatcheries as the novel COPP Assay is confirmed as a user friendly method that saves time and labor in detecting the total *Vibrio* levels in seafood.

BACTERIAL-ALGAL PHYCOSPHERE INTERACTIONS: IS THERE AN EFFECT ON GROWTH AND LIPID PRODUCTION?

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The phycosphere is defined as the region in which organic matter excreted by a phytoplankton cell are in high concentration relative to the surrounding environment. The phycosphere often contains specific bacterial communities that use the relatively high concentrations of nutrients for growth. Recent studies have demonstrated the potential for bacterial-algal interactions affecting microalgal growth and biomass accumulation. The purpose of this study was to evaluate how bacterial-algal phycosphere interactions alter the growth rates and lipid content of microalgae and identify secondary metabolites that are correlated with these changes.

To assess the effect of bacteria on microalgae, we investigated 11 co-cultures of a single strain of the diatom *Phaeodactylum tricornutum* with different species of bacteria isolated from its phycosphere. These co-cultures were compared to an axenic (bacteria-free) culture. Growth rates were determined for all cultures. Cultures that exhibited the highest and lowest growth rates relative to the axenic were tested for lipid/fatty acid analyses and compared. Bacteria were then re-isolated from these co-cultures and maintained axenically. The bacteria were added back to the axenic *Phaeodactylum* culture at the same concentration found in the original co-cultures. The new co-culture were grown in 2.5 L volumes. The growth rates, lipid content, and fatty acid profiles were compared between the new co-culture, original co-culture, and the axenic culture to isolate the effects of the bacteria. A time of flight- mass spectrometer system was used to identify differences in secondary metabolites between co-cultures that had a significant effect of bacteria: algae and the axenic culture.

For the exponential period of growth there is an average decrease of 32% in growth rate between the top and bottom performing co-cultures. Maximum doublings per day decreases by 46% from the top and bottom performing co-cultures. The co-culture that exhibited the highest growth rate was associated with bacteria from the *Muricauda* genus. The co-culture with the lowest growth rate was associated with bacteria from the genera *Arenibacter*. Interestingly both of these genera are from the same family of bacteria, the Flavobacteriaceae.

Most algal species have specific phycosphere associated bacteria, which can have beneficial or detrimental effects on their microalgal hosts. Interactions that increase growth rates and lipid production could improve the production efficiency of biofuels and increase production of essential fatty acids for human health supplements or agriculture/aquaculture feeds. Bioactive compounds that exhibit inhibitory properties could be used to mitigate the effects of harmful algal blooms in coastal waters and fresh water resources.

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WHICH AUTHOR IS WHICH? GENDER AUTHORSHIP POSITION AS A PROXY FOR THE STATUS OF GENDER IN AQUACULTURE LITERATURE

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Examining authorship position in aquaculture facilitates an improved understanding of status of women in the discipline, as authorship is a critical factor in professional success in academia and beyond. In a review of more than eight million papers in the JSTOR Corpus across disciplines in natural and social sciences and humanities, West et al. 2013 found that men predominate in the first and last author positions and women are underrepresented in single-authored papers. Other studies have assessed women authorship in other disciplines such as law and medicine, and found that a gender gap in published literature still persists.

This study applies the large sample size and methodology of West et al. 2013 to the broad discipline of aquaculture, and compares these results to gender authorship in the International Aquaculture Curated Database (IACD) – a compilation of 543 peer-reviewed publications supported by four long-term international aquaculture programs headquartered at Oregon State University and a curated database of aquaculture journals in the Web of Science. Results reveal that the percentage of women authors (13.8%) was similar for the JSTOR aquaculture subsample and the IACD (15.7%), yet significantly lower for that of the Web of Science database (3.7%). Women are not well represented any of the databases, and remain underrepresented as authors in any position in aquaculture journals. To contextualize our findings with the percentage of women graduating in the field, we examined the number of women graduates in agricultural, biological, natural, and social sciences who earned Bachelor's, Master's, and PhD's in the U.S. from 1991-2015. Results from the U.S. Department of Education's National Center for Education Statistics shows that the percent of women graduates each year has increased with women representing more than 50% of graduates in 2015. While this does not represent international graduates, it still provides some contextualization for the proportion of women in the discipline. Learning how authorship has changed in the aquaculture discipline over the last few decades is critical for promoting gender equity for future aquaculture scholarship and the sustainability of the professional discipline.

ORGANIC ACID SALTS, A PROTEASE CMOPLEX AND THEIR COMBINATION AFFECT PERFORMANCE OF COMMON CARP *Cyprinus carpio* FED MEAT-BONE MEAL BASED DIETS

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A nine-week trial was conducted at the Shanghai Ocean University of China to assess the effects of organic acid salts (OAB), a protease complex (PR) and their combination (OAB+PR) on growth performance, body indices and serum biochemical indices of common carp *Cyprinus carpio*. The trial consisted of five dietary treatments: positive control (PC, 6% fish meal - FM), negative control diet (NC, where FM was substituted with meat and bone meal - MBM), and three other NC diets, supplemented with OAB, PR, OAB+PR.

Weight gain (WG, %), feed conversion ratio (FCR) and protein efficiency ratio (PER) between animals fed PC (299 ± 2.3 , 1.73 ± 0.03 , 1.89 ± 0.03 , respectively) and NC (215 ± 4.1 , 1.90 ± 0.04 , 1.74 ± 0.04 , respectively) diets was observed. However, no differences in these parameters were observed in animals fed the PC diets with those fed the OAB, PR and OAB+PR diets.

Table: Growth performance of common carp fed the treatment diets

There were no differences in condition factor, hepato-somatic indices, viscero somatic indices and whole body chemical composition of animals fed the treatment diets except for the body phosphorus content (0.65%) in animals fed the OAB+PR diets, the among the treatments. Among the serum biochemical indices, only total anti oxidative capacity (T-AOC) and malondialdehyde (MDA) content OAB and OAB+PR diets were significantly better than the other diets.

It can be concluded from this study that the blend of organic acid salt and their combination with the dietary protease complex can improve performance of common carp when fish meal is completely replaced with meat-bone meal.

Table: Growth performance of common carp fed the treatment diets

Treatments	WG (%)	FCR	PER
PC, 6% FM	231.8 \pm 3.0b	1.73 \pm 0.03a	1.89 \pm 0.03b
NC, 0% FM, 6% MBM	214.7 \pm 4.1a	1.90 \pm 0.04b	1.74 \pm 0.05a
NC + Organic acid blend	230.8 \pm 3.5b	1.83 \pm 0.06ab	1.84 \pm 0.05ab
NC + Protease	221.9 \pm 8.7ab	1.82 \pm 0.04ab	1.80 \pm 0.03ab
OAB+PR	223.9 \pm 2.9ab	1.84 \pm 0.04ab	1.80 \pm 0.04ab

USING A CONSUMER BRAND TO FACILITATE DISCUSSION ABOUT AQUACULTURE

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Environmentally responsible marine aquaculture will play an increasingly important role in our food supply for the health and wellbeing of people and the environment. However, public perception of aquaculture is poor, fueled by misinformation that is often out of date or provided out of context.

LoveTheWild, an emerging consumer seafood company based in Boulder, CO, has a mission to de-demonize great farmed fish and make sustainable aquaculture one of the most exciting and in demand consumer proteins. The case study will explore LoveTheWild's use of branding, celebrity and influencer endorsements, brand partnerships, shopper marketing, and digital storytelling to deliver scientific content in an easily digestible format and engage consumers in the aquaculture discussion.

SUPPLEMENTING RAINBOW TROUT BROODSTOCK DIETS WITH CHOLINE AND METHIONINE IMPROVES OFFSPRING GROWTH

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The objective of this study was to determine whether supplementing female rainbow trout broodstock diets with the epigenetic modifiers choline, methionine, or a combination affects offspring growth performance.

Four treatment diets were produced by top-dressing a commercially available finfish broodstock diet (38% protein, 10% fat) with 1) choline (final 7400 ppm), 2) DL-methionine (final 12000 ppm) or 3) choline + DL-methionine. The fourth diet was the base-diet top dressed by the nutrient vehicle (distilled water) and served as the control. Six families of female rainbow trout were fed treatment diets at or just below satiation beginning 18 months post-hatch through spawning (n=3 tanks per diet). Eggs were collected and fertilized using milt from a single family of unrelated males. Offspring families were reared individually until they were PIT-tagged at approximately 10 g and comingled in three triplicate tanks. Offspring consumed a single commercially available diet (42% protein, 16% fat) for the majority of the grow-out period. Lengths and weights were recorded every 2 months.

There were no differences in maternal body weight, body condition, egg size, or egg yield at spawning that were attributed to broodstock dietary treatments. There was an effect of broodstock diet on offspring growth performance that was age-dependent (Table 1). Offspring fry from choline-treated broodstock (diet #1) were smaller than fry from other treatment groups through 146 days post hatch (dph, ~11g). However, by 259 dph, offspring from broodstock that received choline (diets #1 and #3) exhibited greater body weights than offspring from broodstock that received the control feed (diet #4). By 377 dph body weights of offspring from methionine-treated broodstock (diet #2) also exceeded controls. Offspring condition factor did not exhibit a consistent response to broodstock diet. These findings provide strong evidence that dietary intervention strategies in broodstock can affect performance of the offspring.

Broodstock Diet	days post-hatch						
	90	146	196	259	316	377	439
1. Choline	3.29 ^c	10.64 ^b	43.9	136.0 ^a	336.6 ^{ab}	596.9 ^a	967.3 ^a
2. Methionine	3.70 ^b	11.43 ^a	44.3	131.3 ^{ab}	322.0 ^{bc}	570.6 ^a	928.3 ^a
3. Choline+Met	3.93 ^a	11.61 ^a	45.8	138.3 ^a	340.7 ^a	600.1 ^a	965.4 ^a
4. Control	3.59 ^b	11.27 ^a	43.5	126.9 ^b	303.9 ^c	534.3 ^b	808.2 ^b
Pooled SEM	0.08	0.30	1.0	3.4	7.5	13.1	27.2

Table 1. Effects of broodstock diet on body weight (g) of offspring throughout development. Different letters indicate a significant difference between mean body weights within the same column, $P < 0.05$.

DEVELOPING A VISITOR LEARNING CENTER FOR PUBLIC AQUACULTURE/AQUATIC EDUCATION

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The state of Alabama has tremendous aquatic resources, including the highest diversity of freshwater fish, freshwater mussels, crawfish, turtles and snails if all of the U.S. states. It is also the second largest aquaculture producer in the U.S. However, this is not common knowledge among Alabama residents. Auburn University has what they believe is the largest freshwater aquaculture research facility in the U.S. and perhaps the world. The E. W. Shell Fisheries Center has over 1800 acres, 313 ponds and over 2000 tanks and aquaria. For many years this facilities interaction with the public has been limited to fish market sales for 4 hours each Saturday. For the last 5 years AU faculty have been working on the development of a visitor education center to be housed at the E.W. Shell fisheries station and it is finally complete. The learning center, now filled with interactive and electronic displays along with live fish is open for the public to see and explore. In 2017, the center provided an exciting interactive adventure for more than 1500 people from 22 states and 17 countries and we expect that number to more than double now the center is complete. The details of the journey to develop the visitor-learning center should be of interest to anyone who is involved in educating the public about aquaculture and aquatic resources.

MACROALGAE GROWTH: ASSESSMENT OF EXISTING MODELS and LIMITATIONS

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A wide variety of growth models have been used to describe the growth of macroalgae. The most common models include:

Specific growth rate (SGR)	$\text{SGR} = \frac{\ln\left(\frac{W_f}{W_i}\right)}{t}$	$W_f = W_i \times \text{Exp}(\text{SGR} \times t)$
Linear growth rate (LIN)	$\text{LIN} = \frac{W_f - W_i}{W_i \times t}$	$W_f = W_i + \text{LIN}(W_i \times t)$ [2]
Daily growth rate (DGR)	$\text{DGR} = \left(\frac{W_f}{W_i}\right)^{1/t} - 1$	$W_f = W_i \times (\text{DGR} + 1)^t$

Where W_f = final weight, W_i = initial weight, and t = time. Growth rates are typically reported as %/day. There is a lack of uniformity in the literature regarding the names used for these models; care must be taken to avoid confusion. This presentation will focus only on macroalgae growth in land-based intensive tumble culture.

Most of the reported macroalgae growth rates are only based on final and initial weights. With only two data points, all of these equations give a perfect fit to the data but tell nothing about the accuracy of the models for other times or conditions. The standard deviations of the predicted values for these three models were estimated from the growth data for *Kappaphycus* spp. presented by Yong et al. (2013). Based on the average growth parameters over the entire 7-day experiment, the linear growth model had lower standard deviations than for the specific growth rate (SGR) or daily growth rate (DGR). The fits based only on the initial and final weights were better than for average growth rates approach. For LIN and SGR, the best fit was produced by growth rates based on linear regression.

Before getting caught up in the details of fitting equations to data, it is best to step back and examine the characteristics of macroalgae growth in intensive tumble culture. When adequate nutrients and inorganic carbon was provided, Demetropoulos & Langdon (2004) found that the specific growth rate of Pacific dulse was a strong function of macroalgae density. An increase in density from 2 to 6 kg/m² resulted in a reduction of the SGR by a factor of 2.8. As a result, growth rate and yields, depend strongly on initial densities and harvest intervals. Comparison of growth data based on different initial densities and harvest intervals is misleading.

It is unlikely that a single conventional growth model will be valid over a wide range of densities or harvest intervals. At low densities, the specific growth rate or daily growth rate may be a good fit. At moderate density where the growth rate starts to drop off, the linear growth model may be a better fit. At high densities where growth rate and yields drop, it is unlikely if any of the conventional models will be valid.

Density-dependent growth is commonly observed in a number of different plant populations. Competition within a population is termed self-thinning. It may be possible to develop a self-thinning model that covers the full range of commercial macroalgae densities.

WHAT IS HAPPENING IN THE PACIFIC WITH AwF

Dave Conley * & Roy Palmer

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The Sustainable Pacific Aquaculture Development Project (PacAqua) is a new 5-year project that aims to improve food security and economic development in the Pacific through sustainable aquaculture by enhancing business acumen among aquaculture operations, reduce aquatic biosecurity risks and increase uptake and adoption of improved aquaculture practices.

The project is funded by the Ministry of Foreign Affairs and Trade (MFAT) under the New Zealand Aid Programme. The project is being implemented by Fisheries, Aquaculture and Marine Ecosystems Division (FAME) of the Pacific Community (SPC).

The project activities will provide business mentoring and training, capacity development and technology transfer in feed, seed and brood stock management to selected enterprises and partners. The project will also build capacity at the national level by training government fisheries staff in areas of biosecurity, feed, seed and brood stock management and other services that underpin private sector and community led aquaculture. There is an emphasis on women and gender activities and improvements.

The project is in response to the recognized need that, to increase and improve economic and nutritional gain from aquaculture in the Pacific, aquaculture be developed on a business-like footing, be it private sector or community led.

GOAL - Food security and livelihoods in the Pacific are improved through sustainable aquaculture demonstrating enhanced business acumen, reduced aquatic biosecurity risks and increased uptake of improved aquaculture practices.

FEMINIZATION OF PROTANDROUS HERMAPHRODITES *Centropomus poeyi* INTO FUNCTIONAL FEMALES USING 17 β -ESTRADIOL

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Several Centropomid species support important recreational and commercial fisheries in the Gulf of Mexico, among them, the Mexican snook *Centropomus poeyi* is a unique species that spends most of its life history in fresh water, but requires to migrate to the coasts to spawn. It is considered endemic to Mexico with a distribution range reported only in watersheds discharging to the Gulf of Mexico. Very limited information has been generated concerning its biology, ecology or population status. In our laboratory we have confirmed the condition of protandric hermaphrodite through histological examination. Based on empirical observations, *C. poeyi* in captivity has advantages over other Centropomids (i.e. large size, high tolerance to handling, grow-out in a wide range of salinities, and fast growth). However, handling broodstock poses complications since females range from 5 to 10 kilograms generating high risk of loss and expenses during maintenance of the spawners. Therefore, the aim of this study was the production of batches of reproductive females at an early age and a small size through induced sex inversion using 17 β -estradiol. For this, we conducted a study where low and high dosages of 17 β -estradiol (10, 20, and 30 mg kg⁻¹) and (40, 50, and 60 mg kg⁻¹) impregnated in the food were assessed. Fish fed with 50 and 60 mg kg⁻¹ of 17 β -estradiol showed 100% feminization, while the control group presented only males (Fig 1). At four years of age, sex-reversed fish remained as females, producing oocytes and were capable of spawning, while fish from the control groups were ripe males with running milt.

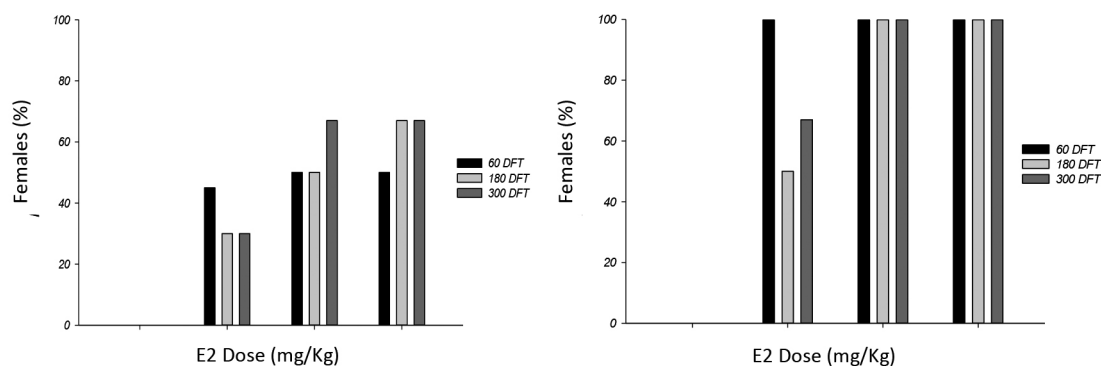


Fig 1. Average percent (\pm SE) of *C. poeyi* females obtained with low (a) and high (b) dosages of E2 impregnated in the food sampled at 60, 180 and 300 days post treatment.

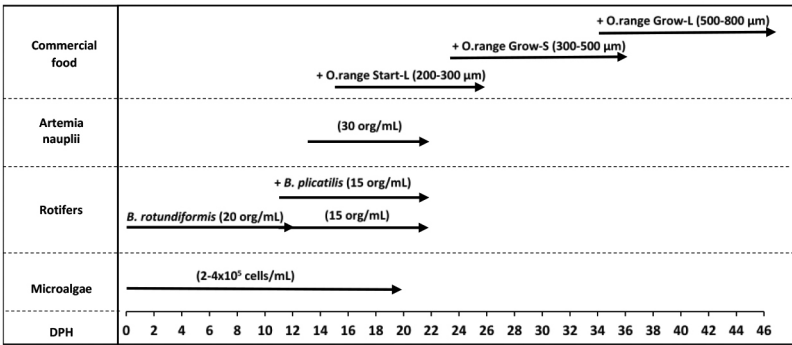
RECENT ADVANCES IN THE CULTURE OF NATIVE MARINE SPECIES FROM THE COAST OF TABASCO, MEXICO: PERSPECTIVES FOR STOCK ENHANCEMENT AND MARINE AQUACULTURE

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Marine fishes represent an important source of food for inhabitants of the coastal region of Tabasco. Their fisheries are the only source of income for several communities and aquaculture is seen as the future for sustained resource management since catch volumes are decreasing at a constant rate. At the Marine Aquaculture Station located in Jalapita Tabasco, we continue our efforts for domesticating native marine species of fish. Broodstocks originated from wild populations are currently been assessed from three species of snooks: *Centropomus undecimalis*, *C. poeyi* and *C. parallelus*. The protocol for induced reproduction is complete including hormone type, dose and spawning timing. Spawning is achieved either with wild broodstock or sex-reversed females. We are currently working on larviculture and feeding regimes. Live food include phytoplankton (*Tetraselmis chuii* and *Nannochloropsis oculata*); zooplankton (*Brachionus rotundiformis*, *Brachionus plicatilis* and *Artemia* spp). In recent years, some species of the family Lutjanidae were incorporated (*Lutjanus griseus*, *Lutjanus analis*, *Lutjanus cyanopterus*), as well as, the sparid *Archosargus probatocephalus*. We have developed strong ties with the aquaculture and fisheries sectors, workshops and seminars have been conducted, particularly oriented towards Centropomid aquaculture, restocking and good fishing practices. Our next challenges are consolidating larviculture, evaluation of growth under culture conditions and massive release of juveniles in the neighboring coastal lagoons.

Figure 1. Feeding regime proposed for *C. poeyi*



GROWTH, DEFORMITIES AND SURVIVAL OF SABLEFISH LARVAE *Anoplopoma fimbria* FED TAURINE-ENRICHED ROTIFERS

Matthew A. Cook*, Jonathan S.F. Lee, Ronald B. Johnson and Frederick W. Goetz

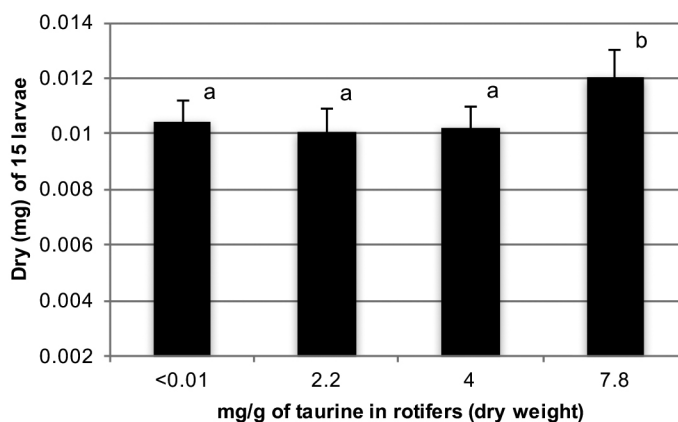
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Sablefish (*Anoplopoma fimbria*) is a high value fish found throughout the northeastern Pacific. There is a worldwide demand for sablefish and a strong interest in the U.S., Canada and Korea to develop sablefish as an aquaculture species. To improve aquaculture protocols for sablefish larvae we studied the effects of increased dietary taurine during the rotifer feeding stage on growth, deformities and survival from first-feeding to juveniles. Malformed fish cannot be used for post weaning nutrition and developmental research or retained for our sablefish broodstock program at NOAA. Reducing sablefish deformities and improving growth and survival during early larval development is important to aquaculture facilities, researchers and consumers.

First-feeding sablefish larvae were fed taurine-enriched rotifers and a non-aurine control (<0.01, 2.2, 4.0 or 7.8 mg/g dry weight) for 14 days. All treatments (n=4) were switched to *Artemia* and then weaned to dry diet. Rotifers were enriched with taurine by placing them in a solution of aerated seawater and taurine at zero 2, 4 and 8 g/L for one hour. Larvae samples were taken for dry weights, lengths and rotifer consumption at several points during development. Samples were also taken to determine deformities after the taurine feeding, at weaning and three months after weaning.

On day 14, the end of the taurine feeding, larvae fed the highest taurine concentration were significantly heavier ($p = 0.0064$) than the other three treatments (Figure 1). Larvae from the high taurine treatment were still significantly heavier at weaning compared to the other treatments ($p = 0.0365$). Survival at weaning was ~10% lower in the control treatment compared to the three taurine treatments but not significantly lower. Rotifer consumption was higher in the taurine treatments on three of the four sampling days compared to the control, though not significantly higher. Total deformities per 100 juveniles was greater in the control by ~15% compared to the taurine treatments, however the result was not significant. Adding taurine to rotifers improves sablefish larvae growth and appears to increase survival to weaning and reduces overall deformities in 120-day old juveniles.

FIGURE 1. Sablefish larvae weight 14 days post first feeding with taurine enriched rotifers.



BUILDING AWARENESS AND SKILLS FOR AQUACULTURE IN K-12 STUDENTS

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Background: Seafood Nutrition Partnership (SNP) is the leading 501(c)3 non-profit organization in the U.S. building awareness of the health and nutritional benefits of seafood. SNP is addressing the country's public health crisis through education programs that inspire Americans to incorporate more seafood and omega-3s into their diets for improved health as per USDA Dietary Guidelines.

The 2015-2020 USDA/HHS Dietary Guidelines for Americans recommends at least two servings of seafood per week and at least 250mg of omega-3s EPA+DHA per day to support heart and brain health as part of a healthy diet. Only 10% of Americans eat seafood twice a week and on average Americans take in 80mg of omega-3s EPA+DHA per day and children take in a lower level than adults.

Program Pilot: SNP launched a Seafood In Schools – Aquaponics Pilot for the 2017-2018 school year. The goals for this pilot are to help the next generation understand the health benefits of eating seafood and learn about aquaculture through a classroom aquaponics system and accompanying instructional materials. Learnings from the first half of the pilot will be presented.

SNP provided 10 schools with classroom sized aquaponics system, aquaponics educational materials for 3rd to 6th grade, and seafood nutrition educational materials for 3rd to 8th grade teachers. SNP designed measurement tools to determine the knowledge gains from this initial pilot.

In the initial pilot, SNP will determine the key success factors that would help identify teachers with the best background such as the support required from the teacher's school, school environment, student demographic, and other factors that arise in the initial pilot phase.

To ensure that an organization receives the maximum benefit from an aquaponics system, our hypothesis is that ideal teachers will be those that teach STEM, Family & Consumer Sciences, and Nutrition; the school may need to approve introduction of this system into the classroom; a small teaching team from teacher, food service, and school operations would be helpful with installation, planning for use of the system in the classroom and link to school foodservice, and maintenance during school and out of school periods.

CULTIVATION OF NOVEL LINEAGES OF THE SEA ASPARAGUS *Salicornia neei* WITH SALINE EFFLUENT OF SHRIMP FARMING IN FIELD PLOTS

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Salicornia neei Lag. is a native species of the Brazilian coast with high nutritional quality and can be cultivated with sea water. Since 2010, a breeding program generated distinctive pure lineages of *S. neei* but their growth under saline irrigation yet need to be evaluated. Plants of f3 and f4 progenies of both BTH1 and BTH2 lineages of *S. neei* (n= 20 per progenies) were grown in two field plots with fine sand soil (6.5 × 3.5 m), which were randomly assigned to be irrigated with 375 L of shrimp farming effluent every other day (T2) and every 4 days (T4) for 22 weeks.

The saline effluent was the main source of nutrients and water for *S. neei* plants. During the growth period, the average values (\pm standard error) of water salinity, pH, nitrate, ammonium and phosphate were 18.50 dS m⁻¹, 8.65 \pm 0.04, \leq 0.03 NO₃-N mg L⁻¹, 0.15 \pm 0.13 NH₄-N mg L⁻¹, 0.30 \pm 0.05 and PO₄-P mg L⁻¹, respectively. The average daily temperature, rainfall and solar radiation were 21.2 \pm 0.4 °C, 2.2 \pm 0.1 mm day⁻¹ and 1835 \pm 26 kJ m⁻² hour⁻¹. Over the last 13 weeks of the experiment, during summer-fall 2015, fell only 20 mm of rain. In this dry period, the average soil moisture of T2 plot (11.0 \pm 0.5%) was significantly (p< 0.001) higher than in T4 (9.4 \pm 0.5%). There were no differences in the average soil electrical conductivities (CE_{1,2}) of the plots (15.1-15.6 dS m⁻¹).

There were no significant differences between the fresh shoot biomass between the two generations of each lineage (Figure 1). However, on average, BTH2-f4 plants showed significantly (p< 0.001) larger shoots (201.2 \pm 17.1 g) than BTH1 plants (f3= 104.0 \pm 8.9 g; f4= 142.6 \pm 12.1 g). No differences were observed in the global averages of individual fresh shoot biomass between the plots T4 (164.7 \pm 8.3 g) and T2 (154.3 \pm 12.9 g). Both lineages of *S. neei* can thrive under low volume irrigation with saline effluent.

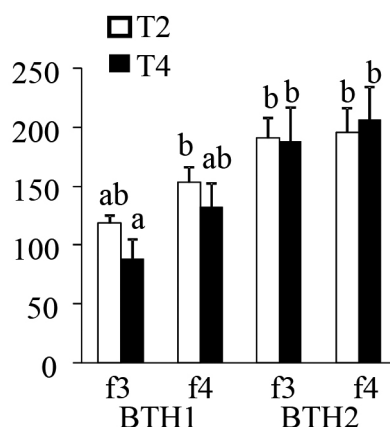


Figure 1. Averages (\pm standard error) of fresh shoot biomass of four progenies of *S. neei* after 22 weeks of cultivation irrigated every other day (T2) and every 4 days (T4) with saline effluent of shrimp farming. Different lowercase letters represent significant differences between the averages (p < 0.05), according to the Tukey test.

HYDROPONIC PRODUCTION OF THE SEA ASPARAGUS *Salicornia neei* WITH RECIRCULATING SALINE WATER FROM A MARINE FISH CULTURE

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The species of the genus *Salicornia* (Amaranthaceae) showed great productive potential when irrigated with saline water and marine aquaculture effluents. Their succulent shoots are sold as ‘Samphire’ or ‘Sea asparagus’ and have high nutritional quality and chemical characteristics for human and animal diets, biofuel production and the pharmaceutical industry. In Brazil, a breeding program generated a pure lineage of the native *Salicornia neei* Lag. denominated BTH2. The aims of this study was to quantify the growth and biomass production of the lineage BTH2 of *S. neei* in a nutrient film technique (NFT) bench irrigated with recirculating saline water from fattening tanks of a culture of cobia (*Rachycentron canadum*), marine fish, in Rio Grande (RS, Brazil).

The NFT was set up on a commercial hydroponic bench, accommodating a total of 120 plants, which received saline water from a water tank containing 450 L, with a flow of 385 L/hour and a daily water renewal rate of 20.5 times. The saline water in the tank was replaced once a week and EC ranged between 42-48 dS/m. The water concentrations of nitrate, ammonium and phosphate ranged between 21.9-34.9 mg NO₃-N L⁻¹, 0.1-0.3 mg NH₄-N L⁻¹ and 0.6-7.2 mg PO₄-P L⁻¹, respectively. *S. neei* plants are perennials and 64 days after being established in the NFT bench each shoot was cut to 4 cm above ground to evaluate plant sprouting and biomass production.

On average *S. neei* shoots originated from sprouts (88 days after initial cutting) showed a shoot height of 26.4 cm, 5.4 branches per shoot with a maximum branch length of 16.5 cm and a fresh shoot biomass of 36.2 g (Figure 1). BTH2 plants surpassed a commercial shoot height of 10 cm adopted internationally for gourmet species of sea asparagus. The potential production of fresh biomass of *S. neei* shoots of lineage BTH2 was from 2.6 kg/m²/year.



Figure 1. Plants of the lineage BTH2 of *Salicornia neei* growing in a NFT bench irrigated with recirculating saline water from fattening tanks of a culture of cobia.

ARTIFICIAL INTELLIGENCE TO GAIN VALUABLE INSIGHT ON AQUATIC ORGANISMS

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Data quantity and reliability has been the main driver for yield optimization in agriculture and most livestock industries. In aquaculture however, accurate and reliable data is hard to obtain since counting and sizing small aquatic organisms mostly still relies on manual methods. These manual methods are time consuming, inaccurate and non-repeatable. Inconsistency in inventory assessments of aquatic organisms leads to mismanagement of feed and poor production performances for aquaculture producers.

In recent years, technologies such as computer vision have been explored with moderate success to provide information about aquatic organisms. However, recent development in artificial intelligence are proving to deliver viable options for efficient development of computer vision based solutions in aquaculture. In this project, an artificial intelligence approach using machine learning and computer vision was used to accurately predict the number of Giant tiger prawn (*Penaeus monodon*) post-larvae in a production setting. Data was gathered using an electronic device that image samples in optimal conditions. A training framework was then used to train and validate a classifying algorithm based on annotated data.

Once trained, the algorithm could count Giant tiger prawn (*Penaeus monodon*) post-larvae with more than 97.6% accuracy and 2.1% standard deviation. Other algorithms were also developed combining different technologies for different species and prediction of the size distribution was also implemented in a similar but more complex way.

Fig. 1. Example of a sample image being processed.

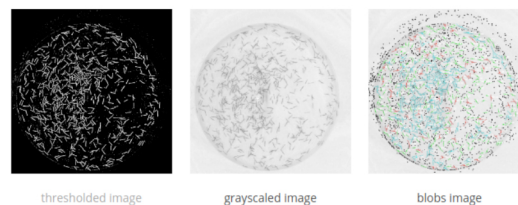


Table 1. Counting accuracy of Giant tiger prawn post-larvae using artificial intelligence.

Mean (%)	Standard deviation	N
97.6	2.1	723

TEXAS PARKS AND WILDLIFE COASTAL CONSERVATION ASSOCIATION MARINE DEVELOPMENT CENTER SOUTHERN FLOUNDER *Paralichthys lethostigma* 2017 CULTURE PRODUCTION UPDATE

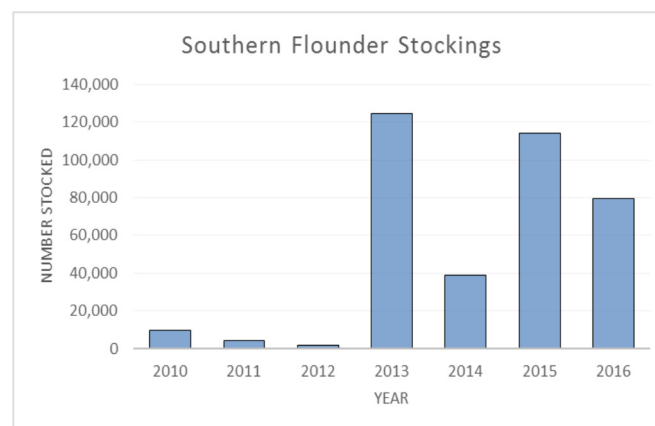
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Texas Parks and Wildlife Department dedicated to conserving the natural resources of the state and managing those natural resources for sustainable usage while providing fishing, hunting, and outdoor recreational opportunities to the public. Stock enhancement of fish, vital to maintaining sustainable populations in the face of fishing pressure, habitat degradation, and other issues, is a primary goal of the Texas Parks and Wildlife Department. Stock enhancement efforts involve red drum, spotted seatrout, and southern flounder. Southern flounder, *Paralichthys lethostigma*, have been cultured for stock enhancement purposes at Texas Parks and Wildlife Coastal Conservation Association (TPWD CCA) Marine Development Center in Corpus Christi, Texas for several years, with updates on culture practices frequently occurring. Improving culture methods may collectively improve growth and survival of cultured southern flounder for stock enhancement.

Texas Parks and Wildlife Coastal Fisheries resource monitoring data indicates that southern flounder populations have been in steady decline along the Texas coast for many years. As a means to supplement southern flounder populations, Coastal Fisheries has initiated a propagation program for purposes of stock enhancement. Methods to rear southern flounder on a large-scale hatchery basis are still in the early stages of development. Additional facilities to culture flounder are in planning stages and scheduled for construction initiation in late 2017. The new facilities will extend the production season for this species from the current four months to at least eight months per year, and incorporate flounder culture advances into routine hatchery operations. To date, 378,091 hatchery-reared southern flounder fingerlings have been stocked into Texas coastal waters since 2006 (Figure).

This presentation will describe current practices, production, and future goals for southern flounder culture at the TPWD CCA Marine Development Center.



POTENTIAL OF FISH EATING BIRDS TO SPREAD VIRULENT *Aeromonas hydrophila*

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Aeromonas hydrophila is a Gram-negative, rod shaped, facultative anaerobic bacterium that is ubiquitous to freshwater and slightly brackish aquatic environments and can cause infections in fish, humans, reptiles and avian species. Recent severe outbreaks of disease in commercial catfish aquaculture ponds have been associated with a highly virulent *Aeromonas hydrophila* strain (VAh) that is genetically distinct from less virulent strains. This strain is responsible for over 3 million pounds of catfish losses per year.

Previous research has shown that Great Egrets, Double-crested Cormorants (*Phalacrocorax auritus*), American White Pelicans (*Pelecanus erythrorhynchos*), and Wood Storks (*Mycteria americana*) can carry and shed viable VAh after consuming fish infected with VAh. These fish-eating birds can serve as a reservoir for VAh and may spread the pathogen while foraging on uninfected catfish ponds.

Therefore, our objectives were to 1. Examine the role of fish-eating birds in the epidemiology and spread of VAh, 2. Evaluate the potential and likely process for colonization of VAh in non-endemic catfish ponds by evaluating transmission using a piscivorous bird (Great Egret, *Arde alba*) model in experimental ponds. We found that VAh survives passing through the GI tract of Great Egrets and viable VAh can be shed at substantial levels for a limited period when birds consume infected fish. We found that both treatment ponds had positive qPCR results for VAh while the control pond remained negative. VAh was detected in fish, pond water, chironomids, invertebrates, snails and mud from treatment ponds. We conclude that fish eating birds that consume fish infected with VAh can spread the bacteria to naïve ponds and cause a disease outbreak.

REPRODUCTION, DEVELOPMENT, AND GROWTH OF CICHLID HYBRIDS (*Cichlasoma synspilum*, *C. urophthalmus*, and *Amphilophus citrinellum*) – TO CONTINUE

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Fertility, survival, and growth of Central American cichlid hybrids and their backcrosses were investigated to address both production in captivity and prediction of success in invading new environments (Texas, Florida). Surprisingly little attention in the scientific literature has been devoted to the fertility and viability of these high priced ornamental fish hybrids that frequently are released or escape to the wild.

The original hybrid (red parrot) has a high survival rate and females reach maturity at one year old. The backcross has low viability ($11 \pm 7\%$ feeding; $n=6$), whereas the parrot/Mayan hybrid (PMH) has comparatively high viability (30% feeding), and a low rate of skeletal deformities. Juveniles were fed with rotifers and *Artemia* nauplii for the first 3 weeks and then transitioned to commercial feed, Otohime). Mean weight of PMH at 28 dpf (326 ± 94 mg) exceeded that of parental species, red parrot hybrid (310 ± 8 mg), *C. urophthalmus* (Mayan cichlid) (291 ± 24 mg) and red parrot backcross (191 ± 40 mg).

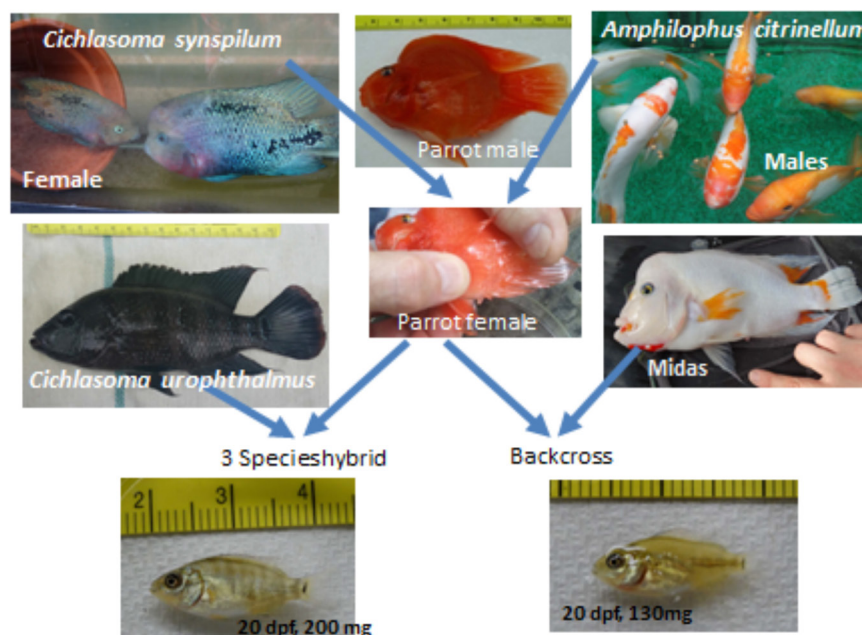


Fig. 1. Hybridization resulting in production of first generation hybrid and a backcross generation of red (blood) parrot and a 3-way hybrid of Mayan cichlid and red parrot (female). Age of juveniles is expressed in days post-fertilization (dpf).

SALINITY EFFECTS ON PHYSIOLOGICAL RESPONSES IN HYBRIDS OF MEXICAN, MAYAN AND MIDAS CICHLID HYBRIDS (*Cichlasoma synspilum*, *C. urophthalmus*, and *Amphilophus citrinellum*)

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Success of invasive fish species to adapt into new environments depends on their tolerance of variable salinity, and non-native cichlids in Florida are a prime example of such challenges. However, because hybridization among cichlids is not uncommon we investigated salinity tolerance among Central/South American cichlid hybrids (Fig.1). A series of experiments assessing both chronic and acute salinity tolerances of different juvenile stages of two hybrid cichlids were conducted.

In the first series of experiments, Mexican x Midas cichlid hybrids, commonly called blood parrot cichlid, were subjected to salinities ranging from 0-28 ppt at three different body sizes (0.09g, 2.0g, and 19.4g) for three weeks followed by an acute challenge. Survival, growth, and feed conversion were recorded throughout all chronic experiments. Blood plasma osmolality and hematocrit were analyzed during experiments where fish size allowed for collection of blood.

A final acute salinity challenge of Mexican x Mayan hybrids and blood parrot cichlid was conducted to compare the salinity tolerances of both hybrid offspring by increasing salinity every 24-h from 0ppt to 20ppt, then to 30ppt, and finally to 35ppt. Blood parrot cichlids experienced loss of equilibrium leading to mortality following the increase to 30ppt at 27-hrs. Although blood plasma osmolality was significantly higher 24-h after increasing to 35ppt in challenged Mexican x Mayan hybrids (385 ± 29 mOsm) compared with controls (327 ± 4 mOsm), no mortality occurred throughout the 72-h trial. This indicates that Mexican x Mayan hybrid cichlids inherited salinity tolerance from Mayan cichlid and in comparison to blood parrot cichlids could pose an increased risk of invasion of both fresh and estuarine environments.

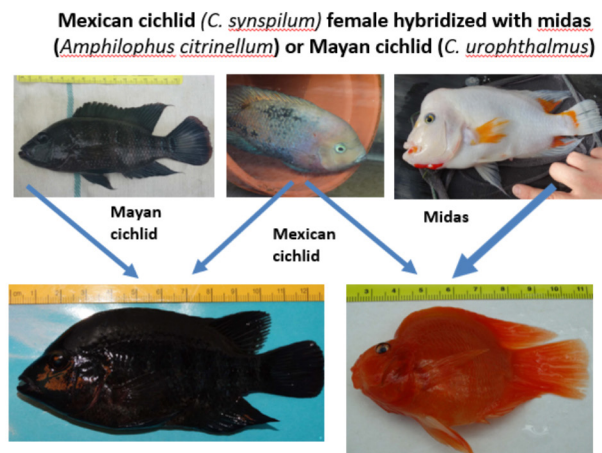


Fig. 1 Parent species and hybrid offspring examined in the experiments.

VOLATILITY DYNAMICS IN AQUACULTURE FISH MARKETS

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Global trade in seafood has more than doubled from 72 billion USD in 2004 to 148 billion USD in 2014 according to Food and Agricultural Organization (FAO) (2016). Aquaculture is the main contributor to this increase in trade, because of high growth in aquaculture supply (Asche, 2008). While wild landings stagnates due to biological limitations, aquaculture production has increased for several species thanks to improvements in technology and logistics. In a recent paper, Anderson et al. (2017) show increasing commoditizing for the main seafood species groups due to increasing scale and trade globally. Their results agree with Tveteras et al. (2012) whom find well-integrated global markets for most groups of species.

In this paper, we assess volatility dynamics in aquaculture markets. Several papers (Dahl and Oglend, 2014; Asche et al., 2015; Dahl, 2017) argue that the advantages in aquaculture production over wild capture provides a stable supply reducing price volatility. Table 1 provides an overview of the species and markets considered in our study. We apply monthly trade data from 01.1990 to 12.2016 and aggregate a value-weighted price index per region and per species. Moreover, we estimate the cross-sectional volatility (Garcia et al., 2010; Goltz et al., 2011) using each product's dispersion from the index mean. This provide an instantaneous estimate with no need to evaluate other parameters.

Previous studies on volatility dynamics in seafood markets, consider volatility spillover. In particular, Dahl and Jonsson (2017a) examine volatility spillover between seafood markets in EU, Japan and US, and find that events like El Niño/La Niña cause peaks in volatility spillover between EU, Japan and US. In a related article, Dahl and Jonsson (2017b) study volatility spillover between aquaculture and wild, and find that wild products typically transmits volatility to aquaculture products. Moreover, they show that it requires a substantial (negative) supply shock to aquaculture production in order to shift volatility spillover from aquaculture to wild. Both articles show time-varying volatility dynamics.

We apply a time-varying copula to study the volatility dynamics between the aquaculture species. The method provides us with information on relationship over time and between the regions and species considered. The article contributes to previous research by adding knowledge about aquaculture fish markets dynamics. Our results corroborates well with previous research and show considerable time-varying dynamics in price volatility.

Table 1 – Aquaculture species and product price series in study. Source: Import prices in EU, Japan and US provided by the Norwegian Seafood Council.

Species	Product forms	Markets
Salmon	Frozen and fresh	EU, Japan and US
Trout	Frozen and fresh	EU, Japan and US
Catfish	Frozen	US
Tilapia	Frozen and fresh	US
Putterfish	Frozen	Japan
Seabass	Fresh	EU

(Continued on next page)

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SURVIVAL AND GROWTH PERFORMANCE OF THREE DIFFERENT SOURCES OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN ON-SHORE TANKS IN LOW SALINITY WATERS OF WEST ALABAMA

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Inland, low salinity shrimp farmers in west Alabama, that produce the Pacific white shrimp, *Litopenaeus vannamei*, have been reporting abnormally low survivals at harvest. This phenomenon is not restricted to one farm, but has been observed across all farms culturing this species in west Alabama. Multiple theories exist as to the cause of increased mortality on Alabama shrimp farms including disease, toxic algae blooms, water quality, shrimp source, and reduced robustness of shrimp in later stages of production. In order to compare the survival and growth performance of shrimp from different sources, shrimp were obtained from three different hatcheries and stocked on the same day in three different 12-tank (700 L each) flow-through systems. Each source of shrimp was replicated four times in each system. Two of the 12-tank systems (TS) were installed on two different pond banks of one farm (Farm1-TS1; Farm1-TS2), and another was installed on a pond bank of a neighboring farm (Farm2-TS). The culture systems operated flow-through by pumping low salinity pond water through the tanks at a flow rate which exchanged water in the tank twice per day. Thirty-four shrimp were stocked in each tank and shrimp were offered a commercial feed once per day. Shrimp in tanks on each system were offered the same amount of feed. Shrimp were supplied with aeration via two air stones and a regenerative blower. Throughout the trial, tanks were sampled periodically to track growth and survival. At the end of the trial shrimp were harvested, counted, and group weighed. A composite sample of hemolymph was taken from shrimp in each tank to determine hemolymph osmolality and blood ion levels. In order to assess survival following stocking, after 30 days tanks were drained to count and weigh shrimp in each tank system. Survival ranged from 85-94%, 95-97%, and 61-81% for Farm1-TS1, Farm1-TS2, and Farm2-TS, respectively. Following 107 days of culture on Farm1-TS1 there were no statistical differences in survival (72.8 – 91.2 %) or final weight (21.2 – 24.6 g) among shrimp sourced from three different hatcheries. Likewise, in Farm1-TS2 no differences in survival (83.1 – 86.8%) or final weight (19.8 – 20.7 g) were observed. At Farm2-TS, following 111 days of culture there were significant differences in survival from shrimp sourced from one hatchery (40.5%) compared to the other two hatcheries (61.0 – 69.8%). However, no significant differences in final weight were observed among treatments at Farm 2 (18.3 – 22.1 g). A large percentage of the observed mortality in the trial occurred in the first thirty days for two of the tank systems (Farm1-TS1 and Farm2-TS). Hemolymph osmolality and analysis of whole body proximate composition are currently ongoing and will be discussed.

ECONOMIC FEASIBILITY IN PRODUCTION AND MARKETING OF BONELESS BIGHEAD AND SILVER CARP MEAT AT A SMALL SCALE

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Bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) are large river planktivores native to China and eastern Siberia. The carp were imported to the United States in the 1970s as a form of phytoplankton control; however, by the 1980s they had escaped to natural waters and began repopulating. Currently, Asian carp inhabit most water bodies in the Mississippi River Basin and have been shown to have a negative impact on native planktivore condition factor. Efforts to control their populations with intensive commercial harvest have been hindered by lack of interest in American markets. The presence of intermuscular “Y” bones creates a serious obstacle for American consumers who prefer boneless fish products. This study determines the cost of deboning Asian carp at small-scale and the professional perceptions of the products.

Multiple bighead and silver carp were filleted and deboned by trained fish processors and data regarding including processing times, fish dress-out yield, etc., were recorded. Dress-out percentages for bone-in bighead carp fillets were consistent with previous literature; however, silver carp dress-out was lower than in previous studies. Silver carp dress-out rates (13.25%) were significantly ($P=0.0215$) higher than for bighead carp (11.19%).

Bighead carp took less time to process ($11:48 \pm 0:29$ minutes for bighead, $13:18 \pm 0:50$ minutes for silver). Optimal labor allocation was two employees deboning and one employee at each of the other stations; this was necessary due to a bottleneck occurring at the deboning station. On average 179.27 ± 0.13 bighead carp were processed per day while only 134.80 ± 0.26 silver carp were able to be processed per day for breakeven prices of $\$13.72 \pm 0.004$ / kg ($\$6.22$ /lb.) and $\$10.06 \pm 0.01$ /kg ($\$4.56$ /lb.), respectively.

Chefs preferred the taste of bighead carp and significant differences were seen in taste scores ($P = 0.0216$). It is unlikely that deboning bighead carp would be a profitable enterprise when selling at the average stated WTP and in no scenario tested did the breakeven price fall below the $\$11.00$ /kg mark common for tilapia and catfish; however, based on this study’s results, deboning silver carp could be profitable. Utilizing optimal labor, all silver carp breakeven prices generated were below the $\$11.00$ /kg mark.

EFFECTS OF SEMI-CONTINUOUS PERACETIC ACID DOSING ON RAINBOW TROUT *Oncorhynchus mykiss* PERFORMANCE, WATER QUALITY, AND OFF-FLAVOR COMPOUNDS IN RECIRCULATION AQUACULTURE SYSTEMS

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Water clarifying and disinfection techniques such as ozonation and ultraviolet irradiation are commonly used in recirculation aquaculture systems (RAS); however, the capital and operating costs of these technologies are expensive. Cost-effective treatment options that maintain fish health and simultaneously improve water quality without impacting nitrification are still required. Peracetic acid (PAA) has shown potential as an effective aquaculture chemotherapeutant that, at certain doses, is compatible with biofiltration and may provide water quality advantages. The comprehensive effect of regular PAA dosing on RAS water quality, fish health and performance, and off-flavor compounds requires further study.

A trial was conducted using six replicated 9.5 m³ RAS; three operated with semi-continuous PAA dosing and three without PAA addition, while culturing rainbow trout (*Oncorhynchus mykiss*). Target PAA doses ranging from 0.05-0.30 mg/L were evaluated at 1-2-month intervals. Concentrated peracetic acid must be handled with caution, and as such, a safe and effective method for handling, storage, and dosing was developed. VigorOx®-SP15 (Peroxychem) was used as the PAA source and was dosed via peristaltic pumps to the inlet headspace of low head oxygenators (LHOs) of respective RAS. A water recycle rate >99% was maintained, and system hydraulic retention time averaged 2.7 days.

Rainbow trout growth performance was not affected by semi-continuous PAA addition at any of the doses evaluated. Preliminary analyses indicate that oxidative reduction potential (ORP) and true color were affected at certain PAA doses. ORP was generally greater in RAS where PAA was added and true color was slightly lower. Nitrification was not negatively impacted. In addition, concentrations of the off-flavor compounds geosmin and 2-methylisoborneol in water, biofilm, and trout fillets were not affected by PAA at the tested doses.

Overall, peracetic acid dosing within the selected concentration range was compatible with rainbow trout production and RAS operation. However, PAA dosing did not create dramatic improvements in true color and did not improve total suspended solids, biochemical oxygen demand, dissolved metals, or ultraviolet transmittance levels, as has been reported in previous experiments when applying a relatively low, non-disinfecting ozone dose. The effect of PAA on ORP is an important finding and indicates potential for continuously monitored ORP to be integrated as an on/off control for PAA dosing in RAS.

THE USE OF AUTOMATED FEEDING SYSTEMS FOR THE PRODUCTION OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*: A RESEARCH PERSPECTIVE

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The continued success of shrimp aquaculture 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. Feed management applications can also dictate the water quality within a pond also resulting in poor performance. Hence, the aim of this study was to compare feed management strategies in pond culture of Pacific white shrimp (*Litopenaeus vannamei*) to evaluate the effects of feeding rates on the growth performance, production, water quality and economic returns. Four feed management techniques were evaluated over a two-year period. In both years a 16-week production cycle using 16, 0.1Ha ponds that were stocked at 17 shrimp/m² or 38 shrimp/m² at the Claude Peteet Mariculture Center, Gulf Shores, Alabama, USA. In both years, feed management up to week 4 was essentially identical then the various feeding protocols were implemented. In year 1 we evaluated 2 treatments with hand feeding using the Standard Feeding Protocol (SFP) and manual feeding twice per day. SFP was 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%. This included a standard ration as well as a 15 % increase to the ration from 8-16 weeks. Two treatments utilized automatic feeders, one treatment included a timer feeder, which fed the SFP+15% divided into 6 feedings/day and the other used AQ1 acoustic demand feeding which allowed up to 120 kg/ha/day based on shrimps feeding activity. Significant increases in final weights were seen using the timer and acoustic feeders (28.66 and 35.91 g respectively) when compared to the SFP and SFP + 15% (23.55 and 24.65 g respectively). The AQ1 treatment also resulted in significantly higher shrimp value (\$21,198/ha) than the other treatments (\$11,776 - \$13,446/ha). No significant differences were seen in survival ($72.2 \pm 5.99\%$), FCR (1.03 ± 0.095), or water quality. In year 2 shrimp were stocked at 38 shrimp/m² and cultured over a 13 week pond production cycle. Four treatments evaluating one hand feeding treatment using our SFP. Two treatments utilized automatic timed feeder delivering feed at 115 and 130% of the SFP. The fourth treatment utilized the AQ1 acoustic demand feeding which is feeding on demand during the day with a 150 kg/ha/day limit. Final weight were 19.74, 25.2, 27.5 and 32.0g; final yield was 4843, 5629, 6416 and 7430 kg/ha and FCR were 1.13, 1.12, 1.07 and 1.24 for our SFP, Auto feeder 115%, 130% and the AQ1 systems, respectively. In general, increasing feed inputs without subsequent increase in the number of feedings did not improve shrimp growth. The use of automated feeding systems (which increase the number of feedings) improved production incrementally with feed inputs. Increases in feed input, application of the technology and training of people to maintain the new system must all be considered when evaluating these technologies.

IDEAL PROTEIN CONCEPT AND ITS APPLICATION IN PRACTICAL DIETS FOR NILE TILAPIA *Oreochromis niloticus*

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Nutritional methodologies applied to formulating cost effective feeds is often geared towards using low protein diets or inexpensive protein sources. However, this can result in an imbalance of amino acid profiles of feed, causing impaired growth, and reduced feed efficiency. Therefore, it is necessary to optimize amino acid balance in diets for fish. Like other fish species, researches on the balance of indispensable amino acid (IAA) and dispensable amino acid (DAA) for promoting maximum growth and protein deposition on Nile tilapia have been limited and inconsistent. Considering the importance of meeting amino acid requirements, this research sought to further optimize amino acid balance of the diets offered to Nile tilapia *Oreochromis niloticus* by applying and validating the use of the ideal protein concept. The efficacy of reduced protein diets and the effects of IAA supplements was evaluated in the first study. The results of this study indicate that the utilization of balanced IAA profiles of feed (under the application of the ideal protein concept) can help the fish to attain better growth performance and feed utilization efficiency. With the use of balanced IAA diets, the percentage of intact protein inclusion of feed can be reduced from 32% to 27.2% without causing impaired growth performance and feed utilization efficiency of fish. Further reduction of intact protein levels to 24.7% and 22.2% of diet, however, induced growth depression which could result from the deficiency of nonspecific nitrogen as a source of energy or limitation of daily IAA intake. In our ingredient matrix, in addition to lysine, methionine and threonine, it was hypothesized that tryptophan, isoleucine, arginine, histidine and valine could also be limiting. Hence, the following study was conducted to confirm a potential deficiency of these IAAs in our matrix of ingredients. These IAAs were individually deleted from the IAA profile of the diet with enhanced IAA (the IAA profile of diet which supported the highest growth of fish in the first study) and DAA supplements. The results illustrated that with the exception of valine, the deletion of the other crystalline IAA supplements (tryptophan, arginine, threonine and isoleucine) did not cause any deleterious effects on growth performance and protein utilization efficiency of fish. Therefore, in addition to lysine, methionine and threonine, valine is limiting in our ingredient matrix and the supplementation of this IAA is necessary to meet the requirement of fish. While tryptophan, histidine, arginine and isoleucine are likely adequate for growth of fish. The supplementation of DAA to spare the use of relatively expensive IAA was also taken into consideration by revising the ideal protein concept. Based on the growth data obtained from this study, it can be concluded that DAA plays an important role in meeting the nonspecific nitrogen requirement of fish. In a low protein diet (22.2%), enhancing IAAs above the requirement (120% NRC or to the IAA profile of the reference diet which supported highest performance of fish) did not help the fish to reach comparable weight gain to fish fed the diet with DAA supplements (4%). The results of this study also indicated the inferior growth of fish fed diet with IAA supplements at 100% NRC requirement and 4% DAA supplements which might result from limitation of daily IAA intake.

RESOURCE USE AND EFFICIENCY IN WHITELEG SHRIMP *Litopenaeus vannamei* PRODUCTION IN INDIA

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Many of the impacts of aquaculture are related to resources embedded in feed and in the production process at the farm. Conservations stand to be made through improved efficiency, however current resource use is not well understood on shrimp farms. Here, resource metric use surveys were administered at 89 *Litopenaeus vannamei* farms in India. Data collected included farm characteristics, resource use, and amendment usage. The average feed conversion ratio for production in India was 1.48. Wild fish use averaged 1606.30 kg/mt of shrimp. Mean total land use was 0.70 ha/mt, mean total energy was 78.47 GJ/mt, and embedded freshwater use was 2183.15 m³/mt of shrimp. Production intensity averaged 7.86 mt/ha/year in India. Embedded resource use decreased with greater production intensity. A great amount of variation in resource use demonstrates that increased production can be achieved in shrimp aquaculture without an increase in resource use through greater efficiency. These results are compared with previous results of similar surveys conducted in Vietnam and Thailand.

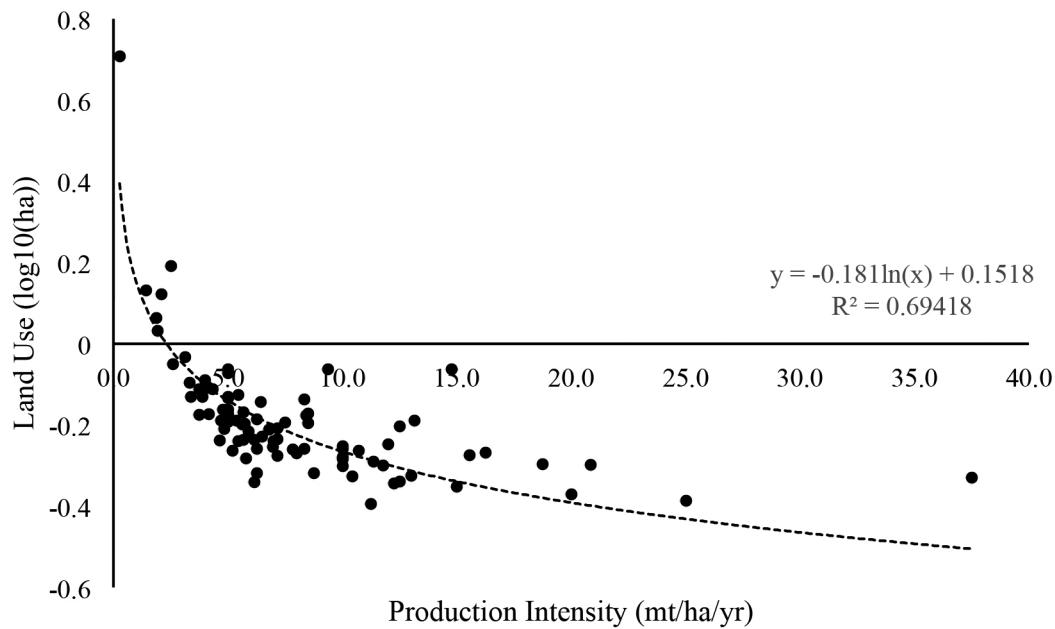


Figure 1. Total land use versus farm production intensity for *L. vannamei* production in India.

RESOURCE USE AND EFFICIENCY IN BLACK TIGER SHRIMP *Penaeus monodon* PRODUCTION IN INDIA

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Many of the impacts of aquaculture are related to resources embedded in feed and in the production process at the farm. Conservation gains stand to be made through improved efficiency, however current resource use is not well understood on shrimp farms. Here, resource metric use surveys were administered at 10 *Penaeus monodon* farms in India. Data collected included farm characteristics, resource use, and amendment usage. The average feed conversion ratio for *P. monodon* production in India was 1.36. Wild fish use averaged 1478.05 kg/mt of shrimp. Mean total land use was 0.83 ha/mt, mean total energy was 60.24 GJ/mt, and embedded freshwater use was 2008.84 m³/mt of shrimp. Production intensity averaged 3.90 mt/ha/year in India. Embedded resource use generally increased with greater production intensity, however total energy use decreased. These results are compared with previous results of similar surveys conducted in Vietnam and Thailand.

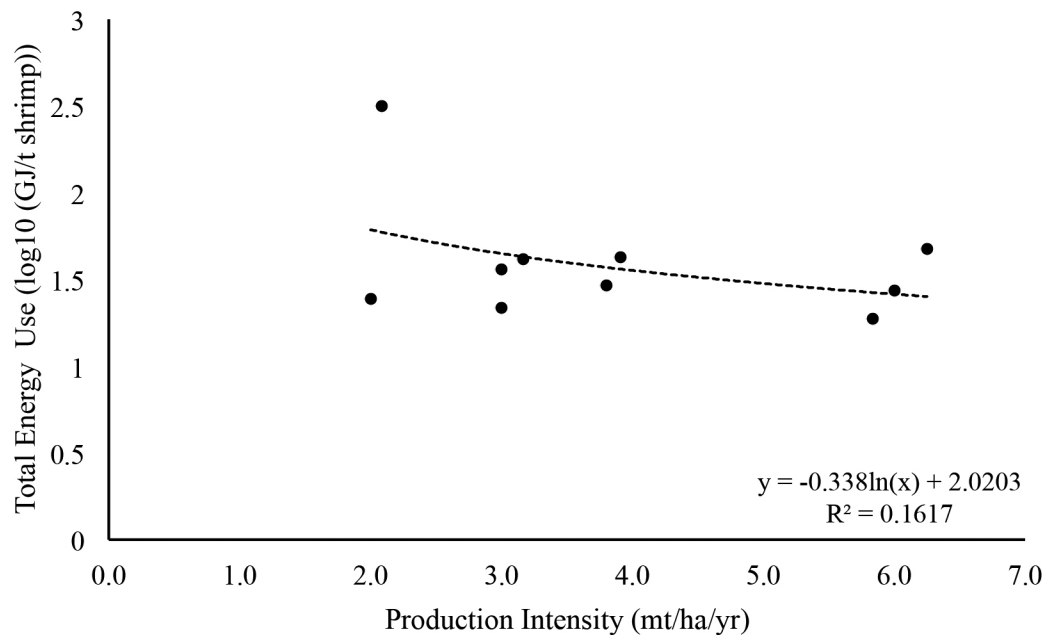


Figure 1. Total energy use versus farm production intensity for *P. monodon* production in India.

EVALUATION OF PARTIAL REPLACEMENT OF FISHMEAL AND FISH OIL BY MICROALGAE EXTRACTS IN DIETS FOR HYBRID STRIPED BASS *Morone chrysops* X *Morone saxatilis*

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Aquaculture production has intensify in recent years resulting in increased demand for ingredients of marine origin in aquafeeds. This has caused steadily increasing aquafeed prices ranging from 20-40%. Many strategies have been implemented to reduce costs of aquafeeds including replacement of fishmeal (FM) and fish oil (FO) with alternative ingredients. Thus, two comparative feeding trials of 6 weeks duration were conducted to investigate the effects of FM and FO replacement with different types of microalgae in the diet of juvenile hybrid striped bass (HSB) (average initial weight of 10.6 g/fish). A control diet was formulated to contain 40% crude protein (CP), with equal contributions from FM and SPC as the protein sources, and 10% crude fat, using FO as the main lipid source. The microalgae evaluated in feeding trial 1 (F1) to partially replace FM and soy protein concentrate (SPC) included lysed extracted *Nanochloropsis salina* (LENS) meal and mixed culture of *Phaeodactylum tricornutum* and *Nanochloropsis salina* (PTNS) meal. In feeding trial 2 (F2), the heterotrophic algae *Arthrospira* sp. and *Schizochytrium limacinum* was used to partially replace FM, SPC and FO. The algae products were substituted to replace either 25% or 50% of the total CP % in F1. In F2, combinations of meals from *Arthrospira* and *Schizochytrium limacinum* was used to replace 10, 20, 30, 40 and 50% of FM and SPC in the control diet.

Results showed that replacing CP in the control diet with LENS and PTNS in F1 had a significant effect within the treatment groups on weight gain (WG) of the fish ($P < 0.05$) (Fig 1). Similarly, substitution of FM, SPC and FO with *Arthrospira* sp. and *Schizochytrium limacinum* had significant effects on WG ($P < 0.05$) (Fig 2); however, no significant differences in feed efficiency ratio (FER) were evident. No significant differences was observed in whole-body proximate composition and fish had 100% survival in both trials. In conclusion, LENS was able to substitute 25% of protein from FM and SPC without adversely affecting WG in F1, and in F2, substitution of *Arthrospira* sp. and *Schizochytrium limacinum* up to 50% of the FM and SPC improved WG of the fish.

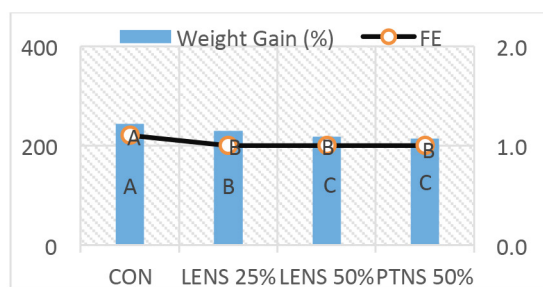


Figure 1. Performance of HSB with partial

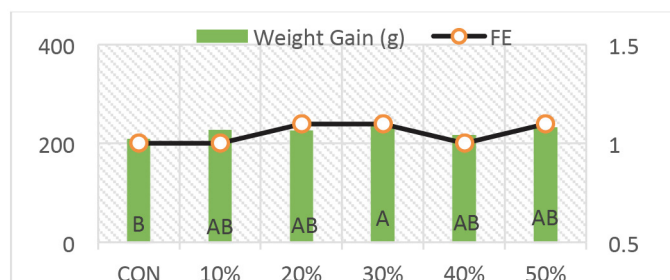


Figure 2. Performance of HSB with partial replacement of FM, SPC and FO. replacement of FM and SPC.

EFFECTS OF NUTRITIONAL CONDITIONING ON GROWTH OF NILE TILAPIA FRY

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Global production of farmed Nile tilapia (*Oreochromis niloticus*) has increased exponentially over the past 30 years. Feed comprises 50-70% of production costs for tilapia, with protein being the most expensive component. We have previously shown that alternate day feeding and pond fertilization improves feed efficiency and increases gut microbe diversity of tilapia grown in ponds with no detriments to the survival rate of the fish. To further these studies, experiments have been performed to determine whether larval nutritional conditioning, the concept that critical events early in life have lifelong effects on growth and health and may modify the gut transcriptome and microbial community in favour of improved food conversion efficiency.

We conditioned newly hatched fry on an initial low protein (25% crude protein) diet versus a typical 48% crude protein diet for various time intervals (7, 14, 21 days) to determine if reducing protein in the diet early in life may subsequently enhance growth or affect protein processing, uptake, and utilization later in life (Fig. 1). Following this initial exposure to low protein, fry were then grown out for up to 49 days on either the 25% reduced protein or the typical 38% protein diet. We show that tilapia fry fed a 25% protein diet for 14 days, followed by either the reduced 25% or 38% protein growout diet, had greater mean weights (Fig. 2) and lengths than fry fed the initial 48% protein diet. There were also no differences in survival rates for fry offered the 25% protein diet when compared to fry offered the 48% protein diet. This study suggests for the first time that tilapia may better utilize dietary nutrients following an early period of low protein exposure. The effects of the reduced protein diet on gut gene expression and microbial content following nutritional conditioning will also be discussed.

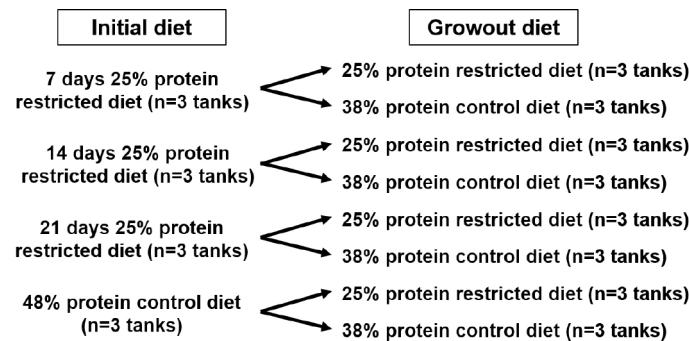


Fig 1. Experimental design indicating protein content of each diet.

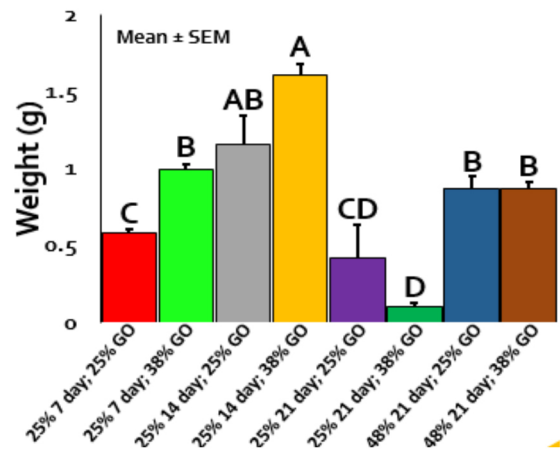


Fig 2. Mean weights of tilapia fry fed protein-restricted diets. Control diet is represented by the brown bar.

MATERNAL EFFECTS ON UNIFORMITY OF GROWTH IN A HYBRID FINFISH

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Uniformity of growth is an economically important trait, as it allows farmers to produce a consistent product and reduces or eliminates the need for labor intensive practices such as grading and multiple harvests. Additionally, uniformity of growth can reduce aggressive behavior between different size classes, thereby reducing stress and increasing the growth and health of fish. We show that in hybrids of zebrafish *Danio rerio* ♀ x pearl danio *Danio albolineatus* ♂ uniformity of growth is influenced by a maternal effect gene.

We first obtained hybrids by crossing five zebrafish dams with one pearl danio (sire 1). Dam 1 produced a family with highly uniform growth, while dams 2-5 produced families with high variability in growth rate (Fig 1). Dams 1 and 2 were crossed with two additional sires (sires 2 and 3) and this difference in uniformity was consistent between sires. Dams 1 and 2 were crossed to an unrelated zebrafish male to produce F_1 females (no difference in uniformity of growth was evident between the two F_1 families). These F_1 females were mated to pearl danio sires and uniformity of the resulting offspring was measured. All five F_1 females derived from dam 2 produced highly variable families (Fig 2). Of ten F_1 females derived from dam 1, four produced highly variable families and six produced uniform families (not different from 1:1, Chi-square test $p > 0.10$). This suggests Mendelian inheritance of a maternal effect gene that influences the uniformity of growth rate in hybrid offspring. Based on our observations, the allele which leads to uniformity of growth is likely dominant, although we cannot eliminate the possibility that it is recessive and the zebrafish sire of the F_1 generation was heterozygous for this allele. Crosses that will discriminate between these possibilities are being evaluated and these results will be presented.

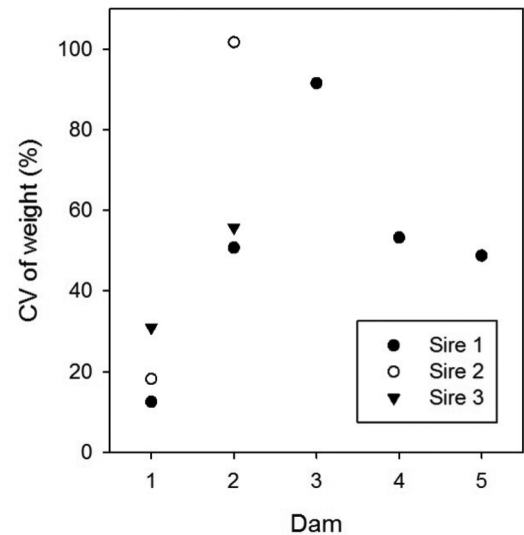


Fig 1. Coefficient of variation (CV) of weight at 21 days post-fertilization (dpf) of hybrid families (n=10 fish/family)

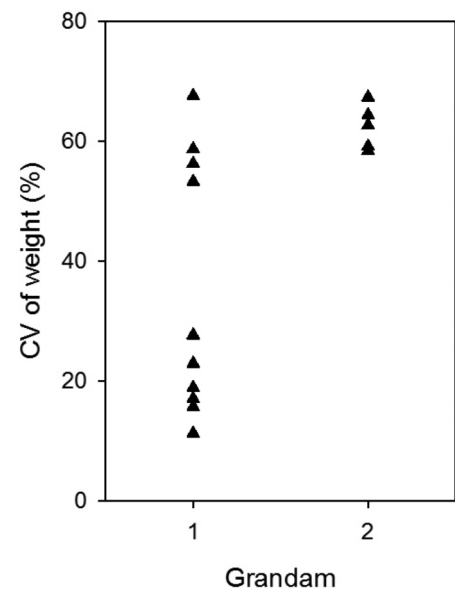


Fig 2. Coefficient of variation of weight at 21 dpf of hybrid families produced by F_1 dams (n=15 fish/family)

ENHANCEMENT OF LAKE STURGEON (*Acipenser fulvescens*) CONSERVATION THROUGH FEEDING MANAGEMENT

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Lake sturgeon (*Acipenser fulvescens*), listed as threatened under the State of Michigan's Endangered Species Act, has high ecological and economical values as a native benthivore. A lot of efforts for enhancing stocking have been implemented to restore wild populations. For current stocking programs, lake sturgeon are raised with raw/live feed, which causes stocking programs to be expensive and difficult to maintain due to special cares required for managing live feed and decreased control on biosecurity. To address these drawbacks, we aim to replace raw feed with formulated feed and develop a biosecured and sustainable feed management program for the culture of lake sturgeon. A series of feeding trials were conducted to study the effects of different feeds (Artemia, formulated feed, combination diet with Artemia and formulated feed) and to determine an optimal feeding rate for the fish based on their survival and growth performance. With the selected formulated feed, the optimal feeding rates for lake sturgeon ranging from 3.6 g to 80 g (body weight) decreased from 7.6 to 3.5% body weight/day. Lipid storage in the whole body of lake sturgeon was significantly ($P < 0.05$) influenced by different feeding rates with a lower lipid level in the fish fed at a suboptimal feeding rate. These results indicate that lake sturgeon can be fed with formulated feed if a proper feed management design is previously implemented during the early life stage. The changes in nutritional status of sturgeon may potentially affect their response to temperature challenges. The results are pending and will be discussed in the presentation.

Table 1. Optimal feeding rate of lake sturgeon fed formulated feed.

Fish Size (g)	Optimal feeding rate (g/kg body weight daily)	Optimal feeding rate (MJ/kg body weight daily)
3-6	76	1.47
8-12	62	1.20
35-80	30-35	0.56-0.69

BALANCING NUTRIENT PRODUCTION AND UPTAKE IN RECIRCULATING AQUAPONIC SYSTEMS

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Aquaponics, the growing of plants using excess nutrients from fish production in recirculating systems, optimizes input utilization efficiency and maximizes system productivity. By using aquaculture effluent that is high in nutrients such as nitrogen that are required for plant growth, there is very little need for outside chemical fertilizers that can be very energy intensive. The growing field of aquaponics is currently based on a single model provided in research literature, with general sizing guidelines for balancing nutrient production from fish and uptake by plants. However, recent preliminary research has shown these guidelines may misrepresent plant nutrient requirements, potentially resulting in aquaponic systems which do not efficiently utilize inputs. Research is needed to appropriately establish a balance between nutrient production and uptake to optimize aquaponic systems for both productivity and economic sustainability.

The University of New Hampshire is addressing the need for the development of the aquaponic industry through farm-scale research under actual production conditions. Currently, aquaponic nutrient research is being conducted in the New Hampshire Agricultural Experiment Station Macfarlane research greenhouses at the pilot-scale. The results from the ongoing research is being used to finalize designs for the construction of three, farm-scale greenhouses dedicated to replicated large-scale production systems operated under actual production conditions for realistic data collection.

Research conducted compares aquaponics to Hydroponics, developing intensive production model in the United States. Currently, tilapia and Boston butterhead lettuce are grown as model crops. The research aims to develop a framework for establishing nutrient production and uptake models for a variety of crops (fish and plants). Data includes the collection of water and tissue samples to establish a nutrient mass-balance between the fish and plant production unit processes. Complete tissue nutrient profiles will be developed in both control (hydroponic) and aquaponic crops. It is pertinent to understand the differences in nutrient requirements based on different crops and varieties, and adjust inputs such as feed into the systems based on this data. The research is ongoing currently. Results to date and system design concepts will be presented and discussed.

FIRST REPORT OF THE PRESENCE OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN TEXAS, USA

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A disease outbreak occurred in shrimp farms cultivating *Penaeus vannamei* shrimp in Texas, USA that caused large-scale mortalities during June 2017. Shrimp samples originated from these farms were tested in Aquaculture Pathology Laboratory in The University of Arizona, Tucson, Arizona. Duplex polymerase chain reaction (PCR) performed using DNA isolated from hepatopancreas tissue and OIE-approved primer sets used to detect acute hepatopancreatic necrosis disease (AHPND) causing *Vibrio* sp carrying binary toxin genes (*pir A* and *pir B*) provided positive results. The presence of DNA containing toxin gene in the samples were further confirmed by another set of OIE-approved primers, AP4. Subsequently PCR was carried out to identify the presence of *Vibrio* species in hepatopancreas tissue. The results showed that the DNA samples isolated from *P. vannamei* hepatopancreas tissue represents *V. parahaemolyticus*. The PCR-based genotyping revealed that the *V. parahaemolyticus* present in the *P. vannamei* Texas samples represent Mexican genotype, and not Asian genotype. The full-length of the binary toxin genes, *pir A* and *pir B*, were amplified by PCR and sequence analysis performed to determine the sequence similarity with known homologous sequences in the GenBank database. Histopathology of *P. vannamei* shrimp originated from the Texas farm revealed characteristic lesions pathognomonic of AHPND. Our results showed *P. vannamei* shrimp originated from Texas farms experiencing large-scale mortalities contained *V. parahaemolyticus* causing AHPND. To our knowledge, this is the first report of the presence of AHPND causing *V. parahaemolyticus* in the US.

DO HOUSEHOLD FISH PONDS IMPROVE FAMILY NUTRITION? A STUDY IN NEPAL

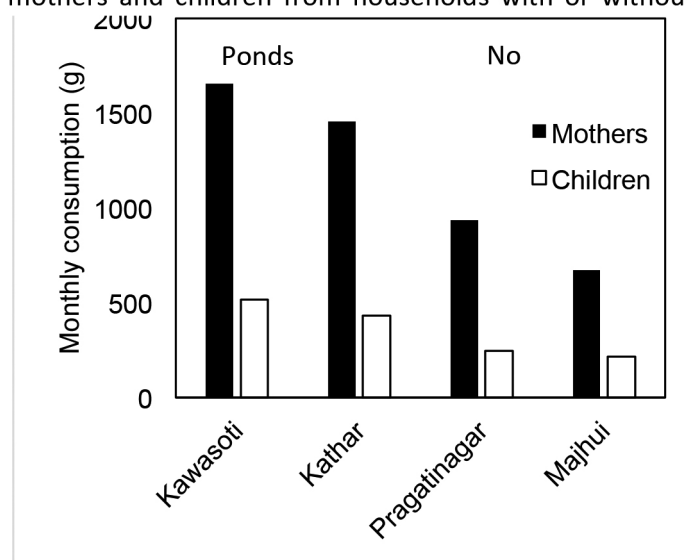
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Malnutrition and micronutrient deficiencies are major concerns afflicting the people of Nepal and other countries in the region. Fish has been recognized as a nutritionally beneficial food source around the world, and small-scale fish culture has recently increased in this region with the inclusion of household ponds in rural communities. This study focused on the value of household ponds by comparing fish consumption and indicators of health for children and women in households with fishponds, to those without ponds. In Kathar, Chitwan and Kawasoti, Nawalparasi, 51 and 55 households, respectively, each including children between the ages of one and five years and owning at least one fish pond were recruited for participation through door-to-door visits. Similarly, in Majhui, Chitwan and Pragatinagar, Nawalparasi, 54 and 55 households, respectively, that did not own fish ponds were recruited.

Mothers from locations with fish ponds consumed significantly more fish than those without ponds (132% more), and also reported 126% higher rates of fish consumption by their children. Owners of household ponds also consumed fish more frequently (97% more) than did households without ponds. Health of children evaluated using details on stunting and wasting indicated that there were no significant differences between households with or without ponds. However, children from our study groups averaged 19% underweight, 18% stunted, and 12% wasted. These values are quite low, compared to 2013 estimates for the entire country for stunted (40.5%) and underweight (28.8%), but not for wasted (10.9%) children, and suggest that the inclusion of fish in child diets may provide essential nutrition to promote healthy growth and development for children in the region.

Figure 1. Monthly estimates of fish consumption by mothers and children from households with or without



RECENT DEVELOPMENTS IN MARINE ORNAMENTAL AQUACULTURE AT THE UNIVERSITY OF FLORIDA'S TROPICAL AQUACULTURE LABORATORY

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Marine fishes represent a small fraction of the total volume of ornamental species sold in the aquarium trade today yet their high retail prices make them a significant contributor to the economic value of the industry. Unlike their freshwater counterparts, many marine ornamental species are still harvested exclusively from wild stocks and the sustainability of this practice has recently been called into question. Development of captive propagation protocols for marine fishes has the potential to facilitate the production of new commodities for the ornamental industry and allay sustainability concerns by bringing a farm raised product to market. Numerous technical impediments still hinder reliable spawning and larval production for many species and continued research is needed to grow this sector of the ornamental industry.

Rising Tide Conservation is an initiative focused on promoting coral reef conservation through the continued growth of the marine ornamental aquaculture industry. As a member of this initiative, the University of Florida's Tropical Aquaculture Laboratory has spent the last seven years researching and refining production protocols for numerous ornamental species of commercial value. Recent research has focused on closing the life cycle for the Pacific Blue Tang and several species of *Halichoeres* wrasses. This presentation will highlight recent advances in culture efforts with these species and examine future directions for the expansion this important research.

PRELIMINARY COST PROJECTIONS FOR PRODUCTION OF THE CALANOID COPEPOD *Parvocalanus crassirostris*

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In the wild, copepods are the dominant prey item for marine fish larvae. Their use as a first feed for production of marine food fish and ornamentals has garnered increased interest from the aquaculture industry. Elevated fatty acid levels and small naupliar sizes make them a superior choice when compared with more conventional live feeds such as rotifers and *Artemia*. Despite the clear benefits associated with inclusion of copepods in marine larval rearing protocols, adoption of such practices has been slow mainly due to technical impediments associated with mass scale culture of the nauplii. Furthermore, there is paucity of data regarding the economic feasibility of commercial scale copepod production and this information is critical for prospective farmers to make informed financial decisions.

The calanoid copepod, *Parvocalanus crassirostris*, has recently emerged as a promising species for commercial culture. Most notably this species was recently used to produce the first captive reared Acanthurids and numerous species of Labrids and Chaetodontids. Over the past five years the Tropical Aquaculture Laboratory at the University of Florida has developed pilot scale systems for production of *P. crassirostris*. This preliminary study focused on elucidating the cost of production for *P. crassirostris* in these prototype systems.

Physical components and operational parameters of the copepod culture systems were initially described and investment costs for all system components including those for water holding, filtration, and microalgae production were determined. Non-cash expenditures and all variable costs were compiled to generate an estimate of operating costs. Both the fixed and variable costs were then used to calculate a cost per unit of copepod nauplii. Results from these analyses will be discussed as well as the implications for production of other copepod species and the economic feasibility of incorporating similar copepod systems into commercial hatcheries.

ECONOMICS OF WILDLIFE CONTROL AND PERMITTING TO LIMIT DAMAGE TO AQUACULTURE

Brian S. Dorr

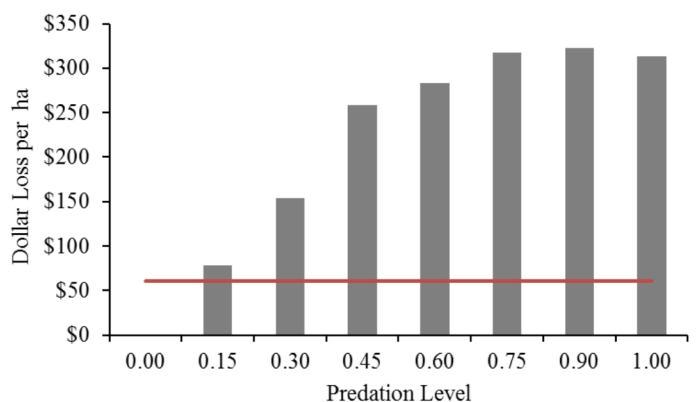
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A wide variety of wildlife can cause significant losses of commercially produced aquaculture products depending on the location and type of farm. However, the most problematic type of wildlife typically are birds. Cormorants, wading birds, pelicans, and even ducks and blackbirds forage at commercial catfish, crawfish, baitfish, ornamental fish, and trout farms. Most birds are protected by state and federal laws, so fish farmers must rely on nonlethal techniques to accomplish control objectives or must obtain special permits. A federal depredation permit allows a farmer to capture or kill birds to reduce damage or to protect other interests such as human health and safety or personal property.

A fundamental aspect of all wildlife control to protect aquaculture resources is do the benefits of control outweigh the costs. There are costs associated with obtaining permits for wildlife control, however these are minor relative to control costs themselves. For example permit costs to control birds on catfish aquaculture in Mississippi are about \$125.00 (U.S.)/year. Whereas average annual wildlife control costs on Mississippi catfish aquaculture farms were estimated at \$15,500 (2013 U.S. dollars). Savings to producers can be substantial for effective wildlife control programs. For trout aquaculture in California annual losses at 2 hatcheries were \$50,000 to \$60,000. After exclusion, production increased 25 to 30% at both facilities and the return on investment was realized within 3 to 4 years. For catfish aquaculture in Mississippi on a 104 ha farm, savings over average control costs could be as high as \$323 per ha annually, depending on farm practices and predation level (Figure 1).

Producers can optimize current control efforts by understanding and considering the costs, and limitations of different techniques and by developing integrated strategies for their use. Wildlife control methods typically fall into a few major categories: exclusion or barriers, frightening and dispersal methods, lethal control, and changes in culture practices. The different aquaculture products and production methods used and the wildlife species causing damage will determine the best methods to use. Other than complete exclusion, most wildlife damage problems require an integrated approach to be successful.

Figure 1. Estimated total dollar loss (\$ U.S.) per ha on catfish aquaculture at varying levels of fingerling (15-18 cm) predation. Production simulated for an enterprise budget for ponds with a 50% harvest of food fish with 50% restocking of fingerlings at 25,000/ha in a multi-batch production system and a breakeven price of \$1.30 (\$U.S./kg) The red line represents average Mississippi producer control costs per ha (2013 dollars).



BIRD DEPREDATION RESEARCH AND MANAGEMENT ON AQUACULTURE: AN OVERVIEW

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Aquaculture in the United States is very diverse with respect to species cultured, annual production, size, complexity, and spatial arrangement of facilities. Similarly, species of predacious birds using aquaculture facilities also vary with many of these industry characteristics. Fish eating birds are highly adaptable, and relatively common throughout the aquaculture industry, and are often associated with fish depredation problems. Suitability of information regarding the impacts of birds to aquaculture varies dramatically by depredating species and industry sector and changes over time.

For example research has shown that Little Blue Herons (*Egretta caerulea*) can negatively impact baitfish aquaculture but more recently scaup (*Aythya spp.*) have become a primary species of concern. Impacts of Double-crested Cormorants (*Phalacrocorax auritus*; Figure 1) to catfish aquaculture are well documented and have been estimated to range between \$6-12 million annually, but the industry has changed dramatically in recent years. Early research indicated Great Blue Heron (*Ardea herodias*) depredations on catfish aquaculture caused significant damage. Newer research however, initiated a paradigm shift in management by demonstrating that some wading birds like the Great Blue Heron and Great Egret (*Ardea alba*) largely eat diseased catfish and consumption of healthy catfish can be limited by specific management efforts. However, disease transmission is still an important but poorly understood issue with these species and others such as the American White Pelican (*Pelecanus erythrorhynchos*). For some aquaculture products such as crawfish, there is little information regarding bird species and their impacts.

These issues highlight the fact that bird depredations are dynamic and evolve with changing demographics of both the aquaculture industry and bird populations. As bird populations and aquaculture change, it is imperative that research identify where and how aquaculture production losses occur and guide science-based management plans to abate production loss.

Figure 1. Double-crested Cormorant (*Phalacrocorax auritus*). Photo provide by USDA, APHIS, WS National Wildlife Research Center.



DOUBLE-CRESTED CORMORANT PREDATION ON CATFISH AQUACULTURE

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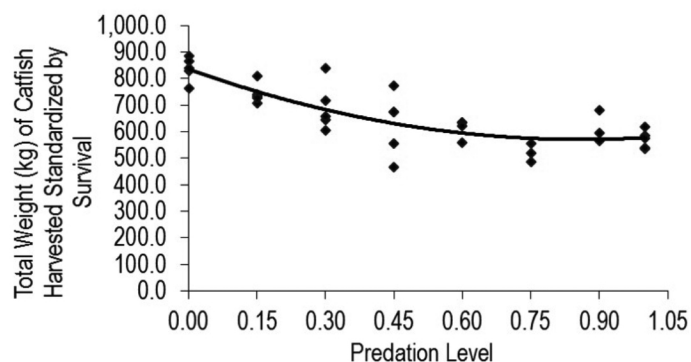
Commercial production of catfish (*Ictalurus spp.*) is one of the largest aquaculture industries in North America. Due to favorable geologic, climatic, and socioeconomic factors, much of this catfish production occurs in the southeastern United States and the southern end of the Mississippi flyway, a major migratory route for birds, including Double-crested Cormorants (*Phalacrocorax auritus*). Cormorant depredation at catfish farms has been extensively studied. Here I provide an overview of some of the research conducted by the National Wildlife Research Center (NWRC) and their collaborators to try and address issues associated with cormorant depredations on catfish aquaculture.

The NWRC estimated that direct predation impacts due to cormorants in western Mississippi alone were estimated at US \$6 to \$12.0 million annually. While these estimates of regional impacts are important to the industry, individual farmers experience losses at the pond level. Trying to estimate impacts at the farm and pond level have proved challenging for a host of reasons.

Research at NWRC has shown an average of about 16 cormorants per day feeding on a 6-ha catfish pond in a single-batch production system over the winter (October–March) could cause a 22% decline in biomass at harvest and negative return to the producer. This reduction occurred even in the presence of buffer prey and accounting for compensatory growth of surviving catfish. Other research evaluated declines in catfish production based on simulated levels of cormorant predation on only stocker size fingerlings in a multibatch system. This research evaluated a scenario in which about 50% of the harvestable catfish had been removed and replaced with stocker size fingerlings. The sale price needed to break even increased with increasing predation on fingerlings up to a maximum of U.S. 14.3¢/kg, largely due to reductions in biomass at harvest due to cormorant predation (Figure 1).

The research conducted at NWRC and elsewhere has shed considerable light on issues associated with cormorant predation and aquaculture. This research has informed management and policy with regard to cormorant depredations. However, much has changed in the catfish aquaculture industry and with cormorant management in recent years and a better understanding of cormorant impacts to catfish aquaculture under current conditions is needed.

Figure 1. Non-linear trend ($R^2=0.68$) in overall weight of catfish harvested standardized for pond specific survival.



A COLLABORATIVE EFFORT TO SUPPORT MARINE ORNAMENTAL FISH CULTURE IN THE COUNTRY OF ORIGIN

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In an effort to ensure that the environmental and monetary benefits of fish production for the ornamental trade benefits the country of origin, a marine fish breeding and hatchery facility in Makassar, Indonesia was established. In addition to the provision of high quality fish with known provenance into the trade, raising fish by small scale aquaculture provides alternate livelihoods for island communities, traditionally reliant on fishing. In order to support these fish culturing efforts a programme of marine ornamental fish (*Amphiprion percula*) breeding was established at the Waltham Centre for Pet Nutrition (WCPN), U.K. in order to develop best practice protocols to be deployed at the facility in Indonesia. The UK led breeding programme primarily focused on 1) the environmental conditions required for larval survival and 2) the importance of larval nutrition *via* live feed supplementation of various commercial and novel algal products.

A local government aquaculture facility in a rural suburb of Makassar (South Sulawesi) was converted to a fish hatchery housing broodstock of various marine ornamental species. Here, broodstock were spawned and larvae grown to juveniles following WCPN protocols. Once juvenile fish were of a suitable size for transportation (2-3 cm), they were moved to a grow-out facility on Pulau Badi, an island ~15 km off the coast of Makassar, *via* car and boat. Islanders who would normally rely on destructive fishing techniques have been supplied with aquatic facilities for juvenile fish grow-out which are then sold into the ornamental industry, providing significant income improvement.

Various obstacles were encountered in applying these methods in Indonesia which may be typical for expanding into such territories. These included issues related to; continuous power to hatchery facilities, local environmental conditions, cultural barriers and education. However, once these issues were resolved and a sustainable programme is established this then promotes alternative more lucrative livelihoods and reduces environmental impact to local reefs of cyanide and dynamite fishing. Further research is now being conducted to assess the potential for dietary supplements to reduce stress responsiveness during transport, thus enhancing welfare of cultured fish.

COMPENSATORY RESPONSES OF LARGEMOUTH BASS *Micropterus salmoides* TO CHRONIC HIGH ENVIRONMENTAL AMMONIA EXPOSURE

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Water borne ammonia is a ubiquitous environmental pollutant that is toxic to all aquatic animals. Understanding how fish cope with common stressors, such as high environmental ammonia (HEA), is very useful to fish physiologists, fishery managers and fish culturists.

Prolonged exposure to HEA is well documented to limit the overall performance of fish. Moreover, the compensatory mechanisms to maintain homeostasis against ammonia toxicity involve a cascade of physiological, metabolic and ion-regulatory responses. Many of these countervailing responses against HEA are reasonably well understood in many freshwater teleosts. However, till date no relevant information exists for largemouth bass *Micropterus salmoides* which possess a high commercial value as culture and sport fish.

Therefore, the present experiment was designed to gain knowledge on the effect of HEA on the biological performance of largemouth bass and various mechanisms implemented by this fish species to cope with toxicity induced by HEA. To begin with the ammonia exposure experiments, we first determined the ammonia lethal dose to largemouth bass. As such, we investigated mortality rate to calculate 96 h LC₅₀ values using Probit analysis. It was calculated to be 22.5 mg/L total ammonia nitrogen (TAN) at pH 7.8 and 28°C. Thereafter, the fish were exposed to 5.5 mg/L TAN (at pH 7.8 and 28°C) which corresponds to 25% of the 96 h LC₅₀ value. The fish were exposed over 28 days and sampled at 3, 7, 14, 21 and 28 days. Control groups (naïve fish) were set up in parallel to the first (3 days), middle (14 days) and the last sampling period (28 days) and were sampled in an identical way as the exposed fish groups.

Experimental protocol consisted of transferring exposed and parallel naïve fish groups to flux chambers (3 L) that allowed the determination of oxygen consumption rates, ammonia flux rates, and urea excretion rates for individual fish. Results show that in response to HEA, ammonia excretion rate were significantly reduced and even ammonia uptake from the environment was evident. We also documented a reduction in oxygen consumption rate in exposed fish relative to the control. Urea excretion rate did not change extensively but tended to be numerically higher in HEA exposed fish. Accumulation of ammonia and ion status (Na⁺, K⁺ and Cl⁻) were also investigated under experimental conditions. Overall, our data set indicate that to combat ammonia toxicity, largemouth bass can modulate compensatory responses differentially in several different physiological systems.

SUPPORTING DEVELOPMENT OF NON-FED, LOW-TROPHIC AQUACULTURE SYSTEMS TO ADDRESS FOOD SECURITY AND ECONOMIC GROWTH IN COASTAL COMMUNITIES IN AFRICA, ASIA, AND LATIN AMERICA

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According to the FAO, approximately half of the world aquaculture production in 2014, including animals and aquatic plants, was achieved without the use of supplemental feeding. Feed inputs are often identified as a constraint to aquaculture expansion, especially in developing countries, making low-trophic species produced in non-fed culture systems well suited to address food insecurity and poverty. Globally, the bulk of non-fed species are cultured in marine and coastal environments, dominated by macroalgae (“seaweed”) and bivalve shellfish.

Sustainable bivalve and algal aquaculture offer a variety of benefits to smallholder farmers and coastal communities in developing countries, including: (1) increased food security and employment; (2) production of a nutritional source of protein, omega-3 fatty acids, and micronutrients; (3) reduced need for inputs (such as feed and fertilizer); and (4) production can be achieved through simple, low-tech culture systems. In addition, the culture of low-trophic species such as bivalves and algae typically have low impacts on the environment and can provide ecosystem services including turbidity reduction, biodeposition of organics, sequestration of carbon, creation of structural habitat/shoreline stabilization, and nutrient uptake. This type of aquaculture also has added benefits for women, who are historically marginalized in the fisheries sector, since these species can be cultured locally with minimal investment and can provide income through the production, marketing, and sales of value-added products.

Bivalve and algae aquaculture however face constraints that may limit adoption in developing countries, including: (1) limited availability of suitable growing environments; (2) user conflict, property rights, and resource management issues in the near-shore environment; (3) degraded water quality, especially around urban areas where demand is highest, leading to human health concerns and restricted access to markets; and (4) the lack of infrastructure preventing safe post-harvest processing, storage, and transport to markets.

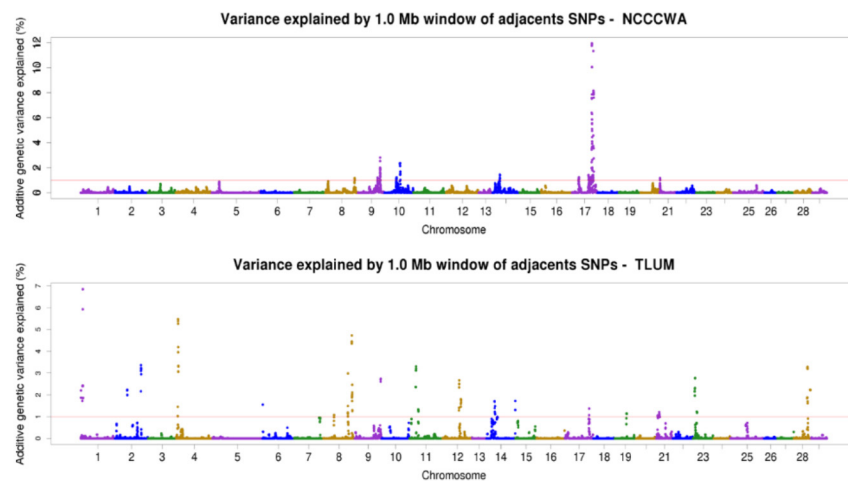
The AquaFish Innovation Lab has addressed these challenges and provided opportunities for the sustainable culture of non-fed, low-trophic species by developing technologies and enhancing human and institutional capacity to alleviate poverty and increase food security in coastal communities in Africa, Asia, and Latin America.

COMPARING TWO POPULATION OF RAINBOW TROUT AGAINST *Flavobacterium columnare*

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Flavobacterium columnare (Fc) is the causative agent for columnaris disease (CD) and this pathogen infects both warm water and cool water aquaculture species. Recently, columnaris has become an emerging problem in rainbow trout farmed within southern Idaho. Herein, we compare 2 production lines of rainbow trout to determine if selective breeding has the potential to be part of an integrated approach for controlling CD. The objectives of this study were to 1) determine family variation in innate resistance to an Fc immersion challenge in 2 populations of rainbow trout, 2) compare and contrast the heritability of disease resistance, 3) examine whether there is a genetic correlation between bacterial cold water disease resistance and CD resistance, and 4) identify quantitative trait locus (QTL) in both populations that influence Fc resistance. The results indicate that resistance against BCWD and CD are heritable (BCWD - $h^2=0.29\pm0.03$ and 0.34 ± 0.08 ; CD - $h^2=0.17\pm0.07$ and 0.35 ± 0.09 , respectively for NCCCWA and TLUM populations) on both populations and favorably genetically correlated. The genetic correlation between the resistance of BCWD and CD were favorably positive in both studied populations (0.17 ± 0.25 and 0.40 ± 0.17 , respectively for NCCCWD and TLUM). These findings are encouraging because they suggest that both traits can be improved simultaneously if genetic selection is applied for only one of those traits. Based on weight single step genome wide association (WssGWAS) of both trout populations, resistance to CD is a polygenic trait being ruled by a few genomic regions explaining variations $>1\%$. However, the SNP windows found to be associated with CD do not explain a proportion of variance high enough for choosing marker assisted selection (MAS) instead of genome-wide selection (GS). In addition, because of few overlapping windows, this information should not be used for selection decisions across populations.



THE ROLE OF INDISPENSABLE AND DISPENSABLE AMINO ACIDS SUPPLEMENTATION IN THE REDUCTION OF DIETARY CRUDE PROTEIN CONTENT IN CHANNEL CATFISH *Ictalurus punctatus* DIETS

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Protein represents about 70% of the dry weight of fish muscle, which is needed for maintenance and growth. In fact Catfish is like other fish species, do not have a protein requirement, but they require a source of nonspecific nitrogen and indispensable amino acids. Usually these nutrients supplied either from a mixture of intact protein sources or by using a mixture of feedstuffs supplemented with purified, crystalline amino acids. These amino acids are utilized for protein biosynthesis and deposition or, used for energy if it supplied in excess. So, catfish feeds should be balanced through the supply of adequate levels of nonspecific nitrogen, amino acids, and non-protein energy in exact proportions. Dispensable amino acids can be synthesized by catfish, but dietary inclusion of these amino acids help to promote growth through saving energy used in their synthesis, and some dispensable amino acids can partially replace some indispensable amino acids.

Growth trial was performed to assess the role of indispensable and dispensable amino acids supplementation in the reduction of dietary protein content in channel catfish *Ictalurus punctatus* diets and their effect on growth response. 9 isolipid (8% experimental diets were formulated with different crude protein levels) 32 control, 30, 28, 26, 24 % CP) with supplementation of Essential amino acids (EAAs) ; Lysine and Histidine to meet EAAs requirements for channel catfish *Ictalurus punctatus* according to NRC 2011 with and without addition of Nonessential amino acids (NEAAs); Glutamic acid and Glycine (2, 4, 6, 8% NEAAs) .

Experimental fish (average initial weight 10.56 g) were randomly distributed into 9 groups, 4 replicate for each 15 fish/ tank in recirculating system, fed experimental diets two times/day % of body weight and weighing bi-weekly for measuring (Mean weight (g), weight gain and feed conversion ratio. Dissolved oxygen, temperature and salinity measure 2times/day while, water samples collect 2 times/week for Total ammonia nitrogen and Nitrite analysis. Also, PH measured by PH meter two times/ week.

The growth results showed that, there is no significant difference ($P>0.05$) in the final means (g), total weight gain(g) and feed conversion ratio (FCR) among fish groups which fed with {32 (control), 30, 28 & 26% CP supplemented with EAAs Lysine and Histidine}. However, fish fed with 24 % CP supplemented with EAAs showed statistically different ($P<0.05$) compared to 32 and 30% CP diets. While the NEAAs supplementation to 24% CP diet with EAAs did not show an obvious contribution in growth response.

IMPACT OF NRAC AQUACULTURE FUNDING IN THE US NORTHEAST

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Through support from the U.S. Department of Agriculture National Institute of Food and Agriculture, the Northeast Regional Aquaculture Center (NRAC) invested \$4.1 million in 32 aquaculture research and extension projects from 2005 to 2014. A multi-disciplinary team of researchers from the University of New Hampshire conducted an evaluation of these projects to assess: their economic impact on the aquaculture industry and overall economies in the Northeast; their effectiveness in solving problems confronting the aquaculture industry; and their effectiveness in securing other research grants.

The evaluation involved three surveys: one with NRAC project leaders; a second with the research, extension, and industry collaborators who were part of the design and implementation of NRAC projects; and the third with representatives from the aquaculture industry throughout the Northeast region. Data from these surveys were used in an estimation of the economic impact of the NRAC-funded projects in the region and in individual states using an IMPLAN input-output model.

Money invested in the 32 NRAC-funded projects benefitted regional Gross Domestic Product (GDP), job growth, and state and local tax revenues (Table 1). A modest investment of just over \$4 million resulted in an increase of almost \$79 million in GDP of NRAC states; 777 new jobs; over \$4 million in state and local tax revenues; over \$9.5 million in federal tax revenues; and nearly \$33 million in additional external grant funding secured, not including matched funds. People engaged in the aquaculture industry rated these projects as having been very important to critically important to the future of the industry. Depending on the assumptions of the model, the overall economic impact of NRAC funding from 2005-2016 is estimated at 5.3 to 21.9 times the initial investment.

TABLE 1. Total Economic Impact of 2005-2014 NRAC-Funded Projects from Verified Sources

	Increase to States' GDP	Jobs	State and Local Tax Revenues	Federal Tax Revenues	Increase in GDP plus Taxes
NRAC Grants	\$7,225,833	67.6	\$358,433	\$895,316	\$8,479,582
Leverage from Grants	\$56,856,275	541.2	\$2,869,380	\$7,167,314	\$66,892,969
Aquaculture Industry Members Surveyed	\$14,547,749	167.9	\$871,839	\$1,477,109	\$16,896,697
TOTAL	\$78,629,857	777	\$4,099,652	\$9,539,739	\$92,269,248

IMPROVING WHITE WORM *Enchytraeus albidus* NUTRITION FOR ORNAMENTAL FISHES

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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. We have been exploring the potential of commercial scale cultivation of white worms. By using no-cost industry wastes, we have been able to produce worms high in protein and lipids, and low in ash. From the aquaculture stakeholder input we received, the ornamental aquaculture industry seems the best fit for white worms, however a feed higher in n-3 long-chain polyunsaturated fatty acid content is desired. In this talk, we will present the latest nutritional studies to enhance fatty acid profiles of white worms and the implications these have for the ornamental industry.

EFFECT OF PROBIOTIC ON OSMOLALITY AND SOME SEMINAL PLASMA IONS OF RAINBOW TROUT *Oncorhynchus mykiss* BROODSTOCK

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Seminal plasma produced by the sperm duct provides an ionic environment that maintains the viability of spermatozoa after their release from the testis. As a result, some parameters such as seminal plasma ions and osmolality can affect sperm quality. Also, according to previous studies, proper nutrition can improve sperm quality. This study aimed to investigate the effect of probiotic on osmolality and some seminal plasma ions of rainbow trout broodstock. For this purpose, 44 males of rainbow trout broodstock with an average initial weight of 1365.7 ± 33.40 g (mean \pm S.E.M) were fed for 12 weeks with diets containing 0, 0.5, 1 and 2 g/kg Bio-Aqua probiotic. During the feeding period, fish divided into four groups (n = 3-4 in each replicate) in a raceway ponds. For each treatment, 3 replicates were considered. At the end of the experiment, seminal plasma was collected after semen centrifugation of three adult males from each replicate. The results of the present research are shown in Table 1. According to the results, the highest level of Ca^{2+} was related to 1 g/kg treatment and was significantly different with 0.5 g/kg and control treatments ($P < 0.05$). The highest level of Mg^{2+} was observed in 2 g/kg treatment and significantly different with control treatment ($P < 0.05$). Furthermore, the highest level of Na^+ was observed in 1 g/kg treatment and significantly different with control treatment ($P < 0.05$). K^+ level was not significantly different among treatments ($P > 0.05$). As well as, a significant difference was observed in osmolality between 0.5 g/kg treatment and control treatment ($P < 0.05$). The results of the present study demonstrated the ability of probiotic to influence on osmolality and seminal plasma ions in rainbow trout broodstock. In this regard, results showed that use of probiotic in rainbow trout broodstock diet can improve sperm quality.

Table 1. Effects of Bio-Aqua probiotic complex on osmolality and seminal plasma ions in rainbow trout (*Oncorhynchus mykiss*) broodstock after 12 weeks (mean \pm S.E.M).

Groups with different alphabetic superscripts in the same row differ significantly at $P < 0.05$.

Parameters	Probiotic levels (g/kg diet)			
	0	0.5	1	2
Ca^{2+} (mmol/l)	0.97 ± 0.01^b	1.01 ± 0.04^b	1.27 ± 0.08^a	1.17 ± 0.03^{ab}
Mg^{2+} (mmol/l)	0.96 ± 0.04^b	1.23 ± 0.20^{ab}	1.45 ± 0.16^{ab}	1.76 ± 0.26^a
Na^+ (mmol/l)	38.00 ± 1.88^b	42.19 ± 1.65^{ab}	46.25 ± 1.11^a	44.68 ± 1.82^a
K^+ (mmol/l)	18.78 ± 0.71	18.24 ± 1.59	16.72 ± 0.32	17.49 ± 0.48
Osmolality (mOsmol)	198.50 ± 3.55^a	180.45 ± 2.33^b	184.83 ± 5.29^{ab}	192.00 ± 2.45^{ab}

CHANNEL CATFISH *Ictalurus punctatus* PRODUCTION IN FLOATING RACEWAYS

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Floating in-pond raceway systems (IPRS) consist of large floating rectangular cages placed into existing ponds. IPRS generally occupy less than 2% of the pond area, have a constant water flow of highly aerated water and grow fish at a high density. Due to the efficient feed conversion ratio (FCR), effective pond water circulation and easier management of fish stock, IPRS can support a higher fish yield compared to conventionally managed catfish ponds. Research at Auburn University has shown that hybrid catfish (female channel catfish, *Ictalurus punctatus* x male blue catfish, *I. furcatus*) can be grown to market size (680 g) in nine months, with survival ranging from 85 to 96%, FCR below 1.60 and gross yield greater than 15,000 kg/ha, while conventionally managed ponds often yield less than 8,000 kg of catfish/ha, with FCR above 2.0.

This study aimed to verify if channel catfish can be raised in IPRS units to a commercial size of 680 g in one growing season at a yield twice as much as the gross yield recorded in conventional catfish ponds in Alabama (7,800 kg/ha/yr). For this, one floating IPRS was placed in each of four 0.4-ha ponds. Raceways were 64 m³ (4.9 m wide, 10.7 m long and 1.22 m deep) in ponds B1 and B2, and 45 m³ (3.05 m wide, 12.20 m long and 1.22 m deep) in ponds B3 and B4. Each IPRS had an airlift device made of a grid with 12 m of diffuser hose placed under a directional hood, propelled by a 1.5-HP air blower. The airlift device was positioned just before the inlet screen and provided raceways with a constant water flow and maintained adequate oxygen levels during the night and early morning hours. IPRS units were covered with a 25 mm net to prevent bird predation. Catfish fingerlings were stocked into IPRS units in April 2017 and fed a 32% CP commercial catfish pellet once or twice a day depending on water temperature and oxygen conditions. The feed was supplied at will until near apparent satiation. Raceways were inspected daily for the presence and removal of dead fish.

Partial production 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 losses of fish in all raceways. Fortunately, fish were still small at the onset of those infections, causing minor losses of biomass. The IPRS unit in pond B4 experienced the highest mortality but fish in this unit also have the highest mean weight and highest estimated biomass compared to fish in other raceways. Full trial results post-harvest will be presented.

Table 1 – Partial production data from the second year study on farming catfish in IPRS. Last data collection was on Aug. 31, at day 127 of the trial.

Ponds (0.4 ha)	B1	B2	B3	B4
IPRS volume (m ³)	64	64	45	45
Number of fish stocked	12,205	10,890	10,809	10,806
Initial mean weight (g)	47.3	41.3	47.7	43.4
Stocking biomass (kg)	517	437	500	452
Number of dead fish removed	941	869	540	2,670
Estimated mean weight (g)	293	287	263	391
Estimated final biomass (kg)	2,928	2,788	2,618	3,035
Feed applied (kg)	3,857	3,720	3,335	4,560
Estimated FCR	1.62	1.61	1.58	1.77
Avg. feeding rate (kg/ha/day)	36.4	35.4	31.2	42.6
Specific growth rate (g/fish/day)	1.94	1.93	1.70	2.74
Estimated standing crop (kg/m ³)	45.7	43.6	58.2	67.8
Estimated standing crop (kg/ha)	7,319	6,971	6,545	7,589
Estimated survival (%)	92.3	92.0	95.0	75.3

COMPARISON OF CACHARA *Pseudoplatystoma reticulatum* AND HYBRID *P. reticulatum* × *P. corruscans* GROWTH CURVES

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Growth curves of animals can be described through non-linear mathematical models, which allow aggregation of weight-age information into a small set of parameters. This study aimed to compare the growth curves of cachara (*Pseudoplatystoma reticulatum*) and hybrid cachapinta (*P. reticulatum* × *P. corruscans*) produced in cages. The experiment was conducted using floating cages placed in a 24-ha hillside pond at a commercial fish farm. A randomized experimental design was established with two treatments (cachara and hybrid) and three replicates, using six cages (13.5-m³ each). Mean weight of fish at the start of the experiment were 75 ± 0.004 g and 85 ± 0.001 g for cachara and hybrid, respectively. The results of the experiment were adjusted for a density of 135 fish per cages. Since the mean weight of the groups differed, the number of fish were adjusted at the onset of the experiment so that the density in kg per m³ remained the same for each cage, irrespective of the group present. The experimental period lasted for 216 days. Student's *t*-test was used to compare between groups for the dependent variables, assuming equivalent variances ($P > 0.05$). The exponential model was used for analysis of the growth curves of the morphometric characteristics of each genetic group. To test the similarity of the models between the two groups we used the likelihood ratio test (LR), which verifies the equality of the parameters between them. In the LR test, four models were evaluated: model 1 (M1) the groups presented initial sizes (A) and different growth rates (K); model 2 (M2) where A was the same and different K; model 3 (M3), the two groups had K equal and A different; model 4 (M4), even A and K. The choice of the model used for each evaluated characteristic was the one that presented the smallest number of parameters and did not differ from the complete model (M1). When the M2, M3 and M4 models differ from the complete model (M1), it was concluded that the parameters were different between the two groups. There was no difference ($P > 0.05$) between the growth curves of the cachara and the hybrid when compared using the characteristics: weight, body height BH, body width BW, head length HL (Table 1). The model used to compare these characteristics was the reduced model (M4). Model 2 was the one that best adjusted for the total length TL in which there was no significant difference in the initial size (A), however, the cachara presented a higher growth rate (B). The complete model (M1) was used for head width HW, where A was smaller and B was larger for this characteristic in the cachara compared to the hybrid. The cachara (*Pseudoplatystoma reticulatum*) presented a higher growth rate for the characteristics TL and HL in relation to the cachapinta hybrid (*P. reticulatum* × *P. corruscans*).

Table 1. Model chosen for the characteristics according to the likelihood ratio (LR) test in the comparison of the growth curve of the two genetic groups cachara (cc) and hybrid cachapinta (hb)

characteristics	A _{cc}	K _{cc}	A _{hb}	K _{hb}	A	K
Weight (g)	-	-	-	-	23.9714	0.0135
TL (cm)	-	0.00414	-	0.00368	17.7661	-
BH (cm)	-	-	-	-	1.4943	0.00530
BW (cm)	-	-	-	-	1.6526	0.00528
HL (cm)	-	-	-	-	5.1557	0.00402
HW (cm)	1.7416	0.00499	1.9445	0.00441	-	-

RANDOM MUTAGENESIS OF *Phaeodactylum tricornutum* AND SELECTION OF CLONES RICH IN NEUTRAL FATTY ACIDS WITH C75

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The application of mutation techniques in microalgae mainly targets the creation of over-producing strains of saturated fatty acids for biodiesel or highly polyunsaturated fatty acids (HUFAs) for human nutrition and aquaculture. There are several antibiotics and herbicides that can block the synthesis of fatty acids in microalgae and their lethal dose for the wild strains can be used to select mutant cells favoring the synthesis of saturated and/or unsaturated fatty acids. Cerulenin is one of these antibiotics, it is produced by the fungus *Cephalosporium ceruleans* and has been successfully used as selector of mutants with high fatty acid content of several microalgal species.

This work reports for the first time the use of a synthetic analog of cerulenin, called C75, to select clones rich in HUFAs of *P. tricornutum* mutagenized with ethyl methyl sulfonate (EMS).

P. tricornutum survival declined with EMS increasing concentrations; the optimum mutagenic dose was 0.15 M. The cytotoxic effect of C75 was dose and time dependent. The lethal dose of C75 was 100 μ M. and successfully selected only mutant cells with significant higher contents of the commercially valuable fatty acids EPA and DHA than the parental strain.

SMALL-SCALE AQUACULTURE HELPS TO IMPROVE NUTRITION AND PROFIT OF RURAL COMMUNITY: A CASE STUDY FROM NEPAL

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Subsistence farming system of crops and livestock is the mainstay for the rural communities in Nepal. Poverty associated with malnutrition is the major problem, especially among rural ethnic communities. Transformation of subsistence system into semi-commercial farming using profitable and innovative technologies is needed to combat household malnutrition and low economic opportunities. Aquaculture is becoming one of the most popular tools especially after the successful launching of small-scale aquaculture, which involved mainly ethnic women of the rural communities (Bhujel *et al.*, 2008). However, how such aquaculture was improving the livelihoods of the rural community was not clear. Therefore, a household survey was conducted during January-April 2017 interviewing 71 women in Kathar village of Chitwan, Nepal to investigate the status and impacts of the aquaculture development project a decade after its completion. Results showed that households with fish farms earn a yearly additional income of \$265 (40% of per capita income) and consumed approximately 46 kg more fish than non-fish farming households (Table 1). Additionally, women with fish farms reported feeling empowered and independent.

Parameters / Indicators		Project Supported Fish Farmers	Non-Project Supported Fish Farmers	All Fish Farmers	Non- Fish Farmers
Number of farmers interviewed	(n)	30	11	42	30
Operative members	(n)	30	9	39	-
Number of ponds	(n)	32	15	47	-
Fish per family	Mean	1.07	1.00	1.05	-
	SE	0.05	0.00	0.03	-
Pond size (m ²)	Mean	423	477	437	-
	SE	110	143	14	-
Production (kg/yr)	Mean	129	109	123	-
	SE	38	81	28	-
Feed efficiency (kg/m ² /yr)	Mean	0.35	0.26	0.33	-
	SE	0.01	0.01	0.00	-
Consumption (kg/yr)	Mean	78	70	76	30
	SE	6	8	5	6
Sales (kg/yr)	Mean	82	74	80	-
	SE	37	25	28	-
Profits (NRs/yr)	Mean	29,954	21,113	27,581	-
	SE	8,366	4,652	6,165	-
Profits (US\$/yr)	Mean	288	203	265	-
	SE	81	45	59	-

DIETARY SUPPLEMENTATION DURING PRODUCTION AND SURVEY OF MICRONUTRIENTS OF TILAPIA (*Oreochromis niloticus*) IN THE U.S. MARKET PLACE

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Tilapia, (*Oreochromis spp.*) contributed to global production of 5, 576,800 MT in 2015. Due to high production there are extensive researches going on regarding nutrient requirements and alternative feed ingredients for Nile tilapia. However, limited research has been devoted to define the optimum micronutrient requirements for this species of fish. Selenium (Se) is a micronutrient that is of a great interest. By incorporating into group of Se containing proteins called selenoproteins this element plays a crucial role in varieties of biological processes. Gluthothione Peroxidase (GPx) is one of the main selenoproteins which is responsible of protecting cell membranes against oxidative damages. Antioxidant activity of Se in human has shown to be protective against 50 diseases (i.e. Kashan’s disease) and can cause liver necrosis and thyroid problems. Se role in preventing oxidative stress and inducing apoptosis is particularly important for the aquaculture industry where immunocompetence and disease resistance can be compromised by intense culture environment. Also, fish fillet quality and shelf-life may be improved by the levels of antioxidant such as Se in pre-slaughter diet.

For these reasons, we conducted two studies. First study was a survey to evaluate the Se status of tilapia in the U.S. market. Market samples were also evaluated for the levels of As, Ca, Cd, Cu, Fe, Mb, Mn, Na, Pb, and Zn. Second study was a fish trial to evaluate alternative sources of Se that can be used to enrich the tilapia fillet. The results from our survey study provided evidence that tilapia fillets (n=12) in U.S. market has Se concentrations of $0.94 \pm 0.12 \mu \text{g}^{-1}$. A seven-weeks fish trial was conducted. Bioavailability of different forms of Se (organic and inorganic) in Nile tilapia (*O. niloticus*) was evaluated using uptake, tissue retention of Se and plasma and liver GPx activity. There was no significant difference between growth of fish fed different diets. However, organic Se fed fish fillets had Se levels of $2.6 \pm 0.19 (\mu \text{g g}^{-1})$ which were significantly ($P < 0.05$) higher than inorganic Se fed fish ($1.01 \pm 0.01 (\mu \text{g g}^{-1})$). Higher Se levels were found in liver and kidney compared to the fillet. However, supplementation with any of investigated Se forms did not affect the GPx activity of liver and plasma. Inorganic source of Se is currently used in aquaculture diets. As expected, concentrations of Se in inorganic Se fed fillets was almost identical to market samples. This study shows that seleno-L-methionine (organic Se) is an excellent Se supplement to deliver a high level of Se in the fillet without causing harm to the fish. Organic species of Se should be used by the aquaculture industry as an alternative to inorganic species. Also, result of market study shows that tilapia in U.S. market are good source of essential elements and do not pose any health risk problem to consumers.

ELEMENTAL COMPOSITION OF TILAPIA, SALMON, AND CATFISH IN UNITES STATES OF AMERICA MARKET: HEALTH AND NUTIRITIONAL ASSESMENT AND HEALTH BENEFIT VALUE of SELENIUM (HBVse)

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The aquaculture industry has experienced rapid growth in an era of health-conscious consumers who consider seafood a healthy alternative to red meats. Elemental food composition data can help the aquaculture industry to improve nutritional value of fillet through dietary intervention. Composition data also can be used for compliance with regulation concerning quality, safety, and labeling and to identify potential contribution of food to diet. Fish has been acknowledged as an essential component of a well-balanced diet and is an important source of minerals. Minerals such as Zinc, Magnesium, Copper, and Selenium are critical part of enzymes that are important for several vital biological processes. However, high concentration of some micronutrients can cause toxicity. Also, there is concern in regards to mercury and other heavy metals exposure due to fish consumption that can counteract the beneficial effects of fish consumption. Mercury in edible portion of fish is mainly present as methylmercury (CH_3Hg) which is highly toxic and have adverse health effect if it is consumed in large quantities. Health benefit value of Se (HBVse) is a risk assessment criterion that has been recently developed that enable concurrent consideration of CH_3Hg exposures and dietary Se intake. HBVse shows both absolute and relative amount of Se and Hg in the diet. Positive HBVse is an indication of no health risk.

In this study, a total of 34 fresh and frozen samples of the commercial catfish, tilapia and salmon available in the USA market were analyzed for levels of As, Ca, Cd, Cu, Fe, Mb, Mn, Na, Pb, Se, and Zn. Samples were purchased from Mid-Atlantic, Midwest Northeast, and Southwest regions of USA. Data regarding type of culture (wild caught vs farmed), country of origin, price and fish condition was recorded upon the purchase if available. HBVse was also calculated for each species of fish. Finally, contribution of each element form consumption of this fish in Dietary Reference Intake was calculated per serving (100 g). Fillet samples were digested using microwave assisted acid digestion and minerals were analyzed using inductively coupled plasma mass spectroscopy (ICP-MS). Methodology was verified by analysis of DORM-4 which is a fish protein standard reference material for trace element analysis. Samples had variable prices based on the region, grocery market, species of fish and types of culture (wild versus farmed). This study shows available catfish, salmon and tilapia in USA market are good source of essential elements. These species do not pose any health risk problem to consumers. All the species analyzed had positive HBVse and mean total mercury level of less than 0.3 ppm. This shows these fish do not pose any health risk associated with mercury toxicity for consumers. This study can help to complete the database regarding level of minerals in commercial fish in USA market. It also confirms the importance of promoting the consumption of fish. Similar studies should be conducted primarily to monitor other quality parameters and other contaminants to better understand the risks and benefit associated with fish consumption.

ECONOMIC BENEFITS OF REDUCED FEED INPUTS AND POLYCULTURE OF TILAPIA WITH MAJOR INDIAN CARPS

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The aim of these studies was to determine if reductions in feed inputs and introduction of native Indian carp, rohu (*Labeo rohita*) and catla (*Catla catla*), can increase economic benefits of tilapia culture in earthen ponds in Bangladesh. Two on-station pond trials were carried out for 150 days at the Fisheries Field Laboratory at Bangladesh Agricultural University. In the first study, ponds consisting of four treatments (T1, T2, T3, and T4) with four replications each were stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*, 5 fish/m²) without (T1) or with (T2) addition of rohu (0.25 fish/m²) and fed a full daily ration of feed (CP, 35% protein; 10%–3% body weight/day). Ponds were fertilized weekly (28 kg N and 5.6 kg P ha/week) in the other treatments and tilapia were grown in the absence (T3) or presence of *Rohu* (T4) at half the daily feed ration as T1 and T2. The survival rates (75–81 %) of tilapia was similar among treatments. The specific growth rate (SGR, %/day) of tilapia was higher in the T3 (1.87 ± 0.00) and T4 (1.85 ± 0.03) than the T2 (1.76 ± 0.05), and T1 (1.71 ± 0.06) groups ($p < 0.05$). Feed efficiency was significantly better in the T3 and T4 groups relative to those treatment fish fed the full ration. Gross production of tilapia was higher in the T4 ($5,385.23 \pm 276.98$ kg/ha) followed by T3 ($5,340.62 \pm 156.47$ kg/ha), T2 ($4,440.99 \pm 440.04$ kg/ha) and T1 ($4,089.83 \pm 518.46$ kg/ha) groups, respectively. Rohu gross production was similar among the T2 and T4 groups. A significantly higher net return (BDT 743,977/ha; benefit cost ratio of 2.92) was found in T3 followed by T4 (BDT 673,750/ha; benefit cost ratio of 2.72), T2 (BDT 286,469/ha; benefit cost ratio of 1.49) and T1 (BDT 226,675/ha; benefit cost ratio of 1.37) groups, respectively.

In the second study, ponds consisting of three treatments (T1, T2, and T3; 4 replicates each) were stocked with sex-reversed Nile tilapia (5 fish/m²) and rohu (0.625 fish/m²; T1), or catla (0.625 fish/m²; T2), or with both rohu and catla (0.32 fish/m² and 0.31 fish/m², respectively; T3). All ponds were fed a half daily ration of feed and ponds were fertilized weekly. Gross production of tilapia was higher in T2 ($7,737.78 \pm 646.51$ kg/ha) followed by T1 ($6,867.11 \pm 570.36$ kg/ha), and T3 ($6,272.23 \pm 183.44$ kg/ha), respectively. Rohu gross production was higher in T1 and catla production was higher in T2. There was no significant difference in net return or benefit cost ratio between treatments.

Based on the higher net return and benefit-cost ratio it may be concluded that pond fertilization with feeding at half ration is substantially more cost effective over standard full feeding for growout of tilapia. Addition of major Indian carps to tilapia culture may also provide further income benefits to farmers as net production of fishes is greater in polyculture than tilapia monoculture systems regardless of the feeding regimen applied. Since tilapia growth was little impacted by feeding at half ration, but tended to grow better when polycultured with either Catla or Rohu alone compared with Rohu-Catla combined, it might be preferential to polyculture tilapia with only one of the carp species. Regardless, the results indicate profits can increase by 200% if tilapia are grown with native Indian carps and provided half the standard ration level typically used for tilapia monoculture.

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 1st, 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 β (E2) 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.

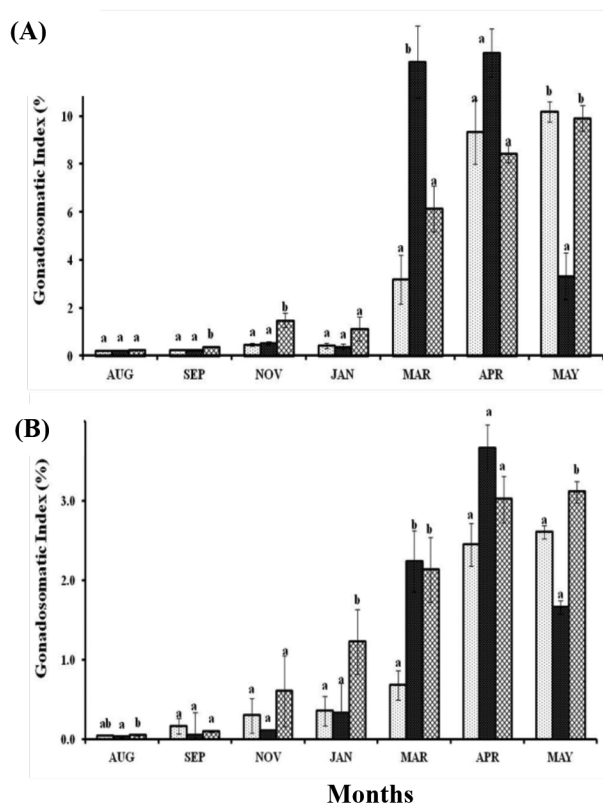


Fig. 1 Variations in gonadosomatic index of females (A) and males (B). One way ANOVA computed difference between values of natural (NP), advanced (AP) and continuous (CP) treatments at level of $P < 0.05$ on same sample point, shown by letters. Natural: □, Advanced: ■, Continuous: ▨. n at each sample point (AUG – APR) = 18 per treatment. n at final sample point (MAY) = 90 per treatment

THE EFFECT OF LINSEED ON GROWTH AND FATTY ACID PROFILE IN GRASS CARP (*Ctenopharyngodon idella*)

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Linseed is an economical plant source of n-3 polyunsaturated alpha-linolenic acid (ALA) which can be supplemental dietary lipid in fish feed particularly carps. In developing countries where fish oil is not an ingredient of fish feed due to its high cost, addition of economical plant oils rich in n-3 PUFA in fish diet can remarkably improve its quality. Present study evaluated the effect of addition of linseed in fish feed on growth and fatty acids profile in grass carp (*Ctenopharyngodon idella*); an important commercial carp in Asia.

Present study investigated the effects of three different diets (A: 5% linseed flour, B: 5 % linseed oil, C: 2.5 % linseed flour + 2.5% linseed oil) on growth and fatty acids profile (C12:0 – C22:6) in grass carp (age = 12 months). Trial continued for 31 days at ambient temperature. Control group was fed with soybean based commercial diet. No significant effect of diet A and C was observed on fish growth and profile of n-3 and n-6 PUFA in total liver lipids. However, treatment group fed with diet B showed a significant increase in condition factor (1.75 ± 0.14 %), feed conversion ratio (FCR) (0.97) and hepatosomatic index (HSI) (0.62 ± 0.1 %) as compared to those in control group. Comparatively high profile of decosahexanoic acid (DHA) (C22:6) were observed in total liver lipids of fish in all three treatments groups (A: 0.31%, B: 0.41%, C: 0.40%) as compared to control group where DHA was not observed in fatty acids profile. Similarly high profile of eicosapentanoic acid EPA (C20:5) were observed in all three treatment groups (A: 0.56%, B: 1.64%, C: 1.35%) as compared to those in control group. Therefore, present study recommends that linseed can be a suitable source of n-3 and n-6 PUFA in carp feed, improving the condition and flesh quality of fish.

Table 1 Comparison of fatty acid profile (%) in total liver lipids of grass carp (*Ctenopharyngodon idella*) fed with three different diets (A: 5% linseed flour, B: 5 % linseed oil, C: 2.5 % linseed flour + 2.5% linseed oil) over the period of 31 days (April 5 - May, 5, 2017) (Cont.: control).

Carbon	Fatty acids	Cont.	A	B	C
C _{12:0}	Lauric acid	1.34	1.32	1.3	-
C _{14:0}	Myristic acid	0.44	0.47	0.15	0.76
C _{16:0}	Palmitic acid	26.1	28.7	38.6	33.6
C _{16:1}	Palmitoleic acid	1.02	0.40	0.31	0.39
C _{17:0}	Heptadecanoic	-	0.73	1.3	0.52
C _{18:0}	Stearic acid	5.49	0.91	1.2	0.50
C _{18:1}	Oleic acid	36.5	39.8	39.1	39.0
C _{18:2}	Linoleic acid	19.87	24.8	12.7	17.2
C _{18:3}	α linolenic acid	4.46	0.57	1.07	1.73
C _{18:3}	β linolenic acid	2.15	0.36	-	2.31
C _{20:0}	Arachidic acid	1.1	0.32	0.35	0.73
C _{20:1}	Condolic acid	0.74	0.33	1.13	1.22
C _{20:2}	Eicosadionic acid	-	-	0.24	-
C _{20:3}	Eicosatronic acid	-	-	-	-
C _{20:5}	Eicosapentanoic acid	0.79	0.56	1.64	1.35
C _{22:0}	Decosanoic acid	-	0.20	0.22	0.23
C _{22:6}	Decosahexanoic acid	-	0.31	0.41	0.40

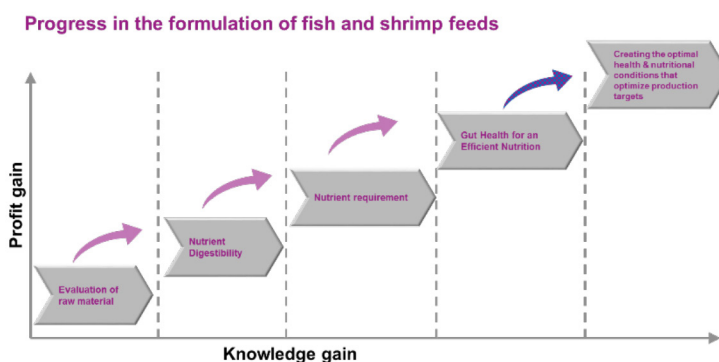
PRECISION AA NUTRITION IS KEY FOR ECONOMIC AND SUSTAINABLE PRODUCTION OF HEALTHY FISH AND SHRIMP

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With the world's growing population, there is a rising demand for high-quality meat, fish and shrimp. Animal feeds play a leading role in the economic production of animal proteins throughout the world. Sustainability of this industry is very much dependent on its capacity to deliver more innovative, effective and sustainable nutritional solutions that best support the performance of animals. With feed costs accounting for up to 80% of the total variable costs of animal production, precision nutrition is becoming increasingly important to stay competitive. Formulation of feeds that accurately match production targets must put knowledge into practice: knowledge of raw material composition, digestibility and animal requirements are needed for a healthy growth. Besides affecting negatively animal growth, raw material that are poorly digested result in high N and P load into the environment. More recently, precision nutrition programs using NIR technology have allowed the nutritional value of feed and ingredients to be determined in a rapid and simple manner and thus help minimize economic losses and improve profitability. Remarkable examples of putting science and technology at the service of the animal meat industry come from the poultry and salmonid industry. During the 1980s and 90s, advances in poultry nutrition included computerised least-cost feed formulation, the development of the ideal protein concept and formulation for digestible nutrients, and an efficient absorption and utilisation of nutrients and energy. Moving from a formulation based on dietary crude protein (CP) to a formulation based on essential amino acids (EAA) was one of the major breakthroughs in formulating animal feeds, allowing nutritionists to meet animal amino acid (AA) requirements while minimizing N release into the environment. As a result, in 1957 a 42-day-old broiler weighed 540 g with a feed conversion (FCR) of 2.6 while today, a broiler of the same age weighs 2.8 kg with an FCR under 1.70. The use of more highly digestible nutrient-density extruded feeds allows farmers to achieve a FCR of 1.1-1.2 for Atlantic salmon grown to market size today. Moreover, the salmonid industry has made considerable progress in reducing the inclusion of marine ingredients from close to 90% in 1990s to less than 20% in 2016. Accurate information about nutrient requirements of salmonids grown under practical conditions have greatly contributed for these achievements. Carp, tilapia and shrimp industries are equally progressing by putting into practice the scientific knowledge generated in the areas of fish nutrition, feed processing technologies, feed development and feed management practices.



INFLUENCE OF OIL AND MINERAL SOURCE ON PERFORMANCE, FATTY ACID PROFILE AND MINERAL PROFILE IN RAINBOW TROUT *Oncorhynchus mykiss*

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An 8 week feeding trial was conducted to evaluate the impact of oil source and mineral source on weight gain, whole body fatty acid profile and whole body mineral analysis of Rainbow trout. Eight isonitrogenous and isoenergetic diets contained soy bean oil and either fish oil or All-G-Rich® as an oil source and inorganic or Bioplex® minerals at a 100, 75 or 50% of recommended NRC levels. The oil source had no impact on weight gain ($p>0.05$). In this study, the fish that were fed the diet containing 100% inorganic minerals had the lowest weight gain compared to the fish fed any level of Bioplex minerals. The fish fed diets containing All-G-Rich had a higher level of omega 3 fatty acid ($p<0.05$) and DHA ($p<0.05$) in the whole body and the omega 6 fatty acid whole body profile was not changed by oil or mineral source. The fish fed the fish oil diet had higher levels of whole-body zinc and selenium compared to fish fed the All-G-Rich diets ($p<0.05$). The fish fed the 50% Bioplex mineral diet had a lower selenium level compared to other diets ($P<0.05$). The growth data from this study indicate that Bioplex minerals can be provided at 50% of the NRC required level and maintain growth. The use of Bioplex minerals at all levels resulted in greater DHA uptake.

PRODUCTION OF WALLEYE *Sander vitreus* AND HYBRID WALLEYE *S. vitreus* x *S. canadense* WITH FORMULATED FEED UTILIZING VARIOUS REARING SYSTEMS AND NOVEL TECHNOLOGIES FOR COMMERCIAL FOOD FISH PRODUCTION

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For over ten years the University of Wisconsin-Stevens Point Northern Aquaculture Demonstration Facility (UWSP-NADF) staff scientists have successfully used a variety of rearing systems, formulated feed, and novel technology to produce purebred walleye, purebred sauger and hybrid (walleye female x sauger male) fingerlings and market sized fish for different uses. Field trials conducted by UWSP-NADF scientists have shown remarkable growth of walleye and 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. We will discuss findings from several past and ongoing research projects that involve novel methodology including; out of season spawning, intensive fry-fingerling production, hybridization, cold banking, recycle systems and more as potential ways to improve walleye aquaculture for the commercial production of this valuable food fish.

EFFECTS OF SUPPLEMENTAL LED LIGHTING ON WATER QUALITY AND PACIFIC WHITE SHRIMP *Litopenaeus Vannamei* PERFORMANCE IN A CHEMOAUTOTROPHIC-BASED HYBRID SYSTEM

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Biofloc-based systems can provide supplemental nutrition to shrimp and have lower startup costs compared to clearwater recirculating aquaculture systems. Indoor biofloc systems have also been shown to respond positively to supplemental lighting. However, nitrification cycles in biofloc based systems can be unstable and lead to a buildup of toxic chemicals in the system including ammonia and nitrite. Hybrid systems combining positive aspects of both biofloc and clear-water systems have been proposed to overcome shortcomings of both system types. This study examined the effects of supplemental LED lighting on such hybrid systems.

Four 1 m³ round tanks were assigned to each of three treatments for a total of 12 tanks. Two treatments had supplemental LED lighting overhead set to 24 hours of lighting (Full Light, FL) and 12 hours of lighting (Partial Light, PL), the third treatment had no supplemental lighting provided (No Extra Light, NL). All treatments were stocked with 250 shrimp at an average weight of 1.2g. All tanks had a settling chamber to remove excess solids from the system and a moving bed bioreactor to provide biofiltration. Settling chambers were run throughout the project but kept at low flow levels early in the experiment to allow biofloc particles to build in the water. All treatments were fed the same amount and each tank was equipped with a 24 hour belt feeder to dispense feed. The DO, pH, temperature, salinity were each measured twice daily and ammonia, nitrite, nitrate, turbidity, chlorophyll, and TSS/VSS were measured weekly.

All systems were harvested after 84 days. We found significant differences in shrimp production between all three treatments. FL systems produced significantly more shrimp than PL or NL systems (4569.5g/tank FL, 3434.6g/tank PL, 3082.8g/tank NL) and FL and PL systems produced significantly larger shrimp than NL systems (25.0g/shrimp FL, 24.2g/shrimp PL, 19.9g/shrimp NL). Feed conversion ratio (FCR) was significantly lower in FL systems compared to PL and NL systems (1.37 FL, 1.83 PL, 2.10 NL) and survival was significantly higher in FL tanks compared to PL tanks (74.1 FL, 57.2 PL, 62 NL). Shrimp growth per week was significantly higher in FL and PL systems compared to NL systems (1.98g/week FL, 1.91g/week PL, 1.56g/week NL). NL systems required a significantly higher amount of NaHCO₃ to raise pH levels over the course of the study than FL and PL systems (222.5g FL, 260g PL, 365g NL). The results of this study show that supplemental lighting in chemoautotrophic hybrid systems can be beneficial to shrimp growth. Further research should investigate whether the extra lighting affected the microbial community, the physiological condition of the shrimp, or both.

THE EFFECTS OF VERTICAL SUBSTRATE AND SHRIMP *Litopenaeus vannamei* DENSITY ON WATER QUALITY AND PRODUCTION DYNAMICS IN INTENSIVE GREENHOUSE-BASED BIOFLOC SYSTEMS

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The use of artificial substrates in shrimp aquaculture has been shown to have beneficial effects on the animals being cultured. Increased growth and decreased FCRs have been recorded in multiple studies. In intensive shrimp production systems the use of substrates has been proposed as a method to allow increased densities of shrimp while reducing negative effects of over-crowding. Substrates also increase the surface area available for periphyton growth that could impact shrimp production and water quality. This study evaluated the effects of substrate and density on water quality and shrimp production in greenhouse-based biofloc systems located in Kentucky, USA.

Four 11.04 m³, wood frame, polyethylene-lined tanks were constructed in each of four high tunnel greenhouses for a total of 16 tanks. Four treatments were created using two factors: substrate and density. The four treatments were: High Density Substrate (HDS), High Density No Substrate (HDNS), Low Density Substrate (LDS), and Low Density No Substrate (LDNS). High density systems had 200 shrimp/m³ and low density tanks had 100 shrimp/m³. Tanks with substrate had an added 18.1m² of surface area created by vertically oriented plastic construction barrier material. The experiment was blocked by greenhouse, such that each of the four treatments was randomly assigned to one tank per greenhouse. All tanks were chemoautotrophic-based biofloc systems with a 470-L settling chamber to control suspended solids levels in the systems. No heaters were used in the experiment, all tanks relied on ambient air and solar energy to maintain water temperature. All systems were fed twice daily at 12:00 and 16:00 and feed amounts were based on initial stocking density. Dissolved oxygen, pH, salinity and temperature were measured twice daily. Total ammonia nitrogen, nitrite, nitrate and turbidity were measured weekly.

Shrimp were stocked at 7.15g, and harvested after 119 days. There were significant ($p < .05$) differences in shrimp production due to density but no significant differences due to substrate. Average weights in high density tanks were 24.1g (HDS) and 24.8g (HDNS) and average weights in low density tanks were 26.4g (LDS) and 25.7g (LDNS). Peak nitrite levels and overall mean nitrite levels were significantly higher in high density treatments compared to low density treatments. Dissolved oxygen levels and pH were significantly lower in high density systems over the course of the study. Results from further shrimp production and water quality analyses are pending.

ECONOMIC ANALYSIS OF EXPERIMENTAL AQUAPONICS AND HYDROPONICS PRODUCTION IN THE US MIDWEST

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The US Midwest has experienced frequent investments into the aquaponics business but very few have remained viable. The focus of production in aquaponics tend to center on the vegetables. There are no established aquaponics business models in the US Midwest that potential investors can examine to help them make informed decisions concerning entry into the aquaponics business. Most of aquaponics operations in the region are small-scale producers. Given that vegetables provide the most revenues from aquaponics production, an analysis of the financial risks associated with aquaponics and hydroponics, would provide useful financial guidelines. This study sought to examine the different systems and scales of production to help answer questions relating to the financial viability under the different production systems and risks associated with the business.

The two experimental systems involved the production of basil, lettuce and cherry tomato in a hydroponics system, and for the aquaponics system, tilapia was added for a 90-day production period. Economic analysis conducted on the experimental systems was then extrapolated for a 10-year project period with a 6% annual discount rate. First, we compare the economic viability using the same vegetable price. Then, we use different vegetable prices for aquaponics production because some farms report premiums for organic vegetables from their operation.

The initial investment in aquaponics is 8% higher than hydroponics due to the use of aquatic heaters, biomedica and media bag, and dissolved oxygen probe. The cost of aquaponics is 11.6% higher mainly from consumables of water testing, fish feed, and fingerlings. In both systems, labor is the more expensive item representing more than 49% of operating costs. The cash flow results are presented in Table 1. Hydroponics and aquaponics showed feasibility in a 10-year production project even using non-organic prices. For hydroponics, all results show very good financial return on investment. A payback of 3.13 shows that the investment can be recovered in a rapid way. For aquaponics, results from non-organic prices are worse than hydroponics. However, with an increase in prices due to organic production, aquaponics generates better revenues than hydroponics. An increase of 19.7% in vegetables prices makes NPV of both systems equal.

Table 1 – Financial results for aquaponics and hydroponics

Results	Hydroponics	Aquaponics			
		Non-organic prices	10% veg. price increase	20% veg. price increase	30% veg. prices increase
IRR (%)	48.7	18.27	32.61	45.83	58.51
NPV (\$)	73,872	20,144	47,447	74,750	102,052
Payback (yrs)	3.13	6.83	4.04	3.25	2.79
Benefit/Cost	1.36	1.11	1.22	1.32	1.43

ANAYLSIS OF THE 2017 NATIONAL AQUACULTURE EXTENSION CONFERENCE

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The 2017 National Aquaculture Extension Conference (NAEC) was held from June 5th – 9th at the Riverside Hotel and Convention Center in Boise Idaho. USDA National Institute of Food and Agriculture's (NIFA) five Regional Aquaculture Centers and the NOAA National Sea Grant Program co-sponsored the conference. Held approximately every five years since 1992, this unique conference has provided a forum for professional development and growth for all levels of experience and years of service in aquaculture Extension education.

Following the conference, 52 evaluation surveys (79% response rate) were collected. The survey addressed conference logistics, quality and utility of the presentations and field trip, an open-ended reflective section on what attendees took away from the conference, areas for focus for the national aquaculture Extension network of over the coming five years and discussions on value, frequency and location for future NAEC conferences.

Forty-seven out of forty-eight participants were in favor of having a NAEC conference every 3 to 4 years, while four gave no response. The one who voted no actually wanted to have more NAEC conferences more frequently. Every three to four years would be a good interval for National Aquaculture Extension conferences. At least two years are required to reserve a venue and plan a conference. An Atlantic coast venue may be an appropriate for the next NAEC.

This presentation will provide results of the Likert scale used to evaluate the conference logistics, quality and utility of the presentations and field trip. A summary of the 2 open-ended questions will be discussed.

ENVIRONMENTAL DESIGN CONDITIONS FOR AN OFFSHORE AQUACULTURE SITE IN THE GULF OF MEXICO

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The development of offshore aquaculture in the Gulf of Mexico (GoM) will require containment systems capable of withstanding extreme storm events. In particular, knowledge of the forcing mechanisms induced by hurricanes on an aquaculture system is needed for effective configuration design and component specification. The objective of this presentation is to provide an example of how to establish extreme design conditions for storms with return periods of 25-, 50- and 100-years. The procedure includes examining environmental data sets from the National Data Buoy Center and the U.S. Army Corps of Engineers to characterize GoM hurricane force winds, waves and water current velocities at a particular site. An application is also presented as described in the American Petroleum Institute publication, API Bulletin 2INT-MET, for use in the design of offshore structures.

HYDRODYNAMIC CHARACTERISTICS OF MACROALGAE GROWN ON A LONG-LINE AQUACULTURE SYSTEM FROM PHYSICAL MODEL TESTS

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The objective of this presentation is to describe the hydrodynamic characteristics of dense macroalgae grown on a long-line aquaculture system from a set of physical model tests. The tests were conducted at the United States Naval Academy in the 116-m x 8-m x 5-m tank facility to obtain drag and inertial forces with a full-scale model. The full-scale model was built considering the viscous force effects described by Reynolds number with geometric and material properties based on samples of *Saccharina latissima* obtained from sites in Maine. The model was built to represent a 1-m section of a long-line with 3-m long macroalgae blades having a total weight of about 15 kg/m. The experimental plan included a series of towing, wave and planar motion mechanism tests with the model mounted in both inline and normal configurations. Measurements obtained from force-block instruments were used to resolve forces related to velocities and accelerations.

PUBLIC PERCEPTIONS OF AQUACULTURE: EVALUATING SPATIOTEMPORAL PATTERNS OF SENTIMENT AROUND THE WORLD

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Aquaculture is developing rapidly at a global scale and sustainable practices are an essential part of meeting the protein requirements of the ballooning human population. Locating aquaculture offshore is one strategy that may help address some issues related to nearshore development. However, offshore production is nascent and distinctions between the types of aquatic farming may not be fully understood by the public – important for collaboration, research, and development. Here we evaluate and report, to our knowledge, the first multinational quantification of the relative sentiments and opinions of the public around distinct forms of aquaculture.

Using thousands of newspaper headlines ($N_{\text{total}} = 1,596$) from developed (no. countries = 26) and developing (42) nations, ranging over periods of 1984 to 2015, we found an expanding positive trend of general ‘aquaculture’ coverage (Fig. 1), while ‘marine’ and ‘offshore’ appeared more negative. Overall, developing regions published proportionally more positive than negative headlines than developed countries. As case studies, government collected public comments ($N_{\text{total}} = 1,585$) from the United States of America (USA) and New Zealand mirrored the media sentiments; offshore perception being particularly negative in the USA. We also found public sentiment may be influenced by local environmental disasters not directly related to aquaculture (e.g., oil spills). Both countries voiced concern over environmental impacts, but the concerns tended to be more generalized, rather than targeted issues. Two factors that could be inhibiting informed discussion and decisions about offshore aquaculture are lack of applicable knowledge and actual local development issues. Better communication and investigation of the real versus perceived impacts of aquaculture could aid in clarifying the debate about aquaculture, and help support future sustainable growth.

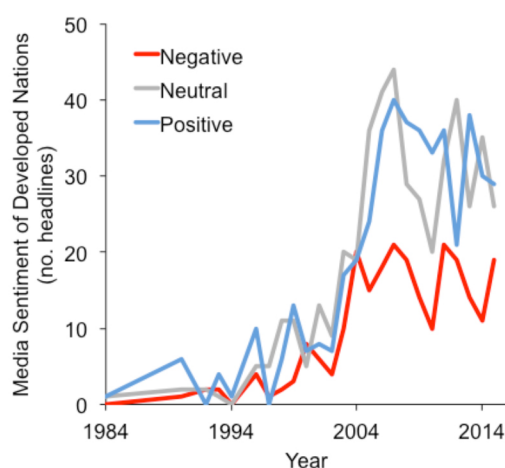


Figure 1. Newspaper ‘aquaculture’ media sentiment. Sentiment over time based on the frequency of newspaper headlines with negative (*red*), positive (*blue*), and neutral (*gray*) titles for developed nations ($n = 1,165$).

TOWARD COMMERCIAL PRODUCTION OF PIGFISH *Orthopristis chrysoptera* FOR THE LIVE BAIT INDUSTRY: EFFECTS OF STOCKING DENSITY ON POND GROW-OUT

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Red claw crayfish Pigfish are a popular marine baitfish in the southeastern United States, and the entire supply comes from harvesting natural stocks. Commercial production could increase supply, expand the season of availability, and reduce pressure on wild populations. Laboratory research has refined many aspects of captive spawning, larval rearing, and the effects of ration and temperature on growth and survival of larvae and juveniles. Here, the effects of stocking density on survival and growth were evaluated during the grow-out phase in pond-based cages.

Fingerlings were reared to a total length of 54 ± 4.3 mm (2.3 ± 0.09 g) from captive spawned adults. They were placed into 1-m³ cages constructed of 12-mm nylon mesh at densities of 100, 250, 500, 750, and 1000 fish m⁻³. Cages were attached to a dock and submerged in a 2-acre pond. Fish were fed a commercial dry diet 3 times per week. Samples of fish were measured weekly for length and wet weight and returned to the cage. Survival was tallied at 7 weeks (end of experiment). The experiment was repeated three times. Salinity, temperature, and dissolved oxygen averaged 16.7 ppt, 27.9°C, and 5.5 mg l⁻¹, respectively.

Mean survival was 88.5% with no significant effect of stocking density ($P = 0.24$), but mean survival at the highest stocking density (83.1%) was less than it was at all other densities (89.8%). Growth rate was affected by stocking density ($P < 0.01$), with slower growth at the three highest densities (Fig. 1). Mean grow-out time to market size (100 mm, 9.5 g) from a stocking size of 54 mm was 5.7 weeks at the lowest density and 8.5 weeks at the highest density.

Separately, a half-acre fertilized pond stocked with 1 million eggs produced approximately 8,000, 100-mm pigfish in 16 weeks.

Stocking density had minor effects mortality and growth rate of pigfish in ponds. Results suggest that pigfish can be cultured at high densities and reach market size quickly.

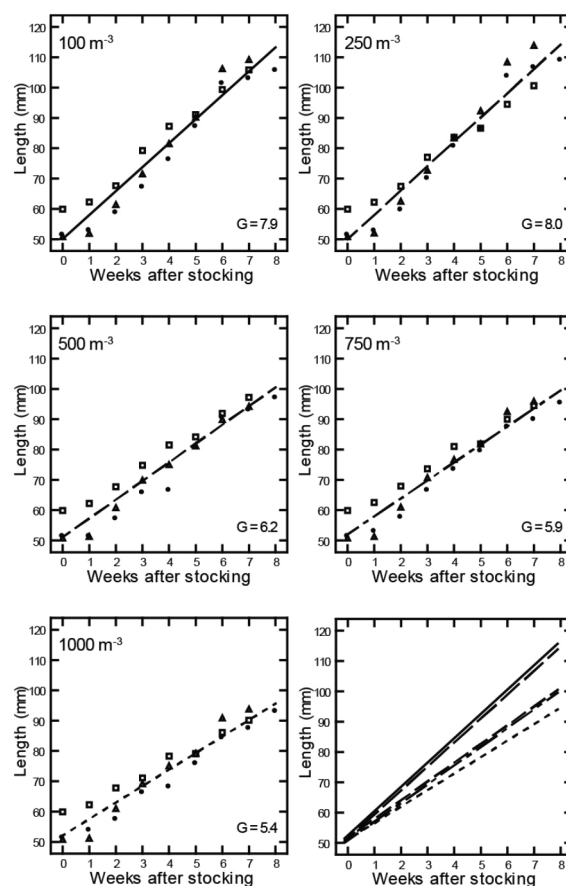


Figure 1. Growth of pigfish in cages at stocking densities of 100 to 1000 fish per m³. G is average growth rate in mm per week.

IMPACTS OF TRADE COSTS ON NORWEGIAN EXPORT OF FARMED SALMON

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Norway accounts for more than half of the global production of Atlantic salmon, and the country's export of farmed salmon amounts to 15 % of the mainland export value (oil and gas excluded). Trade costs, including tariffs, transportation costs, and non-tariff barriers, vary substantially between the wide range of destinations that the more than 80 exporters serve. While trade costs are relatively low to the nearby European Union destinations, they are in general higher to more distant markets that require transportation by plane, higher cross-border transaction costs, and in many cases higher tariffs. More knowledge about how trade costs affect export revenue is important, *e.g.*, in order to assess potential gains from future trade agreements.

Focusing on whole fresh salmon, that makes up 80% of the export value, we utilize a unique set of transaction level custom data (period 2004-2014) that is linked to export firm information. For all destinations we add specific information about trade costs, most important tariffs applied to Norway, distance and transport mode, and the usual gravity variables. In particular, we investigate: 1) characteristics of firms that serve destinations with high trade costs (*e.g.*, productivity, extensive and intensive margin); 2) the association between trade costs and exporters' market share; and 3) how trade costs affect firms' export revenues, decomposed in price and quantity effects.

THE COMPLETE OPEN OCEAN FARMING PLATFORM

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In the past year InnovaSea Systems, Inc. has made significant strides to provide the marketplace with the complete open ocean farming platform. Focusing on the major farming operations; stocking, feeding, treating, mortality removal, harvesting and data collection; the company has tested a variety of products in two separate oceanic conditions.

Until recently forced air was used to deliver feed from the barge to the pen. While air delivery is an efficient method for surface pens, it is inefficient and physically problematic when feeding fish in submerged pens. Engineers at InnovaSea have developed a single point, arterial feeding system allowing a surface barge to deliver feed to multiple submerged fish pens. Feeding is delivered in a timely manner with significantly less fines than traditional methods. To evenly distribute the pellets in the pen a helical feed disperser (Figure 1) is mounted to the central spar.

Aquaculture is an industry of scale and managing costs. InnovaSea has successfully more than doubled their pen volume recently. Rapidly going from 6400 m³ to 7200 m³ to 8000 m³ and finally 14,500 m³, the company has been able to respond to the market's demand for larger pens. All four pen sizes are being successfully farmed on commercial operations in Panama, Mexico and Hawaii.

InnovaSea has completed their initial trials on a submersible gravity pen named the Evolution Pen. Loosely based on traditional surface pens, the Evolution Pen can easily submerge, ship in standard 40 foot containers, and scale up or down in volume with minimal design effort. Designed for regions where there are aperiodic storms, the Evolution Pen will offer ease and security to farmers who do not need equipment designed for harsh open ocean conditions.

Lastly, InnovaSea has recently purchased Amirix, the producers of Real Time Aquaculture technology. Farmers can now view in real time, key data such as salinity, temperature and dissolved oxygen. Real Time Aquaculture transmits the data from the sensors to a central hub wirelessly. This data is then transmitted to either a vessel nearby or a command station on shore. Eliminating cables addresses the frequent problem of fatigue failure at the cable/sensor interface.

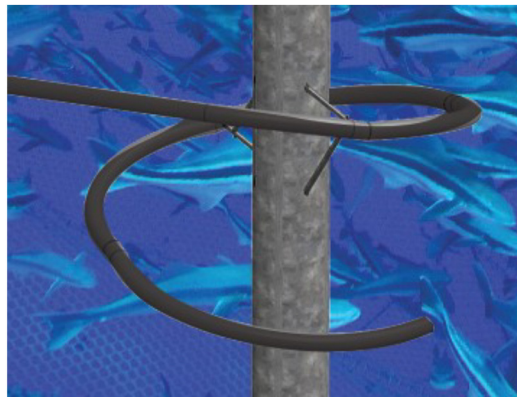


Figure 1: Helical Feed Disperser

PRODUCTION OF ANTIBODY SELECTED FOR DETECTION OF PLASMA IMMUNOGLOBULINS IN STRIPED CATFISH (*Pangasius hypophthalmus*)

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Striped catfish (*Pangasius hypophthalmus*) is an economically relevant fish species that is primarily cultured in southeast Asia, while also becoming a common foodfish around the world. Despite its importance as a major aquaculture species, there have been few studies tailored to understand the normal physiology of this fish, especially when it comes to its immunology. The scarcity of this information often makes diagnostic and management decisions difficult when it comes to addressing fundamental issues of fish welfare. In an effort to help our understanding of humoral immune responses in striped catfish, this work aimed to isolate, purify, and characterize plasma immunoglobulins (Igs). Additionally, the purified Ig from striped catfish was used to produce a polyclonal antibody specific for this catfish species.

The first part of this study consisted of the isolation of striped catfish antibodies. Four catfish (mean weight of 340 g) were kept in a recirculating aquaculture system. Individual fish were immunized on day 0 with 195 μg of the F[ab']₂ fragment of a goat IgG. On days 14 and 28, the fish received a booster with doses of 195 and 100 μg , respectively. Blood samples were collected on days 35, 42 and 49. Striped catfish Ig was separated from fish plasma by affinity chromatography. Purity of Ig was confirmed via SDS PAGE.

The second part of this project dealt with production of anti-striped catfish antibodies. To achieve this, purified striped catfish Ig were concentrated and sent to a commercial vendor that generated these antibodies in rabbits during a period of 72 days. Plasma from rabbits was received, and rabbit IgG was purified via use of protein A-affinity chromatography. Specificity of anti-striped catfish was checked via western blotting, which revealed that this antibody was selective for the heavy chain of the striped catfish Ig. Additionally, an Ouchterlony double-diffusion gel showed the lack of reactivity of this antibody towards different non-*Pangasius* fish species.

The third portion of this work entailed the use of the polyclonal antibody to determine total levels of plasma Ig in striped catfish. A sandwich ELISA was designed and optimized for use of striped catfish plasma (n=69). Data from this assay helped determine the normal values, also termed reference intervals, of Ig in healthy striped catfish to be in the range of 1-7 mg/mL, with a mean value of 3.5 mg/mL.

Findings from this study can help guiding decisions in striped catfish diagnostic and management cases. Moreover, the use of this technology can be also applicable for the purpose of measuring total Ig levels in other fish species.

IMMUNOMODULATION IN NILE TILAPIA (*Oreochromis niloticus*) FOLLOWING A DIETARY SUPPLEMENTATION OF DIFFERENT *Bacillus subtilis* STRAINS

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Tilapia is an economically important aquaculture species cultured around the world, with production only second to carp species. Despite their relative high tolerance to a broad range of stressors, compared to other fish species, major losses still occur as a result of bacterial diseases. Because of the controversy around antibiotic application, the scope has shifted to the use of more environmentally friendly agents, such as probiotics, to increase both immune responses and fish health. Although the number of probiotic studies in tilapia has increased in recent years, much work remains to be done as the mechanisms of action of many probiotic candidates remain to be deciphered in tilapia. This study assessed the effects of two probiotic candidates of *Bacillus subtilis* at the systemic and organ level functionalities of the innate immune response in Nile tilapia.

For this work, sixty non-sexed fish (161 ± 3.9 grams) were split into three groups and were maintained in one recirculating aquaculture system. On day 0, twelve fish per group were tagged intramuscularly near the dorsal fin to follow the individual fish immune responses over time. Each group was fed a diet supplemented with spores of either of two *B. subtilis* strains or a control diet for 51 days. On days 14, 28 and 51, blood was collected from caudal vessels of the fish, and lysozyme content and alternative complement activities were measured in the plasma. On day 51, spleen and anterior kidney samples were collected to measure phagocytic activity, respiratory burst, and immune gene expression of pro-inflammatory cytokines.

Throughout the trial, both plasma lysozyme concentration and plasma alternative complement of tilapia were significantly increased ($p < 0.05$) by varying degrees after dietary supplementation with either probiotic strain, relative to the non-treatment control.

On day 51, there was a trend towards increased phagocytic and respiratory burst activities observed in immune organ derived leukocytes. Consistent with this data, there were elevated levels of pro-inflammatory cytokine mRNA levels in these immune organs. Moreover, the occurrence of these results could be more tightly associated with supplementation of the probiotic strains, given that *Bacillus* bacteria were observed to populate the intestines of the treatment groups.

These results suggest the potential roles of these probiotic candidates to stimulate immune responses of Nile tilapia over time, without impairing the growth of the host.

EVALUATION OF DIFFERENT SOYBEAN SOURCES AS AN INGREDIENT IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Solven extracted soybean meal (SBM) is generated using different varieties of soybeans grown under a range of conditions and then processed at different crushing plants. Due to its competitive cost and availability, it is a popular plant base protein source for shrimp feed formulations. However, there is limited information on the effect to variations in these meals on performances of shrimp. Hence, the present study was designed to determine the effects of different soybean sources on the growth performances of *L.vannamei*. Two growth trials were conducted with iso-nitrogenous and iso-lipidic (350 g/kg protein and 80 g/kg lipid) test diets formulated with twenty-five soybean sources. Trial one incorporated 14 treatments including a soy based diets containing 517 g/kg SBM (eight replicates) and this soy source was then replace with 13 different soybean sources (four replicates). The second trial used the same basal diet and 11 soybean sources (Total 12 diets) with five replicates. Both of the growth trials were operated with the stocking density of 10 shrimps/aquarium in a semi-recirculatory system and the initial weight of shrimp for trials 1 and 2 were $0.23 \text{ g} \pm 0.02$ and $0.67 \text{ g} \pm 0.02$ respectively. During the two trials, shrimp were fed four time/day assuming a FCR of 1.8, over 42 days for trial 1 and 35 days for trial 2. As per the statistical analysis, no significant differences were found for the growth performances of shrimps at trial one except for FCR, while in trial two significant difference were observed in most parameters (Table 1). Individual and combined results of the growth trials will be presented along with data on possible correlations to biochemical composition of the meals.

TABLE 1. Growth performance of Pacific white shrimp (*Litopenaeus vannamei*) fed with twenty-five different soybean sources over growth trial one (42 days) and two (35 days).

Trial 1							Trial 2						
Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC ¹	Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	5.69	5.46	2302	1.73 ^{ab}	85	0.098	1	6.07 ^{ab}	5.40 ^{ab}	811 ^{ab}	1.86 ^{ab}	86 ^{ab}	0.092 ^{ab}
2	5.78	5.54	2283	1.70 ^{ab}	90	0.099	15	5.53 ^b	4.86 ^b	731 ^{ab}	1.93 ^{ab}	92 ^{ab}	0.087 ^{ab}
3	5.54	5.31	2269	1.73 ^{ab}	90	0.097	16	5.36 ^b	4.70 ^b	712.2 ^b	2.02 ^a	96 ^a	0.085 ^b
4	5.94	5.71	2458	1.60 ^b	88	0.101	17	5.44 ^b	4.76 ^b	697 ^b	2.04 ^a	90 ^{ab}	0.085 ^b
5	5.71	5.50	2602	1.64 ^b	85	0.101	18	5.52 ^b	4.85 ^b	717 ^b	1.97 ^{ab}	96 ^a	0.086 ^b
6	5.61	5.38	2365	1.68 ^{ab}	85	0.098	19	6.02 ^{ab}	5.36 ^{ab}	807 ^{ab}	1.81 ^{ab}	88 ^{ab}	0.092 ^{ab}
7	5.58	5.36	2466	1.69 ^{ab}	95	0.099	20	5.97 ^{ab}	5.31 ^{ab}	807 ^{ab}	1.79 ^{ab}	96 ^a	0.091 ^{ab}
8	5.06	4.84	2210	1.97 ^a	80	0.094	21	6.33 ^a	5.66 ^a	851 ^a	1.67 ^b	92 ^{ab}	0.095 ^a
9	5.28	5.05	2231	1.78 ^{ab}	83	0.095	22	5.89 ^{ab}	5.20 ^{ab}	749 ^{ab}	1.84 ^{ab}	90 ^{ab}	0.089 ^{ab}
10	5.34	5.10	2152	1.73 ^{ab}	93	0.095	23	6.08 ^{ab}	5.39 ^{ab}	791 ^{ab}	1.77 ^{ab}	92 ^{ab}	0.091 ^{ab}
11	5.62	5.39	2371	1.71 ^{ab}	80	0.099	24	5.85 ^{ab}	5.17 ^{ab}	764 ^{ab}	1.84 ^{ab}	92 ^{ab}	0.089 ^{ab}
12	5.18	4.96	2259	1.75 ^{ab}	97	0.095	25	5.55 ^{ab}	4.86 ^b	707 ^b	1.99 ^a	80 ^b	0.086 ^b
13	5.42	5.19	2290	1.70 ^{ab}	90	0.097							
14	5.23	4.99	2165	1.80 ^{ab}	85	0.095							
PSE ²	0.39	0.38	217.65	0.13	7.94	0.003		0.37	0.37	60.08	0.14	7.19	0.004
P-value	0.07	0.07	0.23	0.06	0.07	0.067		0.001	0.001	0.001	0.002	0.041	0.001

TGC¹: Thermal Growth Coefficient, PSE²: Pooled standard error; One way ANOVA was used for statistical analysis while values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons

USING GENOMIC INFORMATION TO OPTIMIZE GENETIC IMPROVEMENT IN CHANNEL CATFISH

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Catfish production is the largest aquaculture segment in the US and recent research has been conducted to improve production efficiency. Since 2006 selection has been based on traditional BLUP evaluations. With the availability of genomic information for catfish in early 2017, the first objective of this study was to investigate the feasibility of using genomic selection in US catfish. The second objective was to identify major SNP associated with harvest weight and residual carcass weight.

Phenotypes were available for harvest weight (n=27,160) and residual carcass weight (n=6020), and the number of fish in the pedigree was 36,365. After quality control, genotypes on 54,837 SNPs were available for 2911 animals. Genomic and pedigree predictions were calculated in a 5-fold cross validation approach, using single-trait models. Single-step genomic BLUP (ssGBLUP) was the method of choice for genomic predictions. Ability to predict breeding values was calculated as the correlation between adjusted phenotypes based on complete data and EBV or genomic EBV (GEBV) for each one of the folds. Inflation was assessed as the regression coefficient (b1) of adjusted phenotype on (G)EBV. For the association study, the GEBV were back solved to SNP effects and the percentage of variance explained by each SNP was calculated as SNP effect squared.

Predictive ability for both traits increased 8 points when genomic information was used, compared to traditional evaluations (Table 1). Genomic information also helped to reduce inflation (Table 1), especially for residual carcass weight (b1=0.97). The proportion of variance explained by windows of 20 SNP was at maximum 2.2% for harvest weight and 3.3% for residual carcass weight. Both traits appear to be polygenic with no major SNP. Using genomic information is beneficial in catfish selection because of higher predictive abilities and it also allows estimation of the Mendelian sampling, helping to identify individuals within families, which is not possible with only pedigree information.

Table 1. Average predictive ability and inflation for harvest weight and residual carcass weight.

Trait	BLUP		ssGBLUP	
	Predictive ability	Inflation	Predictive ability	Inflation
Harvest weight	¹ 0.29 ^{0.02}	0.88 ^{0.06}	0.37 ^{0.01}	0.92 ^{0.05}
Residual carcass weight	0.25 ^{0.03}	0.81 ^{0.10}	0.33 ^{0.03}	0.97 ^{0.10}

¹ Average and standard error

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF MUCUS EPIDERMAL NILE TILAPIA *Oreochromis niloticus* AGAINST BACTERIA OF THE GENUS *Vibrio* AFFECTING WHITE SHRIMP *Litopenaeus vannamei*

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The outbreaks of diseases in shrimp farming, has generated economic great losses, mainly caused by pathogenic organism in the countries Latin American. The alternative for counter this problem in the shrimp farming is the system of polyculture, this system generates at shrimp some benefic by the presence of fishes in the ponds with the shrimp. On the other hand, the presence of small molecules related to the immune response has been confirmed, in the epidermal mucus of the some fish important in aquaculture. This studio try to confirm antimicrobial activity of the extracts obtained from the two tilapias varieties of Tilapias *Oreochromis niloticus* and *Oreochromis sp* against pathogenic bacteria of white shrimp *Litopenaeus vannamei*. The Tilapias *Oreochromis niloticus* was proportionated from fry production laboratory, while that the other variety was captured in mats (Adapted to the marine environment) connecting to Navachiste Bay, Sinaloa, México.

The epidermal mucus was collected to perform the acidic, organic and aqueous extractions. We assessed the antimicrobial activity of each of the extracts against *Vibrio parahaemolyticus* strain E9-2 and ATCC 17802, and *V. harveyi* CAIM 1792 using the turbidimetric method. The percentage of inhibition was assessed with the values obtained. A total of eight extracts (table 1) of the two tilapias varieties were obtained. Each one of the extracts was adjusted to the concentration of 1 mg mL⁻¹ of dry weight in distilled water. The antibacterial activity of each one of the extracts was different for each of the strains tested. With the tests carried out in the present study, the presence of antimicrobial activity of the different extracts of the epidermal mucus of the two tilapia varieties was confirmed. So it can be concluded that in each of the different extracts are present antimicrobial agents with activity that could be bacteriostatic, bactericide or bacteriolytic.

Table 1: Extracts of the two varieties of Tilapia *Oreochromis niloticus* and *Oreochromis sp*. percentage of inhibition against the strain *Vibrio parahaemolyticus* E9-2, *V. parahaemolyticus* ATCC17802 y *V. harveyi* CAIM1792.

Keys	Extract	Concentration of extracts (mg/mL)	Volume of extracts	<i>Vp</i> E9-2	<i>Vp</i> ATCC 17802	<i>Vh</i> CAIM 1792
				% Inhibition 6 hours	% Inhibition 9 hours	% Inhibition 9 hours
ATAE	Adapted Tilapia Acid Extract	1	10 µL	58.6	61.5	61.8
APATOE	Aqueous Phase Adapted Tilapia Organic Extract	1	10 µL	-	0.1	-
OPATOE	Organic Phase Adapted Tilapia Organic Extract	1	10 µL	28.7	-	100.0
ATAQE	Adapted Tilapia Aqueous Extract	1	10 µL	4.0	5.2	-
FWTAE	Fresh Water Tilapia Acid Extract	1	10 µL	55.8	69.4	69.4
APFWTOE	Aqueous Phase Fresh Water Tilapia Organic Extract	1	10 µL	-	0.6	-
OPFWTOE	Organic Phase Fresh Water Tilapia Organic Extract	1	10 µL	100.0	-	100.0
FWTAQE	Fresh Water Tilapia Aqueous Extract	1	10 µL	-	2.0	-

TO OR NOT TO POOL?: GUIDELINES FOR POOLING DECISIONS IN AQUATIC ANIMAL HEALTH SURVEILLANCE

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For surveillance purposes (presence or absence of pathogens), testing of pooled specimens from aquatic animals is preferable to testing individuals when the prevalence of infected samples is low and concentration of the target analyte is high. However, use of pooling raises important questions: *for which combinations of prevalence and load can pooling be justified; should pooling recommendations differ for systemic vs. localized infections; are there published data to support recommendations for pooling of 5 specimens?*

A systematic review of published studies in aquatic animals was undertaken using standard search terms in PubMed and CAB Abstracts. OIE-listed and non-listed diseases were included. Abstracts were screened and relevant information was extracted and summarized from the full papers. An expert group was convened to review the collated evidence and develop guidelines for designing pooling studies.

Fourteen relevant papers were identified in the search, many involving qPCR. Most authors concluded that pooling reduced sensitivity compared with testing of individuals. Supporting data for pooling was limited. Only two papers specifically mentioned the concept of pooled sensitivity and pooled specificity. These two outbreak studies provided useful comparative data (virus isolation and qPCR for SAV and ISAV), but no papers reported Ct values.

Pooling is logistically necessary for testing early life stages but is optional in other situations. Scientific evidence about pooling that can withstand legal scrutiny is needed, especially for OIE-listed diseases. The preferred design is field studies with parallel testing of both individual specimens and these same specimens in randomly-created pools that mimic real-life surveillance scenarios supported with the respective cost and sensitivity data. Individual and pooled Ct values should be reported even if the final decision is presence/absence. Analyte loads are likely to be much lower several months after outbreaks and much more reflective of surveillance scenarios. Use of experimentally-infected animals and spiking experiments were considered inferior sources of data to evaluate effects of pooling. Studies are also needed to describe the distribution of Ct values in naturally-infected but healthy aquatic animals, as this information can provide a sound basis for simulation studies of potential effects of pooling and inform laboratory experiments.

EXPERIMENTAL INDUCTION OF *Aeromonas hydrophila* IN TILAPIA FINGERLINGS ON A NOVEL ANTIBODY DIET

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Aquaculture is the fastest growing animal-production sector for food in the world. Health and safety issues are prevalent among aquatic animals during massive growth and production. Disease outbreaks within aquaculture facilities can cause economic losses in the billions. Antibiotics are currently in use in aquaculture as a therapeutic for treating disease. However, over time the use of antibiotics has brought up a new set of issues; antibiotic resistant bacteria/genes and transfer of these to the environment and to humans via consumption. For this reason, efforts are underway to replace antibiotics, while maintaining a safe and high quality environment for the animals.

The up-regulation of interleukin-10 (IL-10), an anti-inflammatory cytokine, has been determined by recent studies as the reason for lack of immune detection by gastrointestinal pathogen invasion. This study focuses on the use of anti-IL-10 egg yolk antibody in tilapia fingerling diet as a neutralization of IL-10, when challenged with pathogen, *Aeromonas hydrophila*. To understand the effectiveness of the anti-IL-10 diet in tilapia fingerlings, the diet was administered for at least 3 days prior to an experimental induction of *A. hydrophila* by both immersion and oral gavage. Method development for experimental-induction is still underway. Survival rates for these preliminary studies were monitored for at least 24h post-induction.

Duplicate trials of immersion were completed using tilapia fingerlings exposed to $1-2 \times 10^6$ CFU/ml of *A. hydrophila* in trial 1 and $7-9 \times 10^7$ CFU/ml in trial 2. Results showed 100% survival throughout all treatments in trial 1. In trial 2, the control diet challenged with *A. hydrophila* treatment resulted in 73.34% 18.85 survival, while the treatment fed the anti-IL-10 diet and challenged with *A. hydrophila* resulted in 50%14.14 survival.

Duplicate trials of induction by oral gavage were also studied to compare the effects of different routes of exposure. The volume gavaged was dependent on fish weight; 0.02ml of *A. hydrophila* (grown in in Tryptic Soy Broth) per gram of fish. In the first oral gavage trial, fingerlings were dosed $1-2.5 \times 10^6$ CFU/g fish of *A. hydrophila*. The survival rates were 96.65%4.74 and 86.65% 18.88 for control diet and anti-IL-10 diet, respectively. The second trial was done by gavaging *A. hydrophila* over various time points on the bacterial growth curve; 2, 4, and 5.5 hours of growth. Concentrations gavaged included 2.78×10^6 CFU/g fish (2h growth), 1.68×10^7 CFU/g fish (4h growth), and 3.72×10^7 CFU/g fish (5.5h growth). The survivability of fish on the 2h culture was 87.5% (control diet) and 75% (Anti-IL-10 diet), on the 4h culture, 87.5% (control diet) and 75% (anti-IL-10 diet), and on the 5.5h culture, 75% (control diet) and 100% (anti-IL-10 diet). Results are preliminary, as method development of these studies are progressing.

WHAT IS HAPPENING IN LATIN AMERICA?

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What is happening with our organisation and Latin America?

Our program started in 2013 with our first Aquaculture Learning Centre and lessons have been learned and we are building our new strategy which is based on creating a Coastal and Estuary Ecosystem

This includes important food security activities based on bi-valve aquaculture.

We have now started a project sponsored by the Australian Government which aims to create a long term sustainable Oyster industry in Tamaulipas. The plan involves bringing two suitable Mexicans (two educational experts assigned by Universidad Tecnológica del Mar de Tamaulipas - UTMart) to Australia for specific training and then having one Australian expert visit Mexico. From the local knowledge and the imported expertise, the project will culminate with workshops and other essential meetings adding to a strategic plan which is a blueprint for similar activities in other seafood

DISPENSABLE AMINO ACIDS OF IMPORTANCE IN FISH NUTRITION

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In recent years it has become increasingly apparent that some amino acids previously classified as “dispensable” or “non-essential” can have significant impacts on fish when supplemented in diets. Included in this group are glutamine, glycine, hydroxyproline and proline. Other amino compounds or derivatives of amino acids such as taurine and creatine, which are abundant in fishmeal, also have gained increased attention as efforts continue to identify feedstuffs to potentially replace some portion of fishmeal in the diets of aquatic species to increase the sustainability and cost-effectiveness of aquaculture. However, many alternative protein feedstuffs are deficient in one or more indispensable as well as dispensable amino acids. Therefore, this laboratory intensified its efforts in recent years to evaluate dietary requirements for various amino compounds with red drum (*Sciaenops ocellatus*), hybrid striped bass (*Morone chrysops* x *M. saxatilis*) and channel catfish (*Ictalurus punctatus*).

The presentation will provide detailed information about the synthesis of the amino acids traditionally classified as dispensable and how dietary supplementation may improve various metabolic responses. In particular, the presentation will review studies in which dietary glutamine supplementation was evaluated in terms of gastrointestinal health and immunological responses of the species noted above. Supplementation of glutamine at 1 to 2% of diet had beneficial effects on intestinal health and various immunological responses including enhanced vaccination efficiency. Additional studies were conducted with hydroxyproline and red drum because that amino acid is a vital component of collagen, and growth of various species has benefited with its supplementation. However, in our studies with red drum, we were unable to confirm beneficial effects of crystalline hydroxyproline supplementation in regards to growth of fish fed diets high in plant-proteins. Changes in hydroxyproline content were found in plasma and tissues at higher inclusion levels, which could have some effect on the texture of fish flesh. Several investigations in this laboratory have established the dietary essentiality of taurine to red drum as well as its potential sparing effect on methionine in other fish species. The positive effects of dietary creatine supplementation on weight gain, feed efficiency, and reduced circulating homocysteine levels of red drum also has been observed in multiple trials. Supplemental creatine also was demonstrated to have osmoregulatory benefits in hybrid striped bass when cultured in brackish water. However, beneficial effects of dietary creatine supplementation on channel catfish and Southern flounder (*Paralichthys lethostigma*) have been more limited. Results from these various studies emphasize the potential importance of a number of dispensable amino acids and other nitrogenous compounds in the nutrition and metabolism of various fish species.

THE PARADIGM SHIFT IN SALMON PRODUCTION: CHALLENGES AND POTENTIAL OF LARGE INDUSTRIAL LAND-BASED PLANTS FOR POST SMOLTS AND MARKET SIZE FISH

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The Aquaculture industry is becoming a driving force for high yield production and sustainable development of the food industry, with a strong focus being made on compact and resources saving solutions, disease free plants and recovery of energy, nutrients and water.

In the last 7 years the salmon production from aquaculture has turned into a global industry, with salmon seen as a commodity product such as chicken and beef. Prognoses for Norway alone shows an increase in salmon production of more than 8% per year to reach up to 3 Million tons produced in 2030. In the world's salmon industry the smolt-concept is in rapid change, with a global trend to produce larger smolts, e.g. from 250 grams up to 1 kg prior to transfer to traditional cages at sea. Such a large fish proves to be more robust than smaller smolts, show reduced mortality, shorter production time in open cages in the sea, and thus fewer problems with salmon lice for example.

Over the last few years, Several projects to produce market size salmon on land, based on RAS technology, has pointed out loudly the long list of risks and challenges of a still “in its infancy” production concept.

The industry trends to go for larger plants (mega post smolts or market size fish plants aiming for 5000 MT yearly capacity or more), larger fish tanks (20 meter diameter and more), larger RAS units (dealing with re-circulated flow rates over 10 000m³/h), together with lower water and energy consumption.

Design of such facilities needs to focus on fish biosecurity, welfare and health, together with optimal layout design, for all fish movements, operators, and ancillary equipment. The initial concept design and business model needs to be supported by a realistic and flexible bioplan.

Challenges, concerns, but also key drivers and possible solutions will be presented and discussed, illustrated from some recent references in Norway, from among the world largest post smolt factories, and proposal on how should be designed compact industrial salmon Mega factories, capable to produce 5 000 MT from egg to 5 kg fish.

BENEFITS VS. COSTS OF ANTIBIOTIC MEDICATED FEED USE VS. WITHHOLDING FEED DURING BACTERIAL OUTBREAKS IN POND-REARED WARMWATER FISH

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Bacterial disease outbreaks in pond-reared fish are frequently experienced during growing season when fish are being fed to achieve maximal growth rate by fall. When there is detection of disease associated with a bacteria susceptible to antibiotics, frequently farmers will treat with medicated feed. This presentation will assess the benefits vs. costs of treating with medicated feed, withholding feed, and unmedicated feed for control of mortality associated with bacteria in pond-reared fish.

Early signs of bacterial disease in fish can range from anorexia to morbidity such as erratic swimming to acute mortality. When the fish are diagnosed, the farmer must decide on treatment options. Should he use medicated feed containing an antibiotic that the bacteria are susceptible to? Should he take the fish off feed to break the fecal/oral cycle and decrease the continual transmission of the bacteria in the pond? Or should he feed unmedicated feed to the appetent fish so that he will have fewer, but larger fingerlings by fall? The advantages and disadvantages of these strategies will be explored in this presentation. A partial budget analysis will be performed to explore the economic effects of specific medicated and therapeutic treatments.

THE EFFECT OF SALINITY ON THE TOTAL ACCUMULATION OF COPPER AND ZINC IN THE MACROALGAE *Ulva fasciata*

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The relationship between macroalgae and metal pollution is critical in the growing industry of aquaculture. Metal pollution can inhibit cultivation and spoil product. Phytoremediation with macroalgae has been explored as a method of metal removal in aquatic systems. Salinity is recognized as an important factor in metal accumulation. However, the impact of salinity on total metal accumulation in macroalgae remains unclear. The impact is further complicated by metals, such as copper and zinc, that function as micronutrients in fine doses, but poison the algae under excessive concentrations. The present study was undertaken to determine the degree to which salinity affects the total copper and zinc accumulation in macroalgae.

To determine the relationship between macroalgal copper and zinc accumulation at different salinity levels, a model organism, *Ulva fasciata*, was cultured at three different salinity levels (15, 25, and 35 parts per thousand) and four metal combinations (50/100, 50/250, 100/100, and 100/250 as parts-per-billion copper/parts-per-billion zinc). Triplicates of each salinity-metal combination - for a total of 36 test units - were cultured indoors for three weeks. Toxic effects were assessed as a function of growth rate at the end of the testing period.

Results and a discussion will be provided in a future update.

GENETIC (CO)VARIANCES IN AHPND RESISTANCE, HARVEST WEIGHT AND SURVIVAL IN *Penaeus vannamei*

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SyAqua Siam Co.

Diseases are a major constraint on the intensive production of shrimps. Conditions in production ponds favor disease development, and epidemics of several previously unreported diseases have occurred and caused severe losses. When elimination, eradication or cultural control is difficult, selective breeding for host resistance to the pathogen may be an attractive option for disease control.

Commercial growers now seed ponds with shrimp populations selected for resistance to Taura Syndrome Virus with excellent results, whilst up to now development of White Spot Syndrome Virus resistant populations has been an elusive goal when selection is made on family means.

Despite, AHPND has been one of the major threats for the shrimp industry, there is no information on the possibility of applying selecting breeding as a measure to control the epidemic nor the effect over other important economic traits like growth and pond survival.

In this paper we present the first results of the genetic (co)variation in resistance to AHPND, harvest weight (HW) and pond survival (S).

To assess the genetic resistance to AHPND 95 families from SyAqua's breeding program were challenged by two different methodologies: a) direct infection with the bacteria and (Bac) and b) using an isolate of the AHPND toxin (Tox).

Results indicate substantial additive genetic variation for all traits with a favorable and moderate genetic correlation between the two AHPND infection methods and between Harvest weight and Pond Survival. (Table 1). Genetic correlations between AHPND resistance and harvest weight and pond survival were not significant different from zero.

Based on these findings we conclude that it is possible to select for AHPND resistance with no effect over other important economic traits and that the toxin might be used as a proxy of bacterial infection.

Table 1. heritability estimates ($h^2_{\pm SE}$) for AHPND resistance induced with Bacteria (Bc) and with Toxin (tox), Harvest weight (HW) and Pond Survival (S) on the diagonal. Genetic correlation between the same traits below the diagonal

individual Trait	AHPND Tox	AHPND Bac	HW	sS
AHPND Tox	0.37±0.17		PS	PS
AHPND Bac	0.58±0.28	0.32±0.18		
HW	0.03±0.22	0.06±0.1	0.58±0.07	
PS	-0.03±0.15	0.09±0.09	0.44±0.25	0.36±0.16

MOLECULAR, TRANSCRIPTIONAL AND FUNCTIONAL INSIGHTS OF ROCKFISH 1-CYS PEROXIREDOXIN

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Peroxiredoxins are antioxidative enzymes that catalyze the reduction of alkyl hydroperoxides to alcohols and hydrogen peroxide to water. To characterize the peroxiredoxin 6 biochemical functions, 1-Cys Prx gene was cloned from *Sebastes schlegelii* (SsPrx6) and carried out the hydrogen peroxide reduction, DNA protection and oxidoreductase activities. Putative open reading frame encoded 221 amino acids with 25 kDa polypeptide and a *pI* of 5.6. A thioredoxin-2 domain and a Prx-specific signature motif were identified. Catalytic triad amino acids, N-linked glycosylation site and peroxidatic cysteine was identified. It shared the highest identity (93.2%) and similarity (98.2%) with the *Cynoglossus semilaevis* Prx6. Multiple sequence alignment revealed the conservation of functionally active peroxidatic cysteines and Prx-specific signature among the other Prx6 counterparts suggesting the common peroxidase activity. Viable cell number was increased by extracellular hydrogen peroxide scavenging activity of recombinant SsPrx6. Moreover, it could reduce the intracellular ROS level in THP-1 cells and zebrafish larvae via extracellular hydrogen peroxide scavenging activity of rSsPrx6. Metal catalyzed oxidative (MCO) stress, cleaved the pUC19 DNA from supercoiled state into nicked state where, rSsPrx6 protein could protect the pUC19 DNA cleavage by MCO system in a concentration dependent manner. The oxidoreductase activity of rSsPrx6 catalyzed the insulin reduction activity with the presence of 1,4-Dithiothreitol (DTT) in a time dependent manner. The *SsPrx6* transcripts were ubiquitously expressed in all examined tissues with highest expression in ovary followed by testis. Bacterial (*Streptococcus iniae* and LPS) and viral (poly I:C) immune stimulated kidney and liver tissues showed significant *SsPrx6* mRNA expression after the post infection. Collectively, SsPrx6 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 SsPrx6 is an active member of rockfish antioxidant and innate immune defense systems.

A 2X2 FACTORIAL STUDY ASSESSING PHOTOPERIOD AND FEEDING RATE ON THE OVERALL QUALITY OF ATLANTIC SALMON *Salmo salar* POST-SMOLTS RAISED IN LAND-BASED FRESHWATER SYSTEMS

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Producing larger Atlantic salmon *Salmo salar* smolts (up to 1 kg in size) in freshwater systems is a novel approach for land-based salmon production. In this scenario, there is a need to establish optimum environmental conditions to maximize smolt quality, through optimum induction of smoltification, reduction in early maturation, and minimization of losses to disease upon transfer to sea cages and thereafter. Photoperiod has been shown to have a strong influence on both smoltification and the initiation of sexual maturation; however, determining the optimum photoperiod regime, in terms of osmoregulatory and reproductive physiological outcomes, during pre-growout freshwater rearing, has not been adequately assessed. Feed restriction during pre-growout has also been shown to reduce early maturation in post-smolts. We investigated two photoperiod regimes: i) LD24:0 (i.e. 24 hours light, zero hours dark), and ii) LDN (simulated natural photoperiod, Bergen latitude) applied to Atlantic salmon post-smolts raised in freshwater systems at 13°C up to 1,000g in mean weight. All fish were reared on LD24:0 from first feeding up to 40g, at which point the fish received a 6-week LD12:12 artificial winter while simultaneously being split into full ration (FR) and reduced (60%) ration (RR) groups. Following the 6-week winter, fish were exposed to 8 weeks of LD24:0 to induce smoltification, after which both the FR and RR groups were further split into either LD24:0 or LDN groups and raised under these conditions with a target final weight of 1,000g in the best performing group. Gill samples were collected at the beginning of the 6-week artificial winter, for gene expression (NKA α 1a and 1b, NKCC, DIO2a) and ATPase activity; gill samples for the same testing were further collected at i) the return to LD24:0, ii) midway through the LD24:0 smoltification window, iii) just prior to splitting the fish into photoperiod treatments, and iv) at 300g. Brain and pituitary were sampled during these latter sampling points, in order to assess brain DIO2b mRNA activity and pituitary gene expression, as well as head kidney to assess immunocompetence through microarray transcriptome analysis of annotated immune response pathways. Plasma was also collected during the three sampling points in the LD24:0 smoltification window, to assess chloride/osmolality, 11-ketotestosterone, cortisol, and thyroxin. Additionally, fish performance (length, weight) was assessed at regular monthly intervals, as well as condition factor, fin condition, and cataracts. At the time of abstract submission, the study is ongoing; the best performing fish are approximately 500g, and many tissue analyses data are still forthcoming. Presently, both photoperiod and feeding regime are independently statistically associated with growth performance, with best performing fish in the FR groups and poor condition factor exhibited in the RR groups. Full results will be presented at Aquaculture America 2018.

ANALYZING THE EFFECT OF PRODUCTION VARIABLES ON THE GROWTH VARIABILITY OF CHANNEL-BLUE HYBRID CATFISH

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The growth variability of channel catfish, *Ictalurus punctatus*, female x blue catfish, *I. furcatus*, male hybrids was studied in both commercial and research settings. Various production variables were, therefore, examined surveyed from traditional ponds of single/multiple batch, split ponds, and in-pond raceway system. Stepwise regression, partial F test, ANOVA (analysis of variance) and residual analysis (i.e., residual vs fitted, Durbin Watson test) were, hereby, performed for data analysis. Preliminary results suggested that the coefficient of variation (CV) (%) was in the expected range of 37-48%, which was quite closer as compared to fingerling stage (23-56%). Mean percentage of undersized (<0.45 kg) and oversized fish (>1.82 kg) were also found in minimal stage, which were at the rate of 9% and 5%, respectively. These were evidenced from a typical population distribution data (N= >54,500), which formed almost a bell shape curve with showing a positive and moderate skewness (value >1) (Fig 1). Preliminary results also showed that aeration, individual weight of stocked fingerling, grading, FCR (feed conversion ratio), feeding and culture period, survival rate, sock size, and harvesting method (partial/complete) could have the probable impact on the growth variability of hybrid catfish production.

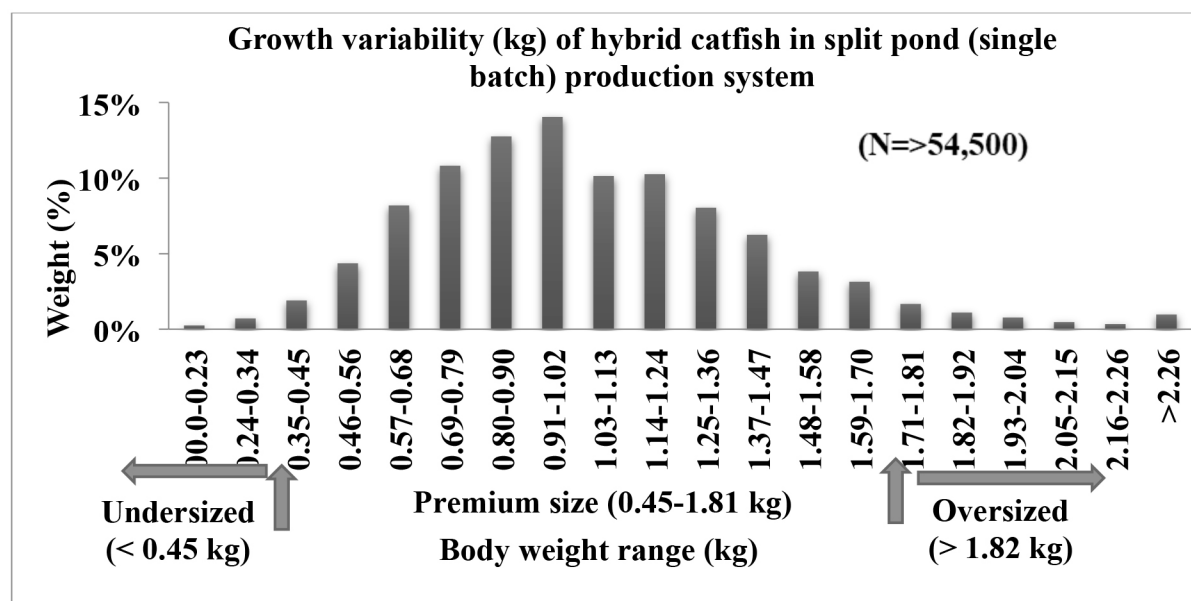


Fig 1: A typical example of growth variation in split pond production system (single batch) in hybrid catfish production (N=>54,500)

ECONOMIC IMPACT OF CHANNEL-BLUE HYBRID CATFISH GROWTH VARIABILITY ON PRODUCTION

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Hybrid catfish (channel catfish, *Ictalurus punctatus*, ♀ x blue catfish, *I. furcatus*, ♂) farming is a prime example of yield intensification, but has witnessed, to some minor extent, growth variability problem. Analyzing the economic impact of such problem is critical in order to understand fish producers' profitability as the fish processors' demand certain premium size fish. A comprehensive industry-wide fish sampling as well as survey were, therefore conducted in Mississippi, Arkansas, and Alabama from 2015 to 2017. In total, 164 ponds were randomly sampled, which included single batch (N=25), multiple batch (N=16), split pond (N= 98) and in-pond raceway system (IPRS, N=25). Out of these IPRS, four raceways were selected from research settings while the rest were selected from research conducted at commercial settings. Two comparative economic scenarios (A, B) were developed using a standard enterprise budget. Preliminary results suggest that the breakeven price above variable cost is in the range of \$1.54/kg to \$2.42/kg if the fish processor pays \$2.62/kg for premium sized food fish (0.45-1.81 kg), but pays 50% less (\$1.31/kg) for undersized (<0.44kg) and oversized fish (>1.82 kg), Scenario A. In Scenario B, the breakeven price above variable cost ranged from 1.83/kg to \$2.95 if the processor pays \$2.62/kg only for premium size food fish, but pays zero (\$0.00/kg) for undersized and oversized fish (Fig 1). Moreover, income above variable cost is positive and significant for scenario 'A' but is marginal or negative for Scenario B. The income above variable and total costs are inversely dependent on coefficient of variation (CV) for growth. Earlier preliminary analysis on growth variability of hybrid catfish showed that CV was in the expected range of 12-56%. Split pond system appears to be the most profitable enterprise as compared to single, multiple batch and IPRS.

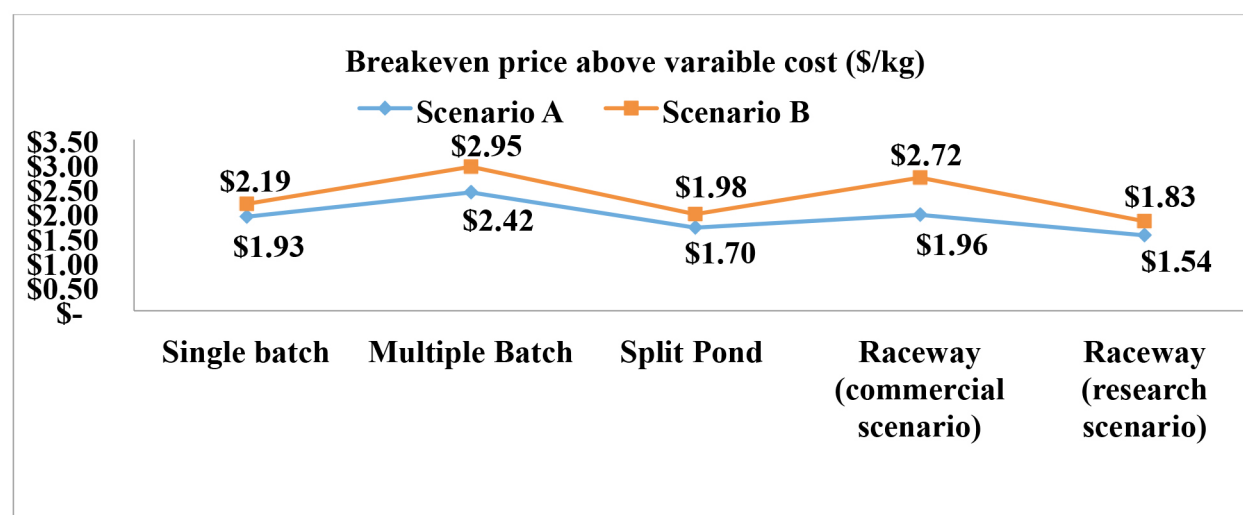


Fig 1: Breakeven price above variable cost (\$/kg) analyzed for traditional ponds of single batch, multiple batch, split-pond and in-pond raceway system (IPRS).

INVESTIGATING POTENTIAL MECHANISMS OF CLOVE OIL (EUGENOL) IN MODEL CRUSTACEANS

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Eugenol is used as topical anesthetic in mammals to remedy pain and to anesthetize fish for short periods. Eugenol was examined for its potential use on crustaceans. The exact mechanisms in the effects are still not fully understood. The effects of eugenol on neuronal activity in sensory and motor neurons in the Red Swamp crayfish (*Procambarus clarkii*), Blue crab (*Callinectes sapidus*) and Whiteleg shrimp (*Litopenaeus vannamei*) with electrophysiological recordings was examined. The neurogenic heart rate in the 3 species was also monitored along with behaviors and responsiveness to sensory stimuli while exposed to eugenol. The activity of the primary proprioceptive neurons was reduced at 200ppm and ceased at 400ppm for both crayfish (i.e., muscle receptor organ) and crab (i.e., leg PD organ) preparations when saline containing eugenol was directly applied to exposed sensory organs. Flushing out eugenol resulted in recovery in the preparations within 5 to 10 minutes. Administering eugenol to crayfish and crabs both systemically by injection and through exposure in their aquatic environment resulted in the animals becoming lethargic. Direct injection into the hemolymph was quicker to decrease reflexes and sensory perception but heart rate was still maintained. Eugenol at a circulating level of 400ppm decreased electromyogram activity in the claw muscle of crabs. Surprisingly, this study found no change in heart rate despite administering eugenol into the hemolymph to reach 400ppm in crabs or crayfish; however, heart rate decreased in shrimp. Excessive use of clove oil can result in overdose and cellular toxicity in humans. Overuse of topical eugenol or ingestion may silence neurons in mammals by the same mechanisms as in our model crustacean animals. The next focus is to determine the mechanism of action by intracellular recordings from neurons to support scant evidence of blocking voltage gated-sodium channels and thus decrease neuronal excitability.

PARTIAL AND TOTAL REPLACEMENT OF FISH OIL WITH FATTY ACID ETHYL ESTERS IN THE STARTER DIETS OF RAINBOW TROUT *Oncorhynchus mykiss*

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Fish oil is a necessary dietary ingredient for meeting the omega-3 polyunsaturated fatty acid (PUFA) requirements in most fish. The rising price of fish oil, as well as the limited harvest of wild fish to meet aquafeed demands, have led to an interest in finding suitable replacements for this PUFA source. Fatty acid ethyl esters (FAEE) are formed as a byproduct of PUFA refinement, and are a potential dietary alternative to fish oil. This investigation examined the culture performance and lipid composition of rainbow trout fry fed experimental starter diets with graded levels of FAEE and fish oil.

Rainbow trout fry (144 ± 20 mg) were randomly distributed to eighteen 20 L flow-through tanks with an initial stocking density of 60 fish/tank. Fish were fed six experimental diets for 62 days on a restricted feeding regime (4-5.5% biomass/day). Fish were weighed and sampled at 31 and 62 days of feeding, after which density was reduced to 40 fish/tank, then 15 fish/tank, respectively. Six experimental diets were formulated to be isonitrogenous with 20% lipids. One control diet was formulated using full-fat menhaden meal and menhaden oil (M. Control), while the other diets used defatted menhaden meal and Alaskan pollock oil and/or FAEE (Organic Technologies, Coshocton, OH). These five diets had 0% (P. Control), 25%, 50%, 75%, and 100% replacement of Alaskan pollock oil with FAEE, and were formulated with analogous fatty acid profiles.

The total growth and feed conversion ratio of fish were significantly different ($\alpha=0.05$) between diet groups in the first 31 days of feeding (phase I; $p<0.0001$), but were not significant from 32 to 62 days of feeding (phase II; Figure). Trends in the data suggest that rainbow trout fry have less efficient growth on diets containing high FAEE concentrations during first-feeding, but that these trends are diminished as fish grew larger (phase II). Additionally, this investigation suggests that dietary FAEE do not affect fish health and survival. Further studies are needed to confirm that any initial growth limitations can be overcome as juveniles/adults.

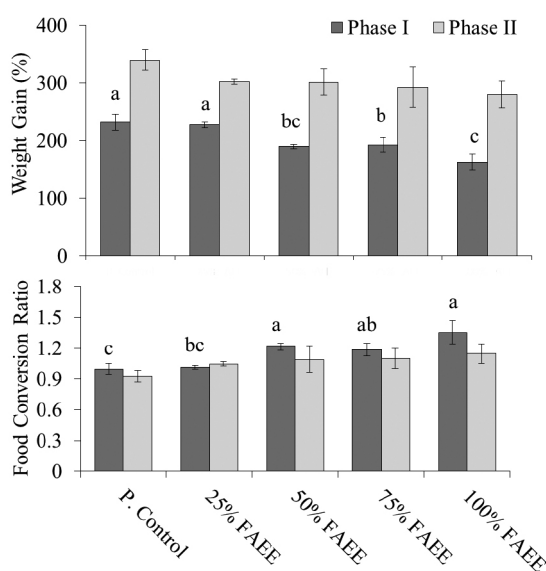


Figure. Weight gain and food conversion ratio of rainbow trout alevins from 1-31 days of feeding (phase I) and 32-62 days of feeding (phase II) on experimental diets. Letters correspond to significant differences at $\alpha=0.05$.

COMPARING THE ENRICHMENT OF LIVE FEEDS WITH α -TOCOPHEROL OR A MIXED TOCOPHEROL PRODUCT AND THEIR EFFECTS ON THE PERFORMANCE OF YELLOW PERCH *Perca flavescens* LARVAE

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Yellow perch is an important commercial and recreational finfish species in the Great Lakes region with considerable potential for aquaculture expansion. The optimization of feeding and nutrition in early life stages is vital for the commercial success of yellow perch production. Live food enrichment with polyunsaturated fatty acids (PUFA) is a commonly used technique in larval finfish culture, and elevated dietary PUFA often necessitates antioxidant supplementation. Concentrated α -tocopherol (α -T) is the most common form of vitamin E used as a dietary antioxidant, however mixtures of all tocopherol forms (α , β , γ , and δ) require less processing and are more sustainably sourced. This investigation examined the culture performance of yellow perch larvae during the first ten days of feeding with rotifers (*Brachionus plicatilis*) and *Artemia* nauplii enriched with PUFA and α -T or mixed tocopherol (mix-T) antioxidant supplementation.

Larvae were reared in a specialized recirculating system with nine 50 L conical tanks. Water was kept at $21.6 \pm 0.4^\circ\text{C}$. Sea salt and microalgae concentrate were continuously added to maintain a salinity of 2.8 ± 0.5 ‰ and a turbidity of 8.2 ± 1.2 NTU. Enrichment emulsions contained 890 mg ω -3 PUFA and no tocopherol (Control PUFA), 235 mg \cdot g $^{-1}$ α -T (25% α -T), or 235 mg \cdot g $^{-1}$ mix-T (25% mix-T; TC-70 Natural Mixed Tocopherols, Organic Technologies®, Coshocton, OH). The lipid and tocopherol composition of live feeds and yellow perch were assessed using gas chromatography and high-performance liquid chromatography.

The treatment groups had statistically significant differences ($\alpha=0.1$) in fish survival and final weight at the end of the feeding period (Figure). The mix-T group had the fastest growth, but lower survival than the α -T group. While differences in survival are statistically significant, all group means were within 5% of each other. These data suggest that tocopherol supplementation to live food PUFA enrichments is beneficial to the culture of yellow perch larvae, and that mix-T may have unclear impacts compared to α -T.

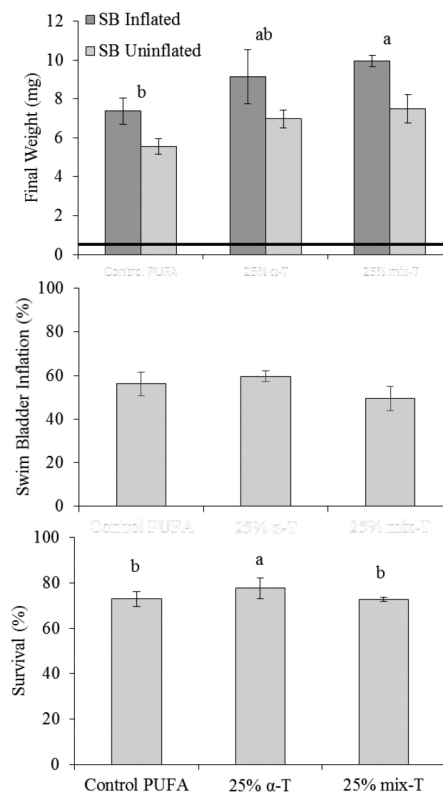


Figure. Survival, swim bladder (SB) inflation, and final weight of yellow perch after 10 days of feeding on rotifers and *Artemia* nauplii enriched with PUFA and no tocopherol (control), α -tocopherol, or a mixed tocopherol product (α , β , γ , and δ). Black bar represents initial size and letters correspond to significant differences at $\alpha=0.10$.

GREENWATER CULTURE AND LARVAL DEVELOPMENT OF THE MELANURUS WRASSE *Halichoeres melanurus*

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The Labridae family is one of the most popular families of fishes in the marine aquarium trade. Wrasses encompass over 500 species, however, less than ten have been successfully cultured in captivity with no current commercial production of ornamental species. Wrasse species within the genus *Halichoeres* are prime candidates for commercial production primarily due to their short larval duration compared to other wrasse genera.

A preliminary study was conducted to describe larval development and culture requirements of the Melanurus wrasse (*Halichoeres melanurus*). Fertilized eggs measuring 0.627 ± 0.013 mm in diameter were stocked in a 125 L tank at a density of 25.2 eggs/L. Larvae (8 ± 3) were sampled and measured throughout development and post metamorphosis (Figure 1). Newly hatched larvae measured 1.590 ± 0.140 mm (notochord length) and lacked a functional mouth and gastrointestinal tract. Initiation of exogenous feeding, swim bladder inflation, and flexion were observed at 3, 10, and 15 days post hatch (DPH), respectively. Completion of metamorphosis was first observed at 37 DPH with a mean standard length of 11.851 ± 0.230 mm. Larval growth appeared linear over the 45-day period. Survival through metamorphosis was 0.54% yielding 17 juvenile wrasses.

To further refine culture methods, a study was conducted to evaluate the effect of algal density on survival and growth of Melanurus wrasse larvae (0-3 DPH). The study consisted of five temporal replicates per *Tetraselmis chuii* algal density treatment (0 cells/mL, 50k cells/mL, 100k cells/mL, and 200k cells/mL). Fertilized eggs were stocked in 13L larval tanks at 10 eggs/L. Larvae were harvested and measured at 3 DPH. Larval survival was significantly ($P > 0.05$) higher in 50k cells/mL and 200k cells/mL treatments compared to the 0 cells/mL treatment, indicating that early microalgae introduction plays a significant role in larval survival before initiation of exogenous feeding. Larval growth did not vary significantly among treatments.

Information gathered from these trials will help to advance commercial aquaculture protocols for the Melanurus wrasse and guide future aquaculture research with additional Labrid species.

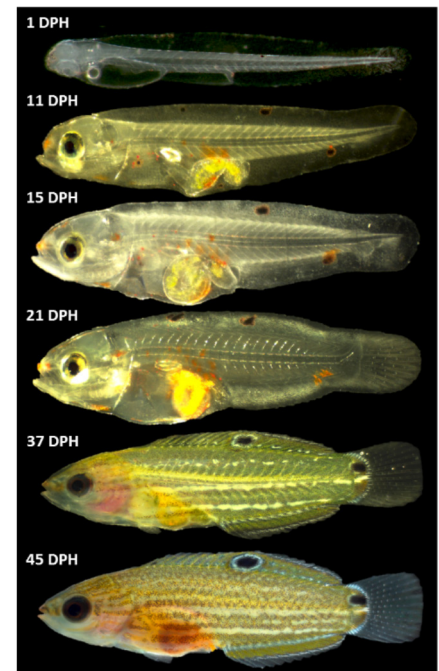


FIGURE 1. *H. melanurus* larval development (not to scale).

THE GENETICS INITIATIVE AT THE OFFICE OF AQUACULTURE: RISKS, BENEFITS, AND FINDING BALANCE FOR MARINE AQUACULTURE

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The NOAA Fisheries Office of Aquaculture established several Science Initiatives over the past decade focused on managing key social (often legal) and scientific barriers to the development of sustainable and viable domestic marine aquaculture, including genetics, feeds, aquatic animal health, shellfish aquaculture, and so on. Here, we provide an overview of the objectives of the Genetics Initiative, including in-house efforts to understand and model the potential risks of marine (including offshore) aquaculture, understand its benefits, and develop management options to find a balance between these risks and benefits. [NOAA's Aquaculture Policy](#) outlines the agency's commitment to addressing the "potential competitive and genetic effects (of escaped fish) on wild species." Consequently, the first objective of the Genetics Initiative was to articulate the genetic risks to wild populations associated with escaped farmed fish, which include the potential for reduced genetic diversity within and among populations and loss of fitness should escapees interbreed with wild fish. These concepts were covered in depth in a comprehensive technical memorandum review of the "[Genetic Risks Associated with Marine Aquaculture](#)" published by Robin Waples, Kjetil Hindar, and Jeffrey Hard in 2012. However, our ability to determine the magnitude of the hazard, the degree of risk, and thus, the appropriateness of any mitigative measures associated with a given escape pattern for a given species was still limited. Therefore, the second objective was to model genetic interactions. NOAA developed the [Offshore Mariculture Escapes Genetics Assessment \(OMEGA\) simulation model](#), in collaboration with ICF International, to provide insight into variables affecting risk; identify information gaps and research priorities; explore options for operational design, management, or mitigation; and inform policy and management decision-making. Current work is focused on a sensitivity analysis toward making OMEGA fully operational and user-friendly and fostering external collaborations to develop model scenarios with data from aquaculture operations worldwide. The third objective of the Genetics Initiative is to understand the benefits and risks of using genetic improvement and find solutions that allow for maximizing benefits and minimizing risks. Key to this goal is understanding how techniques like selective breeding, sterilization, and sex control and emerging technologies like epigenetics and gene editing impact the relative benefits and risks of marine aquaculture. For instance, genetic improvement both helps optimize aquaculture production by increasing the incidence or expression of desirable traits, such as growth and disease resistance, and has potential to mitigate risk by reducing survivability of escapees. A high-level NOAA/USDA technical review is in progress, with current work focused on merging the technical and conceptual risk/benefit trade-offs of various genetic improvement techniques. In sum, the overall goal of the Genetics Initiative is to help develop management options and tools that support conservation goals, as well as a robust marine aquaculture industry in the U.S.

USING A HIGH-PROTEIN BREWER YEAST PRODUCT (DBY50) TO REPLACE FISHMEAL AND SOYBEANMEAL IN PRACTICAL DIETS FOR THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Two 6-week growth trials were conducted to evaluate the utilization of an all-nature high-protein brewer yeast product (DBY50) on growth performance of the pacific white shrimp, *Litopenaeus vannamei* in practical diets. In the first trial, grade levels of DBY50 (0, 6, 12, 18, and 24%) was used to replace fishmeal (FM: 19.12, 14.34, 9.56, 4.79, and 0%). As second series used graded levels of DBY50 (0, 6, 12, 18, and 24%) as a replacement for soybean meal (SBM) referred to as Diet 1-5, and Diet 4 and 6-9, respectively. Each diet was randomly fed to 4 replicate groups of 10 shrimps per diet. After the feeding trial, the result showed that there were no significant differences in final biomass, survival, FCR and limited differences in final weight and weight gain. To confirm the results in the first trail, we repeat Diet 1 and Diet 3-6 as well as a low fishmeal diet containing 2% of a 75% protein product [DBY70 (Diet10)]. Each diet was randomly fed to 4 replicate groups of 30 shrimp per diet. Result showed that there were no significant differences in final biomass, mean weight, survival, weight gain and FCR among all the treatment. Shrimps fed the high fishmeal basal diet exhibited significantly higher weight gain than those offered the diet containing 24% DBY50. There was no difference in performance of shrimp offered low fishmeal diet or the low fishmeal diet containing DBY70. In general, 18-24% DBY50 can be used in practical diets as a replacement for fish meal or up to 24% when replacing soybean meal.

Table 1. Response of juvenile shrimp to diets with grade level of DBY50 to replace FM and SBM for two 42 days growth trail.

Trial 1			Trial 2		
Diet	Weight (g)	WGR ^a (%)	Diet	Weight (g)	WGR ^a (%)
1	4.5 ^{ab}	2496.7 ^{ab}	1	7.5	826.3 ^b
2	4.5 ^{ab}	2606.5 ^b	3	7.3	789.9 ^{ab}
3	4.7 ^a	2770.6 ^b	4	7.2	778.6 ^{ab}
4	3.7 ^b	2028.5 ^a	5	6.7	727.4 ^a
5	4.1 ^{ab}	2197.2 ^{ab}	6	7.2	776.7 ^{ab}
<i>P value</i>	0.015	0.008	10	7.1	764.8 ^{ab}
4	3.7	2028.5	<i>P value</i>	0.207	0.104
6	4.5	2314.1	^a WGR: weight gain rate		
7	4.4	2505.3			
8	4.4	2488.0			
9	4.4	2513.5			
<i>P value</i>	0.081	0.287			

PRODUCTION RISK AND TECHNICAL EFFICIENCY OF TILAPIA AQUACULTURE FARMING IN BANGLADESH

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Tilapia is a relatively fast-growing fish species that has huge potential for production in Bangladesh. Despite its impressive growth, production is highly volatile across farms and location. There are several sources of production volatility; both output risk and/or inefficiency will lead to variation in production. While a number of studies has been focusing on either technical efficiency or the adaption towards risk, few studies have been conducted of the combined effect of production risk and technical inefficiency in aquaculture. By employing a stochastic production frontier model with flexible risk specification, this article quantifies production variability of tilapia fish farming by means of two main possible sources: production risk and technical inefficiency. Further, the analyses identify the factors influencing risk and inefficiency. Knowing the source of the variation is vital for farmers and policy makers in choosing the right strategy to discard the production variation. The empirical analysis is based on cross-sectional data from 339 sample farms. Main findings are that significant production risk and technical inefficiency exists in tilapia farming.

THE EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS ON GROWTH OF JUVENILE (AGE-1) BLACK BUFFALO *Ictiobus niger*

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Although Black Buffalo *Ictiobus niger*, a catostomid species native to the Mississippi River basin, are broadly distributed, populations have been declining. Artificial propagation and culture are a part of many native species recovery plans, however culture methods for catostomids are limited. An important component of culture techniques is the development of formulated diets, and establishment of ideal protein levels. In the case of at-risk species, diet formulations may be based on nutrient requirements of more common species. For catostomids, studies that establish protein requirements in diets are limited, particularly for larger juvenile to sub-adult sizes. Therefore, an experiment was designed to evaluate effects of different dietary protein levels on growth of juvenile Black Buffalo.

A 10-wk feeding trial was conducted to evaluate optimal protein levels in juvenile (age-1) Black Buffalo (mean \pm SE: total length = 218.7 ± 0.76 mm, weight = 148.5 ± 1.6 g). Five practical diets, similar to a commercially available Razorback Sucker Diet (~42% crude protein; Rangen, Inc.), were formulated to contain 45%, 41%, 38%, 34%, and 30% crude protein. Two separate recirculating aquaculture systems (RAS) containing 10 tanks (400-L; 1-m diameter) per RAS (N=20), stocked at 10 fish per tank, were used in this study. Each diet was fed to four randomly assigned replicate tanks. Fish were fed to satiation daily by providing excess feed and removing uneaten feed after 30 minutes. Constant temperature (mean \pm SE: $23.5 \pm 0.1^\circ\text{C}$) and photoperiod (12 h light: 12 h dark) were maintained throughout the experiment.

At the end of the experiment, fish fed a diet containing 41% crude protein displayed significantly greater biomass gain (total weight gain for all fish in the tank) when compared to all other diets (Table 1). Similarly, individual weight gain (percent increase) was significantly higher in fish fed the diet containing 41% protein compared to lower protein diets (Table 1). Interestingly, fish fed a diet containing 45% protein had significantly lower biomass gain compared to fish fed the 41% protein diet (Table 1). Results from this study suggest a diet with ~41% crude protein would produce optimal growth for juvenile (age-1) Black Buffalo.

TABLE 1. Mean (SE) biomass gain and mean weight gain (percent increase) of Black Buffalo fed 5 different diets. Significant differences are indicated by different letters (P<0.1).

Diet	Dietary protein (%)	Survival (%)	Biomass gain (g)	Weight gain (% increase)
1	30	97.5	662 (34) z	45 (4) z
2	34	100	693 (43) z	47 (2) z
3	38	97.5	671 (39) z	45 (2) z
4	41	100	837 (36) y	56 (2) y
5	45	100	686 (29) z	49 (2) yz
P-value			0.0175	0.0297

EVALUATION OF BREWERS GRAINS IN TILAPIA *Oreochromis niloticus* DIETS AS APPLIED TO INCREASING AQUAPONICS SUSTAINABILITY

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Tilapia are a popular farmed food-fish worldwide and currently the most commonly used species in aquaponic production in the US. Tilapia are able to efficiently utilize plant products as feed, lending to both economical and sustainable production. However, the US imports most of the farmed tilapia available to consumers. One key factor is that feed costs for production are greater than 50% in the US. The incorporation of locally available by-products might reduce feed costs, with one potential source being the spent cereal solids left from brewing alcohol. These solids, called Brewer's Grains (BG), are a concentrate of protein and fiber remaining after most of the sugar has been extracted from the mash. Tilapia are unique in their ability to digest fibrous plant materials and are often fed agricultural and industrial by-products in other parts of the world. As breweries continue to thrive in the US, and considering the many small-scale breweries associated with craft beer, there could be locally-sourced material available at a very low cost for inclusion in tilapia diets.

An eight-week feed trial evaluated the effects of adding increasing levels of BG into diets for Nile tilapia (*Oreochromis niloticus*). Ten juveniles (avg. weight 6.6 g) were stocked into each of sixteen 38-L aquaria with four replicates per dietary treatment. Fish were fed three times daily, one of four experimental diets containing either 0% BG, 15% BG, 30% BG or 30% BG with added crystalline amino acids (AA; 30% BGwAA) at 0.35% methionine and 0.35% lysine supplementation. The 0% BG was a control and modeled after a commercial tilapia diet. All diets contained 8% fish meal and were formulated to be isocaloric and isonitrogenous. Added BG replaced conventional soy bean meal and wheat flour in the formulations.

After eight weeks there was no significant difference ($P > 0.05$) in survival among treatments which averaged 95% overall. The average weight (g) and specific growth rate (gain in g/d) of fish fed 0% BG (56.5 and 0.90, respectively) and 15% BG (53.6 and 0.84) were statistically similar, although were both significantly greater ($P \leq 0.05$) than for fish fed 30% BG (48.7 and 0.76) and 30% BGwAA (43.3 and 0.65), with 30% BG being larger than those fed 30% BGwAA. Feed conversion ratio (FCR) was significantly lower (more efficient) for fish fed 0% BG (1.14) than for both 30% BG (1.41) and 30% BGwAA (1.54). FCR for 15% BG (1.32) was smaller than fish fed 30% BGwAA, but not different from the other diets.

BG inclusion rates between 15 and 30% produced growth reduction and decreased FCR, and also indicate reduced palatability in the 30% BGwAA diet. Based on these data, inclusion rates up to 15% resulted in acceptable growth and FCR of juvenile tilapia. The utilization of locally sourced BG may be one step toward lowering production costs in aquaponic production, while also adding to the popular sustainability aspect.

CONSUMER PREFERENCES AND CONSUMPTION PATTERNS FOR FISH IN UGANDA

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The current government policy on aquaculture is promotion of the subsector to complement dwindling capture fisheries supplies from the wild to improve nutrition and eradicate poverty. Much as the government is pursuing this policy there exists limited information on consumer behavior between captured and farmed fish. The objective of this study was to establish consumer preferences and consumption patterns for the two categories of fish. This study was carried out in selected districts representative of Uganda's fish consuming community that is, Nebbi, Kampala, Busia, Kasese, Kisoro and Kabale. A total of 350 consumers were randomly selected and interviewed using a structured questionnaire. Descriptive statistics and regression analysis were used to analyze the data.

The results show that consumers' average age was 33 years, had a household size of 6.74 persons and earning 628,200UGx monthly. Distance to the fish source was 3.49km and 70% of the respondents had eaten fish as a protein source for an average of 23 years. 92.5% bought tilapia which was mainly (62.2%) captured fish. When buying fish, 70% of fish consumers considered fish species as the most important attribute. The majority (55%) of consumers purchased their fish from traditional markets and the rest from roadside markets and landing sites. On average, consumers bought fish about 6 times per month, resulting in total consumption of 13.86kgs. There was a general preference (56%) for fish above 500gm. Many consumers (67.5%) preferred smoked fish and mainly (75%) prepared fish by boiling. 30% of the Consumers indicated that farmed fish in most cases was small size (< 300gm) and bonny. Some considered small size as a deterrent to their taking farmed fish as their purchase choice. 33% testified to have ever tasted farmed fish and noted its soil like smell.

Econometric results show that distance to fish market / supply source, annual household income, education level and perceived quality significantly affected fish consumption patterns. In view of the results, it is recommended that researchers should breed fleshy easy to farm fish species which can grow to 500gm preferred by consumers. In order to address the issue of muddy fish smell, there may be a need to design fish production systems that avoid fish proximity with mud during the production process or have fish flushed with flesh water for the last week before sell to have any debris cleaned from the gills area.

ESTIMATING DEMAND FOR FISH AND ITS PRODUCTS IN UGANDA

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Uganda has one of the highest population growth rates in the world. Along with this goes the high demand for plant and animal protein of which fish is an example. However, much as many studies have been done on fish production and marketing limited information exists that analyzes demand for the fish in the country. Thus this study aimed at estimating fish demand in order to provide policy makers information on which base decisions on estimating supply. The study was carried out in all the four regions of Uganda. A total of 315 respondents were randomly selected and interviewed in both rural and urban areas of Uganda. To minimize on the weaknesses of small sample size and aggregation problem a three stage multiple budgeting framework of household was structured in a simple way. Further still, the study strictly focused on demand projections with respect to income growth and did not consider price and other parameters to keep the study simple. Descriptive statistics was used to characterize respondents.

Respondent average age was 36 years with a monthly income of 650,456UGx (US \$182). The coefficient of food and fish expenditure functions for national, urban and rural areas was found to be positive and significant, indicating that the response of food expenditure to income changes and fish expenditure to food budget changes are substantial. Respondents had a per capita expenditure on food and fish of 558,000UGx (US\$155) and 171,428UGx (US\$ 48) respectively. Income elasticity was positive and less than one for all the fish types but was higher in rural areas than in urban areas. The income elasticity of demand for tilapia was found to be highest amongst fish consumers and is expected to play a dominate role in meeting fish demand. Considering 2010 as a base year, the demand for fish is likely to grow at a rate of 3.45% nationally, 3.33% for rural and 2.34% for urban areas. As such demand for tilapia will grow by 3.25%, Nile perch 3.12% and silver fish 4.37% percent between 2017 and 2040.

The country and other stakeholders need to invest their resources in promoting tilapia production and promoting silver fish consumption to meet this envisaged fish demand in the foreseeable future.

NOVEL AQUACULTURAL APPLICATIONS FOR INDIVIDUAL AND FLEETS OF AUTONOMOUS SURFACE VEHICLES

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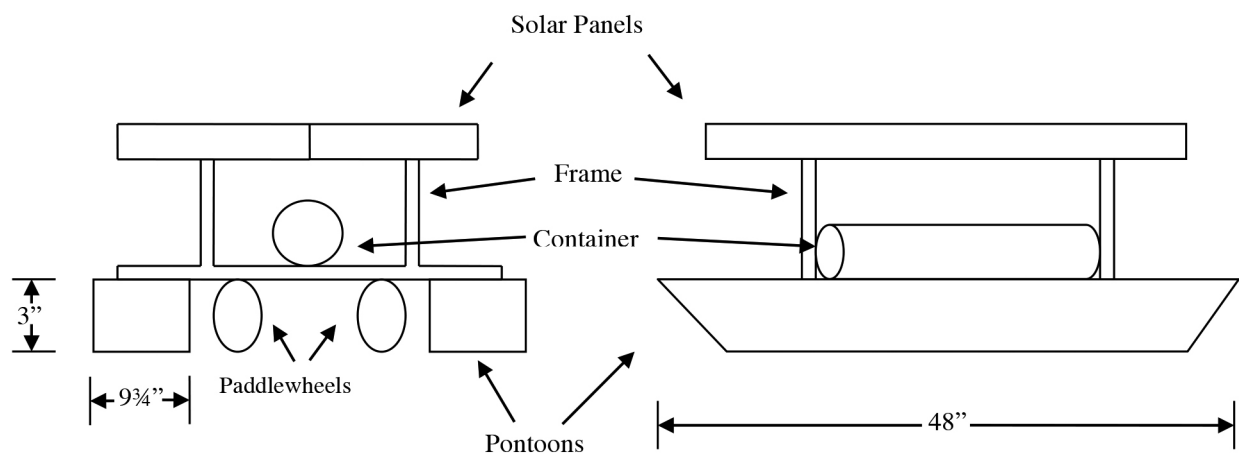
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Autonomous vehicles and fleets of such vehicles have been developed over the last several years. These devices can be programmed for many applications and can be especially useful for jobs difficult for humans to perform, and also to enhance human knowledge and capabilities. Such tasks have been done by single vehicles or small groups; more recently, fleets of coordinating vehicles have been proven effective in tasks such as reducing predatory birds from aquacultural ponds, measuring depth, and tracking temperature and chemical gradients. A fleet of autonomous vehicles may complete these jobs more efficiently than a single boat by covering more area and communicating so that the areas that need the boats can be found more easily.

This fleet was designed so that each boat would be identical. Remote measurements of water quality parameters including salinity, temperature, acidity and nutrients could be useful in extensive aquaculture (e.g. oyster, clam or other extensive applications). Site specific environmental monitoring could be applied to aquatic culture and restoration sites. Pollutant and/or toxin gradient tracking could be done by coordinated boats. Aquatic biomass could be collected by coordinated boats, possibly using less energy resources. Automated water sample collection could be useful in large tanks, ponds and reservoirs, as well as in open lakes or coastal areas. The ability to tap into location via GPS, and capture physical samples or water quality data, as well as photographic or video could be useful in a variety of applications. Discussion of work to date and plans for future work is of interest.

FIGURE 1. The basic layout of a single boat. Multiple boats can communicate and coordinate.



NUTRITIONAL VALUE AND NUTRIENT UTILIZATION OF PRESSURE COOKED PIGEON PEA (*Cajanus cajan*) FORMULATED DIET ON *Clarias gariepinus* (Burchell) FINGERLINGS

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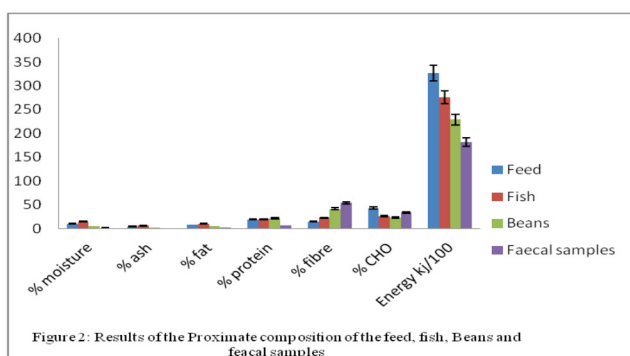
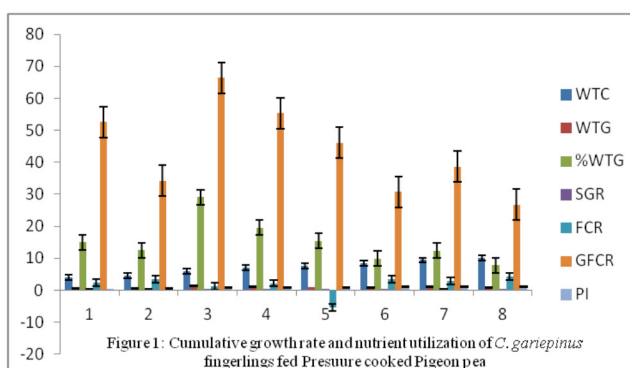
The need to substitute fishmeal in animal feed has necessitated the use of plant derived feedstuffs. However, problems of anti nutritional factors in tropical legumes have limited their widespread usage and direct incorporation into animal feeds. Different processing methods have been devised to remove or reduce the concentration of these factors.

Pigeon pea was subjected to pressure cooking using gas at 100°C for 30min, after which it was oven dried for 7 hours. A 40% crude protein diet was formulated with the dried Pigeon pea and fed to *C. gariepinus* fingerlings of 3.5g mean body weight to evaluate the nutritional value and nutrient utilization on the growth and digestibility. This was aimed at determining how best pressure cooked method is in optimum utilisation of pigeon pea meal for fish production.

Fingerlings were fed twice daily at 4% body weight for 56 days to measure the following parameters weight gain (WTG), percentage weight gain (%WTG), specific growth rate (SGR), food conversion ratio (FCR), gross food conversion ratio (GFCR), protein intake (PI) and survivability.

The fish were weighed and reweighed weekly with feed fed adjusted accordingly and faecal matter collected. The faecal matter was dried in the sun at an ambient temperature and kept for proximate analysis.

The study revealed that pressure cooked pigeon pea seed could be a good protein source for fingerlings, and that it causes a reduction in the anti nutritional factors and can be incorporated into the diet. It should be used in addition with another source of protein, preferably animal protein to enhance better and faster growth of the fingerlings. The prospect of better management practices, based on the utilization and value of Pigeon pea in fish nutrition, bodes well for the aquaculture industry.



HAEMATOLOGICAL AND HEPATIC RESPONSES OF *Clarias gariepinus* TO SUBLETHAL EXPOSURE OF INDUSTRIAL EFFLUENTS FROM OLOGE LAGOON, LAGOS, NIGERIA

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Fish in rivers/lagoons receiving high discharges of effluent from industries has shown a range of alterations related to physiological abnormalities. These effects have been attributed to various estrogenic chemicals known to be present within treated or/and untreated industrial effluents. This report is aimed at assessing sub-lethal effects of the effluents on the haematological characteristics and on the tissues of liver using histological techniques on the early life stages of *C. gariepinus*.

The present study is on the haematological and hepatic responses of *Clarias gariepinus* to sublethal toxicity of industrial effluents (IE) from the environment of Ologe Lagoon, Lagos, Nigeria. The fish were cultured in five concentrations of industrial effluents: 0% (control), 5%, 15%, 25%, and 35%. Trials were carried out in triplicates for twelve (12) weeks. Haematological parameters were: Packed Cell Volume (PCV), Red blood cells (RBC), White Blood Cell (WBC), Neutrophil and Lymphocytes etc; and histological alterations were measured after 12 weeks. Haematological results showed that *C. gariepinus* had PCV ranging from 13.0 ± 1.7 - 27.7 ± 0.6 , RBC ranged from 4.7 ± 0.6 - 9.1 ± 0.1 , and Neutrophil ranged from 26.7 ± 4.6 - 61.0 ± 1.0 amongst others. The highest values of these parameters were obtained in the control and lowest at 35%. Reverse effects were observed for WBC and lymphocytes. The liver shows normal liver cells in the control (0%), but at higher toxic levels, there were: vacuolation, destruction of the hepatic parenchyma, tissue becoming eosinophilic and severe disruption of the hepatic cord architecture. The study revealed that disposal of effluents into aquatic environment affects the health of fishes by impairing normal hepatic functions and hinder vital physiological processes if exposure continues for a long period of time.



Figure 1: External features of *Clarias gariepinus* (Photo by Dr. O.A. Adeboyejo).

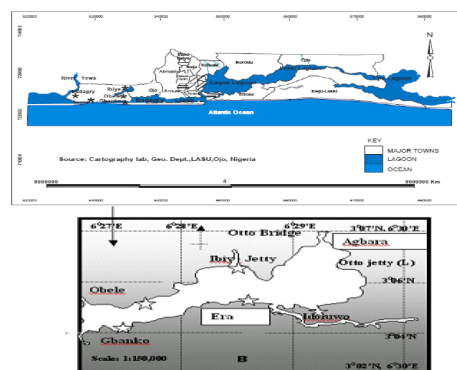
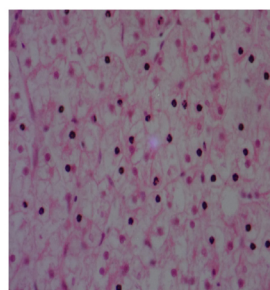


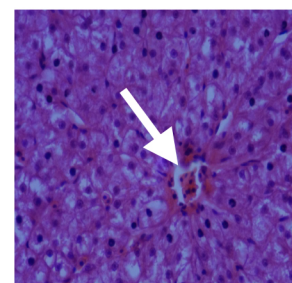
Figure 2: Map of Lagos Lagoon, Nigeria Inset Ologe Lagoon

Conc (%)	PCV (%)	RBC/mm ³	WBC/m ³	Differential WBC counts (%)	
				Neutrophil	Lymphocyte
0	27.7±0.6 ^a	9.1±0.1 ^a	7100±100.0 ^a	61.0±1.0 ^a	34.0±4.6 ^a
5	19.0±1.0 ^b	6.4±0.3 ^b	6333±115.5 ^b	50.7±2.3 ^b	36.3±3.5 ^a
15	17.3±1.2 ^b	5.7±0.4 ^c	8133±208.2 ^c	25.0±4.0 ^c	63.0±1.0 ^b
25	14.7±3.1 ^c	5.0±1.0 ^c	9133±115.5 ^d	32.7±3.1 ^c	55.7±1.5 ^b
35	13.0±1.7 ^c	4.7±0.6 ^d	10800±200.0 ^a	26.7±1.5 ^c	63.0±1.90 ^b
0	92.97±1.66 ^a	9.4±0.4	6300.0±10.0 ^a	175.0±5.0 ^a	88.4±2.1 ^a
5	115.43±3.4 ^b	7.8±0.4	12533.3±39.1 ^b	102.9±20.5 ^b	92.43±1.4 ^b
15	108.73±8.2 ^b	8.07±1.4	23800.0±18.5 ^b	54.33±0.31 ^c	94.63±2.2 ^b
25	115.9±0.9 ^b	7.5±0.15	82000.0±30.0 ^c	96.9±1.85 ^d	96.7±1.5 ^b
35	102.07±2.0 ^b	7.5±1.23	97330.3±50.2 ^d	64.97±3.0 ^e	96.2±1.1 ^b

Note: Dissimilar letters as superscripts indicate that the mean values at the different concentrations differ significantly ($P < 0.05$). PCV = Packed Cell Volume (%), RBC = Red Blood Cell (RBC/mm³), and WBC = white blood cells (mm³).



0% = Showing normal liver cells with no lesions; hepatic cord is normal.



35% = there was widespread vacuolar degeneration and severe disruption of the hepatic cord architecture (see arrows), fusion of the hepatic cord. Adaptive responses are observed.

IMPROVING AQUACULTURE'S VALUE THROUGH ENHANCED NUTRIENT MANAGEMENT

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Interest in locally produced foods and sustainable production methods has increased dramatically in recent years, and for U.S.-grown fish and seafood “local” can translate to anywhere in the U.S. A national study conducted in 2003 found that an interest in healthy, safe, and fresh foods increased the likelihood of buying local foods. In addition, 40 percent of respondents to a 2011 trends survey conducted by the Food Marketing Institute cited knowing a food’s source as a motivation for purchasing local foods. Consumers interested in “healthy, safe, and fresh” are likely to support aquaponics operations if their story is communicated to potential customers through effective marketing efforts.

In an international survey of aquaponics growers, Love et al (2015) found a significant relationship between sales of non-food products from aquaponics farms and the farms’ profitability and suggested that aquaponics growers may want to explore revenue sources other than just vegetables and fish to enhance economic feasibility.

Farmers engaged in aquaculture make a significant investment in feed amounting to some 60 percent of overall production costs. Fish ingest the feed and then excrete some 75 percent of the total, which is often ignored or discarded. Developing an optimized system to utilize the fish waste as a way to generate additional income will improve the profitability of aquaculture and contribute to the industry’s environmental sustainability.

This USDA/NIFA funded project links greenhouse production of tilapia and vegetables through the fish feed and fish excretion to explore possibilities of further use of these wastes to produce additional crops for food and energy. Additionally, we are investigating the possibility of using solid fish waste to produce valuable bio-products, such as lactic acid via fermentation. Adding uses to produce additional saleable items can enhance the overall benefit value stream of the operation and reduce greenhouse fertilizer costs.

This project is also looking to validate food safety practices in combined fish-vegetable production. At present, science-based, aquaponics-specific Good Agricultural Practices do not exist. There have been food safety concerns expressed by consumers about aquaponics produce, with concerns being more common among the high-end consumers who are more willing to pay premium prices. Concerns about harmful bacterial counts in the aquaponic water effluent, adequate testing and monitoring of bacteria, and whether bacteria from the fish production units would get into the vegetables is being addressed in this research. We are sampling for the presence of pathogens throughout the system. By scientifically documenting the presence or absence of pathogens and improving system bio-safety, we can contribute to the food safety goal of these systems.

IMPACTS OF CLIMATE CHANGE ON SNAKEHEAD FISH VALUE CHAINS IN THE LOWER MEKONG BASIN OF CAMBODIA AND VIETNAM

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Truong Hoang Minh, Robert Pomeroy

The productive fisheries of the Lower Mekong Basin of Cambodia and Vietnam are essential to the food security and nutrition of 60 million people. Yet these fisheries, both culture and capture, are susceptible to the impacts of climate change. This paper reports on a study undertaken to examine the vulnerability, as perceived by snakehead (*Channa striata*) fish farmers in Vietnam and fishers in Cambodia, to the impacts from climate change. Perceived impacts on various actors in the value chain are identified, as well as adaptation strategies currently being utilized and planned for the future. Recommendations are suggested to contribute to assisting snakehead farmers and fishers in adapting and preparing for the impacts of climate change.

STATUS OF WOMEN IN DIFFERENT SOCIO-CULTURAL CONTEXT OF AQUACULTURE-BASED SOCIETIES: A CASE FROM BANGLADESH

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Women in Bangladesh are commonly characterized by lower economic, social, cultural and political status, heavy domestic workloads, restricted mobility, inadequate knowledge and skills, low sovereign power and a limited role in household decision-making. However, the situation can be an exception when they are physically and mentally more active and can generate more income. The objective of the study was to measure the social status of women with two different cultural set ups in a southwestern coastal communities of Bangladesh, where mostly Hindu households reside. The study considered 100 Hindu women and 50 Muslim Women. In some cases, the male members of their families were also interviewed. The Muslims are the minority in this area and their cultural perspective does not allow them to work for fish production in the gher**. A Muslim woman who works in the gher is locally considered as 'bad woman'. It could be mentioned here that the gher are located very near from the house (like a yard). It was found that because Hindu culture allows both men and women to work for fish production, they receive more output. When the male members are busy with other farm, non-farm or off-farm work, the female members lead the gher culture. They catch fish whenever needed and guide the harvest from their ponds before selling; the women always keep the required amounts for home consumption. As a result, they consume fish for 15 – 20 days a month. The families of Hindu female labours who work in others' gher, consume more fish than comparatively richer Muslim families who depend on male members to make fish available at home either from the gher or from the market. In case of 'female headed' Muslim households, the local adage holds: 'no male at family, no fish to consume'. It was also observed that due to more fish consumption (their perception), the Hindu women were healthier, more energetic, can work longer in the field and at home. Though they were engaged in work for more hours as their reproductive and productive responsibilities, rarely they mentioned about double burden of workload. Rather they enjoy their works as their economic situation was improving and they receive more cash income. Opposite situation was observed for the other culture followers. The circumstances may conclude about the relationship between cultural views and social status. Therefore, context specific cultural change may improve the socioeconomic status of women in any society.

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** Gher is the pond which is dug on previous agricultural land and currently used for fish culture. In Bangladesh, the term 'gher' is used in south-west coastal areas.

The research is funded by the AquaFish Innovation Lab of the United States Agency for International Development (USAID)

GROWTH AND PRODUCTION PERFORMANCE OF AIR-BREATHING CLIMBING PERCH (*Anabas testudeni*) AND MAJOR CARPS IN POLYCULTURE

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The use of Koi or climbing perch (*Anabas testudeni*) in aquaculture has grown substantially over the past decade in Bangladesh because it has a high market value and is rich in nutrients. Further, being an air-breathing fish, Koi have a strong capacity to tolerate poor oxygen environments. Koi production is currently limited to monoculture systems with intensive use of commercial-grade feeds. Feed constitutes almost 80% of the total costs for producing Koi and thus methods to reduce feed inputs can provide significant economic benefits, particularly if fish are co-cultured with carps that rely primarily on natural pond productivity rather than direct consumption of formulated feeds. Thus, the aim of the present study was to investigate growth and production of Koi when used in polyculture with major Indian carp species, Rui (*Labeo rohita*) and *Catla* (*Catla catla*) relative to that observed in with monocultures. We also examined the effects of combining reduced feed ration and pond fertilization on Koi-carp polyculture.

The experiment consisted of four treatments, with three replicates each (12 ponds; 100 m² area, 1.5 m depth). T1 consisted of a Koi monoculture (5/m²) with full daily feeding while the other three treatments consisted of a Koi-carp polyculture (*Catla*, 0.2/m²; Rui, 0.8/m²; Koi, 5/m²) with full daily feeding (T2), 75% daily feeding (T3), or 50% daily feeding (T4). Additionally, the ponds for T3 and T4 were fertilized weekly with urea and triple super phosphate (28 kg/ha N, 5.6 Kg P/ha) to boost pond productivity. Koi were fed a full daily ration of commercial feed (CP feed) according to current practice (20% down to 5% body weight/day) or a fraction of this based on treatment groups.

After 126 days, the average weight gains for Koi were 54.79, 96.63, 119.6 g, and 120.5g in T1-4, respectively. The body lengths for Koi were highest in T3 (18.22 cm), followed by T4 (17.97 cm), and lowest in T1 (9.5 cm). Average weights of Rui at the end of the study were 273, 298, and 340 g for T2-T4, respectively, while that for *Catla* was 456, 339 and 396 g for T2-T4, respectively. Survival of Koi ranged from 45 - 57% with the highest rate occurring in T4. *Catla* and Rui survival was 67- 80% and 58 - 61%, respectively. The results demonstrate 1) that Koi growth is enhanced when polycultured with carps versus in monoculture, 2) addition of carps to Koi culture improves overall fish production over monoculture alone, and 3) reducing daily ration by 50% along with pond fertilization has little impact on growth of Koi or carps. Based on this research, Koi polyculture with carps is substantially more beneficial in terms of fish production than the current practice of Koi monoculture. This along with reductions in feed ration allow considerable improvement in feed conversion and cost savings with little impact on fish production. (Supported by the AquaFish Innovation Lab – USAID)

NATIONAL VETERINARY ACCREDITATION PROGRAM MODULE 13: AQUATIC ANIMAL HEALTH REGULATIONS AND HEALTH CERTIFICATION

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The goal of the NVAP program is to ensure that private veterinary practitioners who provide regulatory services to U.S. livestock, poultry and aquaculture industries, are adequately trained and well acquainted with regulatory requirements through USDA-APHIS accreditation. In addition to other accreditation requirements, accredited veterinarians are required to successfully complete a required number of 29 training Modules (<http://tinyurl.com/NVAP-Modules>), four of which currently cover aquatic animal health regulatory issues.

Although initiated as a 1896 agreement between the U.S. and Canada to combat equine disease outbreaks, in 1921 the U.S. Department of Agriculture (USDA) formalized the National Veterinary Accreditation Program (NVAP) so private practitioners could assist Federal veterinarians in controlling animal diseases. In 1992 regulations allow standardized procedures and requirements, and uniform administration to be managed nationally by APHIS, but with authorization of veterinarians licensed to practice on a State-by-State basis.

In 2001/2002 an “Animal Health Safeguarding Review by the National Association of State Departments of Agriculture (NASDA) to further redesign and upgrade the NVAP and suggested that “the accreditation program be the core for emergency preparedness and the response plan.” Recommended revisions were published in 2002 (“New Directions for the National Veterinary Accreditation Program,” J. Amer. Vet. Med. Assoc., 22(10): 1470-1472), with revised regulations implemented in 2009.

With accredited veterinarians being the first line of defense against catastrophic disease outbreaks, U.S. has successfully controlled outbreaks of several foreign animal diseases (FADs), including contagious equine metritis, equine piroplasmiasis, epizootics of exotic Newcastle disease and West Nile virus, cases of screwworm and monkey pox, and pandemics of the influenza virus – and several aquatic animal diseases.

Module 13 provides information about the agencies involved in regulating aquatic animal health and trade, with an emphasis on USDA and the role of accredited veterinarians. It also addresses the proper completion of health certificates for farmed aquatic animals and provides resources for obtaining current regulations.



NATIONAL VETERINARY ACCREDITATION PROGRAM MODULE 28: SIGNIFICANT AND EMERGING VIRAL DISEASES OF CARP, KOI AND GOLDFISH

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Module 28 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.



APPROACH FOR DEVELOPING COMMERCIAL AQUACULTURE HEALTH PROGRAM STANDARDS (CAHPS)

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Currently the U.S. commercial aquaculture industry sectors are operating without uniform, consistent or science-based standards for animal health management. U.S. aquaculture industry sectors are burdened with varying health requirements by states and trading partners for animal movement often resulting in expensive yet meaningless animal testing. A risk-based approach to health management provides a framework to verify aquatic animal health, allow for branding, provide leverage for negotiations with trade partners, facilitate safe animal movement and protect natural resources. The National Aquaculture Association (NAA) in collaboration with the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), have developed a non-regulatory framework for the improvement and verification of the health of aquatic animals cultured in U.S. The standards implement the National Aquatic Animal Health Plan (NAAHP) for commercial aquaculture and establishes a model approach for aquatic animal health and pathogen management that may be used by other sectors. The commercial aquaculture health program standards (CAHPS) outline a series of practices that ensure animal health, enhance individual farm biosecurity, and, most critically, describe methods that may assist with branding and promotion, facilitation of animal movement and export and expand markets for the U.S. commercial aquaculture sectors.



ASSESSING THE RISK OF ENVIRONMENTAL HARM FROM AQUACULTURE ESCAPES: A STATE OF SCIENCE ASSESSMENT FOR ATLANTIC SALMON *Salmo salar* IN PUGET SOUND

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The potential for fish to escape from offshore net pens has been realized in virtually every location that they occur. The effects of escapes may be immediate, long-term or virtually non-existent, and will vary based on the species of fish, the number of fish released, fish size and condition, periodicity, geography, the health of native fish populations, and a host of other factors. Competition for feed and habitat resources, predation, pathogen transmission, and spawning disruption are potential ways that escapees can negatively impact wild fish. It has been proposed that when Atlantic salmon (*Salmo salar*) escape from net pens in the Pacific Northwest they exert additional stress onto already threatened and endangered native salmonids. Negative effects on predatory marine mammals have also been suggested.

By integrating information from peer-reviewed research and experiences from a recent escape event, we evaluated the state of science on the potential for escaped Atlantic Salmon to cause environmental harm in the Puget Sound. This assessment is being used to inform regulators, industry, and environmental non-governmental organizations as they work together to determine sustainable management approaches for net-pen aquaculture in Washington State.

RECENT PROGRESS IN THE USE OF LIPOSOMES FOR THE DELIVERY OF LOW MOLECULAR WEIGHT WATER-SOLUBLE (LMWS) NUTRIENTS TO AQUATIC ORGANISMS

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Many key nutrients needed to support the growth and survival of aquatic organisms are classified as low molecular weight, water-soluble (LMWS) substances, including amino acids, vitamins and minerals. These substances are difficult to deliver to aquatic organisms because they are rapidly lost from current, commercially-available microparticulate diets and enrichment products when suspended in seawater. Liposomes are phospholipid-based microparticles that can be produced in a size range appropriate for marine suspension feeds such as rotifers, *Artemia* and bivalve molluscs. Liposomes show high retention of LMWS nutrients when suspended in seawater and can be used to deliver these substances to aquatic organisms with high efficiency.

We have shown that liposomes can be used to enrich rotifers and *Artemia* with several LMWS nutrients, including taurine, iodine, selenium and vitamin C and that these compounds are available and may be beneficial to marine fish larvae. For example, the concentrations of taurine, selenium and iodine can be elevated in rotifers to levels equal or above those typically reported in copepods. Moreover, we have found that rotifers and *Artemia* enriched with these compounds may improve larval performance in a number of species including Northern rock sole (*Lepidopsetta polyxystra*), California yellowtail (*Seriola dorsalis*), white seabass (*Atractoscion nobilis*) and may improve the survival of juvenile seahorses (*Hippocampus reidi*) during periods of elevated stress. Liposomes may have broad application in aquaculture and have the potential to deliver a larvae variety of LMWS substances, including nutrients and pharmaceuticals, to marine fish larvae that should improve the quality and quantity of larvae produced by commercial hatcheries, ornamental fish hobbyists and researchers.

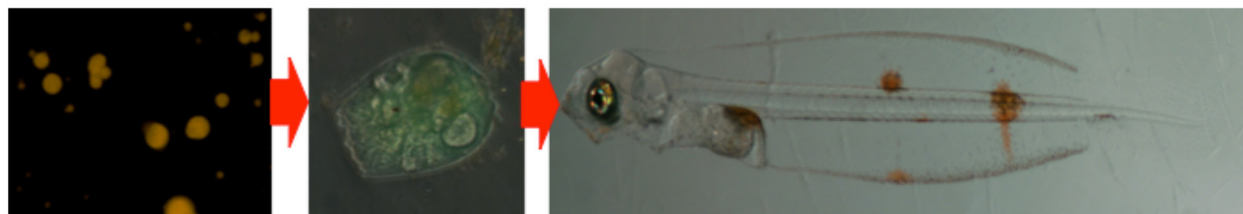


Figure 1. Liposomes containing a wide variety of low molecular weight water-soluble compounds, such as iodine, selenium, vitamin C and taurine can be used to enrich rotifers and *Artemia* resulting in improved nutrition of marine fish larvae.

EVALUATION OF EGG BIOCHEMICAL COMPOSITION AS A PREDICTOR OF SPAWNING SUCCESS IN COHO SALMON *Oncorhynchus kisutch*

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For anadromous fishes such as salmonids, the quality and quantity of food available to adults in the ocean before and during oogenesis can affect spawning success. As relatively little is known about the optimum dietary levels of essential nutrients for normal egg development in Pacific salmon, the present study evaluated the effects of egg nutrient levels on spawning success of returning coho salmon (*Oncorhynchus kisutch*) at two National Fish Hatcheries (NFH's). In fall of 2016, a subset of unfertilized eggs was sampled from 40 returning coho females at Eagle Creek (Columbia River tributary) and Quilcene (Olympic Peninsula) NFH's. Egg weight, Vitamin A, Vitamin E, astaxanthin, proximate composition, and fatty acid profile of eggs were determined. The remaining eggs from each female were fertilized for a total of 40 full sibling crosses and spawning success (e.g. percent eye-up, fry length, fecundity) of each family was determined.

Female weight, fecundity and egg weight differed significantly between hatcheries. All families had relatively high offspring survival. Egg astaxanthin and Vitamin E levels were significantly different between hatcheries (Table 1), as were proportions of several fatty acids such as EPA ($p=0.004$) and DHA ($p=0.010$). The present nutrient profile data provide further evidence for different ocean migratory patterns between coho salmon from the two hatcheries.

Table 1. Vitamin and Astaxanthin levels (mean \pm SD) in coho salmon eggs collected at two National Fish Hatcheries.

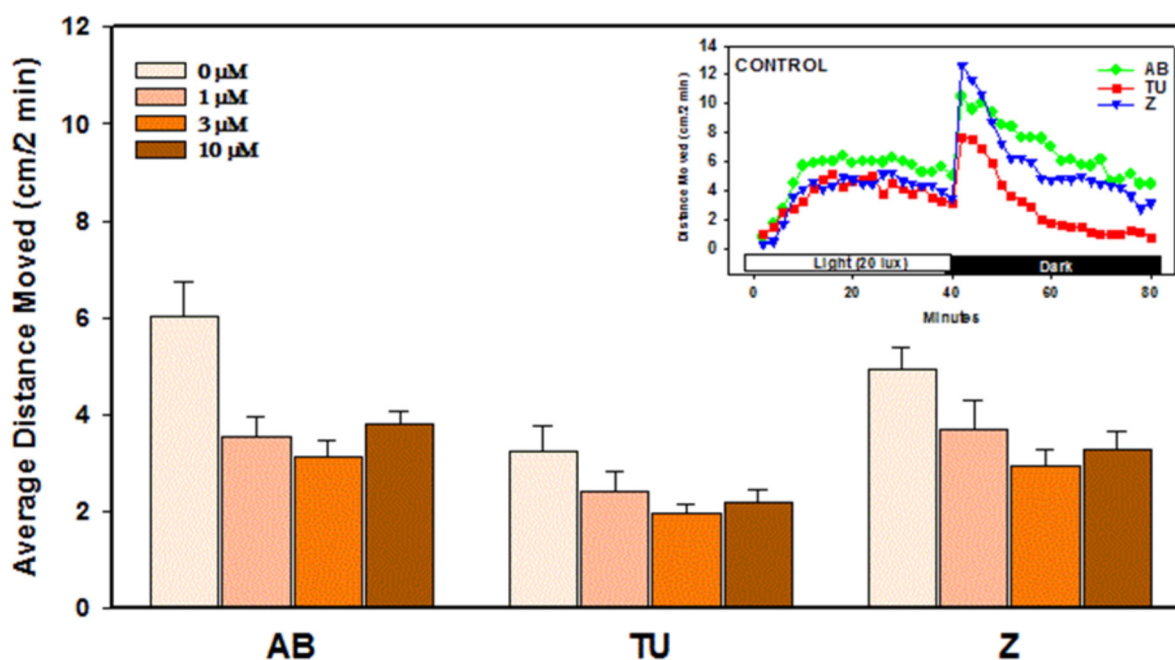
Parameter	National Fish Hatchery		p-value
	Eagle Creek n=20	Quilcene n=20	
Astaxanthin ($\mu\text{g/g}$)	16.6 \pm 3.7 ^b	21.2 \pm 4.03 ^a	0.0009
Vitamin E (IU/100g)	10.9 \pm 1.9 ^a	9.1 \pm 2.3 ^b	0.0113
Vitamin A (IU/100g)	867.1 \pm 213.7	954.9 \pm 202.7	0.2086

COMPARISON OF BASELINE BEHAVIOR AND THE DEVELOPMENTAL EFFECTS OF DIAZEPAM ON LOCOMOTOR ACTIVITY IN 3 STRAINS OF LARVAL ZEBRAFISH (*Danio rerio*)

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Choice of strain is an important consideration in zebrafish husbandry and research. In the scientific literature there is concern that zebrafish strains may behave and respond differently to toxicants. A few studies have compared the baseline behavior of various strains of larval zebrafish (*Danio rerio*), but, to our knowledge, there are no studies on strain-specific responses to developmental neurotoxicants (with the exception of ethanol). The present group of experiments examines both the baseline behavior and the effects of developmental exposure to the anxiolytic compound Diazepam (DIA), in three commonly-used zebrafish strains: AB (AB), Tübingen (TU) and our in-house wild type, out-bred strain (Z). AB and TU embryos were obtained from Zebrafish International Resource Center (Eugene, OR) and reared onsite for breeding stock with the offspring used for testing along with same age Z breeding stock. On day 0, zebrafish embryos of each strain were plated in one of three 96-well plates (n=24-36/strain/plate) for a total of 96/strain across the 3 plates (all strains were represented on each plate). Fish were dosed daily with DIA (0, 1, 3 or 10 μ M final concentration) on days 0-4 post fertilization (dpf) and changed to buffer without DIA on days 5 and 6 (depuration). At 6 dpf locomotor activity was assessed in a 100 minute light:dark testing paradigm to determine if developmental DIA exposure had affected brain development. In general, TU controls had lower locomotor activity in the dark period compared to the AB or Z strain. Developmental DIA treatment depressed locomotor activity in all three strains, and there was no significant difference in the dose-related pattern of hypoactivity among the 3 strains. These data indicate that different strains of larval zebrafish do show locomotor behavior differences but do not show different behavioral responses to developmental DIA exposure. *This abstract does not necessarily reflect official USEPA policy.*



A SPATIAL OPPORTUNITY ANALYSIS TO INFORM AQUACULTURE DEVELOPMENT PLANNING IN SAN DIEGO BAY, CALIFORNIA

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The U.S. marine aquaculture industry is growing at an unprecedented rate, creating challenges for spatial planning and effective environmental management. As aquaculture continues to develop, spatial planning analyses that include biogeophysical and socio-economic data can be used to conduct exclusion, suitability, and opportunity type analyses. We performed a series of robust geospatial analyses for aquaculture screening and siting within and around the highly-used San Diego Bay area of California. Using a centralized geodatabase, created from federal and state data, recommendations for aquaculture development were based on needed environmental conditions for likely candidate shellfish and algae aquaculture species and associated gear types. We first analyze practical operational constraints to determine usable area for shellfish and algae aquaculture at the local scale. Within the usable area, we then consider algae and shellfish biophysical and gear-specific factors, providing further specificity to aquaculture opportunity and recommendations. Farm configuration, aquaculture function, variations in structural stress related to hydrodynamic forces, and socio-economic impacts were not included to date. This future work will increase confidence in estimated aquaculture opportunity. Using the geospatial exclusion and subsequent opportunity analyses approach, we provide a robust example for siting sustainable, resilient aquaculture in the United States.

FROSTBITE CLOWNFISH *Amphiprion ocellaris* BREEDING AND DEVELOPMENT

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The purpose of this project was to establish the quantitative genetics of F1 generations of a Frostbite x Frostbite clownfish cross. We chose the Frostbite phenotype for broodstock as it has the potential to generate Frostbite, Snowflake, Wyoming White, DaVinci, as well as the normal wild-type (i.e., “Nemo”) phenotype from a single Frostbite broodstock pair.

Four egg clutches were examined over the course of 3 months. The first and second clutches were hatched in 10-gal static tanks provided with live rotifers and RotiGrow®, followed by *Artemia* nauplii, then commercially formulated dry feeds. Water changes were conducted every few days. The third clutch was treated similarly but reared in a 20-gal static tank. The fourth clutch was hatched in a 20-gal static tank provided with live rotifers and live *Nannochloropsis*, followed by commercially formulated dry feeds (no *Artemia* nauplii). The 20-gal tank was initially filled with only 10 gal of seawater and then water was added everyday until full. After that, water changes were conducted every few days.

Offspring phenotypes (Table 1) included normal wild-type, DaVincis, Snowflakes, and a number of varieties of Wyoming Whites (including Miami White) and Frostbites (e.g., Frozen, Chilled, Flurry, and Subzero).

We aim to gain the necessary skills required to rear more difficult species within the tropical ornamental trade so as to mitigate the impacts of wild-collection. We believe that in time many in-demand saltwater aquarium species will be capable of being bred in captivity.

TABLE 1. Resulting phenotypes of 4 clutches of a Frostbite x Frostbite clownfish cross

Phenotype	1 st clutch	2 nd clutch	3 rd clutch	4 th clutch	TOTAL
Frostbite	5	6	3	50	64
Snowflake	3	1	0	16	20
Wyoming White	1	2	4	27	34
DaVinci	0	0	3	7	10
Normal wild-type	1	1	1	28	31
TOTAL	10	10	11	128	159

EVALUATION OF A COMMERCIAL - AVAILABLE SOY PROTEIN CONCENTRATE AS AN ALTERNATIVE INGREDIENT IN FEEDS FOR PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

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Changes in production technology and marketing and changes in feed ingredients are key structural transformations necessary for the aquaculture sector to grow. With improved genetic techniques, novel genetic lines are being bred for maximum efficiency over a shorter production period with lower feed conversions. Thus, the correct amount of micro-nutrients present in shrimp diets is crucial. Furthermore, rapid growth of aquaculture worldwide has become increasingly dependent upon the use of external feed inputs and the use of compound aquafeeds. Pressures to reduce fishmeal consumption for sustainability reasons, combined with economic reasons, require intensive research efforts to find suitable candidates for fish meal replacement.

Formulating low fish meal aquaculture feeds may require the use of several supplemental protein ingredients since most feedstuffs have been shown to have significant nutrient and functional limitations that do not allow their use at very high levels in the diets of most aquaculture species. Fishmeal has always been the preferred choice of nutritionists for quality protein, especially in feeds for the youngest ages of shrimp. However, due to the reasons listed above, additional renewable and sustainable protein alternatives need to be evaluated.

An alternative ingredient that can be used in aquaculture feeds to replace fishmeal is Soy Protein Concentrate (SPC). SPC is produced from the defatted flakes by the removal of the soluble carbohydrates. This can be achieved by two methods, either by ethanol extraction or enzymatic degradation. For the current feeding trial, a commercially-available SPC (Nutrivance™, Midwest Ag Enterprises, Marshall, MN) was included in the diets of Pacific white shrimp (*Litopenaeus vannamei*) at 0, 6.15, 7.45, 9.3, 10.5 or 12.4% of the diet as a replacement to fishmeal.

Nutrivance™ SPC is a quality ingredient that can be utilized in diets of Pacific white shrimp to support normal growth rates. Results obtained in the present study showed that Nutrivance™ can be used as a partial replacement of fishmeal in shrimp diets. However, when fishmeal levels are less than 3.25% of the diet, other nutrients (fat and/or cholesterol) may need to be added along with Nutrivance™ to support optimal average daily gain and maintain acceptable survivability. Further work is necessary to determine which nutrients are lacking in shrimp diets when replacing fishmeal with Nutrivance™ soy protein. Economic considerations should also be made when determining how much soy protein concentrate can be used in diets of Pacific white shrimp. Feed formulations and production data analysis for this study will be presented.

EVALUATION OF WHOLE CORN GRAIN MEAL INCLUSION IN COMMERCIAL DIETS FOR PACIFIC SHRIMP, *Litopenaeus vannamei*

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Aquaculture is expanding rapidly throughout the world. The driving forces for this expansion includes the need for additional food resources, and the recognition of marine oils and other products as healthy substitutes for other traditional products. New potential aquatic species are being studied and cultured each year creating a need for specialized formula feeds and feed ingredients. However, one of the major challenges faced by the aquaculture industries worldwide includes sourcing economical alternative ingredients for aquaculture feeds.

The shrimp farming industry is also concerned with widespread occurrence of disease. While disease is often due to bacterial and viral infections, other potential causes of disease are the environment and shrimp feed. In feed, the presence of mycotoxin contamination can be quite common, especially in humid tropical regions where the majority of shrimp are produced today. Mycotoxins are secondary metabolites of molds that can grow on agricultural grain products before harvest and when stored. As the trend to replace fishmeal and other expensive protein sources in aquaculture diets continues to grow, the use of plant-derived alternatives increases, which creates a greater chance of mycotoxin contamination of the feed.

Wheat and wheat by-products currently comprise a large part of commercial shrimp diets. A major reason why corn grain and other corn co-products are not used in shrimp feed is the presence of aflatoxin (and other mycotoxins) in corn around the world, especially in tropical regions. Considering the small incidence of aflatoxin in Minnesota corn coupled with the demonstration that corn and corn co-products could replace wheat in commercial shrimp diets without reduction in performance, a large new market for Minnesota corn could be produced.

In this study, dietary treatments for two trials were arranged in such a way that the major grain ingredient (wheat) would be replaced by a mixture of corn and corn distillers dried grains with solubles (DDGS). The first experiment used a commercial diet formulated with approximately 30% wheat and was compared to a similar diet in which approximately 70% of the wheat was replaced with a mixture of corn and corn DDGS at varying levels (0, 5, 10, 15 and 20% DDGS). The second experiment used a commercial shrimp diet formulated with approximately 30% wheat and wheat by-products (middlings) and was compared to diets using corn and corn DDGS replacing a percentage of the wheat and wheat by-products, as in the first experiment. Results of the trial indicate that corn grain, free of mycotoxins, can be included in diets of Pacific White Shrimp up to 17% and still support normal growth. Further research is necessary to determine optimum levels of corn co-products that can be added to diets that already contain corn grain. Feed formulations and production data analysis for this study will be presented.

EFFECTS OF HABITAT AND FLUCTUATING TEMPERATURES ON SOUTHERN FLOUNDER SEX RATIOS

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Southern flounder (*Paralichthys lethostigma*) exhibit environmental sex determination (ESD), where environmental factors can influence sex and masculinize populations. Flounder aquaculture as well as recreational and commercial fisheries are dependent upon females due to their faster growth and larger size relative to males, so it is important that populations not become male biased. Impacts of the environment on sex occurs during early juvenile development (30 - 65 mm) and sex reversal (masculinization) is limited to the XX female genotype. Previous data show that temperature extremes (18°C and 28°C) and background color can influence sex determination, with suboptimal parameters promoting male development. Ideal conditions at 23°C will produce at most 50% females, with much lower percentages possible due to masculinization of XX females. We also show that ESD in these fish is in part mediated by cortisol, as treatment with this stress hormone masculinizes fish. As such, environmental variables that induce glucocorticoid signaling during sex determination could impact population sex ratios. As temperatures naturally fluctuate in the wild, and in systems that are not temperature controlled (outdoor tanks/ponds), the present study first examined the effects of temperature fluctuations on sex determination in wild populations of southern flounder in North Carolina, USA. We then assessed whether the patterns of temperature variation associated with male bias sex ratios in the wild would reproduce male skewed sex ratios under laboratory conditions.

We show that northern sites (Pamlico River) had near 50:50 sex ratios from 2014-2016 (52%, 37%, and 61% male). The Neuse River, an intermediate location, produced male biased sex ratios from 2012-2016 (88%, 82%, 76%, 59%, and 82% male). In the southern locations, south of the New River, sex ratios were male skewed from 2014-2016 (88%, 86%, and 81% male). The habitats that produced male skewed sex ratios were associated with warmer temperatures, with an average of 4°C max difference between daily temperature readings across habitats. In Swanquarter Bay, a nursery habitat in the Pamlico area with 50:50 sex ratios, the average temperature over the sex determination period was 23.3°C, consistent with the temperature that promotes female development in the laboratory. Hence, we determined whether a 4°C increase or decrease under the naturally fluctuating temperature profile observed at this site would produce male-biased sex ratios with fish reared in controlled systems. We show 98% males in a constant 27°C system, while sex ratios were 83%, 65%, and 100% male in 19°C, 23°C, and 27°C fluctuating systems respectively (Fig. 1). These results indicate that controlled temperature fluctuations in the laboratory produce sex ratios consistent with that found in natural habitats.

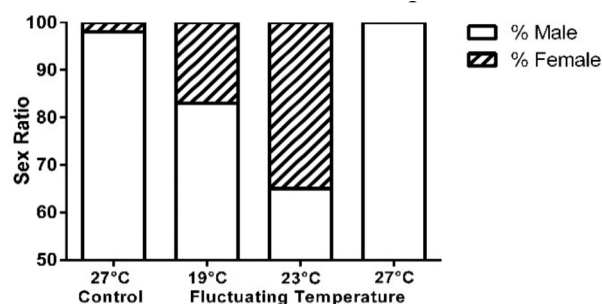


Figure 1. Sex ratios of southern flounder exposed to fluctuating temperature (19°C, 23°C, 27°C) throughout sex determination. Ratios were 98%, 83%, 65%, and 100% male for the 27°C constant, 19°C, 23°C, and 27°C fluctuating respectively. (n = 48/treatment, p < 0.0001)

MARINE FINFISH HATCHERY DESIGN: DESIGN APPROACH AND RESULTING BEAD FILTER APPLICATIONS

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In 2015 HTH engineering was hired to design a phase-one marine finfish hatchery for red drum (*Sciaenops ocellatus*) and spotted seatrout (*Cynoscion nebulosus*) in the U.S. state of Florida. These species are among the most important recreational fishes in the Gulf of Mexico and have been the subject of decades of aquaculture and stock enhancement research. However, rather than simply reinventing the wheel, we instituted a *de novo* design process that operated within the context of Florida's Aquaculture Best Management Practices to achieve our client's objectives.

With no preconceived notions, the design process found an experience-based design approach in the work at the University of Bari Aldo Moro, the Florida Stock Enhancement Research Facility, and USM's Gulf Coast Research Laboratory which resulted in the application of floating-bead, bead filters. These R&D facilities focused on the application of the right marine technology for robust and efficient RAS based operations. The RAS approach was also strongly encouraged by Florida's Aquaculture Best Management Practices developed by various Florida Agencies to encourage the development of Aquaculture in the State and to streamline the environmental permitting process.

Performance objectives include: limited effluent discharge conducive to operation using artificial seawater, low maintenance costs, small footprint, efficient filtration of fine particles, resistance to corrosion, and low pressure – low energy operations.

The design includes seawater pumping, water treatment and recovery for operating quarantine, broodstock, live feeds, and larval rearing systems. The design incorporates Polygeyser Drop Bead Filters and Bubble Wash Bead Filters for mechanical and biological filtration in Pre-treatment, Quarantine and Broodstock Holding/Conditioning RAS. Bubble Wash filters are also used for bay seawater and recovered seawater filtration, and for larval rearing RAS upstream of moving bed bioreactors.

INTEGRATION OF MOLA *Amblypharyngodon mola* IN PRAWN-CARP GHER FARMING SYSTEMS TO INCREASE HOUSEHOLD NUTRITION AND EARNINGS FOR RURAL FARMERS IN SOUTHWEST BANGLADESH

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The fish farmers of the Southwest Bangladesh Khulna region use a combined freshwater prawn (*Machrobrachium rosenbergii*) and carp (*Labeo rohita*) culture in seasonal paddy fields, a practice referred to as “gher farming”. The farmers typically sell the prawns in overseas markets to fetch higher prices, leaving family members (particularly women and children) malnourished from lack of complete protein and vitamins. The present study sought to mitigate this problem by incorporating nutrient-rich Mola (*Amblypharyngodon mola*) for home consumption into traditional prawn-carp gher farming systems. Mola is a nutrient-dense, small indigenous fish that self-recruits and lives off natural pond biota. Hence it can be grown at little additional costs to farmers, yet can provide an important source of nutrition for them.

The first experiment investigated the effect of Mola incorporation on prawn production using the following treatments: a traditional prawn-carp culture (T1), a prawn-Mola culture (T2), and a prawn-carp-Mola culture (T3) with stocking densities of 2, 1, and 0.1/m² for prawn, Mola, and carp, respectively. The biomass produced by the end of the 6-month experimental period is shown in Table 1. Results indicate that production of prawn increases, while carp production is little effected by introduction of Mola. These findings indicate that integrating Mola into prawn-carp gher farming systems has no negative impact on the production of either species and could enhance total seafood production.

A second experiment sought to optimize Mola stocking densities in prawn-carp gher ponds. Three experimental systems were examined containing 2/m² prawn, 0.1/m² carp, and either 1 (T1), 2 (T2), or 4/m² (T3) Mola. A fourth control system (T4) contained only carp and prawn stocked at the same densities. The biomass recorded at the end of the study period was 455.58±14.69, 462.77±15.60, 456.28±13.94, and 362.25±17.84 kg/ha for prawn and 588.11±16.47, 572.19±17.28, 586.75±15.39, and 502.92±16.84 kg/ha for carp in T1-4 while Mola production was 298.55±11.55, 376.21±15.34, and 397.66±18.41 kg/ha in T1-3. Considering the initial stocking weight for Mola in T2 was lower than T4, and final production was similar between the two groups, the results suggest that stocking brood Mola at a density of 2 pieces/m² in prawn-carp gher farming systems provides optimal production of all three species.

(Supported by the AquaFish Innovation Lab – USAID)

Table 1. Production (kg/ha) of prawn, Mola, and Rohu. Values with different letters are significantly different ($P < 0.05$). (mean ± SD)

	<i>Treatment 1</i>	<i>Treatment 2</i>	<i>Treatment 3</i>
Prawn	417.4±12.70 ^b	446.8±11.03 ^a	462.6±12.74 ^a
Mola	NA	308.0±20.33 ^a	255.5±23.08 ^b
Rohu	569.5±25.50	NA	573.5±28.12

DATA MANAGEMENT IN AQUACULTURE: ABIDING BY THE US FEDERAL DATA QUALITY ACT

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Data and information are valuable national and global resources. Although data sharing has increased over the last decade partly because of government and/or peer-reviewed journal mandates, many scientists never publish their datasets to publicly-available repositories and, furthermore, of those datasets that are published, many are incomplete or unusable. As AquaFish Innovation Lab (AquaFish) begins to publish their data in compliance with the U.S. mandate for increased access to federally funded scientific research, our greatest hurdles and lessons revolve around data management. As an interdisciplinary program working in 33 countries in Africa, Asia, and Latin America with nearly 300 partner institutions since 2006, AquaFish presents as a unique opportunity to explore the challenges of managing legacy datasets in international development (e.g., data in several languages, quality control) and the importance and potential impacts of successfully managing such data (e.g., augmented collaboration, decreased administrative costs, increased technology transfers to local communities).

A COMPARATIVE ANALYSIS OF FISH TRADE IN UGANDA: GENDER PERSPECTIVE

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The East African region commonly referred to as the great lakes is richly endowed with many lakes. However, Uganda as a country produces more fish than most of its neighbors in East Africa. Fish is as such traded in Uganda domestically as well as exported to her neighboring countries including Democratic Republic of Congo (DRC). Many studies have analyzed some aspects of the Ugandan domestic fish trade Jagger (2001), Hyuha et al. (2011) and Gordon et al.(2012), but comparisons of cross border fish trade along Uganda / DRC border and gender perspective has not been done. This study sought to fill the knowledge gap in order to feed into policy.

This study was conducted in Central and Uganda and DR Congo border where cross border trade in fish is active. A total sample size of 141(71males and 70 females) was purposively selected. Descriptive statistics and regression analysis were used to analyze the data.

The mean age of respondents was 36 and 37 years for male and females. The males traders had more experienced (13 years) in fish trade than female (9years). As expected, male traders were more educated than female traders (7, 5 years spent in school, respectively). Male Fish traders registered higher gross margins than females. However, the Benefit/Cost analysis showed that females were getting higher returns on investment than males because of the small volumes involved. Regression analysis showed that contract arrangements, number of times information is received and distance to selling locations significantly affected the profitability of the trade.

The results point to the fact that to facilitate trade, contract arrangement is encouraged, while reducing distances to encourage easy access to market is also important. In addition, information dissemination of information about the trade, for instance through mobile phones would be an important avenue to improving returns to fish trade.

A SURVEY OF PROTOZOAN INFECTION OF SOME COMMERCIAL FISHES IN BIDA, NIGER STATE, NIGERIA

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A study was conducted to identify the parasites of fresh water fish species in Bida, Niger State, Nigeria. Ten (10) specimens of *Tilapia*, ten specimens of *Clarias gariepinus* and *Heterobranchus spp.* were obtained from fishermen. The fish were collected and were examined using hand lens and microscope between June, July and August 2016. 13(65.00%) of these were infected. Parasites recovered were *Chilodenella sp.* 11(40.74%) *Cryptobia Iubilans* 2(7.41%), *Ichthyophthirius multifiliis* 01(3.70%) *Trichodina sp.* 08(29.63%), *Cryptobia Iubilans* 01(3.70%) *Camallanus Sp.* 04(14.81%). There was no significant difference in number of fish infected between sex, different group length and weight of the fish species. This study also revealed that the intensity and incidence of infection increased with increasing length, size, and weight of fish species. These findings are similar with the findings of Robert (2000), Mohammed *et al.* (2009), Bichi and Dawaki (2010) and Allumma and Idowu, (2011). The high infection rate in these fishes reveal the sanitary condition of the place, the location of the lake from living place. Trematodes are the most frequently encountered groups of all the parasites group isolated. There is need for more research into the life cycle of these parasites with the aim of reducing or even eliminating the load, intensity and prevalence of the parasites in fish, so as to reduce or even stop the rate of infection to man.

COMPARATIVE STUDY OF SOME PHYSICO-CHEMICAL AND BACTERIOLOGICAL PARAMETERS OF BOREHOLE AND PIPEBORNE WATER IN MINNA METROPOLIS, NIGER STATE, NIGERIA

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Physico chemical and bacteriological analyses of boreholes and a pipe-borne water at Minna metropolis were carried out. Values obtained were averaged for months, seasons, stations and zones. The values were generally low in comparison to set standards by World Health Organization (WHO NSDWQ AND APHA). Temperature was generally above the permissible limits of WHO, NSDWQ AND APHA with mean value ranges from $(25.325 \pm 0.53 - 28.250 \pm 0.27)$. The pipe borne water sources show higher temperature values when compared with boreholes sources in the study area. More so, BOD indicates a mean value between $(0.80 \pm 0.77 \text{ mg/l} - 70.10 \pm 35.89 \text{ mg/l})$ against 5 mg/L by WHO and NSDWQ, although the peak was attained in Tunga from borehole source. Similarly, COD mean value ranged between $(3.00 \pm 0.00 \text{ mg/l} - 175.25 \pm 88.97 \text{ mg/l})$. The only sample that went above the WHO permissible limit was that of Tunga borehole which was reported close to the dump site. EC, TDS, Turbidity, pH, Total hardness, F, NO_3 , DO, Phosphorus, phosphate, mg, ca, Total coli form and E -coli have their mean values below the WHO, APHA, and NSDWQ maximum permissible limits. The study shows that pipe borne water sources is safer for drinking and other domestic uses when compared with bore holes in Minna metropolis.

SURVEY OF THE FISH FAUNA OF RIVER KUNKO, DABBAN, NIGER STATE, NIGERIA

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Six (6) species from five families were sampled from River Kunko at Dabban, Niger State, Nigeria for the duration of four months study period (May – August, 2016). Family Bagridae had the highest diversity of fish species (2) i.e *Clarotes laticeps*, *Chrysichthys aluensis*, and family Cichlidae dominated by weight in shore, surface and bottom water sampling. The gill net mesh size (25.4mm) recorded the highest number of fish (13.57) and the highest weight of the fish caught (112.73) during the sampling period. While, the gill net mesh size (127.0mm) and (177.8mm) recorded the least catch in both number and weight. The analysis of variance, when considering the weight of fish shows significant variance ($P < 0.05$) among species, habitat, station and months. The interactions varied significantly ($P < 0.05$) for the sampling periods, while a non significant variations ($P > 0.05$) exist in the interaction of species and stations during the sampling period. Thus, the fish species relative composition of the river has been established and a management strategy to enhanced productivity of the water body should be established.

WATER QUALITY ASSESSMENT AND BACTERIOLOGICAL STUDY OF SOME SELECTED WELLS IN BIDA METROPOLIS, NIGER STATE, NIGERIA

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Assessment of the water quality and bacteriological study of some selected wells in Bida Metropolis was analyzed sampling water from five different wells from Banwuya road, Zungeru road, Minna road, Suleija road and Bangaie road. The analysis was conducted at Niger River Basin Laboratory Minna, Niger State, Nigeria to determine microbial load and water quality parameters of the samples collected, for the study duration of April to July, 2016. The result of the water quality assessment and bacteriological analysis of the selected wells in Bida Metropolis, Niger State, Nigeria indicated that most of the drinking water sources are contaminated with coliforms and pathogens. The overall analytical result showed that Dissolved oxygen and turbidity have exceeded the permissible limit for drinking water. While, the concentrations of Dissolved oxygen, total alkalinity, total hardness and conductivity decreased across the months, other parameters did not show this pattern. The water from the sampled wells was classified as soft water.

A GEOSPATIAL ANALYSIS OF THE SOCIAL AND ECOLOGICAL TRADEOFFS OF AIR-BREATHING FISH AQUACULTURE FOR DECISION MAKING IN A CHANGING CLIMATE

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Climate change has profound implications for freshwater aquaculture-based production of finfish, especially at small- to medium-enterprise (SME) scales. Many interrelated changes are compounded by the needs of growing urban populations, resulting in complex tradeoffs between human demands and preservation of the natural world. A geospatial analysis can help producers and planners determine the best ways to adapt freshwater finfish aquaculture practices through species diversification and site suitability, providing predictive information and climate resilient management options. The overall goal of this work is to model the complex social and ecological tradeoffs associated with the culture of climate-resilient air-breathing fish species under a changing climate. The model will inform an interdisciplinary decision framework that explicitly considers human dimension with environmental conservation in a landscape setting for helping to improve the climate resilience of freshwater finfish production. With a focus on the air-breathing pangasius catfish (*Pangasius hypophthalmus*) industry in the Mekong Delta, this study employs the use of open-source data and data models to characterize the landscape in terms of suitability and tradeoffs for pangasius catfish farms. Considering the market potential for increased income and impacts on the receiving environment, the model developed will incorporate variables such as degree-growing days, connectivity, and flood potential to provide policy makers, business owners, and other stakeholders with decision-making foresight into the associated social and ecological tradeoffs of various production priorities. Field studies in the region and physiological limits of the pangasius catfish will be used to parameterize the model, providing place-based and species specific information. Ultimately, this work serves as the basis for a map-based decision-support system focused on the cultivation of air-breathing fish to provide data-driven management and policy options.

THE NATURE AND SEVERITY OF HISTOPATHOLOGICAL CHANGES IN AFRICAN CATFISH, *Clarias gariepinus* INFECTED WITH ICHTHYOPHTHIRIASIS

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Majority of cultured fishes are infected by a protozoan ectoparasite, *Ichthyophthirius multifiliis*. With increasing investment in aquaculture and closer examination of factors such as fish diseases that contribute to the risks faced by an aquaculturist, the concept of integrated health protection is very paramount. *Clarias gariepinus*, which grows fast, easily raised in captivity, is popularly cultured in Nigeria. The parasitic infection of *I. multifiliis* mainly in the gill and skin of fish, contribute to the diverse tissue damages. Histopathological changes in the gill and skin of Ich-infected *Clarias gariepinus* was studied. A total of 120 healthy parasite-free post juvenile catfish were disinfected with 0.05% potassium permanganate. The infective stage, theront of *I. multifiliis* was obtained from the wild as a parasite source and then mass produced continuously by serial passage by cohabitation. The fish were randomly distributed into three groups with 20 fish per replicate (group A, Normal control, Group B (infected with 24,000 theronts and group C, 44,000 theronts). The skin and gill tissues (n=3) were excised and quickly subjected to histological processing. Severity of the lesion was progressively classified in three stages of tissue damage. The values considered to be non-parametric were subjected to Kruskal-wallis and Mannwhitney U test using SPSS version 20. The skin and gill of uninfected fish showed normal skin morphology such as intact collagen cells, chromatophores and intact primary and secondary lamellae. Significant differences ($P<0.05$) were observed in their lesion scores between group B and C. Histopathological changes observed in gill of group B and C were aneurysm, edema, epithelial lifting with evidence of trophont accumulation, inflammation, distortion of lamellae. In the skin, hyperemia, abscess formation, edema and inflammatory responses were observed at different degrees between groups B and C. Significant differences ($P<0.05$) in the degree of severity of lesions were observed in groups B and C. The observed tissue damages in the gill and skin of ich-infected fish proved that both routes are mainly compromised when there is an outbreak of Ichthyophthiriasis. Therefore, the need for proper sensitization in promoting aquatic life safety and proper disease management is paramount.

EVALUATION OF DIFFERENT CARBON SOURCES IN THE BIOFLOC PRODUCTION, NUTRITIONAL COMPOSITION AND RELEVANT MICROORGANISMS IN PLASTIC TANKS

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A study was conducted to investigate the different carbon sources (local and synthetic) on the biofloc production, nutritional composition and relevant microorganism for 6 weeks in a zero –water exchange culture tank. The experimental plastic tanks 30 L (A-D) used as biofloc systems (BFs) contained different water sources and inoculums; 50% tap water+50% pondwater+40g of kaolin+65g feed+ 60g corn flour, 100% tap water+40g kaolin+65g feed+ 60g natural ground corn, *Zea mays*, 100% pond water+40g kaolin+65g feed+ 60g rice bran and 100% tap water neither kaolin nor carbon source added+ 65g feed, respectively. The BFs were constantly aerated while the inoculums were maintained. The overall C/N ratio in the BFs was 13.79. Highest quantity ($P < 0.05$) of flocs produced was in BFs A, in comparison to other experimental groups. Elevated levels ($P < 0.05$) of proteins, lipids and carbohydrates were observed in BFs A, in comparison to other groups. The relevant microorganism recorded in all the flocs produced was *Lactobacillus plantarum* (4.2×10^6) and was highest in the BFs A. A linear relationship ($r = 0.86$, $p < 0.0001$) was recorded in the total suspended solids and floc volumes produced in the BFs A, indicated good biofloc formation in 50% pond water and 50% tap water+40g kaolin+65g feed than other experimental groups.

INTEGRATING AGRICULTURE WITH AQUACULTURE: ROLE OF FISH SPECIES AND POND MUDS ON GROWTH, YIELD, AND NUTRITIONAL QUALITY OF SNAKE GOURD AND INDIAN SPINACH

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Integrated farming is becoming imperative for the sustainable production of plants and animals in a manner that is both cost-effective and environmentally friendly. One example is the use of fish farm ponds to grow various vegetables. We showed in a separate experiment that the use of fertilizers in gher farming systems increases fish production by enhancing the growth of natural food sources, thus supplementing formulated feed provided by the farmers. In turn, the combination of fertilizer and feed results in a build-up of organic matter and other nutrients in the pond sediment which could be applied to pond dykes to improve the quality of the soil used for growing vegetables. The country of Bangladesh is currently experiencing a large fertilizer deficit and thus the costs associated with growing crops have risen substantially. As such, the use of pond muds could reduce costs while simultaneously increasing crop yields. The aim of the present study was to investigate the use of pond sediments from different fish culture systems on the growth, yield, and nutritional quality of snake gourd and indian spinach.

We examined the effect of using 0 (M_1), 50(M_2), or 100% (M_3) pond mud from three different aquaculture systems where either prawn and carp (P_1), prawn and Mola (P_2), or all three (P_3) were grown together to cultivate snake gourd and indian spinach. The use of 100% pond mud provided the greatest yield of both indian spinach (Table 1) and snake gourd (Table 2), regardless of what species were grown within the pond. However, gourd yield was typically higher in P_3 while spinach yield was greatest in P_1 . Variations in nutritional content (Ca, P, Fe, S, and vitamins A and C) of vegetables were also observed, although it was not consistently greater within any one treatment. These results indicate that pond muds are a viable alternative to fertilizers and could help to increase crop yields while mitigating agricultural costs for Bangladeshi farmers.

Table 1. Effects of different pond muds on growth and yield of Indian spinach.

Treatment	Plant Height (cm)	Leaf Number	Leaf Length (cm)	Leaf Breadth (cm)	Twig Weight (g)	% Dry Matter	Yield/Plot (kg)
P_1M_1	26.20	18.60	12.90	7.60	80.10	8.40	1.40
P_1M_2	29.80	20.30	12.00	7.00	82.50	8.00	2.03
P_1M_3	29.80	21.60	17.20	12.60	91.40	8.50	2.10
P_2M_1	21.30	12.50	11.70	6.70	69.30	8.30	1.53
P_2M_2	21.10	14.60	10.60	6.30	72.50	9.40	1.63
P_2M_3	24.70	18.30	11.20	8.10	84.20	7.80	1.82
P_3M_1	23.80	12.60	11.60	7.90	75.20	8.10	1.58
P_3M_2	25.10	14.70	14.60	11.70	72.20	7.80	1.57
P_3M_3	29.30	15.10	13.10	8.20	100.60	8.20	1.78
LS	NS	*	**	**	**	**	*

Table 2. Effects of different pond muds on growth and yield of Snake gourd.

Treatment	Fruit Diameter (cm)	Cavity Length (cm)	Cavity Breadth (cm)	Flesh Thickness (cm)	No. of Stripes/Fruit	Yield (kg/plant)
P_1M_1	9.80	37.60	2.99	0.39	10.80	7.01
P_1M_2	10.40	37.90	3.17	0.36	10.70	7.12
P_1M_3	10.50	39.10	3.26	0.50	10.70	7.73
P_2M_1	7.90	25.60	3.43	0.49	9.90	7.37
P_2M_2	9.20	27.60	3.11	0.50	10.20	7.44
P_2M_3	9.60	28.60	3.31	0.5	9.00	7.82
P_3M_1	10.20	36.80	2.86	0.50	9.60	7.39
P_3M_2	10.7	36.7	2.86	0.50	9.90	7.65
P_3M_3	10.80	36.60	3.05	0.49	10.60	7.84
LS	**	**	*	**	**	**

RESPONSE OF TOMATO (*Solanum Lycopersicum*) GROWTH AND YIELD WITH INTEGRATION OF FISH POND MUDS AND MANAGEMENT PRACTICES

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The sustainability of agricultural systems greatly relies on the quality of the soil in which the crops are grown. Thus, employing agronomic practices that enhance various properties of the soil (eg. aggregate stability, particulate organic matter, soil nitrates) can improve crop production. Our previous work showed that pond sediments from aquaculture systems can be used as an alternative to traditional fertilizers to enhance the growth and yield of vegetables. Further, it is known that black polythene can be used as mulch to modify the energy and water balance at the soil surface and create more favourable conditions for plant growth. The use of this material reduces the costs of irrigation and weeding, while the application of pond muds can mitigate fertilizer costs. One of the most important crops worldwide is tomato (*Solanum lycopersicum*), however production varies with season, planting time, climate, soil properties, management practices, and varietal. The present study sought to determine whether employing certain agronomical practices, namely mulching, in addition to using pond sediments from aquaculture systems to enhance soil quality would improve the growth and yield of tomato crops.

The experiments were performed in pond dyke systems in the Dumuria Upazila, Khulna District of Bangladesh and consisted of nine treatment combinations employing pond sediment from different aquaculture systems with one of three management practices (Table 1). Fertilizer was applied to the ponds fortnightly for a total of 14 times prior to being added to the tomato plots. The plots were 1.5 m x 1.5 m while the transplanted seedlings (tomato hybrid cultivar ‘Sathi’) were spaced 50 cm apart. Tomato growth and yield was typically greater in plots containing mud from fertilized ponds (P_1 and P_2), regardless of management practice (Table 2). However, the greatest yield was observed when mud from P_1 was used in combination with T_3 . The results indicate that combining the use of fertilized pond mud with mulching practices could enhance crop production.

Table 1. Experimental Design.

Species	Factor A: Pond Fertilizer			Factor B: Management Practice
	P_1	P_2	P_3	
Prawn	2/m ²	2/m ²	2/m ²	T ₁ Pond mud (100%) T ₂ Pond mud + Mulch T ₃ Pond mud + Mulch + Inorganic fertilizer
Rohu	0.1/m ²	0.1/m ²	0.1/m ²	
Mola	2/m ²	2/m ²	2/m ²	
Fertilizer (kg/ha)	Urea (15) & TSP (7.5)	Molasses (30) & Yeast (0.3)	None	

Table 2. Effect of pond muds and agronomical practices on tomato yield.

Treatment	Fruit Weight (g)	Yield (kg/plant)	Yield (kg/plot)
P_1T_1	95.5 ± 2.5 a	3.3 ± 0.14 cd	28.5 ± 0.29 c
P_1T_2	100.3 ± 6.2 a	3.8 ± 0.05 ab	30.2 ± 0.44 bc
P_1T_3	107.1 ± 4.3 a	4.0 ± 0.12 a	32.3 ± 0.44 a
P_2T_1	98.2 ± 0.8 a	3.6 ± 0.08 bc	28.5 ± 0.29 c
P_2T_2	101.1 ± 1.5 a	3.8 ± 0.04 ab	29.5 ± 0.29 bc
P_2T_3	106.8 ± 1.1 a	4.0 ± 0.8 ab	30.3 ± 0.44 b
P_3T_1	96.4 ± 1.01 a	3.1 ± 0.03 c	24.5 ± 0.29 d
P_3T_2	91.3 ± 2.48 ab	3.1 ± 0.12 d	24.7 ± 0.33 d
P_3T_3	78.9 ± 3.48 ab	2.6 ± 0.11 f	21.8 ± 0.44 e

IMPACT OF FERTILIZER ON PRODUCTION OF PRAWN, CARP, AND MOLA IN TRADITIONAL GHER FARMING SYSTEMS OF POOR RURAL FARMERS IN SOUTHWEST BANGLADESH

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In the Khulna region of Bangladesh, giant freshwater prawns are co-cultured with carps in paddy fields, a practice known as “gher farming”. The farmers, however, are typically unable to invest sufficient amount quality feed due to the associated costs, limiting production and consequently household earnings. We previously established that Mola, a small self-recruiting indigenous fish species of high nutritional value, can be integrated into prawn-carp culture, providing a source of protein and nutrients for farming households to consume without having any negative impacts on prawn production and thus earnings. In the present study, we examined the effectiveness of applying various fertilizers to gher systems in enhancing production of prawn (*Machrobrachium rosenbergii*), carp (*Labeo rohita*), and Mola (*Amblypharyngodon mola*) by increasing natural foods within the ponds. We investigated the following three fertilizer treatments, each of which were applied fortnightly: 15 kg/ha urea and 7.5 kg/ha triple super phosphate (TSP) (T1), fermented mixture of 30 kg/ha molasses and 0.30 kg/ha yeast powder (T2), combination of 50% T1 and 50% T2 (T3), and a control system receiving no fertilizer treatment (T4). The fish were stocked at densities of 2, 0.1, and 2/m² for prawn, carp, and mola, respectively. Prawn was fed 30% crude protein pelleted feed (2-3% of biomass) three days a week. Prawn production was 459.12±14.6, 471.8±15.6, and 508.8±13.9 kg/ha in T1, T2, and T3 respectively, all of which are significantly higher than the control system (396±17.8 kg/ha) (Table 1). Carp production was also enhanced by the addition of fertilizers with total biomasses of 608±16.47, 589±17.3, 633±15.4 kg/ha in T1-3 relative to 547.6±16.8 kg/ha in the control system. Lastly, Mola production was 401± 11.6, 417 ±15.3, and 440.4±18.4 kg/ha in T1-3 which again was significantly higher than that of the control system (348.4±23.3 kg/ha).

These data indicate that the addition of fertilizers to gher farming systems could be a cost-effective way for rural Bangladeshi farmers to enhance production of prawn-carp-Mola cultures. The highest biomasses were recorded in the T3 ponds, suggesting that a combination of urea, TSP, molasses, and yeast would enhance production of all three species and generate the best economic return for the farmers.

(Supported by the AquaFish Innovation Lab – USAID)

Table 1. Production (kg/ha) of prawn, mola and rohu. Rows with different letters indicate significant differences.

Treatments	T1 Urea + TSP	T2 Molasses + Yeast	T3 50% T1 + 50% T2	T4 No fertilizer
Prawn	459.12±14.6 <i>a</i>	471.8±15.6 <i>a</i>	508.8±13.9 <i>b</i>	396.8±17.8 <i>c</i>
Rohu	608±16.47 <i>ab</i>	589±17.3 <i>a</i>	633±15.4 <i>b</i>	547.6±16.8 <i>c</i>
Mola	401± 11.6 <i>a</i>	417 ±15.3 <i>ab</i>	440.4±18.4 <i>b</i>	348.4±23.3 <i>c</i>

ROS GENERATING GENES OF KURUMA SHRIMP *Marsupenaeus japonicus*: XANTHINE DEHYDROGENASE AND ALDEHYDE OXIDASE, AND THEIR GENE EXPRESSION ANALYSES AGAINST PATHOGENS

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Reactive oxygen species (ROS) play important roles in shrimp physiological reaction. Particularly, the bacteria killing mechanism by ROS in macrophages is a key function for biological defense. ROS is generated by xanthine dehydrogenase (XDH) and aldehyde oxidase (AOX), members of the molybdo-flavoenzyme subfamily. Although these enzymes are found in many vertebrates, some insects and plants, little research has been conducted on in crustaceans.

The entire cDNA sequences of XDH (*Mj*XDH: 4,328 bp) and AOX (*Mj*AOX: 4,425 bp) were cloned from *Marsupenaeus japonicus* (kuruma shrimp) using reverse transcriptase-polymerase chain reaction (RT-PCR) and random amplification of cDNA ends (RACE). Quantitative real-time RT-PCR transcriptional analyses revealed that *Mj*XDH mRNA is highly expressed in heart and stomach tissues, whereas *Mj*AOX mRNA is highly expressed in the lymphoid organ and intestinal tissues. Furthermore, expression of *Mj*AOX was up-regulated in the lymphoid organ in response to *Vibrio penaeicida* at 48 and 72 h after injection; in contrast, hydrogen peroxide (H_2O_2) concentrations increased significantly at 6, 12, 48, and 72 h after injection with white spot syndrome virus (WSSV) and at 72 h after injection with *V. penaeicida*. This study is the first to have identified and cloned XDH and AOX genes from a crustacean species and clarified the response to pathogens.

ASSESSMENT OF BLOOD HEALTH, TOTAL PROTEIN CONTENT AND GILLS HISTOPATHOLOGY IN FISH INJECTED WITH PLASTICIZERS, Di-methyl Phthalate AND Di-n-octyl phthalate

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Healthy juveniles of *Ctenopharyngodon idella* were injected intramuscularly with phthalates, Di-methyl-phthalate, (DMP), Di-n-octyl Phthalate (D-n-oP) and mixture of both Phthalates for 3, 6 and 15 days, respectively. Histo-pathological alterations in gills were examined in juveniles of *C. idella* of varied weight (61.00 ± 11.44 g) and length (8.40 ± 0.37). Analysis of condition factor (C.F.) for fish health assessment revealed that C.F values of 6- and 15-days phthalate exposures in both treatment groups showed significant lower mean values as compared to the 3-days exposed group indicating poor meat quality and toxic impact of phthalates. Comparison of total protein content in all the treatment groups showed statistically significantly highest fluctuations in the protein contents. Hematological analysis of *C.idella* revealed significant decrease in Total RBCs count, Hemoglobin concentration, Hematocrit and platelet count in all treatment groups at $p < 0.05$. Significant histological alterations in gills were epithelial lifting, fusion of primary and secondary gill lamellae, epitheliocystis, expansion of basal epithelial cells of gill arch and hyperplasia. HAI_{Gills} for phthalate injected fish followed the order: $HAI_{15-day} > HAI_{6-day} > HAI_{3-day}$.

Table 1. Fish Groups and Doses of phthalates.

Fish Groups		Dose	Exposure Period
Control group	Group A	-	-
Treated group	Group B	$1.5 \mu\text{g L}^{-1}$	3 days
	Group C	$4 \mu\text{g L}^{-1}$	6 days
	Group D	$10 \mu\text{g L}^{-1}$	15 days

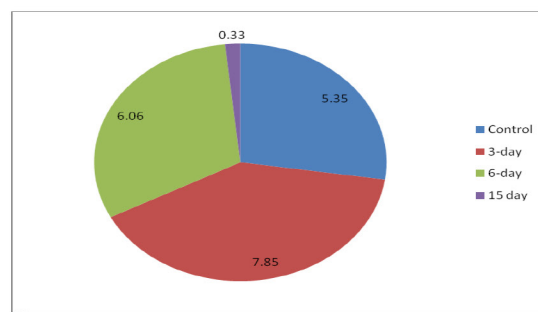


Figure 1. Comparison of total protein contents of *C. idella* in relation to phthalate toxicity.

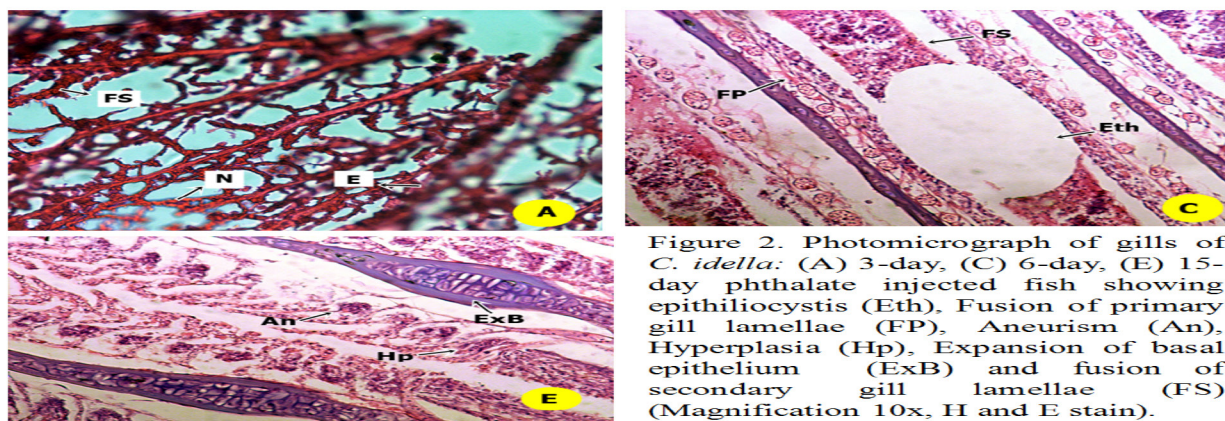


Figure 2. Photomicrograph of gills of *C. idella*: (A) 3-day, (C) 6-day, (E) 15-day phthalate injected fish showing epitheliocystis (Eth), Fusion of primary gill lamellae (FP), Aneurism (An), Hyperplasia (Hp), Expansion of basal epithelium (ExB) and fusion of secondary gill lamellae (FS) (Magnification 10x, H and E stain).

REEVALUATING THE ESSENTIAL FATTY ACID REQUIREMENTS OF NILE TILAPIA, *Oreochromis niloticus*

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A major constraint for the aquafeed manufacturing industry is the availability of cost-effective feed ingredients. Fish oil has long been utilized as an energy dense ingredient with a complete suite of long-chain polyunsaturated fatty acids (LC-PUFA). Although alternative lipid sources are plentiful, they often lack appreciable amounts of essential fatty acids (EFA). Accordingly, we evaluated the growth performance of Nile Tilapia fed diets containing different combinations of n-3 and n-6 C₁₈ polyunsaturated fatty acids (C18 PUFA) or LC-PUFA. Diets varied only in lipid source and composition, containing menhaden fish oil (positive control, “FO Control”), hydrogenated soybean oil (negative control, “EFA-Free Control”), or soybean oil amended with ethyl esters of 18:3n:3 (“ALA”), 18:2n-6 and 18:3n-3 (“C18 PUFA”), 22:6n-3 (“DHA”), 22:6n-3 and 20:4n-6 (“ARA+DHA”), or 22:6n-3, 20:4n-6, and 20:5n-3 (“LC-PUFA”). Diets were randomly assigned to quadruplicate tanks (*N*=4) of Nile Tilapia (10 fish/tank; 25.7 g/fish), fish were fed to satiation twice daily for 7 weeks. At harvest, individual fish weights were collected to analyze growth performance. Data were analyzed by one way analysis of variance, followed by Tukey’s HSD test when omnibus tests indicated significant treatment effects (*P* < 0.05).

As anticipated, fish fed the FO Control experienced significantly greater weight gain (223.4%) compared to those fed the EFA-Free Control diet (147.1%; Figure 1). However, diets containing both C₁₈ PUFA performed numerically superior (390%) compared to diets formulated with all three intact LC-PUFA (365%) (Figure 1). Results from this trial agree with historical data that Nile Tilapia can effectively utilize dietary C₁₈ PUFA.

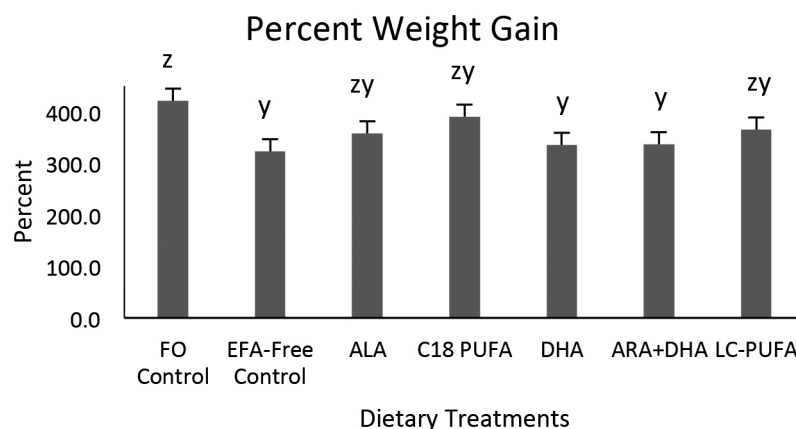


Figure 1. Weight gain (%) of Nile Tilapia fed diets with different EFA compositions. Columns represent least-squares means of triplicate tanks, error bars represent standard error, and columns with different letters are significantly different

REEVALUATING THE ESSENTIAL FATTY ACID REQUIREMENTS OF FLORIDA POMPAÑO, *Trachinotus carolinus*

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Numerous alternative lipid sources have been identified as suitable replacements for fish oil in the production of aquafeeds, however, they often do not provide significant amounts of essential fatty acids (EFA). Accordingly, we evaluated the growth performance of Florida Pompano fed diets containing different combinations of n-3 and n-6 C₁₈ polyunsaturated fatty acids (C18 PUFA) or long-chain polyunsaturated fatty acids (LC-PUFA). Diets varied only in lipid source and composition, containing menhaden fish oil (positive control, “FO Control”), hydrogenated soybean oil (negative control, “EFA-Free Control”), or soybean oil amended with ethyl esters of 18:3n-3 (“ALA”), 18:2n-6 and 18:3n-3 (“C18 PUFA”), 22:6n-3 (“DHA”), 22:6n-3 and 20:4n-6 (“ARA+DHA”), or 22:6n-3, 20:4n-6, and 20:5n-3 (“LC-PUFA”). Diets were randomly assigned to triplicate tanks ($N=3$) of Florida Pompano (8 fish/tank; 47.4 g/fish), fish were fed a fixed ration of 4% body weight per day split between two feedings for 7 weeks. At harvest, individual fish weights were collected to analyze growth performance. Data were analyzed by one way analysis of variance, followed by Tukey’s HSD test when omnibus tests indicated significant treatment effects ($P < 0.05$).

As anticipated, fish fed the FO Control experienced significantly greater weight gain (223.4%) compared to those fed the EFA-Free Control diet (147.1%; Figure 1). Although not significantly different, fish fed the LC-PUFA and ARA+DHA diets gained more weight (173%) than the negative control group (Figure 1). Results from this trial indicate that Florida Pompano benefit from the inclusion of intact dietary LC-PUFA.

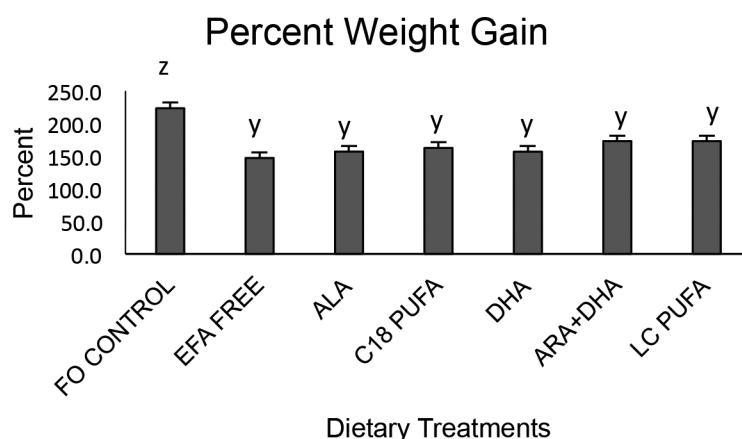


Figure 1. Weight gain (%) of Florida Pompano fed diets with different EFA compositions. Columns represent least-squares means of triplicate tanks, error bars represent standard error, and columns with different letters are significantly different

COMMERCIAL RESEARCH DEMONSTRATION TRIALS (2015-2017) USING IN-POND RACEWAY SYSTEMS TO INVESTIGATE FISH DISEASES ON CATFISH FARMS IN WEST ALABAMA

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The Alabama catfish industry is an important agriculture sector in the state of Alabama. Currently there are 85 commercial catfish farms on 17,500 water acres primarily located in seven counties in west Alabama. Mortality due to disease represents a substantial financial loss to Alabama catfish producers. In 2016, the primary sources of fish kills on Alabama catfish farms were virulent *Aeromonas hydrophila* (vAh), *Flavobacterium columnare*, *Edwardsiella ictaluri*, and toxic algae releases and represent a \$12.3 million annual loss to producers. In order to investigate different approaches to reducing disease losses on commercial farms, a collaborative effort between commercial farmers, industry, and Auburn University was devised using in-pond raceway systems (IPRS) deployed as research/demonstration production units on a commercial farm in west Alabama. In 2015, three IPRS were constructed and placed into three separate catfish ponds on a commercial catfish farm in Alabama. Each IPRS was comprised of 16 individual raceway cells (256 ft³) and were supplied with flow and aeration via a regenerative blower (1.5 HP). Each year, fish were stocked (1000 – 1350 fish per cell) in the late Spring and harvested in the Fall. Prior to stocking, catfish were vaccinated. Fish were fed (32% protein commercial feed) according to established feeding protocols twice per day on a daily basis. Water quality was monitored throughout the trial (dissolved oxygen, temperature on a daily basis; total ammonia nitrogen, total nitrite nitrogen, and pH) on a weekly basis. The Fish Health Diagnostician at the Alabama Fish Farming Center monitored fish health routinely. Mortality was tracked on a daily basis. Experimental treatments are presented in Table 1. At the end of the trial, raceway cells were harvested to determine survival, average weight, and food conversion ratio. Results from all three years of production will be discussed.

Table 1. Experimental treatments for a commercial research demonstration using in-pond raceway systems in 2015, 2016, and 2017.

2015 – Treatments	2016 – Treatments	2017 - Treatments
Control	Control	Control
Oral vaccine for vAh	Oral vaccine for vAh	Injected trivalent vaccine
Probiotic feed	Probiotic feed	Immersion trivalent vaccine without adjuvant
Hybrid catfish	Phytase feed	Immersion trivalent vaccine with adjuvant

EVALUATION OF A TRIVALENT VACCINE FOR *Aeromonas hydrophila*, *Flavobacterium columnare*, AND *Edwardsiella ictaluri* USING IN-POND RACEWAY SYSTEMS IN WEST ALABAMA

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Commercial catfish farming in the state of Alabama has been hit hard in recent years with significant losses due to bacterial disease outbreaks. Since 2015 Auburn University and the Alabama Fish Farming Center in west Alabama have been conducting trials on a commercial farm in Hale county to investigate different methods for controlling disease outbreaks. In 2017, an experimental trial is being carried out to examine three different administration methods (injection, immersion, immersion with adjuvant) for a trivalent vaccine that targets *Aeromonas hydrophila* (vAh), *Flavobacterium columnare*, and *Edwardsiella ictaluri*. These three bacteria account for a \$12.3 million-dollar annual loss for producers in Alabama. Three in pond raceway systems (IPRS) on three different ponds are being used to culture fish for this trial. Each IPRS unit consists of 16 individual 256 ft³ cells (4 replicates per treatment) with air lifts that supply flow and aeration via a regenerative blower (1.5 HP). Channel catfish that were vaccinated at Auburn University were transported to west Alabama and stocked into the IPRS units at 1000 fish per cell in early June and will be cultured until harvest this fall. Water quality (dissolved oxygen, temperature on a daily basis; ammonia, nitrite twice weekly; alkalinity, hardness, and pH biweekly) is being closely monitored along with removal and counting of mortalities in each cell. All disease outbreaks within the cells are monitored and diagnosed by the Alabama Fish Farming Center's diagnostics lab. Throughout the trial catfish are being fed (32% protein commercial feed) twice daily according to established feeding protocols. At the end of the trial catfish in each raceway cell will be harvested, counted, and group weighed. Survival (%), average weight, and feed conversion ratio will be determined. The results for each vaccine administration method will be analyzed to determine which is most effective.

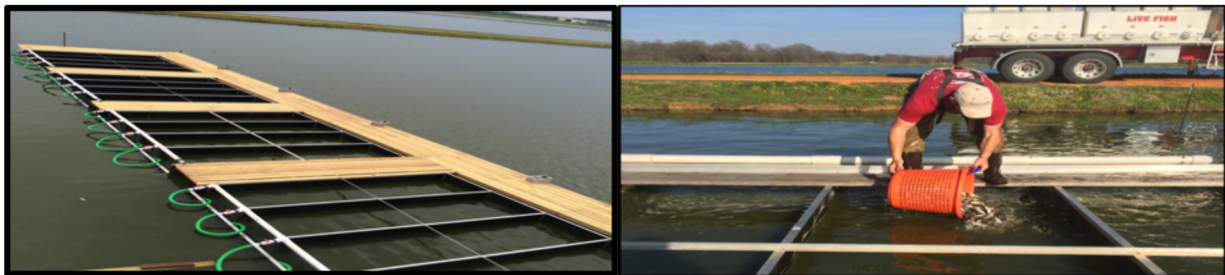


Figure 1. In-pond raceway systems were utilized to conduct a commercial trial using a trivalent vaccine for virulent *Aeromonas hydrophila*, *Flavobacterium columnare*, and *Edwardsiella ictaluri*.

GROWTH PERFORMANCE OF GIFT INFLUENCED BY VARYING CP LEVEL SOY-BASED EXTRUDED FEED AND LIMNOLOGICAL PARAMETERS

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The study was conducted in two earthen ponds with one as control in replicates for 180-days to evaluate the varying Crude Protein (CP) level soy-based diets on the growth performance of Genetically Improved Farm Tilapia (GIFT) in correlation with limnological parameters of the water viz., pH, TDS, Temperature, DO, Transparency and water level. GIFT having average body weight of 110-g was stocked in earthen ponds and was fed with two varying CP levels commercially available soy-based pelleted feed viz., 30% CP for the first 90-days and 22% CP for rest of the period, respectively, while conventional mixture of feed without soybean was used to fed the control pond. Water quality parameters were studied throughout the study period with special emphasis on water temperature management at three different levels of the pond. At the end of research trial after statistical analysis of the data it was observed that there was significant difference among the control and soy-based fed ponds in terms of growth of gift. The average maximum weight gain remained as 158 g during the month of September and the average optimum water quality parameters remained as; Temperature 20-34°C, pH 7.7-8.7, TDS 470-650 ppm, DO 5.25-8.59 ppm, Transparency 22-35 cm throughout the study period.

Growth & Limnological Parameters

Months	Treatments	Average Growth & Water Quality Parameters					
		Temp. (°C)	pH	TDS (ppm)	DO(ppm)	LP (cm)	Wt. G. (g)
June	Control	34	8.7	579	7.12	35	63
	Treatment-I	33	7.7	542	5.45	27	150
	Treatment-II	34	8.2	559	6.44	30	145
July	Control	32	8.5	581	8.59	26	54
	Treatment-I	31	7.9	540	5.93	22	140
	Treatment-II	32	8.2	546	6.41	24	151
August	Control	33	8.4	559	5.63	25	36
	Treatment-I	32	7.8	481	6.89	22	79
	Treatment-II	33	8.1	537	6.67	23	83
September	Control	29	8.7	583	7.62	33	78
	Treatment-I	29	7.7	470	5.25	25	158
	Treatment-II	28	8.2	513	6.54	27	153
October	Control	24	8.5	650	7.12	35	65
	Treatment-I	25	7.9	523	5.45	33	146
	Treatment-II	24	8.2	594	6.44	35	151
November	Control	20	8.4	534	8.59	34	43
	Treatment-I	21	7.8	598	5.93	34	117
	Treatment-II	21	8.1	501	6.41	33	119

Temp.=Temperature, TDS = Total Dissolved Solids, DO = Dissolved Oxygen, LP = Light Penetration, Wt. G. = Weight Gain, g= Grams

EVALUATION OF FOOD SAFETY RISKS IN AQUAPONIC PRODUCTION OF VEGETABLES AND TILAPIA

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Aquaponics is the integration of aquaculture and hydroponics that is now being used as a model for sustainable food production. Because fresh vegetables are usually consumed raw, there are concerns about food safety and zoonotic risks from fish waste in aquaponic production. The purpose of this study was to determine the survival, persistence, and transfer via root uptake of an attenuated *Salmonella* strain in a recirculating aquaponic system (RAS) used for leafy green production in order to gain the knowledge of good agricultural practices specific for aquaponic practitioners to reduce the potential for foodborne illnesses due to product contamination.

A prototype RAS for experimental trials was designed and built at the Center for Aquatic Biology and Aquaculture (CABA) facility at UC Davis (Figure). Initially, the lethal and infective dose of *Salmonella enterica* serovar Typhimurium (aPTVS177) strain to naïve tilapia (*Oreochromis* spp.) fingerlings was determined by intra-gastric challenge. Using two (high and low) non-lethal doses, a second group of fish was challenged and used in a laboratory controlled RAS growing hydroponic lettuce. *Salmonella* was quantified in the system components (tanks, tubing, plant bed substrate), fish waste (feces), and lettuce plants (roots and leaves) using microbiological and molecular analysis. We determined that the three highest inoculum doses (10^8 – 10^{10} CFU) resulted in fish with *Salmonella* positive gastrointestinal tracts and associated tissues. *Salmonella* recovery during phase 2 is shown in Table 1. On day 42 (harvest), lettuce leaves, roots, pots, and rafts were negative; two fish had positive stomach and intestinal tissues. Data from this study will fill knowledge gaps regarding how foodborne pathogens may persist and move through an aquaponic system.

Table 1. *Salmonella* concentration (Most Probable Number, MPN/g) in RAS components

	Samples collected	Day 1	Day 7	Day 14	Day 28*
System 1 (high inoculum)	Plant water (MPN/mL)	240	0.0061	<LOD	<LOD
	Fish water (MPN/mL)	700	<LOD	<LOD	<LOD
	Feces (MPN/mL)	>700	0.45	0.23	>70
	Bioballs (MPN/g)	58	0.047	<LOD	<LOD
System 2 (high inoculum)	Plant water (MPN/mL)	240	<LOD	<LOD	<LOD
	Fish water (MPN/mL)	700	<LOD	<LOD	<LOD
	Feces (MPN/mL)	700	<LOD	0.31	0.071
	Bioballs (MPN/g)	58	<LOD	<LOD	<LOD
System 3 (low inoculum)	Plant water (MPN/mL)	0.0017	<LOD	<LOD	<LOD
	Fish water (MPN/mL)	0.0017	<LOD	<LOD	<LOD
	Feces (MPN/mL)	<LOD	<LOD	24	0.02
	Bioballs (MPN/g)	0.052	<LOD	<LOD	<LOD
System 4 (low inoculum)	Plant water (MPN/mL)	0.0061	<LOD	<LOD	<LOD
	Fish water (MPN/mL)	0.0240	<LOD	<LOD	<LOD
	Feces (MPN/mL)	0.046	<LOD	>70	0.086
	Bioballs (MPN/g)	<LOD	<LOD	<LOD	<LOD
LOD= limit of detection, 0.00082 MPN/g					
*Treatment samples days 35, 42 and controls <LOD					

STABLE ISOTOPE ANALYSIS FOR DETERMINATION OF FEEDING HABITS OF BLUE CRAB *Callinectes sapidus* IN THE DELAWARE BAY

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Stable isotope analysis is becoming a popular method to analyze nutrient cycling which can measure traceability in fisheries. A large, tidal creek – Blackbird Creek – connects to the Delaware Bay and was used to study blue crabs (*Callinectes sapidus*) and water quality for ecological influences and blue crab fisheries sustainability. A total of 25 blue crab samples were collected in various Delaware Bay sites in 2014. Water samples were monitored for temperature, dissolved oxygen, pH, salinity, conductivity and turbidity at those sites within different marsh habitat (Spartina dominant, Phragmites dominant, mixed, agricultural, non-agricultural). Water samples were further analyzed in the laboratory for ammonia, nitrite+nitrate, total nitrogen, orthophosphate and total phosphorus. Blue crabs were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic composition using tissues from the legs and gut. Preliminary data that was assessed in 2014 determined that water quality had no influence on isotopic signature; however, the shoreline habitat influenced the $\delta^{13}\text{C}$ isotope. Preliminary data also discovered that the blue crabs were feeding higher in the food chain, thus changing the fisheries dynamics in this region. Further water and crab samples were taken once every month from July through October 2017 and crab samples are currently being analyzed. Further isotope analysis is being done to confirm our findings in 2014 regarding blue crabs feeding at higher in the food chain. Both years' data will be presented and discussed.

A SCHOOL POND EDUCATION PROGRAM FOR CREATING AWARENESS ON AQUACULTURE IN NEPAL

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Malnutrition among children is a global health concern, especially prevalent in Asia and Africa. Proper nutrition in school age children is essential for maintaining growth, cognitive development and lifelong health. Our objective was to create awareness on the nutritional value of fish as a supplement to regular diets by establishing fish ponds in schools and educating adolescent students on aquaculture. One pond each was established in four schools in the Chitwan and Nawalparasi districts of Nepal. A total of 121 students, including 57 males and 64 females, were trained in this program. Carps and tilapia were provided for each school from nearby government fish hatcheries, and were stocked in each pond at normal densities. The materials necessary to maintain ponds, including feed and fertilizer, were provided to each school. Pre- and post-training tests were conducted for participating students. Results showed the knowledge of students on fish culture and nutritive value of fish was significantly increased ($p < 0.05$). The number of students scoring $< 40\%$ decreased while number of students scoring $61-80\%$ and $> 80\%$ increased after training ($p < 0.05$). Another interesting observation was the increase in consumption of fish (13.1 ± 7.1 times per year) and its frequent inclusion in the diet after training as compared to before training (7.7 ± 3.3 , $p < 0.05$). The reason for this increased consumption was better awareness about nutritive value of fish. Surprisingly, members in 14.5% of participating students families constructed ponds after getting training. Thus, development of school ponds increased awareness on the value of nutrition and fish consumption in rural households by teaching school children and participating teachers about aquaculture.

In the second phase of program two additional fish ponds were constructed, one each in two public schools of Chitwan and Nawalparasi districts. Forty students of grade 8, 9 and 10 and three teachers were selected from each school to provide regular training on different aspects of fish culture along with role of fish in human nutrition. In addition to the new ponds, phase two also included providing water quality testing materials and nets to participating schools from both phases. In addition to student training, informal education activities were also carried out for women groups, which included forming two women's fish farming groups in the school community for each district. A linkage was developed so that the women's fish farming groups could ultimately work with the teachers and students to ensure the long term sustainability of the school ponds.

SUSTAINABLE PEARL FARMING USING NEW TECHNIQUES OF SPAT COLLECTORS IN ZANZIBAR

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Marine pearl culture is an important aquaculture industry in the world. Currently, there is a growing interest in pearl culture production among Tanzanian coastal societies primarily due to opportunity as an alternative income generation activity and also a way of using the ocean in a sustainable manner. Attempts to culture pearls have been successful but in Zanzibar and order to make it sustainable spat collection experiments were initiated in two villages of Bweleo and Nyamanzi. Community were trained on how to use these various spat collection techniques and how to maintain them until the oysters are ready for seeding. Different types of **spat collectors** were used and this will be elaborated in the presentation. The targeted species were *P. margaritifera* and *Pteria* sp. Based on the results of this study, it is possible to obtain good numbers of pearl oyster spats and grow them. It is also possible to produce relatively good quality half-pearls within a short period of 9 months using *P. margaritifera*. The establishment of such an industry would provide much needed alternative income activity among Tanzanian coastal communities while serving in utilizing coastal resources in a sustainable way. The women were also involved in the experiments and ere provided more training on entrepreneurship skills

ADDITION OF A RED MACROALGAE TO ALTERNATIVE PLANT BASED FEEDS FOR JUVENILE SABLEFISH *Anoplopoma fimbria*

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Sablefish is a cold-water marine fish of the Eastern Pacific Ocean with good economic potential for aquaculture. Previous research at our laboratory has demonstrated that sablefish have a dietary requirement for taurine when fed alternative, plant based feeds. Terrestrial plant ingredients are void of taurine, but taurine is present in considerable amounts in some red macroalgae, including Turkish Towel *Chondracanthus exasperates*. Macroalgae also contains many essential nutrients that may be limiting in terrestrial plant ingredients, including trace minerals, omega-3 fatty acids, and essential amino acids. In addition, there appears to be health benefits associated with the use of macroalgae in aquaculture feeds for some fish species. Turkish Towel, cultured intensively in land based tanks at NOAA's Manchester, WA laboratory, averages 24% protein and 0.8% taurine, on a dry weight basis. The current research study aimed to assess the potential benefits of incrementally replacing wheat flour with Turkish Towel in a typical plant based diet for juvenile sablefish, with or without synthetic taurine supplementation. The substitution of wheat flour with Turkish Towel resulted in slight increases in feed taurine concentrations.

Fish growth significantly increased with the addition of taurine ($p < 0.001$), and to a lesser extent, Turkish Towel ($p = 0.058$) to the experimental feeds (Figure 1). Feed efficiency and protein retention were significantly improved with the addition of taurine, but were not affected by Turkish Towel addition. Liver histomorphology was generally normal; however evidence of cellular alteration was present in some fish by the end of the experiment. The number of fish affected was lower among fish receiving feeds containing Turkish Towel. In particular, fish with hepatocellular nuclear pleomorphism and clear cell foci were significantly fewer ($p < 0.05$) among algae fish. The addition of taurine to experimental feeds had no effect on liver histomorphology. Overall, results from this study reaffirm taurine supplementation is beneficial to sablefish receiving plant based feeds, and indicate Turkish Towel may be a promising alternative feed ingredient for cold water marine fish.

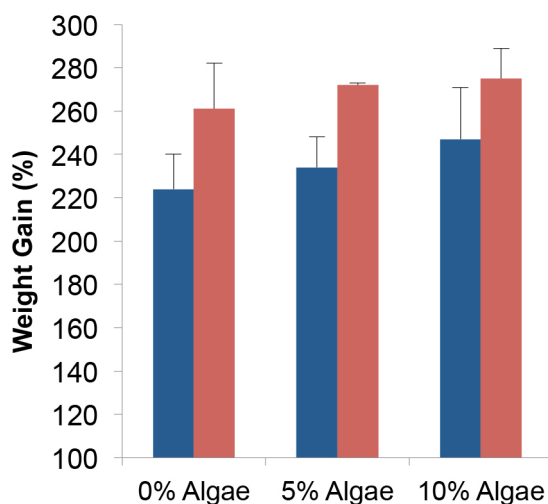


Figure 1. Sablefish growth when fed alternative feeds with no (blue) taurine or 1% (red) synthetic taurine added.

PHYSIOLOGICAL RESPONSES AND MORTALITY OF HYBRID STRIPED BASS *Morone chrysops* x *Morone saxatilis* SUBJECTED TO ACUTE STRESS AFTER BEING FED DIETARY PREBIOTIC AND PROBIOTIC SUPPLEMENTS

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Physiological changes and mortality of hybrid striped bass were monitored following two experimental stressors to study the effects of dietary prebiotic and probiotic supplementation on acute stress responses. Hybrid striped bass averaging 440 g were kept in 1200-L fiberglass round tanks in a recirculating culture system. A basal diet with 42.9 % crude protein and 13.1 % crude lipid (as fed) was formulated. Two supplemented diets with either 2% prebiotic (GroBiotic®-A, International Ingredient Corporation) or 10^7 CFU/g probiotic (Aquablend, BIO-CAT Microbials) and the basal diet were fed to 16 fish in each of two replicate tanks. An acute stress challenge was conducted after 8 weeks of feeding. Fish were maintained under low water condition with aeration for 30 min. Blood samples were collected at 0 (pre-challenge), 30 min (post-stress), and 12 h (recovery). Parameters measured were: hematocrit, blood neutrophil oxidative radical production (at time 0), as well as plasma glucose, cortisol, lysozyme, total protein/immunoglobulin, and osmolality. Experimental fish were given 1 week to fully recover, after which a transportation stressor was applied. Mortality was recorded until fish resumed normal feeding activity.

Results to date showed that: (1) All dietary groups displayed a general decreasing trend in hematocrit following the acute stress challenge ($p < 0.05$). No differences among treatments were found at any sampling time; (2) Prebiotic group had lower blood neutrophil oxidative radical production than both basal and probiotic groups ($p = 0.005$); (3) Lysozyme activity in plasma had a post challenge decreasing trend but no statistical differences were found among sampling times nor treatments. (4) No difference was found in the survival following the transportation stress. But it was worth pointing out that, numerically, the survival results agreed with a previous study in which basal group had more mortality compare to the other treatments, most of which occurred in an earlier post-challenge stage. Analysis of this experiment is ongoing and more results will be evaluated such as plasma glucose, cortisol, and osmolality.

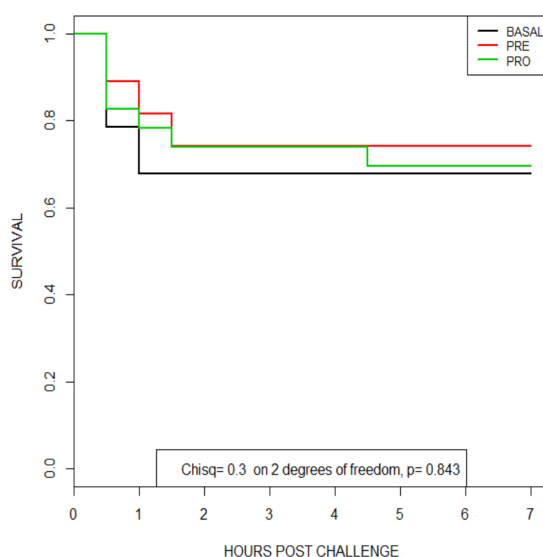


Figure 1 Kaplan-Meier survival analysis of hybrid striped bass subjected to transportation stress (log-rank test)

BRAZILIAN AQUACULTURE CERTIFICATION PROGRAM: AN INSTRUMENT OF FOOD SAFETY

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Global and national aquaculture is growing rapidly (FAO, 2012). The expansion is due to the rising of global demand for fish and byproducts, and increased awareness of the fact that aquatic resources, although renewable, are finite. Therefore, they need to be managed properly. Aquaculture's importance is an undeniable fact, but intensive and disorganized practices can lead to a variety of problems (NAVES, 2014). Product quality can be cited as one of them, for the possibility of contamination by pesticides and microbiological agents. In this context, the past few years have seen many efforts to answer public perceptions and market requirements. Food security standards were increased and international commerce regulations became more strict. Many countries have created policies and regulations. Markets acknowledge that certification is the way to assure that aquaculture products are safe to consume and come from farms that adopt sustainable management practices (NACA, 2007). In Brasil, the National Institute of Metrology, Quality and Technology (Inmetro) has been developing the Brazilian Fish Certification Program (PCPB). This program aims at fostering and collaborating to the sustainable development of the sector through the increase of fish value added and competitiveness, contributing to the country's financial development

Biography

Alessandra Julião Weyandt has over 15 years experience in the field of Quality & HSE Management, she got her PhD in Food Science and Technology from UFRRJ, Master in Animal Products Hygiene and Technological Processing from UFF, Specialization in Quality Control and Food Guarantee UFRJ, Bachelors Degree in Veterinary Medicine UFRRJ. Nowadays she works as a researcher at the at the Conformity Assessment Board - INMETRO. She is also the Aquaculture Special Study Group Coordinator at the Brazilian Association of Technical Standards – ABNT



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Category: Poster presentation

A STUDY ON THE POTENTIAL OF SEAWEED AQUACULTURE BEDS (SABs) FOR ADAPTATION TO OCEAN ACIDIFICATION AND EUTROPHICATION

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Recently there have been ever increasing recognition of kelp forests and seaweed aquaculture beds (SABs) as mitigation and adaptation measures in the context of climate change and cultural eutrophication. We conducted ecophysiological studies of several wild and cultivar seaweed species under low pH and high ammonium concentration in the laboratory. Some physiological parameters, such as pH changes, photosynthetic oxygen evolution rates, nutrient uptake rates, relative growth rates and state of chlorophyll fluorescence, were measured to reveal the effect of acidification and nutrient enrichment. All physiological responses of tested specimens were enhanced under those experimental conditions of elevated CO₂ and nutrient treatments. We speculated that seaweeds could mitigate the stresses of ocean acidification (OA) and eutrophication. In addition to the seaweed mitigation potential of organic carbon sink, the adaptation measures of seaweeds to the OA and cultural eutrophication could be implemented in the coastal waters. We would strongly propose “Seaweed Adaptation Action Plan (SAAP)” by the seaweed aquaculture beds (SABs) based on our experimental results.

DETECTION OF A MICROSPORIDIUM IN FROZEN ARTEMIA

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Hepatopancreatic microsporidiosis, caused by *Enterocytozoon hepatopenaei* (EHP), is an emerging disease of *Penaeus vannamei* and *P. monodon* culture. The disease was originally described in 2003 in *Penaeus monodon* cultured in Thailand, and the etiologic agent was later described in 2009. Since the initial report of this disease from Thailand, EHP has now been reported from several South and South East Asian countries including China, Indonesia, Malaysia, Vietnam, and India. EHP is diagnosed through H&E histology examination of hepatopancreatic tissue from suspected animals, *in situ* hybridization and PCR. The PCR diagnosis of EHP is based on the amplification of 18S rDNA gene using the primers EHP-510F (5'- GCC TGA GAG ATG GCT CCC ACG T) and EHP-510R (5'-GCG TAC TAT CCC CAG AGC CCG A) which generates a 510 bp amplicon. During routine screening of samples for EHP, an approximately 510 bp amplicon was obtained for a frozen *Artemia* sample but not from dry *Artemia* cyst sample. Since EHP has not been reported from either dry cysts and frozen *Artemia* samples, PCR amplification was carried out using EHP-specific primers based on the EHP spore wall gene. No EHP-specific amplicon was obtained for frozen *Artemia* samples.

To determine the identity of the 510 bp amplicon from frozen *Artemia*, amplicons were sequenced. The sequence analysis revealed that the ~510 bp amplicon from frozen *Artemia* origin showed similarity to *Enterocytozoon* *artemiae* that infects *Artemia*. This indicated that the primers based on the 18S rDNA gene is not highly specific for the detection of EHP, especially when frozen *Artemia* samples are screened, and alternative primers such as those based on the EHP spore wall and other genes need to be used for EHP detection in shrimp and frozen *Artemia*.

EFFECTS OF FREEZE-DRIED AND SPRAY-DRIED PREPARATIONS OF MILFORD PROBIOTIC BACTERIAL STRAIN OY15 IN PREVENTING BACTERIOSIS AND IMPROVING SURVIVAL OF LARVAE OF THE EASTERN OYSTER (*Crassostrea virginica*)

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The Milford Laboratory has researched and developed Milford probiotic bacterial strain OY15 (*Vibrio alginolyticus*), from its culture and isolation from the digestive glands of adult Eastern oysters (*Crassostrea virginica*), to pilot-scale applications to all life stages of oyster larvae. Results from larval bioassays have shown that this benign, naturally-occurring bacterium has significant positive effects upon the survival and disease resistance of larvae. OY15 improves survival of oyster larvae by 20-35% when challenged with a known larval shellfish pathogen (*Vibrio corallilyticus*) by stimulating hemocyte immune defense functions, specifically phagocytosis and Reactive Oxygen Species (ROS) release, two critical steps in pathogen elimination in shellfish.

The Milford Laboratory collaborated with Envera LLC through a Cooperative Research and Development Agreement (CRADA), utilizing Envera's specialized expertise in manufacturing and providing beneficial microbial products to the aquaculture industry, to determine if OY15 can be mass-cultured effectively and produced economically in a stable formulation for commercialization and marketing to commercial oyster growers. Envera was successful in large-scale production of OY15 and provided the Milford Laboratory with stable, freeze-dried and spray-dried bacterial formulations. In May of 2017, the probiotic team conducted a larval oyster bioassay to determine if the effects of the OY15 preparations were similar to the beneficial probiotic effects of live OY15 bacterial cells in protecting larvae from bacteriosis and improving larval survival through metamorphosis. These results will provide the insight needed to move forward toward commercialization of Milford Probiotic Strain OY15 and subsequent marketing to commercial shellfish growers in the United States.

A METHOD FOR ESTIMATING THE INNER SHAPE OF CYLINDRICAL NET- FISH CAGE

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The healthy, quality of the cultured species inside a cage lies on extent of fiber net deformation under rough environmental conditions where the cage installed. So, it is important to estimate inner shape of the cylindrical net. This helps to find issues in advance and provide opportunity for design improvement. In this study, a mathematical model proposed for estimating the shape of netting at any current conditions. A cage is composed of flexible structure: netting and rigid structures including floating collar, sinking collar. The extent of the constriction of the net depends on cage structure, and design of the mooring systems, external and internal forces acting on the members of the cage. The vertical extension of the netting structure lies on the weight in water of the sinker. Horizontal extension, bending and constrictions lies on the current velocity and stiffness of the fiber of the netting. Moreover, the deflections of the front half of the net and the rear half net is proportional to the incoming current speed to cage and inner (filtered) current speed. The calculated figures using this model compared with observed values obtained from the flume tank test at same flow current speed ranging from 0.1 to 1.0m/s at 0.1m/s and with other previously proposed models.

METAGENOMIC ANALYSIS OF PROBIOTIC EFFECT ON SHRIMP MICROBIAL COMMUNITY

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Introduction: Culturable bacteria from aquatic environments is estimated to be less than 0.1% of the living bacteria present. A high-resolution account of the complexity and diversity of bacteria in their natural environments is now possible using metagenomics. This relatively new technology provides an opportunity to phylotype and quantitate culture-independent microbial communities. Metagenomics was used to evaluate the biodiversity of bacterial communities in shrimp ponds when treated or not treated with probiotics during the most stressful period of the shrimp lifecycle.

Materials/Methods: Four adjacent five-acre shrimp ponds in the USA were used for this study; two were treated with probiotics and two were untreated. Pond water samples were collected during the last three months of shrimp grow-out, days 74, 85, and 114 post-seeding with juvenile shrimp. Samples were held at 4 °C for less than three days before centrifugation at 10,000 x g for 30 minutes. Pellets were frozen at - 40 °C and sent to Transgenada Enterprises, Inc. (Mesa, AZ) for gDNA extraction and 16s rRNA gene sequencing. Conventional bacterial culturing for *Vibrio spp.* and non-*Vibrio* organisms was carried out and water chemistry performed.

Findings: Twelve samples resulted in over 3.3 million reads. rRNA phylotyping showed three novel organisms, which diverge from their closest reference sequence by more than 2% (**Table 1**). Correlation analysis is underway to assess community composition, quantity, and shifts in prominent bacteria, probiotics, and *Vibrio species*.

Results: Preliminary analysis of these data show a very unique ecosystem with enriched novel bacteria in the water column at different times of the shrimp production cycle. With further analysis, we expect to be able to delineate the ebb and flow of major microbial players and *Vibrio spp.* populations relative to the presence/absence of probiotics.

Table 1. Novel Bacteria by rRNA Phylotyping

Enriched Novel Bacteria	Day		
	74	85	114
<i>Chthoniobacter flavus</i>	x		x
<i>Bdellovibrio bacteriovorus</i>		x	
<i>Spirobacillus spp.</i>		x	

IMPROVING GROWTH PERFORMANCE IN YELLOW PERCH (*Perca flavescens*) JUVENILES FED A SOYBEAN MEAL BASED DIET AS THEIR FIRST FORMULATED FEED

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With the aquaculture industry facing fish meal sustainability issues, plant based protein sources are becoming a requirement at higher inclusion levels in aquafeeds. One possible mechanism of adaptation for fish to utilize alternative protein sources in their diet is nutritional programming. The concept of nutritional programming can be defined as early nutritional events exerted during critical developmental stages that may result in changes expressed later in life affecting growth potential, health and metabolic status. By feeding juvenile fish a soybean meal-based diet as their first formulated feed, it is hypothesized that they will have enhanced growth performance when reintroduced to soybean meal later in life and these modifications will be passed on to from parental phenotypes to offspring.

In this experiment, the first generation of fish (F0) were nutritionally programmed and reproduced annually for three years to determine if their offspring had improved growth performance when given a fish meal or soybean meal-based diet as their first formulated feed. Nutritional programming of the F0 generation consisted of 4 phases; in phase 1 fish were separated into two groups, one group fed a fish meal (FM) based diet (control) and the other group fed a soybean meal-based (SBM) diet (75% replacement of FM protein) for 2 months. In Phase 2, fish were combined in triplicate tanks and all fed a FM diet for 9 months. In Phase 3, 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 during gametogenesis.

In the first year of spawning (2015), eggs from nutritionally programmed (F0) females from both initial dietary groups (FM or SBM) were fertilized with sperm from outside males (n=3) to minimize variability. In 2016 and 2017, F0 females from both initial dietary groups were fertilized with 3 (F0) males from the same first dietary group. Each year, F1 juveniles were reared for ~35-45 days on live feed until they reached a particular size. For juveniles in 2015/2016, they weighed 61 ± 12 mg at the start of phase 1, while juveniles in 2017 weighed 126 ± 27 mg. Siblings were equally divided into 2 tanks and fed either a FM or SBM diet for 2 months. Survival and weight gain were measured at the end of each trial.

Results of the three spawning events over subsequent years continue to show improvement in growth performance, particularly for fish fed the SBM based diet as their initial feed. Figure 1 depicts the relative weight gain after phase 1 among the F0 generation and all F1 sibling groups. A MANOVA was run to determine significance among all groups as well as between the diets. Results showed that differences among F1 generation and offspring groups as well as diet were significant ($P < 0.001$). The interaction between all groups and diet was also significant ($P < 0.05$). Overall, it was determined that crossing nutritionally programmed females and males allows for increased weight gain (%) as seen in 2016 and 2017 offspring. The size of juveniles when transitioned to the formulated feed is important, as 2017 was the largest weight at the time of stocking, which resulted in the best survival.

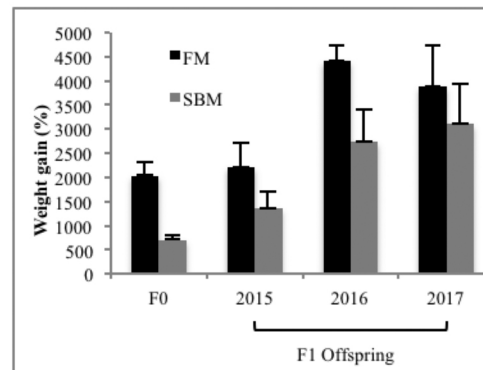


Figure 1: Phase 1 comparison of weight gain (%) among the F0 generation and F1 siblings (repeated in 2015, 2016 and 2017).

APPLICABILITY OF WASTE-REARED *Hermetia illucens* AS AN INGREDIENT IN THE DIET OF NILE TILAPIA *Oreochromis niloticus*

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We conducted an eight-week feeding trial to assess the applicability of Black Soldier Fly Larvae (*Hermetia illucens*) meal (BSFLM) and oil (BSFLO) as feedstuffs in the diet of Nile Tilapia. The BSFL used in this study was reared on dried distiller's grains with solubles (DDGS), and then was processed to produce meal and oil. Three diets were formulated to contain 32% crude protein (CP) and 8% lipid. The control diet (Control) was formulated to derive most of its CP and lipid from fishmeal (FM), soybean meal (SBM), fish oil (FO) and soybean oil (SBO), respectively. The first test diet (BSFLM) was designed to replace all FM and most of the SBM in the Control with BSFLM. The second test diet (BSFLM&O) was formulated identically to BSFLM except for a complete replacement of FO with BSFLO.

Each diet was fed twice daily and to apparent satiation to triplicate groups of 10 juvenile tilapia (49 ± 1.5 g) stocked in 100-L rectangular fiberglass tanks operating as a recirculating aquaculture system. The results (Table 1) revealed statistically significant reductions in feed efficiency ratio (FER) and protein efficiency ratio (PER) for fish fed the BSFL-based diets, while final weight (FW), weight gain (WG), specific growth rate (SGR), survival (S) and condition factor (K) were unaffected ($P > 0.05$). In addition, no significant dietary effects on health-related blood parameters (not shown) of the Nile tilapia were observed.

Our results indicated that BSFL-derived meal and oil evaluated in this study have lower nutritional value relative to FM, SBM, FO and SBO. However, the BSFL-based diets were readily accepted by the fish and supported similar growth rates. Therefore, this study demonstrated that waste reared BSFL-derived feedstuffs are suitable feed ingredients for Nile tilapia. Further studies to optimize BSFL-based diets for cultured fish species are warranted.

Table 1. Production performance parameters of Nile tilapia after feeding the experimental diets for 8 weeks.

	Control	BSFLM	BSFLM&O
FW (g)	149.2±8.4	140.6±6.4	135.5±7.1
WG (%)	204.5±15.0	183.1±13.0	173.8±10.0
SGR (%/day)	1.6±0.1	1.5±0.1	1.4±0.1
S (%)	100.0±0.0	96.7±3.3	100.0±0.0
FER	0.9±0.0 ^a	0.6±0.1 ^b	0.6±0.0 ^b
PER	2.6±0.1 ^a	1.9±0.2 ^b	1.9±0.1 ^b
K (%)	2.1±0.1	2.2±0.0	2.2±0.1

GROWTH POTENTIAL, BODY COMPOSITION AND STRESS RESPONSES IN *Pangasius hypophthalmus* WHEN CULTURED WITH INDIAN AND CHINESE CARPS

Noor Khan* and Summia Perveen

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Present study was focused to record the growth, body composition and stress responses in pangas (*Pangasius hypophthalmus*) when cultured with Indian major carps (*Labeo rohita* and *Cirrhinus mrigala*), Chinese carps (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*) and *Cyprinus carpio*. The experiment was carried out in earthen ponds with an area of 0.03 ha each, for 90 days. There were three treatments and control groups, each having two replicates. Total 400 fish were stocked in polyculture system of different species at different ratios with *P. hypophthalmus*. The fish were weighed, measured at the time of stocking and after every fortnight 10 fish of each species were captured randomly by drag net for monitoring growth indices. The results indicated highest final weight, net weight gain and final length of *P. hypophthalmus* in T₁ compared to other species and higher than T₂, T₃ and control. The FCR and % SGR values of all species in T₃ were found comparatively better than T₁ and T₂ while among species the better FCR was recorded for *P. hypophthalmus*. In case of T₃, the highest % weight gain was observed for *P. hypophthalmus* (39.2%) and common carp (49.2%). Proximate analysis showed non-significant differences among different treatments. Similarly, stress responses were found in polyculture and monoculture with minimum variation. It is concluded that polyculture of pangasius with only Indian or with mixed culture of Indian and Chinese carp proved best combination with reference to weight gain and overall fish production.

SYNERGETIC EFFECTS OF DIETARY PROBIOTICS WITH PREBIOTICS ON GROWTH PERFORMANCE OF JAPANESE EEL *Anguilla japonica*

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A 12-week feeding trial was conducted to investigate the effects of dietary probiotics *Bacillus subtilis* and *Bacillus licheniformis* with prebiotics mannan oligosaccharide (MOS) or fructo oligosaccharide (FOS) on growth performance in Japanese eel, *Anguilla japonica*. Fish averaging 12.8±0.47g (mean±SD) were randomly divided into 5 treatments of three tanks (10 fish/tank) and fed one of the five experimental diets. A basal diet without supplementation was used as control diet (Con), and four other diets were formulated by adding probiotics (10⁸ CFU/g diet) with prebiotics (5 g/kg diet); *B. subtilis* and MOS (BSM), *B. subtilis* and FOS (BSF), *B. licheniformis* and MOS (BLM) and *B. licheniformis* and FOS (BLF). At the end of the experiment at period, weight gain, specific growth rate and hepatosomatic index of fish fed BSM and BSF diets were significantly ($P \leq 0.05$) higher than those of fish fed Con diet. Although, there were no significant differences ($P > 0.05$) among fish fed BSM, BSF, BLM or BLF diets with no significant interactions (two-way ANOVA) of probiotics or prebiotics on growth performance. Therefore, these results indicated that dietary synbiotics consisting of *B. subtilis* with MOS (BSM) or FOS (BSF) could be more beneficial than other synbiotics (BLM, BLF) to the growth performance of Japanese eel.

MICROBIAL POPULATION DIVERSITY IN PACIFIC ABALONE *Haliotis discus hannai* OF DIFFERENT GROWTH RATES

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Microbial community in the intestine of marine organisms might reflect their physiological status. To study the relationship between an internal microbial population and the grow rate, hence the productivity, microbial populations in the intestine of Pacific abalone *Haliotis discus hannai* of different sizes were analyzed by using a culture-independent Illumina Mi-Seq analysis. Here we report the differences in the diversity of microbial populations depending on the sizes of the Pacific abalone. Pacific abalones of 750 days post fertilization cultured in net cage collected were divided two, small and large, size groups. Intestine of each abalone were subject to DNA purification and its quality was assessed by gel electrophoresis. Quality of metagenomic DNAs extracted were tested by using PCR with 27F and 518R primers specific to the 16S rRNA gene.

SUMMARY

- Microbial composition in intestine of Pacific abalone was identified using Illumina MiSeq.
- Different (small and large) sizes of Pacific abalones were subjected to microbiome analysis. Microbial composition in each group was compared by species proportion and alpha-diversity indices. Small size abalone showed a more diverse microbial diversity than large abalone.

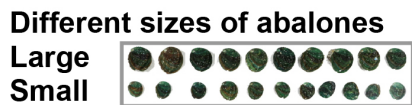


Fig.1. Photograph of Pacific abalones showing 2~6-fold difference in size

Quality control of DNA

Small abalone Large abalone

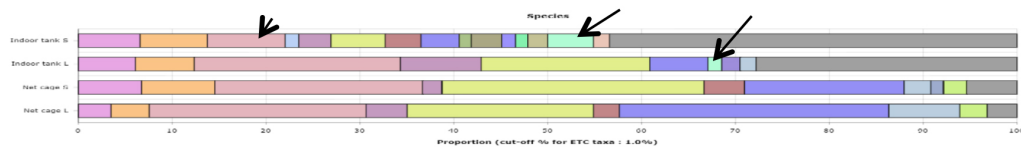
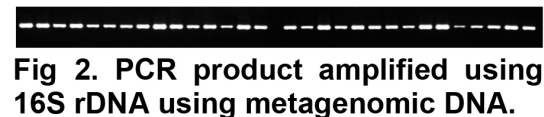


Fig 3. Bacterial composition in the intestine of small and large abalones.

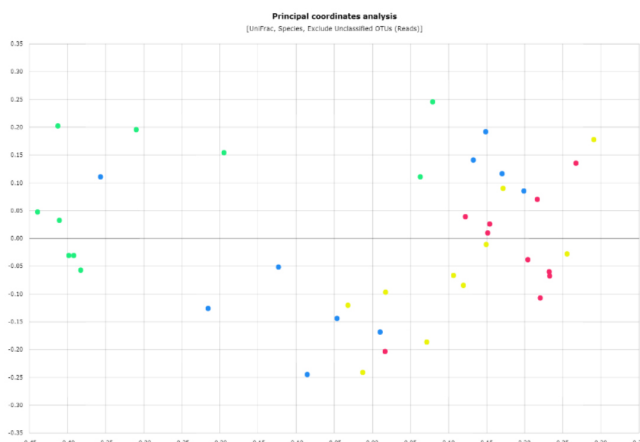


Fig 4. Principal coordinates analysis (PCoA) showing the relationship among samples.

BEAK MICROSTRUCTURE ANALYSIS OF OCTOPUS VULGARIS TO MEASURE REARING STRESS

Inyeong Kwon, Taeho Kim*

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We investigated the relationship between the beak microstructure of the common octopus, *Octopus vulgaris*, and possible stress events caused by culture conditions (i.e., keeping individuals in groups or separately) at 15°C for 15 days versus natural conditions. We used nine juveniles (age range: 55–163 days; weight range: 187.71–456.75 g) caught by local fishermen using octopus traps. The juveniles were separated into three groups (G1: natural conditions, G2: culture conditions as a group, G3: culture conditions with individuals kept separately) to analyze the amount of stress caused by rearing conditions. After the 15-day rearing period, all nine juveniles were anesthetized and then frozen, and the upper rostrum sagittal sections (RSS) of the beaks were extracted. The stress marks were observed using a high-resolution stereo fluorescence microscope (Axiozoom v.16; magnification: 10 – 112X). The mean a coefficient of variation (CV) obtained from the upper RSS readings was 1.61 ± 0.92 . Capture stress was detected in six of the nine individuals (G1, G2, and G3 combined). There was no statistically significant difference in stress between sexes ($P > 0.1$). There was a statistically significant difference in the stress levels detected between G2 and G3 ($P \leq 0.1$), with G1 exhibiting no stress lines after initial adaptation, unlike G2. In conclusion, octopuses kept in separate containers were not affected by stress after initial adaptation.

INTERACTIONS OF AMERICAN WHITE PELICANS AND SOUTHEASTERN AQUACULTURE

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USDA Wildlife Services offices in Arkansas, Louisiana, and Mississippi began receiving complaints concerning American White Pelicans (*Pelecanus erythrorhynchos*) foraging in commercial channel catfish (*Ictalurus punctatus*) ponds in 1990. Because of the relatively shallow pond depth and high fish stocking rates used by most producers, commercial catfish ponds provide a near perfect foraging environment for American White Pelicans. Damage abatement recommendations have consisted of harassment measures similar to those used for other piscivorous birds, issuance of depredation permits, and draining water from fields used as loafing sites. Since 1993, Wildlife Services, National Wildlife Research Center biologists have conducted research to learn more about pelican numbers and movements. Here, I provide an overview of past, current, and future research on American White Pelican interactions with southeastern aquaculture.

INTERACTIONS OF AMERICAN WHITE PELICANS AND AQUACULTURE IN THE SOUTHEASTERN UNITED STATES

D. Tommy King*

USDA/WS National Wildlife Research Center; P.O. Drawer 6099; MS State Univ., MS 39762
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UTILIZATION OF FISH PROCESSING WASTE AS A SUPPLEMENTAL FEED FOR SUSTAINABLE AQUACULTURE PRACTICES

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An island survey conducted by Aquafeed.com LLC in 2014, and commercial fish landings from the Division of Aquatic Resources, Department of Land and Natural Resources (published 2011) estimated 6,500 tons of fish processing waste (FPW) was being generated from Hawaii's commercial landings each year. The removal of FPW from the fish processing companies is costing upwards of \$10,000 per month to dispose. Total catch of fish landed in Hawaii is approximately 60% tuna species and 40% all other fish species. The freshness (low TVN) of the FPW being generated and dumped, suggest that a high quality ingredient for a supplemental feed could be made available for aquaculture feed industry in Hawaii and the Pacific region. The goal of this study was see if we could replace commercial imported fish feed to the islands by generating a simplified semi-moist water stable feed by grinding and cooking the FPW with the additions of a minimal amounts of ingredients such as flour and salt. For an initial study tilapia (*Oreochromis honorum*) were fed one of five diets where a control commercial tilapia feed (35% protein) was replaced with the supplemental FPW feed at 0% (control), 25% FPW, 50% FPW, 75% FPW, or 100% FPW levels. After 8 weeks, fish fed the 25% supplemental FPW feed was significantly superior in weight gain to all the other treatments. The 50% supplemental FPW feed were statistically comparable to the commercial diet in weight gain.

Another 8 week study comparing 25% supplemental FPW feed to commercial tilapia feed (35% protein) and commercial trout feed (45% protein) resulted in the trout feed significantly superior in weight gain. However, there was no significant difference in weight gain between the 25% supplemental and the commercial tilapia feed. A third 8 week trial is underway comparing 25% supplemental feed replacement FPW, 50% supplemental feed replacement FPW and commercial 35% tilapia feed.

With the high cost of disposal for fish processing waste in Hawaii and the outer Pacific islands, utilization of this high quality commercial fishery waste product for supplemental feed could enhance small aquaculture businesses and the local farming industries. Not only would it relieve the land fill/disposal burden for fish processors, FPW is a valuable raw material for the local industries, reducing cost of commercial feeds.

FKCC PRODUCT DISBURSEMENT II: CULTURED MARINE ORNAMENTAL FISH

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At Florida Keys Community College (FKCC), the primary aquaculture product of our Tropical Ornamental Mariculture Technician (TOMT) Certificate Program is trained aquaculture technicians. However, a mass byproduct of the program is cultured marine ornamental fish, including the False Percula clownfish *Amphiprion ocellaris*, the Tomato clownfish *Amphiprion frenatus*, the Gold Stripe Maroon Clownfish, *Premnas biaculeatus*, as well as a number of designer varieties of these species. We have also reared the Orchid Dottyback *Pseudochromis fridmani* and the Blue Neon Goby *Elacatinus oceanops*. Since 2015, our students have produced thousands of fish as a result of our program.

While we are currently developing internal procedures for selling our fish to distributors, wholesales, and retailers, we are also working closely with the Florida Department of Agriculture and Consumer Services, Division of Aquaculture to formally obtain our State Aquaculture Certificate of Registration. In the meantime, we have been donating fish to various K-16 educational facilities within and beyond the Florida Keys, including the Key West Preschool Co-op and the Key West Collegiate Academy charter high school. We have provided bonded pairs of clownfish fish to The University of New Hampshire's Sustainable Fisheries and Aquaculture Club. In addition, we provide researchers at Wartburg College in Iowa with clownfish for research purposes. Clownfish are a good model species of saltwater fish, are easy to rear in captivity, and large numbers can be kept in a small space.

In 2017, FKCC launched its inaugural Jr. Tropical Hobbyist summer camp. Through hands-on interactions in college labs, campers investigated live feeds, water quality, and basic tank care and maintenance. At culmination, each camper built and took home their own aquarium with an FKCC-reared clownfish.

In 2015, we launched our Adopt-a-Clownfish program. Individuals are given the opportunity to take home a clownfish of their own through an optional donation to the College. All proceeds benefit FKCC's Tropical Ornamental Mariculture Program.

Proceeds gained from rehoming FKCC clownfish will enhance the experience of the TOMT students. Currently students pay laboratory fees that cover costs such as fish feeds and system maintenance. Lowering the financial burden on these students incentivizes enrollment and makes our small college a competitor among larger schools and universities. As we refine and advance our production of aquacultured species, we gain the ability to incorporate an aquaculture business model into the TOMT program. By providing a real world example of a successful aquaculture facility, students gain another layer of education. This is quite valuable as many of our students have an entrepreneurial drive and are motivated to begin their own private venture in the marine aquaculture industry.

TABLE 1. Summary of TOMT fish culture

Minimum # of fish disbursed to date	239
# of Educ. Facilities receiving fish	4
# of interested local distributors	2
Min. potential revenue for program/yr	\$10K

ISOLATION OF *VIBRIO* SPECIES AND POTENTIAL ANTAGONISTS FROM OYSTER SPECIES FROM DIFFERENT GEOGRAPHICAL REGIONS

Susan E. Knudson*, Becky L. Rivoire, and Luke S. Keeton

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Vibrio as a genus is phenotypically diverse causing disease in marine life as well as in humans. *Vibrio* spp. have been isolated worldwide, are found in coastal waters and estuaries, and are the most common cause of disease associated with the culture of oyster and other bivalves in hatcheries and in nurseries. Juvenile oysters are most susceptible to vibriosis while adult bivalves do not suffer high mortalities when challenged with *Vibrio* spp. Not all *Vibrio* spp. found in oysters and marine life are pathogenic. Moreover, non-pathogenic bacteria found in the marine environment have the potential to be antagonists against pathogenic bacteria. Vibriosis can be catastrophic to the aquaculture industry. Finding a biological antagonist that has efficacy against *Vibrio* spp. isolated from different geographical regions would be a significant find. In this study, we collected oysters grown in different locations in the United States. Oysters were ground and plated on Thiosulfate-citrate-bile salts-sucrose agar (TCBS) for *Vibrio* isolation and on Tryptic Soy Agar (TSA) for identification of potential antagonists. Interactions were examined between isolated *Vibrio* spp. and potential antagonists using agar inhibition techniques. A subset of the data is summarized (Table 1).

One isolate had activity against 4 different *Vibrio* spp. from the two geographical regions tested. Additional isolates were identified that were active against one or two *Vibrio*. RA34 will be tested against additional *Vibrio* spp. from other regions to determine its probiotic potential.

Table 1. Agar well diffusion results.

	Hawaii		Washington	
Hawaii potentials	<i>V. vulnificus</i>	<i>V. harveyi</i>	<i>V. anguillarum</i>	Unknown
RA19	*P	**N	P	N
RA21	P	N	N	N
RA22	P	N	N	N
RA23	P	N	N	P
RA24	P	N	N	P
RA25	P	N	N	N
RA26	P	N	N	P
RA27	P	N	N	N
RA28	N	N	N	N
RA30	N	N	N	N
RA31	P	N	N	P
RA34	P	P	P	P

*P is a positive interaction and **N is no interaction

DIETARY B-GLUCAN IN SILVER CATFISH *Rhamdia quelen* DIETS AND THE SURVIVAL AFTER A CHALLENGE WITH *Aeromonas hydrophila*

João Fernando Albers Koch*, Janine Di Domenio, Lucas de Figueiredo Soveral, Fernando de Oliveira Roberti Filho and Luiz Carlos Kreutz

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Introduction: Silver catfish (*Rhamdia quelen*) is endemic in South America and has been widely used for aquaculture alone or comingled with other fish species. Fish productivity might be hampered by infectious diseases and, in this scenario, the use of immune modulators such as β -glucans to strengthen defense mechanism has been explored. Here, we evaluated the effects of β -glucans on silver catfish resistance to *A. hydrophila* challenge.

Material and Methods: One hundred and fifty fish (70-90g) were allocated equally into three groups in duplicates. The fish were kept in tanks with continuously water flux and fed *ad libitum* with commercial pelleted food (42% protein). After acclimatization, one group was kept as control (no β -glucan) and the others received 0,01% and 0,1% of β -glucans (MacroGard, Biorigin, Brazil) in the food twice a day for 42 days. Then, the fish were intraperitoneally challenged with *A. hydrophila* (2×10^8 CFU/fish). After 24h, 10 fish from each group were captured for blood sampling aiming to detect bacteremia. The remaining fish were evaluated up to 7 days post-infection to evaluate survival rate.

Conclusion: β -glucans increased silver catfish resistance against *A. hydrophila*

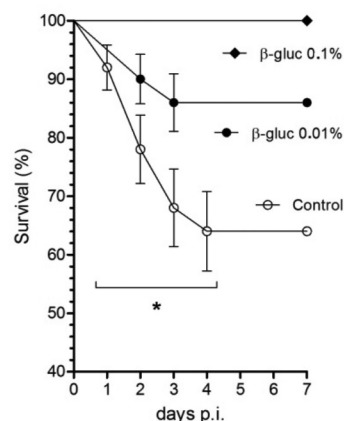


Fig1. Effect of β -glucan on survival rate of silver catfish challenged with *A. hydrophila*. The data is expressed as daily survival rate \pm SEM ($p < 0.05$) of replicates tanks. Significant differences within groups are indicated by asterisk.

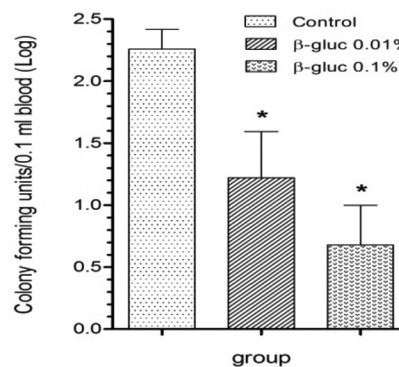


Fig2. Number of colony forming units (CFU) in the blood of silver catfish challenged with *A. hydrophila*. Significant differences from the control group ($p < 0.05$) are indicated by asterisk.

Conclusion: β -glucans increased silver catfish resistance against *A. hydrophila*

ASSESSING THE NUTRITIONAL VALUE OF NOVEL SOYBEAN MEALS IN THE DIET OF LARGEMOUTH BASS *Micropterus salmoides* L.

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Owing to the limited supply, market dynamics, and escalating prices, the aquaculture industry has witnessed a rapid shift from over-reliance on FM towards the use of more sustainable feed ingredients. Among these, soybean meal (SBM) is the foremost protein ingredient currently used in aquafeeds. However, soybean antinutritional factors can cause adverse effects in the fish, reducing production performance and survival, thereby leading to species-specific upper limits for dietary SBM. Novel processing technologies were developed to overcome such nutritional limitations and the resulting novel SBMs have potential for use in aquafeeds. Therefore, the present study aimed at assessing the relative nutritional value of a pool of commercially available SBMs in the diet of largemouth bass (LMB).

A 10-week feeding trial was conducted to evaluate three SBM products as surrogates for fish meal (FM) in the diet of LMB. Following an incomplete 2×3 factorial design including two FM replacement levels (50 and 75%), three SBM types (SBM-A, B and C), and a 35% FM control diet (FM-35), seven isonitrogenous (42% CP) and isolipidic (12% lipid) experimental diets were formulated. Each experimental diet was fed twice daily to triplicate groups of 15 LMB juveniles (15.3 ± 0.32 g/fish) stocked in 110-L glass aquaria operating as a recirculating aquaculture system.

At the end of the feeding trial, survival was above 90% for all treatments and was unaffected ($P > 0.05$) by diet. Feeding rate ranged from 3.4 to 4.1% BW/day and was significant higher for LMB fed the SBM – C diets. Despite higher feeding rate, fish in the SBM-C treatments displayed significantly lower weight gain, and feed efficiency compared to those fed SBM-A and SBM-B diets. Both SBM-A and SBM-B could replace 75% of the FM in the FM-35 diet without affecting the production performance of juvenile LMB. We also found significant ($P = 0.04$) and marginally significant ($P = 0.08$) detrimental effects on final weight (not shown) and weight gain of LMB, respectively, when FM replacement increased from 50 to 75%. These effects were likely caused by SBM-C diets alone since no other dietary treatment differed from FM-35 as analyzed using Dunnett's test ($P > 0.05$). Significant effects of dietary treatments were also observed on some of the analyzed blood parameters of LMB, which will be presented.

Dietary FM can be reduced from 35 to 8.8% replaced by SBM-A or SBM-B without detrimental effects on the production performance of LMB. Additional studies are warranted to further optimize SBM-based diets for this species.

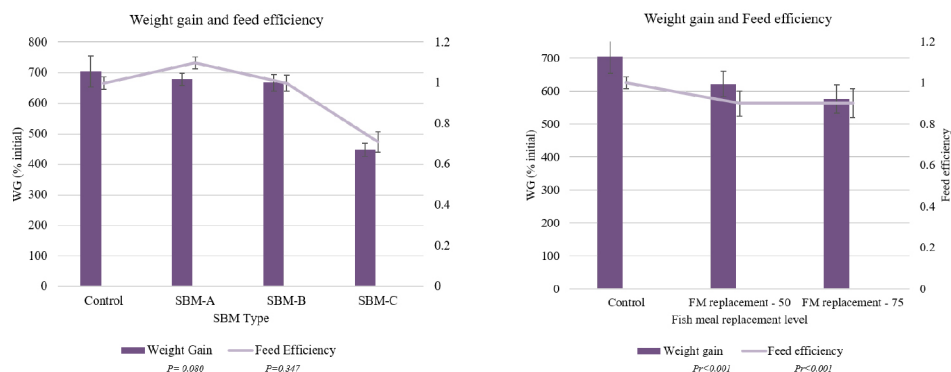


Figure 1. Weight gain and feed efficiency of largemouth bass fed the experimental diets for 10 weeks. No interactions between FM replacement level and SBM type were found ($P > 0.05$). Error bars represent SE.

WOMEN AND THE DEVELOPMENT OF AGRICULTURAL ACTIVITIES IN IVORY COAST: THE CASE OF AQUACULTURE

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Since its independence in 1960, Côte d'Ivoire has made agriculture the pillar of its development. The propaganda remarks of his first president, who states that the country's success is based on agriculture, are an illustration of this. Thus, after the colonial period, several coordinated state actions (including the establishment of agricultural services, training and agronomic research centers, seed distribution, etc.) of the population to areas of agricultural production in search of arable land for the practice not only of export crops but also of food crops.

This race for agriculture has a strong presence of women especially in food production when they do not assist their spouses in the industrial field (Koné, 2003). According to the 2001 National Census of Agriculture (NAS), they accounted for 49.20% of the proportion of farmers in Côte d'Ivoire (compared with 50.80% for men); according to the same source, they were the majority in food production. However, this strong representation of women in the Ivorian agricultural sector is experiencing profound disparities in their distribution within the sector. Sectors are neglected while others are heavily involved. This is particularly true of aquaculture, where women producers have very little presence; nevertheless, defined superficially as the water culture according to ENITA (1998), it is also considered as an agricultural practice.

The general question of the study on aquaculture development is why, unlike other sectors of agriculture, women are poorly represented in the field of aquaculture in Côte d'Ivoire? Its main aim is to show why women are not very active in aquaculture production in Côte d'Ivoire. To achieve this objective, the study is based on documentary research and field surveys carried out in selected areas according to reasoned choices.

Our investigations confirm the hypothesis of the low representation of women in aquaculture production in relation to a diversity of elements that are not favorable to their strong presence in the sector. These factors are the complexity of aquaculture production techniques for a generally illiterate female population, the problems of access to the production spaces to which they are confronted, the absence of an aquaculture tradition that is difficult to surmount for women concerned about the insurance of the daily pittance of the family. On the other hand, our study reveals their strong involvement in the distribution of products from aquaculture.

REPRODUCTIVE ABILITY OF TRIPLOID KOI X GOLDFISH HYBRID FEMALES AND RESULTS OF THEIR CROSSES WITH GOLDFISH AND KOI MALES

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Previous studies have shown that F_1 hybrid females between koi carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) produce diploid eggs due to a transformation of meiosis. Crossing of F_1 hybrid females with males of parental species resulted in appearance of triploid progeny without application of any physical treatment to early embryos. The goal of this study was to evaluate the reproductive ability of backcross triploid koi x goldfish hybrid females, which were obtained by crossing F_1 hybrid females with koi males. These triploids have in their genome two haploid sets of koi and one haploid set of goldfish.

In spring 2017, five triploid hybrid females were individually crossed with goldfish males; two of these females were also crossed with koi males. Ploidy of juveniles in obtained progenies was determined by flow cytometric analysis of DNA content. Pooled data on ploidy distribution of fish from crosses triploid hybrid females with goldfish males are shown in Fig. 1A. Most of the fish were aneuploid with ploidy range from $2.3n$ to $3.0n$ with a mean value about $2.5n$; one fish had a ploidy of $3.6n$. Ploidy distribution of fish from crosses triploid hybrid females with koi males are shown in Fig. 1B. Fish from both progenies had ploidy distributions similar to those observed in crosses with goldfish; one cross had more fish with higher values of ploidy ($3.6n$ - $4.0n$). Since aneuploid fish have in their genomes one haploid set from parental males, the data obtained indicate that triploid hybrid females produced aneuploid eggs with ploidy range from $1.3n$ to $2.0n$, and a modal ploidy level around $1.5n$. Apparently fish with ploidy range $3.6n$ - $4.0n$ resulted from spontaneous suppression of 2^{nd} meiotic division in aneuploid eggs.

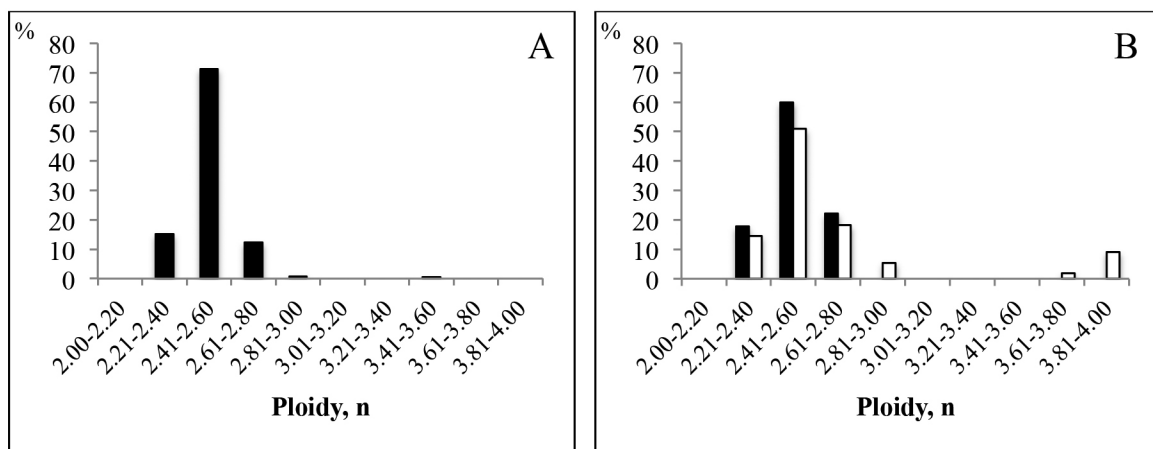


Figure 1. (A) Ploidy distribution of juveniles obtained from crossing triploid hybrid females with goldfish (n=244). (B) Ploidy distribution of juveniles obtained from crossing triploid hybrid females with koi (n=100).

POLYCULTURE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* AND JUVENILE TILAPIA *Oreochromis niloticus* IN INDOOR BIOFLOC AQUACULTURE SYSTEMS

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Biofloc aquaculture systems contain a dense community of microorganisms in the water column which is responsible for maintaining water quality and can provide supplemental feed for animals. These systems use very little water and allow for bio-secure, inland production of marine animals, as salt can be conserved. Shrimp and tilapia have very similar environmental tolerances, making them good candidates for polyculture. Producing two animal crops may allow farmers to diversify production and maximize the utilization of resources. This study examined the effects of adding juvenile tilapia (*O. niloticus*) to biofloc shrimp (*L. vannamei*) production systems; we examined water quality and animal production dynamics.

Eight pairs of two tanks were used with one tank in each pair raised above the other. Shrimp were stocked in all eight of the raised tanks while only four of the lower tanks were stocked with tilapia, creating two treatments: Shrimp only (SO) and Shrimp with Tilapia (ST). Water was constantly pumped between each pair of tanks with a screen preventing transfer of feed. Shrimp were fed a commercial diet while tilapia were not fed directly; they were left to consume biofloc particles in the water column. All shrimp tanks received the same amount of feed. Water quality parameters were measured routinely and the study lasted 76 days.

Nitrite, nitrate, and turbidity were all significantly higher ($P < 0.05$) in the ST treatment compared to SO. There were no significant differences between the two treatments regarding shrimp production. However, the total animal biomass produced was significantly greater in the ST treatment. Likewise, the total feed conversion ratio considering feed inputs and all biomass gained, was significantly lower in the ST treatment.

The results of this study indicate that significantly more biomass can be harvested in a polyculture system with the same nutrient inputs. Producers may have opportunities to operate tilapia nursery systems concurrently with shrimp biofloc production systems, thereby increasing total output. However, the burden of extra biomass on water quality needs to be considered.

	Treatment	
	Shrimp Only (SO)	Shrimp/Tilapia (ST)
Shrimp Weight (g)	10.8 ± 0.3	10.0 ± 0.2
Shrimp FCR	1.3 ± 0.1	1.4 ± 0.1
Shrimp Survival (%)	95.2 ± 0.9	89.7 ± 1.0
Shrimp Biomass (kg)	3.3 ± 0.1	2.9 ± 0.1
Total FCR	1.3 ± 0.1 ^a	1.1 ± 0.1 ^b
Total Biomass (kg)	3.3 ± 0.1 ^a	3.5 ± 0.1 ^b

EDUCATION ABOUT 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 700+ 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 in this key area.

THE CULTURE OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* WITH LIGHT RESTRICTION REARED IN BFT SYSTEMS

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In recent years, new management practices have been studied for production of food shrimp with emphasis on reduced water exchange. The biofloc technology culture system (BFT) contributes to the water quality, through the removal of nitrogen compounds, supplements the shrimp diet and allows the use of high stocking densities. In this system, in this system They may occur changes in water when exposed to long periods of solar insolation changing from a system with green water, to a brown water system, and vice versa. The present study aims to evaluate the bacterial community and zootechnical performance of *Litopenaeus vannamei* in tanks with light restriction in a biofloc 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 ($1.06\text{g} \pm 0.58$) juveniles were stocked at $400/\text{m}^3$ in a nine-35 m^3 lined raceways greenhouse enclosed. Three treatments (three replicates) were tested: T1, 24 hours night; T2, 24 hours day, and T3 12 hours night and 12 hours day. Shrimp were fed with a commercial diet (42% CP, 0.4-1.2mm, Guabi®, Campinas, SP, Brazil), offered on a feed tray (10 cm diameter, 5 mm mesh size, one per tank). At study initiation shrimp were fed 50% their total biomass. Daily rations were adjusted based on shrimp consumption and growth performance. Tanks were kept with no water exchange through the duration of the study. Water temperature, salinity, dissolved oxygen (DO) and pH was recorded twice daily (0800 and 1700) using a YSI 556 MPS meter (YSI Inc., Yellow Springs, Ohio, United States). Water was tested daily for for measuring TA-N. Monitoring of $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4^{+3}\text{-P}$ was done every five days, while alkalinity was measured once a week. Adjustments of culture water pH in all test-tanks were made anytime the levels dropped below 7.2 using 0.05 g of $\text{Ca}(\text{OH})_2$ for each liter of water (added directly into water). Water turbidity was measured once a week using a Turbidimeter (Hach 2100P, Hack Company, Loveland, Colorado, United States). Total suspended solids (TSS) and settleable solids (SS) were measured three times per week. Every week, 60 shrimps were randomly sampled from each tank and individually weighed. At the end of the trial, total shrimp biomass along with individual weights of randomly selected 200 shrimp from each tank were recorded. Water quality parameters were compared by two-way repeated measures ANOVA (system type). Significant differences of $P < 0.05$ was used in all zootechnical performance, Tukey's multiple-range test was applied when significant differences were detected. At this time the study is currently underway and this presentation will summarize the results obtained in this study.

PRECISION GENETICS FOR IMPROVING PRODUCTION TRAITS OF TILAPIA

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Global production of aquacultured tilapia is approaching 6 million tons a year and global sales exceed \$10 billion. In order to support the rapid expansion of tilapia production, we need to continue to innovate. The tilapia industry has been supported by genetic programs that use selective breeding to select for desirable production traits such as enhanced growth rates, improved feed efficiency, increased disease resistance to pathogens, and stress tolerance. These programs have been instrumental for the overall success of the tilapia industry. However, there are some production traits that are not easily addressed by selective breeding. One such trait is fillet yield.

Intrexon, a biotechnology company, has introduced the “FLT” trait into tilapia in order to improve fillet yield. The FLT trait is a recessive gene alteration that does not involve the insertion of recombinant DNA and is not transgenic. This precision breeding approach has successfully increased the fillet yield by 25% (*Figure 1*) without negatively impacting growth rate, feed efficiency, or nutritional quality of the fillets. An increased fillet yield will provide producers an opportunity to either (i) shorten the production cycle while maintaining the same amount of saleable meat, or (ii) maintain the production cycle with more fillet to sell to the market. This technology can be used to improve other production traits and can be used in other species of finfish.

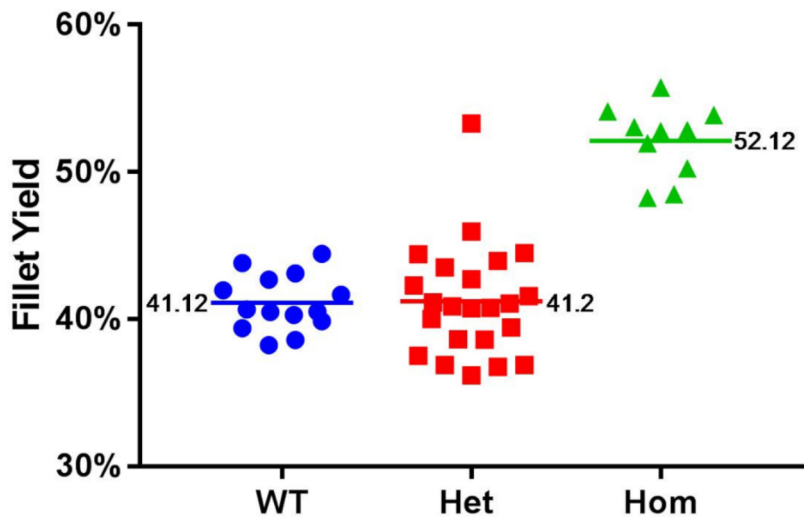


Figure 1. Improved fillet yield of tilapia (WT = wild type [WT/WT], Het = heterozygotes [FLT/WT], and Hom = homozygotes [FLT/FLT]). Representative data from five genetically distinct batches.

NON-GENETICALLY MODIFIED, IMPROVED SOYBEAN MEAL (EnzoMeal™): POTENTIAL PROTEIN SOURCE FOR FISH AND SHRIMP

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Plant proteins currently represent the only economic and sustainable protein alternative to fishmeal and are increasingly being used in commercial fish feed, with the most common being soybean meal, which has a high protein content. Recently, Ohio Soybean Council and its research partners have developed a new technology to reduce the anti-nutritional factors and enhance the protein content from conventional soybean meal (CSBM) without compromising the nutritional value. This product is called improved soybean meal (ISBM) or EnzoMeal™ (EZM). EZM is free from many ANF (anti-nutritional factor), 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 altering the amino acid composition. As such, on a cost per unit protein basis, EZM is likely an appealing protein source for most farmed finfish. Indoor studies in recirculatory systems on Yellow perch (*Perca flavescens*) and Pacific white shrimp (*Litopenaeus vannamei*) showed that 50 - 100% fishmeal protein could be replaced by EZM without compromising the growth and health.

Recently, we have conducted a pond feeding trial to evaluate the nutritional value of two soy products (CSBM and EZM) in the diet of largemouth bass (LMB, *Micropterus salmoides*). Four isonitrogenous (42% crude protein) and isolipidic (12% lipid) were formulated. A control diet (FM40): 40% fish meal, two EZM based diets (EZM60 and EZM80) were formulated to replace 60 and 80% of the FM in FM40 on an isonitrogenous basis, respectively, and an additional test diet (CSBM80) was formulated to replace 80% of the FM with CSBM. All diets were extruded into 4-5 mm floating pellets. Each experimental diet was fed twice daily to apparent satiation to triplicated groups of advanced LMB (71 g mean initial weight) stocked (6000 fish/ha) in 12, 0.05-ha earthen ponds for a 10-month period (Aug 2016 to May 2017). Upon completion of the feeding trial, we found no significant differences in feed conversion ratio (ranging from 1.4 to 1.6), feeding rate (ranging from 1.9 to 2.0 % BW/day), survival (ranging from 82 to 86%), and condition indexes of LMB across dietary treatments. However, LMB fed CSBM80 outperformed ($P < 0.05$) those fed EZM60 and EZM80 in terms of final weight and weight gain, while no significant differences were found among these treatments and FM40. The superior performance of the CSBM-based diet compared to both EZM-based diets was unexpected. One potential speculation for such responses is that the enzymatic removal of oligosaccharides in EZM may lead to the concentration of other anti-nutritional factor (s) that can be detrimental for LMB. Results for blood chemistry parameters indicated that soy-based diets might significantly affect the health and metabolism of LMB. According to our findings, both CSBM and EZM can replace 80% of the FM in the diet of pond-raised advanced LMB without negatively affecting production performance. Overall, the results of these studies enlarge the portfolio of EnzoMeal for application in the aqua feed.

EXPRESSION PROFILE OF ENERGY METABOLISM AND NUTRIENT TRANSPORT REGULATING GENES IN RAINBOW TROUT IN RESPONSE TO FASTING-REFEEDING AND POSTPRANDIAL SAMPLING TIME

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Alternations in metabolism induced by starvation and refeeding result in changes in growth and concomitant bio-chemical and cellular remodeling which is strongly associated with changes in nutrient metabolism and storage, as well as mobilization of body energy reserves. Understanding of the pre/postprandial dynamics of hepatic, intestinal and muscle gene expression in rainbow trout (*Oncorhynchus mykiss*) is still limited. Therefore, the present study was undertaken to develop protocol guidelines for the appropriate fasting and refeeding practices in fish nutrition research for gene expression measurements, and appropriate sampling time after feeding.

Groups of Hagerman-ARS strain juvenile rainbow trout (average weight of 82.4 ± 8.5 g) were subjected to three fasting and re-feeding protocols, and tissues sampled for gene expression measurements. Tanks were assigned in triplicate to each level of each factor with 12 fish per tank (i.e. 51 tanks). A completely randomized, factorial design with two independent variables (preprandial fasting duration and postprandial sample time) was used to assess differences in gene expression in three separate tissues (intestine, liver and muscle) in rainbow trout. Non-fasted fish (Group 1) were compared to two levels of preprandial fasting: a 24 h fast followed by a single meal (Group 2) and a 48 h fast followed by a single meal (Group 3). Postprandial sampling was carried out at 3, 6, 9, 12, and 24 h for all groups. Group 1 fish were fed for 14 days and sampled post-prandially after their final meal. Group 2 fish were fed for 14 days, fasted for 24 h, refed a single meal and sampled. Group 3 fish were fed for 14 days, fasted for 48 h, refed a single meal and sampled.

In intestine, genes encoding for glucose transporters (GLUT1, GLUT4), amino acids (PEPT1) and long-chain fatty acids (FABP2) showed differential expression associated with post-prandial sampling time. At the 3 h and 6 h post-prandial sampling time, all experimental groups showed remarkably different pattern for GLUT4. A similar pattern was recorded for FABP2 at 3 h post-prandial sampling time. In hepatic tissue, the expression level of genes involved in glycolysis, the pentose phosphate pathway and the citric acid cycle changed over time and with preprandial fasting duration. As such, mRNA level of glucokinase (GK: hexokinase IV) in the liver were initially (3h – 6h) low in both fasted groups compared to non-fasted counterparts but gradually augmented overtime and surpassed non-fasted fish significantly at 24 h. Similar to Glucose-6-phosphate dehydrogenase (G6PDH), expression level of cytosolic malate dehydrogenase (MDH) in non-fasted fish were relatively higher than both fasted fish till the end of the experimental period (Fig 1). Expression of genes associated with fatty acid biosynthesis (e.g. Hepatic carnitine palmitoyltransferase CPT1a, fatty acid synthesis FAS, Peroxisome proliferator-activated receptor PPAR α) in hepatic tissues was highly variable over time and affected by preprandial fasting duration. In addition, we also investigated the expression pattern of myogenin (MYOG), Calpain inhibitor (CASTL) and Atrogin 1 (ATRO1) in muscle tissue.

Overall, our study offers a comprehensive analysis at gene level as how rainbow trout copes with events of food deprivation and consequent refeeding.

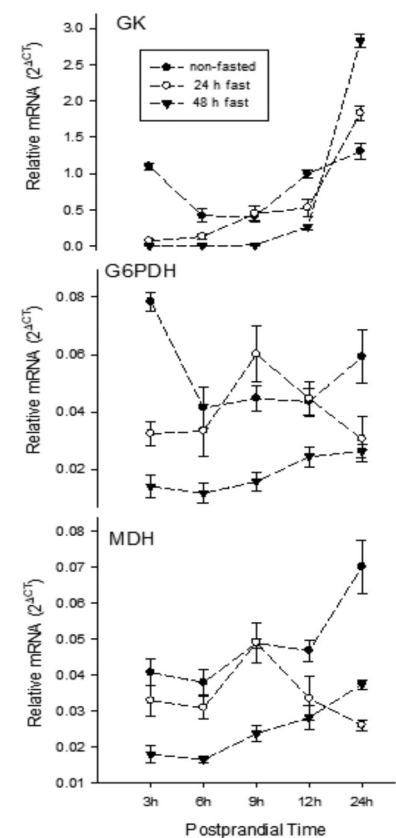


Fig 1: Relative gene expression

SEASONAL VARIATION OF FISH MIGRATION IN SARIAKANDHI FISH PASS

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The importance of open water fish in our socio-economic regime has recently drawn the attention of the policy makers of the country. FCD/FCDI projects mainly serve the agricultural interests, but it interfere fish migration. This inevitably affects the open water fisheries sector as migratory routes. Nursing grounds of many species of fish are hampered and disturbed for these projects also. In order to permit fish migration in rivers, it is necessary to maintain conditions that help migrants reach their spawning grounds. To overcome obstacles, such as hydraulic structures, placed in the path of migrating fish, structures must be designed to assist the fish to pass them. The periodic and directed travel of fish mainly for feeding, breeding and over coming adverse climatic conditions is called migration. Fish passes are constructed to allow normal breeding migration and to ensure natural route of fish movement.

The concept of a fish passes is relatively new in Bangladesh. At present, two Fish passes and two fish friendly structures are constructed. These are Fish Pass in Jamuna to Bangali River at Sariakandi in Bogra, fish Pass in Kawadighi Haor of Monu river in Moulovibazar, fish friendly structure in Lohajong river of Tangail and fish friendly structure at Morichardanra in Chapainawabganj. Fish fry, spawning and hatchling movement from Jamuna to Bangali River was the main objective of Sariakandi Fish Pass Project. The Fish Pass Project of Sariakandi is necessary for the development of the dominant fishes like catfish and small fishes. The structures will also aid in efficient development of the carp fishes. Spawning migration, mainly in carp fish, in the study area was found to begin at the 2nd week of May and continue up to the 3rd week of July. Catfish migrations began at the last week of March and continue up to the 2nd week of June.

Seasonal variation was found in four periods of fish migration; firstly over-wintering or dry season migration, in this period the large size carps and small catfish especially Magur and Shing showed peak migration. Secondly spawning migration season, small size catfishes showed peak migration. Thirdly nursery/ grow out migration season, carps, catfishes and other fishes showed peak migration during this season. And fourthly flood recession migration season, large size carps and catfishes also showed peak migration.

CHEMICAL COMPOSITION OF NILE TILAPIA *Oreochromis niloticus* FINGERLINGS CHALLENGE BY DIETARY FUMONISINS B₁ + B₂

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Toxicity of fumonisin (FB) has been considered for different fish species. In fishes, although many effects of fumonisins have been shown, were not found reports of their effects on the muscle chemical composition and fatty acid profile. Thus, we aimed to evaluate the effects of different levels of fumonisin on chemical composition in carcass of tilapias fingerlings.

A total of 180 sex-reversed male fingerlings of GIFT × Thai breed group, with an average weight of 2.64 g, were used. Fishes were distributed into three fiber cement tanks with a useful volume of 900 L each, with an individual water restoration system (15%/day) and constant aeration through a porous rock coupled to a central air blower. Four hapa net cages were inserted into each tank. Isocaloric and isoproteic diets were formulated considering the concentration of FB₁ + FB₂. Experimental groups were formed. Control diet, no FB; 20 mg FB kg⁻¹ (16.36 mg g⁻¹ FB₁ + 3.64 mg g⁻¹ FB₂); 40 mg FB kg⁻¹ (32.71 mg g⁻¹ FB₁ + 7.29 mg g⁻¹ FB₂); and 60 mg of inclusion of FB kg⁻¹ (49.07 mg g⁻¹ FB₁ + 10.93 mg g⁻¹ FB₂). Carcass was collected after slaughter for fatty acids profile analysis.

Moisture showed quadratic effect of regression ($Y = -8.682x^2 + 20.06x + 68.03$; $R^2 = 0.688$); ashes showed linear effect of regression ($Y = 0.205x + 1.465$; $R^2 = 0.811$); crude protein was significantly lower to the higher dose (FB60 = 15.38 ± 0.54 , $P = 0.0003$) than other treatments (control = 17.82 ± 0.63 , FB20 = 17.07 ± 0.2 , FB40 = 18.16 ± 0.25); and total lipids showed quadratic effect of regression ($Y = 0.140x^2 - 0.806x + 1.796$, $R^2 = 0.868$). Fumonisin reduces the weight gain in fishes to every level of contamination studied. FB60 level decreases crude protein in Nile tilapia fingerlings. Fumonisin alters chemical composition on Nile tilapia fingerlings.

Table 1. Chemical composition of Nile tilapia fingerlings muscle fed with increasing levels of fumonisin B₁ + B₂

	Crude Protein(%)			Moisture		Mineral matter (%)			Total lipids (%)	
Control	17.82	±	0.63	80.53	±	0.26	1.76	±	0.09	1.36 ± 0.093
FB20	17.07	±	0.20	82.36	±	0.22	1.69	±	0.20	1.02 ± 0.172
FB40	18.16	±	0.25	80.20	±	0.14	2.17	±	0.04	0.67 ± 0.008
FB60	15.38	±	0.54	80.76	±	0.56	2.28	±	0.10	1.04 ± 0.069
CV%	2.61			0.42			6.41			10.1
P value	0.0003			0.0002			0.0004			0.0003

Values based on the natural matter.

GLOBALIZATION OF SEAFOOD MARKETS – ANALYZING MARKET INTEGRATION BY DEVIATIONS FROM COMMON PRICES

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Aquaculture has boosted global trade and led to increasing commodification of many seafood products. Along with other developments such as lower transportation cost, improved freezing technology and growth of supermarkets this has induced stronger competition among seafood producers globally (Anderson, Asche and Tveteras, 2010). This is documented in a growing body of studies that estimates competition among wild-caught and farmed seafood products, and for different kinds of fish species. Tveteras *et al.* 2012 discuss markets interactions between fisheries and aquaculture on a more aggregate level using the FAO Fish Price Index.

We have access to over 100 price series from white fish, salmon, shrimp, tuna and pelagic species based on seafood imports to EU, Japan and USA. To tests market integration and delineation among species and products for such a large number of price series we create a common price for each main species groups. We then investigate the degree of integration by how analyzing the deviations of the individual price series from the the common prices. The results indicate increasing market integration over the data period spanning from 1990 to 2017.

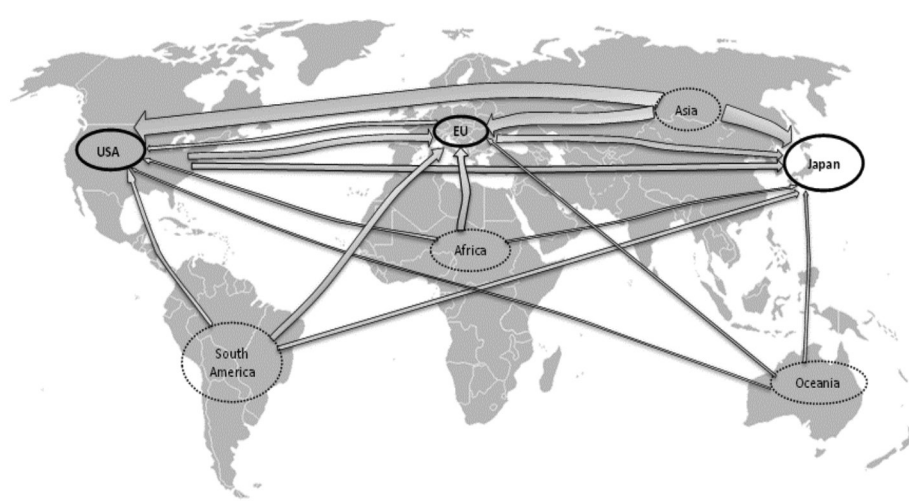


Figure 1 The global seafood trade map indicates the size of the trade flows in 2011 that corresponds to our data set

CULTURE OF PACIFIC DULSE *Palmaria mollis* IN LAND-BASED SYSTEMS

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The red seaweed Pacific dulse (*Palmaria mollis*) is found in the low inter-tidal and subtidal zones on the US West coast. Atlantic dulse, *P. palmata*, has been used as a human food for centuries and is still harvested as an ingredient in local dishes and for health supplements. *P. mollis* contains up to 20 % protein (dry weight) and is a rich source of trace minerals, such as iodine.

Research at the Hatfield Marine Science Center and the Food Innovation Center, Oregon State University, has focused on expanding development of Pacific dulse as a sustainable, healthy human food. Reduction in the economic costs of land-based culture has been achieved by simplification of the nutrient mix used to fertilize the dulse. Batch culture of dulse, without a requirement for a continuous supply of seawater, has proven possible with no adverse effects on growth rates. In addition, successful use of artificial seawater for dulse culture will allow dulse farming at locations distant from sources of seawater but close to urban centers where demand is likely to be highest.

Sensory evaluations of dulse alone and as a food ingredient have been undertaken at the Food Innovation Center. Dulse plants that differed in appearance due to culture conditions and genetic factors, were on average found to be similar in their likeability by panelists, although individual panel members differed in their preferences. Dulse added as an ingredient to salad dressing and rice crackers were highly liked by panelists.

Overall, we have found that dulse can be cultured under batch conditions using artificial seawater and with additions of a simplified nutrient mix. The potential for dulse as a human food or as a component of animal feeds is considerable. Dulse could be a valuable source of protein in a future with limited supplies of freshwater and an increasing human population.

A SEAWEED APPROACH TO SEAWEED AQUACULTURE, IMPLICATIONS FOR THE TECHNOLOGY OF PRODUCTION, PROCESSING AND PRODUCT QUALITY

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Seaweeds constitute the largest single contributor to the current production of aquaculture products and, with a growing human population and corresponding demand for food security, they will play an increasingly important role in meeting the world's food requirements. Seaweeds also supply a suite of ecosystem goods and services and nonfood related products that contribute to the value of marine plant aquaculture. In addition to food *per se*, seaweeds are a source of biomass for fuel production, specialty biochemicals, fertilizers, animal feeds, nutrient scavengers, pH regulation, carbon sequestration and potential habitat for marine animals. As seaweed production scales up, however, there are both technological and ecological challenges that must be balanced to maximize benefits and minimize negative impacts. With any intensive culture system there are both anticipated and unforeseen issues that will come into play. In the case of seaweed, issues of scale become important if one is interested in, for example, the production of biomass for fuel; then there is substantial waste from such endeavors. Intensive monocultures can also promote disease, and in the case of seaweeds eutrophication could compromise the surrounding waters. Further, the concept of integrated multi-trophic aquaculture depends on balancing nutrient flux at a farm scale by understanding the specific plant/animal interactions. This presentation will briefly review the obstacles and advantages of developing seaweed aquaculture, with a focus in the US, from an ecosystem perspective.

EFFECTS OF FEED PROBIOTIC LEVELS ADMINISTERED TO SHRIMP IN A BIOFLOC, NO EXCHANGE TANK SYSTEM

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The incorporation of probiotics in feed can contribute to overall health by increasing immune function which may in turn improve production. The present study examined the effects of topdressing shrimp feed (Zeigler HI-35) with Aquastar®, a probiotic manufactured by Biomin, Inc., on shrimp reared in a biofloc tank system. A four factorial random block design (0, 2, 3, and 5 g/kg) trial was conducted in 1.13 m³ (1.45 m dia) cylindrical HDPE tanks operated with air-driven floc circulation systems. Biofloc from the HBOI-FAU IMTA ex-situ bioreactor (16 L) was used to inoculate the culture medium and one hundred 1.5 g shrimp were stocked (88.5/m³) in each tank. Tanks were maintained with no water exchange and only freshwater was added to offset evaporation losses. Feed was delivered 24/7 using belt feeders. Growth was assessed weekly while survival, growth, and FCR were determined at the study termination (12 weeks). Water quality parameters were measured twice daily (DO, temperature, salinity), daily (pH, SS), or twice per week (TSS, TAN, NO₂, NO₃). Water samples were collected weekly and plated in triplicate on TCBS media. At trial termination water and shrimp intestines were plated onto Marine agar, TCBS, and MacConkey's agar.

Shrimp reached an average weight of 23 g with no statistically significant difference ($P = 0.25$) between treatments (Table 1). Although survival was higher and FCR was lower in the treatment group containing higher levels of probiotic, differences were not significant ($P = 0.056$, $P = 0.060$). There was no significant difference between *Vibrio* populations in water collected from the various treatment groups, however enteric bacterial counts were higher in treatments without probiotic ($P = 0.045$). There was no significant differences between the concentration of *Vibrio* and enteric bacteria in shrimp from the different treatments, although on average shrimp from the control treatment group had a higher percentage of pathogenic *Vibrio* and a higher percentage of lactose fermenting colonies. Taxonomic identification of bacteria has yet to be conducted.

Table 1. Production performance of *Penaeus vannamei* juveniles (1.5.g) reared in biofloc for 12 weeks, fed diets incorporating various levels of Aquastar® probiotic.

	0%	0.2%	0.3%	0.5%
Survival (%)	73+10	67+10	81+6	85+7
Final Weight (g)	23.9+2.6	24.7+1.3	21.9+1.3	22.1+3.0
SGR (% day)	3.3+0.1	3.3+0.1	3.2+0.1	3.2+0.2
Weekly Growth (g)	1.87+0.22	1.97+0.13	1.7+0.11	1.72+0.25
Yield (kg/m ³)	1,318+79	1,216+325	1,415+69	1,563+154
FCR	1.42+0.08	1.61+0.43	1.32+0.06	1.2+0.10

GLUTEN-FREE FISH: DIET AND INFLAMMATION IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Mary E.M. Larkin*, Allen R. Place

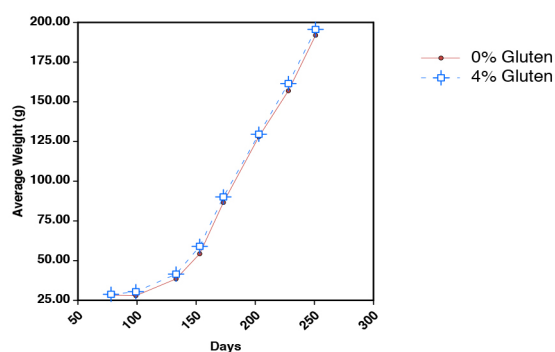
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The aquaculture industry is rapidly expanding to feed a burgeoning world population. The unsustainability of harvesting small ocean forage fish to feed aquaculture fish has precipitated the increased incorporation of plant proteins. A variety of these ingredients including low-fat, high-protein concentrates derived from plants such as wheat (including processed gluten) have been successfully incorporated into fishmeal-free diets for a variety of cultured species.

Though wheat gluten may be well tolerated in some species, several studies conducted by the Place laboratory bring into question its use in formulations designed for feeding of young cobia (*Rachycentron canadum*). Dietary wheat gluten in even small quantities (< 3%) contributed to either sub-par performance or low survival in small juveniles of ~8-10 g starting weight. Suspecting that carnivorous species might be more sensitive to this ingredient, we implemented a dietary study in European sea bass using plant-based formulations with and without added wheat gluten. Due to the lack of fish meal, we supplemented the diet with taurine, which had proven to be an essential amino acid for cobia. European sea bass was chosen for study as another carnivorous species that was easily available and had published genomic data that would allow us to probe for specific immune mediators.

A six-month dietary study showed that European sea bass growth is equivalent on plant-based diets with or without 4% added wheat gluten (see figure). To assess any immune reactions or changes to overall health in response to dietary wheat gluten that did not result in changes in weight, we will probe for markers including C-reactive protein, tumor necrosis factor- α , interleukin-1 β , IgM, and IgT. Changes to blood chemistry indicative of changes to overall health will be evaluated, including aspartate aminotransferase and alanine aminotransferase for liver function and urea and creatinine for kidney function. In addition, we will evaluate alterations to the gastrointestinal tract with regards to molecular markers, histology, and the microbiome.

No Differences in Growth for European Sea Bass (*D. labrax*) Fed Plant-Based Diets With or Without Added Wheat Gluten



LC-PUFAS AND GUT MICROBIAL COMMUNITY OF WILD AND HATCHERY RABBIT FISH (*Siganus guttatus*) LARVAE

Duy V.B. Le^{*,**}, Phuoc N. Nguyen, Kristof Dierckens, Dung V. Nguyen, Nico Boon, Tim Lacoere
Frederiek-Maarten Kerckhof, Jo De Vrieze, Peter Bossier

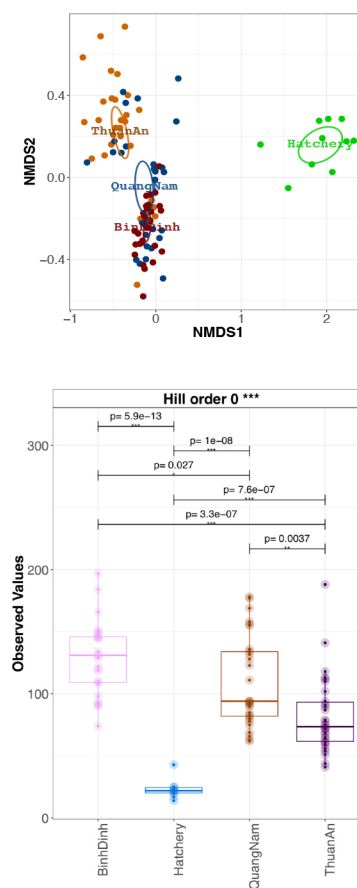
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In this study, the PUFA composition of body tissue and the gut MC of hatchery and wild larvae of rabbit fish at 3 different locations in Vietnam (ThuanAn, QuangNam and BinhDinh) over a period of 3 years were investigated. Results from this study showed that rabbit fish larvae contained high proportion of ARA. The ARA proportion of wild larvae was stable between sampling locations over the three-year period, and no significant differences were detected in the ARA level between wild and hatchery samples. The DHA/EPA ratios in fish larvae were very high and varied between locations. The PUFA composition is within the normal range in the wild. The difference between temperatures at the 3 locations are negatively correlated with the DHA content and the DHA/EPA ratio, but not the EPA content.

The results of Illumina analysis of 16S libraries of wild samples showed that the location affected the gut MC composition. The bacteria that were identified in the rabbit fish gut content were mainly belonging in the Proteobacteria, Bacteroidetes, Fusobacteria, Actinobacteria and Firmicutes phyla. In addition, the gut MC diversity and composition of hatchery larvae, which were fed probiotic-enriched rotifers, was completely different than those in the gut of wild larvae. The probiotic strains (*Lactobacillus plantarum*, *L. fermentum*, *L. brevis*, *Pediococcus acidilactici*, *P. pentosaceus* and *B. subtilis*) were predominant in the hatchery larvae. Next, relative genetic distances between ThuanAn and BinhDinh/QuangNam were bigger than those between BinhDinh-QuangNam. More importantly, sampling location had significant impacts on the MC. The diversity of gut MC increased significantly from the north to the south. In conclusion, the current larval rearing protocol is reliable in terms of PUFA profile and probiotics. The hatchery larvae should not be treated differently in the future.

Figure 1: NMDS (upper figure) and hill order 0 (lower figure) of hatchery and wild larvae gut MC from three locations in three years.



MARINE SALMON NET PENS AND WATER QUALITY PERMITTING

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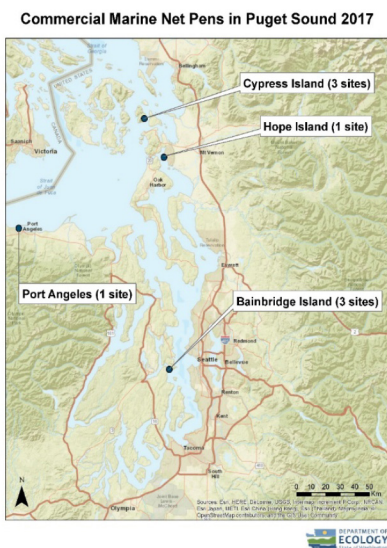
The Washington Department of Ecology (Ecology) Water Quality Program administers the National Pollutant Discharge Elimination System (NPDES) permits. The NPDES permit translates the general requirements of the Federal Clean Water Act into specific provisions tailored to any operation discharging pollutants to waters of the state. Any marine salmon net pen operation that produces more than 20,000 lbs. of fish per year or feeds more than 5,000 lbs. of fish food during any calendar month is required to obtain an NPDES permit.

In 1991, Ecology, with input from representatives of the net pen aquaculture industry, state and local regulators, tribes, and environmental groups, developed a model NPDES permit for net pens. That model permit was used to write and issue 12 individual NPDES permits for existing marine salmon net pens in 1996. The first permits were appealed by environmental groups, and were adjudicated in the courts over the next several years, creating the regulatory framework under which permitting of marine salmon net pens still operates. The eight existing NPDES permits for marine Atlantic salmon (*Salmo salar*) net pens in Puget Sound are based on the original model permits, environmental monitoring information submitted during each permit cycle, state surface water quality and sediment standards, the current state of the science regarding marine net pen aquaculture, and public input.

The NPDES permits include reporting of biomass, feed, and disease control chemicals, effluent limits and monitoring requirements in the sediment and water column, underwater photographic surveys, and operational plans to address pollution prevention, fish release prevention, and accidental fish release response.

With technical assistance from NOAA National Center for Coastal Ocean Science, the history of compliance with water quality standards and sediment standards has been compiled for analysis and will help inform any future NPDES permits for marine salmon net pens.

An overview of permit development and compliance history will be presented, along with an update on the August 2017 fish escape incident, including possible ramifications for future permits, and lessons learned.



COMPARATIVE LYSINE REQUIREMENT OF TWO STRAINS OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

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Lysine is one of the limiting amino acids in formulated fish diets containing plant protein sources and is estimated to be 2.4% of the diet for rainbow trout (NRC 2011). However, genetic selection has increased trout growth rate, even when fish are fed a plant-protein diet. We pose the question: does a fast-growing trout strain require more lysine in the diet than a slow-growing strain? In other animals, it has been shown that nutrient requirements vary in terms of daily feed intake but not necessarily in terms of nutrient content of the diet. It is not known if nutrient requirements vary with fish strain. The objective of this study was to evaluate the lysine requirement of two domestic strains of rainbow trout, a selectively bred fast-growing strain (UI-ARS strain) vs. a randomly bred strain (Hayspur).

Five isonitrogenous (37% digestible protein) and isocaloric (5.0 kcal kg⁻¹ digestible energy) diets were formulated to be supplemented with L-Lysine HCl in increments of 0.40% (1.80, 2.20, 2.60, 3.00 and 3.40% diet). UI-ARS select strain (12.2±0.3 g) and Hayspur non-selected strain (8.54±0.19 g) trout were randomly stocked into fifteen (n=3) 145-L tanks each at 35 fish per tank. Fish were fed to apparent satiation three times daily.

After 12 weeks of the feeding trial, UI-ARS fish had significantly higher weight gain (%) and daily feed intake (g/fish) compared to Hayspur fish. Using the broken-line regression analysis, based on nitrogen retention (%), the optimum lysine level for UI-ARS and Hayspur strains were 2.69% and 2.24% of the diet, respectively. This level was similar to levels needed to support maximum weight gain and feed conversion ratio. Conclusively, these results demonstrate that the fast-growing strain (UI-ARS) consumes more feed and requires more lysine (as a % of diet) than the slow-growing strain (Hayspur) of rainbow trout.

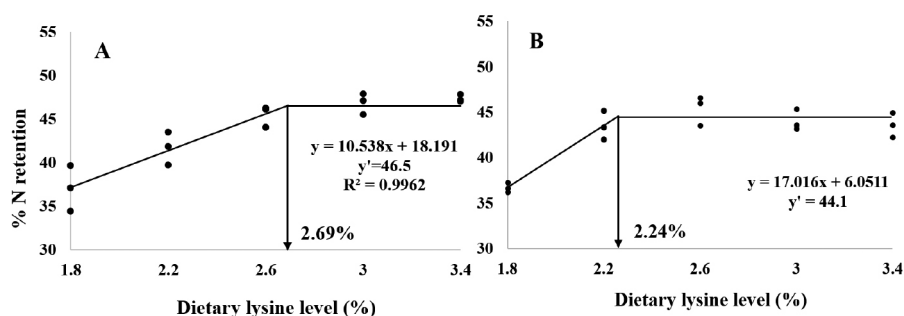


Figure. Broken line analysis of nitrogen retention (%) in rainbow trout fed graded levels of dietary lysine. A: UI-ARS, B: Hayspur

CONTINUOUS SUPPLY OF ROTIFERS AT LOW CONCENTRATIONS FOR LARVAL CULTURE OF RED DRUM *Sciaenops ocellatus*

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Intensive production of live foods is expensive yet is necessary for intensive larval culture of marine fish. Although higher concentrations of live foods, such as rotifers, fed to fish larvae (larvae) provide greater opportunities for larvae to encounter and ingest prey, negative consequences including excess production of rotifers, and liver disease of larvae may result if food consumption is in excess of nutritional needs. A targeted rotifer concentration (rotifer/mL) that leads to efficient rotifer consumption by larvae can be maintained in larval culture tanks by automated pumping of rotifers from cold-storage at concentrations specific to larval age or size. An experiment was conducted to determine the effect of continuous rotifer abundance on ingestion by larval red drum, and their growth, survival, and liver condition. Three levels of rotifer concentrations maintained continuously in larval culture tanks were compared. Rotifer abundance was established at the initiation of the 14-hour photoperiod and maintained continuously throughout by automated pumping and direct tank feeding of rotifers. In-tank rotifer estimates were used to guide adjustments to the rotifer concentration in the larval tanks. Rotifer concentration did not affect survival. Ingestion of rotifers increased with larval age and rotifer concentration, but growth was affected only at the lowest rotifer concentration, 0.5 rotifers/mL. Hepatic lipidosis was evident early in the larval period for rotifer concentrations higher than 0.5 rotifers/mL and persisted into advanced larval stages, indicating a negative association between rotifer ingestion and larval health. The data indicates that larval red drum can be aquacultured effectively by maintaining a relatively low rotifer concentration for intensive aquaculture (0.5 rotifers/mL) in tanks continuously with minimal effects to growth. Although, for production purposes a flexible feeding schedule for intensive larval production may permit a higher rotifer concentration to buffer against rotifer depletion and inter-tank variability, improved fish health and use of resources occur when continuous rotifers supply is closer to the minimum requirements for maximum survival and growth.

RECIRCULATING AQUAPONICS USING SHRIMP, TILAPIA AND VEGETABLES IN A HYBRID SYSTEM

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Recirculating Systems have become more popular in aquaculture installations due to a lack of resources available, and now days also because of the production cost that these resources have increased. Such resources are: Water, Space, Energy, Feed and Waste. Shrimp production has suffered a sanitary problem over the past years due to flow-through systems used worldwide. Recirculating Systems have become more popular in order to increase the biosecurity and get higher yields. Some systems have been developed in fresh water sources and the salinity addition. In this project have been evaluating the management of a poly-culture system using Tilapia, Shrimp and halophytes in an integrated system.

There are different techniques to recirculate water in aquaculture which include: a) Photoautotrophic based systems (also known as algae based systems or green water) which have the advantages of feed reduction on filtration species and low energy consumption compared to other techniques; b) Heterotrophic based systems (also known as Biofloc systems) that consume ammonia to produce a high load of bacteria that could be used for feed production and that conserve energy for temperature increase; Chemoautotrophic systems (or nitrifying systems) that oxidize ammonia to produce Nitrate and that can handle a high density of fish; d) Photo-Chemoautotrophic plant based systems (also known as Aquaponics) where ammonia and other nutrients are converted by bacteria and assimilated by commercial plants. There is a chance to combine some of this techniques together in one system and get more advantages.

At BOFISH farm we have tested and executed in commercial stage, the production of Tilapia, Shrimp and Vegetables in a Hybrid integrated system where a combination of the different recirculating systems has been developed in order to increase sub-product yields and reduce some operative costs such as: feed and energy.



EVALUATION OF BASIL *Ocimum basilicum* VARIETIES GROWN IN AN AQUAPONIC SYSTEM IN HUMBOLDT, CALIFORNIA

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Basil (*Ocimum basilicum*) is the most frequently raised herb in aquaponics because it is a fast growing, good yielding crop with high cash-value. Comparative growth studies of basil varieties in the western United States are lacking. These comparisons are useful to aid aquaponic producers' decision-making to maximize profitability. The objective of this experiment was to compare production of eight basil varieties (dark opal, holy, Italian large leaf, lemon, lime, spicy globe, sweet Genovese, and Thai) in a modified Nelson and Pade, Inc. (Commercial 200) aquaponic system in a greenhouse facility on the Humboldt coast.

The aquaponic system consisted of four 1 m³ fish tanks, each containing approximately 26 white sturgeon (*Acipenser transmontanus*) that averaged 1,608 ± 508 g (mean ± SD), with a total feed input of 1,440 g/day, equal to 60.5 g of feed/day/m² of plant growing area. Effluent from the fish tanks flowed through two AST Endurance™ bead filters and into one degassing tank, and then into two 11.8 m² hydroponic beds. The flow rate through the hydroponic beds was 1,420 L/hour with an average retention time of 1.4 hours. Seedlings were planted at a density of 25 plants/m² in replicated floating rafts. All plants were harvested and weighed two times by the "cut and come again" method. Yields (in kg/ m²) were analyzed using a two-way ANOVA to determine differences among varieties and harvest (using a significance level of $P < 0.05$).

Basil yield was significantly different among varieties ($P < 0.001$), but not between the first and second harvest ($P = 0.627$). Post-hoc pairwise Tukey comparisons indicated that yields of holy were significantly greater than all other basil varieties, by an average of at least 2.8-6.0 kg/m² (Figure 1). Lime yield was the second largest and significantly greater than spicy, dark opal, and Italian varieties.

This experiment suggests holy basil is well suited for maximizing basil yields on the North Coast of California in an aquaponic greenhouse setting. We are in the process of conducting a second trial of our experiment.

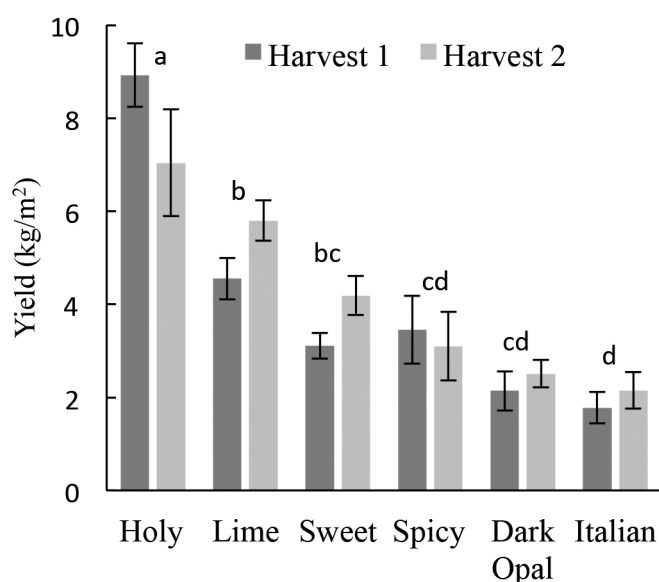


FIGURE 1. Yield (mean ± SE) by harvest of basil varieties for Trial 1. Identical letters denote non-significant differences in variety type.

DAVE LIEBER'S STORYTELLING TIPS

Learn. Practice. Shine.

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DAVE LIEBER, CSP

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EVERY COMPANY HAS A STORY TO TELL Every day, stories of overcoming problems, helping customers, making improvements happen all around you. The best ones — that show ‘universal truths’ about your company — should be shared.

YOUR BRAND SHOULD BE YOUR STORY Only a few years ago, a company needed a website. Now websites are becoming somewhat obsolete. The new medium is story. Through social media, in conversation, through advertising and marketing. Actually, it’s not new. It’s back to our earliest roots in humanity.

YOUR MARKETING — TO BE EFFECTIVE — SHOULD TELL A STORY We start with the facts we want to share, and our goals and motivation for telling the information. Then we construct a story around it.

STORIES MUST HAVE SIGNIFICANCE Stories you tell or write should be more than about something interesting that happened to you. Your stories ought to reveal a universal truth about the human condition — something that brings deeper meaning and triggers heartfelt emotions for members of your audience. If it doesn’t, toss it.

STORIES MUST BE STORIES! They MUST have a beginning, a middle and end. They must have a hero and a villain, although the villain can be a person, place, thing, trend, etc. They must have a plot and a climax with some type of resolution (tying up loose ends) at the conclusion. Stories ought to follow Dave’s V-Shaped Storytelling Formula.

STORIES MUST HAVE A DRAMATIC ARC that keeps the readers/listeners entwined as they progress through the story. Each piece of the tale must lead to the next.

YOUR SALES PITCHES MUST TELL A STORY Don’t sell with the facts. Sell with the emotion. Storytelling taps emotions, heart and ‘brain happiness.’

STORYTELLING WORKS INTERNALLY AS WELL AS EXTERNALLY Messages need not have bullet points and be fact laden. Short stories will be read by more. Charts and numbers on a screen for company communication events make the work dull. Live with stories, told in different ways. The facts will be remembered.

Combine “The Magic V-Shaped Storytelling Formula” with these four main storytelling techniques in both writing and in telling.

Scene by scene construction. Told as if in a movie. Show, not tell.

Dialogue in full. Characters talk instead of being described.

Told with strong point of view. Through the eyes of a character.

Use “status symbols” to bring stories to life.

(Continued on next page)

MORE STORYTELLING TIPS

Consider splitting your story for effect. When you are training, instructing, educating, one technique that works is to take them through your big story, and at the most dramatic moment, break off, promise you will get back to it later. Then quickly go through the instructional portion of the program to satisfy the meeting requirements.

When you can, re-launch the story and do your best to customize it to that industry (based on your research) as you steer your story towards its climax and resolution. (You'll see me do that with YOU!)

Stories must be sparse. Not a single wasted word or phrase. As you tell your stories, think what words, sentences, phrases or sections can be eliminated.

Structure of the story. Following up on previous, look at each part of a story as a floor in a building. And each floor builds upon the one underneath it. Anything that is not absolutely crucial to the building's integrity can be deleted. That's how you cut a 30-minute story to 15 minutes. Or a 5-minute story down to 2 minutes. Hint: Audiences eyes glaze during weaker sections of a story. And ALWAYS keep the parts that draw laughter! Nothing works better than humor. Nothing.

Your story constantly changes. A story evolves. It changes as you become more sophisticated in its telling. You watch audience reactions. You keep what works; you throw out what doesn't. During the storytelling, you will actually hear yourself come up with new lines, new funny parts and descriptions. They pop out of your head in the retelling. If these new pieces work, (you can tell by audience laughter, deeper eye contact or vigorous head nodding) you keep them. These add-ons emerge from your subconscious, your writer's muse.

Listen to your muse! The muse is buried inside each of us. It fosters our hidden storytelling talent, and we ignore it at our own peril. Tap your muse and let it flow.

Excerpt from *The New Journalism*, edited by Tom Wolfe
Published in 1975 by Picador

[The following is included in Dave Lieber's storytelling manual, "The High Impact Writer"]

I discovered this book in a London bookstore on a trip while in college. As soon as I read the opening chapters penned by Wolfe, I immediately realized that this was how I wanted to write. — and speak — for the rest of my life. Let me share the two most important pages:

Tom Wolfe writes:

By trial and error, by 'instinct' rather than theory, journalists began to discover the devices that gave the realistic novel its unique power, variously known as its 'immediacy,' its 'concrete reality,' its 'emotional involvement,' its 'gripping' or 'absorbing' quality.

This extraordinary power was derived mainly from just four devices, they discovered. The basic one was **scene-by-scene construction**, telling the story by moving from scene to scene and resorting as little as possible to sheer historical narrative. Hence the sometimes extraordinary feats of reporting that the new journalists undertook so that they could actually witness the scenes in other people's lives as they took place — and record the **dialogue in full**, which was device No. 2. Magazine writers, like the early novelists, learned by trial and error something that has been demonstrated in academic studies: namely, that realistic dialogue involves the reader more quickly and effectively than any other single device. (Dickens has a way of fixing a character in our mind so that you have the feeling he has described every inch of his appearance — only to go back and discover that he actually took care of the physical description in two or three sentences, the rest he has accomplished with dialogue.)...



Tom Wolfe

(Continued on next page)

The third device was the so-called ‘**third-person point of view**,’ the technique of presenting every scene through the eyes of a particular character, giving the reader the feeling of being inside the character’s mind and experiencing the emotional reality of the scene as he experiences it. Journalists have often used the first-person point of view – ‘I was there’ – just as autobiographers, memoir-ists and novelists had. This is very limiting for the journalist, however, since he can bring the reader inside the mind of only one character – himself – a point of view that often proves irrelevant to the story and irritating to the reader. Yet how could a journalist, writing nonfiction, accurately penetrate thoughts of another person?

The answer proved to be marvelously simple: interview him about his thoughts and emotions, along with everything else. This was what I had done in *The Elec- tric Kool-Aid Acid Test*, what John Sack did in *M* and what Gay Talese did in *Honor Thy Father*.

The fourth device has always been the least understood. This is the **recording of everyday gestures, habits, man- ners, customs**, styles of furniture, clothing, decoration, styles of traveling, eating, keeping house, modes of behav- ing toward children, servants, superiors, inferiors, peers, plus the various looks, glances, poses, styles of walking and oth- er symbolic details that might exist within a scene. Symbolic of what? Symbolic, generally of people’s *status life*, using that term in the broad sense of the entire pattern of behavior and possessions through which people express their position in the world or what they think it is or what they hope it to be. The recording of such details is not mere embroidery in prose. It lies as close to the center of the power of realism as any other device in literature. It is the very essence of the ‘absorbing’ power of Balzac, for example, Balzac barely used point of view at all in the re- fined sense that Henry James used it later on. And yet the reader comes away feeling that he has been even more completely ‘inside’ Balzac’s characters than James’s. Why? Here is the sort of thing Balzac does over and over. Before introducing you to Monsieur and Mad- ame Marneffe personally (in *Cousin Bette*) he brings you into their drawing room and conducts a social autopsy: ‘The furniture covered in faded cotton velvet, the plaster statuettes masquerading as Florentine bronzes, the clumsily carved painted chandelier with its candle rings of molded glass, the carpet, a bargain whose low price was explained too late by the quality of the cotton on it, which was now visible to the na- ked eye – everything in the room, to the very curtains (which would have taught you that the handsome appearance of wool damask lasts for only three years) – everything in the room begins to absorb one into the lives of a pair of down-at-the-heel social climbers, a Monsieur and Madame Marneffe. Balzac piles up these details so meticulously – there is scarcely a detail in the later Balzac that does not illuminate some points of status – that he triggers the reader’s memories of his own status life, his own ambitions, insecurities, delights, disasters, plus the thousand and one small humiliations and the status coups of eve- ryday life, and triggers them over and over until he creates an atmos- phere as rich and involving as the Joycean use of point of view....’

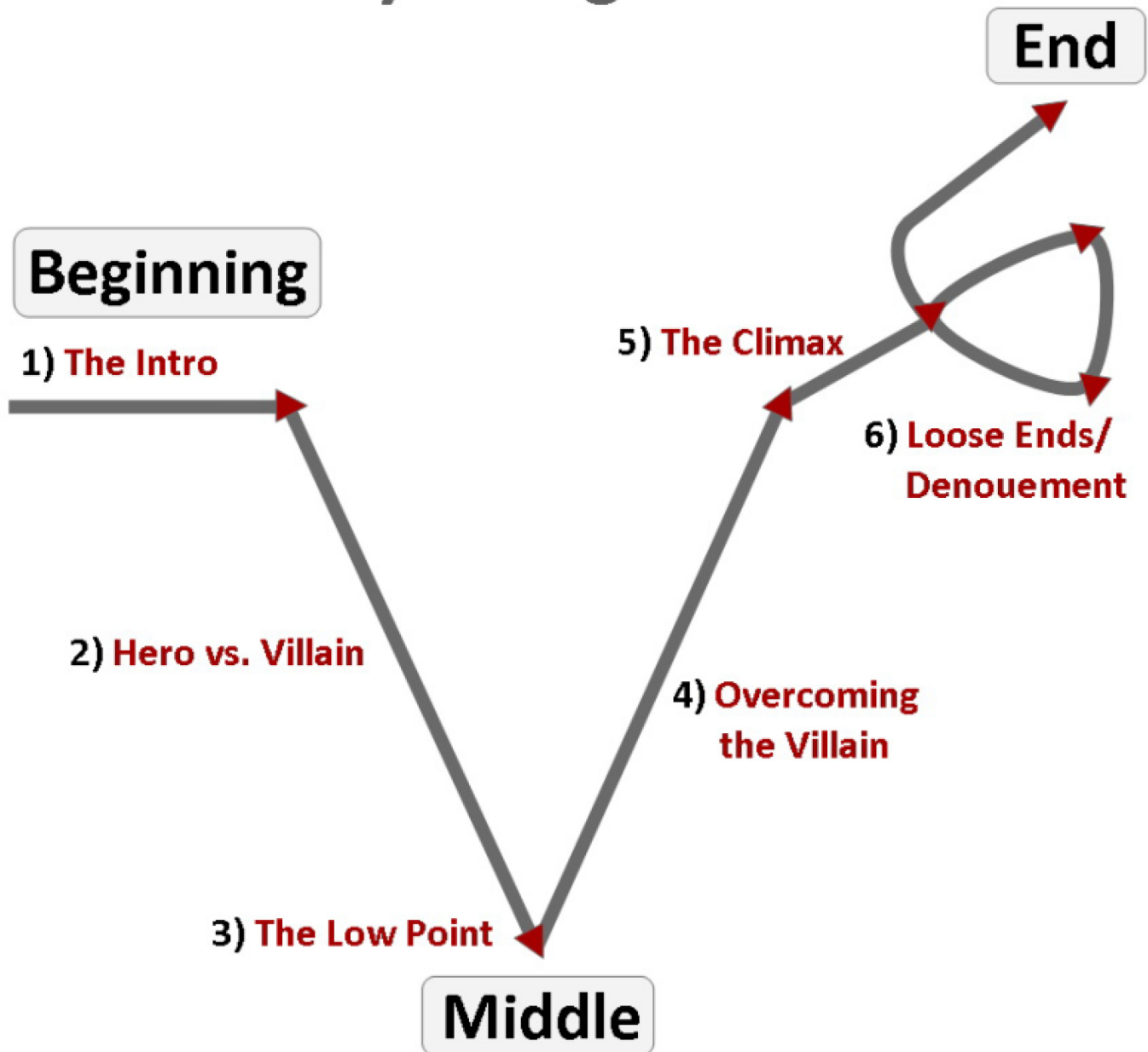
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Dave’s Favorite New Journalism books that show great storytelling

- *The Right Stuff* by Tom Wolfe
- *Fame and Obscurity* by Gay Talese
- *I Want to Thank My Brain for Remembering Me* by Jimmy Breslin
- *Bad Dad* by Dave Lieber (yeah, that’s me!)

Dave Lieber's V-Shaped Storytelling Formula



LONG-TERM GROWTH AND SURVIVAL OF HYBRID BETWEEN *Haliotis discus hannai* AND *Haliotis discus discus*

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Abalone is considered an important commercial species in the shellfish aquaculture industry. Though it accounts for only 3% of the total volume of the farmed shellfish, the species occupies over the half of the total value.

However, over-crowding and prolonged farming activities have caused problems like eutrophication, higher mortality and genetically inferior hatchery stock. As these problems are gradually lowering the productivity, many abalone farmers are looking for ways to shorten rearing period and to improve hybrid seeds with faster growth to overcome mortality.

To develop a hybrid abalone species with faster growth, this study conducted hybridizations between cold water *H. discus hannai* and warm water *H. discus discus* to produce both hybrid and pure hatchery stock and then compared growth and survival between the hybrid and pure seeds which were reared in indoor tanks and then seawater cages.

A 250 day long culture in indoor tanks showed the fastest growth in the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ with 33.9 mm shell length and 4.3 g weight, which was followed by the hybrid *H. discus hannai* ♀ × *H. discus hannai* ♂. After the 250 day long culture in tanks, seeds with about 34 mm shell length were selected from the tanks and transferred to seawater cages to be reared for another 950 days. The sea cage culture also showed the highest growth in the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ with 88.6 mm shell length and 90.5 g weight. Survival rate of the pure *H. discus hannai* ♀ × *H. discus hannai* ♂, the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂, the hybrid *H. discus discus* ♀ × *H. discus hannai* ♂, and the pure *H. discus discus* ♀ × *H. discus discus* ♂ during the 950 day cage culture was 38%, 39%, 31% and 24%, respectively, indicating the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ having the highest Survival rate. Mortality mostly occurred in newly stocked small juveniles in the seawater cages. In conclusion, the comparison of growth and mortality between the hybrid and pure seeds reared in land-based tanks and seawater cages demonstrated that the hybrid *H. discus hannai* ♀ × *H. discus discus* ♂ performed the best.

Table 1. Growth rate of two purebred and two hybrid abalone in the sea cage for 950 days

Species	A	B	C	D
Initial mean body weight (g)	4.6 ± 0.06	4.7 ± 0.08	4.5 ± 0.06	4.5 ± 0.08
Final mean body weight (g)	85.2 ± 4.13 ^a	90.5 ± 4.87 ^a	72.4 ± 3.67 ^b	70.3 ± 3.17 ^b
Weight gain (%)	1742.7 ± 77.5 ^a	1809.2 ± 70.9 ^a	1510.6 ± 44.3 ^{ab}	1466.8 ± 80.4 ^b
Specific growth rate (%)	0.36 ± 0.01	0.37 ± 0.00	0.35 ± 0.00	0.34 ± 0.01
Absolute growth rate (%)	163.9 ± 7.8	158.5 ± 8.9	152.9 ± 14.6	158.9 ± 19.2
Daily growth rate (%)	6.59 ± 0.23	6.78 ± 0.25	5.99 ± 0.19	6.12 ± 0.23
Condition factor	721.1 ± 42.4 ^{ab}	834.3 ± 59.6 ^a	683.4 ± 24.7 ^{ab}	661.0 ± 50.3 ^b

A: *Haliotis discus hannai* ♀ × *H. discus hannai* ♂, B: *H. discus hannai* ♀ × *H. discus discus* ♂, C: *H. discus discus* ♀ × *H. discus hannai* ♂, D: *H. discus discus* ♀ × *H. discus discus* ♂.

NEW TOOLS FOR SELECTIVELY IMPROVING STRAINS OF SUGAR KELP *Saccharina latissima* FOR FOOD AND FUEL

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As a part of ARPA-E's MARINER program, a team of scientists and entrepreneurs is pursuing a selective breeding program to improve the productivity and composition of sugar kelp, *Saccharina latissima*, which could serve as feedstock for biofuels. ARPA-E's and our goal is to develop tools and a pathway toward low-cost (< \$100/DWT) seaweed feedstock that could supply 10% of US transportation fuels. Current markets include human and animal food.

Our project will develop several complementary tools to reach this objective. To facilitate high-throughput creation of family crosses, the NOAA Milford Lab and UCONN are developing cell sorting methods to efficiently isolate and clone gametophytes. USDA/Cornell and HudsonAlpha will employ PacBio and Illumina sequencing to create a deep-sequenced reference genome and establish a variant catalog for our founding populations and families. WHOI, UCONN and GreenWave will oversee field trials of 144 families (from 12 different 'strains') planted in triplicate plots on two farm sites (nearshore and offshore) over two growing seasons. The resulting family phenotypic data will be associated with genetic markers (GWAS), and we will identify variants significantly associated with primary productivity and composition traits. A goal is to develop methods to predict offspring (SPs or sporophytes) performance based upon genotype and breeding values of parents (GPs or gametophytes) as a short cut around extensive and expensive field testing (Figure 1). A separate ARPA-E project will test the potential labor-saving use of WHOI's robotic autonomous underwater vehicles and sensors for conducting phenotypic measurements of family test plots. These will be compared to conventional hands-on field measurements.

Ultimately our project goal is to select sugar kelp best suited genetically to offshore farm environments and possessing qualities of increased dry matter yield per unit area (up to 10% per generation) and improved composition for use as a bioenergy feedstock.

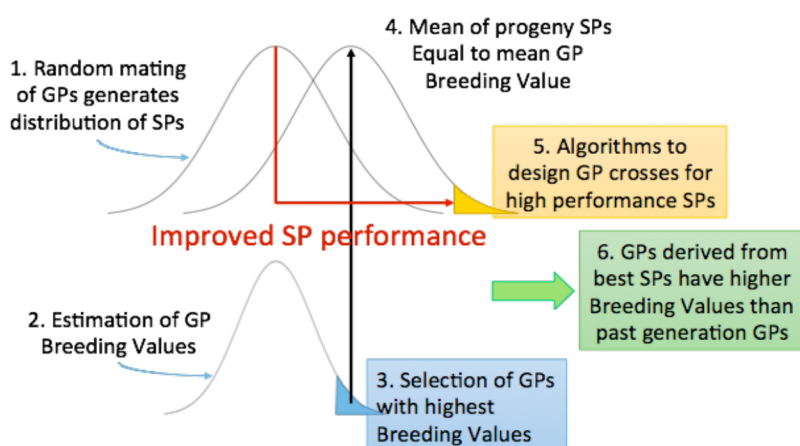


Figure 1. There are breeding advantages that we derive from kelp's biphasic life history. In blue and yellow are the critical elements to improve SP performance within one life cycle. In green is the critical element to improve performance across multiple cycles.

REPLACING *Artemia* IN THE LARVAL CULTURE OF SIX FRESHWATER ORNAMENTAL FISH

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Larvae of freshwater fish produced for the ornamental aquaculture industry are generally given live *Artemia* nauplii as a first feed. While *Artemia* has proven to be invaluable in the development of protocols for culturing various fish species, this live feed organism also has many disadvantages to alternative feeds. These include variable cost and availability, the potential for pathogen transfer, and irregularities in nutritional profile. In light of these disadvantages, research investigating the efficacy of alternative feed items for larval freshwater ornamental fish is important. The present study evaluated survival, growth and homogeneity of growth of six commercially important ornamental fish (Rosy Barb *Pethia conchonius*, Tiger Barb *Puntigrus tetrazona*, Black Tetra *Gymnocorymbus ternetzi*, Redtail Sharkminnow *Epalzeorhynchos bicolor*, Dwarf Gourami *Trichogaster lalius*, and Freshwater Angelfish *Pterophyllum scalare*) fed four different diets for the first fourteen days of exogenous feeding. Diets evaluated were the industry standard first feed, *Artemia*, and three commercially available microparticulate diets: Larval AP-100, Otohime A1, and Golden Pearls. All fish were raised to 14 days after the onset of exogenous feeding, at which point survival and standard length were evaluated. For Rosy Barb, Tiger Barb, Redtail Sharkminnow and Dwarf Gourami, mean survival did not differ significantly. Mean survival of Freshwater Angelfish and Black Tetra, however, varied significantly, with larvae fed Golden Pearls exhibiting the highest survival. The diet exhibiting the highest final mean standard length was more variable across species. This study provides evidence for the successful first feed replacement of *Artemia* with commercially available micro-particulate diets in freshwater ornamental species.

REDUCING OFF-FLAVOUR COMPOUNDS GEOSMIN AND 2-METHYLISSOBORNEOL IN TILAPIA THROUGH DIFFERENT CULTIVATION TECHNOLOGIES

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Off flavor in aquatic products is a rising concern among consumers and researchers. This study compares the effects of four different tilapia cultivation systems, namely tilapia-water spinach integrated system, sewage discharging system, sewage discharging and tilapia-water spinach integrated system with tilapia monoculture system as control on the water quality, tilapia production and off-flavor contents in water and tilapia. Using gas chromatography and mass spectrum (GC-MS) based on microwave assisted distillation followed by purge-and-trap, the off-flavor compounds geosmin (GSM) and 2-methylisoborneol (2-MIB) were determined in water and tilapia tissue. The results showed that the production of the tilapia-water spinach integrated system was the highest among the systems ($P < 0.05$). The contents of ammonia nitrogen and nitrite in the sewage discharging and tilapia-water spinach integrated system were significantly lower ($0.64 \pm 0.06 \text{ mg/L}$ and $0.086 \pm 0.005 \text{ mg/L}$, respectively). In addition, the concentration of 2-MIB and GSM in the tilapia muscle of the sewage discharging and tilapia-water spinach integrated system ($0.31 \pm 0.02 \text{ } \mu\text{g/kg}$ and $0.53 \pm 0.042 \text{ } \mu\text{g/kg}$ respectively) were significantly lower than in the control group ($0.67 \pm 0.022 \text{ } \mu\text{g/kg}$ and $0.87 \pm 0.018 \text{ } \mu\text{g/kg}$ respectively). The results indicated that the sewage discharging and tilapia-water spinach integrated system reduced the accumulation of off-flavor compounds in water and tilapia muscle in the intensive tilapia farming system.

ALKALINIZATION MEDIATED Ca^{2+} INFLUX ACTIVATES SPERM MOTILITY FROM LIVE-BEARING FISH

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Signal regulations for sperm activation of egg-laying fishes have been widely studied, however, such mechanisms for live-bearing fishes are poorly understood, despite the importance of artificial insemination of live-bearing fishes to activities such as development of germplasm repositories via sperm cryopreservation for biomedical research models and imperiled species, and induced spawning of aquarium species. Live-bearing species often produce sperm bundles which are another challenge for mechanistic studies. We studied intracellular calcium (Ca^{2+}) signaling during sperm activation of live-bearing fish using Redtail Splitfin (*Xenotoca eiseni*) as a research model, and established methodologies for studying cell signaling with sperm bundles. Real-time Ca^{2+} imaging analysis with Fura 2/AM was used to investigate intracellular Ca^{2+} signals during sperm activation. We found that the percentage of activated bundles and motility increased with extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_e$) and pH levels ($[\text{pH}]_e$). Intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$) of sperm within bundles significantly ($P < 0.0001$) increased with $[\text{Ca}^{2+}]_e$ (Fig. 1), and such increases stimulated by 10 – 50 mM $[\text{Ca}^{2+}]_e$ were 633 – 1940% higher than 0.5 – 2 mM $[\text{Ca}^{2+}]_e$. This $[\text{Ca}^{2+}]_e$ -dependent increase of $[\text{Ca}^{2+}]_i$ was confirmed in free sperm cells from dispersed bundles. When $[\text{Ca}^{2+}]_e$ remained at 2 mM, $[\text{Ca}^{2+}]_i$ increased significantly ($P < 0.0001$) with $[\text{pH}]_e$, and the $[\text{Ca}^{2+}]_i$ stimulated by 8.5 – 9.5 > pH 8.0 > pH 6.5 – 7.5). Furthermore, we tested the effect on Ca^{2+} signaling of most recognized test agents: non-selective Ca^{2+} channel blockers (CdCl_2 , NiCl_2 , and ruthenium red), a mechano-sensitive channel blocker (GdCl_3), a store-operated calcium channel blocker (SKF-96365), L-type voltage-dependent Ca^{2+} channel (VDCC) blockers (nimodipine, verapamil, and methoxyverapamil), and T-type VDCC blockers (mibefradil and NNC 55-0396). The CdCl_2 (200 μM) inhibited ($P = 0.0304$) Ca^{2+} signals for 25%, but all other blockers failed to inhibit intracellular Ca^{2+} signals. These findings strongly suggest that sperm activation and bundle dissociation of live-bearing fish *X. eiseni* are due a Ca^{2+} influx that can be mediated by increased extracellular alkalization. However, the influx does not appear to be mediated through any commonly known Ca^{2+} channels. This raises the possibility of a novel mechanism at work in *X. eiseni*. Findings from this study can provide a foundation for standardization of the artificial insemination, and further investigation of molecular activation mechanisms using sperm from live-bearing fishes.

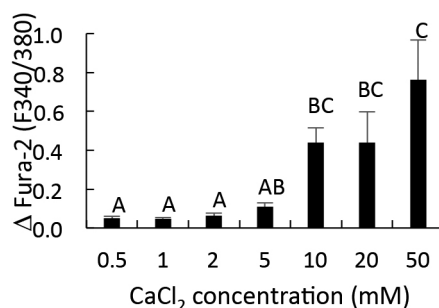


Fig.1 Calcium chloride induced increases in intracellular Ca^{2+} concentration of sperm within bundles.

GERMPLASM REPOSITORIES CAN PROVIDE A NECESSARY TOOL FOR RECOVERY PROGRAMS OF IMPERILED FISHES

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Recovery programs for endangered or threatened species require a combination of various conservation tools such as status assessment, legislation, habitat restoration and management, translocation, and captive breeding. However, limitations of those tools challenge implementation of such programs. For example, long-term efforts such as habitat restoration can take decades, but population sizes and genetic diversity of concerned species can continue to decline in nature before full restoration. Shorter-term efforts such as captive breeding are at risk due to disease outbreaks, administrative discontinuity, high costs, and inbreeding depression. Repositories of cryopreserved germplasm can become a valuable addition to the conservation toolbox to address these challenges by preserving germplasm for future use at a relatively low cost, ensuring integrity of genetic diversity, enabling genetic assessment, and enhancing captive breeding. This tool has been used to improve diversity in recovery of endangered mammals such as the black-footed ferret (*Mustela nigripes*). However, it has been neglected in conservation programs of imperiled fishes. For example, in 86 current recovery plans of endangered or threatened fishes developed by the U.S. Fish & Wildlife Service, only 2 mention cryopreservation, and that was for research purposes. We have developed a recovery plan using Redtail Splitfin (*Xenotoca eiseni*) as a model species to demonstrate a comprehensive strategy to couple germplasm (sperm) repositories with conventional conservation tools. Sperm from *X. eiseni* from wild populations can be cryopreserved on-site in French straws, and the straws shipped to a central repository (Fig. 1). When wild populations are brought to hatcheries, sperm from these stocks can be cryopreserved, genetically characterized, and used when necessary to maintain desired levels of genetic diversity and reduce inbreeding of broodstocks. When historic or translocation habitats are ready, germplasm can be incorporated into live populations in hatcheries for introduction to the habitats. Successful recovery requires a practical conservation plan as well as strong collaborations among people and agencies with specialized expertise and function. A calculator model was developed to assist linkage of expertise with germplasm repositories. Based on desired levels of heterozygosity, inbreeding, and effective population size, the brood sizes from cryopreserved germplasm can be calculated for incorporation into captive stocks can be calculated. For example, if 500 offspring from germplasm repositories are desired per year for 50 years, sperm of 174 males from wild populations need to be cryopreserved into 156 straws, and 6 straws need to be thawed and used to inseminate 125 females per year (if 20% of females are able to produce 20 offspring). Protection and characterization of genetic resources can be integrated into conservation programs through development of interaction with germplasm repositories, and expanded strategies for genetic management.

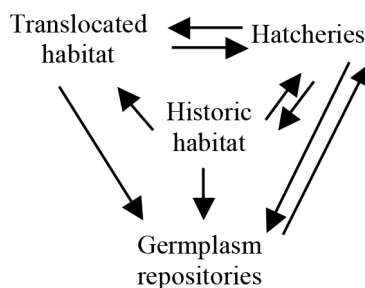


Fig. 1. A strategy to use germplasm repositories in a comprehensive recovery program.

MOLECULAR INSIGHTS INTO LYMPHOCYTE CYTOSOLIC PROTEIN 2 (LCP2); EXPRESSION ANALYSIS OF *Sebastes schlegelii* AFTER ACTIVATING T-CELL RECEPTORS FROM IMMUNE STIMULATION

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Lymphocyte cytosolic protein 2 (LCP2), also known as SLP-76, is a cytosolic adapter protein that nucleates signaling complexes generated by immunoreceptors. It is mostly studied in T lymphocytes and associated with many proteins. These molecules recruited by LCP2 launch the intracellular propagation of molecular signals leading to downstream responses including calcium mobilization, activation of MAP kinases, integrin activation, cytoskeletal reorganization and ultimately full-blown T cell responses. According to in-silico analysis performed, LCP2 protein belongs to the SAM, Atrophin-1 and SH2 super families; possesses inducibly phosphorylated tyrosines in N-terminus, a central proline-rich domain and C-terminal SH2 domain. LCP2 translated sequence consisted of 507 amino acids. Pairwise alignment results showed that highest sequence similarity to *Larimichthys crocea* with 78.3 % identity and 84.0 % similarity and *N*-linked glycosylation sites can be found at ⁴⁸NLSD⁵¹ and ²⁸⁶NRTN²⁸⁹. The LCP2 protein showed signal peptides at 1-20 residues.

Quantitative real time PCR indicated, the highest expression was in blood followed by spleen, gill among fourteen different tissues checked from healthy rockfish. As these tissues actively participated in immune responses, intensive expression of LCP2 can be observed. Then blood and spleen tissues were subjected to the immune challenge with Lipopolysaccharides (LPS), Poly IC and *Streptococcus iniae* to observe variation in the transcriptional pattern of LCP2. According to the results, LCP2 showed a significant response in spleen towards all the stimuli at 12 h to 48 h post infection. Following the T cell receptor engagement to the immune stimulation in the spleen, growth factor receptor-bound protein 2 (Grb2)-related adapter protein 2 (GRAP2) binds to the LCP2 while making a complex with the phospholipase C (PLC) and it binds to linker of activation T cells (LAT1), stabilizing and activating the T cells downstream responses. The LCP2 actively participating in the T-cell related immune responses and it can be identified as an immunologically important gene in Korean rockfish.

EFFECTS OF GENOTYPE AND ENVIRONMENT ON CULTURED CORALS FOLLOWING RESTORATION

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The threatened staghorn coral *Acropora cervicornis* is widely cultured throughout south Florida and the Caribbean for use in restoration. Despite overall success in rearing this species using *in situ* nurseries, outcomes following restoration to natural reefs can be variable, and genotype- and site-specific differences in survival and growth have been reported. Recent work has demonstrated significant phenotypic variability among *A. cervicornis* reared in a common garden nursery, including differences in the rate of extension, branch formation, and calcification, as well as variability in thermotolerance. Selecting a higher proportion of robust genotypes for outplanting could improve overall rates of growth and survival in restoration efforts. However, it remains unclear whether phenotype is conserved following outplanting to natural reefs, particularly across very different reef types.

We conducted a study to determine the effects of both genotype and site on restored *A. cervicornis*. Six genotypes with previously determined phenotypes related to growth and thermotolerance were individually tagged and planted across four sites (two inshore patch reefs, and two offshore forereefs) using marine epoxy. Total linear extension and colony length, width, and height (to estimate volume) were measured for each colony immediately prior to outplanting. After 51 days, colony mortality, length, width, and height were measured. No significant difference in colony survival was found among sites. Growth differed among genotypes (Fig.1; $F=2.278$, $df=5$, $p<0.05$) and was higher at inshore sites compared to offshore sites, ($F=6.469$, $df=1$, $p<0.05$), but the interaction of genotype and site type was not significant.

These preliminary results suggest differences in growth phenotypes are present following restoration. Additional measurements are required to fully determine whether phenotypes following restoration are consistent with those measured in a common garden nursery setting. Lower growth at offshore sites could suggest higher rates of colony breakage compared to more protected, inshore sites. Monitoring will continue for a period of one year to better understand long-term trends and the role of both genotype and site.

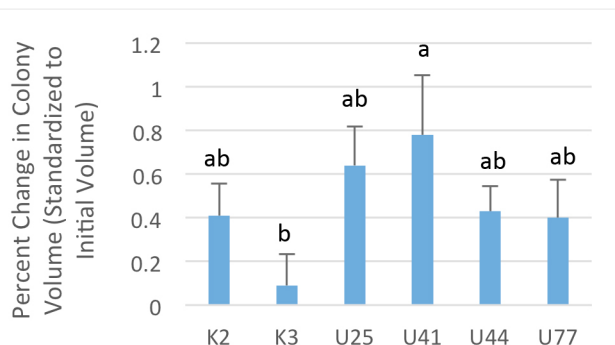


Figure 1. Percent change in colony volume for each genotype, standardized to initial volume of each colony. Letters indicate significant differences among genotypes.

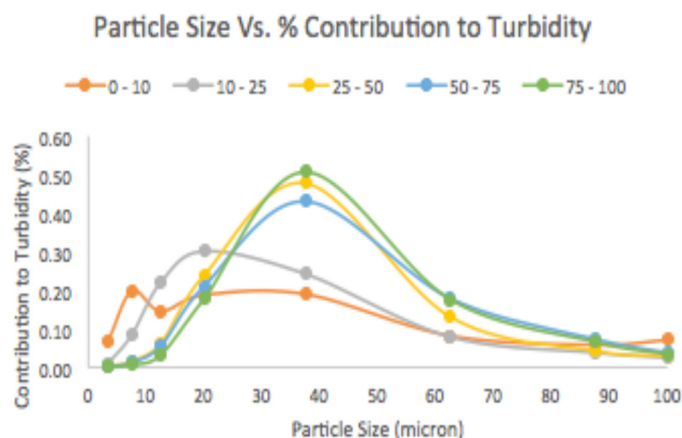
USE OF FOAM FRACTIONATION IN COLLABORATION WITH A LOW-HEAD/LOW WATER LOSS POLYGEYSER BEAD FILTER TO ACHIEVE VISIBLY CLEAR WATER IN MARINE RECIRCULATING AQUACULTURE SYSTEMS

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The yield and efficiency of raising aquaculture in marine systems are highly dependent on the suitability of their environment. In most instances, the source of low water quality is from the culture itself (Boyd & Tucker 1998). This analysis aims to focus on solids management, one element of water quality. The majority of solids produced in a marine system are from either uneaten feed or fish excretion. The presence of these solids provides a medium for bacteria growth, which subsequently results in increased Biological Oxygen Demand (BOD) and the proliferation of disease. To remove these solids, a PolyGeysers, floating bead filter, is the most efficient method. However, removal efficiencies decline from nearly 100% in the 30 – 50 micron size range, and becomes problematic in the 5 – 10 micron range (Malone & Gudipati, 2007).

To overcome this limitation, a foam fractionator can be utilized in addition to the PolyGeysers to remove these micro-particles to achieve visibly clear water. Turbidity, measured in Nephelometric Turbidity Units (NTU), is a measurement of the amount of light scattered when passing through a medium. The human eye can only detect turbid water at turbidity values greater than 5 NTU (Strausberg, 1983), values below that are considered completely clear. A particle size analysis was conducted on several lakes, measuring the amount and size distribution of micro-particles within. A strong correlation between measured turbidity and combined surface area of the particles measured was found. Using this correlation, the effect that a particular particle size has on turbidity can be derived, which can be seen in the figure above. Using measurements taken at various turbidity values, it can be seen that the particle size distribution, and its contribution to turbidity, is typically constant until your turbidity falls below 25 NTU. At this point, particles below 20 microns play a significant role in keeping the water turbid. The effectiveness of the combined use of a foam fractionator and a PolyGeysers bead filter has been proven in a recent 7-month study conducted by AST where shrimp systems went from turbid to clear within a month.



LARVAL SMALLMOUTH GRUNT *Haemulon chrysargyreum* FEED SURVIVORSHIP AND GROWTH RATE ON DIFFERENT COPEPODS *Apocyclops panamensis* AND *Parvocalanus* SPP.

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The marine aquarium trade (MAT) has grown into a major global industry. This rapid growth has illuminated concerns regarding the sustainability of the MAT. Marine ornamental aquaculture (MOA) is a growing part of MAT sustainability, and there is a great demand for captive-bred individuals of high-demand species. Culturing marine species for the MAT has become easier as technologies advance, however the culture of fish can still pose a challenge to aquaculture facilities, specifically the larviculture portion. One of the major bottlenecks for larviculture is the live feed requirement. *Apocyclops panamensis* (Apo), a species of omnivorous cycloid copepod, has recently become popular in aquaculture due to its ability to be cultured on a non-viable algal paste. Culturing Apo is of great value to determine the suitability of this species as a live feed for the smallmouth grunt *Haemulon chrysargyreum*, to then extrapolate to other larval fish species. The goal of this research was to determine the suitability of Apo as a first feed for *H. chrysargyreum* by testing the effect of feeding Apo on larval fish survivorship, growth, and fatty acid composition, compared to fish fed *Parvocalanus* spp. (Parvo) copepods.

All *H. chrysargyreum* were kept at $26.38 \pm 0.46^\circ\text{C}$ until 17 days post hatch (dph). Nauplii concentrations were maintained at $4.64 \pm 3.19/\text{mL}$. *H. chrysargyreum* on the Apo treatment attained a notochord length of 6.68 ± 0.14 mm, whereas *H. chrysargyreum* on the Parvo treatment attained a notochord length of 8.06 ± 0.18 mm. At 17 dph 21% of the fish on the Apo treatment remained, compared to the 44% on the Parvo treatment.

This study is noteworthy because if larval fish can be raised using a copepod that does not require live algae this could reduce costs of production and make more MOA larviculture accessible for a greater number of aquaculture facilities.

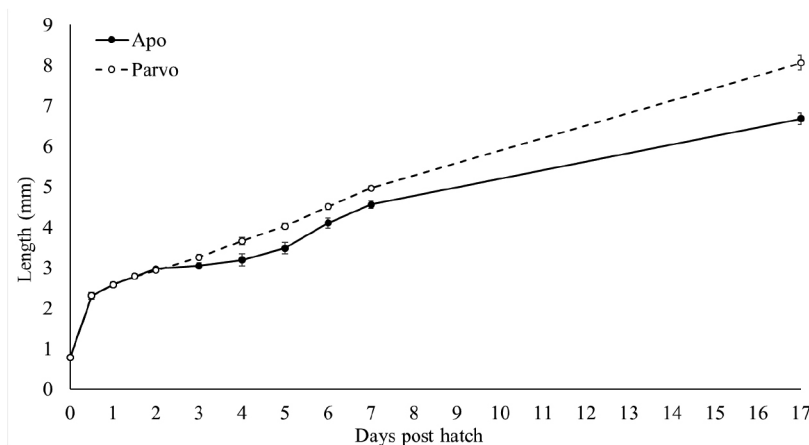


Figure 1. Larval growth rate \pm standard error of the two treatments, Apo and Parvo, throughout the duration of the experiment.

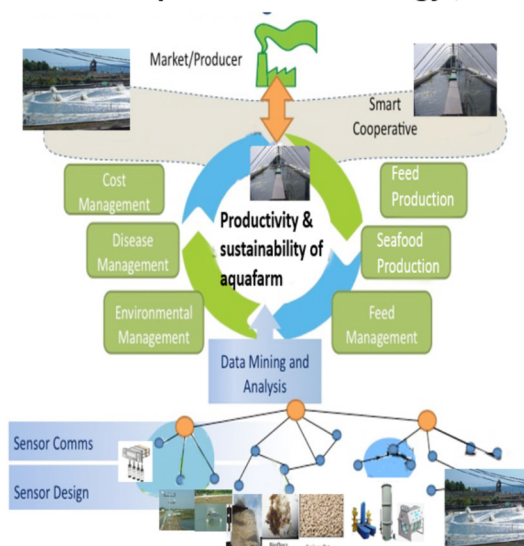
SMART AND PRECISE AQUACULTURE SYSTEM FOR INCREASING PRODUCTIVITY AND SUSTAINABLE PRODUCTION OF AQUACULTURE

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With the rapid global changes in the 21st century, aquaculture should be transformed into a knowledge- based, environment-friendly, high-tech industry in order to remain competitive and sustainable. In this presentation, we will introduce a new aquaculture production model i.e. aquaculture 4.0 which employed modern IoT technology and biotechnology strategies to revolutionize aquaculture. Three sub-systems were developed to fulfill the goal; 1 Advanced monitoring of aquaculture environment by ICT technology, i.e. IoT+ aquaculture system means extensive measuring, monitoring and automatic control by driving various regulators; 2 Automatic species recognition, weight determination and precise feeding system, By using a vision-based catch registration system can automatically recognize fish species and measure the body length and then converts to body weight. Collection of biological data on individual length and weight of specimen has always been an important part of aquaculturists. 3 Tele-diagnosis system for fish diseases. A clinical sign-based diagnosis aid system is applied by sending infected animals images, text description through mobile phone to the diagnosis center. To implement the designed diagnosis process, fish disease database and disease cause/prevention/treatment database were established. Candidate disease by comparing the observed and selected clinical signs to the information on the remote database. Information such as cause, diagnosis, treatment and prevention method of candidate disease are provided as a medical advice through internet to user PC or mobile devices. This system will support fish farmers and veterinarians by provide easy and rapid diagnosis of fish diseases. Since ICT and IoT technology are used to collect information from farmers and biotechnology can provide the solution for the problems of commercial fish farming.

Precision Aquaculture Technology ;PAT



Aquaculture 4.0 –Smart aquaculture system

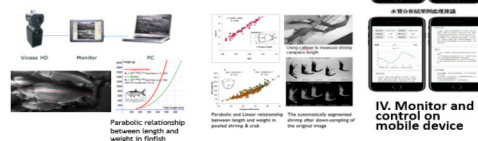
I. Online Environment monitoring and automation system



II. On-line aquatic disease diagnosis system



III. Online growth performance monitoring system



IV. Monitor and control on mobile device

DEVELOPMENT OF MICROSATILLITES FOR PARENTAGE ANALYSIS IN BAY SCALLOPS *Argopecten irradians* SPAWNED FOR RESTORATION

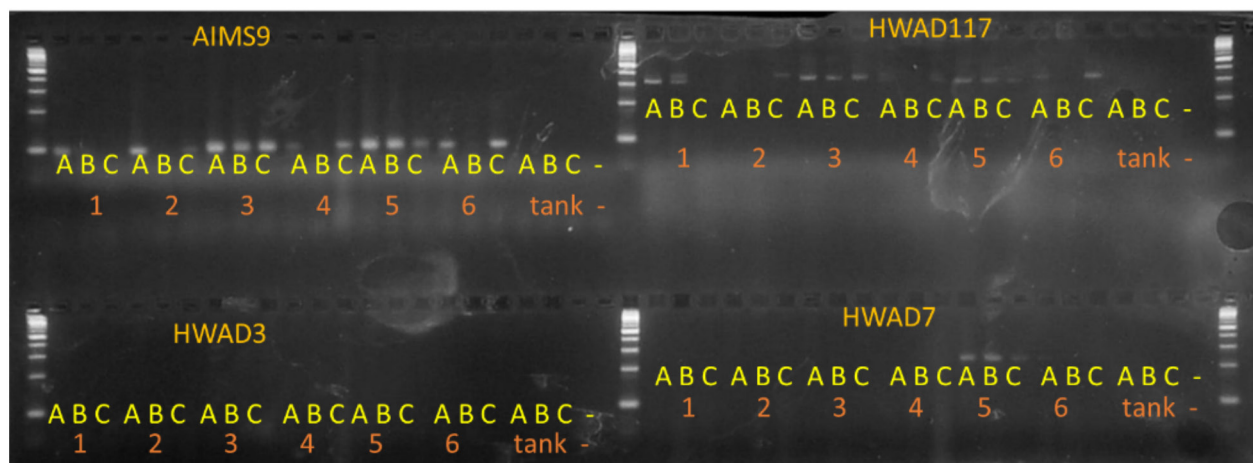
Rebecca Lucas*, Betty Staugler, Chris Dervinis, Matias Kirst, and Josh Patterson

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The bay scallop *Argopecten irradians* is a filter feeding bivalve that lives in seagrass beds. In Florida, this species supports a culturally and economically important recreational fishery. The scallop population has declined due to various factors and aquaculture-based restoration efforts have historically occurred and are ongoing in many areas of the state at a variety of scales. To document the success of restoration and the genetic diversity of cultured scallop larvae, we worked to develop a method for parentage analysis using microsatellites. Such a system would also be valuable for analyses of population-level genetic structure.

Development of the microsatellite system included trial and error determination of field sampling methods. Methods for non-lethal broodstock sampling evolved from the tedious practice of clipping tentacles to sterile cotton swabs of the adductor muscle (Fig. 1). Tissue storage agents evolved from ethanol to LoTEPA buffer, which requires prompt DNA extraction but greatly reduces processing time. Critically for parentage analysis, we established that individual bay scallop larvae can provide sufficient DNA for PCR amplification as early as 7 days post fertilization. Only a handful of the dozen-plus published bay scallop microsatellite loci (target $n = 9$) have proven suitable for analysis. Others produced numerous spurious bands after capillary electrophoresis, making interpretation difficult. Thus, an NCBI-available draft genome for the species was assembled to identify primers. As of November 2017, the new primers are being verified to complete a set suitable for parentage analysis.

Figure 1: Agarose gels testing the sterile cotton swab method of DNA collection. Amplification for four primers (AIMS9, HWAD3, HWAD7, and HWAD117) was tested. Numbers are individual scallops swabbed and letters are technical replicates. The last four lanes of each gel are tank water and no template controls.



MOLECULAR CLONING AND CHARACTERIZATION OF ARACHIDONATE 5- LIPOXYGENASE FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

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Arachidonate lipoxygenases are a heterogeneous family of lipid peroxidizing enzymes with ability to convert the oxygenating polyunsaturated fatty acids to corresponding hydroperoxy molecules. Arachidonate 5-lipoxygenase (ALOX5) facilitates biosynthesis of leukotrienes, mediators of inflammation derived from arachidonic acid (AA). In this study, ALOX5 of big-belly seahorse (*Hippocampus abdominalis*; HaALOX5) were identified and characterized at sequence, transcriptional and molecular levels. Coding sequence of HaALOX5 was 2025 bp that encode respective protein sequence with 674 amino acids in length. *In silico* analysis reveal that homology modelling of HaALOX5 3D structure was resemble to the human ALOX5 monomer. Signal peptide was not detected on the amino acid sequence. The recombinant HaALOX5 catalyzed the conversion of AA to 5-Hydroxyeicosatetraenoic acid (5-HETE). Final product of 5-HETE was detected by RP-HPLC. Phylogenetic tree showed a neighboring evolutionary relationship for ALOX5 vertebrate counterparts, with close assembly to the fish homologs. ALOX5 transcripts was detected in all tissues with highest in intestine and lowest in ovary. In blood and intestine, the mRNA expression of ALOX5 showed similar pattern upon *S. iniae* challenge. In blood, upon Poly (I:C) challenge, expression was significantly elevated after 72 h post injection and upon LPS, similarly increased at 3 h. Our results enriched understanding the modulation of HaALOX-5 mRNA expression under conditions of pathogenic stresses.

EVALUATION OF CASSAVA CHIPS AS AN ALTERNATIVE FEED INGREDIENT IN TILAPIA *Oreochromis Niloticus* AQUACULTURE FROM A GUT HEALTH PERSPECTIVE

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In order to increase aquaculture production while reducing production costs, alternative ingredients must be investigated to reduce the dependency on conventional energy sources in aquaculture feeds. Modulation of the intestinal microbiota of aquaculture species through the use of prebiotics not only benefits the health of the host, but also reduces costs to aquaculture production. Like prebiotics, feed ingredients with high fiber and resistant starch also modulate the intestinal microbiota of the host while providing nutritional value for growth performance. Tilapia (*Oreochromis sp.*) are the most widespread aquaculture species in the world due to their relative fecundity, omnivorous feeding habits, and tolerance of marginal growing conditions, making them ideal study species for alternative feed ingredients. This study investigated the use of sun-dried cassava (*Manihot esculenta*) chips as corn replacement to reduce feed cost while maintaining production levels of tilapia (*O. niloticus* × *O. mossambicus*). Four hundred tilapia fingerlings (~10g initial body weight) were randomly and equally placed in 20 tanks and fed with one of the 5 diets with 0, 4.38, 8.75, 17.5, and 26.25% cassava inclusion in basal diets for 12 weeks. Feed intake and body weight was recorded weekly and intestinal and environmental samples were collected monthly and processed for microbiota analysis using PCR-DGGE, metagenomics and qPCR. Tilapia growth performance was not significantly different among treatments ($P>0.05$), suggesting that cassava can be incorporated up to 26.25% into the tilapia diets without negative impact. Additionally, cassava inclusion into the tilapia diets significantly altered the intestinal microbiota, which can be used as a strategy to modulate gut health of Tilapia.

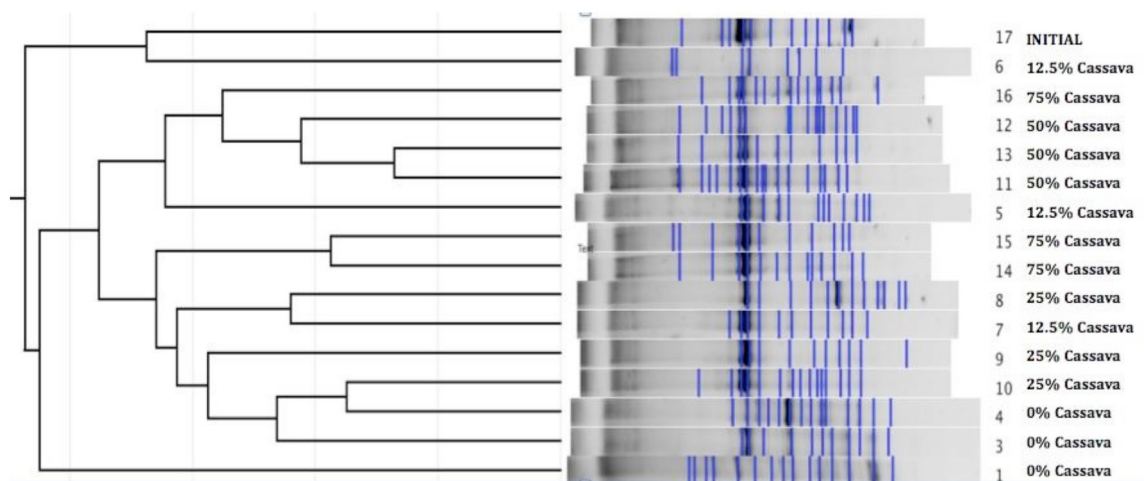


Figure 1. Results of the PCR-DGGE analysis of cassava samples replacing 0%, 12.5%, 25%, 50%, and 75% of corn in the diet of tilapia.

DEVELOPMENT OF TECHNIQUE FOR MASS PRODUCTION OF HOUSEFLY (*Musca domestica*) MAGGOTS

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Aquaculture in Tanzania has remained subsistence partly due to lack of quality affordable aquafeeds. This is because conventional sources of protein such as fishmeal and oil seed cakes are scarce and costly. Housefly maggots are locally available, affordable and contain high protein, thus potential alternative protein source. However, a limiting factor is lack of appropriate culture technique for mass production of maggots with convenient harvesting. Thus culture unit was designed composed of a plastic enclosure with 40 cm diameter and 21.5 cm height consisting of two chambers (Plate 1).

The top chamber served as a culture unit where the substrate was placed. The bottom chamber served as harvesting unit and was separated from the top chamber using a 2 mm nylon mesh. The mesh allowed dropping of maggots into harvesting unit due to photosensitivity of maggots when exposed to light upon opening of the culture unit. Five substrates in triplicates namely, cattle manure, poultry manure, pig manure, cattle offal and kitchen leftovers were used to assess suitability of the developed system as well as the culture conditions. About 2.5 kg of substrate and 250 gm of attractant (mixture of blood, small pieces of meat debris and rotten eggs) were placed into culture unit and houseflies allowed to lay eggs. Harvesting was done from day four where maggots were cleaned with water, blanched and then weighed. Prior to harvesting, temperature was recorded. Substrates were refreshed weekly for the whole trial period of three weeks. Harvesting maggots was more convenient as they were easily collected from the harvest chamber after opening lid of the culture unit. Cattle offal resulted in significantly higher maggot yield while least was from cattle manure ($p < 0.05$) (Table 1).

Temperature was significantly higher in poultry manure ($p < 0.05$); however, there was no relationship between temperature and yield, $r = .191$, p (one-tailed) $> .10$. Thus cattle offal where easily available is most suitable substrate for culturing housefly maggots. Efforts are now underway to upscale this production technique for production of larger volumes of maggots.



Plate 1: Side and top view of culture containers and housefly maggot cultures

Table 1: Yield and temperature from different types of substrates

No	Substrate	Yield (g)	Temperature (°C)
1	Cattle Manure	21.57 ± 0.21^d	32.66 ± 3.50^b
2	Poultry Manure	40.83 ± 0.67^b	41.60 ± 3.45^a
3	Pig Manure	27.25 ± 1.29^c	34.66 ± 3.25^b
4	Cattle Offal	50.47 ± 0.43^a	33.46 ± 5.05^b
5	Kitchen Leftovers	27.24 ± 0.33^c	34.86 ± 4.45^b

EFFECTS OF WATER PARAMETERS ON PHYTOPLANKTON ABUNDANCE IN PANGASIUS *Pangasianodon hypophthalmus* AND TILAPIA *Oreochromis niloticus* PRODUCTION

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Phytoplankton and zooplankton retort rapidly to any alterations in nutrient changes in water bodies indicating the growing nutrient pollution. Plankton analysis gives an overview of the environmental condition of the water body. On one hand where phytoplankton serves as food source, on the other hand some forms causes fish poisoning due to release of toxins during blooms (Boyd, 2004). The present study was carried out to analyze the water quality and plankton diversity of 4 selected pangasius (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*) ponds from two different regions of Bangladesh; Bogra and Khulna.

Monthly sampling was done for a period of 12 months, July 2016 to June 2017. Samples were collected from one pangasius pond and one tilapia pond from each region in triplicate. Samples were analyzed for physico-chemical parameters. Quantitative analysis of plankton was done on Sedgewick-Rafter counting chamber (S-R cell). Qualitative study of plankton was done to identify plankton up to genus level.

Plankton populations in the water of the experimental ponds were found to be consisted of 91 genera belonging 94 species under 13 planktonic groups composed of 9 groups of phytoplankton and 4 groups of zooplankton (Table 1).

Carl Pearson's correlation coefficients were calculated for abundance with different water parameters. Temperature, nitrate, phosphate, ammonia were positively correlated and pH, DO, transparency were negatively correlated with the plankton abundance.

Results shows that the water is fairly good for fish culture. Nutrients of water enhanced the plankton growth which can lead to bloom. Therefore, it needs some corrective measures to maintain the water chemistry of the pond which will help to keep the plankton community in control.

Table 1: No. of species identified during 12 months of study period in two regions

Group	Bogra		Khulna	
	Pangasius	Tilapia	Pangasius	Tilapia
No. of species				
Phytoplankton				
Bacillariophyceae	14	14	15	14
Chlorophyceae	19	19	10	10
Chrysophyceae	1	-	1	-
Conjugatophyceae	-	-	2	2
Cryptophyceae	-	-	-	-
Cyanophyceae	9	9	8	8
Dinophyceae	1	1	1	1
Euglenophyceae	4	4	3	2
Mediophyceae	-	-	2	3
Zooplankton				
Copepoda	2	2	2	2
Cladocera	5	4	5	4
Rotifera	5	5	5	5
Crustacean larvae	1	1	2	2
Total	61	59	56	53

BOOSTER SPROUTING: A NEW ERA OF SAVING TIME AND SPACE IN AQUAPONICS

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Aquaponics was developed as an alternative to extensive farming, benefitting growers by saving land, water, and providing food. Different aquaponics systems use water in a similar manner, but one common challenge is to maximise the efficient use of space as defined by production per square meter. In this context, a promising system has been developed that uses a vertical tower; in the form of an aeroponic system, in contrast to more traditional horizontal aquaponic plant production systems.

The aim of this research was to test the difference in growth between sprouted and booster sprouted lettuce (*Lactuca sativa*) with rainbow trout (*Oncorhynchus mykiss*) (50 fish/tank) in 3 identical vertical towers (Figure 1). Booster sprouted refers to plants that were grown in an aquaponic system in a greenhouse for the first 25 days then transferred to the towers.

This experiment took place over a period of 42 days, at the University of Western Australia Field Station, Aquaculture and Native Fish Breeding Laboratory, Shenton Park, Western Australia, Australia.

Results showed the advantage of booster sprouting in a greenhouse compared with regular sprouting in an aquaponics system. The initial weight of lettuce was 1.2 g/20 plants whilst the final average weight of plants sprouted in the traditional manner (Figure 2) was 8.9 g and 209 g for booster sprouted ones (Figure 3).

As space requirements at early stages of plant growth is much smaller, these findings help in finding an efficient way to save time and space while also maximising production.



Fig.1 Vertical Tower



Fig.2 Regular sprouted Lettuce



Fig. 3: Booster sprouted Lettuce

EVALUATION OF TWO SALT TREATMENTS FOR HOLDING MARKET SIZE LARGEMOUTH BASS *Micropterus salmoides* AS A FOOD FISH

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Distributing locally grown food fish live may provide aquaculture producers in Kentucky a more profitable market opportunity than selling fish as a commodity. Routinely holding fish live in recirculating systems with minimal loss will be required for this pathway to be successful. In this study investigators evaluated the use of salt in recirculating systems for holding unfed market size largemouth bass. Two experiments were conducted and two treatments per experiment were evaluated: 1) 0 and 4 parts per thousand (ppt.) NaCl; 2) 4 and 8 ppt. Water quality was monitored daily, and blood chemistry was analyzed at 0, 48, 96, 144, and 192 hours after stocking. Blood chemistry was measured with the Abaxis Vetscan VS2 analyzer.

In experiment 1 (0 & 4 ppt. NaCl), total ammonia nitrogen (TAN), un-ionized ammonia (UIA), nitrite and nitrate increased over time. There was a decrease in pH of the water after hour 0. Differences between treatments were found for TAN, UIA, and pH. In the blood, Albumin, Globulin, and Total Protein increased from hour 0, then remained elevated throughout the experiment. Phosphorus and the enzyme Alanine Aminotransferase (ALT) decreased over time, while blood glucose was highest at hour 0, then decreased and stabilized. Treatment effects were found for blood sodium and plasma cortisol.

In experiment 2 (4 & 8 ppt. NaCl), nitrate increased over time; pH of the water decreased at hour 0; and treatment effects were found for nitrite, TAN, UIA, and pH. Blood chloride and sodium increased over time, while calcium, potassium, and the enzymes Alkaline Phosphatase (ALP) and ALT decreased. The enzyme AST and blood CO₂ changed over time as well. Blood glucose was greatest at hour 0, then decreased and stabilized. Treatment effects were found for chloride and CO₂.

There was no apparent weight loss or mortality in either experiment. In both experiments water pH, blood glucose, ALT, and decreased and nitrate increased. There were salinity treatment effects for TAN, UIA, pH, and sodium in both experiments.

WQ	EXP 1	EXP 2	Time	Salinity
TAN	=	=	* +	**
UIA	=	=	* +	**
Nitrite	=	=	* +	*
Nitrate	=	=	** +	
pH	=	=	** -	**
Blood				
Albumin	=	=	* +	
Globulin	=	=	* +	
Total Protein	=	=	* +	
Glucose	=	=	** + -	
Cortisol	=	=		*
Sodium	=	=	* +	**
Calcium	=	=	* -	
Potassium	=	=	* -	
ALT	=	=	** -	
ALP	=	=	* -	
Phosphorus	=		* -	
AST		=	*	
CO ₂		=	*	*
Chloride		=	* +	*
= parameter measured in that experiment				
* = sig in one experiment			+ = increase over time	
** = sig in both experiments			- = decrease over time	
+ - = increases then decreases over time				

ASSESSMENT OF NEW YEAST PRODUCTS AS FEED FOR RAINBOW TROUT *Oncorhynchus mykiss*

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Fuel ethanol plants generate a co-product stream known as condensed distillers solubles (CDS). Alternative methods of adding value to CDS were developed by concentrating the nutrients from this stream in the form of the yeast *Saccharomyces cerevisiae*. The present study began assessment of these novel yeast feed products for aquaculture.

Yeast was produced from CDS by either centrifugation of the spent yeast of ethanol fermentation (Sedicanter) or by propagation of a proprietary strain of yeast grown on CDS (Aerobic); each source was either dried (Untrted) or subjected to a patent-pending process and then dried to produce UltraMaxTM. A 12-week juvenile trout feeding trial was conducted with test diets containing 42% crude protein and 16% crude lipid and various yeast sources included as 20% of the diet. All diets were hand fed to quadruplicate tanks of trout (initial weight 20 g) to apparent satiation thrice daily. Fish were group-weighted every 3 weeks to monitor performance. At the end of the trial, all fish were weighed and measured for size distribution curves. Ten fish from each treatment were sampled for biological indices (hepatosomatic index, spleen-somatic index) and histological evaluation (liver, kidney, distal intestine).

Based on the combined data, it is concluded that UltraMaxTM shows significant potential as a rainbow trout and aquaculture feed ingredient.

TABLE 1. Animal performance of rainbow trout fed different sources of *Saccharomyces cerevisiae* yeast from fuel ethanol process streams.

Treatment	g of Feed Offered /Tank	Weight Gain (g / fish)	SGR (% BW / day)	Gain:Feed (g / g)
Commercial yeast control	1688 ^a	58.2 ^a	1.91 ^a	1.15 ^a
Sedicanter yeast (untrted)	1740 ^{ab}	55.0 ^a	1.83 ^{ac}	1.27 ^b
Sedicanter UltraMax TM	2119 ^c	73.3 ^b	2.32 ^b	1.02 ^c
Aerobic yeast (untrted)	1813 ^b	58.8 ^a	1.95 ^c	1.18 ^{ab}
Aerobic UltraMax TM	1974 ^d	69.5 ^c	2.22 ^d	1.04 ^c
Positive Control (no yeast)	1986 ^d	75.5 ^b	2.40 ^b	0.93 ^d
S.E. of the mean	31	2.2	0.03	0.01

Means in each column with unlike superscripts differ ($P < 0.05$)

IMMUNE STATUS AND GROWTH RESPONSE OF NILE TILAPIA, *Oreochromis niloticus* TOWARDS OXYTETRACYCLINE SUPPLEMENTED FEED

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Among many antibiotics used in the field of aquaculture, oxytetracycline is extensively supplemented in feed with the aim of diseases prevention and growth promotion. The current experiment was therefore designed to evaluate the effectiveness of two levels of oxytetracycline i.e. 100 and 200 mg/ Kg of feed supplementation on growth and immune status of Nile tilapia (*Oreochromis niloticus*). Twenty fish per aquaria were randomly divided into three experimental treatments as control T_0 (without oxytetracycline), T_1 and T_2 with 100 and 200 mg oxytetracycline/ Kg of feed respectively. The experiment was designed in completely randomized design along with three replicates. The experimental feed having 35% crude protein and selected levels of antibiotic was used at the rate of 7% of body weight given twice a day. Parameters of development/ growth for example total weight (g) and total length of body (cm) were noted down every week. Immune response in terms of different hematological parameters such as RBCs ($10^6/\mu\text{L}$), WBCs ($10^3/\mu\text{L}$), Platelets, Hematocrit (%), Hb (g/dL), MCV (fl), MCH (pg), MCHC (g/dL) of the sampled fish of each treatment were evaluated at the end of experiment. Different physico-chemical parameters of water like pH, DO and temperature $^{\circ}\text{C}$ (mg/L) were also monitored every week. The response towards growth remained significant between the values of weight gain (g) for T_0 , T_1 and T_2 at the end of experiment. Total weight gain's (g) mean average values in T_2 was $1.96 \pm 0.02\text{g}$ followed by T_1 $1.64 \pm 0.03\text{g}$ and T_0 $0.83 \pm 0.06\text{g}$. The deviation among length gain (cm) of treatments was also significant in T_0 , T_1 and T_2 . Total length gain's (cm) mean average value in T_0 at the end of trial was $0.22 \pm 0.02\text{cm}$ in T_1 and T_2 was $0.36 \pm 0.01\text{cm}$ and $0.57 \pm 0.02\text{cm}$ respectively. The results of immune response after blood analysis at the end of trial revealed that the concentration of RBCs ($10^6/\mu\text{L}$) in T_0 was 0.11 ± 0.01 ($10^6/\mu\text{L}$) while concentration was higher in T_1 0.79 ± 0.01 ($10^6/\mu\text{L}$) and T_2 0.36 ± 0.02 ($10^6/\mu\text{L}$). The WBCs ($10^3/\mu\text{L}$) concentration in T_0 was 9.14 ± 0.02 ($10^3/\mu\text{L}$) while increased in T_1 42.85 ± 0.20 ($10^3/\mu\text{L}$) but decreased in T_2 18.23 ± 0.25 ($10^3/\mu\text{L}$). Platelets concentration was increased in T_1 845 ± 2.89 and T_2 279 ± 1.73 as compared to T_0 90 ± 0.85 , Hematocrit (%) concentration also increased in T_1 $3.39 \pm 0.34\%$ and T_2 $4.09 \pm 0.06\%$ as compared to T_0 $0.91 \pm 0.01\%$. Hemoglobin (g/dL) counts decreased in T_1 9 ± 0.03 (g/dL), and T_2 4 ± 0.29 (g/dL) as compared to T_0 12.50 ± 0.20 (g/dL). MCV (fl) decreased in T_1 43.03 ± 0.55 (fl), as compared to T_0 86 ± 0.58 (fl) and T_2 115 ± 1.73 (fl). MCH (pg) count increased in T_0 24.50 ± 1.26 (pg), but decreased in T_1 23.05 ± 0.04 (pg) followed by T_2 22.90 ± 0.63 (pg). MCHC (g/dL) concentration increased in T_1 46 ± 2.31 (g/dL) and T_2 44.83 ± 1.42 (g/dL) as compared to control T_0 33.17 ± 1.01 (g/dL). By the results of experiment it is evident that inclusion of oxytetracyclin in feed showed a significant and positive effect on growth and immune response in Nile tilapia. Although the highest growth is shown in T_2 (200 mg oxytetracyclin/ Kg of feed) but better immune parameters along with good growth was exhibited by T_1 (100 mg oxytetracyclin/ Kg of feed).

MODERN BIOTECHNOLOGIES OF AQUACULTURE IN THE SOUTH OF RUSSIA

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The Southern Federal District of Russia (SFD) is characterized by the most favourable natural and climatic conditions for fish farming, and is the region where all directions of production should be developed: pond fish farming, fish farming in warm waters of power plants, trout farming in the foothills, pasture fish farming in lakes, estuaries, and water storage reservoirs. The volume of production of fish farming in the South of Russia in 2016 increased to 73.7 thousand tons (in general in the country – 173.9 thousand tons). The main fish farming objects are carp, herbivorous fish species, trout, paddlefish, and sturgeon species, and rarely farmed objects are buffalo, catfish, and tilapia.

Promising directions of aquaculture development in the southern regions of Russia are:

- pasture aquaculture – one of the promising directions to obtain products of aquatic life when using bioproduction capacity of water bodies;
- pond aquaculture – one of the directions using pond areas and extensive technologies to obtain commercial fish products;
- industrial forms of fish farming (in cages, tanks, RAS, and other fish breeding capacities), which have received intensive development in the SFD in recent years;
- recreational aquaculture – the cultivation of objects for sport and recreational fishing;
- mariculture (of great potential in the SFD) – cultivation of marine hydrobionts.

Intensive technologies of cultivation of marketable fish and fish stocking material, with the release of 17-24 metric centners/ha, should be used in the southern regions of Russia. The main objects of pond fish farming in the southern regions are carp and herbivorous fish species. In recent years there has been a trend of broadening species diversity of farmed fish both at the expense of native fish species (tench, pike, catfish, crucian carp, pike-perch, and perch) and the use of previously acclimatized species: channel catfish, so-iuy mullet, paddlefish, and buffalo.

Commercial fish farming in Russia is developed within the following areas: cage fish farms in warm waters and in water bodies with natural water temperature; tank fish farms using fresh, brackish, and salt water; fish farming in RAS, which is of special attention (both the development and improvement of technologies of hydrobionts' farming).

Research organizations have developed and successfully implemented a number of scientific and technical solutions, new biotechnologies of sturgeon breeding at fish farms in the South of Russia. High plasticity and adaptability of sturgeon species allow using practically all types of farms, including cages (warm water and marine), ponds, tank systems, and RASs for commercial sturgeon breeding. A comprehensive biotechnology for the production of environmentally friendly sturgeon fish products, which also allows eliminating climatic risks, has been developed at *SSC RAS*.

The “green” technologies have become widespread in recent years as they envisage the general environmental management (control over water and air pollution, industrial wastes), energy production from renewable sources (solar energy, biofuel), and reduction of harmful emissions into the atmosphere.

Thus, fish farming technologies in the South of Russia can both quantitatively increase the production volumes of live fish and reduce the costs within a short-term period. The introduction of new aquaculture objects and expansion of the range of farmed fish species in the southern regions of Russia will allow increasing the production potential of fish farming.

BIOTECHNOLOGY OF JOINT AQUAPONICS FARMING OF AQUACULTURE AND AGRICULTURE OBJECTS USING BIOPREPARATION TO IMPROVE THE EFFICIENCY OF CROPS CULTIVATION IN THE ARTIFICIAL MULTI-TIERED SYSTEM

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Development of new aquacultural and agricultural technologies plays significant role in the era of energy conservation and environmental priorities. Aquaponics is a technology that can provide population with natural and eco-friendly products. Fish and plants, cultivated via aquaponics technology, are currently covering only 7% of total Russia's food sector. However, in last several years this share is growing, forming a positive trend. During last two years, researches on the joint cultivation of hydrobionts and plants were held in an experimental aquacomplex of SSC RAS. These researches were embodied in the development of experimental integrated system of joint cultivation of hydrobionts and crops. The main goal was to create a biotechnology that allows to produce eco-friendly fish products and green plants via aquaponics method. Optimal aquatic and other parameters were defined and sustained in the integrated water supply system. These parameters were held in line with biological needs of all involved species and plants previously chosen for the experiment.

Bester (a hybrid of beluga and sterlet), clarion catfish and tilapia were farmed in the experimental system. During incubation process, high growth rates were reported. The survival rate indicator was at the point of 96-98%. After 4 months of Tilapia cultivation in a multi-tiered system, the average weight of each object was 194.14 ± 83 g while an average initial mass varied 21.26 ± 1.6 g. The absolute weight gain was 110.69 g, with the average daily increase of 1.7 g/day. Average daily growth rate was 1.3%, with the coefficient of mass accumulation 0.06 pt., and the Fultin's condition factor of 2.52 ± 0.15 pt.

The experiments on the joint cultivation of fish and crops such as tomatoes, cucumbers, bell peppers, strawberries, lettuce and parsley were conducted. Previous studies have shown that plants with a short growing period (45-60 days) are most suitable for farming via aquaponics method. Others with longer vegetative period accumulates an excessive amount of nitrates, exceeding MPC norms, which negatively influences the quality of the product. In this regard, the objective was to apply methods of growth stimulation and nitrate reduction for plants with a long growing season, such as tomato. A biopreparation based on the isolate of the *Serratia ficaria* strain were used during the experiment on tomato. The *Serratia ficaria* strain is not only restrains the development of pathogenic diseases in agricultural crops and stimulates plant growth, but also increases the concentration of vitamin C in fruits as well as reduces the accumulation of nitrates. Biopreparation based on the *Serratia ficaria* strain is completely safe for humans, animals and the environment. Treatment of plants with liquid form of *Serratia ficaria* was performed every 10 days. The obtained experimental data were compared with growth rates of plants from the control group. The liquid form of *Serratia ficaria* strain facilitates the intensive development of tomato. The increase in the length of the stalk in the experiment was 162.8% higher than in the control group. This indicates a significant stimulation of the development of agricultural plants during cultivation in water recirculation system using a preparation based on the *Serratia ficaria* strain. At the same time, the nitrate level did not exceed MPC norms during the experiment.

DESIGN OF MOBILE PHONE APPLICATION TO FACILITATE INFORMATION SHARING AMONG FISH FARMERS IN UGANDA

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Mobile phones have the potential to provide solution to the existing information asymmetry in aquaculture. Uganda's aquaculture sector suffers from low growth rates and low productivity. Issues in access to market and fish production information are weak points at every stage of the aquaculture-supply chain. For small farmer-based economy like Uganda, access to information can possibly enable better incomes and productivity to the farmers. This paper through focus group discussions with fish farmers in Mpigi, Wakiso, Kalungu, Gulu, Lira, Kole and Jinja, has tried to 1) Identify the type of mobile phones (either smart of basic phones) owned by fish farmers 2) identify the critical information needs of fish farmers that can be used in the designing of a mobile application service that will enable fish farmers, input suppliers, fish traders, and fish farmer's association leaders access fish farming and market information 3) assess the impact of mobile application on information access, fish farmer's income and aquaculture productivity.

The information got will guide Public agencies, non-governmental organizations, researchers and cellular service providers in facilitating the design and use of mobile phone applications as a means to guide, coordinate, and instruct fish farmers in Uganda. The study showed that majority of fish farmers prioritized information packages on feeding (how to mix feeds on farm); market prices; pond construction, preparation, stocking, management and harvesting; fish health issues and predator control that should not be missed out on the mobile App. Farmers were also concerned about the quality of information, timeliness of information and trustworthiness of information that will be on the app since these are three important aspects that could help them meet their needs and expectations.

STATUS OF THE AQUACULTURE AND PUBLIC RESOURCE DEPEDATION ORDERS TO ADDRESS RESOURCE DAMAGE ISSUES ASSOCIATED WITH CORMORANTS

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Over the past 40 years a significant rise in the population of the double-crested-cormorant (*Phalacrocorax auritus*) a long-lived fish eating bird has led to cormorant-society conflicts associated with commercial and natural resources such as aquaculture, property and fisheries. Before 1998, the only authority for authorizing lethal take of depredating cormorants by the U. S. Fish and Wildlife Service (USFWS), the Federal agency responsible for overseeing species protected by the Migratory Bird Treaty Act, was through the issuance of depredation permits under the existing federal regulations 50 C.F.R 21.41. In the 1990's, DCCO's became a management priority for the USFWS as conflicts, real or perceived, continued to escalate. Following extensive National Environmental Policy Act and rule making process review the USFWS in cooperation with USDA Wildlife Services recommended new options to address cormorant conflicts. The Aquaculture Depredation Order (AQDO) was issued in 1998. A 2003 Final Environmental Impact Statement led to the Public Resource Depredation Order (PRDO) and updates to the AQDO. The AQDO and PRDO affected 13 and 24 states respectively, providing producers and management agencies increased flexibility including the use of lethal control while maintaining sustainable cormorant populations.

In accordance with NEPA procedures, the orders were reviewed and renewed via Environmental Assessments (EA) in 2009 and 2014. However, in May 2016 the orders were vacated when a federal judge concluded that the USFWS did not adequately consider the impacts on cormorant population and failed to consider additional alternatives under the EA issued in 2014. Without the orders and adequate NEPA documentation the USFWS stopped issuing or renewing depredation permits for cormorant take in states previously covered by the orders. Decisions by the USFWS regarding pending permit applications and processes for the take of cormorants at aquaculture facilities, the protection of natural resources, private property and human health and safety need to be made. Thus, the USFWS in cooperation with WS has developed an EA to comply with the NEPA process and determine if proposed actions may significantly impact the environment. The EA proposes depredation permits across 37 central and eastern states as the mechanism to manage cormorant conflicts. Information on the cormorant management EA status and content, resource damage, management outcomes and the future in regards to cormorant management will be presented.

HERBIVOROUS MARINE FINFISH CULTURE – THE COMPELLING CASE FOR KYPHOSIDS

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Marine finfish aquaculture is dominated by carnivorous species, requiring diets high in proteins and oils, often fulfilled through wild-sourced fish products. Reliance on wild fish resources is a scalability and sustainability obstacle, driving research (including nearly a decade of work by Kampachi Farms) to identify alternative ingredients and reformulate feeds for ‘carnivorous’ fish. However, a more direct means of circumventing these obstacles remains largely unexplored – herbivorous marine finfish that do not demand fishmeal and fish oil in their diets.

Kyphosids (chubs or rudderfish) are esteemed food-fish among the Pacific Islands, and there are species native to much of the world, including all of the U.S.’s prime mariculture development regions. Preliminary research with *Kyphosus vaigiensis* (Brassy chub) has shown them to be amenable to larval rearing in the hatchery, resist skin flukes, yield commercially attractive growth rates, and produce a highly-appealing product with up to 28% lipid (by dry weight).

In June 2017, a successful spawn yielded insight into broodstock husbandry protocols, first feeds, and early larval development milestones.

Grow-out studies of juvenile *K. vaigiensis* confirm an attractive growth rate and FCR for production on low-cost commercial herbivore feeds. The successful culture of these high-quality herbivorous marine finfish could provide a new, sustainable, healthy, and low-cost option for fish farmers and seafood consumers.



Figure 1. *K. vaigiensis* larva, 5 days posthatch

ORNAMENTAL FISH INDUSTRY IN INDIA - ISSUES RELATED TO PRODUCTION AND MARKETING OF FRESHWATER ORNAMENTAL FISHES

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Ornamental fish trade in India can be categorized into export trade and a domestic trade. The export trade is solely depended on the natural recourses of India which contributes only a share of 0.3% to global ornamental fish export. India is listed last amongst all the S-E Asian countries that are major exporters of ornamental fish in the world. It is stagnating at this level for many years with very little possibility to improve as mainly wild varieties of ornamental fishes of India are in demand in international market. The list of important varieties of freshwater species is listed in the paper. Of the 300 species of fresh water fishes of the Western Ghats of India, about 155 have ornamental value. The natural stocks of most of these varieties have drastically declined and the technologies of their mass propagation are established only for a few species. The list of species for which the captive breeding technology is developed is also presented in the paper. The *Sahyadria denisonii* a famous fish of Western Ghats of India that mainly contributed to ornamental fish export from India is now listed as endangered in the IUCN Red List of threatened species. It may sound disappointing to a countryman that country is not contributing significantly to international ornamental fish trade but it is worth noting that the domestic aquarium trade has emerged as a fast developing economic activity in India since the beginning of 21st Century. This is because the hobby of aquarium keeping in country has grown many folds during last two decades. The total value of trade has increased from 550 million INR (7.8 million US\$) to 3000 million INR (42.85 million US\$) during the last decade. It is because of increasing number of aquarium hobbyists which is 2% of a total of 50 million (2015-16) middle income group house-holds (MIGHh) in country. A high growth of domestic trade was a mixed function of increasing number of MIGHh, change in behavior of Indian buyer's to spend a part of income on hobby, easy & cheaper availability of aquarium products and promotional effort by government (Jain & Mercy 2017). The results of SWOT analysis done on the production and marketing of ornamental fishes of India is presented in the paper.

WATERMEN AND FARMERS: THE PLACE OF TRADITIONAL FISHERMEN IN MARYLAND OYSTER AQUACULTURE

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Maryland's commercial aquaculture industry continues to grow as more individuals become involved in leasing areas of public waters and farming shellfish. As part of an ongoing project, we are learning to better understand "who" is getting involved in Maryland oyster aquaculture, and in particular, to what extent the state's commercial wild oyster harvesters, or watermen, are participating in the business. Using an approach of participant observation and semi-structured interviews we have observed that, while watermen are moving towards oyster aquaculture, their motivations for doing so, level of involvement, perceived success, and attitudes toward aquaculture vary individually.

Of these nuances, differences among level of involvement and how a waterman has incorporated aquaculture into his or her livelihood portfolio may be particularly relevant to the management of the state's public fisheries. Thus far, most watermen involved in shellfish aquaculture have added it to an already diverse livelihood portfolio, and, for those who have used it to replace another income-generating activity, they rarely eliminated their involvement in the public oyster fishery. Few watermen interviewed have shifted into aquaculture as their full-time, sole livelihood activity.

Perceived success is also important, as this opinion may influence others to adopt or reject aquaculture. Several features of Maryland's oyster aquaculture industry may color watermen's perceptions of success. The newness of the modern industry is one factor. Although oyster aquaculture has existed in Maryland for a century, 2009 legal and regulatory changes only recently made it a viable option for many. As such, many watermen involved in aquaculture have held leases for fewer than five years and may not yet feel that they have made a return on their investment. This runs counter to earning wages daily through their traditional work of harvesting public resources, including oysters, blue crabs, and other catch. Related to perceived success, an additional theme involves the assumption that one must be well off in order to do well in aquaculture. Accurate or not, this perception may keep watermen away.

Understanding the involvement of commercial watermen in Maryland shellfish aquaculture is important to better realize the direction of both Maryland's private and public oyster industries and provide more effective information for management. This project will help detail what aspects of oyster aquaculture may deter watermen from participating in this fishery, and how to facilitate greater success for those already involved.

EVALUATION OF SEXUAL MATURATION AND REPRODUCTIVE ABILITY OF TRIPLOID YELLOW PERCH *Perca flavescens* AND VIABILITY OF PROGENY

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The sexual maturation and reproductive ability of triploid Yellow perch was evaluated. Triploid (3n) and diploid (2n, control) siblings were produced in the spring of 2014. Triploidy was induced using heat shocks (29°C) applied at 5 minutes post fertilization for 25 min duration. Ploidy was then determined using flow cytometry and triploidy was induced at a rate of 87% in the heat-shocked group, while control siblings were 100% diploid. Fish were grown to sexual maturation and sexed based on morphological characteristics during the 2017 spawning season (April-June). Spermiation was observed in all diploid control males. Triploid males were non-spermiating; however, mature testes were observed upon dissection. Male and female triploid and control fish were then sampled and gonads were dissected for histological analyses (results are forthcoming).

Eggs from both triploid (n=3) and diploid (n=2) females were stripped and *in vitro* fertilization was performed using a single unrelated diploid male during spring 2017. Difference in average GSI between triploid ($16.4 \pm 2.9\%$) and diploid ($20.9 \pm 3.7\%$) females was not significantly different ($p=0.3115$). Fertilization rate was determined at the 2-4 cell stage. Fertilization rate from the 2nx2n control cross ($91.6 \pm 3.9\%$) was not significantly higher ($p=0.1665$) than the 3nx2n cross ($67.9 \pm 18.3\%$). Embryos from each female were isolated during incubation (16-18°C).

Eyed-stage embryos from each female's progeny were randomly sampled for ploidy determination by flow cytometry. Triploid-diploid crosses yielded 80-100% aneuploid progeny and all sampled embryos were deformed to various degrees. The control crosses yielded 100% diploid progeny. Embryos were force-hatched and post-hatch larval survival was counted. Post-hatch survival of control progeny ($15.0 \pm 1.4\%$) was significantly higher ($p=0.0023$) than that of aneuploid progeny ($4.6 \pm 1.1\%$). In order to quantify the deformities within the aneuploid groups, progeny from each female were separated into swimming and non-swimming. Non-swimming individuals made-up 20-100% of the aneuploid groups. We attempted to raise the aneuploid progeny; however, high mortality was observed within the first 10 days of larval rearing in the aneuploid groups (94-100%), while control larvae only experienced 32-38% mortality.

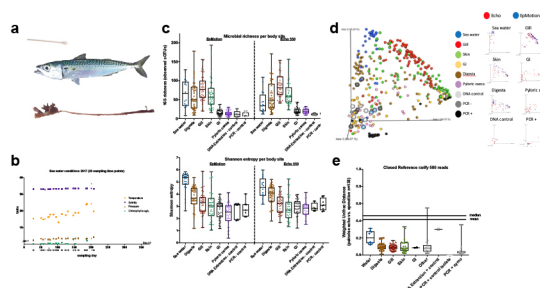
DEVELOPMENT OF A MODEL MARINE FISH: HIGH THROUGHPUT GENOME AND MICROBIOME ANALYSIS OF PACIFIC CHUB MACKEREL, *Scomber japonicus*, ACROSS SEASONS AND BODY SITES

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Although the potential of developing marine aquaculture production in coastal areas is promising, the realization has been encumbered by concerns of environmental degradation, including genetic contamination of wild stocks through escapement, and indigenous fish disease transmission. Because of the high costs and feasibility challenges with carrying out the research to test these questions, the fate of marine offshore finfish production in the US and elsewhere is in limbo. High value species like yellowtail, *Seriola lalandi*, and bluefin tuna, *Thunnus sp.*, are targets of marine pen culture, but few data are available on understanding the natural genomic variation of these fish. Since host-associated microbes make up over 50% of the cellular composition and 100 times the genomic content of most vertebrates, we set out to *describe* the hologenome (Fig 1a) of multiple body sites (gill, skin, digesta, gastrointestinal tract, and pyloric caeca) of the Pacific Chub Mackerel, *Scomber japonicus*, across 20 samplings over 7 months from the Scripps Institute of Oceanography (SIO) pier in San Diego (Fig 1b). Currently there is no genomic assembly for Pacific Mackerel (*Scomber japonicus*) so we sequenced the genome with several technologies including long-read nanopore technology. Using 60x coverage of Illumina short read sequencing we have estimated the diploid *Scomber japonicus* genome to be 750 Mb with 100 Mb of high copy number repeats such as the ribosomal DNA and centromeres. Based on the current genome sequencing the individual we are sequencing has low (1%) heterozygosity. To assess the microbiome, we developed a low cost, miniaturized (5 ul), high-throughput (384-sample) microbiome library preparation method with the Echo-550 using this microbiome timecourse. Alpha diversity, as measured by microbial richness and evenness (Shannon entropy), were not significantly different within sample types across PCR methods, but were significant as compared across body sites (Kruskal-Wallis, $P < 0.0001$) (Fig 1c). Gill samples were rich in microbial diversity but dominated by multiple *Shewanella sp.* sub-operational taxonomic units (sOTUs) and skin samples were enriched in *Photobacterium* and *Rickettsia*. Digesta had the highest evenness across body sites, a higher richness than the GI or pyloric caeca containing various sea water associated microbes like *Synechococcus* and *Pirellulaceae* along with putative GI associated *Vibrio sp.* and *Photobacterium sp.* The GI and pyloric caeca had the lowest alpha diversity (Fig 1c) across body sites and were both dominated by mycoplasma. The gill and skin microbiomes of the mackerel are most similar to sea water compared to GI-associated microbiomes (Fig 1d) yet each are enriched in unique microbes found in low numbers or absent from the ocean. While this finding has been suggested and shown in mammals, this is the first example in fish. Beta diversity was not significantly different when comparing the two methods (Fig 1e), but did differ significantly when comparing across sample types. Various microbes across the body sites (gill = 12 sOTUs, skin = 4 sOTUs, digesta = 8 sOTUs, pyloric caeca = 2 sOTUs) were correlated to seasonal variation of water temperature (Spearman correlation). *Synechococcus* and *Rhodobacteraceae* were positively associated with water temperature and prevalence on the gill, skin and digesta. No microbes were correlated with length, mass, or condition factor of the mackerel. We have described the Pacific mackerel wild type microbiome across multiple body sites and seasons demonstrating the abiotic and biotic effects driving the microbial communities and providing context for future disease transmission studies.

Figure 1. Microbial diversity associated with (a) five body sites from 46 *S. japonicus* sampled across (b) 20 time points from January 2017 to August 2017. (c) Alpha diversity of the body site associated microbiomes as measured by microbial richness and Shannon entropy. (d) Beta diversity as measured by closed reference unifracs rarefied to 500 reads. (e) Technical variation in beta diversity distances (bray-curtis) between different PCR methods is lower than total variation



EFFECT OF AN EXTRACT OF PLANT ORIGIN ON IMMUNITY MARKERS GENE EXPRESSION AND THE PROTECTION AGAINST IN VITRO INFECTION WITH *P. Salmonis*

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Background: NatControl™ is a natural extract of plant origin of the family *Acanthaceae*, standardized in specific labdane diterpenoid. Its immunostimulating properties have been studied in several models, but their effect on fish has been little studied. Therefore, this study aims to determine the *in vitro* properties of the extract in SHK-1 macrophages cells from *Salmo salar*.

Methods: The effect of NatControl™ on the expression of innate immunity markers associated with antibacterial and antiviral mechanisms in SHK-1 cells (derived from *Salmo salar* cephalic kidney) was evaluated. For this, a stimulation kinetics was performed with the product and the levels of IL-12 and IFN- γ were quantified by RT-qPCR. Also, the protective effect of this formulation was evaluated by an *in vitro* challenge with *P. salmonis* strain LF-89 (10^4 and 10^6 bacteria / mL). The percentage of cytotoxicity was calculated by a colorimetric assay that measures LDH enzyme release into the culture medium of the treated and infected cells.

Results: Our results show that 5 nM and 10 nM NatControl™ exhibit the greatest effect on the expression of IL-12 and IFN- γ in SHK-1 cells at 2 h and 12 h stimulation times. Also, against the challenge with *P. salmonis*, a protective effect was noticed on cells pretreated for 6 h with NatControl, lasting until day 20 post-infection. It should be noted that the cells treated with the respective treatments with NatControl™ presented negative values of toxicity at the beginning of the experiment, which could be due to a protective effect of the products on cell viability.

Conclusion: We suggest a potential immunostimulating effect of NatControl™ on SHK-1 cells and a protective effect against infection with *P. salmonis*. It would be interesting to develop future studies to deepen the immunostimulating and protective effect of this compound in fish models in response to infections with *P. salmonis*.

Our special thanks to HP Ingredients for giving us samples of NatControl™

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 CO₂, 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 different matrices (n=3) control, 2-D, 3-D, and “advanced” 3-D, in yielding the highest amount of algal biomass (g AFDW/m²/day) and greatest ability to remove nutrients. Once the most effective mesh material was determined, a subsequent experiment was conducted to determine the effect of different harvest schedules. Four different harvest intervals (4, 7, 10, and 14 days) were chosen to examine their effectiveness in yielding the highest amount of biomass. 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 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.

INTRODUCING THE MARICULTURE SPATIAL ATLAS: AN AQUACULTURE SPATIAL SCREENING TOOL FOR THE U.S. EXCLUSIVE ECONOMIC ZONE

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With over 85,000 miles of marine coastline encompassing more than three million square nautical miles, the U.S. Exclusive Economic Zone (EEZ) is the largest in the world. Aquaculture development within the U.S. EEZ is limited, however, to only a few shellfish and no finfish farms. To increase investor confidence and find the most suitable space for development, NOAA is working to build spatial planning assets for sustainable screening and siting of offshore aquaculture (shellfish, finfish, and algae). In partnership with the Department of Energy's ARPA-e MARINER program, NOAA is developing the Mariculture Spatial Atlas (MSA), a dynamic screening tool for aquaculture in the U.S. EEZ (0 – 200 nm). This tool accesses data from a robust set of aquaculture relevant spatial data at the most suitable temporal resolution, a map viewer, data download capabilities, and a dynamic spatial reporting function designed to return a customized report for a user-defined space within the U.S. EEZ. The customized report is designed to inform exploration and the aquaculture permitting process providing analytical information on natural resources (e.g., sensitive habitats, protected species), commerce and defense (e.g., principle ports, shipping fairways, military zones), energy and minerals (e.g., wind planning areas, sediment resources, pipelines), and biophysical and oceanographic profiles at surface and at depth (e.g., light attenuation, nitrates/phosphates, current speed) for an area of interest. This dynamic marine spatial atlas will be one of the most comprehensive screening tool developed for aquaculture (and other ocean uses) to date. This presentation will provide an overview of the data resources and architectural structure of the MSA and provide a pre-release demonstration.

WAVE EXPOSURE MODELLING TO SUPPORT AQUACULTURE SITING

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Wave energy forecasting is an essential exercise to understand its impact on engineering and biological structures. A wind wave-forecasting model, Wave Exposure Model (WEMo), was designed specifically for shallow water areas in confined water bodies with minimum effects from open ocean swells. WEMo explicitly uses wave generation, shoaling and nonlinear dissipative processes as wave breaking and bottom friction to calculate wave energy. The model works in a Geographic Information System (GIS) format in association with ArcGIS. A newer version of WEMo will soon be released with added functionality of handling batch datasets and faster processing that could make it a suitable tool for aquaculture applications.

Wind wave energy is an essential component in siting new or expanding aquaculture facilities. Surface conditions such as waves and high currents during storms can add stress to aquaculture gear leading to structural failures and detrimental effects on production schedules and operational costs. WEMo could provide a wind wave energy regime for siting a potential aquaculture operation at various temporal and geographical scales. Output from WEMo along with other information will be a part of aquaculture siting tools provided by NOAA for shellfish or finfish aquaculture around the country. WEMo wave energy profiles will also give aquaculture industry and engineers a means to calculate added stress due to waves on aquaculture structures, including nets, bags, lines, anchors, and other ground tackle.

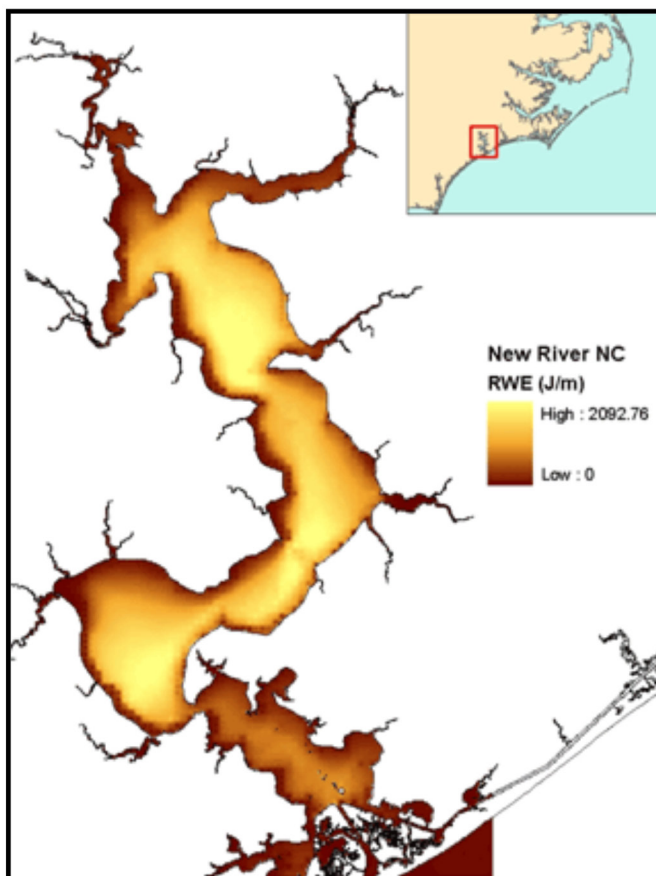


Figure 1. Representative wave energy (RWE) modeled along estuarine shorelines within the New River, North Carolina.

EMPLOYING PRACTICAL METHODS OF MEASURING SOLIDS IN BIOFLOC DOMINATED SHRIMP PRODUCTION RACEWAYS IN A COMMERCIAL APPLICATION

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Measuring solids in biofloc culture systems is important for several reasons. In general, high solids levels are indicative of poor culture conditions possibly resulting from overfeeding or inadequate solids removal. This excessive organic material can encourage the development of pathogenic bacteria and also increase oxygen demand. High solids concentrations can cause gill fouling, leading to respiratory distress or even death. On the other hand, reducing solids levels too low will inhibit nitrification. It is therefore necessary to maintain solids levels within a targeted concentration. The two most common types of solids discussed relative to biofloc systems are: settleable solids (SS) and total suspended solids (TSS). Methods for the measurement of both are described in detail in the APHA *Standard Methods for the Examination of Water and Wastewater*. However, as described both methods are beyond what may be practical on many farms. The purpose of this presentation is to (1) share one farm's solutions/adaptations for practical solids measuring in a biofloc system, and (2) hopefully stimulate discussion for further development of field expedient methods for the measurement of solids at the farm level.

As previously stated the *Standard Methods* methodology for the measurement of SS and TSS are beyond what some managers would consider practical. In the case of SS the major constraint is time. As described this method requires one hour per sample. We have assumed that the bulk of the solids we are interested in will settle within the first 10-15 minutes. In addition we may employ several methods to enhance the settling process, such as tapping, twisting, or slow stirring. As for TSS, the *Standard Methods* not only requires a lot of time but also some specialized equipment and materials not readily available. In our case TSS measurements are obtained indirectly using a turbidity probe (ProDSS, YSI Inc., Yellow Springs, OH). Using the instrument, turbidity readings are taken on at least six water samples from different culture tanks. Corresponding water samples are analyzed by a local laboratory (City of Cape Coral Environmental Resources Laboratory, Cape Coral, FL). The laboratory TSS values and measured turbidity values are entered into the instrument's software, which creates a correlation between the two data sets. The instrument uses this correlation to derive TSS estimates in mg/L from turbidity readings. The combined results of the two methods (SS and TSS) are used to manage solids concentrations of culture water within targeted levels.

The methods described in this presentation, while admittedly less accurate than those prescribed in *Standard Methods*, allow measurements to be taken more frequently. In other words we are less concerned with the absolute accuracy of the results as much as timeliness and the ability to detect and manage changes in the system from day to day. This presentation will include actual data to demonstrate the effectiveness of these two methods for controlling solids in biofloc dominated raceways.

TRIUMPHS, TRIALS, & TRIBULATIONS OF SPF SHRIMP BROODSTOCK IN ASIA

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Historically, the black tiger prawn, *Penaeus monodon*, has been the most common shrimp species cultured in Asia. However, about 15 years ago, an increasing number of Asian shrimp farmers began stocking their ponds with non-indigenous Pacific white shrimp, *Litopenaeus vannamei*, and the dominance of *P. monodon* started to fade. According to the FAO, production of farmed *L. vannamei* in Asia increased from 2,310 metric tons (MT) in 2000 to 3,156,948 MT in 2015. This represents a 136,564% increase in *L. vannamei* production during this 16-year period. In contrast, production of farmed *P. monodon* in Asia increased only 14% during the same period, from 623,194 MT in 2000 to 709,601 MT in 2015. This dramatic species shift can be attributed to a number of factors, including the decline in availability and quality of wild *P. monodon* broodstock and poor growth and survival of *P. monodon* grown in ponds. Importantly, while the production of *P. monodon* in Asia remained stagnant, broodstock suppliers in the United States began exporting Specific Pathogen Free (SPF) *L. vannamei* which were selectively bred for rapid growth and high survival, as well as for tolerance to Taura syndrome virus. The use of SPF *L. vannamei* broodstock in Asian shrimp hatcheries catalyzed the dramatic species shift away from *P. monodon* and resulted in significant financial benefits to shrimp farmers using postlarvae produced from these broodstock. Increased profits resulted from shrimp farmers being able to stock their ponds with healthy postlarvae which were free of the major pathogens plaguing the shrimp farming industry. In addition, because the postlarvae came from selectively bred broodstock, farmers benefited from shrimp that exhibited relatively fast growth and high survival, and *L. vannamei* were tolerant of much higher stocking densities than *P. monodon*.

Despite these benefits, there are a number of challenges associated with the use of SPF broodstock. Imported SPF *L. vannamei* broodstock are more expensive than locally produced, pond-reared broodstock, so some hatchery operators opt for the cheaper alternative. Although there are no published data indicating the extent to which pond-reared broodstock are used in Asia, the quantity may be high in some countries. The negative consequences of using domestic, pond-reared broodstock in the hatchery can be severe due to their unknown health status. Infected broodstock can spread pathogens both horizontally and vertically in a hatchery, resulting in infected postlarvae which are then sent to the farms for growout. In addition, pond-reared broodstock, as well as imported SPF broodstock, are of unknown pedigree to the hatchery operator, and the mating of close relatives will cause "genetic erosion" resulting from the rapid accumulation of inbreeding when closely related broodstock are mated. Offspring produced from such broodstock may exhibit inbreeding depression which manifests in reduced fitness (i.e. lower pond survival, increased susceptibility to pathogens, lower reproductive performance), especially in stressful environments. There are a number of possible solutions to these challenges, including the establishment of public-private partnerships to better control both the health status and genotypic quality of broodstock available to the industry.

NUTRITIONAL EVALUATION OF PROCESSED SOYBEAN MEAL IN DIETS OF GENETICALLY MALE TILAPIA (GMT)

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Replacement of unavailable high cost fishmeal by the identification of alternate high quality protein source can serve as the efficacious shift to the development of sustainable, economical and viable aquaculture production.

The present study was conducted to evaluate the nutritive potential of processed soybean meal (SBM) diet as a substitute to expensive fishmeal for Genetically Male Tilapia (GMT). Experimental fish (n=25) were stocked in fiberglass aquaria (Dimensions: L, 1ft 18 inch× W, 2 ft.) and was fed with experimental diets (dehulled SBM (T2) and non-dehulled SBM (T3)) at 4% of fish wet body weight. After 75 days of study period, the increase in weight gain because of consumption of T2 and T3 SBM was recorded as 626.55 and 595.76 respectively. The experimental fish fed with T2 diet showed significant higher growth than that of T3 diet fed fish group. The feed conversion ratio (FCR) was statistically highly significant for fish fed T2 with a mean value of 1.44 as compared to T3 fed fish that was 1.58. Protein Efficiency Ratio (0.70) and (0.63) and Specific Growth Rate values (1.82) and (1.77) were found significantly different between treatments.

Proximate analysis was performed to ascertain significant differences in meat quality parameters of fish fed with T1 diet as compared to T3. A statistically highly significant difference was observed in crude protein content of the fish fed with T2 (66.90) as compare to T3 diet (65.0) followed by T1 (60.95). Fish Fed with T3 (34.10) showed significantly high values of ash content as compare to fish fed with T2 (32.95) followed by T1 (29.20). Moisture content of the fish fed with T3 (3.35) showed significantly higher values than those of the fish fed with T2 (2.65) and T1 (2.85) No significant difference in fibre and phosphorus contents were found in fishes fed with experimental diet.

(Continued on next page)

Table 1 (A) Fish Feed Formula for the preparation of DSBM and NDSBM.

	DSBM	NDSBM
Soybean Meal	5.43	6.03
Maize	4.61	3.78
Soy oil	1.31	1.54
Gluten 60%	0.93	0.93
L-Lysine	0.53	0.53
Limestone	0.20	0.20
MDCP	0.19	0.19
L-Methionine	0.04	0.04
Supplement	0.02	0.02
Salt	0.02	0.02
Isoleucine	0.005	0.05
Total	13.32	13.30

SBM: Soybean meal; MDCP: Mono di-calcium phosphate

Table 2. Proximate Analysis of Genetically Male Tilapia (GMT).

	T1	T2	T3
Crude Protein	60.95±0.05 ^c	66.90±0.10 ^a	65.0±0.00 ^b
Crude Fiber	6.40±0.10 ^a	6.20±0.20 ^a	6.50±0.20 ^a
Moisture	2.85±0.15 ^b	2.65±0.15 ^{ab}	3.35±0.15 ^a
Ash	29.20±0.20 ^c	32.95±0.50 ^b	34.10±0.10 ^a
Phosphorous	0.405±0.15 ^a	0.405±0.05 ^a	0.405±0.04 ^a

Values are expressed as mean ± S.D.

Data with different superscript letters in a row are significant at P<0.05

Table 3 Assessment of Growth Parameters of fish Fed with different diets.

	T1	T2	T3
Initial w.t	1.32±0.83 ^a	1.43±0.17 ^a	1.46±0.05 ^a
Final w.t	11.36±0.9 ^a	33.74±0.3 ^b	30.50±0.05 ^c
Weight gain	205.61±17.9 ^a	626.55±3.8 ^b	595.76±10.2 ^b
% weight gain	773.22±6.1 ^a	2254.09±263.6 ^b	2072.88±118.3 ^b
Initial Biomass	32.91±2.1 ^a	36.56±4.1 ^a	35.72±1.3 ^a
Final Biomass	238.52±20.01 ^a	781.70±2.0 ^b	701.45±1.1 ^c
Initial length	46.35±1.6 ^a	44.20±1.6 ^a	46.55±0.2 ^a
Final length	112.20±1.7 ^a	118.85±2.6 ^a	117.35±4.1 ^a
Length gain	112±1.70 ^a	117±2.55 ^a	118±4.05 ^a
FCR	2.05±0.40 ^a	1.44±0.005 ^b	1.58±0.01 ^c
SGR	1.24±0.01 ^a	1.82±0.06 ^b	1.77±0.02 ^b
PER	0.49±0.009 ^a	0.70±0.002 ^b	0.63±0.005 ^c
K Initial	1.34±0.2 ^a	1.69±0.01 ^a	1.42±0.06 ^a
K Final	0.80±0.03 ^a	1.62±0.5 ^a	0.98±0.2 ^a

Values are expressed as mean ± S.D.

Data with different superscript letters in a row are significant at P<0.05

Table 4. Enzyme analysis results of Genetically Male Tilapia (GMT) fingerlings

	T1	T2	T3
Phytase	0.41±0.012 ^a	0.42±0.01 ^a	0.42±0.004 ^a
Amylase	0.43±0.003 ^a	0.44±0.01 ^a	0.45±0.004 ^a
Lipase	6.55±0.35 ^b	8.55±0.25 ^a	7.4±0.10 ^b

Values are expressed as mean ± S.D.

Data with different superscript letters in a row are significant at P<0.05

At the end of the trial the fishes were slaughter and gut samples were collected for enzyme analysis. Results of enzyme analysis revealed that non-significant difference was observed for phytase and amylase activity among the treatments. Statistically high significant difference ($P < 0.05$) for lipase activity was found in T1 (6.55) and T2 (8.55) whereas nonsignificant difference was found between T1 (6.55) and T3 (7.4).

In conclusion, the Genetically Male Tilapia (GMT) showed better growth performance with Dehulled SBM diet due to its best nutrient composition, bioavailability and acceptability. The findings of present study suggest that Dehulled SBM diet has found slightly better than the non-dehulled SBM can be used in feed of genetically male tilapia culture on commercial basis.

THE COASTAL AQUACULTURE PLANNING PORTAL: AN OVERVIEW OF TOOLS FOR PLANNING AND SITING AQUACULTURE IN THE COASTAL ZONE

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Aquaculture development activities in the U.S. are increasing in both nearshore and offshore environments. Planning and siting of both economically and ecologically sustainable aquaculture is difficult as evidenced by a high failure rate. Planning efforts are needed at multiple spatial scales including local, regional, and national. Tools and approaches that support the decision process for future site suitability and development of aquaculture infrastructure are in demand. To assist coastal managers and industry, we are compiling tools from around the U.S. that have relevance to aquaculture planning. We have developed the Coastal Aquaculture Planning Portal (CAPP), a portal comprised of over 40 tools organized into four categories including shellfish aquaculture planning and siting, finfish aquaculture planning and siting, environmental interactions, and environmental modeling. Examples of tools include a list of aquaculture siting mappers for state and federal waters, seascape impact viewers, storm water calculators, sealevel rise viewers, habitat priority planners, cultured species factsheets, and protected species interactions guidelines.

EFFECTS OF POND WATER REPLACEMENT AND STOCKING DENSITY ON WATER QUALITY, PLANKTON ABUNDANCE AND GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*)

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To determine optimum water replacement rate for fish pond production, we investigated the effects of pond water replacement and stocking density on water quality, plankton abundance, fish growth rate and yield. Nile Tilapia (*Oreochromis niloticus*) fingerlings with average body weight of 0.4 g were stocked into four 300 m² earthen ponds. Each pond was partitioned into six equal parts using fishnets. Three parts of each pond were stocked with 2 fingerlings/m² and the other three parts with 4 fingerling/m². Each pond was fertilized using 10 kg of biogas slurry three times a week and fish were fed at a feeding level of 10% of their body weight daily for 3 months. For the first two ponds 50% of water was replaced weekly and for the other two ponds water was not replaced. Water quality, plankton abundance and tilapia growth performance were monitored throughout the 90 day experimental period. The results show significantly higher ($p \leq 0.05$) conductivity (168.9 10.7 $\mu\text{S}/\text{cm}$) and nitrate ($\text{NO}_3\text{-N}$) (5.0 ± 0.5 mg/l) concentrations in ponds with no water replacement while significantly higher ($p \leq 0.05$) dissolved oxygen concentration (9.9 mg/l) was observed in ponds in which 50% of water was replaced weekly. Higher plankton (both phytoplankton and zooplankton) abundance was observed in ponds with lower stocking density and 50% water replacement than in ponds with higher stocking density and no water replacement. Stocking density influenced fish yield but water replacement did not affect yield. The average net yield for higher stocking density was superior ($4,891 \pm 565$ kg/ha/yr) to that of lower stocking density ($2,105 \pm 485$ kg/ha/yr). Although stocking density of 2 fingerlings /m² gave higher growth performance than that of 4 fingerlings/m², statistically no significant ($p > 0.05$) differences were observed with regard to growth rate, specific growth rate and survival between the two stocking densities and water replacement regime. Our data suggest that water replacement can improve the productivity of Nile Tilapia (*Oreochromis niloticus*) in earthen ponds, however, for good results more studies are needed with a wider range of both stocking density and water replacement regimes.

THE EFFECT OF NEW ISOLATE PROBIOTICS ON GROWTH AND SURVIVAL OF LARVAL TILAPIA (*Oreochromis niloticus*)

J. L. Myer*, H. J. Schreier, E. Schott and D. McIntosh

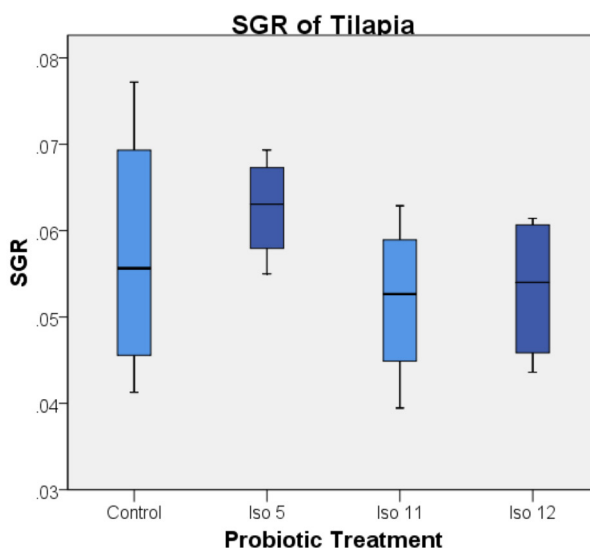
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Heavy stocking, medication and general mismanagement of fish health has led to a need for alternative options to keep aquatic stock healthy. Industry is keenly interested in options that will enable them to maintain the health of their animals. Probiotics have been shown to help with immunity, gut health, growth, and mitigation of stress. Earlier research has identified several probiotic strains of bacteria and demonstrated their ability to inhibit growth of certain fish pathogens in disc diffusion assays. These probiotics were harvested from brackish water fish and are believed to be useful for both marine and fresh water species.

For my research, select strains of the probiotic bacteria were evaluated to see if they can enhance growth and survival of tilapia. Fish were separated into 16, 1-L experimental containers at a density of 25 larvae/L and assigned to one of four treatments (three probiotics and a control). Assigned probiotic treatments were applied by dosing the culture water every other day to maintain a probiotic concentration of 10^6 colony forming units. Fish were allowed to grow under experimental conditions until survival reached 10% in any experimental container, after which time surviving fish were counted, and group weighed by container.

There was no statistically significant difference in larval survival or growth ($p = 0.355$, and $p = 0.459$, respectively from a one-way ANOVA) among the treatments. FCR and SGR also showed no significant differences ($p = 0.579$ and $p = 0.507$ respectively from a one-way ANOVA). Differences in water quality were analyzed with a GLM repeated measures and found to not differ significantly among the treatments; ammonia ($p = 0.384$), nitrites ($p = 0.504$) and nitrates ($p = 0.546$). While there was no significance in growth and survival, the lack of heavy mortality when dosed shows that further research is needed.

Future research will focus on testing the growth and survival of additional aquaculture species including, rainbow trout, striped bass and clownfish. Successful completion of this research will provide insights into the development of additional probiotic species that may prove useful in boosting immunity of target species, promoting gut health, improving growth, and mitigating stress.



SUITABILITY OF BASIC WATER QUALITY CONDITIONS FOR AQUACULTURE IN UGANDA

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In Uganda, aquaculture is a promising commercial venture with untapped potential to provide fish for food as well as an alternative livelihood. However, less attention has been given to water quality as a way of improving aquaculture production in Uganda. The main objective of this study was to assess a suitability status of the quality of Ugandan water sources for Nile tilapia and African catfish farming. Water samples were collected from pond water sources in 20 districts in four regions (Central, Eastern, Northern, and Western parts of Uganda) which were representative of the different climatic zones in Uganda. Water samples were analyzed for specific conductivity, total hardness, calcium hardness, pH, and total alkalinity, and compared by one-way ANOVA with post-hoc multiple comparison. The pH was rather high in some waters and especially in the Eastern region. Waters with pH consistently above 8.5 or 9.0 are not suitable for aquaculture. Total hardness and calcium hardness were usually within the optimum range of 50-150 mg/l, total alkalinity was also within optimum range of 50-150 mg/l apart from the central region. Nevertheless, some water had less than 30 mg/l total alkalinity and pH of less than 6.5, therefore should be treated with lime. Specific conductivity concentrations were all above desired range of 150 μ S/cm. There were statistical differences in the total alkalinity and specific conductivity concentrations of the northern region from the other regions ($F = 4.18$, $p = 0.0075$. $F = 4.15$, $p = 0.0078$, $n = 30$ at 95% confidence interval, respectively).

Water parameter	Region			
	Central	Eastern	Northern	Western
Total hardness (mg/l)	50.3 \pm 11.40 (6.3-258.6)	89.0 \pm 16.62 (21.5-502.9)	102.7 \pm 22.49 (12.7-533.8)	66.0 \pm 13.35 (5.5-332.3)
Calcium hardness (mg/l)	20.9 \pm 4.97 (0.0-109.0)	43.3 \pm 5.94 (8.1-133.6)	34.0 \pm 5.86 (3.2-114.2)	31.1 \pm 7.38 (0.0-140.0)
Total alkalinity (mg/l)	43.2 \pm 8.99 (6.0-69.8)	80 \pm 9.16 (7.9-238.1)	106 \pm 20.72 (13.7-441.1)	55.5 \pm 12.95 (6.4-329.3)
Specific conductivity (μ S/cm)	174.7 \pm 29.16 (38.4-557)	298.2 \pm 46.89 (79.0-469.0)	351.1 \pm 71.70 (53.3-1377.5)	157.6 \pm 19.52 (25.9-383.9)
pH	7.3 \pm 0.20 (5.9-10.6)	9.8 \pm 0.34 (7.0-13.0)	8.3 \pm 0.24 (5.2-13.0)	7.0 \pm 0.19 (5.3-9.4)

PROFITABILITY ANALYSIS AND TECHNICAL EFFICIENCY ESTIMATION OF TILAPIA PRODUCTION IN CHINA

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Tilapia (all Cichlids) production has emerged as the second largest cultured species by over 85% countries in tropical and subtropical countries. A rapid growth rate of 13.4 percent was observed in 2008 (FAO 2010) and this particular species is expected to become a leading global animal protein source. Economic analysis of this particular species is relevant in determining investment decision making among potential investors, and providing a learning experience to developing nations like Uganda about the Best Management Practices (BMP) and new innovations implemented by Chinese tilapia farmers in this particular business venture.

Data collection was carried out in southern coastal areas of China covering 6 provinces of (Guangdong, Guangxi, Fujian, Hainan, Yunnan and Hebei provinces) with major focus on 3 culture models of (monoculture, polyculture and integrated). The study was carried out between June to November 2016 and a total of 50 tilapia farmers purposively selected were interviewed using a structured questionnaire. The study focused on two objectives that included profitability indices using input and output Analysis, technical efficiency estimation was calculated based on Cobb Douglas production model.

All the results were calculated based on 1 Mu (=667m²) as the average production area. Positive Gross margins (GM) and Net farm incomes (NFI) were observed, an indication that tilapia farmers were in position to recover all the production costs. Tilapia production was economically viable with positive Benefit Cost Ratio (BCR) of 1.6 in monoculture and 1.0 among the polyculture farmers. The breakeven yield averaged to 767.9 kg of tilapia for farms to recover total production costs without any profits. Farmers that produced small quantities of tilapia were expected to sell their fish at relatively higher prices to breakeven. Positive returns on investment (ROI) of 1.5 to 1.0 were observed in monoculture and polyculture respectively. Findings on technical efficiency estimation indicated that farmers operated at 82% mean efficiency indicating that there was still room to expand tilapia production using the current level of inputs and technologies. And this efficiency was highly influenced by fish feeds (55%), Electricity (30%) and seed (11%). However land lease negatively influenced technical efficiency by (-0.16%). Farmer specific variables revealed that education 29%, Aquaculture training 10% positively influenced farmer level OF technical efficiency. The gamma value of 0.11 was observed and it was less than 1 an indication that technical inefficiency was as a result of random stochastic effects (noise) beyond farmer's control. Which led to the rejection of the null hypothesis at 1% significant difference. And significant difference of 23% in technical efficiency among tilapia farmers was observed. In conclusion efficient feed utilization is paramount to reducing the current feed conversion ratios (FCR) of 1.4, and also lowering production costs to improve efficiency. Farmers should ensure production of quality fish (organic) will attract more global consumers. There is need to engage/attract the youths and women into aquaculture production to close the labor and gender gaps with in the aquaculture sector.

EVALUATION OF DIFFERENT PROCESSING METHODS ON THE NUTRITIONAL COMPOSITION AND CERTAIN ANTI-NUTRITIONAL FACTORS IN PEAS *Pisum sativum*

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Green pea (*Pisum sativum*) is an annual leguminous crop grown in varied weather condition, however it prefers temperate climate and well drained sandy soil. It is a nutritious legume which contains high protein about 25%, amino acids, carbohydrate, Vitamins A and C, and minerals such as calcium and phosphorus. Apart from being an important source of food, pea, (*Pisum sativum*) seeds play a role as an alternative feed ingredients to soybeans as plant protein source in the diet formulation for marine and brackishwater fish and crustaceans. The proximate composition of peas is found to be comparable with other grain legumes such as lentils and beans (*Vicia faba*) and cereals.

Peas are known to contain anti-nutritional factors, such as trypsin inhibitors (TIA), tannins, phytic acid, saponins which are reported to be responsible for decreasing protein quality, amino acid availability and adversely affect the nutritive value of the seed. TIA are present in many legumes in varying amounts while phytic acid in the seeds is usually stored in the form of phosphorus. Tannins are polyphenolic compounds that bind enzymes and other proteins to form insoluble complexes while on the other hand, saponins were considered secondary plant metabolites.

Therefore, this study was conducted in order to evaluate the different processing methods on the nutritional and anti-nutritional factors of green peas. Raw peas (A) which serves as control were subjected to the different feed processing methods into dehulled green pea (B), autoclaved green pea (C) and soaked green pea (D) samples.

The results of the effect of the different processing methods on the proximate composition and anti-nutritional factors of green peas are presented in Tables 1 & 2.

Table 1 Proximate composition of raw and processed green pea samples (%)

Parameters	A	B	C	D
Moisture	10.21	10.69	4.74	5.67
Crude Protein	19.39	22.31	21.56	20.66
Crude Fat	1.06	1.08	1.30	1.18
Crude Fiber	5.91	1.23	6.77	5.17
Ash	3.16	3.16	2.79	3.11
NFE*	70.48	72.22	67.58	69.88

*Nitrogen Free Extract

Table 2. Effect of processing methods on anti-nutritional factors of *Pisum sativum*

Methods	TIA	Tannin	Phytic	Saponin
A	7.39	3.81	421.10	0.571
B	6.99	4.42	310.07	0.623
C	6.62	4.42	368.30	0.407
D	7.05	4.06	396.73	0.525

OPPORTUNITIES AND CHALLENGES IN PROCESSING AND PRESERVATION OF SUGAR KELP

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Sugar kelp (*Saccharina latissima*) is marine macro-algae and is a rich source of fibers, vitamins, minerals and antioxidants. It can be used as food products for various health benefits. However, due to high moisture content (~92%), it is highly susceptible to microbial attack and enzymatic deterioration. Sun drying is one of the oldest techniques for food preservation, it is very slow, requires clear weather conditions. 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 presentation is to analyze various opportunities and challenges for kelp processing and preservation. In our laboratory, we work on optimizing the drying conditions 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.

This drying conditions (Figure 1) were correlated with the kelp product properties (Figure 2) to study the storage and shelf-life of few bioactive compounds. This important information is essential for developing an innovative technology focused on clean, energy efficient and closed drying system for producing top-notch and local finished products for American consumers. This presentation will focus on various process and product parameters to be considered for sustainable kelp processing and preservation.

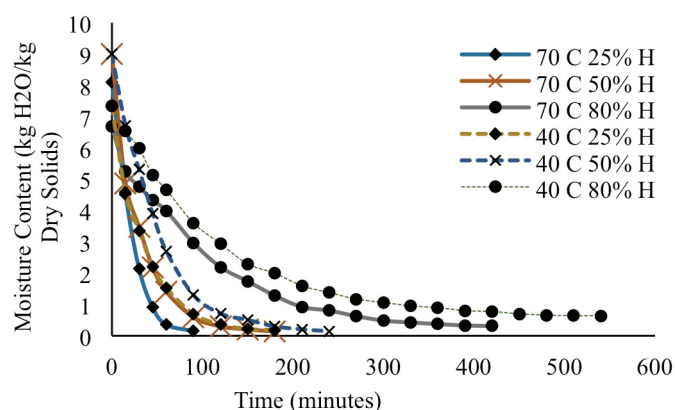


Figure 1. Effects of temperature and humidity on moisture content of sugar kelp

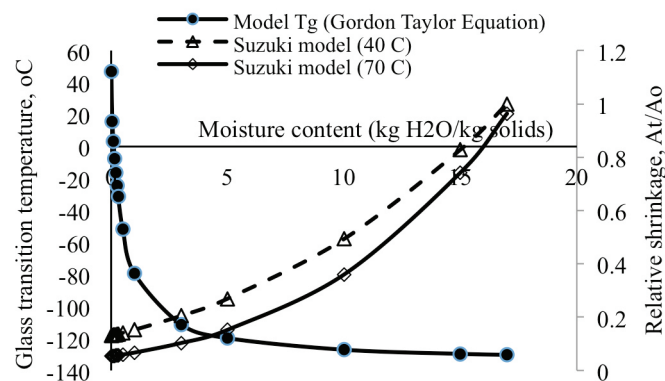


Figure 2. Plot of glass transition, humidity and moisture content of kelp

***Macrobrachium rosenbergii* AND THE AQUARIUM MARKET**

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Freshwater shrimp farmers in Virginia benefit from direct sales of an iced product to consumers at the harvest site on the day of harvest. These transactions result in a cash transfer to the farmer, and a decrease in regulatory issues due to no processing. Additional revenue can be generated due to an expanding interest in the aquarium trade for display uniqueness offered by the large shrimp. This demand has been shown by individual aquarists and business displays. *Macrobrachium rosenbergii* can be maintained in tanks or large aquaria at low density by farmers after harvest. Water quality is important and managed using standard filters, heaters and air pumps with dissolved oxygen concentrations >5 ppm, minimal ammonia levels and temperature kept approximately 20°C. Structure (netting, PVC pipe, plastic mesh) should be provided for shrimp to isolate. Some water exchange is necessary. Low temperature limits growth and molting, but allows feeding activity. Initial returns to farmers were >\$8.00 per blue claw male.

KEY FACTORS NEED TO BE CONSIDERED FOR SHRIMP DISEASE MANAGEMENT: FEED AND BEST FARM MANAGEMENT PRACTICES

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The shrimp industry has grown over the past two decades with the development of husbandry practices that allow for efficient use of resources in their live production. However, this has not been without challenges. Like many confined animal feeding systems, unless properly managed, risk and incidence of disease can be devastating to the economic survival of a farm or the industry. Diseases such as Acute Hepatopancreatic Necrosis (AHPND), White Feces Syndrome (WFS) and Enterocytozoon Hepatopenaei (EHP) disease are some recently emerging disease that have resulted in huge economic losses. To date, best management practices to control these diseases is not confined to any one solution but is a multi-factorial approach consisting of environmental controls, pond management and using ingredients in formulations that promote healthy nutrition.

Healthy nutrition extends beyond formulating the diet based on nutrient and digestibility values for individual ingredients. The shrimp feed industry should contribute a more effective and strategic approach by formulating shrimp diets with highly digestible and functional protein ingredients. These ingredients contain less anti-nutrients/contaminants, limit excretion of undigested nitrogen and phosphorous into ponds for improved feed conversion rate (FCR) as well as improve gut health for better digestion and healthier shrimp. Imp ponds and proper feeding management together with highly pure/digestible protein ingredients used for shrimp feeds would limit the releasing of undigestible nitrogen and phosphorous in your shrimp ponds are all effective and strategic solutions for White Feces Problem ! In this paper; therefore, the aspects of best farm management practices, feed characters and diet formulation approaches with highly digestible protein and functional ingredients as well as healthy nutrients will be discussed in details for better shrimp disease management.

INVESTIGATING THE EFFECTS OF DIFFERENT FEED AND PROBIOTICS ON THE SURVIVAL AND GROWTH OF GOBY (*Oxyeleotris marmorata* Bleeker 1852) 3-45 DAYS OLD

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This study was conducted to increase survival and growth in the period from 3 to 45 days old. The results showed that supplemented probiotic combining with enriched rotifers accelerated the growth of goby fry period 3 - 15 days old. At 15 to 30 days of age, the use of enriched *Artemia* at the beginning of the experiment (15 dph) combined with the addition of *Moina* on 25 dph (NT5) significantly increased the survival rate of marble goby. *Moina* and *Tubifex* as food gave the best performance of marble goby at 30-45 days of age.

TABLE 1. Growth of marble goby at 15 days old

Treatment	Average length (mm)	Average weight (mg)
Control	5,22 ^a ± 0,16	0,82 ^a ± 0,07
T1	5,25 ^a ± 0,18	0,96 ^b ± 0,03
T2	5,22 ^a ± 0,13	0,82 ^a ± 0,02
T3	5,55 ^b ± 0,18	1,05 ^c ± 0,04

TABLE 2. Growth of marble goby at 30 days old

Treatment	Average length (mm)	Average weight (mg)
Control	6,61 ^a ± 0,23	3,57 ^a ± 0,47
T1	7,20 ^b ± 0,23	3,88 ^b ± 0,50
T2	7,70 ^c ± 0,16	4,66 ^c ± 0,32
T3	7,81 ^d ± 0,14	4,66 ^c ± 0,38
T4	8,02 ^e ± 0,15	5,20 ^d ± 0,44

TABLE 3. Growth of marble goby at 45 days old

	Treatment					
	T1 <i>Moina</i>	T2 <i>Tubifex</i>	T3 <i>Moina</i> + <i>Tubifex</i>	T4 Pelleted feed	T5 Pelleted feed+ <i>Moina</i>	T6 Pelleted feed+ <i>Tubifex</i>
Average length (mm)	22,1±0,3 ^a	19,7±0,3 ^b	22,1±0,4 ^a	17,6±0,4 ^c	21,0±0,3 ^{ab}	20,1±0,3 ^b
Average weight (mg)	191,8±3,2 ^{bc}	186,8±4,5 ^c	207,3±4,0 ^a	168,2±2,4 ^d	200,8±3,0 ^{ab}	182,5±3,0 ^c
SGR _L (%/day)	5,6±0,1 ^a	4,8±0,1 ^c	5,6±0,1 ^a	4,1±0,1 ^d	5,3±0,1 ^{ab}	5,0±0,1 ^{bc}
SGR _W (%/day)	17,8±0,1 ^{bc}	17,6±0,2 ^c	18,3±0,1 ^a	17,0±0,1 ^d	18,1±0,1 ^{ab}	17,5±0,1 ^c

SUSTAINABLE SABLEFISH FEED USING FULL FAT SOYBEANS AND FISH TRIM

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Heated (HSB) and unheated (SB) full fat soybeans, soy protein concentrate (SPC), and trim waste from Pacific whiting were incorporated into feeds for marine sablefish. The three feed treatments were formulated with equal amounts of soy protein. Feeds containing HSB and SPC performed significantly better than feed containing SB (Fig. 1). After feeding to satiation for 8 weeks histomorphologic evaluation of distal intestine 2 cm from rectum was conducted by Fish Vet (Portland, ME). Varying levels of inflammation were measured for all three treatments (Fig. 2). Fisher's exact test showed significant difference ($P = 0.013$) in mononuclear cell infiltration of fish fed heat treated full fat soybeans (HSB) versus unheated full fat soybeans (SB). Fish receiving HSB feed had significantly lower intestinal inflammation than SB fish (Fig. 3 and Fig. 4). HSB performed as well as SPC and is a much lower cost ingredient.

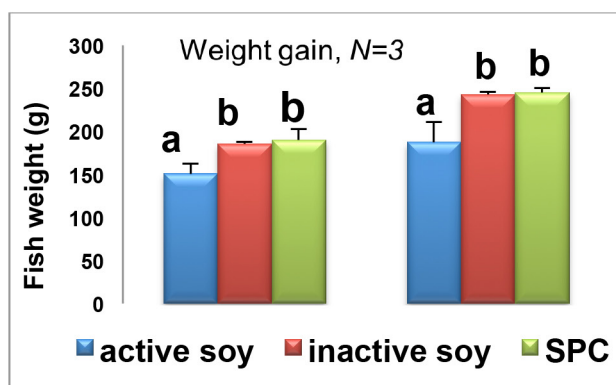


Figure 1. Weight gain.

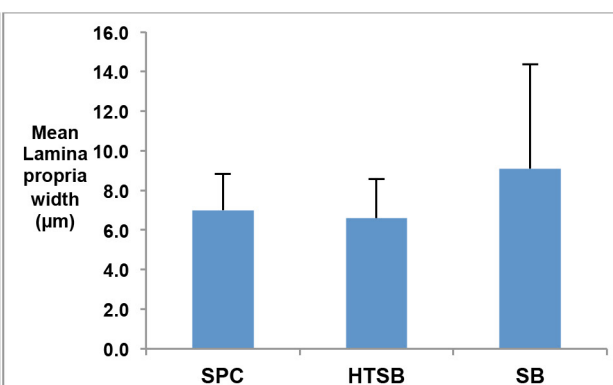


Figure 2. Lamina Propria Width.

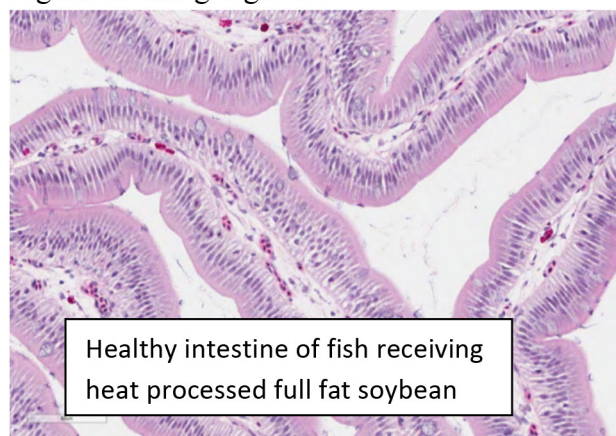


Figure 3. HSB treatment.

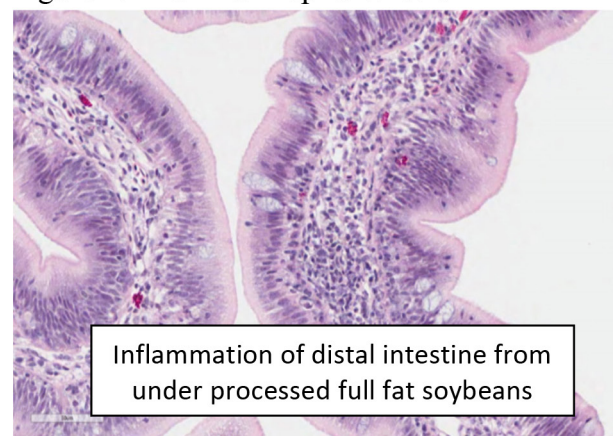


Figure 4. SB treatment.

SEX AND COLOR SEGREGATION IN NILE TILAPIA CROSSES OBTAINED FROM DIFFERENT STRAINS OF YY MALES AND NORMAL XX FEMALES

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All-male tilapia production for market size overcomes the disadvantages of mixed sex rearing by increasing meat yield and size uniformity at harvest, and eliminating possibility of uncontrolled reproduction. As global production of Nile Tilapia *Oreochromis niloticus* continues to rise, ongoing efforts to produce all-male seedstock should be evaluated to improve efficiency, increase profitability, and responsible use of our genetic resources. The YY male technology, a form of genetic sex regulation for production of all-male seedstock, may be considered more acceptable by consumers than hormonally treated fish for human consumption. The goal of this study was to investigate sex and color segregation of commercially available YY males from two sources crossed with three strains of normal XX females.

Broodstock (with ratio of 1 male : 3-4 females) were placed in four independent recirculating tank systems (water temperature 28-29 °C), fed 0.5 – 1% body weight/day, and females were checked for eggs/fry every two weeks. Putative XY progeny (5 – 7 months old) were placed in 500 mg/L MS-222™; the color phenotype was recorded, and fish were dissected to identify testis or ovary. The percentage of males varied from 88 to 100% (Table 1). The YY males of the same origin could produce different percentages of males in crosses with different female strains. The red color phenotypes in crosses 1 to 3 segregated into two phenotypes: 100% red color appearance (no black pigmentation), and highly variable blotched phenotype (red background color with varying area coverage of dark pigmentation). Cross 4 of two dark lines resulted in dark fish only (Table 1). Additional crosses will be analyzed and comparatively raised as part of an integrated approach for identifying a fast growing red tilapia. The data collected so far show that expression of black pigmentation in fish with red background color is very variable and special studies on investigation of inheritance of this trait are needed.

Table 1. Percentage of males and Color Distribution in Crosses of Nile Tilapia YY Males (sources of fish used in the study are denoted by letter superscripts).

Cross No.	Origin and Color of Parents		No. of Fish Analyzed	% of Males	Color Distribution, %		
	YY Males	Females (Color-Strain)			Red	Blotched	Dark
1	Red ^a	Red (Lake Manzala) ^c	189	100	12	88	0
2	Red ^a	Dark (Ismailia Canal) ^d	112	94	3	97	0
3	Red ^b	Blotched (Hybrid) ^{de}	102	88	45	57	0
4	Dark ^b	Dark (Ismailia Canal) ^d	330	99	0	0	100

^a Miami Aqua-culture, Inc. (Boynton Beach, FL); ^b Fishgen Ltd. (Swansea, Wales); ^c University of Oklahoma (Norman, OK); ^d Louisiana Specialty Aquafarm (Robert, LA); ^e Ismailia Canal Dark Strain x Red Strain of Unknown Origin.

EFFECTS OF REPLACING ANIMAL MEAL WITH DIFFERENT ADVANCED SOY PRODUCT AND CORN PROTEIN CONCENTRATE ON THE GROWTH AND DISTAL INTESTINE HISTOLOGY OF FLORIDA POMPARO *Trachinotus carolinus*

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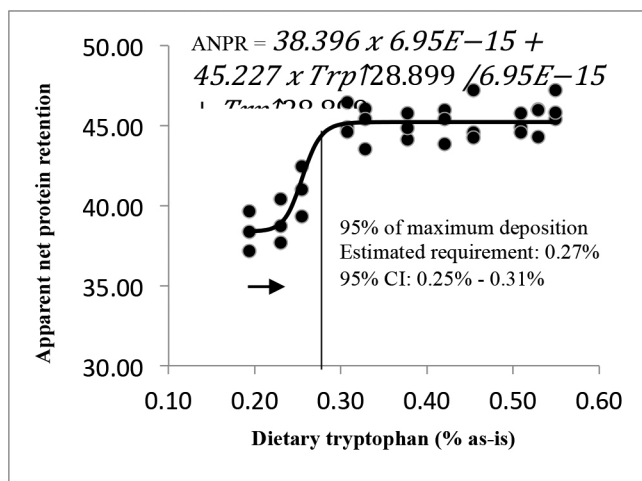
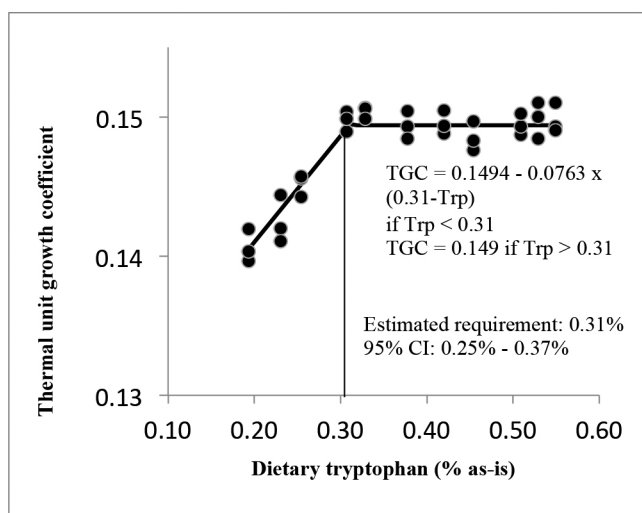
The present study was designed to evaluate the utilization of porcine meal in combination with advanced soy products (enzyme-treated soy and fermented soy) or corn protein concentrate (CPC) to improve the nutritional quality of plant-based diet on growth performance, body composition and distal intestine histology of Florida pompano *Trachinotus carolinus*. Four experimental diets were formulated to be iso-nitrogenous and iso-lipidic basis, to contain 40% crude protein and 8% lipid. A reference diet (PBMD) contained 150 g kg⁻¹ poultry by-product meal (PBM) and 495 g kg⁻¹ soybean meal (SBM), and three test diets were formulated replacing PBM with 15 g kg⁻¹ of CPC (CPCD) and replacing all SBM and PBM with 535 g kg⁻¹ fermented soy (FSBMD) and 451.3 g kg⁻¹ enzyme-treated soy (ESBMD). All test diets were supplemented with porcine meal (PM, 38 g kg⁻¹ of the diet) to serve as a hydroxyproline source. Diets were fed to apparent satiation to triplicate groups of Florida pompano juveniles (mean weight 8.06 ± 0.22 g). After 8 weeks of feeding, fish fed CPCD and ESBMD performed equally well in terms of final body weight, thermal growth coefficient and percentage weight gain in comparison to fish fed PBMD ($P < 0.05$). Voluntary feed intake (VFI) of fish fed FSBMD was significantly lower compared to other treatments. No significant differences were observed in protein, fat, ash, phosphorus, sulfur, potassium, magnesium, calcium, sodium, and zinc contents of whole pompano body across all the treatments. The results obtained in the present histological study showed no significant differences in the thickness of serous layer, muscular layer, and submucous layer among treatments. Fish fed CPCD showed a significant widening of LP with an increase of cellular infiltration and higher presence of goblet cells compared to other dietary treatment, while no significant difference in these histological features between fish fed PBMD, CPCD and ESBMD. Based on these results, PM in combination with 451 g kg⁻¹ enzyme-treated soy or 150 g kg⁻¹ of CPC and SBM can totally replace PBM in the practical diets without any adverse effects on growth, nutritive parameters and several distal intestine health parameters of juvenile Florida pompano.

TRYPTOPHAN REQUIREMENT IN SEMI-PURIFIED DIETS OF JUVENILE NILE TILAPIA *Oreochromis niloticus*

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Alternatives to high-cost protein supplements in formulated diets for Nile tilapia are desirable to reduce feed costs and increase profits in modern farming operations. As tryptophan can be limited in selected alternative ingredients of fish meal, efforts to replace fish meal with other alternative ingredients have a possible risk of creating a Trp deficient diet. To further optimize practical diets for Nile tilapia with respect to IAA requirements, this study was conducted to confirm and refine the tryptophan requirement which is a potential limiting amino acid in our matrix of ingredients. Twelve iso-nitrogenous and isoenergetic diets containing 30% protein and 8% lipid were formulated to meet the nutritional requirement of juvenile Nile tilapia (*Oreochromis niloticus*) with the exception of tryptophan. The basal diet (24AAR) was assigned to meet the respective requirement of indispensable (IAA) based on NRC recommendations for Nile tilapia. This diet contained 24% protein in which 22.22% derived from intact protein and 1.78% from crystalline amino acid (CAA). L-tryptophan was then added to a basal diet at 0.04% increment to produce tryptophan levels ranging from 0.21% to 0.61% of the diet. Diet 30AA which supported the best performance of Nile tilapia in previous work was used as a reference diet. Each diet was fed to three replicate groups of juvenile Nile tilapia (7.9 ± 0.1) in a recirculation system for eight weeks. Saturation kinetic model, broken line models with linear or quadratic ascending portions were used to evaluate dose-response relationship of thermal-unit growth coefficient, apparent net protein retention, tryptophan retention against dietary tryptophan. Akaike weights were calculated and used for model selection in addition to the model's overall R^2 . The tryptophan requirement of juvenile Nile tilapia was estimated at 0.31% (0.25-0.37%), 0.33% (0.26-0.39%), 0.25% (0.24 - 0.25%), 0.27% (0.25-0.31%) of the diet for optimum growth, tryptophan deposition, feed efficiency and apparent net protein deposition (95% of maximum value), respectively.



DIGESTIBILITY AND AMMONIA EXCRETION RATES OF LOW-COST NURSERY DIETS FOR NILE TILAPIA (*Oreochromis niloticus*) FRY

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In Ghana nursery facilities are generally missing from the aquaculture production chain and this compels fish farmers to purchase fish directly from the hatcheries at very small sizes to stock in grow-out ponds which sometimes results in high mortalities. Some farmers have adopted the practice of nursing fish before stocking in grow-out ponds in Ghana but formulated commercial feeds are prohibitively expensive. This research is thus a preliminary study to evaluate the potential for using mixtures of soybean and copra meals as partial replacements of fishmeal in feeds for Nile tilapia (*Oreochromis niloticus*) fry/fingerlings.

Nutrient digestibility and postprandial ammonia excretion rates were examined. Three iso-nitrogenous (48%), and isolipidic (7%) diets (Diet 1, 2 and 3) were formulated with the oilseed mixtures as partial replacements to fishmeal (Table 1) and with a fishmeal-based commercial diet serving as a control. Diets were randomly assigned to triplicate groups of fish (Initial mean weight $0.89\text{g} \pm 0.14\text{ g}$) for the digestibility trials which spanned a total of nine (9), days. The partial inclusion of oilseed meals did not significantly affect apparent protein and lipid digestibilities although ash and dry matter digestibilities were significantly reduced ($p < 0.05$). The inclusions of the plant proteins caused an increase in ammonia excretion (Fig 1). Overall, the oilseed diets showed some potential although further refinements of ingredient might be required to improve somatic growth, dry matter digestibility and reduce ammonia excretion rates.

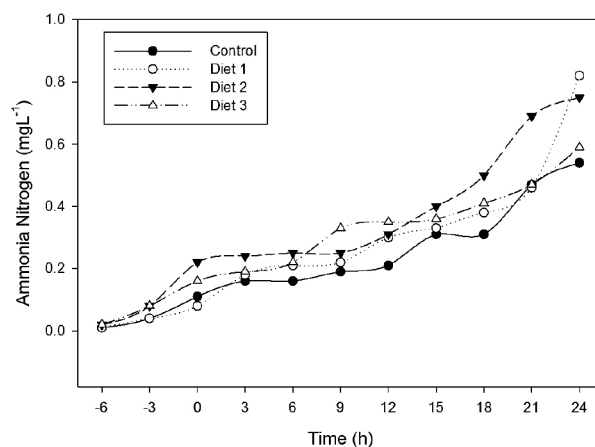


Fig. 1: Ammonia excretion rates of the fish fed the different diets

Table 1: Formulations of the experimental diets

	D1	D2	D3
FM	48.60	40.30	32.45
SBM	32.50	42.47	50.98
FCM	8.10	7.45	7.15
WB	6.80	5.78	5.42
PO	2.00	2.00	2.00
BIN	1.00	1.00	1.00
VM	1.00	1.00	1.00

FM: Fishmeal; SBM: Soybean meal; FCM; Fermented copra meal; WB: Wheat bran; PO: Palm oil; BIN: Binder; VM: Vitamin mix

EVALUATION OF LOW-COST NURSERY DIETS FOR NILE TILAPIA (*Oreochromis niloticus*) FRY: DIGESTIBILITY AND AMMONIA EXCRETION RATES

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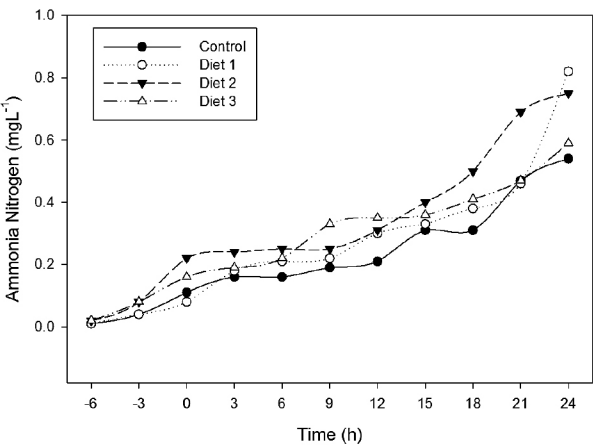


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HAEMATOLOGY AND SERUM BIOCHEMISTRY OF *Clarias gariepinus* FED DIFFERENT LEVELS OF RAW AND STEAM-HEATED *Moringa oleifera* LEAF MEAL DIETS

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The use of plant protein sources in aquaculture to substitute fishmeal is becoming popular however, the effect of these plant protein sources on the blood of fish should be known. The study examined the haematology and serum biochemistry of *Clarias gariepinus* fed two levels of raw and processed *Moringa oleifera* leaf meal diets, respectively. *M. oleifera* leaf meal substituted fish meal at 0 % (control), 15 %, 30 % (raw) and 15 %, 30 % (steam heated) in the five different diets. A total of 300 *Clarias gariepinus* fingerlings mean weight 9.20 ± 0.08 g were randomly distributed into 15 plastic aquaria tanks of 750 liters' capacity at 20 fish per tank in triplicate treatments and were fed twice daily at 9.00 hrs and 17.00 hrs for 12 weeks. The results showed that the differences in packed cell volume, red blood cell, white blood cell and haemoglobin concentration were statistically ($p > 0.05$) not significant between the control and the treatment diets. Fish serum enzymes were statistically ($p > 0.05$) similar for the control and the treatments but blood cholesterol value of the treatments were insignificantly lowered. It is concluded that the use of *M. oleifera* leaf meal either raw or steam-heated as a substitute at 15 % for fishmeal have no significant changes ($P > 0.05$) on the haematology and serum biochemistry, implying no negative impact on the health status of *C. gariepinus*.

F0 FOUNDER PRODUCTION OF AVTR-V1A2 KNOCKOUT STRAIN IN CHUB MACKEREL USING GENOME EDITING TECHNIQUE

Hirofumi Ohga*, Koki Shibata, Satoshi Kai, Ryo Sakanoue, Hajime Kitano, Kohei Ohta, Naoki Nagano, Keishi Sakaguchi, Satoru Kuhara, Kosuke Tashiro, Sangwan Kim, Tetsushi Sakuma, Takashi Yamamoto, Koichiro Gen, Atsushi Fujiwara, Yukinori Kazeto, Takanori Kobayashi, Michiya Matsuyama

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The chub mackerel (*Scomber japonicus*) is one of the most commercially important fish worldwide. In recent years, we standardized the full life cycle aquaculture of this species due to high consumer demand. However, high depletion during juvenile period stemming from cannibalistic behavior is still a serious problem. Here, we report the F0 founder production of arginine-vasotocin receptor (AVTR-V1a2) knockout strain by using TALENs. In the same study, reports showed that AVTR-V1a2 is involved in aggressiveness of teleost fish. The final vision of this project is to produce “manageable fish” for aquaculture efficiency.

Partial *avtr-v1a2* sequence was obtained from genome and TALENs were designed to break S-S bridge. In preliminary trial, 100 ng/uL concentration of TALEN mRNA pairs were microinjected into fertilized eggs. In heteroduplex mobility assay (HMA) analysis, all individuals showed mutation. Moreover, random sequence analysis on PCR products from their genome showed 43.9% mutated cell rate. On May and June 2017, TALENs were microinjected into 1,296 fertilized eggs, 37 of which survived to adulthood. In embryo and juvenile of F0 founder, mutation of *avtr-v1a2* was confirmed in over 80% individuals.

This work was supported by Council for Science, Technology and Innovation (CSTI), Cross-ministerial Strategic Innovation Promotion Program (SIP), “Technologies for creating next-generation agriculture, forestry and fisheries” (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).

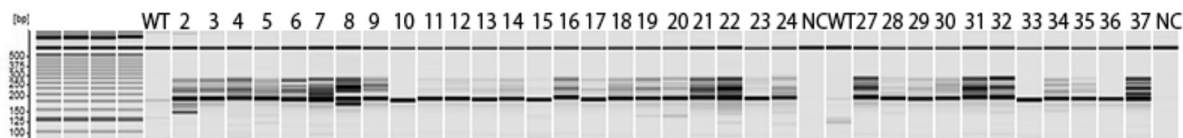


FIGURE 1. HMA analysis in AVTR-V1a2 knockout F0 founder. Multiple heteroduplex bands were shown in PCR amplicons from each TALEN-injected embryo, whereas a single band was shown from each wild type (WT) and 3 TALEN-injected embryos (10, 33, and 36). Mutations were induced in over 80% individuals.

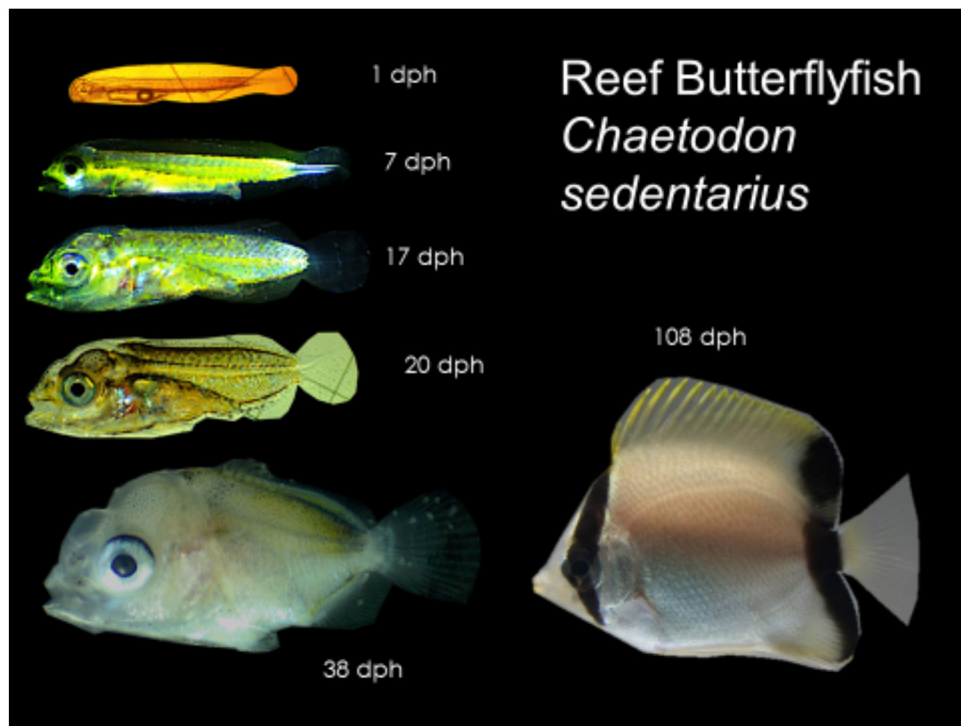
SUCCESSFUL CAPTIVE SPAWNING AND CULTURE OF REEF BUTTERFLYFISH (*Chaetodon sedentarius*)

Cortney L. Ohs*, Jason S. Broach, Isaac S. Lee, Andrew T. Palau

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The Reef Butterflyfish (*Chaetodon sedentarius*) is native to the Caribbean and is marketed to the marine ornamental trade. Captive spawning and culture to metamorphosis have not been previously documented and research regarding all phases of aquaculture is not available.

A bonded pair was obtained from Marathon, FL in 2015. After three months of conditioning, the pair began regularly spawning 3000-5000 eggs every two to three days. On this successful run, 3000 eggs were stocked into a black, 440 L fiberglass tank. Beginning 4 dph, larvae were fed *Parvocalanus crassirostris* copepod nauplii twice daily and enriched *Brachionus plicatilis* rotifers once daily. At 20 dph, newly hatched *Artemia* were added to the diet. At 43 dph, dry diet was added. Average total densities fed daily were 5.3, 8.6, and 0.2 per mL for copepod nauplii, rotifers, and *Artemia*, respectively. At 17 dph larvae had inflated swim bladders. The last mortality was recorded at 36 dph. Metamorphosis was completed by 75 dph and three juveniles were obtained. This study is the first report of the Reef Butterflyfish (*Chaetodon sedentarius*) being successfully cultured in captivity.

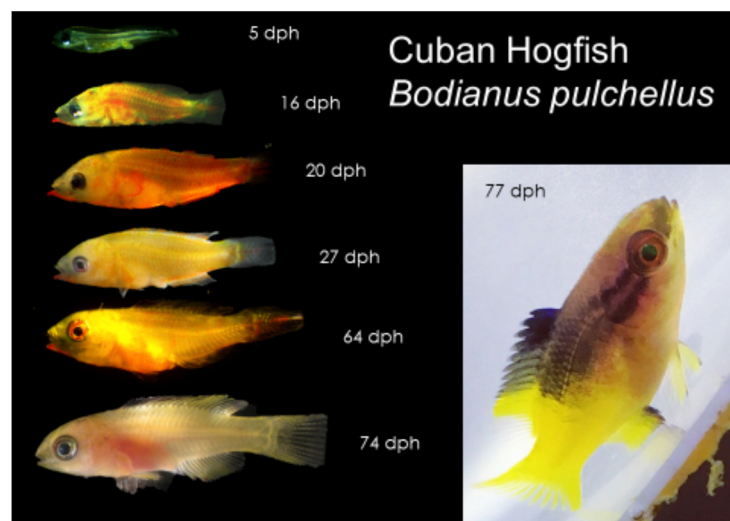


SUCCESSFUL CAPTIVE SPAWNING AND CULTURE OF CUBAN HOGFISH (*Bodianus pulchellus*)

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The Cuban hogfish (*Bodianus pulchellus*) is native to the Caribbean and is marketed to the marine ornamental trade. Captive culture and metamorphosis has not been previously documented and research regarding reproduction, larval culture, and production protocols does not yet exist. Twenty-four adult Cuban Hogfish were stocked into a 2700 L circular tank in January 2016. The first spawns were recorded in October of that year. Two successful culturing attempts were completed, one in November 2016 from a spawn of 3500 eggs and one in February 2017 from a spawn of 4500 eggs. Eggs from each of the two spawns were stocked into 104-L circular tanks with black sides and white bottoms. Larvae hatched within twenty-four hours at 27°C and were fed copepod nauplii (*Parvocalanus crassirostris*) twice daily and enriched rotifers (*Brachionus plicatilis*) once daily beginning at first feeding. Copepod nauplii were discontinued at 36 dph while rotifer feedings were discontinued at 24 dph. Newly hatched *Artemia* were added twice daily beginning at 15 dph. Average total densities fed daily throughout both culture attempts were 10-15, 10-20, and 0.2-1 per mL for copepod nauplii, rotifers, and *Artemia*, respectively. Flexion occurred by 15 dph and metamorphosis occurred by 80 dph. Two juveniles were obtained from the larval culture attempt with the first spawn and 25 juveniles from the second spawn.



GENETIC POPULATION STRUCTURE OF THE INVASIVE TIGER SHRIMPS (*Penaeus monodon*) FROM NIGERIA COASTAL WATERS

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In the last decade, the giant black tiger shrimp (*P. monodon*) suddenly became an invasive species of interest to the global research community. Since they invaded Nigeria, there has been no information on phylogenetics and phylogeography of this non-indigenous species as far as we know. This invasive species could help to improve food security and molecular studies could help to aid in the selection of founder stocks for hatchery management or effective aquaculture practice. Therefore, this research aims to study the molecular diversity of *P. monodon* and assess the population structure and migration pattern within the Nigerian coastal waters.

Tiger shrimp samples were obtained from Ten locations covering five coastal states of Nigeria vis-à-vis Lagos Makoko (LM), Lagos Apapa (LA), Lagos Takwabay (LT), Lagos Tincan (LC), Lagos Folu (LF), Ondo Aiyetoro (AO), Bayelsa Brass (BB), Rivers Bonny (RB), Rivers Kaa (RK) Akwa Ibom Ibeno (AB). Pleopod samples were preserved and transported to United States Geological Survey for molecular analysis. A total of 192 DNA samples were used for microsatellite study. Out of 28 United States invasive tiger shrimp microsatellite markers used for screening the Nigerian invasive samples, 11 markers with highest PIC values and best amplification result were selected for the population studies. A high degree of allelic polymorphism was observed with an average of 12 alleles and total of 140 alleles. The Pmo-1 marker had the highest number of alleles. Among populations, expected heterozygosity (H_e) was highest in RB population and lowest in LT population. Principal Coordinate Analysis of Fst and Nei's genetic distance showed that majority of the populations clustered together, with the AB and OA populations being more genetically distant. However distance values were not significant which may suggest isolation by distance effect on these populations. This study shows an inherent high genetic diversity typified by allelic richness in the gene pool, possibly contributing to the survival and establishment of tiger shrimps in the Nigerian coastal waters. However, lack of significant genetic distance values among populations reflects their homogeneity, suggesting a limited number of founding individuals.



Figure 1: Map of Nigeria showing the sampling stations along the South-West and South-South coastline.

BREEDING AND GROWTH PERFORMANCE OF THE PROGENIES FROM THE CROSSES OF *Pangasianodon hypophthalmus* (Sauvage, 1878) AND *Clarias gariepinus* (Burchell, 1822)

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This study was designed to evaluate the culture potential of the offspring from the novel crosses between Asian catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) and African catfish *Clarias gariepinus* (Burchell, 1822). ♀*C. gariepinus* × ♂*P. hypophthalmus* (Clariothalmus) gave better hatchability than ♀*P. hypophthalmus* × ♂*C. gariepinus* (Pangapinus), however, both were lower than the pure crosses.

Based on the phenotypic characters, a larger proportion of the offspring of Clariothalmus were Panga-like, while only 12% were Clarias-like. However, all offspring of Pangapinus were all Panga-like. The Panga-like Clariothalmus exhibited shared features from both parents hence much more phenotypic divergence from both parents. The remain 12% of the Clariothalmus offspring (Clarias-like) and all the Pangapinus progenies (Panga-like) on the other hand were indistinguishable from the maternal parent.

After sixteen weeks of culture, the Clarias-like Clariothalmus offspring had better growth than the other crosses including the pure sibs. However, cannibalism and aggressive behaviour was higher in this group of fish compared to other. Hence, culturing Panga-like Clariothalmus separately from the Clarias-like progenies may be a management practice of interest in commercial production of the hybrids.

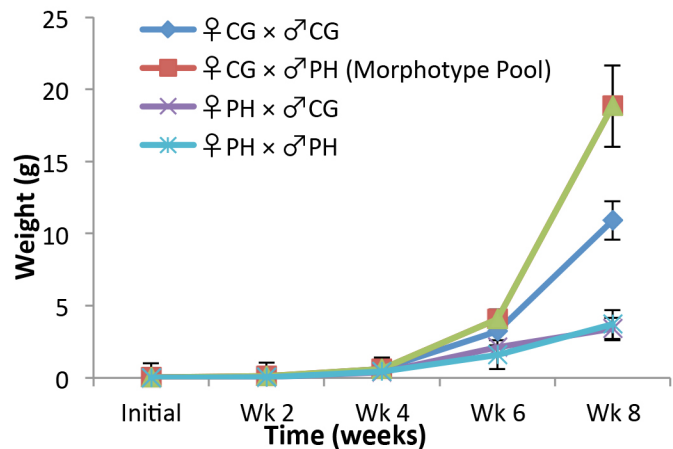


Fig 1: Weekly growth of progenies from pure and reciprocal crosses of *P. hypophthalmus* and *C. gariepinus*.

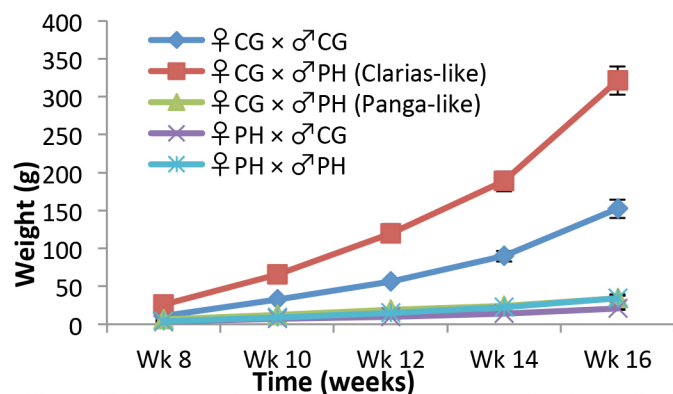


Fig 2: Weekly growth of progenies from pure and reciprocal crosses of *P. hypophthalmus* and *Cl. gariepinus*.

AN EVALUATION OF SUNDRIED CASSAVA PEEL MEAL AS PARTIAL REPLACEMENT FOR MAIZE IN THE DIETS FOR *Clarias gariepinus* FINGERLINGS

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Maize is the conventional source of energy in fish diets, especially in the developing world. However, maize has become costly as a result of its relevance in human nutrition and competition from other livestock feed industries; for which reason its use in fish feed is becoming increasingly unjustified (Tewe, 2004). There is therefore the need to explore cheaper alternatives to reduce the cost of fish feed. Cassava peel, a major by-product of the cassava processing industry; representing about 10-13% of tuber weight (Oladunjoye *et al.*, 2010), is readily available in countries like Nigeria where cassava is cultivated and processed into food for human consumption. A major drawback to the use of cassava peel in fish diets is its content of hydrocyanic acid (HCN) which can inhibit several enzyme systems, depress growth through interference with certain essential amino acids among other harmful effects (Oresegun *et al.*, 2016). Acceptability and utilization of cassava peel in fish diets will therefore depend on the extent of its HCN detoxification. This study investigated the performance implication of feeding graded levels of sundried cassava peel meal to *Clarias gariepinus*.

Four isonitrogenous (35% crude protein) diets were formulated in which sundried cassava peel meal (SCPM) replaced maize at graded levels of 0, 25, 50 and 75%. The diets were designated as control, 25S, 50S and 75S and fed to triplicate groups of *Clarias gariepinus* (average weight, 5.33 ± 0.02 g) fingerlings for 56 days. The weight gain (14.82 ± 1.35) of the group that received 25S was significantly ($P < 0.05$) better than those that received 50S (11.87 ± 0.37) but statistically similar to the control and 75S. There was a decreasing trend in the packed cell volume, haemoglobin and red blood cell with inclusion levels of SCPM. The serum total protein of the group that received 25S and 75S were similar and significantly higher than the control and 25S.

The results showed that sundried cassava peel meal can be incorporated in the diets for *Clarias gariepinus* fingerlings as a replacement for maize at 25% replacement level without compromising growth and nutrient utilization.

Table 1: Composition (%) of experimental diets

INGREDIENT	CD	25S	50S	75S
Fish meal	15	15	15	15
Groundnut cake	30	30	30	30
Soybean	22.19	21.82	21.44	21.05
Maize	24.81	18.89	12.78	6.49
SCPM	-	6.29	12.78	19.46
Others	8	8	8	8

Table 2: Growth performance and nutrient utilization of *Clarias gariepinus* fed diets containing graded levels of sundried cassava peel meal

Parameters	CD	25S	50S	75S
FWG(g)	17.63 ^{ab}	20.15 ^b	17.17 ^a	17.67 ^{ab}
MWG	12.33 ^{ab}	14.82 ^b	11.87 ^a	12.30 ^{ab}
SGR	2.14 ^a	2.37 ^b	2.10 ^a	2.13 ^a
FCR	1.86	1.69	1.80	1.79

PARASITES OF *Oreochromis niloticus* OBSERVED IN THE FISHERIES UNIT OF THE NIGER DELTA UNIVERSITY TEACHING AND RESEARCH FARM

¹ Omovwohwovie, E.E, ²Adeyemo, A.O and² Fineroad, L. Blessing O.C., Preye O. And Egwono S.O.

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Parasites of *Oreochromis niloticus* was studied at the Teaching and Research Farm of the Niger Delta University. The prevalence, abundance and intensity of infection were determined. The prevalence of *Dactylogyrus parasitae* in *Oreochromis niloticus* showed the highest value of 65%, followed by Cestode, ligula 60%, *Ergasilus* 10% and Protozoa and Nematode 1.66% with the least prevalence. The intensity of *Dactylogyrus* obtained was 2.79,while Cestode,(ligula)had 1.10,, *Ergasilus* spp 1.33, Protozoa and Nematode 1.2 respectively..

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EFFECT OF HYPOXIA ON NEUROPEPTIDE GENE EXPRESSION IN CHANNEL CATFISH

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Maximizing appetite is critical for intensive production of catfish; a reduction in feed consumption means a longer production cycle with higher mortalities. When the minimum dissolved oxygen (DO) concentration falls below 3.0 mg/L there is a decrease in feed consumed. The primary objective of this project was to examine regulation of the neuropeptides urotensin I (UI) and corticotropin-releasing factor (CRF) during a short bout of hypoxia that replicated the daily conditions of a catfish production pond. Dissolved oxygen concentration was maintained in control aquaria at 95% saturation. Hypoxia tanks were maintained at 95% saturation, dropped to 20% saturation for 12 hours then returned to saturation in replicate aquaria. Fish were sampled at 0, 6, 12, 18, and 24 hours. Venous PO₂ was the same after 6 hours of hypoxia, 60% lower in the hypoxia group after 12 hours of hypoxia, and the same 6 hours after the DO returned to saturation. Gene expression for CRF did not significantly change during hypoxia at any measured time point. There was a significant increase in Urotensin I gene expression (1.6-fold higher) in the hypoxia group 18 hours after the onset of the experiment but fell to pre-hypoxia level by 24 hours. At the time points sampled, the anorectic neuropeptide UI was significantly upregulated six hours after a 12-hour bout of hypoxia. This data suggests that urotensin I may act to decrease appetite after periods of hypoxia.

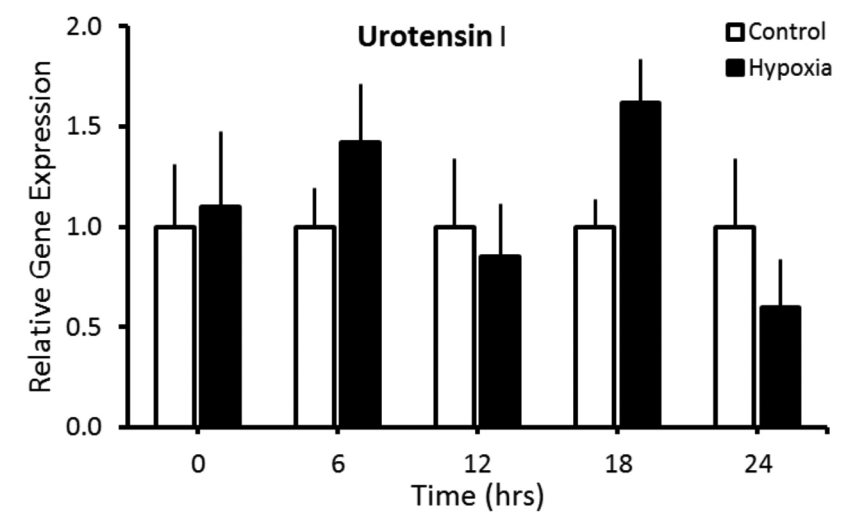


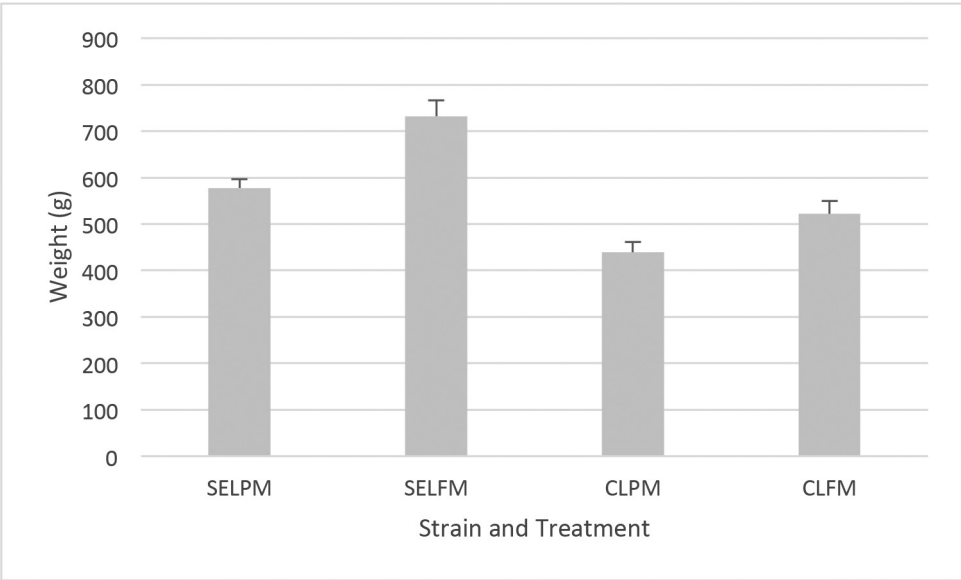
Figure 1. Relative gene expression of urotensin I in channel catfish during normoxia and hypoxia treatments.

EVALUATION OF THE ACUTE AND CHRONIC IMMUNE RESPONSE OF SALMONIDS AFTER BEING FED A PLANT PROTEIN-BASED FEED

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Even though fish meal and fish oil have proven to be ideal protein and oil sources for aquaculture, as production continues to grow, the scarcity of these fish-derived products makes them less than ideal as protein and oil sources in aquaculture feeds. The use of plant proteins and oils are seen as economical and sustainable replacements. However, intestinal inflammation and other symptoms are known to occur when carnivorous fish species are fed feeds with partial or complete fishmeal replacement using certain plant-based protein sources. To decipher the immunological and stress responses in these species, we fed a domesticated stock of rainbow trout (CL), and a strain of rainbow trout that has been selected to utilize soy-based feeds without developing enteritis (SEL), either a fishmeal or plant-based feed for 7 months. Significant differences were found for weight gain between the groups (Figure 1). Samples of gill, spleen, kidney, intestine and liver were taken at 3 and 7 months of feeding for RNA isolation and gene expression analysis. Furthermore, subgroups of these fish were all subjected to a stressor at 3 and 7 months, and the same tissues were sampled to determine the effect of diet and strain on stress responses. Obtained samples were analyzed for the expression of genes involved in either the cellular or humoral immune response and stress response.



HEMATOLOGICAL RESPONSES OF RAINBOW TROUT TO CHEMICAL SEDATION USING TRICAINE, EUGENOL, AND METOMIDATE

Cheyenne E. Owens*, Madison S. Powell, T. Gibson Gaylord, James D. Bowker

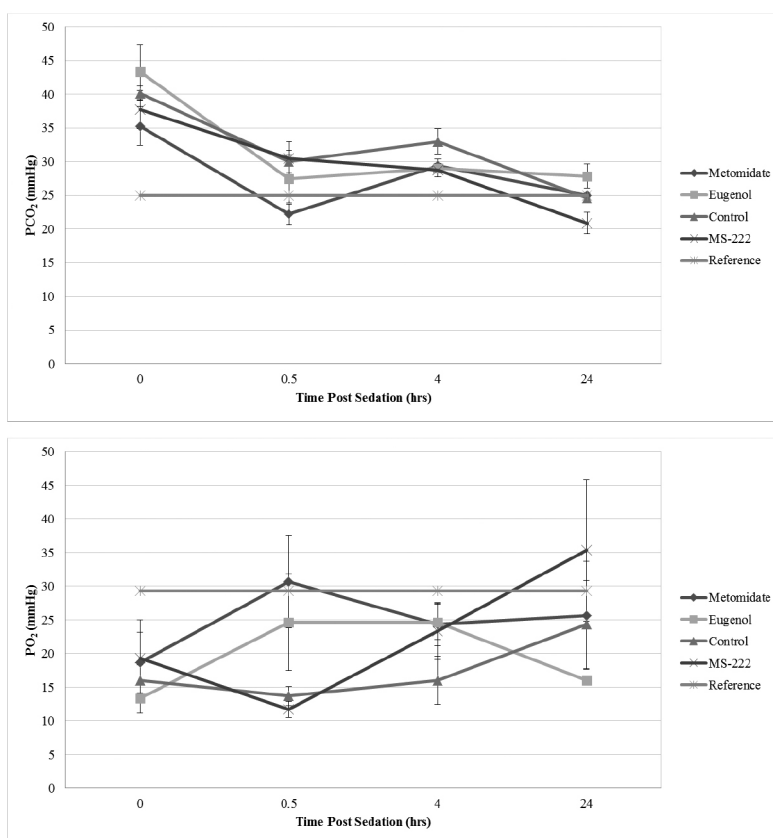
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Fish sedatives are used by fisheries professionals during routine transport and handling of fish to minimize stress, however sedation itself can induce departures from resting physiology. The present study was conducted to assess the hematological responses of rainbow trout following sedation with tricaine methanesulfonate (MS-222), eugenol, or metomidate hydrochloride.

Twelve rainbow trout (26.2 g) were group sedated then placed in a recovery tank. Three fish per sedative were bled at 0 minutes, 30 minutes, 4 hours, and 24 hours post sedation. Control fish were handled similarly but were not sedated. Each treatment was conducted in triplicate; three fish from the reference population were used for baseline. Whole blood was analyzed for pH, PCO_2 , HCO_3^- , TCO_2 , PO_2 , and lactate using CG4+ i-STAT cartridges and hematocrit, hemoglobin, glucose, chloride, sodium, and potassium using 6+ i-STAT cartridges. Remaining blood was centrifuged and plasma stored at -80°C for cortisol analysis.

While no significant treatment effects were found, time effects were found in all hematological responses ($P < 0.05$, PO_2 : $P < 0.1$). Interaction effects were also observed: PCO_2 was higher in MS-222 fish ($P < 0.05$) and control fish ($P < 0.1$) at 0.5-hr post sedation than metomidate fish (Figure 1). PCO_2 was also higher in eugenol fish ($P < 0.1$) at 0-hr post sedation than metomidate fish. PO_2 was lower in MS-222 fish ($P < 0.1$) at 0.5-hr post sedation than metomidate fish and lower in eugenol fish ($P < 0.1$) at 24-hr post sedation than fish sedated with MS-222 (Figure 2).

Hematological data seemed consistent with the generalized stress response and suggest minimal differences between handled and sedated rainbow trout exposed to a mild stress event.



GROWTH, IMMUNE RESPONSE, AND DISEASE RESISTANCE OF JUVENILE RAINBOW TROUT FED DIETS CONTAINING CRICKET MEAL, AND MEALWORM MEAL

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Insect meals may provide an alternative protein source to fish meal in salmonid feeds. Insect meals contain chitin, which can have low digestibility but has been shown to have an immune-stimulatory benefit to fish.

An 8-week feeding trial was conducted to determine whether cricket or mealworm meal could be used as a fish meal replacement in rainbow trout diets. Fish meal was replaced within a control diet at 25%, 50% or 100% of its inclusion level (FMR) with either cricket meal or mealworm meal. Two additional diets were formulated by supplementing the control diet with practical grade chitin from shrimp shells at rates mimicking chitin levels in the 25% and 100% FMR cricket meal diets. All nine diets were formulated on a digestible protein basis. Diets were randomly assigned to triplicate tanks of 5.5g initial weight rainbow trout, and the fish were fed twice daily to apparent satiation. Kidneys were sampled at 2 and 8 weeks in order to compare innate immune response genes. Subsamples of fish were challenged at 2- and 8- weeks post-feeding by 0.1 ml injection with 10^8 cfu/ml *Flavobacterium psychrophilum* and mortality was monitored for 28 days.

No significant differences in final weight, percent increase, FCR, HSI, or VSI were observed due to diet (Table 1). Mortalities were >80% for all treatments and no significant differences due to diet were observed at two-weeks post-feeding. No mortalities were observed in the 8-week challenge. Proximate composition and innate immune response will be discussed.

These findings suggest that with proper formulation, insect meals can replace fish meal within rainbow trout diets. No negative effects of chitin on fish growth were observed; however, no immune benefits were observed when exposed to *F. psychrophilum*.

Diets	Final Wgt (g)	% Increase	FCR	HSI	VSI
Fish Meal (Control)	55.4 ± 0.8	882 ± 21	1.00 ± 0.00	1.6 ± 0.2	12.1 ± 0.1
Control + Low Chitin	54.1 ± 0.6	865 ± 3	0.95 ± 0.02	1.5 ± 0.0	11.0 ± 0.8
Control + High Chitin	52.9 ± 0.8	836 ± 13	1.00 ± 0.01	1.4 ± 0.1	11.0 ± 0.6
25% FMR (Cricket)	54.8 ± 1.1	876 ± 27	0.97 ± 0.01	1.3 ± 0.1	11.8 ± 0.2
50% FMR (Cricket)	54.9 ± 1.7	881 ± 30	0.99 ± 0.01	1.6 ± 0.1	11.2 ± 0.7
100% FMR (Cricket)	54.4 ± 0.8	873 ± 8	0.97 ± 0.02	1.6 ± 0.0	11.9 ± 0.2
25% FMR (Mealworm)	55.5 ± 1.3	883 ± 26	0.97 ± 0.01	1.5 ± 0.2	10.8 ± 0.7
50% FMR (Mealworm)	54.0 ± 0.8	869 ± 12	0.95 ± 0.01	1.4 ± 0.1	11.4 ± 0.3
100% FMR (Mealworm)	53.4 ± 0.4	856 ± 9	0.97 ± 0.01	1.8 ± 0.0	12.2 ± 0.2

INVESTIGATION OF THE SUITABILITY OF INSECT MEALS AS PROTEIN SOURCES FOR RAINBOW TROUT

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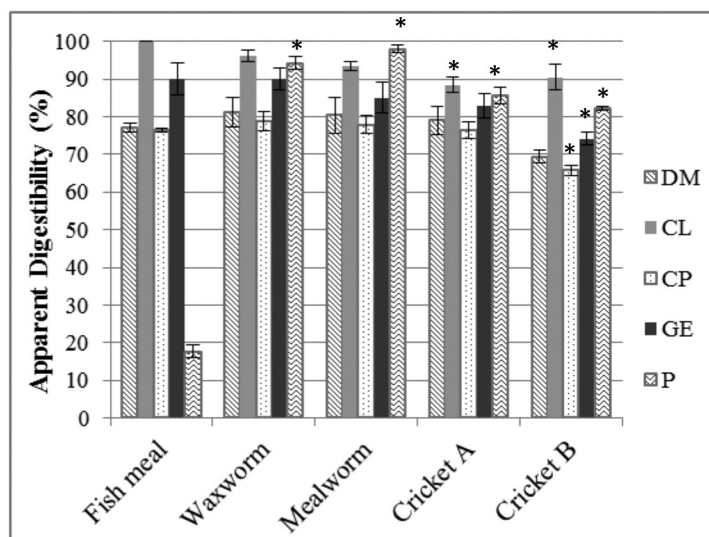
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In recent years, interest has grown among fish culturists for cost effective and sustainable alternative protein sources to fish meal. As a part of the natural diets of salmonid, insects could provide a promising protein source in prepared feeds. A digestibility trial was conducted to determine the apparent digestibility coefficients (ADCs) of key nutrients in insect meals in order to evaluate their suitability as alternative protein sources for rainbow trout feeds.

Fish were fed either a reference diet or one of five test diets created by blending the reference diet with each of the test ingredients (Menhaden fishmeal (MFM), two cricket meals (A and B), waxworm meal, and mealworm meal) in a 70:30 ratio (dry-weight basis). Twenty-five, 370-g Rainbow Trout were stocked per 140-L poly tanks (N= 3/diet), with water temperature maintained at 15°C, and hand-fed twice daily to satiation one week prior to collection of fecal samples. Fecal samples were collected by manual stripping, freeze dried and three collections pooled per replicate tank.

Cricket meals A and B contained similar crude protein (CP) and lipid (CL) levels (68 and 69% CP and 17.1 and 16.6 CL%, respectively). Mealworm meal contained 56.5% CP and 27.7% CL and waxworm meal contained 32.5% CP and 54.2% CL.

The apparent digestibility coefficients (ADCs) of dry matter (DM), CL, CP, gross energy (GE), and phosphorus (P) are shown in the figure. The protein ADCs were 81.6% for Cricket A and 79.5% for Cricket B; the latter of which was significantly lower than MFM (87%, $P<0.05$). The protein ADC for mealworm meal was 83.5% and waxworm meal was 82.8%. Both cricket meals A and B had significantly lower lipid ADCs (88.5% and 90.6%, respectively) than MFM (100%, $P<0.05$). The lipid ADC for mealworm meal was 93.5% and waxworm meal was 96.1%.



NORTHEAST CLIMATE HUB 360° OF CLIMATE EDUCATION: A VIRTUAL TOUR FOR SUSTAINABLE AQUACULTURE PRACTICES

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The Northeast Climate Hub, building on capacity within USDA, delivers science-based knowledge and practical information to farmers and forest landowners in Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Maryland, Delaware, West Virginia and District of Columbia. The USDA Northeast Climate Hub and all land grant partners have worked together on the production of a robust, detailed, and innovative digital experience that can engage people in climate informed decision-making, and immerse the user into a digital field ‘visit’ that feels “as if you were there”. Before adopting a new practice or making an operational change, farmers and foresters often want to first see the new technique in action. As climate change uniquely impacts all managed lands and waters in both rural and urban settings, technology-transfer and knowledge sharing can help to convey new ideas and techniques. Innovative demonstration strategies can help us do so more effectively than ever before. This network showcases a variety of climate change adaptation and mitigation strategies that have been implemented across public and private lands and waters in the Northeast. This web-based network is interactive so that users can “visit” the sites from their own computer, tablet, or mobile phone. The virtual network showcases key practices on existing on-the-ground farm and forest sites using innovative 360° photography and videos.

Virtual tours of various agricultural activities at selected sites and farms have been used to develop educational materials to the public. Rutgers University features living shorelines and oyster castles and application of aquaculture technique while University of District Columbia features aquaponics for tilapia culture in the high tunnel system. West Virginia State University is developing a video featuring a high tunnel with aquaponics and aeroponics and in summer 2018, Delaware State University will be developing a video on near shore oyster culture.

The virtual demonstrations serve as educational experiences that we hope will generate greater interest and understanding about climate change issues, and a larger appreciation and respect for those addressing them. We envision that this project will help build a bridge to climate adaptation strategies. Field visits are a powerful teacher, and we believe virtual field trips can achieve similar results with greater accessibility. In order to engage more people in climate informed decision-making, this project immerses users into digital field ‘visit’ that feels as if you were there. These educational efforts provide necessary information farmers and resource managers need to sustain their production and prepare them for fast changing climate conditions and disasters.



*Pictures by Frank Marengi,
Brian Reckenbeil and UDC
Aquaponics at East Cap Urban Farm.*

MONITORING TOTAL BACTERIA AND *Vibrionaceae* IN EASTERN OYSTERS (*Crassostrea virginica*) EXPOSED TO VARIOUS WATER QUALITY STRESSES

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Consumption of uncooked oysters is known to cause serious health conditions due to the bioaccumulation of contaminants, especially during the warmer months. As filter feeders, oysters ingest bacteria along with their food sources, primarily phytoplankton, from their surrounding habitats. Ensuring seafood safe for human consumption is always a concern, since oysters can be consumed raw, and disease causing organisms, environmental contaminants, chemicals, toxins, and even physical hazards such as glass, wood, soil, and metals can enter thorough feeding and be retained in the oysters.

One of the biggest concerns with consumption of raw oysters is the risk of the bacteria, *Vibrionaceae*. Contracting these bacteria through consumption of raw oysters can lead to fever, chills, nausea, vomiting, diarrhea, shock, skin lesions and in severe cases can lead to death. In this study, Eastern oysters (*Crassostrea virginica*) were exposed to various environmental stresses including high nitrate, low salinity, low and high suspended silt and clay in water in a controlled laboratory setting. The colonization of naturally occurring total bacteria and *Vibrionaceae* in Eastern oysters were compared to the control oysters and relationships between the environmental parameters and total aerobic bacteria and *Vibrionaceae* levels were investigated. The five trials, approximately 300 Eastern oysters from the Broadkill River in Lewes, Delaware were used, 60 oysters for each trial. Baseline *Vibrio* levels in the oysters were detected using the Colony Overlay Procedure for Peptidases (COPP) assay prior to the treatments. The remaining two hundred forty oysters were randomly placed into 16 – 2 L sterile containers filled with 1.5 L of 1 μ m filtered seawater, each housing three to four oysters. Of the total 16 containers; three containers included a low salinity treatment of 10 - 15 ppt seawater, three containers included a salinity of 20 - 25 ppt seawater and referred as a control, three containers had a high nitrate concentration (0.30 mg/L) via addition of 0.3 g of potassium nitrate (KNO_3), and the last six containers included high suspended solid treatment of 1,000 mg/L and a low suspended solid treatment of 100 mg/L prepared by using the settled silt particles and organic material from the Broadkill River.

Correlations between water quality, total aerobic bacteria, and *Vibrionaceae* were examined. Within the control treatments, nitrite and total suspended solids were strongly related to total aerobic bacteria ($R^2 = 99.9\%$ and 92.9% , respectively), while total nitrogen and total reactive phosphorus were strongly related to *Vibrionaceae* ($R^2 = 93.1\%$ and 92.8% , respectively). The increased nitrate treatments showed a strong relationship between total aerobic bacteria and nitrate and nitrite ($R^2 = 99.4\%$ and 99.9% , respectively), whereas *Vibrionaceae* was strongly related to total nitrogen and total reactive phosphorus ($R^2 = 94.6\%$ and 79.3% , respectively). Strong relationships were observed between total aerobic bacteria and nitrate and total suspended solids ($R^2 = 90.5\%$ and 92.5% , respectively) and between *Vibrionaceae* and total suspended solids ($R^2 = 89.0\%$) in the low salinity treatments. Low salinity treatment oysters consistently yielded the highest total aerobic bacteria and *Vibrionaceae* levels, followed by the high nitrate treatment oysters, the high average total suspended solids treatment oysters, and the control treatment oysters. Although the salinity treatments had the highest total aerobic bacteria and *Vibrionaceae* levels, the data was weakly related to the nutrients. Therefore, this study confirms the high aerobic bacteria and *Vibrionaceae* levels are primarily salinity dependent.

EFFECT OF STOCKING DENSITY ON FRY SURVIVAL AND GROWTH OF SAHAR *Tor putitora*

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Sahar (*Tor putitora*) is a high value indigenous fish species of Nepal. We achieved success in induced spawning of sahar using synthetic hormone in the sub-tropical climate of Nepal. An experiment was conducted for 90 days to determine the optimum density of sahar for fry rearing. Sahar fry of average initial weight 0.28 to 0.32 g were stocked at four different stocking densities in 2 m² nylon hapas fitted in concrete tanks. The treatments were: 5 fish/m² (T₁); 10 fish/m² (T₂); 15 fish/m² (T₃); and 20 fish/m² (T₄). Each density treatment was replicated thrice. Fish were fed with commercial pelleted feed (32% CP) twice daily at 5% of the biomass. In situ water temperature, pH and dissolved oxygen were monitored weekly at 6.00-7.00 am.

The mean harvest weight and daily growth rate of fingerlings were highest in T₁, intermediate in T₂, and lowest in T₃ and T₄ ($p < 0.05$; Table 1). The condition factor, specific growth rate and survival rate were not affected by stocking density ($p > 0.05$). Temperature, pH and dissolved oxygen ranged from 28.5-31.5 °C, 7.3-9.6 and 6.0-9.5 mg/L, respectively. The present results demonstrated that sahar fry can be successfully reared up to density of 20 fish/m² in nylon hapa with good survival and growth, although overall growth and harvested weight were considerably higher in the lowest density treatment.

TABLE 1. Growth and survival of sahar fry during the experimental period of 90 days (Mean±SE). Mean values with different superscripts in the same row are significantly different ($P < 0.05$)

Parameter	Treatments			
	T ₁ (5 fish/m ²)	T ₂ (10 fish/m ²)	T ₃ (15 fish/m ²)	T ₄ (20 fish/m ²)
Mean stocking weight (g)	0.30±0.01 ^a	0.29±0.02 ^a	0.32±0.02 ^a	0.28±0.02 ^a
Stocking number (fish/hapa)	10.00±0.00	20.00±0.00	30.00±0.00	40.00±0.00
Average harvested weight (g)	6.13±0.33 ^a	4.34±0.98 ^{ab}	2.97±0.55 ^b	2.84±0.56 ^b
Harvest number (fish/hapa)	9.67±0.33	19.00±0.58	29.33±0.33	36.33±1.20
Daily growth rate (g/day)	0.08±0.04 ^a	0.05±0.01 ^{ab}	0.04±0.01 ^b	0.03±0.01 ^b
Specific growth rate (% BW/day)	1.46±0.03 ^a	1.27±0.15 ^a	1.06±0.11 ^a	1.10±0.14 ^a
Survival (%)	96.67±3.33 ^a	95.00±2.89 ^a	97.78±1.11 ^a	90.83±3.00 ^a

SUSTAINABLE OYSTER AQUACULTURE, WATER QUALITY IMPROVEMENT AND ECOSYSTEM SERVICE POTENTIAL IN MARYLAND, CHESAPEAKE BAY

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The United States has an \$11.2 billion seafood trade deficit. NOAA is targeting expanded shellfish aquaculture to close this gap while also recognizing water quality benefits of increased oyster populations. In the Maryland portion of the Chesapeake Bay, the number of oysters harvested by aquaculture has increased tenfold since 2010 (Figure 1). Oysters are well known for their ability to effectively remove nutrients from the water column through filtration and assimilation into tissue and shell, as well as through oyster farm related denitrification. This recognition has led to approval of harvested oyster tissue for use as a nutrient Best Management Practice (BMP) in Chesapeake Bay to help jurisdictions meet mandated nutrient reductions. There has been interest in compensating oyster operations for the nutrients removed through the Maryland Nutrient Trading Program established in 2010. With the approval of the oyster tissue BMP, payment to growers is possible once approved by the trading program pending development of a payment mechanism. This study is designed to evaluate the potential nutrient removal via sustainable oyster aquaculture, the value of the nutrients removed, and the potential payment to growers for the nutrient removal service their oysters provide.

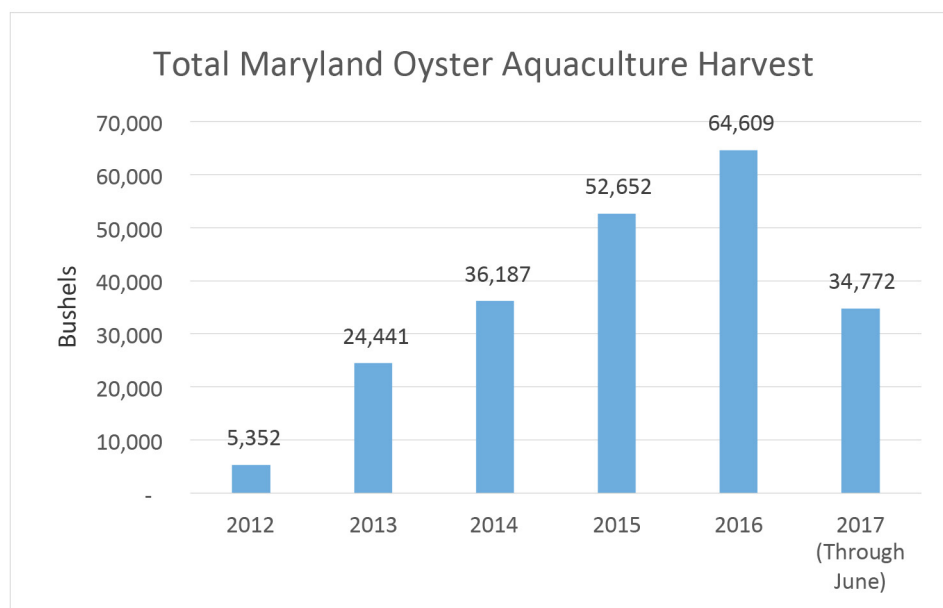


Figure 1. Oysters harvested from aquaculture in Maryland have increased dramatically in the last 4 years. Through June, 2017 harvests are approaching 2014 levels and are expected to increase over 2016 harvest levels.

COMPARISON OF THE PRODUCTION OF BIBB LETTUCE *Lactuca sativa* REARED IN INDOOR AQUAPONIC SYSTEMS USING LARGE MOUTH BASS *Micropterus salmoides* VS. NILE TILAPIA *Oreochromis niloticus*

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Aquaponics is a sustainable food production system that combines hydroponic plant growth with fish rearing in a recirculating aquaculture system. Nile tilapia (*Oreochromis niloticus*) have been primarily raised in these systems due to their fast growth rates and ability to tolerate poor water conditions. As aquaponics becomes more popular in temperate climates, farmers are looking for alternative fish species more compatible with cool temperature culture and have a higher market value. However, fish feed is species specific and the major contributor of available nutrients for cultured plants; indicating a need to look at differences in nutrient availability and plant production between cultured species. The largemouth bass (*Micropterus salmoides*) is a high-value fish that is amenable to culture in more temperate conditions. This study evaluated largemouth bass as an alternative species for the production of Bibb lettuce (*Lactuca sativa*) and Red Russian kale (*Brassica napus*) in aquaponic systems.

Researchers evaluated plant growth factors stocked with Nile tilapia and largemouth bass (LMB) in aquaponics. Fish were stocked at approximately 1,400 g per system and fed five percent body weight per day. Tilapia and LMB were fed a floating feed containing 32% and 45% protein, respectively. Bibb lettuce and Red Russian kale were evaluated in successive planting cycles. At harvest total plant biomass in each system was recorded. Sixteen representative samples from each system were collected and analyzed for plant and root weight, number of leaves, leaf surface area, root/shoot ratio, and chlorophyll content. Plant and water samples were collected and further analyzed for micro and macro nutrient content.

Results indicated that growth of Bibb lettuce in aquaponics with LMB was similar to growth of lettuce grown in systems with tilapia. However, Red Russian kale had significantly greater growth in systems stocked with tilapia than LMB. More data will have to be analyzed to determine differences in the outcome between the two plant crops. Due to results from Bibb lettuce, there may be potential for LMB to be used as an alternative to tilapia in aquaponic systems.

STATUS OF LAND-BASED CULTURE EFFORTS TO SUPPORT SEXUAL REPRODUCTION IN THREATENED CARIBBEAN CORALS

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Seven Caribbean coral species are currently listed as Threatened under the U.S. Endangered Species Act. A major priority for recovery is mitigating threats to these corals such as disease, land-based pollution, ocean acidification, and increased ocean temperatures leading to more frequent bleaching. The past five years have seen rapid development of aquaculture as a complimentary tool to produce coral biomass and increase genetic diversity while regional and global threats are concurrently addressed. Ocean-based coral nurseries now generate tremendous coral biomass for reef restoration through asexual propagation. However, to meet genetic diversity goals, it will also be important to develop reliable techniques for sexual reproduction. Numerous groups are currently engaged in such efforts.

For the past several years the Florida Aquarium has been leading one such effort in partnership with the Coral Restoration Foundation and the University of Florida, among others. Coral larvae collected from the 2017 spawns of *Orbicella faveolata* (Fig. 1), *Acropora cervicornis*, and *A. palmata* were settled ex-situ at the Center for Conservation in Apollo Beach, Florida. Nine-week post-settlement survival rates for the three species range from 69-71% and are the highest achieved at this facility to date. This talk will provide details of current techniques and lessons learned over the years for ex-situ sexual propagation of these species.

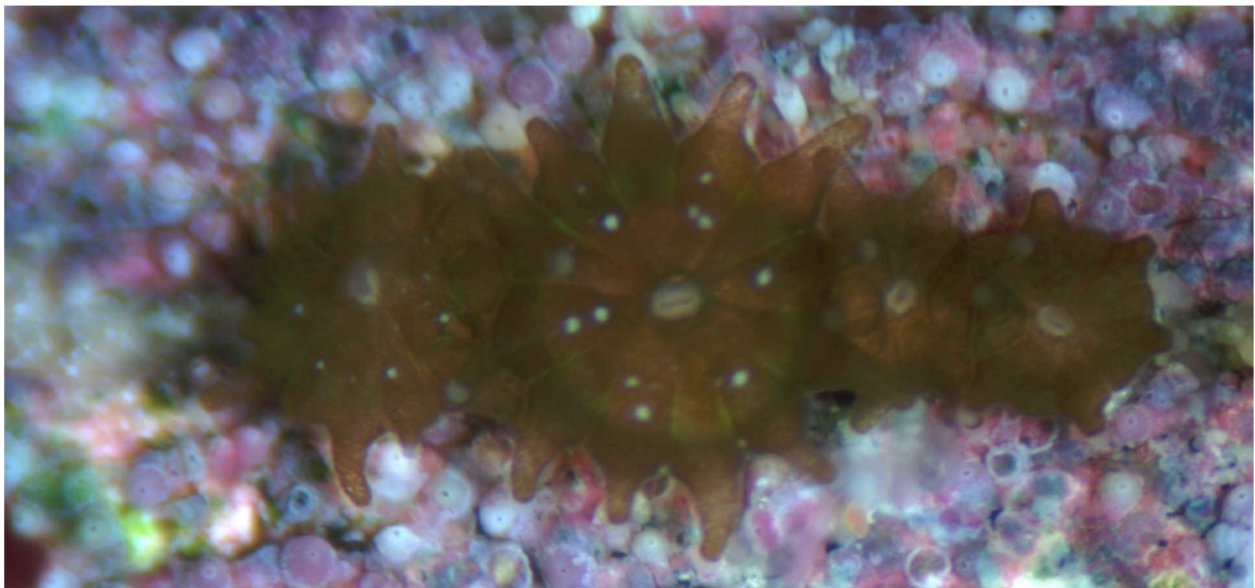


Figure 1. *Orbicella faveolata* polyps at 6-weeks post-larval settlement. Photo: Keri O'Neil

INCREASING ACCESS TO CLIMATE-SMART AGRICULTURE: AQUAPONICS IN JAMAICA

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In Jamaica, climate-related events have been identified as major threats to the agricultural sector, which represents about 7% of GDP and employs about 18% of the country's population. As climate change threatens economic opportunities for small-scale farmers, it is imperative to introduce new business models and technologies that will enable farmers to adapt and thrive. To address these challenges, INMED Caribbean initiated a multi-year project using commercial-scale aquaponics to promote water efficiency, build resilience, and enhance livelihoods for small-scale commercial farmers supplying the local market.

The project goal is to increase economic opportunities for small-scale farmers while reducing vulnerability to climate change and minimizing adverse environmental impacts. The strategy involves implementing an agri-business development program focused on making aquaponics a commercially viable technology for small and medium-scale Jamaican farmers, including women, youth and disabled farmers, while improving their market access and resilience to climate change.

The project will equip farmers with the technical knowledge and basic business planning and management skills needed for commercial farm operations. This will be done through a training and loan prequalification program consisting of interactive online modules, hands-on workshops, and internships.

Once participants have successfully completed the online training program, including the completion of a business plan, they are eligible to apply for loan financing with a pre-selected group of financial institutions. Once approved for a loan, farmers begin build-out of their systems with INMED support; receiving intensive hands-on training and close monitoring to support productivity.



Figure 1. Medium commercial-scale INMED aquaponics system

ONTOGENY OF DIGESTIVE ENZYMATIC ACTIVITY OF PACIFIC RED SNAPPER *Lutjanus peru* LARVAE

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The Pacific red snapper (*Lutjanus peru*) (Nichols & Murphy, 1922) is an important species to the fisheries along the Pacific coast of Mexico. In recent years there has been an increasing interest from both the private and public sectors in developing a sustainable culture program for this species as a viable economic alternative in the region. However, larviculture trials of Pacific red snapper have been characterized by very low survival during the first days of development. Therefore, several aspects related to feeding, nutrition, growth and early development have been studied in our laboratory. Recently, the development of the digestive tract was described and now we present the ontogeny of the digestive enzymatic activity. This information will be essential in implementing new feeding schedules, introducing new prey types or selecting the most favorable day for early weaning during larval rearing.

Larvae were obtained from a single hormonally induced spawn of captive broodstock and were transferred and reared in twelve 180-L fiberglass tanks equipped with a seawater recirculating system. Larvae were fed using live prey following a feeding schedule based on live prey. An average of 20 larvae were collected at 3, 8, 11, 15, 18, 22 and 25 days after hatching (DAH). Whole larvae were homogenized (FastPrep-24™ 5G), centrifuged (Eppendorf 5430R) at 15,000 x g and 4 °C for 15 minutes and the supernatants were stored at -80 °C until use. Activities of main digestive enzymes were assessed by fluorometry (trypsin, chymotrypsin, amylase, lipase and phosphatases alkaline and acid) and acid proteases by photometry. Readings were performed in triplicate using either a Varioskan flash fluorometer (Thermo Scientific®) or a Jenway 6505 spectrophotometer. Results are expressed in specific and individual activity from three replicates and three measurements.

All enzymatic activities were detected from hatching. An increment in the enzymatic activity with development was observed. Higher individual activities of all enzymes were observed between 18 and 22 DAH, except for amylase. Amylase showed high activity from 3 DAH and a late decrease at 25 DAH. These patterns of enzymatic activity during development have been reported in other marine fish larvae. The decrease of amylase activity and the increment of the other pancreatic enzymes are considered a marker for maturation of the digestive function and a transition to a juvenile-like digestion. In conclusion, total enzymatic equipment for this species is complete between 18 and 25 DAH, suggesting that weaning during larviculture trials of this species may be possible at this early age.

ENDOCRINE AND GOBLET CELLS DISTRIBUTION IN THE INTESTINE OF BRAZILIAN FISH TAMBAQUI AND HYBRID SPOTTED SORUBIM

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Endocrine cells are a highly specialized mucosal cell subpopulation involved in the producing of peptide hormones. CCK-8 stimulates pancreatic and bile secretions and NPY is the most potent orexigenic factor known. The main secretory product of goblet cells, mucin, forms a mucus layer coating the gut epithelium acting as the front line of innate host defense. The distribution of endocrine cells producing peptide hormones and goblet cells along the intestine of tambaqui *Colossoma macropomum* and hybrid spotted sorubim *Pseudoplatystoma reticulatum* × *Leiarius marmoratus* were assessed in this study. Intestine samples from 5 from tambaqui and 4 from hybrid spotted sorubim were collected in different segments of the midgut and hindgut.

Endocrine cells CCK-8+ and NPY+ were identified using immunohistochemistry. The goblet cells producing acid and neutral mucins were stained by Alcian Blue (AB) and Periodic Acid Schiff (PAS), respectively. Average number of ECs per mm² epithelial mucosa was obtained from ANOVA and Tukey's test. S.E.M: standard error mean and p-value set at $p < 0.05$ (Fig. 1). For tambaqui, the endocrine cells CCK+ were more uniformly distributed along the intestine, except for the lower density at CP (pyloric caeca). Endocrine cells NPY+ density was higher in T1 and T2 (71%). Goblet cells AB+ highest density was recorded in T2 (37%) while PAS+ had wider distribution from T1 to T3 (27%, 31%, and 26%, respectively). For hybrid spotted sorubim, the density of endocrine cells CCK+ and NPY+ were higher in the midgut on P1 (60%) and P1 and P2 (73%, likewise tambaqui), respectively. Goblet cells AB+ and PAS+ were more concentrated in the hindgut, PP accounting for 45% and 53%, respectively. Endocrine and goblet cells density were different and related to the anatomical features of the digestive tract in each fish. Although particularities exist among species, it seems that there is a distribution pattern of cells type that can be justified by the feeding habits.

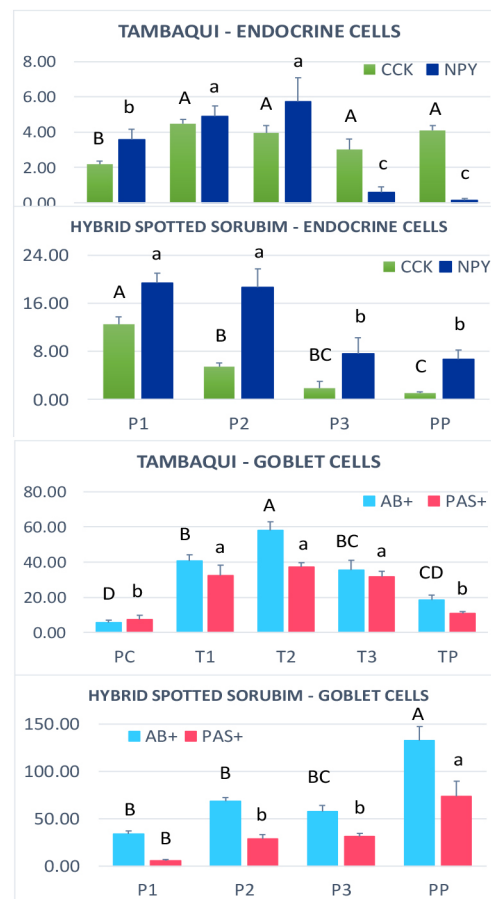


Fig. 1. Cells distribution along the intestine expressed in average number of cells per mm² of mucosal

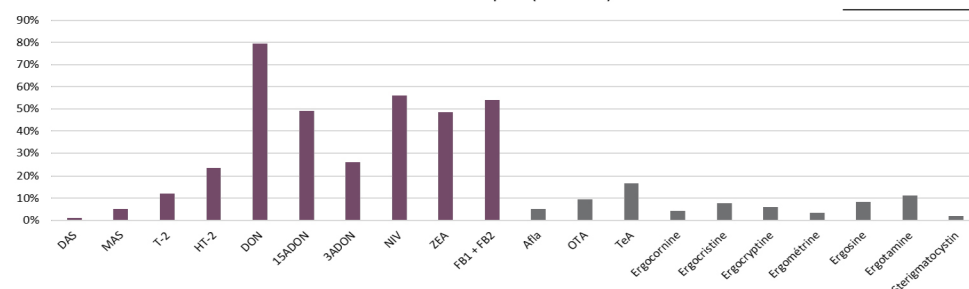
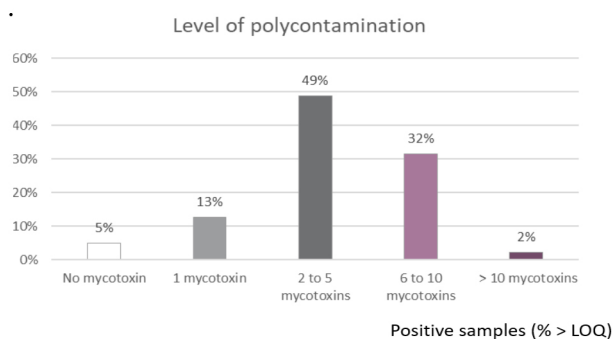
OLMIX SURVEY 2016: THE NATURAL CO-OCCURRENCE OF MYCOTOXINS IN FEEDSTUFF

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There is an increasing trend to use more and more plant ingredients in aquaculture feeds. And with it the risk of mycotoxin contamination also increases. These toxins are produced as secondary metabolites by fungi and can have serious detrimental effects on fish and shrimp. Ranging from mortalities in severe cases, to economic impacts such as reduced growth, reduced immunity, decreased feed efficiency. They can be caused by a single mycotoxin, or as is more common, by more mycotoxins working together synergistically causing even more damage. The contamination of raw materials is common under certain environmental conditions such as humidity, temperature and presence of insects. Accurate measurement of concentration of mycotoxins in the feed is difficult to determine due to its heterogeneous distribution. A representative sample is the accumulation of small portions (sub-samples) from many different locations over and around of the feed heap.

The natural co-occurrence of fungal metabolites in feed ingredients sampled from 42 different countries (Europe, Asia and South America) was studied using an LC-MS/MS based multi-mycotoxin method, between 01/2013 to 31/2016. All analyzed maize samples were found to be contaminated by different mycotoxins. Polycontamination was found in 82% of the tested samples (49% of the samples were contaminated with 2 – 5 mycotoxins, 32% with 6 – 10 and 2% with > 10 mycotoxins). Only in 5% of the samples no mycotoxins were found. Prevention of mycotoxin formation through agricultural practices in the field is becoming of paramount importance. Moreover, due to its dynamic nature, the potential presence of fungal metabolites from the field and/or feedstuff storage is variable, innovative strategies with a wide spectrum to deal with mycotoxin risk in aquaculture feed is strongly recommended.



Feedstuff samples	n
Corn	784
Corn by-products	46
Wheat	503
Wheat by-products	81
Soybean (grain and meal)	110
Sunflower	16
Rapeseed	10
Cotton meal	2
Rice bran	1
Total	1553

THE INTERFERON INDUCED PROTEIN-35 kDa NEGATIVELY REGULATES RIG-I CASCADE GENES IN ROCK BREAM

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Viral diseases are a serious threat to both natural and cultured fish populations. They may lead to massive economic losses to the global aquaculture industry. IFNs play a major role in the antiviral defense mechanism of the innate immune response in vertebrates. Interferon induced protein-35 kDa (IFP35) is an antiviral protein induced by Type II IFN (IFN- γ). In this study, we have identified IFP35 from the previously constructed rock bream cDNA database (RbIFP35) and characterized with the online bioinformatics tools. Moreover, physiological features of the RbIFP35 were evaluated through a spatial distribution where the immune related responses were evaluated through an immune challenged experiment. Moreover, the effect of RbIFP35 towards RIG-1 antiviral signaling was observed *via* evaluating the modulation of selected genes in the RIG-1 antiviral signaling pathway including; MAVS, IRF3 and IRF7. Here, we have transfected RbIFP35 into rock bream heart cell lines, treated with Poly I:C and isolated the cells in several time points. Then we have extracted the total RNA and made the cDNA for the qPCR analysis. The full length cDNA sequence of RbIFP35 contained an ORF of 1113 nucleotides which consists with 371 amino acids. Interestingly, *in silico* analysis showed that the RbIFP35 sequence contains two characteristic Nmi/IFP 35 domains as previously found in rockfish. Additionally, RbIFP35 shared 70% of identity with *Larimichthys crocea* and also closely cladded with IFP35 from *Larimichthys crocea* in the phylogenetic tree. According to spatial distribution a ubiquitous expression was observed among all the selected tissues of healthy rock bream fish but with different magnitudes. The highest expression was observed in the gill tissue, followed by liver and blood. Transcriptional distribution in liver tissue showed that *RbIFP35* significantly up-regulated against different stimuli including; Poly I:C, rock bream Irido virus, *Edverdisella tarda* and *Streptococcus iniae*. The highest expression of *RbIFP35* against Poly I:C, RBIV and *S. iniae* was observed at 48 h p.i. where the highest expression against LPS was observed at 6 h p.i. However, intense expression against *E. tarda* was observed at 24 h p.i. revealing the modulation of *RbIFP35* was differ according to the nature of the pathogenesis. Additionally, cell stimulation assay showed that *RbMAVS*, *RbIRF3* and *RbIRF7* were down-regulated by the transfected RbIFP35 in the rock bream heart cells. Collectively, we could conclude that RbIFP35 is an important immune related gene in the rock bream which may important for the innate immunity of the rock bream.

FOUR CASPASE GENES FROM RED LIP MULLET (*Lizahaematocheilus*); CHARACTERIZATION AND IMMUNE RESPONSES AGAINST *Lactococcus garvieae*

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Caspases are a group of conserved cysteine proteases which play vital role in cellular apoptosis. Mammalian caspases are again subdivided into three functional categories as initiator caspases (caspase 2, 8, 9 and 10); executioner caspases (caspase 3, 6 and 7) and inflammatory caspases (caspase 1, 4, 5, 11 and 12). In this study we have selected four caspases from red lip mullet including caspase 3, 7, 8 and 9. Initially, we have characterized all the four caspase members using bioinformatics tools. The tissue specific mRNA expression was then analyzed using healthy rockfish juveniles. An immune challenge was designed in order to determine the immune related responses of these caspases against selected immune stimuli. According to the ExPASy ProtParam tool MuCas3, MuCas7, MuCas8 and MuCas9 consist with 298 aa, 310 aa, 493 aa and 434 aa respectively. Domain architecture analysis revealed that all these four members possess CAsC domain which is important for their biological function. According to spatial distribution a ubiquitous expression was observed among all the selected tissues of healthy mullet fish including, blood, head kidney, spleen, liver, gill, intestine, kidney, brain, muscle, skin, heart and stomach but with different magnitudes. Interestingly, the intense expressions of all the four caspases were observed in the blood tissue. Highly modulated expression profiles were observed in the immune challenge analysis by all the four caspase members. MuCas3 and MuCas7 started to up-regulate at 6 h p.i. against LPS and poly I:C. With regarding to the *L. garvieae* MuCas3 and MuCas7 started to up-regulate at 24 h p.i. Interestingly, MuCas8 and MuCas9 has started to up-regulate at 6 h p.i. against LPS where both MuCas8 and MuCas9 has been up-regulated at 24 h p.i. against poly I:C and *L. garvieae*. Collectively, in silico analysis revealed that MuCas3, MuCas7, MuCas8 and MuCas9 sequences are well conserved among the homologs and it shared relatively higher homology with teleosts. Additionally, the modulation of the transcription patterns of all four caspases against immune stimuli suggested that these caspases involve with the immune responses in red lip mullet.

CHARACTERIZATION AND IMMUNE RESPONSES OF SELECTED THREE TUMOR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTORS (TRAFs) FROM BLACK ROCKFIS (*Sebastes schlegelii*) AGAINST IMMUNE STIMULI

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Tumor necrosis factor (TNF) is a multifunctional cytokine that plays important roles in cell survival, proliferation, differentiation and death. They are the prototypic members of the TNF ligand family which is mainly crucial in the regulation of inflammatory processes. Additionally, Black rockfish is a highly valued aqua crop mainly in the Asian-Pacific region because of its characteristic features including tolerance to low-water temperature, high survival rate and the fast growth. However, due to the massive culturing systems, the prevalence of infectious diseases was found to be drastically increased over the time. In this study we have characterized and studied the expression of three TRAFs including TRAF 2, 3 and 4 from black rockfish. *In silico* characterization was conducted using bioinformatics tools. Phylogenetic analysis was conducted to determine the evolution of these three genes. The expression analysis of three *TRAF* transcripts was conducted by performing quantitative real time PCR (qPCR). The results of pairwise alignments revealed that RfTRAF 2 shared 93% of the highest identity with *Larimichthys crocea* where RfTRAF 3 and RfTRAF 4 shared their highest identities with *Lates calcarifer* with 97% and 95% respectively. Interestingly, all three rockfish TRAF members were cladded with fish counterparts. According to the spatial distribution blood tissue shows the highest expressional level in all three genes and followed by gill tissue. In addition, post-bacterial infection kinetic expressions of all transcripts were examined in blood tissue. All three TRAF transcripts showed their highest upregulations at 12 h post infection against *Streptococcus iniae*. Additionally the highest upregulation of RfTRAF 2 against poly I:C was observed at the 24 h post infection where RfTRAF 3 and RfTRAF 4 were obtained their intense expression at 12 h post infection. Collectively, the importance of these TRAF members in immune related functions was elucidated through the immune challenged results.

TWO BCL-2 FAMILY MEMBERS FROM BIG BELLY SEAHORSE (*Hippocampus abdominalis*); CHARACTERISATION AND IMMUNE RELATED RESPONSES AGAINST BACTERIAL AND VIRAL STIMULI

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Apoptosis is an essential mechanism that involved in several biological functions including, developmental sculpturing, removal of unwanted cells and tissue homeostasis. Bcl-2 (B-cell lymphoma-2) family proteins are key regulators of the programmed cell death. Functionally this family can be classified as either pro-apoptotic or anti-apoptotic groups. Herein, we have characterized two cDNA and their translated proteins of Bcl 2-like 1 and Bax from *Hippocampus abdominalis*. The seahorse Bcl 2-like 1 (HaBcl2-L1) and Bax like (HaBaxL) full-length cDNAs were identified from previously constructed seahorse cDNA library. *HaBcl2-L1* and *HaBaxL* mRNA expression analysis were conducted by performing quantitative real time PCR (qPCR). Heart, gill, liver, spleen, kidney, intestine, stomach, skin, muscle, pouch, brain, testis and ovary were carefully dissected from the healthy juveniles. Besides, gill was isolated after immunization with *Edverdiselly tarda*, *Streptococcus iniae*, LPS and Poly I:C. The ORF of HaBcl2-L1 encodes for a putative protein of 232 aa, with a deduced molecular weight of 25.7 kDa polypeptide and a pi of 4.85 where the ORF of HaBaxL encodes a putative protein of 204 aa, a molecular weight of 23.4 kDa and a pi of 5.19. Interestingly, both HaBcl2-L1 and HaBaxL possessed Bcl-2-like conserved domain which is essential for their anti or pro-apoptotic function. Pairwise alignment analysis revealed that HaBcl2-L1 was shared the highest identity with *Stegastes partitus* (82%) where HaBaxL was shared the highest identity with *Larimichthys crocea* (81%). A ubiquitous expression was observed in both *HaBcl2-L1* and *HaBaxL* in selected seahorse tissues and their highest expression was observed in the gill tissues. Temporal distribution against *E. tarda*, *S. iniae*, LPS and poly I:C revealed a late responses (72 h p.i.). Finally, it could be suggested that HaBaxL and HaBcl2-L1 play important role in the defense mechanism of big belly seahorse.

TREATMENT OF AQUACULTURE WASTE LAGOON EFFLUENTS USING RECIRCULATING POLYGEYSER® TECHNOLOGY

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Facultative lagoons are widely used in the United States to treat agricultural and domestic wastes. While satisfactory for the treatment of aquaculture sludge, digestion ponds in most situations, lagoons cannot meet stringent ammonia standards required when discharging to impaired surface waters. Recirculating PolyGeysers have been developed to assure oxygen delivery required for organic and ammonia reduction from lagoon discharges. These units aerate and filter the waste stream 10-20 times prior to discharge which assures polishing of BOD and ammonia down to permitted levels.

The effectiveness of the RCPG to treat facultative lagoon effluents was studied over the course of a 5 month pilot scale test at a municipal lagoon in central Mississippi. A trailer mounted RCPG 10, a 10 ft³ model, was used during this test. The RCPG 10 is capable of throughput flows ranging from 1-10 gpm, and recirculates upwards of 100 gpm. This 1:100 to 10:100 flow ratio ensures sufficient oxygen transfer rates through the bead bed. During this study, ammonia, BOD, and TSS concentrations being discharged from the lagoon were seen to be as high as 15, 30, and 50 mg/L respectively. The unit continued to produce concentrations that met the new discharge requirements of <1/<3/<30 (Ammonia/BOD/TSS). BOD conversion rates averaged 1.5 to 2 kg/m³-day. VTRs ranged from .750 to 1.2 kg/m³-day with TAUs being calculated upwards of 2.5 kg/m³-day. These findings bode well for the Recirculating PolyGeyser being a simple, energy efficient solution for a problem that is plaguing the growth of aquaculture in the United States.



DEVELOPMENT OF PRESERVATION PROCESSES FOR REFRIGERATION-STABLE SEA VEGETABLES: PHYSICOCHEMICAL AND MICROBIAL SAFETY ASPECTS

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In recent years, harvesting and farming of seaweeds in US coastal regions, including Maine, has increased rapidly as the economic potential and ecological benefits of farming these crops have been recognized. According to the Maine Aquaculture Impact Report recently published by the Aquaculture Research Institute, Maine's seaweed industry produced ~54,000 pounds of product in 2014 having a farm gate value of over \$500,000. A large majority of the farmers who responded to the survey anticipated increasing production by more than 51% by 2020. However, lack of suitable processing infrastructure and knowledge of how to appropriately process harvested sea vegetables into safe, value-added food products have already been identified as significant bottlenecks to the sustainable growth of the sea vegetable industry in the Northeast.

In this study, we investigated the utility of fermentation or dry salting for the production of minimally processed seaweed products with extended shelf life potential. Within processing method, physicochemical characteristics (color, texture) and changes in relevant microbial populations (lactic acid bacteria, aerobic mesophiles, fungi, coliforms) were monitored. We developed a seaweed and cabbage "sauerkraut-style" product and assessed the effects of sea vegetable species (sugar kelp or winged kelp) and seaweed to cabbage ratio (75:25, 50:50, 25:75) on product quality over 60 days of refrigerated storage. We also used dry salting at high (20% and 18% wt/wt) and low (5%, 3% and 2% wt/wt) concentrations to extend the refrigerated shelf life of winged kelp up to 90 days.

Fermentation, as measured by change in pH and population of lactic acid bacteria, was more efficient in samples made with winged kelp compared with sugar kelp samples of the same ratio. Fermented sugar kelp products also yielded lower shear force values than comparative winged kelp formulations, indicating a softer texture. The coliform population in fermented samples appeared to have originated in cabbage and decreased throughout storage in cabbage/winged kelp samples. Cabbage/sugar kelp products did not behave similarly, with the 75% sugar kelp sample showing a net increase in coliform levels during storage. The quality characteristics of dry salted products were maintained well during storage. Salt concentration did not affect significant differences in texture or microbial load. All microbial counts remained < 4 log CFU/g for the duration of the study.

Preliminary results indicate significant promise for the utilization of both fermentation and dry salting to produce value-added products from farmed seaweeds. Additional data in regard to safety in the event of pathogenic contamination as well as consumer liking are needed. These aspects are currently under investigation.

GROWTH PERFORMANCE, SURVIVAL, AND PROCESSING CHARACTERISTICS OF HYBRID CATFISH FED PRE AND PROBIOTICS

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There has been a lot of interest in the use of pre and probiotics to increase growth and improve disease resistance in the catfish industry. This study aimed to evaluate a commercially available prebiotic and probiotic under conditions simulating commercial production in hybrid catfish. The dietary treatments consisted of: 1) a nutritionally complete basal diet, 2) the basal diet supplemented with 2% Grobionic®-A (International Ingredient Corporation, St. Louis, MO), 3) the basal diet supplemented with the probiotic Aquablend® (BIO-CAT, Troy, VA), and 4) the basal diet supplemented with both Grobionic®-A and Aquablend®. The four diets were fed to hybrid catfish (initial weight, 37.1 ± 0.1 g/fish) in 0.04 ha ponds for approximately five months. Results showed that weight gain, feed conversion ratio, food intake, and survival were similar among treatments. Carcass, fillet, and nugget yield were not significantly affected by treatment. A comparison of fillet proximate composition found no differences in protein, fat, or ash among treatments. An economic analysis revealed no benefit to feeding prebiotics or probiotics alone or in combination with one another to hybrid catfish. While interest in feeding pre and probiotics may remain, the results of this study do not support their use in the catfish industry.

UPDATE TO THE ATLANTIC SALMON BREEDING PROGRAM AT THE NATIONAL COLD WATER MARINE AQUACULTURE CENTER

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The USDA-ARS National Cold Water Marine Aquaculture Center (NCWMAC) in Franklin, ME has been supporting the U.S. coldwater marine aquaculture industry for the past thirteen years by developing a genetically improved North American Atlantic salmon. The St. John's River stock was chosen as the focal strain in the program because of fast growth, certification of North American origin, and widespread utilization by industry. The objectives of our research program have been to: 1) develop a selection index for important traits such as carcass weight, cold tolerance, fillet color, fat content, and sea lice resistance, 2) evaluate and validate the usefulness of incorporating genomic information into the salmon breeding program, and 3) evaluate the usefulness of a lumpfish (*Cyclopterus lumpus*) selective breeding program.

Our selected and unselected (control line) Atlantic salmon are evaluated with the assistance of industry partners in net pens to simulate commercial conditions. We have observed an increase in growth by approximately 15% for each generation while survival has been similar between groups of fish. Two of the most important traits for consumers are omega-3 fat content of the fillet and the color of the fillet. We have observed that crude fat, as measured in the Norwegian prime cut (just behind the dorsal fin) ranges from 7 to 13% (wet weight) for all measured year classes. Total omega-3 fatty acid content in families ranges from 5 to 15% of the total fat with an average of 12.8%. The amount of EPA and DHA in a single portion of the fillet averaged 2-3 g, which is the industry standard. Astaxanthin and canthaxanthin concentrations ranged from 1.1 to 4.1 µg/g of fillet. Future year classes will be evaluated to assess progress in these two traits.

Selection for resistance to sea lice has been an important component of the breeding program since 2015. At that time evaluations of phenotypic family based resistance were standardized and conducted across all families in the breeding program. Currently evaluations are based on replicated small scale infections. Based on conservative models, the heritability seen across our populations is 0.20. In the fall of 2017, the first year class of families screened under the new program were spawned. The offspring of this spawning will be evaluated in 2019. In addition to the current challenged based screening, efforts are being made to develop genetic markers and tools to estimate the genetic breeding values. Furthermore, a lumpfish selective breeding program has also been developed as a means to control sea lice infection.

PELLET FEED IMPROVEMENTS THROUGH VITAMIN C SUPPLEMENTATION FOR SNAKEHEAD FISH *Channa striata* CULTURE IN VIETNAM

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In Vietnam, snakehead fish is considered as one of the most valuable cultured fish. However, enhancement of fish health is an important issue for snakehead culture, because bacterial disease is a serious problem and vertebral anomalies have also been observed. Our objective was to determine optimal vitamin C levels in feed for snakehead fish culture. Several benefits have been attributed to ascorbic acid (AA) supplementation in fish such as growth, survival, reduction of skeletal deformities, disease resistance and stress response.

Laboratory feeding trial: The study was conducted to find out the optimal dietary vitamin C levels in terms of the growth performance of snakehead fish and economic aspects. The 8-week growth trial included six treatments with five replications each: 0, 125, 250, 500, 1000 and 2000 mg vitamin C (L-ascorbate-2-monophosphate) equivalent kg^{-1} diet. The diet, based primarily on soybean meal (SBM) and fish meal, contained 45% protein, 9% lipid and 4.2 Kcal.g⁻¹. Eighty snakehead fingerlings (6.24 ± 0.17 g.fish⁻¹) were assigned randomly in 0.5 m³ composite tanks with continuous aeration. Fish were fed twice daily. Growth rates and protein efficiency ratio were significantly greater in treatments with vitamin C supplementation compared with control treatment and optimal growth was obtained at 500 mg.kg⁻¹. Red blood cell counts and lysozyme in treatments with vitamin C supplementation tended to rise significantly proportional to the concentration of vitamin C. White blood cell counts in treatments with vitamin C supplementation were 2.1-3.6 times higher than those in the treatment without vitamin C supplementation. A bacterial challenge following the feeding trial showed best survival for fish fed AA levels of 250 mg.kg⁻¹ or more.

On-farm pond trial: Based on our laboratory results, effects of AA on snakehead in ponds to simulate farm conditions was tested using the following treatments: (i) commercial feed; (ii) commercial feed plus hand mixed AA at 500 mg.kg⁻¹; (iii) commercial feed plus hand mixed AA at 750 mg AA.kg⁻¹; (iv) commercial feed plus hand mixed AA at 1000 mg AA.kg⁻¹; (v) SBM diet without AA; (vi) SBM diet plus 500 mg AA.kg⁻¹; (vii) SBM diet plus 750 mg AA.kg⁻¹; (viii) SBM diet plus 1000 mg AA.kg⁻¹. The experiment was conducted in two experimental ponds (only SBM diet without AA placed in one pond and the rest in the other pond) with three replicate hapas each. Stocking density was 150 fish.m² and culture period was 5 months until market size was attained. Growth rates and protein efficiency ratio were greater in treatments with vitamin C supplementation compared with control treatment. Production cost was lowest for fish fed AA levels of 500 mg.kg⁻¹.



THE SELECTION OF A TEMPERATURE MODE OF FREEZING DURING CRYOPRESERVATION OF INCONNU OOCYTES

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At present, the use of methods of low temperature preservation remains an attractive and rapidly developing direction of conservation of rare and endangered species. Deep freezing of ova and embryos of fish is one of the central problems in the field of cryobiology of this class of animals. The rate of freezing during cryopreservation of cells is important. It has been ascertained that when the cooling is slow enough, the cells can rapidly lose water by osmosis. On the other hand, if the cooling is too fast, the extracellular solution decreases more rapidly than the intracellular one, and, as the result, the latter will eventually form intracellular ice crystals that proved to be fatal to cells.

In connection with the above the goal of the research was to determine the most suitable method of cryopreservation of inconnu eggs – either an ultra-fast one or a slow one.

The study was carried out at Alexandrovsky Fish Hatchery in Astrakhan Region in November 2016. Eggs from two inconnu females were used as research object. Obtaining of inconnu eggs was performed according to the method of draught. The mixture of triglycerides was used as a cryoprotectant. Ova were mixed with the cryoprotectant and placed in Eppendorf vials with their further placement in the Dewar glasses. Slow freezing was carried out at the rate of 2°/min to $t = -70^{\circ}\text{C}$ with subsequent immersion in liquid nitrogen ($t = -196^{\circ}\text{C}$). Cryopreservation of inconnu eggs by the method of ultra-fast freezing was performed by quick immersion of glass samples in liquid nitrogen (1,500 °/min).

The thawing of eggs, cryopreserved by the method of ultra-fast freezing, indicated that 1/3 of the oocytes after thawing was with a damaged shell. It shows that there was an internal restructuring in the oocytes – fat and yolk vacuoles have merged, in contrast to the native eggs, which vacuoles fill the cell uniformly and evenly.

When thawing the eggs, stored by the method of slow freezing, it was ascertained that the oocytes were externally non-damaged/whole, with only a few having a damaged shell. When zooming one can see that all eggs have internal adjustments, but only single eggs were with the restructuring at the initial stages.

Thus, the implemented experiment indicated that cryopreservation of inconnu eggs by the method of slow freezing allows obtaining a greater number of (visually) undamaged oocytes after thawing.

It is likely that to get the expected result, the cryopreservation of the native inconnu eggs is recommended according to the slow freezing method. Due to the fact that the experiment was conducted using only two females, it is necessary to conduct further studies in this direction.

The study is implemented within Agreement No. 14.607.21.0163 of 03.10.2016 (the Unique Identifier RFMEFI60716X0163).

ONTOGENY AND MORPHOLOGY LIMITS OF INTRACOHORT CANNIBALISM DURING THE EARLY-LIFE STAGES OF DOURADO *Salminus brasiliensis*

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Dourado is a native fish species with great potential for aquaculture in South America. Nevertheless, the main bottleneck for its rearing propagation is the high incidence of intracohort cannibalism, hindering the juvenile production. Therefore, the present study detailed the ontogeny and determined the morphology limits of intracohort cannibalism during the early life stage of dourado. The outcomes of this study provide the bases for the development of novel techniques of cannibalism reduction for the species.

Replicated small-scale larvicultures of dourado were performed in 5×30L aquaria in order to assess the ontogeny of the cannibalistic behaviour. A large-scale larviculture was carried-out in parallel, from where periodic fish samples were collected and measured for morphological traits in order to construct predictive models of the limits of cannibalism. Finally, these models were then cross-validated with empirical data collected from a predation experiments where cannibals were challenged with progressively increment of prey sizes.

Cannibalism was extremely intense in the first days of life, removing $85.7 \pm 3.8\%$ of the original population by 05 days after hatching (DAH). The vast majority of predation acts within this period was orientated tail-first. However, its effectiveness reduced down as fish grew older, becoming fully inefficient for fish older than 07 DAH (Figure 1). From this moment onwards, cannibalism turned exclusively head-orientated, but less intense. By 10 DAH, cannibalism removed $97.7 \pm 0.5\%$ of the original population. Morphological models predicted that younger cannibals are able to ingest prey larger than their own sizes (Figure 2). However, as cannibals grew larger, this maximum ingestible prey size reduced down to around 80% (Figure 2). The alternative model ($TL_{prey} = 0.705TL_{cannibal} + 5.650$; where TL is total body length) better matched the empirical results as it considered the inter-individual variability in the morphological traits and therefore better represents the realistic limits of cannibalism for this species.

Funding: São Paulo Research Foundation (FAPESP). Post-Doctoral Fellowship (2015/12210-5).

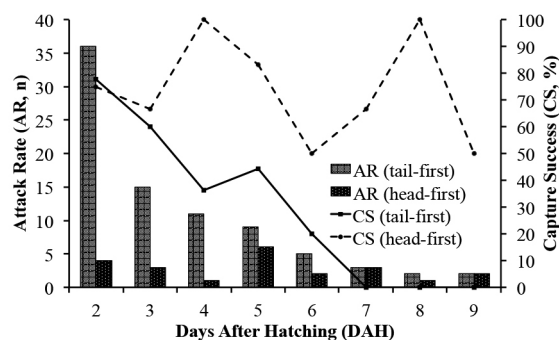


FIGURE 1. Cannibalistic behaviour of dourado. Tail-first cannibalism: cannibals attack and ingest prey tail-first. Head-first cannibalism: cannibals attack and ingest prey head-first. Values represent the pooled data obtained from 5 replicates (initial density of 15 larvae L⁻¹).

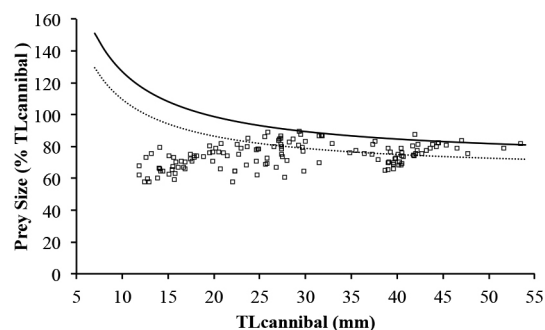


FIGURE 2. Maximum prey size for dourado cannibals. Regression lines include: Standard model (dotted line) based on average BD and MW; Alternative model (solid line) based on minimum BD and maximum MW. Squares are the ingested prey size in the predation experiment.

MassCOAST: A NEW SHELLFISH AQUACULTURE SITING AND PERMITTING TOOL FOR MASSACHUSETTS

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Siting and permitting of shellfish aquaculture operations is a key hurdle that prospective growers must overcome to start or expand operations. Permitting in Massachusetts is particularly difficult, as shellfish aquaculture licensing is under municipal jurisdiction, and growers must obtain a range of additional federal, state, and local approvals. The Massachusetts Coastal Oyster Aquaculture Siting Tool (MassCOAST) was developed to assist prospective growers with the siting and permitting process. This GIS-based, online tool provides key environmental and legal data for consideration by prospective growers, local officials, and state regulators, as well as a step-by-step explanation of how to use these data during site selection. This presentation will introduce the tool and consider how it may be a useful model for other states, regions, and industry sectors.

MEASURING THE ECONOMIC IMPACTS OF AQUATIC ANIMAL DISEASES: THE CASE OF CATFISH FARMING

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Aquatic fish diseases create economic hardships among fish farming households and owners and operators of backwardly- and forwardly-linked businesses. Economic recovery requires long-term remedial measures to restore healthy and productive fish populations and allow the resumption of harvesting, processing, distribution, and consumption of fish products. Estimates of the adverse economic impact of aquatic animal diseases are necessary information to justify the implementation of appropriate government programs for the fish farming industry.

Economic impact analysis is an attempt to estimate the change in economic activity in a specified region, caused by a particular business, organization, policy, program, project, activity, or other economic events, e.g., disease outbreak. Total economic impact is the sum of direct, indirect and induced impacts. Direct effects express the economic impacts in the sector in which the expenditure was initially made. Indirect effects result from changes in the economic activity of other industrial sectors which supply goods or services to the industry being evaluated. Induced effects are the result of personal consumption expenditures by industry employees.

Economic impact analysis estimates output or sales, employment or jobs, labor income, value added and tax revenues. The income, value-added, and output impacts are expressed in dollars for the year specified by the user. Output or sales are the gross sales by businesses within the economic region affected by an activity. Labor income includes personal income including wages and salaries and proprietors' income or income from self-employment. Employment impacts are expressed regarding a mix of both full-time and part-time jobs. Value-added is the contribution made to the value of aquaculture products at each stage of harvesting, processing, and distribution.

The overall goal of this project is to develop a methodology for assessing the economic impacts of aquatic animal diseases and estimate the adverse effects of aquatic fish diseases. The major tasks involved in evaluating the adverse economic impact of aquatic animal diseases are as follows:

1. Identify the types and causes of mortalities of aquatic animals.
2. Compile estimates of mortalities associated with aquatic animal diseases.
3. Compile estimates of annual production and values of aquatic animals.
4. Estimate direct economic losses due to aquatic animal diseases
5. Measure the economic impacts of aquatic animal diseases.

ANTIBIOTIC SUSCEPTIBILITIES OF BACTERIA ISOLATED FROM THE SAME FISH IN CASES EXAMINED AT THE KENTUCKY STATE UNIVERSITY FISH DISEASE DIAGNOSTIC LABORATORY

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This study analyzes differences in antibiotic susceptibilities among isolated bacterial colonies of the same species from a single fish from 10 different cases diagnosed at the KSU Fish Disease Diagnostic Laboratory (FDDL). For each case, four identical bacterial colonies isolated from a single fish will be tested for susceptibility to oxytetracycline, florfenicol and sulfadimethoxine + ormetoprim antibiotics. It is hypothesized that consistency in susceptibilities to all three antibiotics will be found among all four isolates from the same fish. In other words, if one isolate of the same bacterial species from one fish is sensitive to all three antibiotics, then the other three isolates will also be sensitive to them. This would give credibility to recommendations made by FDDL to use these antibiotics when treating for the infection with medicated feed. If, on the other hand, differences exist in antibiotic susceptibilities among bacterial isolates (with the same identification) from the same fish, then confidence in FDDL recommendations would be compromised. In other words, antibiotic susceptibility results would be dependent on which bacterial isolate happened to be chosen, and results would not have been the same if another isolate had been chosen. Results of this study will either confirm/validate FDDL protocols (especially if consistency in antibiotic susceptibilities is found among bacterial isolates) or show that FDDL protocols may need to be modified to achieve more accurate and consistent results in antibiotic susceptibilities and subsequent recommendations to our laboratory clientele to treat with medicated feeds containing these antibiotics.

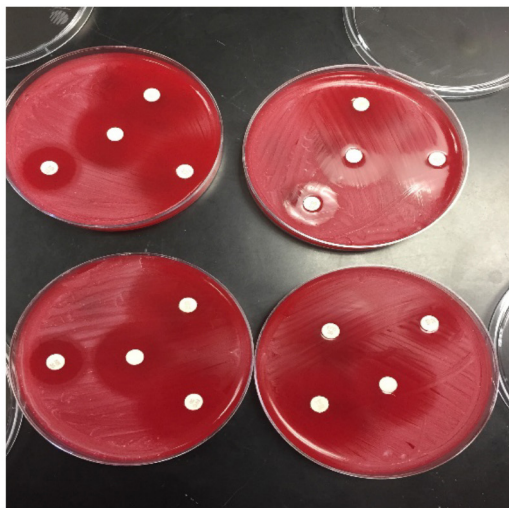


Figure. Measurement of Zones of Inhibition on Mueller-Hinton agar plates for antibiotic susceptibility.

QUALITY ASSESSMENT OF SCALLOP *Mimachlamys varia* AS A VALUABLE SOURCE OF BIOACTIVE COMPUNDS FROM THE ITALIAN MEDITERRANEAN COASTS

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In Europe, the exploitation of *Mimachlamys varia* occurs in small quantities through artisanal fisheries (dredges and trawls) that fail to meet the domestic demand, causing a considerable damage and disturbance to benthic communities and associated nursery habitat. To overcome these problems there is great interest to develop sustainable scallop culture, but the choice of new candidate species presupposes also the acquisition of basic knowledge such as their nutritional value. The objective of this study was to provide information about the nutritional quality of *M. varia*, cultured on suspended cages in the Gulf of Taranto (Ionian sea). For this purpose the monthly assessment of biochemical composition of *M. varia* edible portion were determined.

Scallops were cultivated in a pilot plant suspended cages at a depth of 6-9 m, in Mar Grande of Taranto (Mediterranean sea: 40° 25' 54" N, 17° 14' 22" E) close to a mussel farm. From August 2014 to July 2015, scuba diver collected samples monthly. All analyses were conducted in triplicate. Moisture, crude protein, and ash contents were determined by AOAC (2002) methods. Lipids were extracted using Bligh and Dyer method and quantified by gravimetry. Proteins were analyzed by Kjendahl method. FA profile was obtained by gas chromatography.

Significant seasonal changes were observed in moisture, ash, protein and lipid content content (ANOVA, $p < 0.05$). Despite the inter-annual variability of proximate composition, the results showed that the species may be considered as food item with interesting dietetic properties due to their good proteins content that ranged from 12.9 in July to 10.3 g/100g in December and low lipid content (within a range of 0.72-1.15 g/100g, June and September, respectively). Both the amount of lipid (rather low) and the proportion of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids found in these bivalves contribute to a healthful diet.

The relatively high levels of n-3 PUFA and lower amounts of n-6 PUFA provide a high n3/n6 ratio, favourable for human health, in all months examined.

The highest ratios of n3/n6 and PUFA/SAFA ratios, which are indicators of lipids nutritional quality in food, were always higher that recommended level, therefore the consumption of this species could be beneficial to human health.

Table 1. Seasonal changes of proximate composition of the edible portion of *Mimachlamys varia* reared in the Gulf of Taranto.

	Aug	Sept	Oct	Nov	Dec	Jan
Moisture %	81.25 ± 0.5	82.19 ± 0.1	82.29 ± 0.4	82.64 ± 0.2	81.30 ± 0.8	81.36 ± 0.3
Ash %	3.73 ± 0.2	4.59 ± 0.3	4.52 ± 0.1	4.92 ± 0.2	5.69 ± 0.3	3.80 ± 0.0
Protein g/100g	12.60 ± 0.3	11.28 ± 0.5	11.84 ± 0.3	10.84 ± 0.3	10.37 ± 0.2	11.92 ± 0.2
Lipid g/100g	0.97 ± 0.1	1.16 ± 0.1	1.12 ± 0.1	1.03 ± 0.1	0.80 ± 0.1	1.09 ± 0.1
	Feb	Mar	Apr	May	Jun	July
Moisture %	81.30 ± 1.3	80.74 ± 0.3	82.53 ± 0.4	82.50 ± 0.6	83.47 ± 0.32	79.98 ± 0.4
Ash %	4.27 ± 0.2	4.91 ± 0.1	4.87 ± 0.4	5.03 ± 0.3	5.46 ± 0.10	5.65 ± 0.3
Protein g/100g	10.67 ± 0.9	12.35 ± 1.1	11.64 ± 0.3	11.35 ± 2.5	10.81 ± 0.39	12.93 ± 0.1
Lipid g/100g	1.11 ± 0.0	1.15 ± 0.1	0.87 ± 0.1	0.84 ± 0.1	0.72 ± 0.04	1.04 ± 0.1

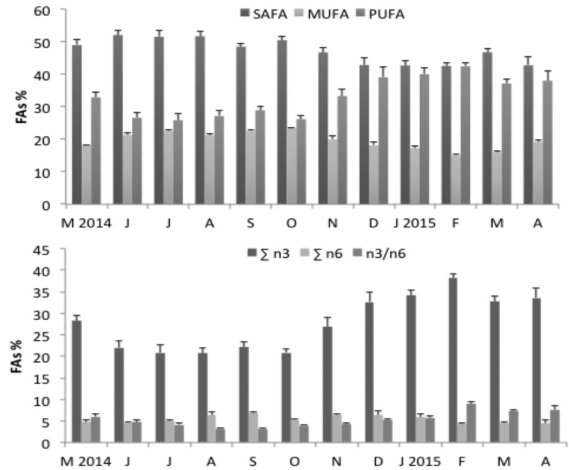


Fig. 1. Seasonal changes in fatty acid content (% of total fatty acids) and nutritional quality indexes of *Mimachlamys varia* (mean value ± sd).

DISK ABALONE IRAK4 INVOLVED IN POTENT IMMUNE RESPONSES DURING PATHOGENIC STRESS, BUT IMPAIRS MYD88 MEDIATED NF- κ B ACTIVITY

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Disk abalone (*Haliotis discus discus*) is one of the commercially important marine gastropod species in eastern Asia including Korea. However, overall abalone production has been markedly affected by mass mortalities due to the pathogenic infections. Invertebrates, including abalone are totally depend on innate immune system, because they lack well developed adaptive immunity. Therefore comprehensive study on abalone innate immunity is mandatory to establish novel disease management strategies. In present study, Interleukin 1 receptor associated kinase 4 (IRAK4), a critical member of Myeloid differentiate primary response factor 88 (MyD88) dependent Toll like receptor (TLR) signal transduction pathway was identified from disk abalone (designated as AbIRAK4) and functionally characterized.

The AbIRAK4 comprised with typical IKK structural features including N-terminal death domain and C-terminal protein kinase domain, similar to its mammalian counterparts. Expressional analysis revealed that *AbIRAK4*, mRNA constitutively expressed at all the early embryonic stages of disk abalone analyzed. In the un-challenged abalones *AbIRAK4* was ubiquitously expressed in all the tissues analyzed with the highest expression levels in the hemocytes. Significantly up-regulated mRNA expression of *AbIRAK4* was detected in hemocytes and gills after challenge with Gram-negative *Vibrio parahaemolyticus*, Gram-positive *Listeria monocytogenes*, viral hemorrhagic septicemia virus (VHSV), LPS and poly I:C. Overexpression of AbIRAK4 in HEK293T cells unable to induce the activities of NF- κ B and AP-1 responsive reporters. Interestingly, AbIRAK4 significantly diminished abalone MyD88-2 and MyD88-X mediated activation of NF- κ B and AP-1 responsive reporters in mammalian cells. Collectively, these findings suggest that AbIRAK4 is evolutionary conserved in invertebrates and actively involved in eliciting an early innate immune responses. Although, AbIRAK4 is mediating MyD88 dependent TLR signaling, it functions in different way compared to that in mammals.

EVALUATION OF GENETIC PARAMETERS AND DEVELOPMENT OF AN OPTIMAL, COMMERCIALY-VIABLE SPF BREEDING STRATEGY FOR *Litopenaeus vannamei*

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Introduction and Objective

Litopenaeus vannamei (Pacific White Shrimp) is a widely-cultured species in Asia and Latin America. In order to improve and adapt production of *L. vannamei*, numerous public and private organizations have developed domestication or selective breeding programs of varying complexity. A breeding program is an integration of population management (at the Nucleus Breeding Center), phenotypic or molecular data collection, and the use of available analysis and selection tools. The system needs to be optimized given the defined breeding goals and resource constraints. Balancing these components is not a clear-cut decision. This study involves post hoc or a posteriori analysis of existing breeding data and seeks to determine how a for-profit breeding program can best be optimized regarding the structure, data collection, data analysis, and trait selection to maximize performance gains.

Methods

Multiple generations of pedigree and phenotypic data from over 1,000 full-sib families will be analyzed. Data will be consolidated and organized, and undergo various quality-control measures. Analysis will be conducted to estimate (co)variance components by restricted maximum likelihood (REML) procedures, and breeding value estimates by best linear unbiased prediction (BLUP) or most appropriate method. Linear Mixed Models and data fitting strategies for BLUP (individual data vs. family means) will be evaluated to determine the best or most appropriate methods. Statistical analysis will be conducted using Genstat (18th Edition, VSN Intl., Hemel Hempstead, UK). Data analysis will begin with the most basic animal model including a single trait and single random (animal) effect, as follows:

$$y = Xb + Za + e$$

More complex models will then be utilized to confirm appropriate effects and the best model will be chosen to continue analysis and estimations of genetic parameters. Based on results of analysis, recommendations will be made regarding routine statistical analysis procedures and how to best optimize the breeding program to achieve maximum genetic gain, given operational, facility, and budget constraints.

Results

Results regarding estimation of breeding values and trends for growth traits will be presented.

A UNIQUE PROTEIN PLATFORM TECHNOLOGY TO MEET THE NEEDS OF AQUACULTURE

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As the population and the wealth of developing nations grow, the need for protein is expected to increase dramatically. Aquaculture represents one of the most realistic opportunities to meet this anticipated demand and therefore significant growth in the industry is in need innovative technologies that are sustainable and environmentally conscious. Creating feed that produces healthy fish while at the same time providing the quality products that consumers are expecting is one of the challenges that KnipBio is tackling.

KnipBio's single cell protein (SCP) technology platform enables a high quality protein alternative to fish meal or soy protein concentrate in aquafeeds, as well as providing high value molecules like antioxidant carotenoids, and immune-enhancing properties that lead to healthier fish and shrimp. KnipBio Meal (KBM) products lower the cost of high-value biomolecules and improve the health of aquatic animals on the farm to make operations more profitable.

In this presentation, KnipBio will provide an update on its technical progress including the expansion of its R&D activities and the current status of the scale-up program as we are entering into a commercialization phase to meet our customer's demand.

DEVELOPMENT OF GENOMIC RESOURCES TO AID U.S. COMMERCIAL ABALONE PRODUCTION AND RESTORATION CULTURE

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Commercial abalone production has greatly expanded over the past decade to become a thriving global industry due to high market value and demand. In the U.S., most commercial production operates in California utilizing three native west coast species: red abalone (*Haliotis rufescens*), green abalone (*H. fulgens*), and pink abalone (*H. corrugata*). Abalone are one of the few groups of species where culture production dominates the commercial market as a result of increasing demand and declining natural stocks. This decline in wild populations due to overexploitation and disease have even led to two U.S. abalone species, the white and black abalone, being placed on the endangered species list. While genomic resources have greatly improved production for most livestock, agriculture, and aquaculture species (where developed), few genomic resources exist for the west coast abalone species. These species differ in commercially important traits that are key to culture expansion in California and improved production efficiency (e.g., growth rate, disease resistance, thermal tolerance). Restoration culture for white and black abalone could also be improved by development of these resources, for example, by evaluating genomic diversity and culture-biased selection to guide breeding and outplanting decisions. To help advance aquaculture practices for the U.S. abalone industry, we are in the process of developing a genomic toolkit for these six abalone species. This included generating a high-quality de novo genome assembly for red abalone (*H. rufescens*), and conducting resequencing of five other abalone species native to the U.S. west coast: green (*H. fulgens*), pink (*H. corrugata*), pinto (*H. kamtschatkana*), black (*H. cracherodii*), and white (*H. sorenseni*) abalone. Additional sequencing on cultured and wild white abalone was conducted to investigate signals of culture-driven selection. Results, to date, on these projects will be presented.



Figure 1: Culture white abalone (*H. sorenseni*) at the NOAA Fisheries, Southwest Fisheries Science Center in La Jolla, California (Photo credit: Michael Ready).

THE STATUS OF AQUAPONICS IN GCC REGION AND THE SOCAIL, ENVIROMENTAL, ECONOMICAL IMPACT OF AQUAPONICS IN SUADI ARABIA

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Aquaponics has been growing lately big times in GCC (Gulf Cooperation Council) region from governments and individual. Saudi Arabia has been doing aquaponics since year 2000 on a governmental level. UAE follow shortly after. Now there are many aquaponics farms all over GCC countries and there become huge support as loans and regulations from governments.

Since that Saudi Arabia is not an agriculture country, we have done a theoretical study on what if Saudi Arabia used aquaponics instead of regular farming. we found that the Saudi Arabia agriculture GDP will raise 101%. which means it will goes from around 3% to 6% with less land and natural resources.

GENE EDITING OF REPRODUCTIVE HORMONES TO STERILIZE CHANNEL CATFISH, *Ictalurus punctatus*

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Sterilization of cultured fish is the most effective strategy to prevent unwanted genetic communication between domestic and wild populations, which is essential for an environmentally-responsible aquaculture industry. In this research, we sought to reversibly produce sterile channel catfish (*Ictalurus punctatus*) by knocking out of reproduction critical genes, the gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) genes, using the zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) technologies.

CEL-I assay and sequencing were applied to identify the mutation of gene-edited parents as well as their first-generation progeny. Spawning trials of the fish with outstanding secondary sexual characteristics were utilized to evaluate fertility. Hormone therapy was conducted to restore reproduction.

GnRH and FSH genes were successfully mutated in channel catfish using TALENs technology, while LH gene was mutated using ZFNs technology. LH gene-edited channel catfish showed normal gonadal growth, but had difficulty in ejaculation and ovulation. Less than 25% of the males fertilized the eggs, and all females failed to ovulate. Majority of the GnRH and FSH gene edited fish were infertile. The rest were fertile and displayed normal gametogenesis and reproductive performance. However, these fish had lower ($p < 0.05$) hatching rate of embryos compared to the control fish. The mutations were transmitted to the F1 generation through nature spawning. Hormone regime with luteinizing hormone-releasing hormone analog (LHRHa) and human chorionic gonadotropin (hCG) administration restored the production and increased embryo hatching rate for some of the GnRH, FSH and LH mutated fish (Table1).

A and B refer to the spawning rate and the average of the embryo hatching rate before hormone therapy. C and D refer to the spawning rate and the average of the embryo hatching rate after hormone therapy. The superscript letters, a and b, refer to the females and the males.

TABLE1. Summary of spawning rates and hatching rates before and after hormone therapy of channel catfish (*Ictalurus punctatus*).

	Genotype			
	GnRH	FSH	LH	Control
A	<20% ^a	<34% ^a	0 ^a	60% ^a
	<50% ^b	<40% ^b	<25% ^b	60% ^b
B	66% ^a	1% ^a	0 ^a	74% ^a
	2.8% ^b	0.5% ^b	1% ^b	74% ^b
C	<57% ^a	<70% ^a	<40% ^a	71% ^a
	<67% ^b	<78% ^b	<33% ^b	67% ^b
D	72% ^a	35% ^a	50% ^a	42% ^a
	72% ^b	35% ^b	50% ^b	42% ^b

EVALUATION OF PRODUCTION SYSTEMS FOR RAISING CHANNEL CATFISH FINGERLINGS

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There is little research-based information on the survival, growth, yield, costs and risk associated with on-farm production of fingerling channel catfish. This study is an attempt to provide experimental data that allows the standardization of production methods for channel catfish fingerlings using different production systems. Intensively aerated ponds and split pond systems have been demonstrated as a means to increase production in the catfish industry compared to traditional level pond culture techniques but to our knowledge those systems have not been evaluated in fingerling production.

Three production systems are being evaluated for raising channel catfish fingerlings, which include: traditional earthen ponds, high aeration earthen ponds, and split pond system. Three replicates were used for each treatment, for a total of nine 0.1 acre ponds. Ponds were stocked with 80,000 fingerlings per acre, and fish were cultured for 90-days. Fish were fed twice per day, using a high quality protein feed (50% crude protein, 17% crude fat). Water quality parameters including dissolved oxygen, temperature and pH were recorded twice per day; ammonia, nitrites, and chlorides were monitored twice per week. Growth was evaluated every other week by sampling all ponds. Survival, growth, yield, and costs of raising channel catfish fingerlings in traditional ponds, heavy aeration ponds and split ponds will be discussed.

AN OVERVIEW OF THE STATUS AND POTENTIAL OF THE MUD CRAB FISHERY IN COASTAL BANGLADESH: PROSPECT, STRATEGIES, APPROACHES & CHALLENGES

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Public awareness of the potential of the Mud crab fishery in coastal areas of Bangladesh has been particularly strong, in recognition of climate resilience and the particularly high export value of crabs. Endemic crabs show promise for cultivation, with potentially excellent economic and nutritional rewards. Despite such favorable prospects, the crab culture industry has remained undeveloped, and is restricted almost entirely to the fattening or growout of wild-caught juveniles; hatchery technology has not yet advanced sufficiently to deliver a reliable and abundant source of seedstock. The high aquaculture and export potential of the sector hinges on favorable biological, social and economic factors; coastal interest and international demand and price are compelling industry incentives. From a commercial perspective, efforts to close the life cycle of the burrowing mud crab *Scylla serrata* have had only limited success, and the hatchery and nursery phases of mass culture remain serious bottlenecks to mass culture. The current study has focused on the underlying perceptions and circumstances of crab fishery in coastal Bangladesh, as relevant to the potential for continuing aquaculture development. Our analysis focused on qualitative opportunities, approaches and impediments, using Strengths, Weaknesses, Opportunities and Threats (or SWOT) analysis. This was conducted to explore issues integral to the development of the crab sector toward sustainable growth of the industry in coastal Bangladesh. Interviews, focus groups, and a workshop were carried out in support of the analytical process. Evaluation and analysis of primary and secondary data revealed several unifying trends, such as consistently escalating price and demand, biological tolerance of variable and at times adverse environmental conditions, relatively low levels of investment required for farming, good benefits, appealing polyculture and integrated farming characteristics, availability of suitable resources, and nearly optimal climate and water quality conditions in coastal Bangladesh. Impediments to industry growth include a daunting dependence on wild-caught juvenile crabs. Challenges include the lack of suitable hatchery operation, limited technical knowledge, unavailability of specialized feeds, shallow and somewhat unstable domestic markets, susceptibility of young crabs to mortality, scarcity of technical and market support, and inadequate strategic policy. These must be considered together with promising incentives for industry development: high market potential, farming interest, prospects for employment generation, nutritional quality, and apparently robust business opportunities. Continuous extractive practices and compromised habitat quality have led to significant depletion of wild stocks, complicating a value chain already troubled by indiscriminate patterns of harvest. Market competition from imported crabs must be factored into plans for industry growth and development; these and other threats impose limitations on the capacity for sustainable expansion of the mud crab industry in coastal Bangladesh. Some recommendations are also detailed in the interest of adopting a responsible integrated coastal fisheries management approach and strategic policy development. This planning approach is based on anticipation of technological, environmental, regulatory and commercial management factors. Important guidance to industry development can be gained by considering past regional successes and failures with other crustaceans. For example, the remarkably successful growth of the mitten crab (*Eriocheirs sinensis*) industry in China may have value as a model for the mud crab industry in Bangladesh.

SUPPLY CHAIN ANALYSIS OF CARP IN TERAI, NEPAL

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In order to examine the supply chain of carp (Rohu *Labeo rohita*, Naini *Cirrhinus mrigala*, Bhakur *Catla catla*, Silver carp *Hypophthalmichthys molitrix*, Bighead carp *Aristichthys nobilis*, Grass carp *Ctenopharyngodon idella* and Common carp *Cyprinus carpio*) from farm to plate, a study was carried out in three districts of Terai, Nepal namely Makwanpur, Chitwan and Nawalparasi districts. Altogether 102 respondents (20 grow out farmers, 9 fish traders and 5 consumers in each district) were selected randomly for survey from three districts. The survey was carried out from May to September 2016. Data were collected by using semi-structured questionnaire interview of respondents, key informants and field observation. Data were analysed by using One Way Anova followed by Duncan's Multiple Range Test. Market survey showed that carp is sold in two forms, live and fresh forms. Four types of supply chains in live carp and seven types of supply chains in fresh carp were identified. The longest chain included middleman, wholesaler and retailer as intermediaries between farmer to consumer whereas shortest chain was directly from farmer to consumer without intermediaries. Supply chain of live carp was comparatively shorter than fresh carp to minimize fish loss due to handling stress. Grow out farmers in Nawalparasi earned significantly ($P < 0.05$) higher profit of 156 ± 1 Rs/kg than Chitwan and Makwanpur due to higher selling price and low variable costs incurred. In live carp supply chain, middleman received higher margin (34-36%) in three districts whereas in fresh carp supply chain, retailers earned higher profit in Makwanpur (50%) and Nawalparasi (56%), and middleman in Chitwan (50%). Unless infrastructure is well developed for fish marketing, unnecessary intermediaries should be eliminated to reduce post harvest loss.

INVOLVING WOMEN IN FIELD-TESTING OF A PERIPHYTON ENHANCED AQUACULTURE SYSTEM FOR NUTRITION SECURITY

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An on-farm trial of carp polyculture was carried out with participation of women farmers from Sundardeep Women Fish Farmer's Cooperative (15 farmers) in Chitwan District and Mishrit Fish Farmer's Cooperative (22 farmers) in Nawalparasi District to field-test the enhancing effect of periphyton on feed ration and fish production. Farmers stocked 6 carp species and 2 small indigenous species (SIS) in ponds. Farmers were divided into two groups. One group fed their fish with dough of rice bran and mustard oil cake with 50% feeding, while the other group installed bamboo substrates in their ponds and fed their fish with half the amount of the feed used by the first group. Farmers netted and weighed fish monthly to check fish growth and calculate ration. Farmers were provided with a book to record fish that were consumed, sold, or died. Final harvest was done by netting fish in December 2015 after eight months of culture. The netted fish were counted, weighed, and returned to the pond as the farmers wanted to keep fish for their biggest festival, "Maghi", in mid-January. In aggregate, 84% of farmers consumed fish at home, and 40% of farmers sold carps. About 95% farmers sold carps at the pond site, while 5% sold in nearby local markets. In case of farm site sales, both men and women were involved, whereas selling at local markets was solely done by women. The trial showed that culturing carps and SIS with 50% feeding rates and with bamboo substrates in ponds resulted in 22% higher fish production compared to culture of carps with normal feeding. More interestingly, the gross margin of the half-fed, periphyton enhanced carp polyculture was almost double that of the normal fed polyculture system. Periphyton enhanced Carp-SIS polyculture has a potential of enhancing family nutrition and women empowerment among small scale women farmers.

REDUCED FREQUENCY FEEDING REGIMES IN SUNSHINE HYBRID STRIPED BASS (FEMALE *Morone chrysops* x MALE *M. saxatilis*)

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Different feeding strategies were conducted in an attempt to improve the feed conversion ratio (FCR) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*, HSB) while maintaining good growth and reducing the feeding frequency under optimal water quality and temperature conditions. The HSB (initial weight 416 ± 7.9 g, mean \pm standard deviation) were fed for 88 days under two different feeding methods (automated belt feeding and *ad libitum* hand feeding) and two different frequencies (3 days and 5 days per week). High protein level floating pellets were used during the experiment (45% protein, 16% fat, Zeigler Brothers, Inc.). Each experimental group was replicated three times in flow-through, outdoor tanks (N = 150 fish each tank). At the end of the trials the fish were weighed and the FCR was calculated for each group. There were significant differences in mean total weight fed per fish, initial feeding rate (percent body weight), and final feeding rate by one-way analysis of variance (ANOVA) and Tukey-Kramer Honestly Significant Difference *post hoc* test ($P < 0.05$) (**Table 1**). There were no statistical differences in average initial weight, final weight, daily weight gain, or FCR between the treatment groups. Differences were not detected in the FCR between the different groups, however the means of the tank replicates were quite variable ranging from 1.71-2.49. The mean FCR was approximately 20% higher in fish that were fed more frequently (5 days per week) compared to those that were fed less frequently (3 days per week). Although not statistically significant, this increase in FCR may have an economic impact in commercial operations. Hence, reducing the frequency of feeding may improve the FCR of sunshine bass and lower the overall cost of feeds required to raise the fish, especially as they get larger during Phase III (final growout) production.

Table 1. Summary data of the hybrid striped bass growth trials including initial weight, initial feeding rate (FR % body weight), final weight, final FR, weight fed, and FCR. *Means in a column with different letters are significantly different (ANOVA, Tukey HSD, $p < 0.05$).

Variable	Feeding method and Frequency			
	Hand x 3	Belt x 3	Hand x 5	Belt x 5
Initial weight (g/fish)	414 ± 5	422 ± 10	416 ± 10	410 ± 4
Initial FR (% body weight)*	$1.28 \pm 0.19b$	$1.96 \pm 0.16a$	$1.01 \pm 0.11b$	$1.30 \pm 0.21b$
Final weight (g/fish)	609 ± 78	593 ± 37	595 ± 8	592 ± 35
Final FR (% body weight)*	$2.16 \pm 0.32a$	$1.98 \pm 0.10ab$	$1.46 \pm 0.22b$	$1.54 \pm 0.53b$
Daily weight gain (g/fish)	2.22 ± 0.85	1.94 ± 0.33	2.01 ± 0.17	2.04 ± 0.42
Total weight fed (g/fish)*	$316 \pm 50a$	$370 \pm 47ab$	$406 \pm 13ab$	$437 \pm 25b$
FCR	1.71 ± 0.35	2.16 ± 0.34	2.28 ± 0.21	2.49 ± 0.60

THE POTENTIAL USE OF ENVIRONMENTAL STRESSORS FOR MASCULINIZATION IN TWO ORNAMENTAL FISHES

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Sex determination in teleosts is highly diverse and sexual phenotypes may result from a combination of both genetic and environmental sex determination (GSD and ESD). Manipulation of the larvae's environment during the undifferentiated labile period has the potential to result in skewed sex ratios. Environmental sex reversal is believed to be linked to the stress response axis and cortisol release. Sexually dichromic species, such as the Rosy Barb *Pethia conchonius* and Dwarf Gourami *Trichogaster lalius*, make excellent model species for sex differentiation research. Current research efforts focus on understanding the role and interaction of environmental stressors on sex differentiation in these two species.

A suite of experiments have been conducted to elevate endogenous cortisol levels in developing larvae by either directly exposing them to cortisol or manipulating environmental factors such as stocking density, temperature, or salinity in such a way to elicit a natural increase in endogenous cortisol levels. Whole body cortisol levels, survival, growth, and sex ratios of replicate tanks were determined and analyzed for each experimental treatment. To date, experimental treatments have resulted in significant differences in growth and survival, but not resulting sex ratios. Results from cortisol assays have confirmed increased stress levels in specific treatments.

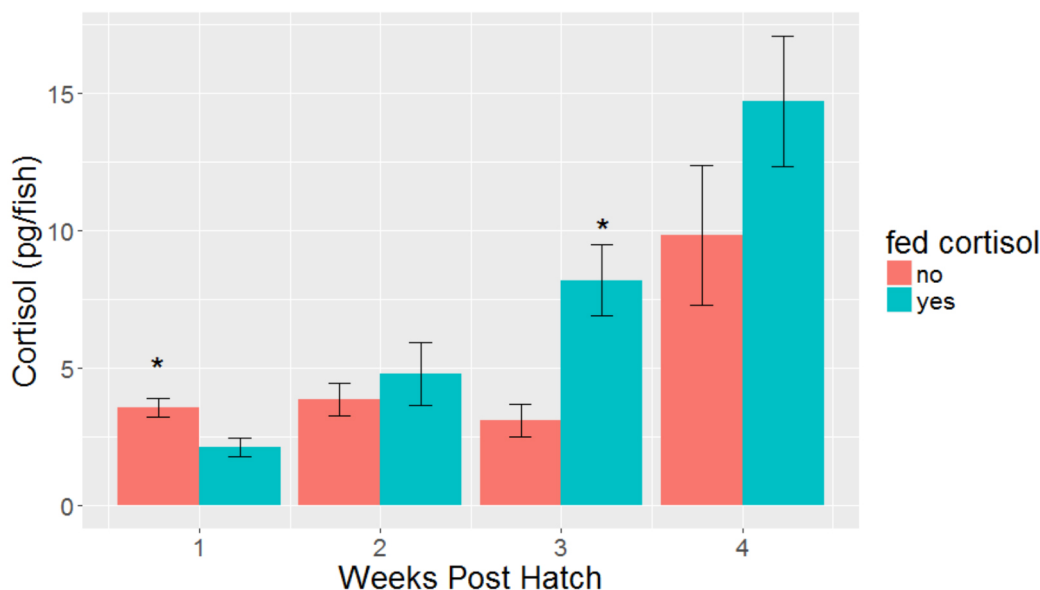


Figure 1: Cortisol levels (pg/fish) of Dwarf Gourami larvae that were fed cortisol feed (0.2 mg/g feed) during the 1st, 2nd, 3rd, or 4th week post hatch.

CLCA IS A METALLOPROTEASE INDUCED DURING PATHOGENESIS AND IS A POTENTIAL THERAPEUTIC MOLECULE TO DEAL WITH PISCINE DISEASE

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Bacterial disease by *Flavobacterium columnare*, and *Aeromonas hydrophila*, cause significant economic loss to the aquaculture farmers. The stringent regulations on the use of antibiotics and low efficacy of available vaccines, creates the need for new solutions. Epithelial tissues play barrier and defensive functions in different organs to protect the fish from pathogens. Bacterial and parasitic infections damage the epithelial tissues and make the fish. Lot of studies have shown the role of epithelial junctional complexes in barrier function and a correlation with immune response. Our goal is to identify cost-effective molecules that can be used in fish feed or water as therapeutics to prevent and treat bacterial and parasitic diseases.

CLCAs are predominantly expressed in the epithelium and endothelium of chordates and is required for maintaining epithelial integrity. We have shown that CLCA2 interacts with EVA1, a protein that is expressed on epithelial and immune cells, both CLCA2 and EVA1 form a complex with TJ protein ZO-1 and CLCA2 alone forms a complex with beta-catenin. Both CLCA2 and EVA1 expression directly correlate with epithelial differentiation and loss of either protein results in cells to lose cell-cell junctions and undergo EMT and thereby compromise barrier function. On the other hand, there are ample studies showing the presence of proteases and metalloproteases in mucus. They directly kill the pathogens either by cleaving the bacterial proteins or by activating the innate or adaptive immune response, or producing antimicrobial peptides. Interestingly, we find that CLCA2 is a self-cleaving metalloprotease. It has a metal binding motif HEXXH which is conserved across species, including zebrafish. There is an auto cleavage site near amino acid 700. We found that Zn^{+2} catalyzed CLCA2 cleavage (Fig 1) and the E to Q mutation in HEXXH abolished its proteolytic activity (Fig 2) (unpublished data). Thus, CLCA2 is a Zn^{+2} dependent, self-cleaving metalloprotease expressed at cell-cell junctions. Our proposed model for CLCA2 is that along with maintaining epithelial integrity, during stress/pathogenic insult, CLCA undergoes self-cleavage, and activates immune cells and mucus secretion. Therefore, we speculate that sCLCA could be a potential therapeutic for piscine bacterial diseases.

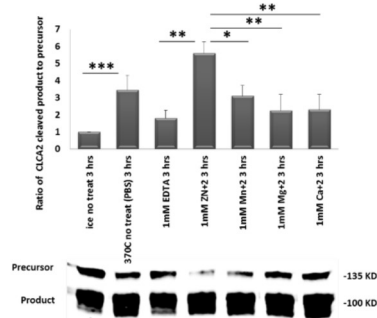


Figure 1. Zinc catalyzes CLCA2 cleavage which was inhibited by metal chelator EDTA. (Ramena et al.,

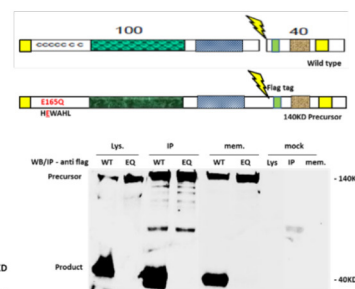


Figure 2. E165Q mutation in the HEXXH amino acid motif of CLCA-N domain inhibits CLCA2 cleavage. (Ramena et al., unpublished data)

FISH ECO-GENOTOXICOLOGY: MICRONUCLEUS ASSAY IN FISH ERYTHROCYTES AS IN SITU BIOMARKER OF FRESHWATER POLLUTION

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Waste disposal from industry and urban structures in Asian rivers has resulted in deposition of variety of new toxic chemicals and organic compounds. Such activities have endangered the existence of ecosystems and their inhabitants. Changes in genome caused by genotoxic agents led to mutations and pose burden to the populations of fish species. Toxicants those induce genetic damage involve everlasting monitoring and before time detection. The unremitting input of toxicants into the freshwater bodies has led to the advancement in techniques for evaluation and monitoring the fate such ecosystems. Fishes are marvelous model animals for genotoxicological studies and provide early warnings for toxicants induced environmental alterations and degradations. Fish species may also be used to estimate the possible effects of toxicants to produce carcinogenic and teratogenic effects in human.

Owing to white meat production *Labeo rohita* have vast economic importance but its population has been reduced drastically in River Chenab due to pollution. Atomic absorption spectrophotometry showed merciless toxicity level of Cd, Cu, Mn, Zn, Pb, Cr, Sn and Hg. Comet assay results indicated significant ($p < 0.05$) DNA fragmentation in *Labeo rohita* as $42.21 \pm 2.06\%$, $31.26 \pm 2.41\%$ and $21.84 \pm 2.21\%$ DNA in comet tail, tail moment as 17.71 ± 1.79 , 10.30 ± 1.78 and 7.81 ± 1.56 , olive moment as 13.58 ± 1.306 , 8.10 ± 1.04 5.88 ± 0.06 , respectively, from three different polluted sites of the river. Micronucleus assay showed similar findings of single micronucleus induction (MN) as $50.00 \pm 6.30\%$, double MN $14.40 \pm 2.56\%$, while nuclear abnormalities (NA) were found as $150.00 \pm 2.92\%$. These higher frequencies of MN induction and NA were found to be the cause of reduction of 96% of population of this fish species in experimental area of the River Chenab. This fish species has been found near extinction through the length of the river Chenab and few specimens in rainy seasons if restored by flood die in sugarcane mill season. Due to sweeping extinction *Labeo rohita* showed the highest sensitivity for pollution and could be used as bioindicator and DNA fragmentation in this column feeder fish species as biomarker of pollution load in freshwater bodies.

PRODUCTION OF STOCKER-SIZE HYBRID TILAPIA IN AN OUTDOOR BIOFLOC PRODUCTION SYSTEM

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Production efficiency per unit volume of water can be improved when stocking rate is adjusted to allow rapid growth of the culture organism to the desired target weight. Fingerlings often are grown to stocker size (ca. 100-150 g/fish) at high stocking rates and then the population is thinned to allow continued rapid growth to market size. Little information is available for production of stocker-size tilapia grown in a biofloc technology (BFT) production system. The objective of this study was to quantify the production function and impacts on water quality of stocking sex-reversed fingerling hybrid tilapia (*Oreochromis aureus* F X *O. niloticus* M) at 100 – 300 fish/m² in 50-fish/m² increments to grow stocker-size fish in outdoor BFT tanks. Fingerlings (12.9 g/fish) were stocked into each of nine continuously aerated HDPE-lined tanks (4.7 m², 3.6 m³) at their randomly assigned stocking rate for this 78-day study. Each tank was equipped with a 74-L settling chamber to remove solids. Fish were fed to apparent satiation twice daily with a commercially formulated diet (44% protein, 15% lipid decreasing to 30% protein, 6% lipid). Fish in each tank were sampled to monitor growth at approximately 30-d intervals. Water quality measurements were made weekly. At harvest, tilapia survival among tanks was high (mean = 94.5%). Gross fish yield (y) increased linearly with stocking rate (x) ($y = 0.0476x + 16.197$, $R^2 = 0.763$), and ranged from 20.1 to 30.5 kg/m³. Mean individual weight (y) at harvest decreased linearly from 155.2 to 81.4 g/fish as stocking rate (x) increased ($y = 180.89 - 0.3455x$, $R^2 = 0.929$). Mean daily feed ration and total feed fed ranged from 251 to 382 g/m³/d and 19.6 to 29.8 kg/m³, respectively, and increased linearly with stocking rate ($P = 0.003$, $R^2 = 0.734$ and $P = 0.003$, $R^2 = 0.734$, respectively). Feed conversion ratio (feed dry weight basis) ranged from 1.00-1.13 and was independent of stocking rate ($P = 0.959$, $R^2 = 0.000$). Stocking density impacted some of the evaluated blood serum parameters (peroxidase activity, lysozyme activity, and cortisol levels) as well as other blood parameters (hematocrit, hemoglobin, and differential blood cell counts). The overall effect of stocking density on measured blood parameters will be discussed. Results suggest that hybrid tilapia grow rapidly over a wide range of stocking rates, and that high yields of stocker size tilapia can be achieved in 78 days.

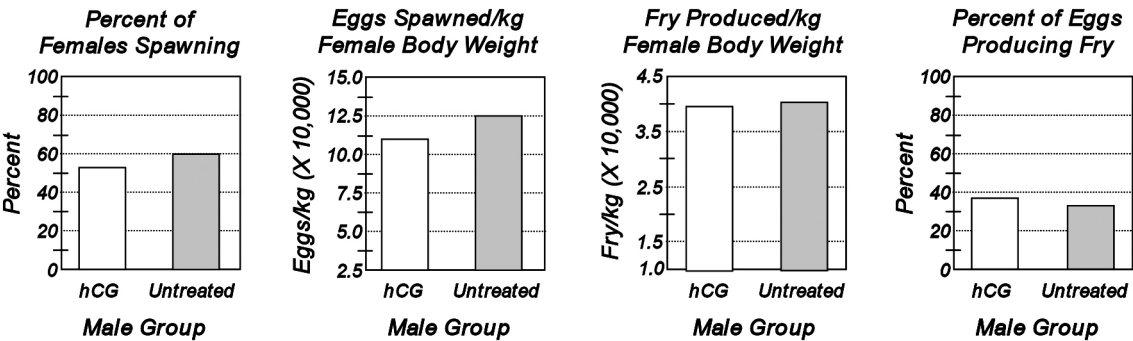
METHODS OF DOMESTIC STRIPED BASS *Morone saxatilis* SPAWNING THAT DO NOT REQUIRE THE USE OF ANY HORMONAL INDUCTION PROCEDURES

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We previously identified organized reproductive group spawning methods of domestic striped bass (*Morone saxatilis*), where several untreated female striped bass volitionally spawn in tanks with several male striped bass treated with 165 IU/kg of human chorionic gonadotropin (hCG) to hydrate semen for better dispersal through the water column. In those trials, fish successfully spawned to produce millions of fertile eggs and fry using photothermal and water salinity manipulations with limited hormone treatments. We demonstrate here that hCG is not required at all for captive spawning of domestic striped bass using the organized reproductive group spawning method and that this hormone-free method of spawning is comparable to that of treating males with hCG in regards to egg and fry production (**Figure 1**). Group spawns (N = 2) that each included 19-20 hCG induced males (age 3 years) placed in tanks with 19-20 untreated females (age 4 years) produced between 8.4 and 9.7 million eggs and 2.8 to 3.1 million swim-up fry. Group spawns (N = 2) that each included 19-20 untreated males placed in tanks with 19-20 untreated females similarly produced between 4.8 and 11.4 million eggs and 1.0 to 3.8 million swim up fry. The percent of eggs harvested that produced fry in all four trials was between 20.8% and 36.6%, with an overall average of about 1/3 fry yield from eggs harvested. In all 4 trials an average of 50% of the female fish spawned. We conclude that 6th generation domestic striped bass males and females successfully spawn together *en masse* to produce fry at commercial scale regardless of whether they are induced with hCG or not. This is a revolutionary advancement toward providing a consistent striped bass fingerling supply that does not rely on specialized expertise for staging or strip spawning fish.

Figure 1. Summary data of striped bass group spawning trials. Bars represent the average values for N = 2 trials of each group (hCG treated males and untreated males; females in all trials were untreated).



DEVELOPMENT OF EXPERIMENTAL AQUAPONICS UNITS IN MYANMAR

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The country of Myanmar (Burma) has been developing and changing at a rapid rate since the military handed power back to the civilian government in 2010. With the transfer of power came deregulation and opportunity for development. Through a USAID grant in collaboration with the University of Arizona and Auburn University began a project aimed to develop a sustainable seafood industry for Burma. Working with the Myanmar Fisheries Federation, the University of Yangon and Patheingyi University as well as dozens of Burmese fish farmers, the project took a multi-faceted approach to build the industry from the ground up. While there were many projects going on within the greater scope, my role was to help students and faculty at the University of Yangon through demonstration and the building of numerous experimental aquaponics systems.

While there were obstacles in acquiring the traditional materials to build aquaponic systems, we settled on a design based on available materials. The system we went with was a floating raft system that could be built with locally available materials. No polyethylene or other plastic tanks were available, so we chose fiberglass tanks for the grow beds and fish tanks and designed biofilters with plastic trash bins and crushed clam and oyster shells donated from a nearby fish farm. Since many Burmese individuals are self-sustaining, we wanted to demonstrate that individual aquaponics systems could be built without having to order parts or materials from out of country. In addition, we wanted to allow students to demonstrate potential capabilities for aquaponics within Myanmar in comparison to hydroponics. Direct comparison between systems with and without fish will show the sustainability and potential cost savings for investment in aquaponics.

EFFECTS OF RELATIVE HUMIDITY AND ATMOSPHERIC OXYGEN ON HATCHING SUCCESS OF AMERICAN ALLIGATOR *Alligator mississippiensis*

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Alligator producers in Louisiana obtain their farm stock by collecting eggs from nests on public and private lands under a permit system managed by the Louisiana Department of Wildlife and Fisheries. Eggs are available only once per year (May-June), so it is critical that farmers maximize egg-hatching success. Some farmers maintain higher than normal oxygen concentrations during egg incubation to improve hatching success, though it is not known if this practice is effective. This study was conducted to determine the effects of relative humidity and normal (21%) oxygen vs. elevated oxygen concentration on egg hatching success.

Two-hundred-sixteen alligator eggs collected at Rockefeller Wildlife Refuge (Grand Chenier, Louisiana) were distributed among 12 incubators (18 eggs per incubator) and maintained at 31.13 ± 0.05 °C (88.0 ± 0.1 °F) for 55 days under one of four treatments involving two levels of relative humidity (RH) and two atmospheric oxygen (O_2) concentrations: (1) 80% RH and 21% O_2 ; (2) 80% RH and 28% O_2 ; (3) 93% RH and 21% O_2 ; or (4) 93% RH and 28% O_2 . Three incubators were assigned to each of the four treatments.

Relative humidity averaged $79.9 \pm 0.1\%$ and $93.5 \pm 0.4\%$ in the low humidity and high humidity treatments, respectively. Hatching success of eggs in the 93/21 and 93/28 treatments averaged $83.3 \pm 5.5\%$ with no significant difference ($P > 0.05$) between treatments (Fig. 1A). Hatching success of eggs in the 80/21 and 80/28 treatments, combined, totaled 1.8% — only two of 108 eggs in the six low-humidity incubators hatched. Body weights (Fig. 1B) and body lengths (Fig. 1C) of hatchlings in the 93/21 and 93/28 treatments also were not significantly different ($P > 0.05$).

Results indicated that 80% relative humidity reduced egg hatching success to nearly zero, regardless of atmospheric oxygen concentration, while 93% relative humidity produced good hatching success. Elevated atmospheric oxygen concentration (7% above ambient) had no effect on egg-hatching success under the conditions of this study

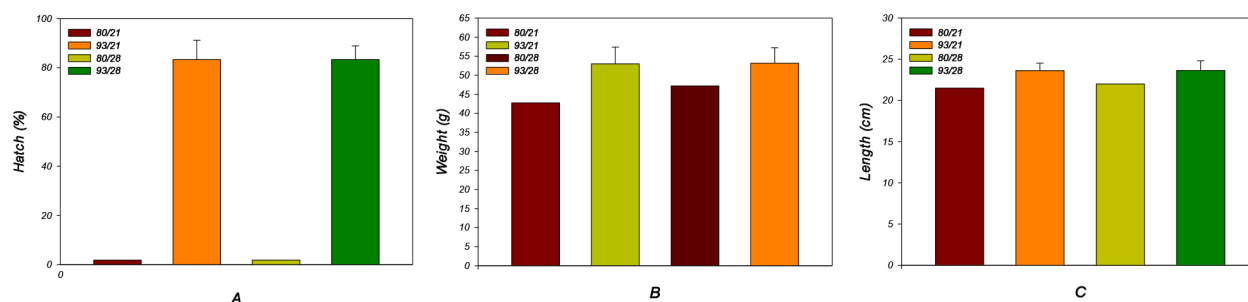


Figure 1. Percentage hatch (plot A), body weight (plot B), and body length (plot C) of alligators obtained from 216 eggs incubated at 31 °C for 55 days at two relative humidity levels (80% and 93%) and two atmospheric oxygen concentrations (21% and 28%).

PROPHYLACTIC USE OF AKUAPRO™ AND COPPER SULFATE HAS POTENTIAL TO PROTECT LARGEMOUTH BASS AGAINST COLUMNARIS DISEASE

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A research trial was initiated with largemouth bass (*Micropterus salmoides*) to test the efficacy of AkuaPro™, copper sulfate or a combination of the two to prevent columnaris outbreaks, their effects on gill histopathology and whether treatments would positively impact survival rates. Arkansas largemouth bass producers constantly combat columnaris disease, caused by *Flavobacterium columnare*. Columnaris disease is prevalent when largemouth bass fingerlings are stocked at high densities during the feed habituation process and when they are held in vats for extended time periods prior to being sold. AkuaPro™ (source: Imerys, Georgia, USA), copper sulfate and the combination of the two (copper sulfate and AkuaPro™) were tested as a prophylactic treatment against columnaris in largemouth bass. Fish were stocked at 5 fish/ L and treated with either 1 ppt AkuaPro™, 3.5 ppm copper sulfate or not treated (control) once per week or using a combination of 3.5 ppm copper sulfate on day 1 and 1 ppt AkuaPro™ on day 3. Fish samples were brought to the UAPB Lonoke Fish Disease Diagnostic Laboratory. Gills, fins, and tissues of treated and non-treated fish were examined microscopically for the presence of columnaris both before and after prophylactic treatment. Fish from all treatments were collected for histological examination. The study is currently on-going, however, preliminary data suggests fish treated with AkuaPro™, copper sulfate or their combination are experiencing fewer incidences of columnaris and have higher survival rates during feed habituation process and holding periods in vats.

BROODSTOCK COLLECTION AND CAPTIVE SPAWNING PROTOCOLS FOR ALMACO JACK *Seriola rivoliana*

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Almaco jack *Seriola rivoliana* have been identified as an important marine finfish for aquaculture development in the Gulf of Mexico Fishery Management Plan due to their fast growth, high market value, and adaptability to intensive culture conditions. These fish are also known as bar jacks, longfin yellowtail, almaco amberjack, and Kona Kampachi. The main bottlenecks associated with commercial aquaculture production include: 1) susceptibility to parasitic and bacterial pathogens; 2) an inconsistent supply of juveniles resulting from poor spawn quality and low hatchery survival. Therefore, the goals of the project included designing protocols to maintain broodstock health; identifying photo-thermal conditions needed for the maturation and spawning of high quality eggs and larvae.

In early 2017, a total of 42 adult almaco jacks were captured in the Gulf of Mexico approximately 150 km offshore and transported to Mote Aquaculture Research Park (MAP) located in Sarasota, Florida. All fish were vented and placed in square totes (working volume 760 L⁻¹) which were continuously supplied with new water from the boat's live well and pure oxygen was supplemented during the transport back to shore. A live hauler was used to transport the fish from Madeira Beach, FL to MAP. Upon arrival, fish were divided into two populations and a health management plan was initiated. During the quarantine period, the fish underwent a hypo-salinity treatment where salinities were decreased from 35 to 15 ppt over the course of 7 days. Approximately 45 days later, the salinity was increased to 35 ppt and an evaluation was conducted to confirm the successful removal of any external parasites. Fish are currently being fed a fresh frozen diet (70% thread herring and 30% squid) and maintained in two separate indoor recirculating tank systems (volume 28 m³) equipped with photo-thermal control, bio-filter, solids filter, and UV sterilization. Fish began spawning daily (volitionally) without hormone induction following the initial photo-thermal conditioning regime (26°C and 13 hr of light). To date, a total of 2.8 million eggs have been collected with a 68% mean fertilization rate. Progress regarding the development of captive maturation, spawning, and broodstock health management protocols will be discussed.

COMPARISON OF AQUI-S 20E AND MS-222 AS ANESTHETICS IN CLOWNFISH (*Amphiprion Ocellaris*)

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Clownfish (*Amphiprion ocellaris*) is a popular marine ornamental fish. Here, we studied the ability of the anesthetic Aqui-S 20E to induce anesthesia in the marine clownfish. We monitored post-recovery behavioral changes, including food intake. Aqui-S 20E is a clove-oil derived anesthetic currently pending FDA approval for use in the US aquaculture industry. We compared AQUI-S 20E at the recommended dosage of 220mg/l and MS-222 at a dosage of 75mg/L. The induction time and recovery time were recorded and post-recovery behavior was monitored for 24 hours after the fish was returned to its holding tank. Neither anesthetic had a negative effect on food intake the day following anesthesia. Both anesthetics were effective in inducing anesthesia at the recommended dosage. Recovery with Aqui-S 20E was also slower than with MS-222 (5.71 min vs. 2.01 min). Aqui-S 20E is an effective alternative to MS-222 as a fish anesthetic.

PARTICLE DEPOSITION ANALYSIS TO INFORM ENVIRONMENTAL MODELING FOR RED DRUM AQUACULTURE IN THE GULF OF MEXICO

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Environmental modeling of ocean net-pen aquaculture is driven by predicting how solid materials fall through the water column to the seafloor. Model parameters of sequential ocean flow vectors and the mapping of the benthos all contribute to calculating where the falling solids stop moving and interact with seafloor. One crucial model input parameter is the velocity at which fecal materials fall through the water column by gravity. Salmon fecal settling velocities, density, and shape factor drag coefficient have been investigated on multiple occasions and are the default values in major net pen models since salmon producers are the primary model consumers. It is anticipated that net pen aquaculture will expand into the Gulf of Mexico cultivating warmer water species than salmon such as red drum. We are investigating red drum fecal settling velocities and mass fractions using various feed types and fish sizes to better understand if and how settlement behaviors are different from salmon. To do so, we have constructed a variable speed flume apparatus capable of measuring settling velocities. Here, we present the first data from the fecal settling velocity trials and provide preliminary comparisons to salmon values.

CHANGES IN TEMPERATURE, SALINITY, AND PH AFFECT GROWTH AND ECDYSONE LEVEL IN MARINE MYSID

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The opossum shrimp, *Neomysis awatschensis* (Crustacea: Mysidae) is a small crustacean native to coastal regions of South Korea. In this study, we measured in vivo ecdysone (i.e. 20-hydroxyecdysone) levels by employing competitive Enzyme Immunoassay (EIA) method from 10 developmental stages which are comprised by embryo, nauplioid, postnauplioid, and adult stage. Furthermore we analyzed potential correlation between environmental fluctuations (i.e. changes in temperature, salinity, and pH) and subsequent modulations in ecdysone levels as well as morphological parameters. Briefly, the ecdysone contents were drastically increased from late nauplioid to postnauplioid stages. The highest temperature treated (25°C) significantly increased growth parameters and ecdysone content, while mortality slightly increased compared to the 20°C-exposed mysids. In the case of salinity, both growth parameters and ecdysone content showed high levels in the 30 psu-treated mysids. Significant low levels in survival, growth parameters, and ecdysone content were observed in the groups treated with relatively low salinity (e.g. 5, 10, and 15 psu). When mysids exposed to different pH conditions, low levels in survival, growth parameters, ecdysone contents were observed in pH 7.0-exposed mysids. Our results suggest that ecdysone level is crucial for mysid growth and the levels are strongly associated with culture conditions in *N. awatschensis*.

EVALUATION OF THE REPRODUCTION OF *Neocaridina davidi* FED WITH PRACTICAL AND COMMERCIAL FEEDS

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Freshwater prawns of the genus *Neocaridinas* are the most indicated for those who want to begin producing ornamental shrimp. The present study evaluated the reproduction of the Red Cherry, *Neocaridina davidi*, fed with practical diets and commercial feeds. A total of 96 animals were used, subdivided into 12 aquariums, and after one week of acclimatization began to receive commercial feed with 35% CP (T1), 55% CP (T2) 50% CP (T3), and practical diets created from cooked vegetables 3% CP (T4). At the end of the experiment, the first reproduction, number of eggs produced, offspring and survival were evaluated. The results demonstrate that the first reproductions occurred in the T2 treatment, which also showed the highest fertility and the highest survival. Although the T4 treatment did not present problems with growth and weight gain, this treatment did not reproduce during the experimental period. The results indicate that the practical diet used by hobbyists can be adopted as a dietary supplement, but it does not replace animal protein. Balanced and proper feeds for juveniles of marine shrimps help to maintain the broodstock, promote good survival and a favorable number of offspring.

PRODUCTIVE PERFORMANCE OF THE PRAWN *Macrobrachium rosenbergii* IN A RECIRCULATING SYSTEM IN DIFFERENT DENSITIES

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The freshwater prawn *Macrobrachium rosenbergii*, has been the subject of several studies on its biology, making it economically possible and feasible for cultivation. This species is the most used in shrimp aquaculture projects worldwide, being the only one commercially farmed in Brazil. The objective of the present study was to analyze the weight gain and survival of *Macrobrachium rosenbergii* in different stocking densities. The prawns were reared in a recirculation system and subjected to four treatments (T1, T2, T3 and T4), each with six replicates. The densities used were 50, 100, 150 and 200 prawns/m², respectively. The initial mean weight used was 0.10521g. The results indicate that the T3 treatment presented the best result in relation to survival, however, the T1 treatment had the best weight gain with a final mean weight of 1.44 ± 0.18g. The data show that the survival rate above 65% supports further studies to improve the cultivation of *M. rosenbergii* in the recirculation system.

PERFORMANCE ANALYSIS OF GLOBAL VALUE CHAINS: A PROPOSAL OF INDICATORS FOR AQUACULTURE IN BRAZIL

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Tilapia is the most farmed fish in Brazil, with several poles of production dispersed in all regions of the country, each presenting significant differences in terms of production volume, processing and input structure, governance and division of value added between the agents. Therefore, the research purpose is to understand what factors influence for such differentiation of economic results between different poles of production and their respective value chains.

Based on the Global Value Chain (GVC) approach, this research aims to define a set of indicators and then to construct an index capable of comparing the performance of different tilapia production zones, with reference to the six dimensions of the CGV. In spite of making possible a holistic analysis, the six dimensions of the GVC (i.e. Input-output structure; Governance; Upgrading; Local institutional context; Industry stakeholders) are essentially based on qualitative and subjective data, which makes difficult comparisons between different value chains. Thus, the first challenge of this research is to develop indicators that correspond to these dimensions of the GVC analysis. From these indicators, mostly qualitative, will be elaborated a quantitative index capable of measuring the performance and/or potential performance of the production zones of tilapia and their respective agents.

The research methodology predicts the use of fuzzy logic in order to mathematically translate the qualitative /subjective information that will be collected in the field with the value chain agents and consequently to consolidate a quantitative index that is able to compare producers and value chains. As a result, it will be possible to identify the factors that influence the economic performance of producers and their value chains. In addition, this model of performance analysis of global value chains could be used in other aquaculture chains or even in other economic sectors, supporting strategic orientation at the level of public policies or of companies and producers.

STATE OF SCIENCE ASSESSMENT: HOW FARMING SHELLFISH IMPACTS SUBMERGED AQUATIC VEGETATION

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Americans have developed what seems to be an insatiable appetite for oysters in recent years and the aquaculture industry has taken notice. In response to market opportunity, well-established oyster farms are expanding operations alongside an array of entrepreneurial growers. Many state and federal regulatory agencies are seeing a significant increase in applications for shellfish farming permits or leases. When planning for and managing aquaculture-industry development, coastal managers and regulators must weigh environmental benefits and costs along with a growing number of other activities within the coastal zone. One such conflict that is often addressed in regulatory review includes the interactions between shellfish aquaculture and submerged aquatic vegetation (SAV). Due to the importance of SAV as a protected resource and essential fish habitat, 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. This presentation will provide an update on the status of a national state-of-science assessment on shellfish aquaculture interactions with SAV, findings thus far, and a timeline for completion.

POND CULTURE OF THE ENDANGERED PUGNOSE SHINER (*Notropis anogenus*)

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Pugnose Shiners are endangered in New York State and Canada and threatened in Minnesota and Wisconsin. This project was initiated by the Fisheries & Aquaculture Program of the State University New York (SUNY) to demonstrate the feasibility of utilizing pond culture techniques to produce Pugnose Shiners for restoration purposes. The New York State Department of Environmental Conservation, and the U.S. Fish & Wildlife Service were partners in these studies.

This project was initiated in the spring of 2015, following field studies and disease certification of Pugnose Shiners collected from Sodus Bay, Lake Ontario. Sodus Bay shiners were utilized for brood-stock, because they are the last remaining New York “lake” population of Pugnose Shiners, which are genetically distinct from the St. Lawrence River population.

On 24 June 2015, 65 adult brood-stock Pugnose Shiners were placed into a .57 acre pond within the SUNY Cobleskill Experimental Pond Complex. The pond had a maximum depth of 2.95 m and was vegetated with aquatic plants similar to the collection site in Sodus Bay (filamentous algae, curly pondweed, water milfoil, broad-leaved pondweed, and common stonewort). Maximum summer temperature was in the low 20’s °C, pH 6.2-6.7, and conductivity ranged from 241-245.

Stocked Pugnose Shiners spawned their first summer producing hundreds of juveniles. By December 2015, the pond cultured population was 41 mm long, just 1 mm shorter than the spawning population of Pugnose Shiners collected from Sodus Bay in May. Growth was reduced during the first year of production (spring 2016-2017), as the pond reached its carrying capacity. The average size of Pugnose Shiners harvested on 25 April 2017 was 30.4 mm, 12 mm shorter than the average size harvested the previous spring.

During the first year of production, 7,400 pond reared Pugnose Shiners were harvested and stocked into Chaumont Bay, Lake Ontario. Harvest of pond reared Pugnose Shiners will increase over subsequent years, so that the size of fish stocked is closer to spawning size.

This project demonstrated the feasibility of pond culture of Pugnose Shiners, and its potential for producing thousands of juveniles a year for restoring extirpated wild populations. Culture techniques refined by this project can be used to restore Pugnose Shiner populations in New York, Minnesota, Wisconsin and Canada.

ON THE DRIVERS OF COST CHANGES IN THE NORWEGIAN SALMON AQUACULTURE SECTOR: A DECOMPOSITION OF A FLEXIBLE COST FUNCTION FROM 2001 TO 2014

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One of the main success factors behind the growth of salmon aquaculture has been productivity growth and cost reductions (Asche, Guttormsen, & Rasmus, 2013). However, the industry has now entered a more mature stage where further cost reductions appears to be more difficult to achieve. To investigate to what degree this is the case, this paper investigates cost changes in the Norwegian Salmon aquaculture sector using a panel data set of salmon firms. Particularly, it explores how cost changes can be attributable to scale economies, technical change, production expansion, and input prices. Moreover, technical change can be separated into four different components: Pure technical change, non-neutral technical change, scale augmentation effect, and capital augmentation effect. We aim to decompose the drivers behind cost changes in the industry for the period 2001-2014. We applied a restricted translog cost function with a flexible technical change structure that allow us to estimate technical change on a yearly basis with the objective to identify patterns and trends in cost behavior.

Our estimations identify three main drivers on costs changes: First, feed prices seems to drive the increase in costs. We found that feed demand is highly inelastic and present low substitutability leaving producers with low adjustment possibilities to feed price shocks. Second, there is presence of technical regress with a significative impact on cost behavior. Furthermore, the decomposition of technical regress allow us to estimate that more than 90% of technical regress is explained by its pure component. This result indicates that technical regress is neither affected by availability of inputs or production expansion but by external factors that are not explicitly incorporated in the cost equation. Third, the increase of other costs that are not directly associated with production inputs suggest that that external negative conditions, such disease spreads have become a main source of inefficiency for the industry. This may be an indicator that after all, aquaculture is still a relatively young industry with several challenges to overcome.

References

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HISTOPATHOLOGICAL LESIONS OF THE ISOPOD *Riggia puyensis* Rodríguez-Haro et. al. 2016, ON THE ARMORED CATFISH *Chaetostoma breve* AND *Chaetostoma microps* (Siluriformes: Loricariidae) FROM ECUADOR

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Chaetostoma Steindachner is a loricariid genus of suckermouth armored catfish from Central and South America. In a survey on parasites of fish from Ecuador an isopod belongs to Cymothoidae was found. Fishes were collected using a cast net in the Puyo and Bobonaza Rivers (Province of Pastaza, an Amazonian region of Ecuador). Eighty-six individuals of *Chaetostoma breve* and 122 of *Chaetostoma microps* were inspected by the naked eye, and only the parasitized were carried to the laboratory for examination. Each of the parasitized fish has a single female isopod encapsulated in the abdominal cavity of the host and maintaining an opening towards the outside through a small pore. The aim of this study was to analyze the damage that *Riggia puyensis* inflicts on its hosts through the evaluation of histological sections. For this, infected fish were fixed by immersion in 10% buffered formalin, and processed for histological studies. The samples were sectioned at 3-4 µm and subsequently stained by the Hematoxylin-Eosin and Masson's trichrome. Histological examination revealed local hypertrophia in the skin due to irritation caused by the parasite to the small pore level. In the epidermis, pyknotic cells were observed and loss of the normal architecture with rupture in some sectors of the epithelium. Also, an infiltration of numerous inflammatory cells, mainly lymphocytes, and macrophages, was detected as part of the host's inflammatory response. In the site where the isopod is supported, atrophy in the epithelium of the abdominal cavity wall was observed. Evaluation of histological sections by light microscopy showed that alterations in the abdominal cavity were consistent with a chronic process.

ROLE OF INTRACELLULAR Ca^{2+} SIGNALING IN THE REGULATION OF MOLTING IN THE BLUE CRAB *Callinectes sapidus*

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The soft crab industry is limited by a supply of pre-molt crabs that is both seasonal and unpredictable. Controlled induction of molting could provide an abundant and predictable supply of soft-shelled crabs, a benefit to the soft crab industry and to consumers. Development of such a method is dependent on understanding of the endocrine mechanisms that regulate natural molting. Cycles of growth and molting in crustaceans are triggered by cholesterol-derived molting hormones (ecdysteroids) released from paired endocrine glands (the Y-organs) located in the anterior cephalothorax. During much of the molting cycle, the levels of ecdysteroids in hemolymph are kept low by the action of a peptide molt-inhibiting hormone (MIH), produced in the eyestalks. While the removal of MIH suppression during pre-molt coincides with increased ecdysteroidogenesis, there is evidence that an additional positive stimulus in the form of an intracellular Ca^{2+} signal also plays a significant role. To better understand Ca^{2+} signaling in Y-organs, our lab investigated the proteins involved in regulation of intracellular Ca^{2+} . These include Ca^{2+} pumps, e.g., plasma membrane calcium ATPases (PMCAs) and sarco/endoplasmic reticulum calcium ATPases (SERCAs). We used a PCR-based cloning strategy (RT-PCR followed by 3'- and 5'-RACE) to clone a full-length cDNA encoding a putative SERCA protein from the Y-organs of the blue crab (*Callinectes sapidus*). SERCA transcript levels in Y-organs were then determined using quantitative PCR. Transcript abundance was assessed after Y-organs were activated by eyestalk ablation, and throughout a natural molting cycle. The results are consistent with the hypothesis that stage-specific changes in SERCA expression occur in response to increased intracellular Ca^{2+} and are not a causative factor in promoting ecdysteroidogenesis. Identification of the stimulus that drives the increase in intracellular Ca^{2+} is critical to an understanding of cellular mechanisms that regulate ecdysteroidogenesis in crustacean molting glands, and may provide insights that allow development of a method for controlled induction of molting.

FERMENTING RICE BRAN AS A CARBON SOURCE FOR BIOFLOC TECHNOLOGY SYSTEMS IMPROVED WATER QUALITY AND AFRICAN CATFISH, *Clarias gariepinus*, JUVENILE PRODUCTION

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Biofloc technology (BFT) is an in situ water management strategy that relies on exogenous carbon source additions, such as sugars, glycerol or starch, to increase the carbon to nitrogen (C/N) ratio that favors heterotrophic bacterial growth. These bacteria convert otherwise toxic nitrogen into potentially consumable biomass. While starches are relatively inexpensive and readily available, their low water solubility can delay the production of bioflocs, thus leading to water quality deteriorations. A potential solution could be degrading the non-starch polysachharide content. Moreover, it is known that more carnivorous speceis, such as African catfish (*Clarias gariepinus*) are poor collectors and consumers of bioflocs, but starches may produce larger bioflocs that may be more easily consumed that could lead to better growth.

A 42-day study was carried out to examine the effects of raw (RRB), enzymatically treated (EnRB) or fermented rice bran (FerRB), in a biofloc-based system housing *C. gariepinus* juveniles (9.6 g). The enzyme used in the EnRB and FerRB treatments was a proprietary product (WHITE CAP™; Baxel Co, Ltd., Thailand). Water quality, biofloc production/proximate composition, and subsequent effects to growth, feeding efficiencies, body proximate composition, and liver histopathology to triplicate groups were measured. A control treatment was a static system.

The FerRB had higher crude protein, water solubility and total soluble sugars as well as substaintially reduced crude fiber than RRB. Biofloc productivity were similar among biofloc treatments, but using FerRB significantly increased biofloc crude protein and ash while causing the lowest nitrogenous waste levels. Catfish growth and whole-body crude protein was significantly higher with FerRB compared to the control, indicating some biofloc consumption.

Previous reports showed that regardless of the carbon source that included sucrose, molasses, glycerol or rice bran, BFT led to no growth improvement in *C. gariepinus*, likely due to this fish being inefficient biofloc collectors/consumers. This is the first study demonstrating BFT can improve catfish growth, likely due to the enhanced solubility of rice bran possibly increasing biofloc size to hence facilitate consumption. Research should continue if this protocol can similarly apply to other carnivores.

Table 1: Growth performance, feeding efficiencceis and survival of catfish reared with biofloc technology with differently treated rice bran or the control after 42 days.

Parameter	Control	RRB	EnRB	FerRB
SGR (%/day)	2.42 ± 0.10 ^b	2.58 ± 0.05 ^{ab}	2.27 ± 0.20 ^b	2.85 ± 0.08 ^a
FCR	0.99 ± 0.05 ^{ab}	0.92 ± 0.07 ^{ab}	1.10 ± 0.13 ^b	0.82 ± 0.03 ^a
PER	2.36 ± 0.12 ^b	2.53 ± 0.04 ^{ab}	2.17 ± 0.23 ^b	2.85 ± 0.10 ^a
Survival (%)	96.00 ± 2.31	94.67 ± 1.33	90.67 ± 2.67	100.00 ± 0.00

DETECTING PISCIVOROUS BIRDS FROM AQUACULTURE OPERATIONS

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The cost of bird damage to aquaculture is hard to quantify. In addition to predation, fish stock might be related to cultural, mechanical, or environmental elements. The costs of control, however, are becoming available from grower surveys, and estimates of loss can be projected from food habit studies. The damage to aquaculture in Europe and North America, based on average food intake of fish-eating birds, are tens of millions of dollars per year; and in Israel, a few millions of dollars per year. Adding to the direct damage from predation, there is a potential spread of disease by birds. However, there is not sufficient information because the complexity of the different facilities and their setting of the average aquaculture operation that prevent a controlled disease studies. Taking it all together, the damage caused by these fish-eating birds can cause the Israeli pond-base aquaculture sector to crash.

As a result of tens of years of urbanization while drying large areas or shallow water bodies, causing the loss of natural habitats for the fish-eating birds, the aquaculture earthen ponds became a rescue haven for the migrating birds. Meanwhile, as a result of people awareness and the increasing value of the bird-watcher tourism there is a significant increase in the fish-eating birds' populations. Israel's unique location on the path of global bird migration between the Europe and Africa intensifies the conflict between aquaculture and fish-eating birds because in the transition seasons the population of fish-eaters has increased significantly.

Many ways to deal with the fish-eater birds were tried, both in Israel and in the world by a) prevention such as nets and cable stretched over the fish ponds, which are costly; b) direct human presence by ground harassment with vehicle patrols, dogs, destroying nesting colonies and disrupting birds at their resting sites during the day and night; c) using devices such as reflecting tapes, lights, eyespot balloons, scarecrows made of hunting birds, pop-up scarecrows with exploders, water spray devices, pyrotechnics, automatic exploders, alarm or distress calls,. These methods, most of the time, had only a short-term success. With the time, the use of traps and firearms has become illegal as a result of bird-watchers and public pressuring the governments to protect the birds.

Therefore, it became obvious that more research needs to be done, for example, the necessity to consider aspects such as the species, species and size of the fish, the size and depth of the pool, and the environment. More studies have been carried out in an attempt to implement technologies for the removal of harmful birds and advanced means of observation and monitoring to cope with the habituation of birds. These systems are based on computing technologies, image processing, and robotics. A review of the latest use of these advanced technologies that was introduced lately to the aquaculture industry in Israel will be presented.

RENEWABLE ENERGY FOR OFFSHORE AQUACULTURE

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Currently, there are no commercial finfish operations in U.S. federal waters, located between 3 and 200 miles (5 to 322 kilometers) offshore. Offshore aquaculture farms must have a reliable supply of energy. The global market for offshore wind energy is rooted in Europe. Even though Europe still dominates the market with 90% of global installed capacity at the end of 2016, Asia significantly increased its offshore deployment in 2015 and 2016. European experience and market data indicate progress in offshore wind technological innovation and market cost reduction. Data from 2017 are projections from the development pipeline of announced projects already under construction that indicate likely deployment levels of over 4,000 MW. This forecast includes more than 1,000 MW in both Germany and China, as well as more than 700 MW in the United Kingdom. Industry-wide confidence that the U.S. offshore wind market is emerging has increased because of decreasing global costs and stronger state policy commitments. The big story in U.S. maritime wind power this year was the start of commercial operations with its first offshore wind project, the Block Island Wind Farm with 30-MW BIWF. Thus, currently, the market in the U.S. developed increasing investment activity, as the U.S. States along the East Coast have advocated supporting offshore renewable wind energy development along their shores. Thus, the developers submitted proposals with the hope to increase the growth of such initiative. Therefore, in 2018, we expect to see a significant growth of the offshore segment beyond the state-level policies while also hoping to see the emergence of innovative partnerships amongst offshore wind, oil and gas companies seeking to gain from the technology that each brings to the table.

While the wind was gusting and solar shone over the last three decades, wave and tidal power made only a small splash. Now they could be the new wave of renewables, as ocean energy projects are being floated globally. Some scientists argue that even if using only one percent of Japan's ocean is capable of generating close to 10GW, which is similar to the energy provided by ten nuclear power plants. It seems that if offshore renewable energy operations are operating around the globe, they could generate enough power to meet global electricity needs. In 2013, there were about 100 pilot wave energy projects worldwide, experimenting with different methods and tools to capture and convert energy from the water. Now, marine generated power is gearing up to go commercial. In the United Kingdom, wave and tidal energy have the potential to deliver twenty percent of their electricity needs at a 30-50GW installed capacity. For example, Marine Power Systems (MPS), a wave power technology company based in Wales, UK, has unveiled during October 2017 its quarter-scale, prototype WaveSub wave energy generator, marking its move to a new, sea-based phase of its development.

EFFECT OF IRON-AMINO ACID CHELATE SUPPLEMENTED FISH FEEDS ON GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) IN AN AQUAPONIC SYSTEM IN KENYA

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Aquaponics is an environmentally friendly production system involving reuse of waste and nutrients in production of fish and vegetables. This study investigated the effect of iron bis-glycine supplement in fish feeds on growth performance of mono-sex Nile tilapia (*Oreochromis niloticus*) and spinach (*Spinacia oleracea*) in a small aquaponic system. The study was conducted at the University of Eldoret for 119 days. Complete randomized design was used. Supplementation rates in fish diets constituted 30g Fe kg⁻¹, 20g Fe kg⁻¹, 10g Fe kg⁻¹ and 0g Fe kg⁻¹ respectively. Nile tilapia fry with a mean weight of 0.475 ± 0.025g were stocked in 12 aquaria in an aquaponic system. The 30g Fe kg⁻¹ treatment resulted in the highest fish growth performance with final weights of 11.606 ± 0.55g, an SGR of 2.516 ± 0.01 and a good FCR of 1.10 ± 0.107 compared to the 0g Fe kg⁻¹ treatment that exhibited the lowest mean weight of 4.354 ± 0.295g, an SGR of 1.744 ± 0.02 and a higher FCR of 2.081 ± 0.797. In the carcass composition analysis, 30g Fe kg⁻¹ treatment exhibited a significant difference ($p < 0.05$) for higher ash content, crude protein and lower crude lipids (16.350 ± 0.03%, 65.607 ± 0.74% and 12.20 ± 0.256%, respectively) while treatment 0g Fe kg⁻¹ showed lowest ash content, crude protein and crude lipids (10.59 ± 2.12%, 59.671 ± 0.676% and 18.20 ± 0.465% respectively). The hemoglobin and hematocrit levels were higher at 30g Fe kg⁻¹ (45.090 ± 0.704 % and 15.630 ± 0.935g dL⁻¹) and lower at 0g Fe kg⁻¹ treatment (29.773 ± 0.213% and 9.9244 ± 0.071g dL⁻¹). We recorded a decrease in glucose levels with increased supplementation of iron amino acids chelates. The 0g Fe kg⁻¹ demonstrated higher glucose levels (26.8 ± 0.0289mg dL⁻¹) and lower levels at 30g Fe kg⁻¹ (13.433 ± 0.169 mg dL⁻¹). These results revealed that 30g Fe kg⁻¹ iron amino acid chelate supplementation had better nutritional attributes as feedstuff for *O. niloticus* growth than the two other dietary treatments. Iron amino acid chelate supplementation at appropriate levels of concentration may be desirable in complete diet formulations for enhancement of physiological efficiency and accumulation of body proteins for growth efficiency. The study recommends the incorporation of iron amino acid chelate in on-farm formulated diets where complete diets are not easily accessible for small scale farmers.

CULTURING TILAPIA IN GREENHOUSE-ENCLOSED INTENSIVE RECIRCULATING AQUACULTURE SYSTEM IN BRITISH COLUMBIA, CANADA

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Warm-water tilapia (*Oreochromis niloticus*) is becoming an important commercial freshwater fish in Canada. Viva Aquaculture and Seafood Distribution Ltd. (“Viva”) in collaboration with The University of British Columbia (UBC) has engaged in sustainable tilapia aquaculture production to supply the strong market demand in Greater Vancouver Area in British Columbia, Canada. To overcome technological uncertainties which cannot be removed using standard practice and to develop a tilapia aquaculture system suitable in a Canadian climate, the general project objective is to intensify production of healthy tilapia in sustainable, environment-friendly, and in a less expensive way in greenhouse-enclosed land-based facility without affecting the surrounding environment by generating “zero waste”. The research team in collaboration with the aquaculture industry has been developing sustainable tilapia aquaculture system in cold Canadian climate using biological principles. Beneficial nitrifying bacteria are used to convert toxic nitrogenous wastes into non-harmful compounds, which are absorbed by selected aquatic vascular plant and microalgae. Innovative bio-engineering design recirculates clean water and conserves heat to maintain suitable condition for tilapia culture. “Zero waste” system is being developed to make fish production more sustainable, profitable, and environment-friendly. Initial research results have shown encouraging outcomes during the first year of project implementation. It is expected that the innovations will lead to lower production cost, increase economic benefits, improve fish quality, and make the aquaculture production of live tilapia for the market more sustainable and environment-friendly.

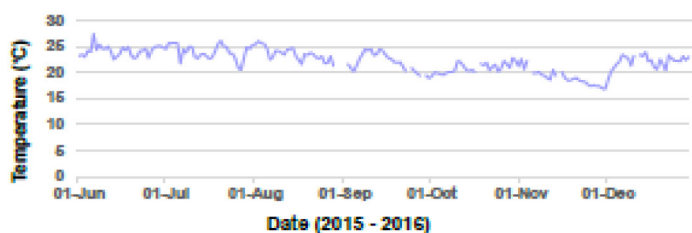


Fig. 1. Temperature fluctuation in culture tanks.

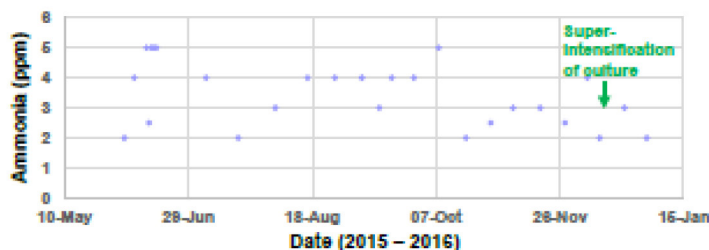


Fig.2 Ammonia fluctuation in culture tanks.



Fig. 3. 30,000 L Culture Tank

EVALUATING THE ECONOMIC FEASIBILITY OF PIGFISH (*Orthopristis chrysoptera*) AQUACULTURE FOR THE TEXAS BAITFISH MARKET

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In 2014, marine recreational angling generated approximately \$1.3 billion in sales and 11,000 jobs in the state of Texas. From 2013 to 2016 the average annual harvest of marine species for bait in Texas was 1.27 million pounds of bait (live-weight) worth approximately \$5.25 million dockside. Aquaculture of marine baitfish for recreational fishing purposes has the potential to both decrease pressure on baitfish stocks by decreasing the need for wild harvest and enhance working waterfronts through the development of new businesses and jobs. Pigfish (*Orthopristis chrysoptera*) possess a number of traits that make the species an ideal candidate for baitfish aquaculture. Pigfish mature at a small size (20-35 cm in length) so breeding stock can be maintained in smaller tanks. The species is tolerant of crowding, handling, and varying environmental conditions. In addition, pigfish is already a popular live bait choice for Texas anglers. In this research, we examine the economic feasibility of pigfish aquaculture for the Texas marine baitfish market.

The analysis evaluates both production costs and market demand for baitfish in Texas. The production analysis incorporates the results of research being conducted at both the University of Texas Marine Science Institute (spawning and rearing) and at a commercial aquaculture facility in Texas (grow out). The physical research examined: broodstock feeding protocols for optimizing egg production and quality, optimal rearing temperature for larvae, optimal water conditions for survival and growth of larvae, and optimal stocking densities for outdoor grow-out to market size. Data from the research trials will be used to estimate production and transport costs associated with potential commercial operations. Market demand for baitfish in Texas will be estimated using Texas Parks and Wildlife Department trip ticket data from 2009 to 2016. The trip ticket data provides information on the species, quantity, price, and timing of bait fish purchases by Texas bait dealers. This data will be combined with the production data to estimate the potential profitability of commercial scale pigfish aquaculture in Texas. In addition, Monte Carlo simulations will be used to evaluate the robustness of profitability estimates to changes in survival and growth rates, key input costs, and output price.

SELECTIVE PRODUCTION OF C₁₀ HYDROCARBONS BY MICROPLANTLET SUSPENSION CULTURES OF THE MACROPHYTIC MARINE RED ALGA *Ochtodes secundiramea*

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This study demonstrated the selective production of a single C₁₀ hydrocarbon, the acyclic monoterpene b-myrcene, by photosynthetic, clonal microplantlet suspension cultures of the macrophytic red alga *Ochtodes secundiramea* (Cryptonemiales, Rhizophyllidaceae). Microplantlets were cultivated within an airlift photobioreactor under continuous medium perfusion with enhanced nutrients (nitrate, phosphate) and 3500 ppm CO₂ in the aeration gas to provide nutrient-replete conditions. The removal of bromide (Br⁻) and vanadate (VO₄³⁻) from the medium shut down bromoperoxidase-catalyzed halogenation, so that only the common precursor to halogenated monoterpenes, b-myrcene, accumulated within the biomass. Nutrient-replete cultivation enabled near-exponential biomass production to density of 6 g DW/L, with specific growth rate of 5% per day during the 36 day cultivation period at near-saturation light intensity. Myrcene was produced during biomass production, and the specific rate of b-myrcene production within the biomass exceeded the specific rate of biomass production, so that b-myrcene accumulated within the biomass. Increasing the mean light intensity from approximately 60 to 95% of light saturation linearly increased the growth rate, but did not increase the specific rate of b-myrcene production. At near saturation light intensity, the specific rate of b-myrcene production was 7% per day with yield of 40 mmol/g DW after 36 days. Although the yields of b-myrcene were low (0.03 wt% in biomass), the potential for high biomass density, ease of microplantlet separation, and high selectivity of hydrocarbon biosynthesis suggest that microplantlets of terpenoid-producing red macroalgae have promise as a new platform for advanced hydrocarbon biofuel production from CO₂, provided new strategies can be developed to enhance monoterpene yield.

EFFECT OF DIETARY PREBIOTICS ON BIOFLOC BACTERIAL PROFILE AND PACIFIC WHITE SHRIMP *Litopenaeus vannamei* PRODUCTION AND HEALTH

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Prebiotics are generally regarded as safe compounds that confer beneficial effects on intestinal bacteria of cultured animals when provided as dietary additives. However, the effect of prebiotics on bacteria of biofloc particles (BFP), as well as their effect on production and health of Pacific white shrimp *Litopenaeus vannamei* cultured in a biofloc technology system (BFT), a system that relies highly on bacteria composition, needs to be further examined.

A 31-day feeding trial was performed to evaluate the effects of dietary inclusion of the prebiotics fructooligosaccharide (FOS), galactooligosaccharide (GOS), inulin (INU) and mannan-oligosaccharide (MOS) and the non-prebiotic water-soluble disaccharide sucrose (SUC) on the bacterial profile of BFP and *L. vannamei* production and health, as indicated by the total hemocyte count (THC). Shrimp weighing 2.0 ± 0.5 g were stocked into each tank (0.457 m x 0.457 m x 0.280 m) at a density of 344 shrimp/m³. Tanks containing an independent heater, an automatic 48-h feeder, and two air stones were filled to 20-cm depth with 28 ppt seawater at $30.0 \pm 1.0^\circ\text{C}$. Each tank was maintained as an individual BFT with two phases. During first phase (day 0-7) and during the second phase (day 8-31), autotrophic and heterotrophic bacteria dominance were promoted, respectively. Only during the second phase, shrimp were fed with 23% protein content experimental diets containing a 3% inclusion level of either FOS, GOS, INU, MOS or SUC. A 23% protein content control diet (CTL) with no additive inclusion was also used only during the second phase. Each diet was provided to each tank according to its treatment in replicates of 4 tanks per treatment.

The bacterial profile comparison, based on denaturing gradient gel electrophoresis, of the dietary treatments and a BFP sample collected on day 6 (autotrophic bacteria dominance; ATr), showed that the ATr and CTL were different from each other, they were different from the prebiotics and SUC treatments, and the SUC and prebiotics treatments were similar (Figure 1). Mean weight gain, survival, feed conversion ratio (FCR) and biofloc level showed no significant ($p \leq 0.05$) differences among treatments (Table 1). A significant ($p \leq 0.05$) increase was observed in the THC of shrimp fed diets containing MOS (Table 1).

In conclusion, the dietary prebiotics and SUC changed the bacterial communities present in BFP. The bacterial communities of the prebiotic and SUC treatments were similar probably because these additives were similarly available to the bacteria. The higher THC of shrimp fed the MOS treatment is a promising result that needs to be evaluated in shrimp subjected to stress conditions.

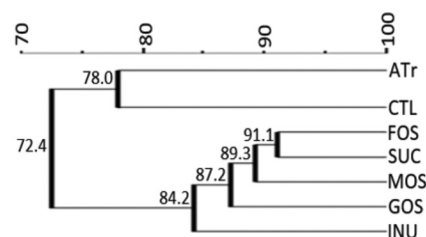


Figure 1. Bacterial profile dendrogram of the BFP collected during ATr and from the different dietary treatments.

Table 1. Weight gain, survival, feed conversion ratio, biofloc level and hemocyte count of shrimp fed the control and experimental diets.

Treatment	Weight gain (%)	Survival (%)	FCR	Biofloc (mL/L)	THC*
Initial	-	-	-	-	1.3 ^b
CTL	216	95.9	1.3	10.4	2.1 ^{ab}
FOS	202	96.3	1.3	11.4	1.8 ^{ab}
GOS	187	97.5	1.5	9.9	2.2 ^{ab}
INU	200	98.8	1.4	9.6	2.8 ^{ab}
MOS	242	100	1.2	9.2	3.1 ^a
SUC	218	96.3	1.3	11.7	1.8 ^{ab}

* Different letters indicate significant ($P < 0.05$) differences according to Tukey's HSD.

DIETARY METHIONINE REQUIREMENT OF LARGEMOUTH BASS *Micropterus salmoides* L.

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The use of alternative protein sources in aquafeed formulations increased faster than amino acid requirements have been defined for most cultured fish species. Methionine can be the first limiting amino acid in alternative-protein based diets and its deficiency leads to reduced fish performance and increased nutrient load into receiving waters per unit mass of fish produced. Production of largemouth bass (LMB) for food-fish markets has increased but information on amino acid requirements for this species remains limited. Thus, we conducted a 9-week feeding trial to assess the dietary methionine requirement of LMB.

A semi-purified basal (Basal) diet was formulated to contain 40% crude protein (CP), 12% lipid, 0.5 g/100 L-methionine (Met) and 0.23 g/100g L-cysteine (Cys). Crystalline Met was supplemented to the Basal diet in additive amounts of 0.15 g/100g originating six additional diets with Met ranging from 0.65 to 1.4 g/100g. Each diet was fed twice daily to apparent satiation to triplicate groups of 20 LMB juveniles (5.9 g/fish initial weight) stocked in 110-L glass aquaria operating as a recirculating system. Water quality was maintained within acceptable ranges for LMB and a 12h photoperiod was maintained using fluorescent lights controlled by timers. Fish in each aquarium were group-weighted at 5 and 9 weeks of feeding.

Survival of LMB fed the Basal diet was 83.3% and that of fish fed the other diets ranged from 90 to 98.3%. A four-parameter saturation kinetics model (SKM) was used to fit the resulting data from the feeding trial and the dietary Met concentration producing 95% of maximum theoretical response was depicted as the requirement. In the presence of 0.23 g/100g Cys, the dietary Met requirement for 95% of maximum weight gain and FE of LMB was estimated to be 0.77 and 0.74 g/100g (Fig. 1), corresponding to 1.83 and 1.76 g/100 of dietary CP, respectively. The dietary Met requirement value determined in this study will aid in the formulation of nutritionally-balanced and cost-effective diets for LMB.

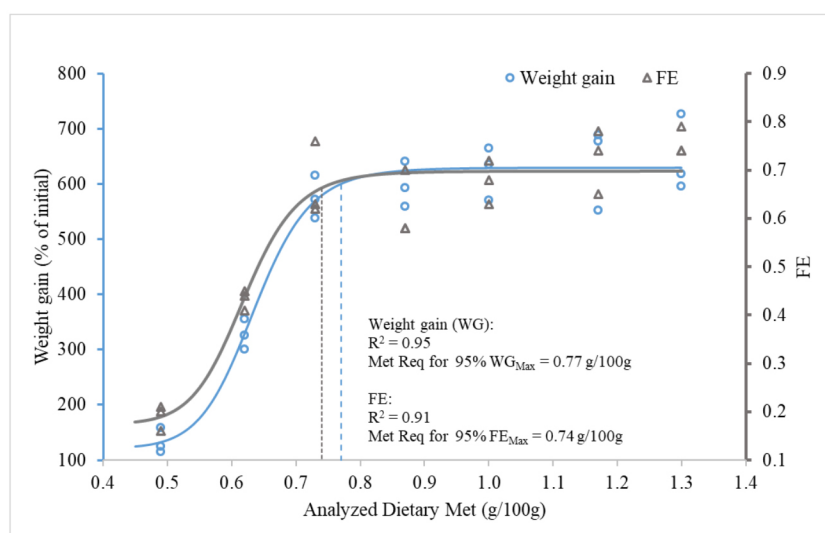


Figure 1. Methionine requirement of LMB for 95% of maximum theoretical weight gain and FE.

MINI-AQUAPONICS: ENGINEERING A MORE SUSTAINABLE WORLD THROUGH EDUCATION AND IMPLEMENTATION

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Aquaponics is a method of sustainable agriculture utilizing the symbiotic relationships between fish, beneficial bacteria, and plants to grow food. The purpose of a mini-aquaponics system is to serve as a learning tool to educate the public through Extension workshops and spread this growing practice. The overall project goal was to engineer a miniature aquaponics system that is cost-effective and well designed to be used for Iowa State University (ISU) Extension workshops.

In order to spread the practice of aquaponics and sustainable agriculture throughout the state, the project must be interesting and appealing to the public. A workshop was conducted in January 2017 using a mini-aquaponics version 2.0 prototype, where participants built their own benchtop scale system. Participants were surveyed through a standard evaluation form as well as through an online survey. Feedback from workshop participants was used to engineer a more desirable and economical system. This project is currently in its third phase - "Mini-aquaponics 3.0" (Figure 1), which is designed to improve functionality and structural integrity, while maintaining relatively low material and operating cost.

An economic cost-benefit analysis was conducted using projected production estimates calculated using existing crop yield data from previous aquaponic research at ISU. Energy inputs, construction costs, and feed expenses for one year were also calculated and factored into the cost analysis. Economic benefits were calculated for two common crops, lettuce and basil, using current retail market prices for similar quality hydroponic product and predicted crop yields from previous ISU experiments. The results of this analysis suggest that annual food-cost savings for the at-home user should be greater than the total input cost for construction, supplies and maintenance. This innovative system will contribute to the advancement of public education of sustainable aquaculture practices.



Figure 1. Mini-aquaponics version 3.0

COMMERCIAL EVALUATION OF A PROBIOTIC MIXTURE (SANOLIFE PRO-W) TO ENHANCE SURVIVAL, GROWTH, AND PRODUCTION OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* CULTURED IN INLAND PONDS OF WEST ALABAMA AND FLORIDA

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Semi-intensive pond production of the Pacific white shrimp, *Litopenaeus vannamei*, has been established in west Alabama and Florida for a number of years. Farmers in west Alabama have been utilizing water derived from an inland low salinity (2-10 ppt) aquifer to successfully raise marine shrimp. In recent years, survival and yield have been reduced compared to previous production seasons. Poor survival at harvest has not been restricted to just one farm and has been reported by multiple farms in several states including Alabama, Florida, and Texas. Farmers have hypothesized that the increase in observed mortality may be due to disease, toxic algae blooms, water quality, shrimp source, or reduced robustness of shrimp in later stages of production. In order to investigate a probiotic as a potential solution for improving survival and yield at harvest, three shrimp producers (two in Alabama and one in Florida) evaluated the use of a probiotic mixture (Sanolife Pro W) which contains a high concentration (5×10^{10} cfu/g) of two strains of bacteria *Bacillus subtilis* and *B. licheniformis*. In Alabama, one shrimp producer (AL-Farm 1) designated eight ponds for the study (4 control, 4 treatment ponds) while the other Alabama farm (AL-Farm 2) and Florida farm (FL-Farm) both utilized six ponds for the trial (3 control, 3 treatment ponds). In the control ponds no probiotic mixture was provided. In the treatment ponds, 80 grams of probiotic per acre-m of water was administered prior to stocking and each week thereafter until harvest. Both control and treatment ponds were subjected to the same management practices except for presence or absence of probiotic treatment. Dissolved oxygen and temperature were tracked daily, while total ammonia nitrogen, nitrite nitrogen, and salinity were measured weekly. pH was determined in situ weekly in the morning before 800 hr and again in the afternoon between 1600 and 1700 hr. In Alabama, major genera of blue green and golden algae were also tracked every two weeks throughout the trial. Shrimp and pond water were sampled and evaluated for major pathogens at the beginning, middle, and end of the experimental trial. The production trial is currently ongoing and ponds will be harvested in September and October of 2017. Shrimp survival, final weight, yield, food conversion ratio, hemolymph osmolality, and major hemolymph ions will be determined following harvest of production ponds. Results will be utilized to evaluate whether a probiotic mixture affected water quality and production of shrimp raised in semi-intensive ponds.

GENOMIC RESOURCES FOR THE SPOTTED SEATROUT *Cynoscion nebulosus*

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The spotted seatrout (*Cynoscion nebulosus*) is a coastal sciaenid exploited by major recreational fisheries in the northern Gulf of Mexico and the southeast United States. Aquaculture of this species is being developed both for fisheries stock enhancement and to promote commercial production for local markets. Genomic tools are needed to perform the tasks involved in the genetic management of wild populations during stock enhancement. Critical tasks include estimation of the effective size of wild and captive stocks, monitoring of the contribution of cultured fish to breeding stocks through parentage analysis and genetic tagging, and assessment of adaptive variation and local adaptation of wild population to define management units. Genomic tools will also be precious when developing domestication and breeding programs for commercial aquaculture.

In this project a draft reference genome sequence was developed using Illumina (one sequencing lane of v4 paired-end sequencing on the HiSeq2500® platform) and PACBIO sequencing (7 SMRT cells, sequencing, 4 additional SMRT cells on the Sequel platform in progress). A total of 402M paired-end Illumina raw reads were generated with 92% of the reads showing quality scores greater than 30. The coverage of the initial PACBIO 7 SMRT cells was insufficient for assembly. The assembly of illumina reads produced from illumina sequencing spans a total of 595MB corresponding to approximately 75% of the spotted seatrout genome with an average coverage of approximately 50X and a N50 value of 5,715. The hybrid assembly will be re-attempted with the increased PACBIO dataset. The Illumina assembly was used as a reference to map double-digest Restriction Site Associated DNA (dd-RAD) Tags and characterize associated Single Nucleotide Polymorphisms (SNPs) in parents and offspring from one full-sib outbred family. Progeny were sampled at 14 days post hatch (n = 132) and at 195 days post hatch (n = 105). Libraries generated using the double-digest RAD-sequencing protocol were sequenced in multiplex on three lanes of the Illumina®HiSeq2500 platform producing an average 1.55 M filtered reads per individual. A total of 4,400 filtered SNPs were assigned to linkage groups using the software LEPmap. Linkage groups are being ordered to produce a high density map of the spotted seatrout genome and anchor genome contigs.

STRESS PHYSIOLOGY OF ECHINODERMS IN AQUACULTURE

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Invertebrates, such as sea urchins and sea cucumbers, have been widely consumed throughout Asia for centuries and are of growing importance as a resource in the United States. They are both a source of high quality protein and of valuable pharmaceuticals. Sea cucumbers, especially, have been found to have a variety of anticancer and regenerative properties, as well as many other valuable medical and pharmaceutical attributes. Sea urchins have long been used as a research model and as a food source. Due to these various factors, the natural population has become the of a growing fishery system. However, as is commonly found in fisheries when the demand increases, the natural population becomes overexploited, and eventually, the population collapses. As the sea cucumber has recently become of great importance as an export from the United States to various countries, it is essential that measures be taken to prevent overexploitation, while at the same time growing and maintaining the market. In order to prevent overexploitation and to grow and maintain the market, it is expedient to establish aquaculture facilities that can successfully raise these invertebrates for both research and consumption. One of the ways of doing this is to establish inland intensive aquaculture facilities that avoid the problems associated with raising animals on the coast, particularly loss to predation. Though intensive aquaculture provides the clear benefits of preventing overexploitation of the natural population and producing large amounts of product, animals raised in this way are vulnerable to a variety of stressors. These sources of stress include crowding, handling, and fluctuations in salinity and temperature. If an animal is stressed, its immune function decreases, and its vulnerability to diseases increases. Since there is currently no effective treatment to disease in echinoderms, the authors examined the impacts of handling and salinity fluctuations on the physiological and immunological responses of sea urchins and sea cucumbers. The sea urchins were much more susceptible to stress and decrease immune functions due to lowered salinity and handling than the sea cucumbers were. Although sea cucumbers did show negative effects, none of them was significant.

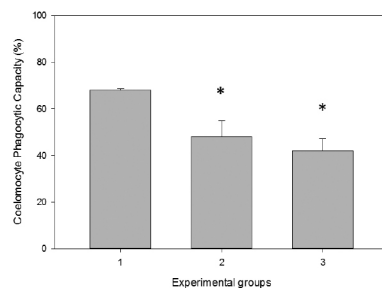


Figure A

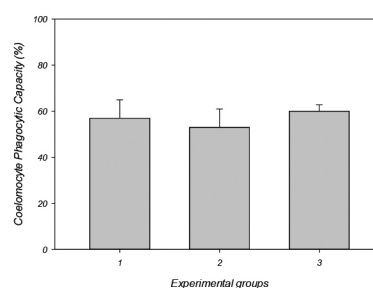


Figure B

Figures: Phagocytic activity of sea urchins (A) and sea cucumbers (B) phagocytic cells.

SOLID WASTE TREATMENT FOR SALTWATER RAS: ANAEROBIC DIGESTION AND BIOMETHANE PRODUCTION

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The recirculating aquaculture system (RAS) treats and eliminates fish farming wastes such as ammonia and carbon dioxide for reuse of water for fish culture. Solid wastes are also removed from system water but usually for the discharge without the treatment. Collected solid wastes should be treated to reduce organic load in RAS effluent to increase RAS sustainability and promote RAS aquaculture. Options that have been applied in some facilities are, for example, to generate fertilizer by composting the wastes and to use for landfill; however, it won't be the case for salt-containing sludge of marine RAS. Another alternative is on-site conversion of the solid waste to biomethane, called anaerobic digestion. This approach reduces sludge organic volume to fuel grade biogas that can be used as a heating source at the facility offsetting the operational costs. One of the problems for this approach with fish sludge from aquaculture systems is the low C:N ratio resulted from the high protein content of both the uneaten feeds and the fish excreta reducing the efficiency of bioconversion.

Our group at IMET has successfully developed consortium of anaerobic microorganisms that are capable to degrade low C:N fish wastes and effectively convert to biomethane using selective enrichment approach. Solid waste treatment and biomethane production using an integrated up-flow anaerobic sludge blanket (UASB) reactor in marine RAS have been demonstrated using enriched consortium. In this report, we describe the development of methanogenic consortium that efficiently converts biomass to methane by sequential transfers of bacteria with the target substrate under the defined anaerobic conditions. Enriched halotolerant microbial consortium is optimal for low COD:N ratios typical of RAS solid wastes and does not require supplemental materials such as organic carbon or nutrients for aquaculture solids bioconversion. We characterized five predominant phylotypes identified in the microbial consortium and isolated them in pure culture. Of which, two isolates are anaerobic fermentative bacteria *Dethiosulfovibrio* and *Fusobacterium* spp. Both species hydrolyze and ferment proteins, peptides and amino acids. The other three isolates are an acetate-utilizing methanogenic archaeon, *Methanosarcina* sp., and two hydrogen-utilizing methanogenic archaea, *Methanogenium* and *Methanoplanus* spp. We evaluated bioconversion rates of re-constituted microbial consortium with five isolates that resulted in equivalent to the original enriched consortium, suggesting those isolates can be used as seeding inoculum for marine RAS solid waste digester. In addition, evaluation of inoculum in the integrated anaerobic digester in marine RAS to accelerate start-up phase and maintain highly active and stable microbial community in UASB during intensive European sea bass production will be discussed.

EGYPT COMPARATIVE EUROPEAN SEABASS WILD COLLECTED, NIOF MARINE HATCHERY AND GAFRD K21 MARINE HATCHERY PRODUCED FRY GROWTH AND PHYSIOLOGY

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Egypt, the 10th world aquaculture producer is also the 1st Mediterranean Sea, Arab and African aquaculture producer (FAO 2016). Egypt marine aquaculture still depending on fry collected from natural resources (95.437 million fry, only 0.797 million fry of them recorded as Seabreams and Seabass fry); although Egypt had 6 hatcheries producing 8.906 million fry, only 3.500 million were Seabass fry in 2015 (GAFRD 2017). Not enough or misbalanced or low quality live feeds and microdiets affecting negatively marine hatcheries larval productivity and quality (Salem 2014; Salem and Ibrahim 2017).

The present study conducted in Fish Physiology Lab., Fisheries Division, National Institute of Oceanography & Fisheries (NIOF), Qayet Bay Castle, El-Anfoshy, Alexandria, Egypt. The present study was conducted using one thousand and five hundreds fry from 3 sources and transferred to the lab., the 1st fry group were collected from the wild habitat in El- Madya region, El- Behira Gov. from the wild fry capture fisheries., the 2nd fry group were 94 (dph) day post hatching produced in the Marine NIOF Hatchery, El- Anfoushy, Alexandria from induced spawning and the 3rd group fry were 94 dph produced in the Marine GAFRD Hatchery, Km 21 Abou Talat, Alexandria, Egypt from induced spawning. Fry samples were randomly collected to statistically determine the length, weight growth and physiology performances.

The comparative effects of European seabass (*D. labrax*) fry sources (wild and two hatcheries) and its impacts on length and weight growth parameters; total length in cm, standard length in cm, body width in cm, total weight, gutted weight in gm, hepatosomatic and vesrosomatic indexes. The wild collected fry achieved the best significant ($P < 0.05$) results in all this growth parameters while the NIOF marine hatchery produced fry achieved the best significant ($P < 0.05$) results in all this physiology parameters.

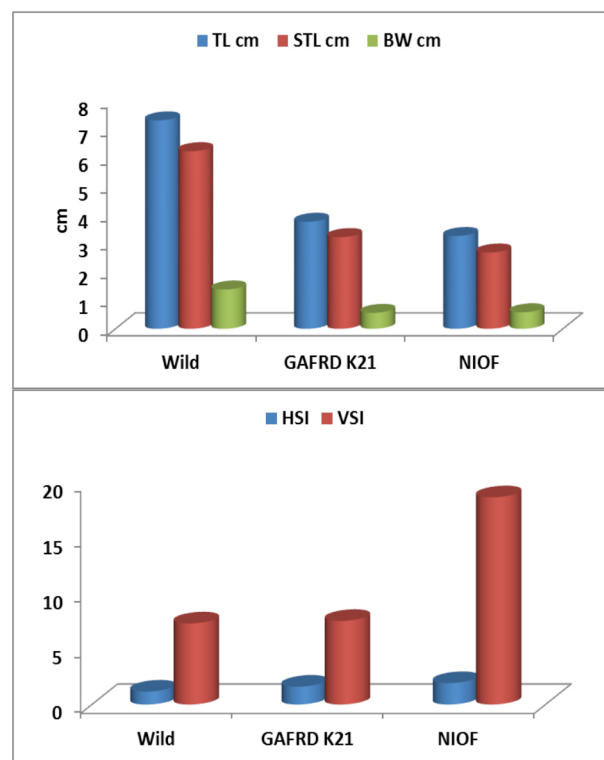


FIGURE (1): The length growth parameters, hepatosomatic and vesrosomatic indexes of wild collected and hatcheries produced European seabass (*D. labrax*) fry.

DIETARY ORANGE PEEL AS A GROWTH PROMOTER FOR NILE TILAPIA (*Oreochromis niloticus*)

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Sweet orange (*Citrus sinensis*) is a plant member of the Citrus family and principally cultivated in subtropical regions. Citrus mainly used regularly for juice and jam production which bring huge amount of by-product, such as peels. The peels contain a considerable concentration of ascorbic acid much more than the juice, and that the peel additionally contain higher concentrations of active materials. Many studies revealed the positive role of herbs that contain different bioactive components which may affect digestive processes in a positive way by enhancing enzyme activity, improving digestibility of nutrients and food absorption, consequently resulting in enhancing of fish growth.

There are no studies concerning the effects of orange peels on fish particularly as feed additives to promote the growth. Therefore, the aim of the present study was to assess the sweet Citrus peels in terms of growth induction of Nile tilapia fingerlings.

Fish were divided into 4 groups and fed for 60 days with four experimental diets (3 supplemented diets with 1 g kg⁻¹ diet (OP1), 2 g kg⁻¹ diet (OP2) or 4 g kg⁻¹ diet (OP4) of orange peel and a control group). At the end of the experiment, the main growth, feed utilization parameters and proximate body composition were investigated.

The results showed that weight gain (WG%) of fish fed on OP1 and OP2 diets was significantly higher than those fed on other diets ($P \leq 0.05$) (table 1). Moreover, Feed conversion ratio (FCR) was also affected by the feed additive, where FCR in fish fed the orange peel supplemented diets was lower than in fish fed control diet (table 2). It could be concluded that OP can efficiently act as a growth promoter for Nile tilapia fingerlings to a certain limit.

Table (1): Effects of different levels of orange peel on growth performance of Nile tilapia

Treatm ent	Initial weight	Final weight	Weight Gain
OP1	4.96±0.24 ^a	17.11±0.16 ^a	12.15±0.39 ^a
OP2	5.48±0.015 ^a	17.73±0.43 ^a	12.25±0.43 ^a
OP4	4.99±0.047 ^a	15.64±0.62 ^b	10.65±0.66 ^b
Control	5.21±0.21 ^a	13.90±0.12 ^c	8.69±0.14 ^c

Table (2): Effects of different levels of orange peel on feed utilization of Nile tilapia

Treatment	Feed Intake	FCR	PER
OP1	17.99±.12 ^a	1.48±.038 ^b	2.47±.06 ^a
OP2	18.38±.42 ^a	1.50±.02 ^b	2.34±.03 ^a
Op4	16.80±.608 ^a	1.54±.08 ^b	2.37±.12 ^a
Control	16.95±.85 ^a	1.95±.129 ^a	1.85±.12 ^b

THE ROLE OF INDISPENSABLE AND DISPENSABLE AMINO ACIDS SUPPLEMENTATION IN THE REDUCTION OF DIETARY CRUDE PROTEIN CONTENT IN CHANNEL CATFISH *Ictalurus punctatus* DIETS

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Protein represents about 70% of the dry weight of fish muscle, which is needed for maintenance and growth. In fact Catfish is like other fish species, do not have a protein requirement, but they require a source of nonspecific nitrogen and indispensable amino acids. Usually these nutrients supplied either from a mixture of intact protein sources or by using a mixture of feedstuffs supplemented with purified, crystalline amino acids. These amino acids are utilized for protein biosynthesis and deposition or, used for energy if it supplied in excess. So, catfish feeds should be balanced through the supply of adequate levels of nonspecific nitrogen, amino acids, and non-protein energy in exact proportions. Dispensable amino acids can be synthesized by catfish, but dietary inclusion of these amino acids help to promote growth through saving energy used in their synthesis, and some dispensable amino acids can partially replace some indispensable amino acids.

Growth trial was performed to assess the role of indispensable and dispensable amino acids supplementation in the reduction of dietary protein content in channel catfish *Ictalurus punctatus* diets and their effect on growth response. 9 isolipid (8% experimental diets were formulated with different crude protein levels) 32 control, 30, 28, 26, 24 % CP) with supplementation of Essential amino acids (EAAs) ; Lysine and Histidine to meet EAAs requirements for channel catfish *Ictalurus punctatus* according to NRC 2011 with and without addition of Nonessential amino acids (NEAAs); Glutamic acid and Glycine (2, 4, 6, 8% NEAAs) .

Experimental fish (average initial weight 10.56 g) were randomly distributed into 9 groups, 4 replicate for each 15 fish/ tank in recirculating system, fed experimental diets two times/day % of body weight and weighing bi-weekly for measuring (Mean weight (g), weight gain and feed conversion ratio. Dissolved oxygen, temperature and salinity measure 2times/day while, water samples collect 2 times/week for Total ammonia nitrogen and Nitrite analysis. Also, PH measured by PH meter two times/ week.

The growth results showed that, there is no significant difference ($P>0.05$) in the final means (g), total weight gain(g) and feed conversion ratio (FCR) among fish groups which fed with {32 (control), 30, 28 & 26% CP supplemented with EAAs Lysine and Histidine}. However, fish fed with 24 % CP supplemented with EAAs showed statistically different ($P<0.05$) compared to 32 and 30% CP diets. While the NEAAs supplementation to 24% CP diet with EAAs did not show an obvious contribution in growth response.

BALANCING THE LOW PROTEIN DIETS WITH MULTIPLE AMINOACIDS SUPPLEMENTATION AT DIFFERENT LEVELS WITH AND WITHOUT USING OF NEAAs IN CHANNEL CATFISH *Ictalurus punctatus* DIETS

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The rapid expansion of the world aquaculture production leads to increase the demand for fish meal as it considered the ideal protein source for fish. On the other hand, the problem of the high prices of protein sources. Therefore, the optimal dietary requirements of amino acids and alternative protein or amino acids sources have had a big interest in nutrition researches. Either using of alternatives protein sources or reducing the dietary protein in fish feed formulation can result in an imbalanced AA profile of feed that can affect growth performance of cultured species. Also, deficiency in some amino acid may have occurred, which has known as limiting amino acids.

This research was performed to reduce the dietary intact protein content in channel catfish (*ictalurus punctatus*) diet and balancing the amino acids profile by using multiple crystalline EAAs/IAAs supplementation at different levels 100, 120 & 140% compared to channel catfish EAAs requirement which stated in NRC 2011. Also, to evaluate the effectiveness of crystalline amino acids comparing to intact protein amino acids (amino acids pound protein). Besides that, assessment the contribution of NEAAs/DAAs supplementation to the low protein diet. 9 isolipid (8%) experimental diets were formulated with different crude protein levels) 30%IP control, 24IP+EAAs up to control diet+NEAAs, 24IP+EAAs 100% NRC, 24IP+EAAs 100% NRC+NEAAs, 24IP+EAAs 120% NRC, 24IP+EAAs 120% NRC+NEAAs, 24IP+EAAs 140% NRC, 27IP+EAAs 120%, 30IP+EAAs 120% NRC).

Experimental fish (average initial weight 23.68 g) were randomly distributed into 9 groups, 3 replicate for each 15 fish/ tank in recirculating system, fed experimental diets two times/day % of body weight and weighing bi-weekly for measuring (Mean weight (g), weight gain and feed conversion ratio. Dissolved oxygen, temperature and salinity measure 2times/day while, water samples collect 2 times/week for Total ammonia nitrogen and Nitrite analysis. Also, PH measured by PH meter two times/ week.

The growth results will be presented in conference.

EFFECT OF DIETARY LIPID SOURCE ON DIGESTIBILITY AND HEALTH PARAMETERS IN CICHLIDS

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Cichlids constitute a family of teleost of primary economic importance: hundreds of species are popular ornamental species while tilapia species provide food and/or revenue to people in numerous countries worldwide. Dietary lipids are important nutrients, as a source of dietary energy as well as of precursors of physiologically active molecules (e.g., hormones, eicosanoids). Consequently, the dietary lipid profile influences growth, health, and metabolism of the individuals. However, the demand for fish oil, which has been the traditional source of essential fatty acids, has increased while global production has remained stagnant. Consequently, the search for alternative lipid sources that would support the energy and health needs of fishes has been a major focus in fish nutrition.

In this study, 7.8g Nile tilapia juveniles were fed one of eight diets formulated with fish, squid, algae, fungal, flax, camelina, canola, or soy oils and grown for 10 weeks. Blood samples were collected and analyzed for hematocrit and serum alkaline phosphatase (SAP), alanine transaminase (ALT) and aspartate transaminase (AST) activities as well as bilirubin, cholesterol, triglycerides and bile acids. In addition, digestibility coefficients of the various lipid sources were determined.

There were no significant differences among diets in growth rates (Fig. 1, overall average final body weight 80.6 ± 5.5 g; mean \pm standard deviation), feed efficiencies or feed intake. Blood chemistry also revealed that none of the measured parameters were significantly impacted by dietary lipid source (Fig.2). The results will be further interpreted considering the digestibility data.

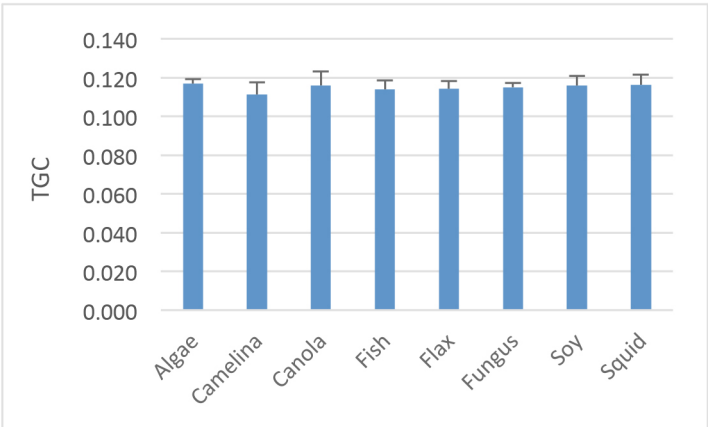


Figure 1: Average growth rates (TGC) of juvenile tilapia fed various lipid sources (p=0.0954)

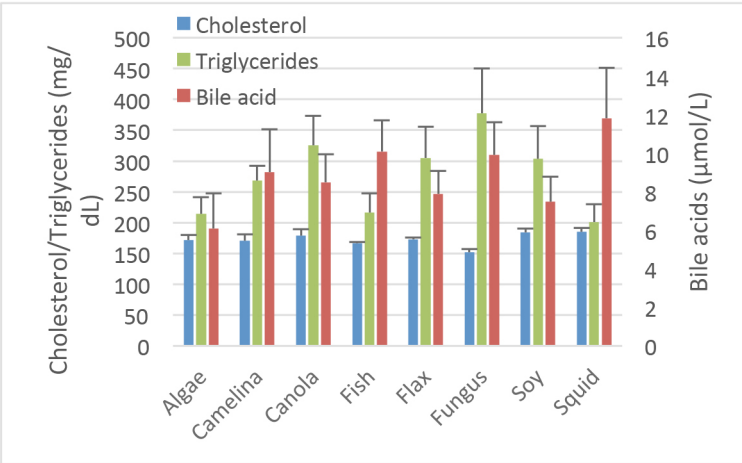


Figure 2: Serum cholesterol, triglycerides and bile acids in juvenile tilapia fed various lipid sources. (p>0.05)

VETERINARY CARE IN AQUACULTURE

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What can aquatic veterinarians do for you and your product? Veterinarians are a critical, and often underutilized, resource in aquaculture production. With the FDA changes to VFDs in 2017, many producers have needed veterinarians where they haven't before. Even if you have no need for a VFD, a relationship with your local aquatic veterinarian can be very beneficial for you and your animals.

Aquatic veterinarians are not a new specialty. Aquaculture veterinarians have been around since the first aquaculture systems were implemented. The specialty has been growing and diversifying, with more veterinarians now available to assist in the treatment of fish species. From private practice to government and everything in between, there is a veterinarian who is available to work with you. The following organizations have been formed to help you find an aquatic veterinarian near you:

World Aquatic Veterinary Medical Association (International) – <http://wavma.org>

Fish veterinarian database: <http://aquavetmed.info>

American Association of Fish Veterinarians (USA) – <http://fishvets.org>

Veterinarians can assist with many different aspects of aquaculture production. Though their primary goal is fish health, they can make recommendations for biosecurity and handling protocols, suggest methods to increase yield by minimizing fish stress, and provide yearly inspections in addition to providing medications when necessary. Veterinarians are here to support your business and should be included in production whenever live animals are included.

METHODS TO MITIGATE DISEASE SPREAD THROUGH AQUACULTURE SYSTEMS

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Managing disease outbreaks can require time, money and energy. Using pre-emptive strategies to mitigate the possible spread of disease can be relatively cheap and easy. No matter the species or disease in question, simple changes throughout the production facility can have important impact on your bottom line.

Fish, and other aquatic organisms, live in a continually contaminated environment. It is impossible to completely sterilize their environment to limit the spread of disease. However, decreasing their stress level goes a long way to improve their immunity. The stress response in fish is very similar to the stress response in mammals. The hormone cortisol is released in events of acute or chronic stress and results in decreased immune function over time. Decreased immune function leads to more frequent disease and parasite outbreaks, potentially resulting in loss of product to morbidity and death.

The most significant contributor to fish stress is water quality. This can be one of the hardest aspects of production to manage depending on your setup and water source. No matter the source of your water, be sure to test it independently from your fish systems. All of your systems need to be tested regularly and more frequently for systems that are undergoing or recently completed chemical treatment. Stocking density can severely affect water quality between systems and needs to be carefully considered when moving animals in confined containers.

Stress from transport and handling can also have major impact on fish health. Fish are not made to be handled in any fashion, so limit the amount of times fish are moved between systems or physically handled for measuring or vaccination. Consider different methods of transport between systems. Could your transport be improved to limit stress? Try to think through the eyes of a fish to see if taking on new technology could benefit your final product.

Immune stimulants are becoming more readily available in aquaculture. Studies involving vitamin C, beta glycans and allicin are showing positive signs in feeding trials. Consider adding an immune stimulant to your fishes' feed in anticipation of a stressful event (i.e. handling, transport, etc) for the best result.

There are many potential benefits to anticipating fish stress and limiting it where you can. True, not all sources of stress can be eliminated, but limiting them will yield larger, healthier product with a robust bottom line.

RECOGNIZING DISEASE IN FISH

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The most important job of a producer is to manage disease. Early diagnosis and treatment is paramount to high survival rates and effective management. Recognizing early signs of disease in fish can be difficult in certain infections. Depending on the disease, you may see physical or behavioral signs, and being able to pick them out early in an infection is the most important aspect of good fish health.

Physical signs of sick fish are relatively easy to spot. This includes spots, tears, growths, color change or missing parts. A wide range of diseases can affect a fish's outward appearance. Many of the diseases commonly found in aquaculture are secondary to stress, and no stressor is more common than water quality. Water quality is the most important aspect of fish health. Like the air we breathe, the water a fish swims in is paramount to its health and longevity. At the start of any disease outbreak or suspicion, water samples should be tested immediately before any other action other than quarantine is taken.

Spots on fish can present in a wide array of colors and appearances. Some spots can be disease specific, such as "White Spot Disease," also known as Ich, short for the parasite, *Ichthyophthirius multifiliis*. Green spots on fish, usually caused by opportunistic algae growth, indicate a loss of immunity at a spot in the skin. This is caused by trauma to a small piece of skin or scale that is dead but remains on the fish. Red spots usually indicate the start of an ulcer or bruise. Ulcers are very serious since they interfere with the protective barrier of the outer skin, allowing more bacteria and parasites to enter the fish. Bruises can be secondary to flashing, a behavior caused by skin ectoparasites. Other spots are usually dependent on the species and species-specific diseases.

Behavioral signs of disease can include body posture, buoyancy, swimming or appetite. Body posture involves how the fish holds itself in the water. A healthy fish is parallel to the surface of the water and symmetrical bilaterally. Sick fish may list to one side or have their head or tail elevated. Buoyancy is where a fish sits in the water column. Both positive and negative buoyancy disorders are common in fish. Swimming may be erratic and disoriented. A fish's appetite is a good measure of its overall health. A fish that does not want to eat is a positive indicator that disease is present.

Many factors go into monitoring fish for good health. Knowing your fishes' normal behavior is important in determining when disease is present. Catching disease early and treating appropriately is very important to good fish health and yields.

COMMON PARASITES OF FRESHWATER AQUACULTURE SPECIES

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Unfortunately, fish exist in an environment that is constantly contaminated. Just from their own wastes alone, no fish can exist in a disease-free environment. This includes the parasites that opportunistically feed on fish from the outside and inside. When a fish is healthy, their immune system is able to keep parasite levels low, but they are never fully eradicated except in isolated systems. However, due to stress from handling, transport, water quality or numerous other causes, parasites can take advantage of a pause in a fish's immune function and proliferate rapidly.

Fish parasites exist both externally and internally. Depending on your species and aquaculture setup, you may see one type more often than the other. All parasites require early diagnosis in order to prevent spread and possible fish morbidity and mortality. Physical signs of parasitic disease can include flashing, scale loss, bruising or decreased appetite with external parasites and failure to thrive and increased morbidity and mortality with internal parasites.

External parasites are very common in all fish species. Most are small enough to only be seen with a microscope on low power (40x). Larger parasites, such as *Argulus* spp (fish lice) and *Lernaea* spp (anchor worms), are large enough to be seen with the naked eye. Simple skin/mucus scrapings are sufficient to diagnose an ectoparasite infection. Common external parasites include *Ichthyophthirius multifiliis*, Trichodina, Ichthyobodo, monogenean trematodes (flukes), Chilodinella, and others. Many of these parasites require easy water-based treatment. Some systems where infections are common may consider prophylactic parasite treatment.

Internal parasites are more common with aquaculture species in contact with wild populations. It is not uncommon to find parasites in wild-caught fish with no physical symptoms. However, in fish being raised for consumption, internal parasites may result in decreased body condition and failure to thrive. Both internal and external parasites may show increased morbidity and mortality, and increased secondary bacterial and fungal infections. Internal parasites will usually require a feed-based or injectable medication for treatment. Diagnosis can be made upon necropsy or fresh fecal samples.

No matter the parasite, fish can become stressed, leading to decreased immune function. This can open the door to many more infections and disease spread. If you suspect a parasite infection, take immediate steps to correctly diagnosis the problem and start treatment early. Early diagnosis and treatment is the best thing to protect your fish investment.

WATER QUALITY IN FRESHWATER FISH SYSTEMS

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Water quality is the most important aspect of fish health. Managing your water can be difficult for systems that are constantly turning over high volumes of fish, but should always be considered in management practices. Areas of water conservation may have increased difficulty with keeping good water at manageable prices. Taking steps to ensure good water quality can improve your fish health, longevity and yields.

The most important aspect of water quality is your nitrogen cycle. This cycle converts ammonia into nitrite, and finally nitrate. Ammonia is the primary waste product from fish, being excreted from the gills and gastrointestinal system. At high levels, ammonia is highly toxic to fish, as is its primary waste product, nitrite. Nitrate is the safest nitrogen compound and is used by plants and algae as a food source. However, even at high levels, nitrate can have the same toxicity as ammonia.

Nitrogen-fixing bacteria colonies will do the work of conversion for you; you just have to give them a place to live. Biological filters have many designs, but the most significant part is your biomed, the structure on which your good bacteria can grow and thrive. This structure includes bio balls, mats, strapping, brushes, and essentially any porous surface with adequate water flow. These filters must be maintained regularly to ensure that old, dead bacteria are removed and water is able to easily flow through the filter.

pH and kH are essential in good fish health. Optimal pH will depend on your fish species. kH measures the buffering capacity of your water, also known as alkalinity. Your gH, or total hardness, will play a part in your kH. You need a sufficiently high kH in order to keep your pH stable. If your species can tolerate a wide range of pH, it is important that a stable pH is maintained. pH is based on a log 10 scale, so a pH change from 5.0 to 6.0 is a 10x decrease in the hydrogen ion concentration. The hydrogen ion concentration of your water can have a significant impact on many different biological processes in fish, most importantly those functioning in the gills.

Water parameters must be tested regularly and depending on your setup, may be required weekly, to daily to hourly. Choose a reliable test kit that multiple staff know how to use correctly. Additional testing may be required for new setups or possible contamination.

NOAA'S COLLABORATIVE ENGAGEMENT TO ADDRESS THE SIGNIFICANT BARRIER OF PUBLIC PERCEPTION IN AQUACULTURE

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Expert analyses show that marine aquaculture can be a resource-efficient, environmentally sustainable form of food production that can play a significant role in increasing seafood supply, improving human nutrition, and creating jobs. However, most of the public still has limited understanding of aquaculture and may encounter information that can be out of date, inaccurate, or incomplete. Public perception of aquaculture is a significant barrier to marine aquaculture development in the United States. Integral to NOAA's aquaculture mission is advancing public understanding of marine aquaculture practices; the associated environmental, social, and economic challenges and benefits; the health benefits of eating seafood; and the science, services, and policies NOAA has to offer in support of aquaculture.

Many stakeholders want to understand the impacts of aquaculture, including an assessment of the risks and the benefits based on the best available science. This is especially important for coastal communities, where aquaculture is a leading employer and part of community culture and history. NOAA's Office of Aquaculture has a responsibility to provide the public with factual, scientific evidence information about aquaculture. This can only be achieved through collaborative engagement with the public we serve. The Office of Aquaculture is currently engaged in ongoing outreach efforts that engage the public with accurate information about the state of marine aquaculture research and management and key initiatives by NOAA and its partners. These efforts include a website redesign, video series for educators and students, CELC network outreach as well as collaboration within NOAA to achieve common goals.

ANALYSIS OF GONADAL DEVELOPMENT, BY BLOOD AND HISTOLOGICAL TESTS IN WILD ORGANISMS OF RED SNAPPER *LUTJANUS COLORADO*, ON THE NORTH COAST OF THE MEXICAN PACIFIC

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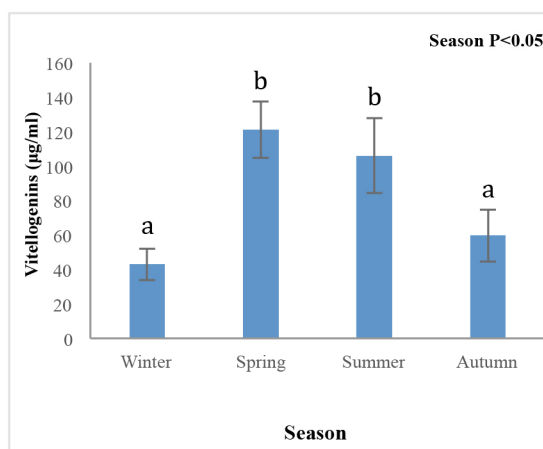
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In the north of the Sinaloa coast, the red snapper, *Lutjanus colorado*, is considered one of the most important fish species because of its commercial and nutritional value. 206 organisms were stacked during the four seasons (winter, spring, summer and autumn) of 2016, from the Sinaloa coast, the blood samples of 1.5 ml were extracted from each fish. The following biochemical parameters in blood were analyzed: protein (PT), cholesterol (COL), triglycerides (TRG), glucose (GLU), vitellogenins, and hematocrit. Red cell (RBC), white cell (WBC) and thrombocyte (TBC) were estimated. Also, the gastric repletion index (GI), gonadosomatic index (GI), hepatic index, and condition factor (K) were obtained. The values of blood samples from wild red snapper were correlated with fish size and season. Fish body weight and total length (LT) ranged from 81.3 to 2,040 g, and from 17.5 to 52.7 cm, respectively. The IRG (3.16 ± 0.25) and K (1.27 ± 0.01 %) indexes were higher in summer, whereas the higher GI and HI values were obtained in autumn, with averages of 0.11 ± 0.01 and 1.8 ± 0.12 %, respectively. For PT and COL, the higher concentrations were observed in autumn, with averages of 7.54 ± 0.44 g/dL and 166.60 ± 7.8 mg/dL, respectively, whereas the higher values of TRIG and GLU were obtained in summer and spring, with averages of 185.23 ± 10.8 and 46.56 ± 2.23 mg/dL, resp. For HTO and RBC the higher values were observed in summer, with averages of 43.10 ± 0.98 % and $2.68 \pm 0.04 \times 10^6$ células μl^{-1} , while WBC and TBC in winter and fall were higher, with averages of $8.08 \pm 0.69 \times 10^3$ and $32.17 \pm 1.08 \times 10^3$ células μl^{-1} . The highest value of vitellogenin per season was obtained in spring (121.22 ± 16.95 $\mu\text{g} / \text{ml}$). In relation to the determination found in organisms of 25 cm (116.72 ± 29.37 $\mu\text{g} / \text{ml}$) and with respect to the weight found in organisms of 587 g (101.40 ± 16.30 $\mu\text{g} / \text{ml}$). The obtained results contribute to better understand on the reproductive physiology of the red snapper from the wild, and the development of its culture in captivity.



WATER QUALITY MAINTENANCE IN GROWTH OF JUVENILES OF THE SPINY LOBSTER *Panulirus argus*, IN RECIRCULATION SYSTEM

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Recirculation aquaculture systems (RAS) are a set of structures that treat lower quality water from the culture tanks, and return the same water with quality, free of metabolites derived from the cultivation without interruption, not requiring water exchange with the environment. The objective of this research is to maintain the water quality in a recirculation system for the growth of juveniles of the spiny lobster *P. argus*.

The research was carried out in RAS with culture tanks with an area of 1.5 m² and volume of 1 m³, sand filters, micropore cartridge filters, skimmer, biofilter with nitrifying bacteria, biofilter with macroalgae and ultraviolet system, maintaining an average flow of 1 m³ h⁻¹. Six tanks with 20 juveniles/ tank, initial mean biomass of 10.67 g and average initial length of the cephalothorax of 22.12 mm were used, during the period of 126 days. The feed was *Artemia* sp., shrimp and mussels. The biometric control was done every 14 days with the average biomass gain rate per tank of 161.60%, average individual biomass gain rate of 310.70%, mean increase rate of cephalothorax of 57.95%, and survival of 65%. The measurements of the physical and chemical variables of the culture water were carried out in culture tanks, biofilter with nitrifying bacteria, and biofilter with macroalgae. We used a multiparameter probe to measure the pH, O₂D, temperature, salinity and conductivity daily, and a photometer to measure NH₃+NH₄⁺, NO₂⁻, NO₃⁻, hardness, alkalinity and PO₄⁻ weekly. We performed the Shapiro-Wilk normality test ($\alpha=0.05$). For the samples with non-normal distribution ($P < 0.05$), NO₂⁻ and hardness the Kruskal-Wallis test ($\alpha = 0.05$) was performed. For remaining variables with normal distribution ($P > 0.05$), ANOVA was calculated. When the null hypothesis was rejected, the Tukey test ($\alpha = 0.05$) was applied.

The mean values and statistical analysis of the system water variables are shown in table 1.

The system maintained the quality and stability of the water variables within the parameters required by the cultivated species, with significant variation only in O₂D, due to the higher consumption in biofilter with nitrifying bacteria.

Table 1- Medium values of temperature (°C), O₂D (mg L⁻¹), pH, salinity (g L⁻¹) conductivity (μS cm⁻¹), NH₃+NH₄⁺ (mg L⁻¹), NO₂⁻ (mg L⁻¹), NO₃⁻ (mg L⁻¹), hardness (mg L⁻¹ de CaCO₃), alkalinity (mg L⁻¹ de CaCO₃) and PO₄⁻ (mg L⁻¹) in the culture tanks – CT, biofilter with nitrifying bacteria – NB and biofilter with macroalgae – BM.

Variables	RAS components		
	CT	NB	BM
Temperature	27,15a	27,20a	27,40a
O ₂ D	4,76a	3,57b	4,16ab
pH	7,49a	7,44a	7,48a
Salinity	33,69a	33,70a	33,68a
Conductivity	53.186,17a	53.371,78a	53.288,63a
NH ₃ +NH ₄ ⁺	0,71a	0,33	0,54a
NO ₂ ⁻	5,47a	4,28a	5,08a
NO ₃ ⁻	5,62a	6,79a	5,18a
Hardness	331,42a	305,75a	341,13a
Alkalinity	70,69a	70,72a	70,79a
PO ₄ ⁻	12,64a	12,02a	11,46a

SPINY LOBSTER *Panulirus argus* FARMING IN AQUACULTURE WATER REUSE SYSTEM, USING DIFFERENT TYPES OF ARTIFICIAL SHELTERS

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In addition to the overexploitation caused by fishing, the commercial lobster production through aquaculture has been facing a number of challenges, including the development of attractive and nutritionally complete foods, disease control and the definition of structures for culture. In the environment, lobsters need structured habitats for their protection and survival. Considering the characteristics of the lobster species of the genus *Panulirus* and the importance of the shelter for their survival as well as the environmental advantages of the water reuse systems, the objective of this study is to cultivate juveniles of the *P. argus* species in different types of shelters in an aquaculture water reuse system.

The experiment lasted 126 days and was carried out in a recirculation system composed of 6 culture tanks with an area of 1.5 m² each, and a filtration system. 120 lobsters were cultivated with initial mean biomass of 9.91 g and initial mean length of the cephalothorax of 21.98 mm, with 20 individuals/tank. Two different treatments were performed: T-I using bricks as a shelter and T-II using PVC fittings, with 3 tanks and 60 lobsters per treatment. The lobsters were fed with *Artemia* sp., *Litopenaeus vannamei* and mussel of the species *Perna perna*. The biometric control was performed in all individuals through tagging every 14 days, and retagged after each ecdise. The mean values of the water variables were: temperature of 27.02 °C, dissolved oxygen 3.98 mg L⁻¹, pH 7.38, salinity 33.16 g L⁻¹, conductivity 52,353.72 µS cm⁻¹, total ammonia 0.56 mg L⁻¹, nitrite 4.16 mg L⁻¹, nitrate 5.40 mg L⁻¹, phosphate 12.48 mg L⁻¹, hardness 314.24 mg L⁻¹ of CaCO₃ and alkalinity 73.83 mg L⁻¹ of CaCO₃. The Shapiro-Wilk normality test ($\alpha = 0.05$) was performed for data analysis. For non-normal values ($p < 0.05$), the Mann-Whitney test ($\alpha = 0.05$) was used to compare the averages.

The data of the biometric variables were analyzed by comparing the average of the 2 treatments, and are shown in table I.

There was no statistical difference in the data of any of the biometric variables analyzed. The use of shelters is of vital importance to the success of lobster farming and, despite the lack of statistical evidence in the final result of this research, the complexity of the shelter, with regard to the options of different dimensions, seems to be better for maintenance of the species *P. argus* of lobster in captivity.

Table I: Biomass gain rate by tank (%) and juveniles (%), cephalothorax growth rate (%) and survival (%) in T-I bricks and T-II PVC fittings treatments.

Treatments	Biometric variables			Survival (%)
	Tank biomass rate (%)	Juvenile biomass rate (%)	Cephalothorax growth rate (%)	
T-I	241,20±62,90	316,31±32,58	57,04±4,65	81,67±10,41
T-II	201,79±55,44	332,17±64,06	62,26±4,71	70,00±8,66

TOWARDS SUSTAINABLE FISH-FREE AQUAFEEDS: EVALUATING MICROALGAL CO-PRODUCT FOR REPLACEMENT OF FISH MEAL IN AQUACULTURE DIETS FOR NILE TILAPIA (*Oreochromis niloticus*)

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Aquaculture, the world's fastest growing food sector, demands 70% of global supplies of fishmeal and fish oil for aqua feeds. Over-reliance of aquaculture feeds on fishmeal, fish oil, and plant-based protein (e.g. soy) is unsustainable financially, environmentally, and socially. Our overall goal is to combine different marine microalgae to achieve fish-free diets. We report here our recent study to develop aquaculture feed formulae using co-product left over after commercially raising microalgae to produce a nutraceutical.

Data from our current experiment showed excellent growth, feed conversion, and survival of tilapia fed a diet replacing 33% of fish meal with a promising marine microalga, *Nannochloropsis oculata* (Na.) co-product. We first determined digestibility of its nutrients for tilapia. We then conducted a feeding experiment with Na. co-product to determine the optimum level of fishmeal substitution for growth of tilapia and their fillet's fatty acid composition. We quantified digestibility by comparing nutrient composition of feed and fish feces using standard methods; and we conducted a three-month growth trial starting with juvenile tilapia using experimental diets (Table 1). Results show that co-product had significantly lower digestibility values for crude protein (73.5% vs. 81.1%) and methionine (64.1% vs. 88.1%) than the whole cells. However, we detected the highest lysine digestibility in the Na. co-product (81.1%). Results of the growth trial with Na. co-product showed: comparable and higher final weight, weight gain, specific growth rate (SGR), and protein efficiency ratio (PER) in Na.0 and Na.33 diets; and FCR, feed intake, and survival rate were not significantly different among diets (Table 2). Although Na. co-product contains higher amounts of protein compared to Na. whole cells (49.7% vs. 37.7%), high levels of anti-nutrients (fiber, non-starch polysaccharide-NSP, and trypsin inhibitor) may have depressed digestibility and growth at higher Na inclusion levels.

We are now testing combinations of other microalgae with Na. co-product to fully replace fishmeal and fish oil in tilapia feed. Adding NSP and protease enzymes could improve nutrient digestibility and growth. Our next experiment will explore adding enzymes to Na Co-Product diets in order to enhance nutrient digestibility and growth.

TABLE 1. Experimental Diet Set-Up

Digestibility Experiment	Growth Experiment
4 replicate tanks/diet	3 replicate tanks/diet
Reference diet	Reference diet (Na 0)
Na Whole- cells diet	Na replaces 33% of fishmeal (Na 33)
Na Co-product diet	Na replaces 66% of fishmeal (Na 66)
	Na replaces 100% of fishmeal (Na 100)

TABLE 2. Initial weight, final weight, weight gain, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), feed intake, and survival rate of tilapia fed experimental diets.

	Diet				P value
	Na.0	Na.33	Na.66	Na.100	
Initial weight (g)	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	0.17
Final weight (g)	33.8 ± 2.4 ^a	28.6 ± 0.9 ^a	24.30 ± 1.3 ^b	25.9 ± 1.5 ^b	0.01
Weight gain (g)	31.9 ± 2.4 ^a	26.6 ± .90 ^a	22.3 ± 1.3 ^b	23.9 ± 1.5 ^b	0.01
FCR	1.12 ± 0.1	1.26 ± 0.03	1.58 ± 0.17	1.55 ± 0.11	0.06
SGR	3.4 ± .1 ^a	3.2 ± 0.0 ^a	2.9 ± 0.0 ^b	3.1 ± 0.7 ^b	<0.01
PER	2.4 ± 0.2 ^a	2.1 ± 0.5 ^a	1.7 ± 0.2 ^b	1.7 ± 0.1 ^b	0.02
Feed intake (g/fish)	35.4 ± 0.7	33.5 ± 0.3	34.8 ± 1.7	36.9 ± 1.8	0.39
%Survival rate	98.0 ± 4.2	94.7 ± 4.4	96.7 ± 0.7	96.0 ± 3.1	0.53

INTERNATIONAL EFFORTS TO PROVIDE A WELL-TRAINED AQUATIC VETERINARY WORKFORCE TO ASSIST AQUACULTURE INDUSTRIES & OWNERS

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With increasing impacts of diseases on aquaculture production in all countries, the need for a well-trained aquatic veterinary workforce (including veterinarians and para-veterinarians – veterinary technicians or nurses, and non-veterinary “aquatic animal health professionals) has become a global imperative. Numerous educational efforts are underway ensure that sufficient numbers are available to support aquaculture industries, producers, governmental agencies and a myriad of supporting industries that provide services or products to prevent, control or eradicate diseases. Without this infrastructure, sustainable and economically viable aquaculture will simply not thrive.

A number of International and National veterinary organizations have, or are developing processes to determine, evaluate, harmonize and accredit veterinary education throughout the world. the educational needs within veterinary degree-earning curricular, and extracurricular continuing education and professional development (CEPD) programs, to ensure an adequate veterinary workforce to meet contemporary and societal needs. These include, but are not limited to the World Veterinary Association (WVA), the Council on International Veterinary Medical Education, the World Organization for Animal Health (OIE), the North American Veterinary Medical Education Consortium (NAVMEC), the Federation of Veterinarians of Europe (FVE). Although organizations all are addressing aquatic veterinary education to some degree, two are focusing on ensuring aquatic are addressed in veterinary curricular and CEPD programs – the World Aquatic Veterinary Medical Association (WAVMA), and the International Partnership on Aquatic Veterinary Education (i-PAVE).

The WAVMA Aquatic Veterinary Certification (CertAqV) Program uses to certify veterinarians who have Day-1 competency to practice aquatic veterinary medicine (equivalent to competencies required of individual receiving a veterinary degree), has identified 9 core subjects. To build on WAVMA and other veterinary organization’s efforts, i-PAVE has embarked on a multi-year project to verify and validate the aquatic veterinary knowledge, skills and experience (KSEs) needed in any country, using a DACUM/SCID Process. This process for **D**eveloping **A** **C**urricul**U**M (DACUM), that includes a **S**ystematic **C**urriculum and **I**nstructional **D**evelopment (SCID) for validating any educational curriculum, involves workshops in N. and S. America, Europe, Africa, and the Asia-Pacific, to verify the KSEs needed, using veterinarians actively practicing aquatic veterinary medicine. Each workshop is then followed by a survey of every veterinary school in each region, to determine which of the core aquatic veterinary subjects are actually covered in veterinary school curricula, or veterinary CEPD programs.

Preliminary information from previous surveys of N. American and European veterinary schools, and completed DACUM workshops, suggests that North America, Australia/New Zealand and Western Europe currently may have sufficient educational opportunities to train an adequate aquatic veterinary workforce. However, many veterinary schools need to include important aquatic veterinary subjects to ensure all subject-matter needs are fulfilled.

THE UNIVERSITY OF ARIZONA'S AQUACULTURE PATHOLOGY LABORATORY WEST CAMPUS RESEARCH FACILITY: AN ICONIC SHRIMP RESEARCH FACILITY IN NORTH AMERICA – HISTORY, ACHIEVEMENTS AND THE FUTURE

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Since the inception of the University of Arizona's Aquaculture Pathology Laboratory and West Campus Agricultural Center in 1989, the APL-WCAC research facility has been an integral part of the history of this iconic laboratory. The APL-WCAC research facility is conveniently located over 200 miles from the nearest ocean which has proven to be the ideal place to study penaeid shrimp and other crustacean pathogens without the risk of introduction into their natural habitat. Over the course of the past 28 years, the APL-WCAC lab has helped to develop pathogen resistant stocks, developed challenge methods for multiple pathogens which aid in the selection of superior stocks, tested numerous products against viral and bacterial pathogens, run primary quarantine to aid in the diversity of the gene pool and studied new and emerging pathogens in live animals. The APL-WCAC lab has also been responsible for helping to train graduate students, supplied shrimp for the annual short course, participated in the research that resulted in numerous publications and patent disclosures and helped to train visiting professionals. In the coming years, the APL-WCAC lab will continue to support the new director and clients in the every changing detection methods and emerging pathogens.

EARTHY AND MUSTY OFF-FLAVOR EPISODES IN CATFISH SPLIT-POND AQUACULTURE SYSTEMS

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The interest and use of variations of partitioned aquaculture systems (PAS) by the southeastern U.S. catfish farming industry continues to grow. Split-pond systems, one type of PAS, are designed to improve management of dissolved oxygen levels and fish waste products (e.g., ammonia) compared to conventional earthen ponds that have been used for many decades. While recent studies of split-pond systems have focused on design, water flow rates, and management practices, there has not been any assessment of the occurrences and intensities of common “off-flavor” episodes in these split-ponds.

For the current study, pond water samples were collected from split-ponds at a commercial farm in west Alabama and at a research facility in west Mississippi approximately every 3 to 4 weeks during the fish grow-out period (May to November) during 2014 and 2015. In addition, catfish fillet samples were obtained in late summer and autumn from these same split-ponds. Concentrations of the “earthy” and “musty” off-flavor compounds geosmin and 2-methylisoborneol (MIB), respectively, in water and fillet samples were determined. Sensory analysis was also performed on catfish fillets collected during 2014.

The highest geosmin and MIB concentrations detected in split-pond water during the study were 898 and 22,651 ng/L, respectively. Geosmin and MIB concentrations in split-pond water were often higher in summer and autumn, and concentrations were similar to those reported previously for conventional ponds. The highest geosmin and MIB concentrations detected in fillets during the study were 1,712 and 29,851 ng/kg, respectively. Concentrations of these compounds in fillets and the description and intensities determined via sensory analysis were similar to those reported previously for off-flavor catfish from conventional ponds. Accordingly, catfish farmers using split-ponds can utilize similar management approaches as those used to mitigate earthy and musty off-flavor problems in catfish raised in conventional ponds (e.g., applications of algicides).

ASSESSING THE NUTRITIONAL VALUE OF NOVEL SOYBEAN PRODUCTS IN THE DIET OF ATLANTIC SALMON *Salmo salar* L.

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Atlantic salmon are one of the top consumed species around the world, but the Atlantic salmon feed industry relies on fish meal (FM) as one of the primary dietary protein sources. FM has become an expensive and scarce ingredient and its continued utilization in aquafeeds at high levels is unfeasible. Although soybean meal (SBM) is the foremost protein feedstuff currently used as a surrogate for FM in aquafeeds, salmon are highly sensitive to antinutritional factors (ANF) present in conventional soybean meal (CSBM). However, with processing technologies, ANF in CSBM can be effectively reduced or completely eliminated improving its nutritional value. One of these technologies resulted in EnzoMeal™ (EM), a product with fewer or undetectable levels of important ANF. Therefore, the objective of this study was to compare the effects of EM inclusion into soybean-based diets for Atlantic salmon (*Salmo salar*).

A 10-week feeding trial was conducted at NOFIMA's land tank facilities in Sunndalsøra, Norway. Four experimental diets (42% crude protein and 20% crude fat) were designed to replace FM with three soybean products (SBP): CSBM (Control), EM, and soy protein concentrate (SPC). Non-vaccinated Atlantic salmon smolts were stocked into 12 tanks (triplicate tanks per diet) at 60 fish per tank (10.89 kg/ m³), and fed ad libitum. Our results showed significant differences in production performance of Atlantic salmon in response to the different dietary SBP (Table 1). Atlantic salmon fed the diets containing 10% SPC and EM (SPC10_EM10) or 20% SPC (SPC20) displayed significantly higher final weight, weight gain, and thermal growth coefficient than those fed the CSBM20 diet, but did not differ ($P > 0.05$) from fish fed the diet containing 20% EM (EM20). There were no significant differences in feed intake or feed efficiency among treatments. Processed SBM (EM and SPC) supported higher gut health compared to CSBM, which led to distal intestine soy-induced enteritis. Histology and nutrient digestibility data will also be presented. These results indicate that SPC and EM are more suitable sources of protein than CSBM in the diet of Atlantic salmon.

Table 1. Production performance parameters of Atlantic salmon after 10 weeks of feeding.

	CSBM20	EM20	SPC10_EM10	SPC20	One -Way ANOVA (Pr > F)
FW	440.8 ± 12.5 ^b	475.7 ± 13.7 ^{ab}	499.2 ± 5.9 ^a	500.5 ± 4.0 ^a	0.009
WG	142.7 ± 6.9 ^b	161.9 ± 7.5 ^{ab}	174.9 ± 3.3 ^a	175.6 ± 2.2 ^a	0.009
TGC	2.8 ± 0.1 ^b	3.1 ± 0.1 ^{ab}	3.3 ± 0.7 ^a	3.3 ± 0.0 ^a	0.009
FI	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	0.071
FE	1.2 ± 0.0	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.0	0.165

FW = final weight (g), WG = weight gain (% of initial), TGC = thermal growth coefficient, FI = feed intake (% of BW/day), FE = feed efficiency. Means within a row with different superscript letters differ significantly (Tukey HSD).

EVALUATION OF THREE INDUCING AGENTS FOR ARTIFICIAL PROPAGATION OF LARGEMOUTH BASS *Micropterus salmoides*

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Human Chorionic Gonadotropin (HCG), Ovaprim, and a new spawning agent, GnRH IIa (D-Arg6-Pro9-NHet), were evaluated for artificial propagation of Largemouth Bass. Four year old brood stock were reared on an artificial diet in 0.1 acre ponds, provided forage, and segregated by sex prior to the spawning season. Mean female weight among 54 fish was 1125 ± 34 g and mean male weight among 54 fish was 921 ± 29 g. All fish were given a single intramuscular injection early in the morning and examined for a response the following day. HCG was applied at a rate of 4000 IU/kg for females and 2000 IU for males. Ovaprim and GnRH IIa was applied at a rate of 0.5 ml/kg and 0.1 mg/kg in a single injection to both male and female brood stock, respectively. Each female was assigned an individual net enclosure. Fish that freely expressed eggs in volume were selected for stripping and anesthetized in MS-222. Fish were patted dry and eggs from each female were stripped into a dry bowl, weighed, sampled, and then combined by treatment prior to fertilization.

The respective response rate for HCG, Ovaprim, and GnRH IIa was 83%, 67%, and 50%, with a mean response of 26.6, 23.8, and 17.6 grams of eggs/kg brooder (Table 1). Regardless of spawning agent, it was not possible to strip milt from males to fertilize eggs. Males were euthanized, the testes were extracted, macerated in 0.85% NaCl solution, and the suspension was used for fertilization. Number of fry/jar was estimated volumetrically by taking three samples of a known volume from a total volume of 10 L and counting individual fish in each sample. Mean fry yield/hatching jar was only 5.2% in the HCG treatment, 5.0% in the Ovaprim treatment and 3.4% in the GnRH IIa treatment.

Table 1. Summary of experimental results (Mean \pm SE).

Treatment	Total yield of eggs (no.)	Mean Latent Period (hr.)	Response Rate	Mean Response (g/fish)	Weight of eggs/kg female brooder (g)	Mean Fry Yield (%)
HCG	295,506	33.4 ± 0.55	83%	31.2 ± 4.6	26.6 ± 3.6	5.2 ± 0.9
Ovaprim	240,532	34.4 ± 0.74	67%	26.8 ± 5.2	23.8 ± 4.1	5.0 ± 1.4
GnRH IIa	105,744	34.7 ± 0.73	50%	19.0 ± 5.5	17.6 ± 8.2	3.4 ± 1.2

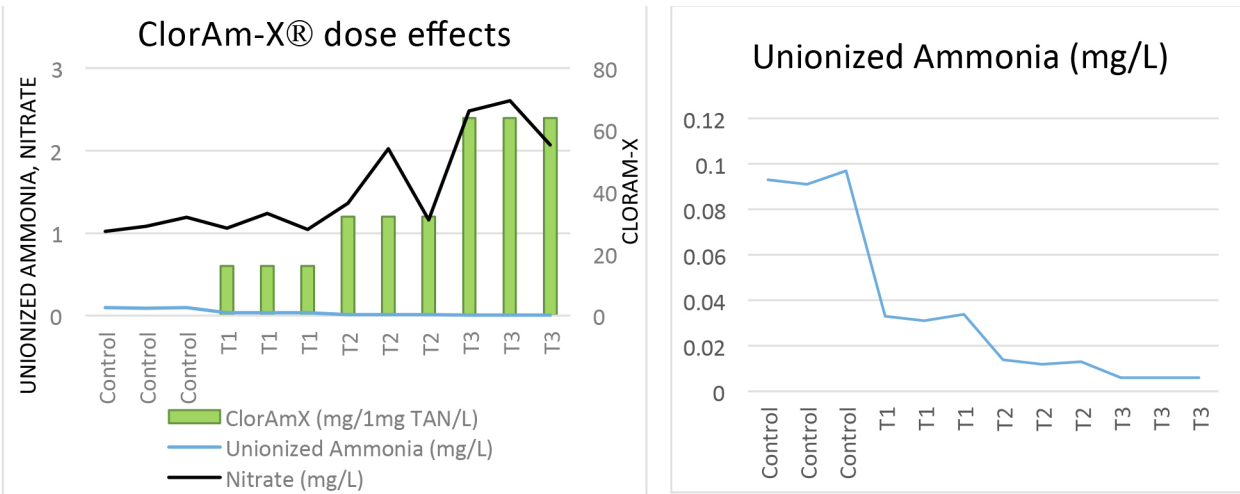
EFFECT OF SODIUM HYDROXYMETHANESULFONATE ON AMMONIA AND RELATED WATER QUALITY PARAMETERS

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A Sodium Hydroxymethanesulfonate product (ClorAm-X®, Aquatic Resources Inc., USA) was used in water from a recirculating holding system tank after 8 days long Channel Catfish holding experiment with Total ammonia nitrogen (TAN) 15.8 mg/L. Three treatments; T1-50% the recommended dose (15.95 mg/L of ClorAm-X® for 1mg/L of total ammonia), T2-recommended dose (31.9 mg/L of ClorAm-X® for 1mg/L of total ammonia) and T3-150% of recommended dose (47.85 mg/L of ClorAm-X® for 1mg/L of total ammonia) were applied in 10 liters of water, each with three replications. Three replications of control without ClorAm- X application were also used. 30 minutes post application, TAN, Nitrite, Nitrate, unionized ammonia and pH were measured using Hach kits, spectrophotometer and pH meter.

Results show that the product is effective in reducing TAN and unionized ammonia in concentration dependent manner. Nitrite and pH were not affected by treatments but nitrate seems to increase slightly with increasing dose of ClorAm-X®. Increase of nitrate may be of concern only in seriously heavy doses of the product. Thus, Sodium Hydroxymethanesulfonate may be used in reducing ammonia content in recirculating holding systems where ammonia buildup is one of the major issues. Further research on use of Sodium Hydroxymethanesulfonate in various aquaculture systems may provide new insights on its usefulness.



FISH SKIN MICROBIOME AS A TOOL TOWARD UNDERSTANDING AND IMPROVING FISH WELFARE

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The welfare of farmed fish is influenced by environmental and management factors. Fish skin is one of the sites of fish immune system and a major route by which infections can be acquired. The bacterial variability of gilthead seabream (*Sparus aurata*) skin in healthy and diseased conditions was characterized, using a high-throughput DNA sequencing method. The fish skin microbiome in both healthy and diseased conditions was compared in a controlled environment, using UV-treated water and non UV-treated water. Swab samples were collected from fish lateral lines, abdomen area and gills, before (T_0), and 24 hr. (T_1), one week (T_2) and three weeks (T_3) after an experimentally induced infection by exposure to *Vibrio harveyi* followed by netting fish out of the water (handling stress). The results showed a similar bacterial composition present initially (T_0) in the different sites sampled, which included six main constant bacterial communities (families). These bacteria families were mostly similar to those present in the surrounding environment (water). The skin bacterial composition changed when the fish were confronted with bacterial infection followed by a handling stress (T_1), resulting in variations in fish skin bacterial communities. Such changes presumably played an important role in fish health deterioration. Furthermore, when skin microbiome occurring prior to fish challenge, was not re-established, fish mortality was observed in both non UV-treated and UV-treated water treatments (60% and 100%, respectively). The importance of a balanced bacterial community appears quite evident when non UV-treated water was compared with UV-treated water (general practice in aquaculture). 30% of the total initial communities (OTU's) present on fish skin were unique to fish that eventually survived the challenge in comparison to the bacterial communities in those that did not survive (Fig 1). These results suggests the importance of a balanced epithelial microbiome on fish health status and the need to preserve this balance.

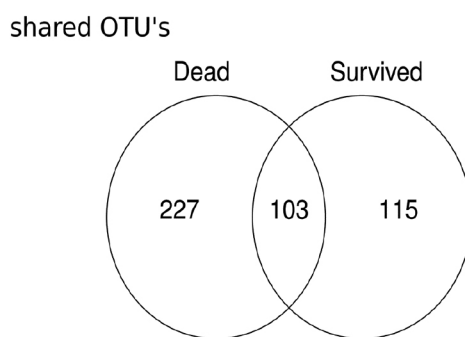


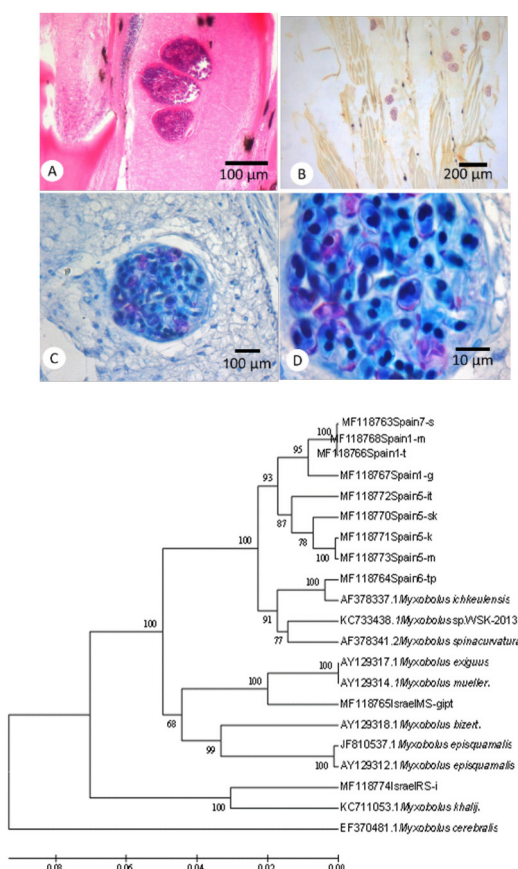
Fig 1: Venn diagram for the shared and unique bacterial communities at T_0

HIGH DIVERSITY OF *Myxobolus* SPP. (MYXOZOA) IN AND BETWEEN JUVENILE *Mugil cephalus* INDIVIDUALS

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Grey mullet, *Mugil cephalus* is an important aquaculture species in the Mediterranean region. In this study we report high variability of *Myxobolus* Bütschli 1882 spp. (Myxosporea) in juvenile grey mullet received at our lab from Israeli aquaculture farms. The examined *M. cephalus* fingerlings originated from wild caught a. Ebro River Delta in Spain, fingerlings imported to Israel and b. River estuaries in northern Israel (Mediterranean Sea). Infections with *Myxobolus* spp. were recorded from both batches. Here we present light microscopy, electron microscopy and molecular analyses of the different *Myxobolus* spores found in multiple sites in these fish, as well as histological attributes of the infections. 18S rDNA sequence analyses indicated the presence of different species of *Myxobolus* in the examined grey mullet fingerlings. Clear differences in both spore morphology and 18S rDNA sequences was found between the *Myxobolus* spp. when the wild grey mullets from Spain ("Spanish") were compared to locally caught wild grey mullet fingerlings ("Israeli"). Nevertheless, spore morphology was similar amongst all *Myxobolus* recovered from the Spanish fish, while their 18S rDNA sequences revealed species variability, between different infected organs (gills, tail, and internal organs) originating from the same fish as well as between individual fish from the same batch. The absence of records of these *Myxobolus* spp. in the GenBank database compounds the diversity of *Myxobolus* spp. which can be found on a single grey mullet host. It is noted that commercially caught wild fingerlings and imported stocks may contribute to this diversity by introducing and spreading new variants from remote geographical regions.



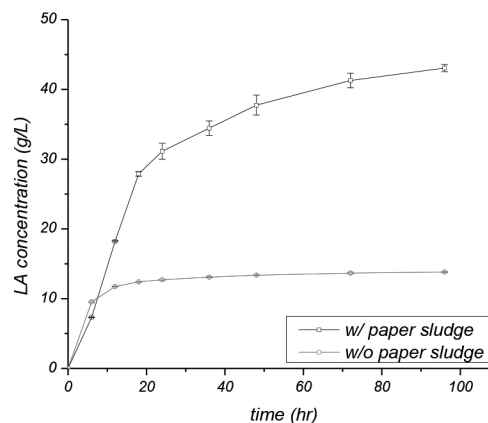
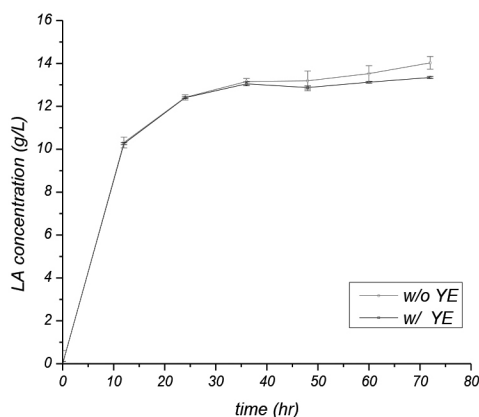
LACTIC ACID PRODUCTION FROM SOLID CATFISH WASTE (MANURE)

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The aim of this work was to study the solid waste (manure) produced by catfish as a potential feedstock for the production of lactic acid via fermentation. The solid waste contains high levels of nutrients for lactic acid bacteria which eliminated the need for external nutrient source such as yeast extract, peptone and/or corn steep liquor. Simultaneous Saccharification and Co-Fermentation (SSCF) was applied using enzyme and *Lactobacillus pentosus* (ATCC-8041). Different loadings of enzyme and solid waste were tested to find the optimal operation conditions for SSCF. The lactic acid concentration achieved at 15% solid waste was 35.7 g/L. Solid catfish waste could also combine with other solid waste, to increase the final lactic acid concentration. When the mixed feedstock of fish waste and paper mill sludge was used, the lactic acid concentration increased to 43.1 g/L at 12% equivalent solid loading. The catfish waste was proved to be a potential feedstock to provide both carbon and nutrient source for LA production, which could be used as a sole substrate or could be combined with other lignocellulosic materials.



EVALUATION OF SKIN AEROBIC BACTERIAL FLORA IN MARKET SIZE (300 – 500 g) OF RAINBOW TROUT IN AMOL CITY FARMS

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Most of fish bacterial infections are caused by secondary pathogens, namely they are present in water and pond precipitations. If the condition change the typical conditions to increase of such bacteria or fish immune system suppression occur, many of diseases by secondary pathogens will be observed. Some of this type bacteria is also regarded as food toxicities in man. Therefore pond bacterial flora is important. In this research, rainbow trout aerobic skin bacterial flora were evaluated in seven fish farms along Haraz River in Amol city in Iran. Thirty five market size (300-500 g) rainbow trouts were collected from these facilities and were cultured for aerobic bacteria in 13 and 37°C for the psychrophilic and mesophilic bacteria, respectively. Identified aerobic mesophilic bacteria are: *Staphylococcus aureus*, *Aeromonas hydrophila*, *Staphylococcus epidermidis*, *Bacillus cereus* besides psychrophiles such as *Pseudomonas aeruginosa*, *Proteus mirabilis*. But some grew on both mesophilic and psychrophilic temperatures that are: *E.coli*, *Staphylococcus saprophyticus*, *Pseudomonas mirabilis* and *pasteurella multocida*.

The most and the least prevalent bacteria were *E. coli* (present in all skin samples) and *Pasteurella multocida* (only in 33.3% of the 4th rainbow trout farm), respectively.

Generally, bacterial load was increased from upstream (farm 7) to downstream (farm 1) farms. In below tables body surface bacterial flora of rainbow trout in each farm is showed briefly.

	Aerobic mesophilic bacteria (37°C)							
	<i>E.coli</i>	<i>A. hydrophila</i>	<i>S. aureus</i>	<i>S. saprophyticus</i>	<i>S. epidermidis</i>	<i>B. cereus</i>	<i>P. mirabilis</i>	<i>p. multocida</i>
Farm 1	+	+	+	+	+	+	+	-
Farm 2	+	+	+	+	+	-	-	-
Farm 3	+	+	+	+	+	-	+	-
Farm 4	+	+	+	+	+	-	-	+
Farm 5	+	+	+	+	+	+	+	-
Farm 6	+	+	+	+	+	+	-	-
Farm 7	+	+	+	+	+	+	-	-

	Aerobic psychrophilic bacteria (13°C)				
	<i>E.coli</i>	<i>S. saprophyticus</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>P. multocida</i>
Farm 1	+	-	+	+	-
Farm 2	+	-	+	-	-
Farm 3	+	-	+	+	-
Farm 4	+	+	-	-	+
Farm 5	+	+	+	-	-
Farm 6	+	+	+	-	-
Farm 7	+	-	+	-	-

TILAPIA AQUACULTURE IN NEPAL: HISTORY FROM INTRODUCTIONS TO FARMERS PONDS

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The government of Nepal introduced Nile tilapia (*Oreochromis niloticus* “Chitralada” strain) in 1985 from Thailand, yet tilapia remained inside the fence of government farms until 1996 when research on tilapia began at the Institute of Agriculture and Animal Science (IAAS), Tribhuvan University, Nepal. In 2004, a new strain of Nile tilapia (GIFT – genetically improved farm tilapia developed by ICLARM and WorldFish) was introduced from the Asian Institute of Technology, Thailand to the Fisheries Research Center, Tarahara, part of the Nepal Agricultural Research Council (NARC). NARC subsequently initiated research in its Tarahara and Begnas Research Stations. Then in 2009, IAAS Rampur imported GIFT tilapia from WorldFish, Bangladesh. Lately in 2012, Himalayan Aqua Agritech introduced red tilapia from Nam-Sai farm Thailand and are maintained at AFU farm Chitwan and NARC farm Parwanipur.

Involvement of IAAS Aquaculture Program with the USAID-supported Aquaculture CRSP began in 2001 and has continued under the AquaFish Innovation Lab (also partly funded by USAID) through the present time. In 2010, IAAS transitioned to a new university structure with the establishment of Nepal’s Agriculture and Forestry University (AFU) in Chitwan. At AFU, increasing public and private interest in tilapia, has resulted in increased emphasis on tilapia research. AFU has conducted a number of projects both at university and farmers’ ponds, largely through AquaFish support, and shared the outcome of this research during workshops with government development and extension officials as well as NARC research scientists. The AFU-AquaFish collaboration has so far succeeded in farm trials at government farms and in farmer’s ponds at Dayanagar Rupendehi, the fish farming pocket area in collaboration with the Directorate of Fisheries Development (DoFD), Nepal. This has led DoFD to include verification trials using tilapia in government farms along with a program for private hatchery development of mono-sex fry production, as part of their annual plan. The AFU fish hatchery and a private hatchery have started production of mono-sex fry to distribute to private farms.

Small-scale famers of AFU’s on-farm research sites in Kathar, Chitwan and Kawasoti, Nawalparasi have begun farming tilapia and selling them in local markets. The AFU aquaculture farm produces tilapia for local sale. Some bigger farmers in Chitwan are producing tilapia with carps, which have a long history of cultivation in Nepal. Farmers in the Morang District of eastern Nepal are culturing tilapia. Danger of unwanted introduction of mixed-sex tilapia in natural waters has been the concerns for government and aquatic diversity conservationist. Currently, tilapia represents a major portion of the wild catch in Phewa and Begnas lakes of Pokhara though it was not intentionally introduced. Four percent of fish farmers are reported to be involved in tilapia farming, and tilapia contributed 1.5% of total aquaculture production in 2014-2015. Controlled culture of mono-sex tilapia in ponds has been the emphasis of AFU. We believe that incorporation of Nile tilapia in polyculture with carps, and tilapia monoculture will increase in future Nepalese aquaculture.

FINDING AN IDEAL FASTING PERIOD PRIOR TO THE LIVE FISH TRANSPORTATION: ASSESSMENT OF AMMONIA EXCRETION DYNAMICS AND AEROBIC METABOLISM TRADE-OFF

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The ability to transport live fish to the market to be sold as fresh product, to re-stock in aquaculture facilities or to deliver to processing plants for slaughter, is a challenge for aquaculture producers and marketers. Fish are routinely fasted prior to live transport to reduce metabolic rate and excretion. Prolonged periods of fasting often diminish the performance of transported fish and limit the ability to recover in their new environment. In this scenario, an ideal fasting period would be determined when fish metabolisms become low enough to keep oxygen consumption as well as ammonia production minimal, while still being able to regulate ammonia-transport so that it enables them to cope with ammonia stress in aquaculture facilities.

Therefore, in this study we have focused on finding the ideal pre-transport fasting period by evaluating temporal ammonia dynamics and associated metabolic trade-off. The experimental fish species, common carp (*Cyprinus carpio*) were progressively fasted up to 14 days. Temporal effect of fasting on oxygen consumption rate (MO_2), ammonia excretion rate (J_{amm}), plasma ammonia (T_{amm}), plasma ions, branchial Na^+/K^+ -ATPase (NKA) and H^+ -ATPase activity, as well as branchial mRNA expression of NKA, H^+ -ATPase, Na^+/H^+ exchanger (NHE-3) and Rhesus (Rh) glycoproteins were assessed. Results show that MO_2 and J_{amm} were considerably depressed from 4-6 days of fasting onwards. This coincided with the onset of higher resting plasma T_{amm} from day 6 onwards, possibly because in fasted fish the basal expression levels of Rhcg-a and Rhcg-b were only maintained up to 6 and 8 days respectively, after which a down-regulation was recorded. Plasma $[Na^+]$ and $[Cl^-]$ were temporarily reduced during 4-8 days of fasting, while an augmented $[K^+]$ was evident. The transcript level of NHE-3 was raised in 12–14 days fasted fish, which along with up-regulation in Na^+/K^+ -ATPase activity and mRNA expression facilitated the recovery of $[Na^+]$ to control level. First signs of energy store depletion in liver, especially glycogen, were recorded from day 8 onwards. Overall, these data suggest that the beneficial reduction of metabolic rate (MO_2 and J_{amm}) started at 6 days of fasting. Interestingly, at this time fish were still able to regulate ammonia transport efficiently, and did not compromise their energy stores yet. Therefore, we propose 6 days as an ideal fasting period before transport.

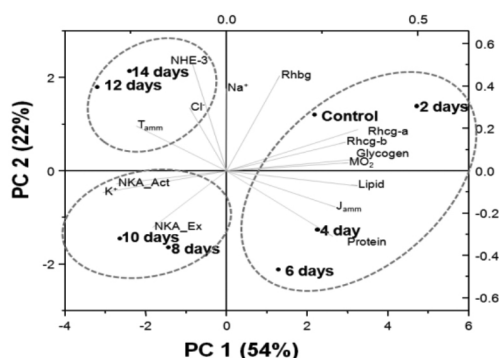


Fig. Principal Component Analysis representing the contribution of parameters

GROWTH PROMOTING EFFECT OF SUSTAINED SWIMMING IN COMMON CARP *Cyprinus carpio*

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Induced swimming has the potential to improve the growth and feed conversion efficiency of fish. However, these premises are not explored extensively for the aquaculture fish species. Therefore, the present study was conducted to reveal the effect of swimming exercise on promoting the growth rate and augmenting physiological capacity of common carp (*Cyprinus carpio*). This species is widely distributed worldwide and extensively used for aquaculture and is therefore of great commercial and ecological importance.

The trials were conducted with common carp juveniles to determine whether constant swimming at different speeds has an influence on growth, metabolic and physiological indices endpoints. The trial lasted for 28 days and was undertaken in three large (1600 L) recirculating aquaculture system called raceways flume (with flow created by an impeller driven by an electric motor). Three groups of 100 fish (mean weight of 4.87 g) were assigned to a constant swimming regime at different speeds: 0 (control), 1.5 and 2.5 body lengths per second (BL/s). Additionally, once in a week, 8 randomly selected fish per group were transferred to Blazka-type respirometers (3.9 L) for evaluation of swimming performance. The results showed a significant increase in the body weight gain for the group swum constantly at 2.5 BL/s compared to both control (0.0 BL/s) and 1.5 BL/s group at the end of week 1, 3 and 4 (Fig. 1). Contrary to the weight gain, there were no significant differences between the groups in critical swimming speed (U_{crit}) and oxygen consumption rate; although there was a trend of increasing oxygen consumption and decreasing U_{crit} in each group during the course of the trial.

Energy budget (hepatic and muscular) showed no difference between experimental groups, however following the week 4 there was a significant increment for protein content in liver of 2.5 BL/s group. Quantitative real-time PCR (qPCR) based expression level of a set of growth genes revealed clear regulatory roles in relation to swimming speeds. The mRNA transcript levels of growth hormone receptor as well as insulin-like growth factor were significantly up-regulated in hepatic tissue of 1.5 and 2.5 BL/s groups. Overall, our study revealed a growth promoting effect of sustained swimming in common carp, and provides a prospective of implementing swimming as a tool to increase fish production in commercial aquaculture system.

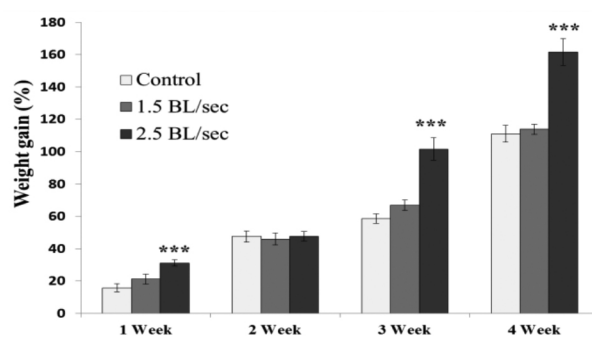


Fig 1. Weight gain in response to swimming speed

UPDATE ON AQUAPONIC FOOD PRODUCTION AND WORKFORCE TRAINING AT SANTA FE COMMUNITY COLLEGE

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Santa Fe Community College has been offering aquaponic education and production experience to students for the past four years in New Mexico. Each year the facilities continue to expand, offering new opportunities to train the future aquaponic workforce and entrepreneurs. Multiple production units currently exist including DWC, Vertical and Ebb and Flow systems using tilapia, barramundi, and goldfish effluent as the main nutrient source for plant production. Work is ongoing with reuse of mineralized fish wastes, creating zero-discharge systems. Unique production models are being evaluated in partnership with private businesses. A new 12,000ft² greenhouse was recently erected which will host hydroponic, aquaponic and algae production systems. Students learn aquaponic application and management from small, DIY production units to larger semi-commercial scale systems. The program provides one of the few opportunities in the world to attain college credit for courses specialized specifically on integrating fish and plants in a symbiotic system.

PRODUCTION OF GREATER QUANTITIES OF FEMALE SOUTHERN FLOUNDER FOR FOODFISH CULTURE AND STOCK ENHANCEMENT

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Gender determination of Southern Flounder is subject to internal factors (genetics, circulating sex steroids, stress hormones) but is also subject to modification by external factors (temperature). Warming trends in coastal waters have significantly skewed the sex ratios of Southern flounder in many areas. For example, a recent survey found sex ratios of Southern flounder to be skewed 16 males: 1 female in Galveston Bay, Texas. Flounder hatcheries and producers are often left to the mercy of Mother Nature due to difficulties associated with heating or cooling large volumes of water. Temperatures as little as 1°C greater than 22°C or less than 21°C during the period of sexual differentiation typically result in much larger percentages of males than females. This skewed sex ratio leads to limited successes in Southern flounder culture as males grow slowly and are difficult to rear to marketable size. Large-scale commercialization as a cultured foodfish, or production of females in quantities necessary to impact wild populations through stock enhancement, has not been realized for Southern flounder.

The majority of flounder produced for 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. To resolve this bottleneck, we evaluated the effects of UV irradiation of sperm followed by exposure to either pressure or temperature shock to suppress meiotic division and create heterozygous meiotic diploid offspring. The meiogynogenic juveniles can then be sex-reversed to become functional males by rearing at high temperatures or feeding methyltestosterone during the period of sex determination. The resulting XX functional males can then be spawned with normal females to produce 100% genetically female offspring. Creation of meiogynogens is only necessary one time, as when new XX functional male broodstock are needed, all-female progeny from the original meiogynogens can simply be sex-reversed using temperature or feeds containing hormones.

Two controls and two experimental treatments were evaluated to assess gamete quality, fertilization rate, embryo development, hatch rate, and total larvae produced. Control 1 served as the 'normal' control with untreated milt and eggs, while control 2 served as an irradiated control to ensure irradiated sperm could not result in embryo production. Treatment 1 utilized irradiated milt followed by 8,500 psi hydrostatic pressure shock, while treatment 2 utilized 4°C cold shock to induce meiogynogenesis. While both experimental treatments resulted in the production of meiogynogens, the 8,500 psi hydrostatic pressure shock yielded greater hatch rates and total larvae produced from the same spawns. Creation of meiogynogenic Southern flounder larvae currently appears to be a viable method to improve production for this species.

WHAT'S WRONG WITH OFFSHORE AQUACULTURE PERMITTING? ... AND HOW TO MAKE IT RIGHT

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The Fisheries Management Plan (FMP) for offshore aquaculture in the Gulf of Mexico was approved by the Gulf of Mexico Fisheries Management Council in 2009. The Rules for implementation of this FMP were finally set in place in January 2016. Since then, not a single applicant has – to our knowledge – stepped forward to initiate a permit process. Something, clearly, is wrong.

The Ocean Stewards have recommended that the Department of Commerce institute five primary changes to the current regulations, to begin to set things right, and to lay the foundation for America's offshore aquaculture industry. These recommendations are :

1. a single agency – NOAA - to take the lead for ocean aquaculture permitting;
2. specific ocean areas to be designated as appropriate for aquaculture, where projects do not significantly conflict with other ocean user-groups, and where permit applications might be expedited;
3. an overarching EIS for ocean aquaculture that would identify all potential significant impacts from a generic net pen fish farm, and describe the required monitoring and mitigatory measures for such projects, to ensure that some uniform standards are applied, and to expedite applicant reviews;
4. a timely review process to be established for commercial aquaculture projects; and
5. a simplified process to be put in place for expedited review and approval of experimental aquaculture projects.

This presentation reviews the underpinning rationale behind each of these recommendations. The presentation also discusses moving forward with small-scale demonstration projects in GoM waters as a precursor to larger commercial permits, to help to allay commercial and recreational fishing interests' fears about the impacts of net pens, and to show the Fish Aggregating Device benefits that can arise from providing structure offshore.

The Ocean Stewards Institute asserts that we have a moral responsibility to grow more of our seafood here, in America's 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.

SELECTIVE SUPPRESSION OF ALGAL BLOOM IN AQUACULTURE PONDS WITH SODIUM CARBONATE PEROXYHYDRATE

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Massive algal bloom formation in aquaculture ponds is a major water quality issue worldwide. The majority of these blooms are formed by cyanobacteria (blue-green algae) which oftentimes produce toxins, and are also accountable for the off-flavor in pond-raised fish species. Nutrient reduction is arguably the best strategy to control harmful algal bloom; however this is a lengthy process. Consequently, there is an urgent need for effective intervention techniques that can rapidly as well as selectively restrain the proliferation of cyanobacterial blooms without degrading overall water chemistry.

We examined the effectiveness of Sodium carbonate peroxyhydrate (SCP, trade name Pak 27®) to selectively suppress noxious cyanobacteria in the aquaculture ponds. SCP is a relatively new hydrogen peroxide based active compound (27% H_2O_2). Overall, the aim of present study was to- (i) determine the efficacy and most optimal dose of SCP to selectively control the target noxious algal bloom (cyanobacteria) and (ii) monitor the dynamics of toxins (microcystin) and H_2O_2 residue after SCP application. To determine the appropriate dose of SCP (quantified as H_2O_2) for full-scale aquaculture ponds (0.1 acre each, bloom was raised using fertilizer), a mesocosm experiment was conducted wherein a series of circular enclosure (75L) were installed in the experimental ponds, and treated with a concentration of 0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/L H_2O_2 . Changes in the strongly dominated cyanobacterium *Planktothrix* sp. abundance, chlorophyll a content and eukaryotes green algae density were monitored daily till 10 days. The results suggest that H_2O_2 concentration ranging from 2.5- 3.5 mg/L can selectively suppress the cyanobacteria, therefore, we used 2.5 and 3.5 mg/L for full scale pond study.

Following pond treatments, cyanobacterial population declined by 80% in 3-5 days in a dose-dependent fashion (Fig.1). Our results also revealed that microcystin concentration decreased at a similar pattern as the cyanobacterial density, suggesting that H_2O_2 could rapidly oxidize microcystin soon after the cell lysis. However, non-targeted biota including the green algae and zooplankton community were adversely affected by 3.5 mg/L H_2O_2 . The added SCP (2.5mg/L H_2O_2) actively degraded in 3 days leaving no measurable traces of H_2O_2 , while persisted till 5 days for higher dose (3.5 mg/L). Our results suggests that SCP at a concentration of 2.5 mg/L H_2O_2 can selectively mitigate harmful cyanobacterial bloom from the aquaculture ponds and leaves no long term traces of H_2O_2 in the water.

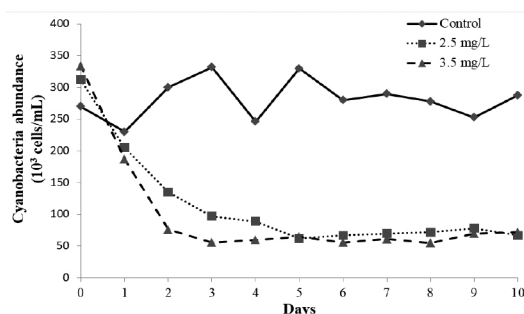


Fig 1. Changes in cyanobacterial abundance

GLOBAL TRANSCRIPTOME PROFILING OF MARINE FISH IN RESPONSE TO ENVIRONMENTAL CHANGE: SALINITY AND AMMONIA INTERACTIONS

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Salinity fluctuation is one of the main factors affecting the performance of marine fish. In addition, water borne ammonia may occur simultaneously with salinity stress. The adaptive capacities to cope with these environmental cues alone or in combination are extensively addressed in fish at physiological, biochemical and (sub) cellular level. To date, studies revealing the global response at transcriptome level is still lacking.

The objective of the present study was to elucidate genome-wide transcriptional responses underlying reduced seawater salinity challenge and ammonia toxicity as single and combined factor in juvenile European seabass (*Dicentrarchus labrax*). This is a marine teleost whose juveniles migrate between open sea and estuaries or lagoons, and thus often challenged with hypo-saline environment. European seabass is one of the most preferred fish species for aquaculture; also possess high commercial and ecological value. By using RNA next generation sequencing approach we intend to identify the genes and the associated pathways that are likely to be involved specifically as well as common to ammonia and salinity challenge. Fish were progressively acclimated to normal seawater (32 ppt) and reduced seawater salinity (10 ppt). Following acclimation to different salinities for two weeks, fish were exposed to high environmental ammonia (HEA, 20 mg/L ~1.18 mM representing 50% of 96 h LC₅₀ value for ammonia) for 12 h, 84 h and 180 h. RNA sequencing (Illumina HiSeq platform) of brain tissue samples from the experimental conditions yielded 12490 contigs of which 2128 contigs were significantly modulated in any of the treatment (salinity, ammonia or combination). Low salinity resulted in the up-regulation of 370 and down-regulation of 832 contigs, whereas in HEA exposure 122 contigs were up-regulated and 270 contigs were down-regulated. While looking for the combinatorial effects, we found that 74 contigs were commonly up-regulated and 94 were down-regulated in both these treatments. A total of 11,578 protein-coding contigs were identified based on sequence similarities with known proteins. Gene enrichment analysis revealed that genes differentially expressed in response to salt stress were highly represented in ion-regulation (typically Na⁺ transporter), cell adhesion and communication, transmembrane signalling receptor, Na⁺/K⁺-ATPase and oxidative stress. Transcripts involved in amino acid biosynthetic/metabolic process (typically glutamate synthase, glutamate receptor signalling), anti-oxidant defense system (peroxidase activity), neurotransmitter and ion-regulation were highly represented in response to ammonia toxicity. During the combined exposure of salinity stress and ammonia- ion transporters, oxidative and anti-oxidant related genes were highly expressed. Overall, our data highlighted that the compensatory responses incited at transcript level against salinity stress were improved during the combined effect of hypo-osmotic stress and ammonia. It suggests that ammonia exposure ameliorates protective responses during reduced sea water salinity (10 ppt).

DEVELOPMENT OF EXTENSIVE LARVAL CULTURE AND GROW-OUT PROTOCOLS FOR COBIA *Rachycentron canadum* TO SUPPORT COMMERCIAL PRODUCTION ALONG THE GULF COAST

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The proposed project will directly address major constraints that currently limit development and expansion of the U.S. food and agricultural systems through the development of novel techniques in cobia (*Rachycentron canadum*) aquaculture. Cobia is a high priority species for aquaculture along the Gulf of Mexico due to high market demand (US \$37.40/kg of fillet), fast growth rates, low-post larval mortality, and excellent feed conversion rates. Increased aquaculture production and availability of this species is a necessity to help meet increasing domestic and international demand for seafood, especially high value yet sustainable species such as cobia. In order to increase availability, we must develop new production protocols and methods for cobia that move production from the current intensive, technology-laden, re-circulation oriented protocols currently used in the U.S., to extensive outdoor culture systems that are widely available along the gulf coast.

The project objectives are to 1) develop economical, on-farm methods for size-graded, live zooplankton harvest from fertilized ponds to feed cobia larvae in hatchery tanks, 2) assess potential of extensive larval culture grow out techniques for cobia larvae in fertilized earthen ponds, 3) determine the economic and environmental feasibility of cobia production in earthen pond systems along the Gulf Coast using abbreviated grow out periods in order to avoid low-temperature fish die-offs, and 4) Determine mean growth potential of cobia in outdoor pond systems during an eight or nine month grow out period.

The proposed project will investigate two novel, extensive larval culture production techniques for cobia and two novel fingerling grow-out methods to simplify and expand production to outdoor pond systems. Both stages of culture, larval and grow-out, will utilize natural food production from fertilized pond systems and will produce market-sized fish under local environmental conditions without the need for indoor heated systems. Current aquaculture producers have expressed great interest in this project and have partnered with TAMU to conduct on-farm trials using the proposed study protocols. These sister studies in commercial settings will provide a great deal of additional data.

EVALUATION OF cGnRH IIa FOR INDUCED SPAWNING OF *Epalzeorhynchos bicolor* AND *Synodontis nigriventris*

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Reproductive dysfunction is a common impediment to the commercial production of many ornamental fish species. Use of exogenous hormone preparations may help to overcome failure of final oocyte maturation and induce ovulation. Ovaprim® (sGnRH α), is currently the preferred spawning aid used by the ornamental fish industry, however, spawning success using this GnRH subtype may be variable across taxa. An alternative gonadotropin releasing hormone analogue, cGnRH IIa, has been recently shown to exhibit increased biological activity and reliability for induction spawning protocols. This study evaluated the efficacy of two spawning aids, Ovaprim® and cGnRH IIa, on a number of quantitative and qualitative measures of spawning performance in *Epalzeorhynchos bicolor* and *Synodontis nigriventris*, two commercially valuable ornamental fish species.

Female broodstock that exhibited >50% germinal vesicle migration from collected biopsies were used in the study. Three cGnRH IIa doses of 50, 100, 200 μ g/kg, a single Ovaprim® dose of 10 μ g/kg and a propylene glycol control were evaluated. All treatment groups were also administered an equal concentration of a dopamine antagonist (5 mg/kg domperidone). Spawning aids were administered intramuscularly via injection near the base of the dorsal fin. Injection regimes differed between the two species tested but mirrored accepted commercial production protocols. At predetermined time periods, light pressure was applied to the coelom to detect successful ovulation. Females were then stripped and the eggs weighed and fertilized. Embryos were stocked into 1L hatching containers from which a subsample was drawn 1.5 – 2 hours later and photographed to determine fertilization success. Hatching success was determined from an additional 50 embryo subsample stocked into 150 mL screen bottomed specimen cups.

Results of contingency tables indicated no significant differences in ovulation success for either *E. bicolor* or *S. nigriventris* ($P = 0.499$ and 0.071). Total fecundity standardized as eggs per gram body weight ($P > 0.159$), fertilization and hatching success ($P > 0.314$ and $P > 0.296$) were also not significantly different among the four spawning aid treatments in either species. Results from this study indicate that cGnRH IIa performs equivalently to the industry standard Ovaprim® for induced spawning of both *E. bicolor* and *S. nigriventris*.

MOLECULAR INSIGHTS AND FUNCTIONAL ANALYSIS OF MANGANESE SUPEROXIDE DISMUTASE IN REDLIP MULLET *Liza haematocheilus*

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Manganese superoxide dismutase (MnSOD) is a nuclear-encoded antioxidant enzyme which is called as metalloenzyme. The main function of this enzyme is dismutation of the toxic superoxide anion ($O_2^{\cdot -}$) into less toxic hydrogen peroxide (H_2O_2) and oxygen (O_2). Structural analysis of mullet MnSOD (*MuMnSOD*) was practiced by using different bioinformatics web tools. Pairwise alignment results revealed that protein sequence matched to *Larimichthys crocea* with a 95.2% sequence identity and 98.8% similarity. Results of the phylogenetic analysis of *MuMnSOD* showed a close relationship with *Fundulus heteroclitus* and *Austrofundulus limnaeus* MnSODs. Multiple sequence alignment showed that the SOD Fe-N domain, SOD Fe-C domain, and Mn/Fe SOD signature were highly conserved among the other examined MnSOD orthologs. Hence, results of multiple sequence alignment suggested that the catalytic function of MnSOD may well conserve among its orthologs. Quantitative real-time PCR exhibited, the highest *MuMnSOD* mRNA expression in blood followed by liver, kidney, heart and muscle tissue among 12 different tissues from healthy mullet fish. Since blood is a main tissue which transports oxygen into host cells, generation of reactive oxygen species (ROS) in the blood is greater than the other organs. Therefore, the mullet antioxidative defense system may activate and produce more *MuMnSOD* transcripts against the formed $O_2^{\cdot -}$ in blood. Hence, expression level of the MnSOD might be high in order to combat these ROSs. Highest *MuMnSOD* expression was observed against *Lactococcus garvieae* at 6 hours post infection in head kidney. *L. garvieae* is a serious pathogenic bacterium which coursed haemorrhagic septicemia in mullet fish. When pathogens attacked immune tissues, ROS generation is increased due to the respiratory burst. Hence, antioxidant defense mechanism of mullet may activate and expression of *MuMnSOD* might be enhanced due to high ROS level. Xanthine oxidase assay (XOD assay) revealed the ROS-scavenging ability of purified recombinant protein (*rMuMnSOD*). The optimum temperature and the pH for XOD activity were 25°C and pH 7 respectively. Relative XOD activity was significantly increased with the dose of *rMuMnSOD*, revealing its dose dependency. However, the activity of *rMuMnSOD* was inhibited by potassium cyanide (KCN). It might be due to binding of CN^- ions into active sites of the MnSOD and replaced the Mn^{+} ions. Inclusively, results of the present study revealed that *MuMnSOD* act as an antioxidant enzyme and as an immune-related substance in the mullet fish.

RETHINKING RAS: DEVELOPING NUTRIENT BALANCE WITHIN RECIRCULATING AQUAPONIC SYSTEMS

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Aquaculture produces 44% of the 167 million tons of seafood consumed annually worldwide. Capture fisheries continue to decline at a steady rate, while the aquaculture industry has grown nearly 6% annually in the last decade. However, excess nutrient-rich effluent produced by typical aquaculture systems leads to eutrophication of water sources, requiring extensive wastewater remediation. Aquaponics offers a new solution to mitigate this costly strain on production by combining fish farming with plant production. Past waste-solids research on recirculating aquaculture systems (RAS) suggests that the nutrient load in the captured solids meets and may exceed the demands of terrestrial plants. Using these waste products in aquaculture systems eliminates the need for exogenous, expensive, and often synthetic nutrient supplementation to achieve adequate plant growth. Additionally, this decreases water treatment expenses while also generating extra profit from sale of the produce, ultimately lowering net production costs. This research focuses on further quantifying both soluble and particulate components of tilapia based RAS effluent for bioavailable macro- and micro- nutrients. We will then compare the resultant profile with typical requirements of various food crops to ensure sufficient fertilization. Characterizing the chemical differences within plant tissues from our aquaponic systems against those produced via terrestrial farming will confirm efficient nutrient transfer and non-inferior plant tissue growth. Further research will include characterizing species-specific effluent differences to more precisely match fish varieties to desired crops within individual systems. In conclusion, converting recirculating aquaculture systems into recirculating aquaponics systems, through our understanding of balanced supply and demand of nutrients derived from fish waste, allows for a more sustainable and economically viable model of integrated agricultural production.

INSULIN-LIKE GROWTH FACTORS AS MARKERS FOR GROWTH RATE IN U.S. LARGEMOUTH BASS POPULATIONS USED FOR FOODFISH PRODUCTION

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Largemouth bass are increasingly becoming more popular as a foodfish within the United States aquaculture industry, in part because pond-bank prices have been rising making it a more profitable species for farmers. Many of the private growers are located in the North Central Region (NCR) of the United States and rely on the Northern largemouth bass, *Micropterus salmoides*, subspecies for foodfish production. A significant limitation to largemouth bass foodfish production in this region is time to market. Many strategies, mostly focused on nutrition, have been attempted to increase the rate of weight gain and reduce time to market.

Because of its recent and rapid rise in popularity, largemouth bass have had little or no genetic manipulation for foodfish production. Most, if not all, of the focus on improving largemouth bass genetics has gone towards recreational angling and selection for large fish with no significant concern for fish age. For largemouth bass produced in the NRC, selective breeding has the potential to be one of the most effective methods of achieving market size at a faster rate than current stocks. This would translate economically to more money in the pockets of the growers and increase potential output to meet growing market demands.

As a first step toward the development of breeding programs focused on growth rate in Northern largemouth bass, growth data was obtained from source hatcheries and modeled to estimate age at market size. From these results, the predicted top two and bottom two performing populations were compared in a growth trial, which included the assessment of insulin-like growth factors I and II as putative biomarkers for growth, both at the genomic and transcriptomic levels. Significant IGF gene polymorphisms were detected across the populations; however, their distribution did not correlate with growth differences. On the contrary, faster growth rate was observed to be negatively correlated to muscle IGF II expression, suggesting its potential as a predictive biomarker to rapid growth.

This research combined statistical, genetic, and molecular techniques as a multi-step approach toward improving largemouth bass stocks for foodfish production. Studies such as this can be used as the building blocks towards genetic improvement of cultured largemouth bass populations, and yield results that can be put into action immediately to assist growers get the most out of current stocks.

FEEDING REGIMES FOR LARGEMOUTH BASS AT LOW WINTER TEMPERATURES

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Over the last few decades, largemouth bass (LMB; *Micropterus salmoides*) research has expanded as a result of an increase in the food fish market. LMB researchers have investigated pond intensification, improvement of genetics, out-of-season spawning, indoor recirculating aquaculture systems, and alternative feed ingredients, among others to decrease costs and improve production. During consultation with LMB farmers, it became clear to UAPB Extension personnel that there were concerns about the effects of harsh summer and low winter temperatures on the growth of their fish. Particularly, they were uncertain about feeding regimes during these seasons. In order to address the winter feeding issue, the UAPB Lonoke Fish Health Services Laboratory responded by developing a low winter temperature trial to obtain preliminary data on the feeding frequency necessary in order to either improve growth or at least limit weight loss over winter. In this trial, juvenile LMB (19.7 ± 0.4 g) were stocked into a 908 L recirculating system with nine aquaria (38 L each, 10 fish/tank). Samples of fish prior to the study and at the end of the study were frozen for future lipid analysis. The study ran for 93 days and temperature was maintained at approximately 7.1°C throughout the trial. Fish were fed a commercial LMB diet (Skretting, 45% protein, 18% lipid) at 1% of their body weight every 3 days, every 7 days, or every 14 days with three replicates per treatment. Water quality remained within acceptable levels necessary for LMB and survival was 100% in all aquaria. Feeding at least once a week contributed to weight gain while maintaining acceptable feed conversion ratios (Table 1). Feed is often the largest cost on a fish farm and improved winter feeding regimes should assist farmers in deciding the correct amount of feed to sustain or improve growth over winter.

Table 1. Mean (\pm SD) harvest data of LMB (19.7 ± 0.4 g) following a 3 month feeding rate study (fed 1% BW every 3, 7, or 14 days). Mean values with different letters within a row are significantly different ($P < 0.05$). FCR = (total g fed / total BW gained in g). FCF = $(W/L^3) \times 100,000$ and SGR = $((\ln W_f - \ln W_i)/93) \times 100$.

Production parameter	Feeding rate			P value
	Every 3 days	Every 7 days	Every 14 days	
Final weight (g)	22.8 ± 0.9^a	21.1 ± 0.6^a	18.6 ± 0.6^b	0.0001
Final length (mm)	116.5 ± 1.4^a	116.4 ± 0.5^a	116.2 ± 1.1^a	0.9442
Weight gain (%)	15.5 ± 0.8^a	7.2 ± 1.6^b	-5.9 ± 2.1^c	<0.0001
Survival (%)	100 ± 0^a	100 ± 0^a	100 ± 0^a	
FCR	2.0 ± 0.1^a	1.7 ± 0.4^a	-1.1 ± 0.3^b	<0.0001
FCF	1.4 ± 0.1^a	1.3 ± 0.1^a	1.2 ± 0.0^b	0.0016
SGR (%/d)	0.2 ± 0.0^a	0.1 ± 0.0^a	-0.1 ± 0.0^a	<0.0001

WATER AND NUTRIENT MANAGEMENT AND SAVINGS IN CLOSED-LOOP AQUAPONIC TILAPIA/VEGETABLE PRODUCTION

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Integrated co-cultivation systems, such as aquaponics, have the potential to revolutionize aquatic food and protein production on a large scale and reinvigorate the aquaculture industry. When recirculating aquaculture systems (RAS) and controlled environment agriculture (CEA) are integrated together to form aquaponic systems, water and nutrient use efficiencies improve, costs per system decrease, environmental contamination decreases, and profitability can improve. The conservation of water and nutrient resources through re-use and re-tasking of initial fish feed is the central keystone for a sustainable domestic aquaculture business sector.

Auburn University School of Fisheries, Aquaculture, and Aquatic Sciences operates a closed-loop, commercial-scale aquaponics system producing tilapia and a variety of vegetables. The incoming water for the 50,000-gallon tilapia system is flowed by gravity from an 11-acre reservoir. Nutrients, mainly nitrogen eventually converted to nitrate, in the form of commercial fish feed are fed daily to 5,000 to 6,000 Nile tilapia, representing the first use of water and nutrients. Nutrient rich water is used a second time to water 2,880 meters squared of vegetables in a 96' x 30' commercial greenhouse. Excess water and nutrients are caught and used a third time to fertilize outdoor raised beds.

Water volumes and nitrate levels were measured prior to usage at each of the three usage points, 1) entering fish tank, 2) prior to watering plant greenhouse, 3) prior to watering raised beds. Water and fish feed were assigned an initial cost. All re-used and re-tasked water and nitrates were subtracted from the initial cost of both water and nutrients to ascribe savings to the overall system. Water use efficiencies were used to compare across production methods for both tilapia and vegetables separately. The following formula was used to calculate a water use efficiencies for plants and fish: Total water volume delivered (gal/m²) divided by marketable fruit or fish yield (number/m²), and expressed as gal/fruit. A water use efficiency for the combined system was calculated using the following formula: Total water volume delivered for the initial fish culture (gal/m²) divided by the combined total marketable yield of fruit and fish (kilogram/m²), and expressed as gal/kg.

Nutrient use efficiencies were estimated with the following formula: Total weight of nitrogen used (g/m²) divided by the marketable fruit yield (number/m²), and expressed as g/fruit. Nutrient use efficiencies were calculated separately for fish and plants and combined. Results were compared across production methods for both tilapia and vegetables.

The results show savings of both water and nutrients due to increased water and nutrient use efficiencies.

ORGANOLEPTIC CHARACTERISTICS AND SENSORY ACCEPTANCE OF FILLETS OF TILAPIA (*Oreochromis mossambicus*) CULTURED IN FRESHWATER OR SEAWATER

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Hawaiian Gold Tilapia (*Oreochromis mossambicus*) of the same family and age, fed the same diet, were grown in clear freshwater (0 ppt) or seawater (32 ppt) until near market size (~1 lb). The animals' fillets were then analyzed for proximate and fatty acids composition (Table 1), and their organoleptic characteristics (Table 2) were judged by a panel of 15 participants in a blind test. For the proximate composition of the tilapia fillets, only the ash content was significantly different between fresh and seawater. The fatty acids composition of the fillets reflected the diet composition, with no significant statistical differences. Also, no significant differences in organoleptic characteristics were detected by the panelists. These results demonstrated that fillet fatty acids composition will reflect the diet fatty acids profile, the ash content of the animals' fillet is negatively correlated to the water salinity due to osmoregulatory requirements, and finally, in clear water systems, the salinity does not affect the organoleptic characteristics of the fillet.

Table 1 – Proximate and fatty acids composition (g kg⁻¹) of the tilapia diet and fillets of animals reared in freshwater (FW) and saltwater (SW) (n=3, *P*>0.05).

Proximates	Diet	Fillet FW	Fillet SW	<i>P</i> -value
Dry matter	90.1	100.0	100.0	--
Crude protein	34.3	84.9	87.3	.085
Crude fat	8.5	3.9	3.6	.677
Ash	9.4	7.2*	5.8*	.008
Fatty Acids				
C8:0	0.72	0.00	0.01	.184
C10:0	0.63	0.00	0.00	--
C12:0	4.93	0.02	0.03	.251
C14:0	5.37	0.14	0.15	.932
C16:0	24.11	1.08	1.07	.949
C18:0	3.68	0.34	0.35	.764
C18:1n-9	6.43	0.33	0.29	.559
C18:2n-6	8.70	0.15	0.14	.786
C18:3n-3	2.41	0.03	0.03	.768
C20:4n-6	0.34	0.09	0.06	.060
C20:5n-3	1.98	0.10	0.09	.789
C22:6n-3	1.41	0.39	0.26	.172

Table 2 - Organoleptic test average scores (1 to 5) of cooked tilapia fillet grown in freshwater (FW) or seawater (SW) from 15 panelists (n=15) with Student's *t*-Test results (*P* < 0.05).

Characteristics	Fillet FW	Fillet SW	Student's <i>t</i> -Test Results
Scent/Aroma	3.8	3.7	3.73 ± 0.99; <i>t</i> (14) = .459; <i>P</i> = .650
Appearance	3.9	3.7	3.77 ± 1.08; <i>t</i> (14) = .664; <i>P</i> = .512
Taste	3.7	4.0	3.87 ± 1.33; <i>t</i> (14) = -.807; <i>P</i> = .426
Texture	3.9	3.7	3.80 ± 1.39; <i>t</i> (14) = .625; <i>P</i> = .537
Aftertaste	3.5	3.7	3.63 ± 1.21; <i>t</i> (14) = -.609; <i>P</i> = .548
Overall	3.9	3.8	3.87 ± 1.25; <i>t</i> (14) = .400; <i>P</i> = .692

SOME ASPECTS OF THE BIOLOGY OF *Rana esculenta* FROM MAKURDI, NIGERIA

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Frogs are eaten in many countries of Asia, Africa and Latin American; Some of this countries have engaged in the commercial trade of frogs and frogs product, supplying market in the European Union (EU) and the United States of America (USA) (Lannoo et al., 1994; Mohnke, 2011). While frog farming plays an increasing role in meeting the global demands for frogs products in other parts of the world, Nigerians however obtain frogs mainly from the wild to satisfy local and international demands. Generally, the exploitation of wild frogs to sustain trade (mainly *Fejervarya cancrivora* and *Limnonectes macrodon*) has been described by experts as far from sustainable (Mohnke, 2011; Bickford pers. comm. 2010; Laufer et al 2008), hence the need to intensify effort to attempt culture in captivity. Understand the biology of this animal is a key to culturing them in captivity which is the focus of this study. A total of 115 *Rana esculenta* were sampled from puddles, with their Morphometrics data recorded. Sex ratio of 2:1 were observed for female to males. Female frogs were found to be bigger than the males with maximum length of 23.29cm and weight of 120.1g. GSI was also higher in female (3.45) compared with male (0.31). However male were observed to be in better condition (K=1.03) compared to the females (K=0.91) High correlation were observed between most of the morphometric parameters measured during the study for both male and female. Female frog were observed to have a positive allometric growth while male frogs had negative allometric growth. More detail study on the reproductive biology of frog is needed to better understand their culture potential in captivity.

Table 1: Morphometric parameters of Frogs

Parameters	Gravid Female	Un-Gravid female	Male	V
B.Weight	81.89 ± 5.61 ^c	120.1 ± 11.2 ^a	101.37 ± 7.29 ^b	0
Snout vent	9.01 ± 0.19 ^c	10.07 ± 0.31 ^a	9.58 ± 0.19 ^b	0
T. length	20.16 ± 0.35 ^c	23.29 ± 0.44 ^a	21.50 ± 0.32 ^b	0
GSI	3.45 ± 0.14 ^a	ND	0.31 ± 0.03 ^b	0
K	0.91 ± 0.03 ^c	0.93 ± 0.03 ^b	1.03 ± 0.11 ^a	0

Mean in the same column with different superscripts differ (P<0.05)

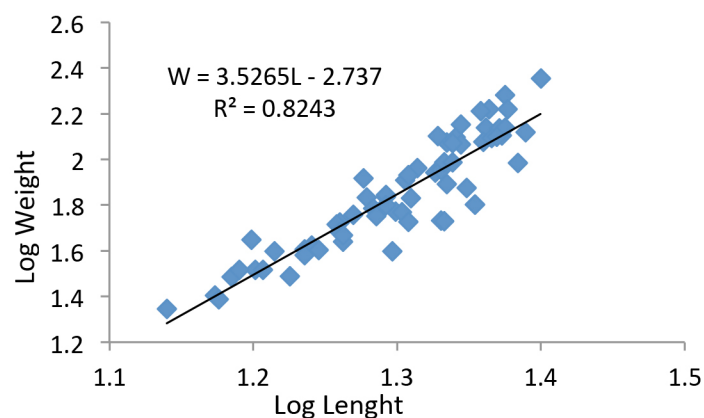


Figure 1: Length-Weight Relationship of Gravid Female frog

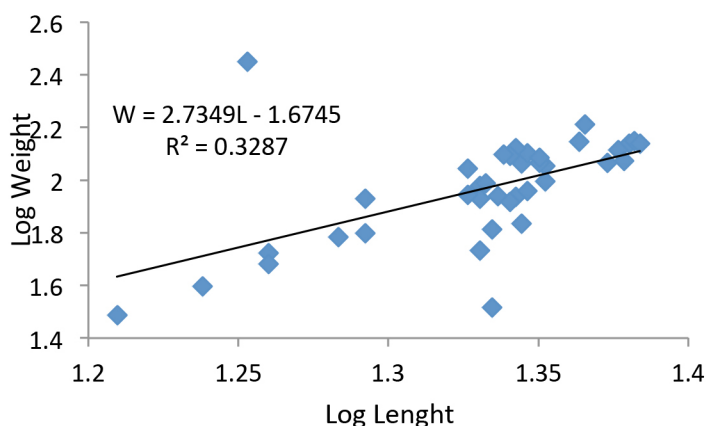


figure 3: Length-Weight Relationship of Male frog

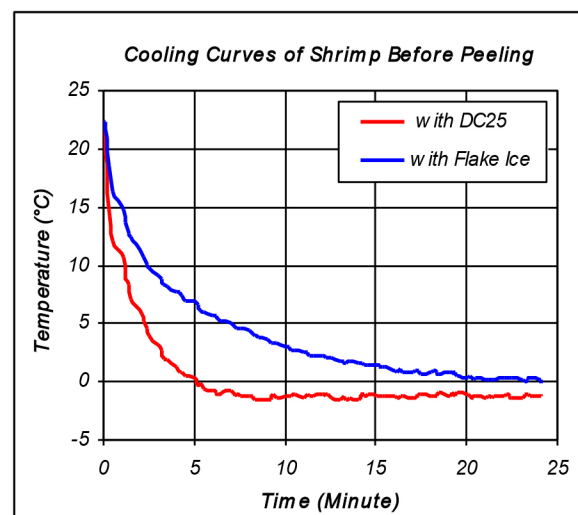
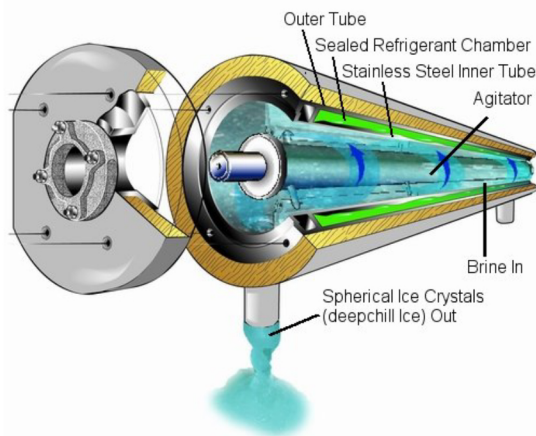
OPTIMIZING SHRIMP PRODUCTION USING DEEPCHILL - AN ADVANCED COOLING TECHNOLOGY FOR SEAFOOD PRESERVATION

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After fifteen years of research & development Sunwell Technologies of Toronto, Canada has created a powerful cooling medium commercially known as “DeepChill™ Variable-State Ice”. DeepChill has been recognized as the most advanced and effective means of product cooling available for food product processing and storage applications. It is comprised of billions of ice “micro-crystals” suspended in a liquid solution. These ice crystals are typically between 0.1 and 1 mm in diameter, have the flow characteristics of water and the cooling capacity of ice. It is an unparalleled cooling medium due to its high heat transfer surface when it contacts a product that is to be cooled. It can be utilized as a very liquid solution with a virtually undetectable ice concentration to a heavy “slush” containing a moderate ice content and ultimately even as a dry “crystal ice”.

This paper will present comparative test data on shrimp cooling using DeepChill. It will also present industry applications from client sites for aquaculture examples.



Primary uses of DeepChill™ technology for seafood cooling include:

- Fishing vessels
- Fish and seafood processing plants
- Aquaculture industry

USE OF ELECTROPHYSIOLOGICAL TECHNIQUES IN EVALUATING EFFECT OF TAURINE DEFICIENT DIET ON OLFACTORY FUNCTION IN SABLEFISH (*Anoplopoma fimbria*)

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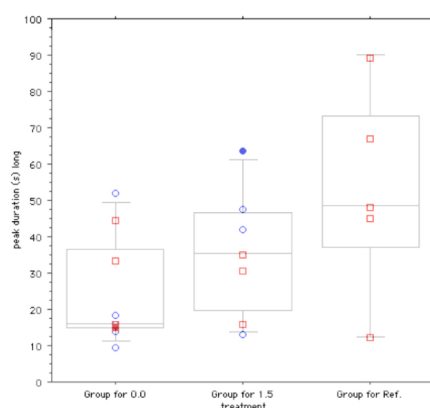
The sense of smell is important to fish, connecting them to the environment by relaying chemical information that plays a role in triggering behaviors associated with feeding, predator avoidance, reproduction and migration. Understanding how fish respond physiologically to different odors can help inform what components are beneficial in inducing these behaviors, and how factors such as diet and ambient water chemistry may affect the functioning of the olfactory system.

Electrophysiological techniques make it possible to measure physiological responses to various substances. Amino acids are essential nutrients and are important stimulants of searching and feeding behaviours. Sensitivity to different types of amino acids has been shown in fish and varies among species. Using the electro-olfactogram (EOG) and the electroencephalogram (EEG), it is possible to measure the neurological responses to these compounds at the receptor sites in the olfactory bulb.

Development of alternative, terrestrial plant-based feeds raises issues with the proper balance of essential amino acids. Taurine functions very similarly to an amino acid and is absent in plant proteins. The figure shows an example of how electrophysiological techniques can be used to study a diet lacking in taurine.

In this pilot experiment, Sablefish were fed diets with either 0% or 1.5% taurine, or a reference diet. Fish receiving the diet with 0% taurine tended to have EEG responses that were shorter in duration than diets with taurine.

Taurine plays a part in a variety of physiological functions. Altered olfaction responses from taurine deficiency may be an indicator of further systemic problems.



Scatterplot and box plots of EEG peak duration for 10^{-4} M L-Serine in different diet groups. ANOVA of one-way, log transformed EEG duration, $p = .12$.

A METHOD TO MEASURE BEHAVIORAL RESPONSE TO AN OLFACTORY STIMULUS

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A behavioral analysis method was developed to measure the effectiveness of various feed attractants as well as study the difference in olfactory-stimulated, behavioral response of fish fed alternative diets either lacking in taurine, or with taurine added.

Feeds are being developed that replace traditional fish-derived protein with plant protein. Currently, aquaculture feeds formulated without fish protein do not perform as well as those that are. Some additives however, may be able to increase food acceptance and growth. A multi-chambered, circular tank is divided into six segments by water currents so that test substances can be contained within one segment yet fish can move freely between them (Figure 1). Individual fish placed in this system were monitored by recording location in the tank segments before and after introduction of a putative chemoattractant. Presence of the fish in the exposure segment was then compared between pre and post-exposure recordings. Squid extract was used to test the effectiveness of the experimental design.

Without squid extract present, fish tend to swim in circles around the periphery of the tank. With the squid extract present, fish changed behavior and spent more time closer to the center of the tank. The amount of time fish spent in the central sections of the tank was compared with and without odor present (Figure 2.).

In conclusion, this radial tank design has been validated as an effective platform for behavioral attractant studies. A reference odorant has been identified that is comparable to already published attractant work. Experimental protocols have been established and the groundwork laid for further experiments with a variety of odorants.

Figure 1

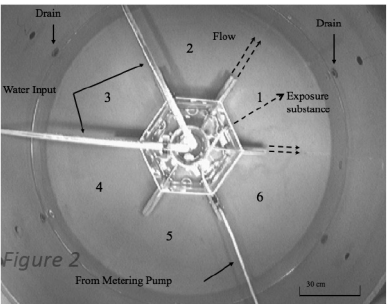
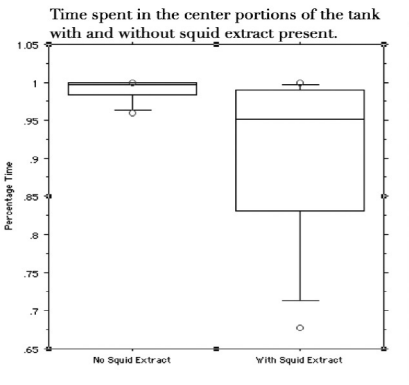


Diagram of experimental tank.



CATABOLISM OF L-4-HYDROXYPROLINE IN TISSUES OF HYBRID STRIPED BASS (*Morone chrysops* x *M. Saxatilis*)

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L-4-Hydroxyproline (Hyp) is one of several bioactive factors to improve the growth of fish. Traditionally, it has been considered to have limited nutritional value, but is now recognized as a substrate for the synthesis for glycine in mammals. At present, little is known about Hyp utilization in fish species. Therefore, the present study was conducted with hybrid striped bass to investigate Hyp metabolism in various tissues. Slices of skeletal muscle, liver, proximal intestine, kidney, stomach, heart, spleen, gill and brain obtained from juvenile hybrid striped bass were incubated in 26 °C for 2 h in 1 ml oxygenated Krebs-Henseleit bicarbonate buffer (pH 7.4, with 5 mM D-glucose) containing 0, 0.5, 2 or 5 mM Hyp. After a period of 2 h incubation, 200 µl of 1.5M HClO₄ was added into the incubation medium to stop the reaction, and the acidified solution was neutralized by 100 µl of 2M K₂CO₃. The extract was analyzed for amino acids using our HPLC method.

Results indicated that glycine was produced from Hyp by fish skeletal muscle, liver, intestine, heart, kidney, spleen, and stomach. Small amounts of aspartic acid, glutamate, serine, and glutamine were generated from Hyp from these tissues. In contrast, fish gill and brain had little or no ability to convert Hyp into any amino acids. Our findings provide a new metabolic pathway for utilization of collagen and Hyp by hybrid striped bass (carnivores).

Table 1. The concentration of glycine in tissue plus medium after 2 h incubation

	After 2 h incubation (nmol/mg tissue)				
	No incubation	0mM Hyp	0.5mM Hyp	2.0mM Hyp	5.0mM Hyp
Muscle	3.02	---	4.62	10.88	12.44
Liver	2.76	3.67	21.75	37.52	34.53
Intestine	0.94	9.72	25.60	33.50	38.50
Heart	1.20	2.36	2.92	2.67	3.43
Kidney	1.02	6.19	8.13	11.05	10.43
Spleen	2.42	8.89	18.45	20.62	18.66
Stomach	0.57	4.52	4.31	4.32	8.08
Gill	1.38	1.64	---	1.99	1.78
Brain	0.87	1.29	1.18	1.49	---

OXIDATION OF ENERGY SUBSTRATES IN ENTEROCYTES OF HYBRID STRIPED BASS (*Morone chrysops* x *M. Saxatilis*)

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In most mammals, enterocytes are major users of glutamine and glutamate to product ATP, citrulline and arginine. It is unknown whether this is also true for fish species. The present study was conducted with enterocytes from hybrid striped bass (HSB) to determine oxidation of energy substrate. Isolated enterocytes were incubated at 26 °C for 30 min in in oxygenated Krebs–Henseleit bicarbonate buffer (pH 7.4, with 5 mM d-glucose) containing 2 mM L-[U-¹⁴C] glutamate, L-[U-¹⁴C] glutamine, L-[U-¹⁴C] alanine, L-[U-¹⁴C] aspartic acid, L-[U-¹⁴C] leucine, or L-[U-¹⁴C] palmitate, or a trace amount of D-[U-¹⁴C] glucose. In parallel experiments, the incubation medium contained in a mixture of unlabeled substrates [glutamate, glutamine, alanine, aspartic acid and leucine (2 mM each) plus 5 mM D-glucose] with one tracer. ¹⁴CO₂ was collected to calculate the rates of substrate oxidation and ATP production from those nutrients.

Results indicated that in the presence of glucose or a mixture of substrates, the rates of oxidation of glutamate and ATP production from glutamate by the enterocytes were much higher than those for other amino acids, palmitate and glucose. Compared with palmitate and glucose, fish enterocytes preferred to use amino acids to produce ATP. There was no synthesis of citrulline or arginine from glutamate or glutamine in these cells. We conclude that glutamate was the primary energy substrate in HSB enterocytes. Together, amino acids contributed to about 87% of ATP production in the presence of a mixture of substrates. Our novel findings aid in better understanding of protein nutrition in fish.

Table1: Production of CO₂ and ATP from the oxidation of a nutrient by enterocytes of HBS in the 5Mm unlabeled glucose or a mixture of unlabeled substrate

Medium mixture	Nutrient						
	[U- ¹⁴ C]Glutamate	[U- ¹⁴ C]Glutamine	[U- ¹⁴ C]Alanine	[U- ¹⁴ C]Asparagine	[U- ¹⁴ C]Leucine	[U- ¹⁴ C]Palmitate	[U- ¹⁴ C]Glucose
	(2mM)	(2mM)	(2mM)	(2mM)	(2mM)	(2mM)	(5mM)
CO₂ production for single substrate¹	9.16 ± 0.58 ^a	1.04 ± 0.04 ^c	0.81 ± 0.06 ^{cd}	1.00 ± 0.09 ^c	0.08 ± 0.01 ^e	1.59 ± 0.13 ^b	0.66 ± 0.02 ^d
ATP production for single substrate	41.23 ± 2.61 ^a	4.69 ± 0.20 ^c	4.03 ± 0.28 ^{cd}	3.76 ± 0.32 ^{cd}	0.47 ± 0.04 ^e	10.51 ± 0.84 ^b	3.31 ± 0.10 ^d
CO₂ production for mixture substrate²	4.12 ± 0.2 ^a	0.23 ± 0.02 ^d	0.51 ± 0.02 ^c	0.75 ± 0.07 ^c	0.06 ± 0.01 ^e	0.53 ± 0.03 ^b	0.08 ± 0.01 ^e
ATP production for mixture substrate	18.55 ± 0.90 ^a	1.02 ± 0.07 ^d	2.54 ± 0.11 ^c	2.82 ± 0.25 ^c	0.36 ± 0.07 ^e	3.54 ± 0.20 ^b	0.38 ± 0.04 ^e

Data, expressed as nmol CO₂/10⁶ cells after 30 mins incubation and nmol ATP/10⁶ cells after 30 mins incubation are mean ± SEM, n=9

¹Oxidation of a labeled nutrient in the presence of 5mM unlabeled glucose

²Oxidation of a labeled nutrient in the presence of a mixture of unlabeled substrate (2mM Glutamate, 2mM Glutamine, 2mM Alanine, 2mM Aspartic acid, 2mM Leucine, 2mM Palmitic acid and 5mM Glucose)

^{a-e} Within a row, means not significant difference (P<0.05), as analyzed by one-way ANOVA

GREEN FEED IN THE MARINE FISH FARMING

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Global catch fishery is said to be oppressed to its limit (Boyd & Schmittou, 1999), further implying aquaculture might be the only solution to the world demand for fishery products. The applied term aquaculture in this paper refers to the one used by NOAA (2008); breeding, rearing, and harvesting of plants and animals in all kind of water environments, including but not limited to ponds, rivers, lakes, and the ocean.

According to Shamshak & Anderson (2008, p. 74) aquaculture has over the past 20 years been the fastest growing food sector with an average annual growth rate of 8.7%. It further represents approximately 37 % (Shamshak & Anderson, 2008, p. 73) of total fisheries production worldwide. But even though aquaculture has taken off, the practice has its critics. The industry must counter criticism about the lack of sustainability. If the industry is able to successfully do this, the farming technique can more easily fulfill its potential role as a world food supplier (Boyd & Schmittou, 1999). But first the aquaculture industry needs better environmental management for a continued growth.

One practice that needs to be curtailed is the choice of using unsustainable (limited) and expensive fishmeal and fishoil. The challenge is to identify more environmentally friendly and cheap substitutes for the unsustainably fishmeal and fishoil. Several trials have been made to reduce the quota of unsustainable fishmeal in farming the deep blue, and where e.g. feed has been substituted to one extend by soybeanmeal. Nonetheless, it is crucial that the substitutes for fishmeal and fishoil maintain both the quality and quantity of production that the original products achieve. Also, further importance and essentials must be paid to make these practices transparent to the industry's stakeholders.

Pittenger et al., (2007, p. 98) has shown that advances in both feed formulation and feed management on a farm level have led to increased fish growth, reduced production costs, and reduced feed conversion ratios but where research is still in progress to continue developing alternative feed ingredients. Of importance is to note that even though progress has been made in identifying substitutes for fishmeal and fish oil, there is currently no commercially available product that can completely substitute for fishmeal and fishoil (Ibid).

A fish farm needs to efficiently deal with the environmental issues it causes, or the effects will be deleterious. This thesis shows that less use of fishmeal (substituted by Soybean Protein Concentrate) can improve waterquality in some parameters used in this thesis. Further this thesis shows how sustainable benchmarks (with respect to watermetrics) efficiently can be managed and communicated to the industry's stakeholders by business managers.

A farm managed with environmental awareness and a willingness to share the experiences in the process of finding a more sustainable production method (also referred as the case farm in this thesis) is; Kona Blue Water Farm, HI, USA.

IMMUNOSTIMULANT AND DIGESTIVE ENZYME ACTIVITY OF POMEGRANATE PEEL (*Punica granatum*) AQUEOUS METHANOLIC EXTRACT IN RAINBOW TROUT FINGERLINGS (*Oncorhynchus mykiss*)

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The immune stimulating, digestive enzyme activity and hematological effects of pomegranate peel (*Punica granatum*) aqueous methanolic extract in rainbow trout fingerlings (*Oncorhynchus mykiss*) was evaluated. Four different concentrations of pomegranate peel (0 (Control), 250, 500 and 1000 mg kg⁻¹) were added to the basal diet and the fish were fed the diet during 60 days. Every 20th day of the study, immune responses of fish were determined. In the study respiratory burst was significantly decreased in group 500 and 1000 mg kg⁻¹ compared to control ($P < 0.05$). No differences was observed between control and 250 mg kg⁻¹ group on 20th day of the study. On 40th day of the study respiratory burst was significantly increased in group 250 mg kg⁻¹. At the end of the study the highest value of respiratory burst was observed in 500 mg kg⁻¹ group. Myeloperoxidase activity (MPO) was increased in group 250 mg kg on 20th day of the study. At the end of the study all experimental groups MPO activity was significantly decreased compared to control. Lysozyme activity was increased in all experimental group compared to control. At the end of the study, amylase was significantly decreased in all experimental groups compared to control. Pepsin was increased 250 mg kg⁻¹ group but not in the other experimental groups. Trypsin activity was also decreased in group 250 mg kg⁻¹ and the other groups showed no differences compared to control. WBC count was significantly decreased in 1000 mg kg⁻¹ group. HGB was increased in 250 mg kg⁻¹ group. Generally no differences was observed in all the other group's hematological values FCR was decreased in group 500 mg kg⁻¹ but SGR was also significantly decreased compared to control. The results indicate that the humoral immune responses of pomegranate aqueous methanolic extract showed a stimulator effects.

RECOMBINANT SUBUNIT VACCINES AGAINST PISCINE FRANCISELLOSIS

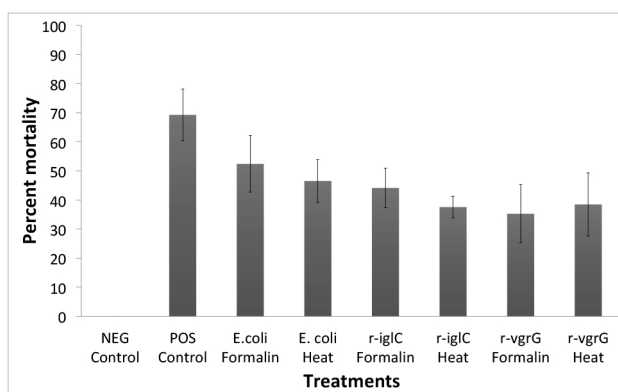
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Francisella noatunensis subsp. *orientalis* (*Fno*) is an emerging bacterial pathogen of marine and fresh water fish with worldwide distribution. Fish francisellosis is a severe sub-acute to chronic granulomatous disease with high mortalities and high infectivity rates in cultured and wild fish. To date, there is no approved vaccine for this widespread emergent disease. To better characterize immunodominant *Fno* antigens, proteomic analyses was performed using serum collected from laboratory challenged Nile tilapia (*Oreochromis niloticus*). Western blot analysis consistently detected antigens between 20-30 kDa. Further analysis identified 19 proteins including both housekeeping and virulence-related proteins. We hypothesized that some of the identified proteins could be used as recombinant subunit vaccines against piscine francisellosis. In this study, the *Fno iglC*, and *vgrG* were cloned into an *Escherichia coli* expression vector, inactivated with formalin or heat, and used to immunized Nile tilapia fingerlings via intracoelomic injection. Thirty days post-immunization, fish were challenged with wild-type *Fno*. Naive tilapia vaccinated with r-*iglC*, and r-*vgrG*, and subsequently challenged with wild type *Fno* presented lower mortality when compared to non-vaccinated controls. This information demonstrates that defined recombinant subunit vaccines have the potential to be used as a safe prophylactic practice against piscine francisellosis.

Mean percent mortality of tilapia vaccinated with different vaccine treatments by intracoelomic injection, or mock vaccinated with PBS (Controls) and challenged 4 weeks later with WT *Francisella noatunensis* subsp. *orientalis*. Fish were vaccinated with: formalin inactivated *Escherichia coli* vector, heat inactivated *E. coli* vector, formalin inactivated *E. coli* vector expressing recombinant *iglC*, heat inactivated *E. coli* vector expressing recombinant *iglC*, formalin inactivated *E. coli* vector expressing recombinant *vgrG*, or heat inactivated *E. coli* vector expressing recombinant *vgrG*. Four weeks post-immunization fish were challenged with 10^5 CFU/ml of WT *Fno*. Mean percent mortality was calculated 30 days post-challenge with WT. Each bar represents the mean percent mortality \pm standard error of three tanks (15 fish/tank).



TROPICAL ORNAMENTAL MARICULTURE TECHNICIAN PROGRAM AT FLORIDA KEYS COMMUNITY COLLEGE: A CASE STUDY FROM CAMPUS TO CAREER

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Mariculture has revolutionized how people obtain food, manage the environment, and mitigate species over-exploitation. At Florida Keys Community College, I was enrolled in the Tropical Ornamental Mariculture Technician (TOMT) Certificate program, which taught me how to breed, raise, and sustain an array of ornamental species, with a focus on the clownfish *Amphiprion ocellaris*. The program led me to an internship at American Mariculture Inc., an aquaculture farm that provides *Litopenaeus vannamei* (Pacific White Shrimp) to a variety of restaurants and consumers.

The internship added a level of professionalism to my experience by enabling me to expand my knowledge and skills in a food aquaculture setting. Beyond the classroom, it strengthened my understanding and speaking of Spanish, enhanced leadership and management skills, heightened understanding of genetics, data collecting, and recording, and conformed skills and duties to a large scale food operation.

The knowledge and skills I gained from the TOMT classes (Table 1) and the Aquaculture Learning Laboratory at FKCC directly fostered my success as an intern at AMI shrimp farm. The two experiences vastly expanded my education, and ultimately molded me into a competent mariculture technician in a diverse array of settings. As a result of my hard work and academic foundation, I was offered a full time position at AMI when I graduate in December 2017. I will be accepting this offer along with advancing my education by concurrently pursuing a Bachelors degree at a local University.

TABLE 1. TOMT classes taken at FKCC and knowledge/skills obtained.

Survey of Mariculture	Best Management Practices	Mariculture Systems and Design	Nutrition for Mariculture	Diseases and Parasites in Aquaculture	Tropical Ornamental Mariculture
<ul style="list-style-type: none"> • Introduction to a variety of systems • Understanding fundamentals • Production scope • Sustainability • Field trips to diverse facilities 	<ul style="list-style-type: none"> • Biosecurity • Laws, rules, standard procedures, & their benefits • Business management strategies • Environmental responsibility 	<ul style="list-style-type: none"> • High & low density systems • Diversity of systems & fundamental components • Hydrodynamics • Filtration 	<ul style="list-style-type: none"> • Variety of live feeds & care • Enrichments • Development, ingredients, and feed properties • Ontogenetic transitions 	<ul style="list-style-type: none"> • Prevention • Diagnostics • Identification • Treatment • Water Quality • Pathogens • Clinical workup 	<ul style="list-style-type: none"> • Aquatic animal husbandry • Grow out & early life stages • Variety of tropical species

GROWTH PERFORMANCE AND PROXIMATE COMPOSITION OF *Clarias gariepinus* REARED ON MEAL USING CHICKEN EGG SHELL AS REPLACEMENT FOR DI-CALCIUM PHOSPHATE

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The growth performance and proximate composition of *Clarias gariepinus* reared on chicken eggshell meal as replacement for di-calcium phosphate (DCP) were investigated for 12 weeks. One hundred and fifty juveniles were fed 0% (control), 25%, 50%, 75% and 100% replacement levels meal. At the end, the diet with the highest mean weight gain (MWG) was 25% diet (35.54 ± 5.69) and lowest was 75% diet (23.72 ± 2.60). Highest protein efficiency ratio (PER) was 25% diet (1.48 ± 0.18) while 75% diet had lowest value (1.08 ± 0.04). In the feed conversion ratio (FCR), 100% diet had highest ratio (1.7450 ± 0.1217) while 0% diet had lowest value (1.5518 ± 0.0411). Proximate analysis showed that 25% diet had the highest crude protein (33.38%) value and 0% diet had lowest (24.15%). In conclusion, DCP could be substituted with chicken egg shell in *C. gariepinus* feed up to 25% level of substitution for efficiency and without negative effects on the growth of the fish.

In fish farming, nutrition is critical because feed represents 40-50% of the production costs (Allan *et al*, 2000). It is, therefore, very crucial to find an alternative (Jauncey and Ross, 1982) to reduce feeding cost, and to make aquaculture a viable and attractive venture. However, feed additives such as DCP can be sub-classified as therapeutic or prophylactic, growth promoters, simple chemical additives, palatability enhancers and non-nutritional additives (Wilson and Brigstocke, 1981). The feed additive, shells of chicken egg which was used in this study can be classified as a nutritive additive which has the capacity to add calcium to fish. Calcium which is important in fish diet helps in bone formation. Hence, it can be made readily available for fish in their feed (Wilder, 2000). Proximate composition generally comprises the estimation of moisture, protein, fat and ash contents of the fresh fish body.

Results obtained from the growth response and nutritional utilization (Table 1) and proximate composition (Table 2) are below.

Table 1 : Growth and nutrient parameters of the fish in various tanks.

Parameters	T ₀	T ₂₅	T ₅₀	T ₇₅	T ₁₀₀
Final weight	52.11±3.68 ^a	56.51±6.22 ^a	47.16±8.0 ^a	39.82±3.55 ^b	48.10±9.23 ^a
Initial weight	16.01±3.65 ^a	20.91±4.24 ^a	17.40±3.40 ^a	16.10±1.82 ^a	20.41±4.84 ^a
MWG	34.05±2.01 ^a	35.54±5.69 ^a	29.76±5.00 ^a	23.72±2.60 ^b	27.62±4.50 ^a
TFI	52.11±3.09 ^a	56.20±6.18 ^a	46.61±7.92 ^a	40.90±1.41 ^b	48.16±8.06 ^a
AFI	0.7539±0.441 ^a	0.8029±0.0961 ^a	0.6659±0.1151 ^a	0.5843±0.0201 ^b	0.6880±0.1154 ^a
SGR	1.530±0.2154 ^a	1.429±0.2011 ^a	1.4289±0.9601 ^a	1.296±0.1163 ^b	1.2314±0.0901 ^b
RGR	193.98±42.35 ^a	175.10±52.52 ^a	172.31±18.23 ^a	148.31±19.78 ^b	137.09±14.98 ^b
FCR	1.5518±0.0411 ^a	1.5930±0.1680 ^a	1.5725±0.1111 ^a	1.7352±0.1550 ^b	1.7450±0.1217 ^b
PI	24.30±0.1513 ^a	24.04±2.04 ^a	24.31±2.11 ^a	22.02±1.95 ^b	21.85±1.50 ^b
PER	1.39±0.05 ^a	1.48±0.18 ^a	1.23±0.21 ^a	1.08±0.04 ^b	1.27±0.21 ^a

Means with the same superscripts along the row are not significantly different ($p > 0.05$). PI = Protein intake, PER = Protein efficiency ratio, MWG = Mean weight gain, TFI = Total feed intake, AFI = Average feed intake, SGR = Specific growth rate, RGR = Relative growth rate, FCR = Feed Conversion Ratio

Table 2 : Proximate analysis of *Clarias gariepinus* fed with chicken egg shells

	Tank	Moisture(%)	Ash	Crude Protein(%)	Crude fibre(%)	Fat(%)
T0	Control	46.50	5.70	24.15	1.98	2.79
T1	25%	45.30	6.20	33.38	1.20	1.01
T2	50%	44.20	6.00	27.69	1.12	2.88
T3	75%	42.40	7.30	31.56	1.42	2.92
T4	100%	43.60	5.90	28.81	1.06	2.80

GENETIC CHARACTERIZATION OF WILD AND HATCHERY-PRODUCED *Ostrea lurida* IN PUGET SOUND, WA

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Body: Efforts to restore the Olympia oyster (*Ostrea lurida*) along its historic distribution in the Puget Sound, WA include population enhancement with hatchery-produced seed. Recent genotype-by-sequencing data suggest genetically distinct populations between sub-basins, and very high allelic diversity, with over 10,000 single nucleotide polymorphism (SNP) loci. In an effort to minimize genetic selection within the hatchery, current practices include collecting broodstock annually, breeding via mass spawn within genetically distinct populations, collecting brooded larvae over an extended period of time, and measuring genetic diversity. Results from 2010 and 2011 microsatellite data informed these hatchery practices. This project examines new data from 2014 and 2015 cohorts, focusing on patterns of allelic diversity between wild broodstock and the hatchery-produced F1 individuals. These results and future annual genetic testing will continue to inform hatchery practices for restoration groups and commercial aquaculture facilities that produce this native oyster.

NUTRITIONAL PHYSIOLOGY OF MAHI-MAHI (*Coryphaena hippurus*) AND THE IMPACTS OF FEEDING ON SWIM PERFORMANCE

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The mahi-mahi (*Coryphaena hippurus*) is a species of high economic and ecological value found throughout the world's tropical and subtropical oceans. Demand for this species as table fare has continued to rise over the past decade, while wild-catch levels fluctuate on seasonal and annual bases. Such conditions have attracted increased attention on development of commercially-viable aquaculture techniques and technology for mahi-mahi. As a fast-growing highly fecund migratory pelagic species, mahi-mahi must balance multiple metabolic demands concurrently to survive in the wild. Research has shown that this species consumes substantial quantities of food during the juvenile and adult stages of growout in captivity, in some cases over 20% bodyweight in single feeding events. However, to date there is no data on the nutritional physiology of adult specimens of this species. Such information is essential to not only understanding the overall physiology of the species but also to optimizing the culture of this species in captivity. Whole fish and squid are commonly used for feeding mahi-mahi broodstock in captivity, while manufactured pelletized diets have also demonstrated effectiveness in pilot-scale commercial trials of juveniles and adults. Given the substantial contribution of feed costs to overall operating expenses in fed aquaculture enterprises, it is critical to understand the nutritional physiology of species being cultured. Whole-animal physiological studies examining the metabolic costs associated with digestion, typically referred to as postprandial metabolism or specific dynamic action (SDA), provide high-resolution data on diet-specific metabolic responses that can be used for improving feeding efficiencies and diet formulations. Results from studies examining the postprandial metabolic response of feeding both natural diets (i.e. forage fish/squid) and manufactured pelletized diets will be presented, along with results investigating the impacts of feeding on swim performance in this species. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

TRADE MARGINS FOR NORWEGIAN SEAFOOD EXPORTS

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Seafood trade continues to increase rapidly, and is an important driver for the growth in aquaculture production. However, while it is well known that markets differ with respect to ability to pay, culture, preferences and a number of other factors, little is known about what makes some firms and products successful in specific markets, and why some firms export to only a few countries while others export to many.

In this paper, we employ detailed export statistics for the 24 most valuable seafood products that together makes up 94 % of the total export value of fish and crustaceans, molluscs and other aquatic invertebrates from Norway, the world's second largest seafood exporter. We decompose the export value of each product category into several components, and investigate how the components correlates with different trade costs. An augmented gravity model is employed to estimate the effects from different trade costs on each trade margin.

We find that total export value for fresh products are more negatively affected by trade costs than export value of conserved products. The negative effect from increased transportation costs is twice as large for fresh products as for conserved products. For fresh products, this negative effect is mainly driven by a reduction in the number of shipments as well as by smaller shipments. Further we show that per-shipment costs have a different effect on fresh and conserved products. Increased per-shipment costs results in smaller shipments of fresh products, and in larger shipments of conserved products. The perishable nature of fresh products is important for how fresh products are affected by traditional trade costs. Conserved products can be shipped in large bulks without loss of quality, while fresh products need more timely delivery to the destination market to ensure the highest quality.

PERACETIC ACID: THE LONG ROAD TO INTRODUCTION OF THIS DISINFECTANT INTO U.S. AQUACULTURE

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Peracetic acid (PAA) is a promising disinfectant for biosecurity in the US aquaculture industry to prevent disease outbreaks from fish pathogens. PAA is a stabilized mixture of acetic acid, hydrogen peroxide and water that breaks down quickly to water and vinegar. It has replaced chlorine in some industries. The U.S. Environmental Protection Agency (EPA) first registered PAA as an antimicrobial in 1985 for indoor use on hard surfaces (e.g., hospitals). Registrations have been expanded to include: sanitation in food/beverage plants, agricultural premises, wineries/breweries, greenhouse equipment, animal housing, as well as commercial laundries, prevention of bio-film formation in pulp/paper industries, and as a disinfectant for wastewater treatment. PAA is approved for use in Denmark, Germany and Norway as a water disinfectant, and our labs international collaborations have studied its effectiveness to many pathogens including *Ichthyophthirius multifiliis*, *Saprolegnia* spp., and various bacteria. On June 26, 2017, the EPA accepted registration of a commercial PAA compound (VigorOx® SP-15 Antimicrobial Agent) for: 1) sanitizing surfaces of harvesting equipment used in the aquaculture industry, and 2) cleaning and disinfecting fish culture tanks and raceways when water is drained and fish are not present. This is the first approved use of PAA in U.S. aquaculture! We will discuss this long road to an EPA aquaculture registration and provide data on its uses in the aquaculture industry.

PERACETIC ACID: A NEW TOOL FOR FACILITY BIOSECURITY

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Peracetic acid (PAA) is a promising disinfectant for biosecurity in the US aquaculture industry to prevent disease outbreaks from fish pathogens. PAA is a stabilized mixture of acetic acid, hydrogen peroxide and water that breaks down quickly to water and vinegar. It has replaced chlorine-based products in some industries. The U.S. Environmental Protection Agency (EPA) first registered PAA as an antimicrobial in 1985 for indoor use on hard surfaces (e.g., hospitals). Registrations have been expanded to include: sanitation in food/beverage plants, agricultural premises, wineries/breweries, greenhouse equipment, animal housing, as well as commercial laundries, prevention of bio-film formation in pulp/paper industries, and as a disinfectant for wastewater treatment. PAA is approved for use in Denmark, Germany and Norway as a water disinfectant, and our labs international collaborations have studied its effectiveness to many pathogens including *Ichthyophthirius multifiliis*, *Saprolegnia* spp., and various bacteria. On June 26, 2017, the EPA accepted registration of a commercial PAA compound (VigorOx® SP-15 Antimicrobial Agent) for: 1) sanitizing surfaces of harvesting equipment used in the aquaculture industry, and 2) cleaning and disinfecting fish culture tanks and raceways when water is drained and fish are not present. This is the first approved use of PAA in U.S. aquaculture! We will discuss this long road to an EPA aquaculture registration and provide data on its uses in the aquaculture industry.

DIFFERENCES IN GASTROINTESTINAL GLUCOSE TRANSPORTERS BETWEEN CARNIVOROUS RAINBOW TROUT *Oncorhynchus mykiss* AND OMNIVOROUS NILE TILAPIA *Oreochromis niloticus* IN RESPONSE TO PULSE CROPS

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Due to rising cost in fishfeed, fishmeal could be at least partially replaced with starch instead of proteins. Our group has investigated the use of pulses which have a high content of slowly-digestible carbohydrates and protein. High starch diets are commonly used in omnivorous species, but this may be detrimental in carnivorous species. There is a paucity of studies to support this concern and none investigating basic mechanisms of carbohydrate absorption in any fish species. Therefore, the present study was conducted to identify glucose transporters in different gastrointestinal regions in carnivorous Rainbow trout (*Oncorhynchus mykiss*) versus omnivorous Nile tilapia (*Oreochromis niloticus*). Transporters were characterized in fish fed commercial diet (baseline study) or in fish after an 8-week growth trial comparing diets with pulse or corn starch inclusion (0-200, or 0-300 g/kg inclusion, respectively, for trout and tilapia).

Since pulse starches are digested primarily to glucose monomers, before absorption, we characterized glucose absorption through the sodium-dependent glucose transporters (SGLTs) using Ussing chamber techniques, qRT-PCR, and pharmacological inhibitors in pyloric caeca/proximal intestine, midgut/mid-intestine, and high-gut from both trout and tilapia.

In trout, pyloric caeca and midgut, baseline glucose transport kinetics were similar to mammalian SGLT1 (a high-affinity, low-capacity transporter) which was confirmed with inhibitors and qPCR. In contrast, atypical kinetics combined with qPCR data suggests a novel SGLT transporter (low-affinity, high-capacity) in trout hind-gut. In contrast to trout, the kinetics, inhibitor, and qPCR data in all three tilapia intestinal sections resembled a combination of SGLT1- and SGLT2-like transporters. In the growth trial, both fish species grew equally well or better with high starch inclusion, but glucose transport physiology was dramatically altered or non-detectable throughout the gut. This study confirms trout and tilapia grow well with high pulse starch inclusion. However, further work is needed to better understand how high starch diets and glucose is handled in these two fish species.

Baseline Kinetics for sodium-dependent glucose transport

Tissue	Rainbow Trout		Nile Tilapia	
	V _{max} ($\mu\text{A}/\text{cm}^2$)	K _m (mM)	V _{max} ($\mu\text{A}/\text{cm}^2$)	K _m (mM)
Pyloric Caeca/Proximal Intestine	$1.8 \pm 0.20^{\text{A,B}}$	$2.7 \pm 0.66^{\text{A}}$	$33.1 \pm 1.0^{\text{A}}$	$1.4 \pm 0.3^{\text{A}}$
Midgut	$1.5 \pm 0.01^{\text{A}}$	$1.4 \pm 0.03^{\text{B}}$	$37.9 \pm 1.3^{\text{A}}$	$1.9 \pm 0.4^{\text{A}}$
Hind-gut	$2.8 \pm 0.08^{\text{B}}$	$14.8 \pm 0.89^{\text{C}}$	$53.5 \pm 2.3^{\text{A}}$	$4.0 \pm 0.7^{\text{A}}$

V_{max} (μA) and K_m (mM) (\pm SEM) represented for each tissue type in Rainbow Trout (colon: n=26, midgut: n=18, pyloric caeca: n=12) and Nile Tilapia (n=36). Letters represent significance as $p < 0.05$.

POTENTIAL FOR POLYCULTURE OF TILAPIA *Oreochromis niloticus* AND FRESHWATER PERCH *Anabas testudineus* WITH PANGASIOUS CATFISH *Pangasius hypophthalmus* IN THE HYPOSALINE WATERS OF SOUTHERN BANGLADESH

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Pangasius catfish (*Pangasius hypophthalmus*), tilapia (*Oreochromis niloticus*), and freshwater perch (*Anabas testudineus*; locally known as *Koi*) are three of the most successful freshwater aquaculture species in Bangladesh. We previously established that *Pangasius* can be cultured in hyposaline waters, allowing for use of coastal waters affected by salinity encroachment. In the present study, we assessed the potential for culturing *Pangasius* with the higher valued tilapia and freshwater Koi in hyposaline waters of coastal Bangladesh as a means of improving the food security and economic viability of these salinity affected region's poor communities. The experiment was conducted in 16 farmer's ponds in the Patuakhali district of Bangladesh at a salinity of 5-6 ppt. Four culture types were examined (Table 1) with fish being fed commercial pellet feed (CP 28-30%) twice daily at a total rate of 10% down to 3% body weight per day.

The study revealed that Koi can be cultured in saline waters as high as 6 ppt and that the highest yields could be obtained through solo culture of *Pangasius* (T1). However, due to the higher market value of tilapia and greater increases in body weight of both *Pangasius* and tilapia, the largest profit came from the co-culture of these two species (T2). This was followed by co-culture of *Pangasius* with Koi (T3). Conversely, the lowest yield and profit resulted from the culture of all three species together (T4). Thus, the results suggest that tilapia is the optimal candidate for polyculture with *Pangasius* in hyposaline waters with a *Koi* polyculture providing the second greatest profit margin. Adoption of such aquaculture practices in coastal Bangladesh could increase earnings and improve the livelihoods of the people inhabiting these areas.

(Supported by the AquaFish Innovation Lab – USAID)

Table 1. Production parameters for cultures of *Pangasius*, Tilapia, and *Koi*.

Parameters	T1	T2		T3		T4		
	Pangasius	Pangasius	Tilapia	Pangasius	Koi	Pangasius	Tilapia	Koi
Stocking (/m ²)	4	2	2	2	2	2	1	1
Initial weight (g)	6.52±0.03	6.52±0.03	0.54±0.01	6.52±0.03	0.50±0.02	6.52±0.03	0.54±0.01	0.50±0.02
Final weight (g)	637±6.41	687±6.60	282±6.12	646±8.12	169±6.50	672±7.63	276±4.21	123±4.85
Weight gain (g)	630±3.66 ^a	680±2.39 ^b	282±3.87 ^c	642±1.45 ^a	171±1.20 ^c	665±2.29 ^a	275±1.94 ^c	122±2.40 ^c
Survival rate (%)	94.38±0.95 ^a	94.58±0.41 ^a	90.83±1.10 ^a	94.17±0.41 ^a	87.92±1.10 ^b	92.50±1.14 ^a	87.5±1.14 ^b	85.83±2.21 ^c
SGR (% per day)	3.05±0.003 ^a	3.11±0.003 ^b	4.23±0.009 ^c	3.07±0.003 ^a	3.89±0.006 ^c	3.09±0.003 ^{ab}	4.21±0.006 ^c	3.67±0.011 ^c
FCR	1.35	1.30		1.33		1.33		
Yield (kg/ha)	22292±141 ^a	12837±62	5064±123	12065±52	2980±53	6138±87	2431±12	1022±13
		17901 ± 66 ^b		15045 ± 73 ^c		9590±65 ^d		
Profit (USD/ha)	3607.37 ^a	6259.76 ^b		4126.71 ^a		2852.53 ^c		

WASTEWATER & TEXTILE INDUSTRY EFFLUENT TOXICITY: DECLINING TRENDS IN LIPID PROFILE IN THE GONADS OF *LABEO rohita*

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The study was designed to investigate the comparison of lipid profile in the gonads (ovaries/testes) of *Labeo rohita* exposed to wastewater and textile industry effluents. Fingerlings of major carps were procured from fish seed hatchery and acclimatized to laboratory conditions for two weeks prior to experiments. After acclimatization fish were divided into three groups one for control and others two as experimental. LC50 for 96 hours was determined for *Labeo rohita* (150.40 ml/l) in Textile industrial effluent and (227.93 ml/l) in sewage water. The sublethal concentrations were of textile industrial effluent (120, 100 and 75 ml/l) and for sewage water (220, 200 & 175 ml/l), respectively for three months. The morphometric parameters were measured fortnightly. The fingerlings were fed @3-4% body weight. Physico-chemical parameters of water (Temperature, DO, EC, TSS, TDS, and pH) were measured on daily basis. After the completion of experimental estimation of total lipids, cholesterol, phospholipids, free fatty acids and glycerides was done by using different protocols.

In case of *Labeo rohita*, when exposed to textile industrial effluents ovaries showed the remarkable decline in the amount of lipid contents as the total lipids were measured 48.90 mg/g in control group while 32.66, 28.69 and 25.63 mg/g in sub-lethal concentrations T¹, T² and T³ respectively. Cholesterol 2.812 mg/g in Control group, 2.398, 1.960 and 1.712 mg/g in T¹, T² and T³ respectively. The mean of Phospholipids was 1.208 mg/g in control and 0.982, 0.732 and 0.566 in T¹, T² and T³ respectively. 4.196 mg/g Free Fatty Acids was in Control and 4.082, 3.758 and 3.322 mg/g in T¹, T² and T³ respectively. Similarly, the Glycerides were 40.69 mg/g in control group but 25.20, 22.24 and 20.03 in T¹, T² and T³, respectively.

Whereas in exposure to sewage effluent, the ovaries of *Labeo rohita* also showed the decreased amount of contents of lipids as follows: Total lipids was 48.90 mg/g for control group and 38.35, 36.32 and 32.77 mg/g in S¹, S² and S³ respectively. The mean of Cholesterol was 2.812 mg/g in Control group, 2.590, 2.224 and 2.196 mg/g in S¹, S² and S³ respectively. The mean of Phospholipids was 1.208 mg/g in control group and 1.164, 1.058 and 0.846 were in S¹, S² and S³ respectively, 4.196 mg/g of Free Fatty Acids were in Control group and 4.120, 3.900 and 3.478 mg/g in S¹, S² and S³ respectively. Similarly, the Glycerides were 40.69 mg/g in control group but 30.39, 29.12 and 26.25 mg/g in S¹, S² and S³ separately.

Comparison of Means of Biochemical Parameters in the Ovaries of *Labeo rohita* after Exposure to Sub-lethal Concentrations of Textile Industrial Effluent

Groups	Total Lipids	Cholesterol	Phospholipids	Free Fatty Acids	Glycerides
Control	48.90±0.218a	2.812±0.021a	1.208±0.010a	4.196±0.083a	40.69±0.125a
T ₁	32.66±0.283b	2.398±0.068b	0.982±0.046b	4.082±0.054b	25.20±0.129b
T ₂	28.69±0.201c	1.960±0.041c	0.732±0.013c	3.758±0.024c	22.24±0.129c
T ₃	25.63±0.045d	1.712±0.012d	0.566±0.022d	3.322±0.017d	20.03±0.010d

DNA DAMAGE IN MAJOR CARP INDUCED BY ACUTE EXPOSURE TO POLLUTED DRAIN WATER

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In this study, Chakbandi drain's composite water concentrations were collected from the selected sites in the month of April, May and June, 2016 and applied to fingerling's of three fish species i.e. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria. After determining the LC_{50} , the sub-lethal dilutions i.e. 20%, 25%, 30%, 35% and 40% of drain water were tested for three month's acute toxicity trial.

All the water quality parameters i.e. DO, pH, temperature, conductivity, TDS, salinity, TSS, BOD and COD and concentration of selected heavy metals (Cu, Cr, Mn, Cd, Co, Ni, Sn, Hg, Zn and Pb) recorded from drain water were found above the permissible limits as described by FAO/ WHO.

During current research, the DNA damage in fish peripheral erythrocytes was quantified by using different categories (Type I, Type-II, Type-III and Type-IV) of damaged cells that were arbitrarily defined according to the tail length (size) of the comets and percentage of damaged cells was calculated. Maximum frequency of DNA damaged cells was recorded in erythrocytes of *Cirrhinus mrigala* when compared with *Labeo rohita* and *Catla catla*. *Cirrhinus mrigala* showed maximum average tail DNA length and average tail moment than *Labeo rohita* and *Catla catla*.

Maximum mean total no. of cells with micronuclei and maximum micronuclei frequency was identified in erythrocytes of *Cirrhinus mrigala* than *Labeo rohita* and *Catla catla*. Conclusively, DNA Fragmentation is found to be reliable for the assessment of environmental pollution. Moreover, findings of this study are helpful

as an early warning for environmental monitoring strategies and for the evaluation of eco-toxicological impacts of pollutions reaching in aquatic fauna particularly fish and indirectly to human populations.

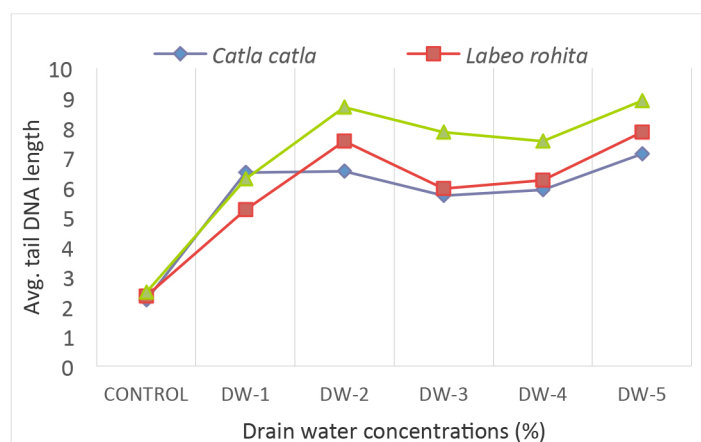


Figure: Line graph representing average tail DNA length in the three fish species

MEMBRANE BIOLOGICAL REACTORS TO REMOVE NITRATE, DIGEST BIOSOLIDS, AND ELIMINATE WATER FLUSHING REQUIREMENTS WITHIN REPLICATED RECIRCULATION SYSTEMS CULTURING RAINBOW TROUT

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Nutrients, particularly nitrate (NO_3^-), can accumulate to very high levels within low exchange recirculation aquaculture systems (RAS) and negatively impact a number of cultured species. To prevent the harmful effects of nitrate accumulation and to dispose of concentrated waste biosolids, many RAS are operated with makeup water as high as 0.5-5% of the total recirculating flow. This means that a fish farm capable of producing 1,000 mt/yr requires a minimum of 800 m³ of daily makeup water even with relatively low water usage; most existing farms would operate with at least twice this makeup flow. While RAS designs use much less water than typical flow-through fish farms, these water requirements and the associated water withdrawal and point-source discharge permits can still limit RAS technology implementation.

Membrane biological reactors (MBRs) are a promising and scalable technology that could allow complete closure of the RAS, decreasing both the water withdrawal and point-source discharge requirements. MBRs have been widely used to remove most of the nutrients, solids, and organics from municipal, industrial, and agricultural wastewaters. Using a series of membranes plumbed in parallel, an MBR operates with both anoxic and aerobic stages within an activated sludge so that complete nitrification and denitrification can occur. In previous research, we found this technology effective at removing solids and nutrients from RAS effluent while aerobically stabilizing the system waste into non-malodorous biosolids suitable for composting or land application as a nutrient-rich soil amendment. The MBR removed nearly all of the nitrogen (< 3 mg/L), phosphorus (< 0.1 mg/L), biochemical oxygen demand (< 1 mg/L), suspended solids (< 1 mg/L), and heavy metals from RAS wastewater. Given these low nutrient levels, it follows that the MBR's permeate is likely well-suited for reuse in a fish culture system. Because the bacterial biomass in biosolids accumulates during MBR treatment, some of this biomass must be removed periodically in a process termed "biosolids wasting." As a result of the requirement to remove these biosolids, we estimate that MBR's could allow for a nearly 10-fold reduction in water flushing requirement over the already tight water recirculation loops in many RAS.

In the present study, six replicated RAS are being used to determine if an MBR can be integrated within a RAS to control nitrate accumulations at levels < 100 mg/L, while simultaneously treating all of the biosolids backwashed or flushed from the primary solids capture processes in the system. This research will also determine if these MBR-containing RAS that operate with almost no makeup water flushing can sustain salmonid growth, welfare, and product quality.

UTILIZATION OF CRYSTALLINE AMINO ACIDS BY PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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In view of the variability in recommendations for essential amino acid (EAA) and the use of amino acids supplements in shrimp diets, this study was designed to determine the efficacy of crystalline amino acids (CAA) in a practical shrimp feed formulation. The basal diet was designed to contain 30% protein and 6% lipid. The primary protein source of the basal diet included: 5% fishmeal, 37% soybean, and 5% gelatine. Intact protein was incrementally reduced to produce diets with 28, 26, 24, and 22% protein. In a second series of diets, CAA were supplemented to the reduced protein diets to return the diets to 30% protein. Thus, producing a series of diets containing 30% protein with 2, 4, 6, and 8% crystalline amino acids. The trial was conducted twice to confirm results. In the first trial, the shrimp (mean initial weight 0.25 ± 0.02) were stocked at 10 shrimp per tank and there were four replicates per diet run over a seven week period. The second trial was stocked at 15 shrimp per tank (mean initial weight 0.30 ± 0.01) run over a six week period. Data within treatments for both trials were variable, resulting in limited statistical differences. From a biological standpoint, percentage weight gain (PWG) appeared to decrease with protein level of the diet (R^2 -value, trial 1: 0.30, trial 2: 0.34). The addition of CAA resulted in a similar trend but did not appear to improve performance. Mean final weight (MFW) also appeared to have a decrease with protein level of the diet (R^2 -value, trial 1: 0.44, trial 2: 0.38). None of the diets with supplemented CAA appeared to have reached the same mean final weight or PWG as the basal diet. It also appeared that the higher the percentage of CAA that were supplemented a decrease in the mean final weight and PWG was observed. There was a high variation observed in the growth performance of the shrimp. The FCR for the treatments was also very poor. Another trial is being run comparing a fishmeal based diet to a soybean meal based diet with and without amino acid supplementation at two different protein levels and will be reported.

TABLE 1. Growth performance of Pacific white shrimp (*Litopenaeus vannamei*) fed with experimental diet for seven weeks (trial 1) and six weeks (trial 2)

Diets (protein+CAA supplement)	MFW (g)		FCR		Survival (%)		PWG	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
30%	5.46	5.56	2.13	1.41	87.50	96.67	2156.02	1789.70
28%	5.42	4.60	2.20	1.72	82.50	97.33	2030.86	1412.97
26%	4.87	4.63	2.36	1.70	97.50	97.33	1898.64	1461.78
24%	4.75	4.58	2.48	1.75	87.50	96.00	1832.84	1413.24
22%	4.73	4.41	2.47	1.80	92.50	97.33	1802.53	1355.05
28+2%	5.39	4.83	2.23	1.63	80.00	96.00	2117.55	1490.19
26+4%	4.94	4.89	2.35	1.60	90.00	92.00	1961.18	1561.04
24+6%	4.60	4.69	2.55	1.68	92.50	94.67	1765.18	1448.06
22+8%	4.62	4.49	2.55	1.77	92.50	97.33	1735.85	1412.93
PSE	0.22	0.25	0.11	0.10	5.06	2.54	121.70	96.57
P-Value	0.23	0.99	0.18	0.74	0.80	0.15	0.49	0.94

PSE: Pooled standard error. ANCOVA (SAS analysis 9.3) was used for statistical analysis.

BLUE ECONOMY INITIATIVES AT THE PORT OF SAN DIEGO: EXPLORING REGIONAL OPPORTUNITIES FOR AQUACULTURE AND BLUE TECHNOLOGY

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The Port of San Diego was established by state legislation as a trustee of the land and water around San Diego Bay for the promotion of fisheries, commerce, navigation and recreation. Inherent to The Port's mission is to utilize its various assets in leading and accelerating the Blue Economy around San Diego Bay. Water dependent business is a long and proud tradition at The Port and its sustainable future is critical to our region's long term success of water dependent fisheries and technologies. To this end, the Port has been exploring opportunities to advance emerging aquaculture and blue tech businesses, as a way to diversify its portfolio of business lines and assets, while strengthening alignment with its mission and Strategic Plan.

Aquaculture is a growing opportunity for new business development in Southern California. The aquaculture opportunity is being driven by the need to support development of sustainable domestic marine aquaculture industry. The U.S. currently imports over 91 percent of the seafood it consumes. In economic terms, these imports consistently contribute to a nearly \$14 billion domestic seafood trade deficit each year. Globally, 50 percent of seafood produced comes from aquaculture. Domestically only three percent of seafood produced is from aquaculture. While there is a clear food production component to this demand, aquaculture offers multiple co-benefits, such as fisheries enhancement, ecosystem restoration, mitigation banking, bio-fuel/medical purposes, bioremediation, and education and outreach.

Since 2015, the District has been conducting studies, planning and completing pre-development work to support and inform aquaculture opportunities including:

- Coastal marine spatial planning to inform future development for aquaculture
- Baywide feasibility study to assess infrastructure capable of supporting aquaculture
- Research to support the development of permit ready infrastructure
- Stakeholder outreach for the San Diego Ocean Planning Partnership
- Research to support development of an Aquaculture Technology Park

In 2016, the District established a Blue Economy incubator and strategic investment fund to assist in the creation, development, and scaling of new business ventures on San Diego Bay, focusing on aquaculture and blue technology.

Ports can and are increasingly playing a critical role in the development of aquaculture, given their expertise, as well as the availability of land and water. As the state-legislated trustee of tidelands and submerged waters of San Diego Bay, developing sustainable domestic aquaculture helps fulfill the District's public trust responsibility to promote fisheries and commerce, as well as aligning with its mission to enhance and protect the environment

GROWTH AND SURVIVAL OF LARGEMOUTH BASS WHEN FED DIETS CONTAINING BLACK SOLDIER FLYLARVAE

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Largemouth bass (LMB), *Micropterus salmoides*, are one of the most popular sport fish in the United States and are commonly cultured for use in stocking programs. Aquaculture production of LMB is on the rise, increasing from 136 farms to 176 farms in 15 years. Most of this production is done in ponds. For the last two years, we have been testing the viability of using LMB in small-scale aquaponic systems. Diets of these fish are particularly relevant due to their high protein demand, and can represent a significant portion of total production costs. There is significant interest from small-scale aquaculture and aquaponics producers to use black soldier fly (BSF) larvae as a food source for their fish, as BSF larvae are easy to produce on-site using food waste. However, not much research has been done to quantify the effect of feeding black soldier fly larvae to aquacultured fish.

The intention of this study is to assess growth and survival of juvenile LMB when fed diets containing various levels of BSF larvae meal. LMB were produced on-site at the DSU Aquaculture Research and Demonstration Facility (ARDF) as part of a larger restoration stocking initiative. Broodstock were taken from Phillips landing on the Nanticoke River and moved to the ARDF in March 2017 for spawning in ponds. Fry were transferred from broodstock ponds and allowed to grow until they were 5-10 cm. 180 fingerlings were moved indoors to a recirculating system for feed training. Once they were feed trained LMB were stocked into a rack system containing 24 18-L tanks connected to a common filtration system at a density of 5 fish/tank.

Individual tanks were randomly assigned to one of six treatments (0, 20, 40, 60, 80, 100), corresponding to inclusion levels of BSF larvae into the diet. Feed for the study was prepared in-house by combining ground BSF larvae and commercial fish feed (42% protein, 16% fat) to desired inclusion levels. BFS larvae and the commercial fish feed were ground, and dried to a constant weight before mixing. Water was added to the dry ingredients to create dough that could be formed into pellets. Experimental diet pellets were again dried to a constant weight before use.

Fish in the experimental tanks will be fed twice daily, with rations adjusted based on fish weight every two weeks. To ensure that the conditions are adequate, temperature, dissolved oxygen and pH will be checked daily while ammonia and nitrite will be tested weekly. After eight weeks surviving fish will be counted and weighed to ascertain survival, SGR and FCR.

A FISH FOR ALL SEASONS: INCREASING AQUACULTURE'S CONTRIBUTION TO GLOBAL FOOD SUPPLY

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Human health and socio-economic development are intimately tied to food access and food security. In a world capable of producing sufficient food to meet the entire dietary nutrient needs of all its people, income plays a determinant role in dictating who has access to food or not, with under-nutrition and malnutrition still negatively affecting the health and well-being of many of the world's poorest nations. This article 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. The data show that fish and fishery products play an essential role in human nutrition, constituting the major source of dietary animal protein consumed within the Asian region and within many lower income countries within the African region.

BLUE REVOLUTION HAWAII: PROPOSAL FOR A PACIFIC INTERNATIONAL OCEAN STATION

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World fisheries agencies have long warned of the depletion of wild fish stocks in our oceans due to overfishing to meet rapidly growing human demands for seafood. The potential of aquaculture for providing safe, sustainable seafood supplies to the world has long been recognized. Seafood aquaculture already supplies total yields about equal to caught fish, but conflicts with near-shore and on-shore usages, navigation lanes, commercial fisheries, and feed and waste discharges in local waters are imposing limits to its expansion.

The obvious solution is to develop ocean aquaculture in deep, voluminous ocean waters farther from shore. But the proof of economically sustainable and environmentally protective best practices is needed before commercial operators will undertake the risks of deep ocean aquaculture operations.

Blue Revolution Hawaii is advocating the deployment of the Pacific International Ocean Station (PIOS) in Hawaiian Exclusive Economic Zone (EEZ) waters 35-75 miles offshore on the lee side southwest from the Hawaiian Islands. PIOS would be a pilot test station for the eventual deployment of commercial ocean resources production platforms in ocean waters.

The 35-75-mile-band of EEZ ocean waters provides a 20,000 square-mile region along the southwest side of the Hawaiian Islands that may ultimately be designated for commercial-scale ocean resources production operations. This area lies in the vacant zone between existing Hawaii-based day-boat and longline fishing operations and is situated far from near-shore usages, navigation lanes, and marine mammal and reef preserve areas.

The PIOS Station would be designed to support a test fleet of submerged fish-growing cages for growing pelagic species of fish from fingerlings to harvesting. A semi-buoyant core platform (similar to oil/gas drilling platforms) would be elevated above storm-surge wave heights and would provide housing and operations space for up to 200 scientific researchers, ocean monitoring personnel, and fish cage operations workers. An outer line of tethered ocean wind turbines can provide electricity for on-platform activities.

The PIOS Station research would include ongoing monitoring and data-gathering of climate, marine life and ocean environment, and would test, design and validate best practices for sustainable and environmentally protective operations for deep ocean aquaculture and other ocean resources production.

GWAS ANALYSIS OF QTL FOR RESISTANCE AGAINST *Edwardsiella ictaluri* IN F2 INTERSPECIFIC HYBRID CATFISH

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Infectious diseases pose significant threats to the catfish industry. Enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* is the most devastating disease for catfish aquaculture, causing huge economic losses annually. Channel catfish and blue catfish exhibit great contrast in resistance against ESC, with blue catfish being highly resistant. As such, the interspecific hybrid backcross progenies provide an ideal system for the analysis of resistance QTL. In this study, we conducted GWAS analysis to locate genomic regions associated with ESC resistance by selectively genotyping F2 backcross families with the catfish 690K SNP arrays. Two genomic regions on linkage group (LG) 1 and LG23 were determined to be associated with ESC resistance as revealed by a mixed linear model and family-based association test. A number of genes within QTL have known functions in immunity, making them potential candidates as disease resistance genes. For instance, seven genes on LG1 (*nck1*, *agtr1*, *trpc1*, *abil*, *apbb1ip*, *actr3b*, and *vav3*) and three genes on LG23 (*mrc1l*, *prkcq*, and *gata3*) were involved in immune-related functions. These genes mainly function in signaling pathways of phagocytosis and T-cell activation, suggesting their roles in disease resistance.

This study demonstrated the power of GWAS analysis for the identification of QTL in the hybrid system. We previously reported one QTL in LG1 using fourth generation of backcross families, which was validated here in the F2 backcross families, suggesting that this QTL is operating in various populations of a broad genetic background, making it useful for application in marker-assisted selection.

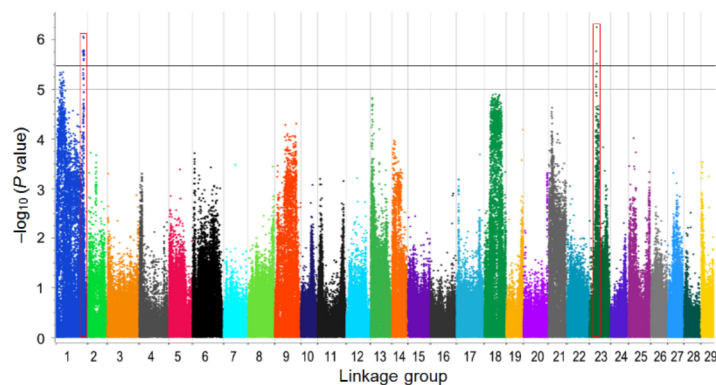


Fig. 1 Manhattan plot of genome-wide association analysis for ESC disease resistance generated from QFAM. The black solid line indicates the threshold P value for genome-wide significance. The grey solid line indicates the threshold P value for significance of “suggestive association”. The red box represents the shared QTL using two methods.

UNRAVELING THE MECHANISM BEHIND PROBIOTIC BENEFITS IN RECIRCULATING AQUACULTURE SYSTEMS

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Increased knowledge of microbial management issues in recirculating aquaculture systems that subject fish larvae to high abundances of opportunistic pathogens has led to a burst in research investigating the effect of beneficial bacteria supplement, or probiotics, on fish growth and survival, particularly at the larval stages. Often, studies identify potential benefits of the probiotic to the system, including measuring increased survival, but few studies have elucidated on the potential mechanism behind this improvement. It is not enough to know that a probiotic is working, but specific reasons for its success should be identified in order to understand how to better improve the effects of the supplement in the system, and to understand how to transfer the technology to other systems and fish species.

This study aimed to compare results from two separate probiotics trials, where probiotic was administered either directly via the rearing water only, or via the rearing water and live feed, versus a control without probiotic treatment. Common snook *Centropomus undecimalis* were reared to approximately 30 dph using standard protocols. Administration of probiotic generally doubled larval survival (Fig. 1A), and subsequent transport stress tests indicated improved survival in fish having been treated with the probiotic (Fig. 1B). This benefit could not be described by increased larval growth or variations in standard water quality measurements (nitrate, nitrite, ammonia, pH, dissolved oxygen).

Investigations into the microbiota composition of the rearing water and larvae indicated large differences between experimental trials (performed in different years), with no consistent patterns of microbial groups affected by probiotic administration. Water and larval bacterial communities overall were more significantly impacted by trial day than treatment, even when comparing treated tanks and larvae versus the control. This presentation will dive deeper into the bacterial community compositions and predicted functions during these trials, relating differences over time and treatments to potential influences on survival.

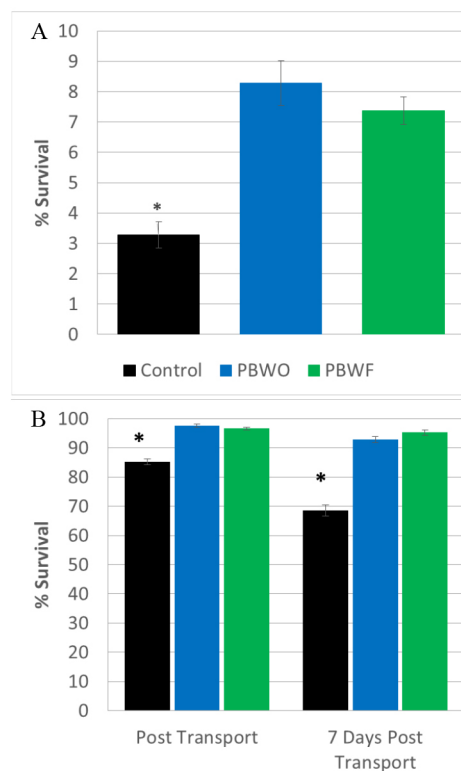


Figure 1. Survival of common snook A) at the end of the probiotics trial, and B) immediately and 7 days following transport.

COMBINING ABILITY OF CHANNEL CATFISH FEMALES AND BLUE CATFISH MALES FOR TOLERANCE OF LOW OXYGEN IN THEIR PROGENY USING A COMPLETE FACTORIAL MATING DESIGN

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In the United States, catfish production is the main pillar of the aquaculture industry in terms of finfish production. While many species of catfish are raised for food, the hybrid catfish is quickly gaining popularity among farmers for its disease resistance as well as its increased performance. Relatively little is known about hybrid catfish in comparison to the channel catfish, which is the most commonly raised food fish in the U.S. Using a complete factorial mating design, we tested 60 to 80-day old fry from 37 different families of hybrid catfish for resistance to low levels of dissolved oxygen. Variation for dissolved oxygen resistance was observed among the families and combining abilities are currently being calculated. These results will be influential in developing genetic improvement plans for hybrid catfish which focus on increased tolerance and resistance to low dissolved oxygen.

A GLOBAL SPATIAL ANALYSIS TO INFORM AQUACULTURE DEVELOPMENT TO RESTORE ECOSYSTEM HEALTH

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Habitat loss, nutrient pollution and other stressors have diminished coastal ecosystem health globally, but bivalve and seaweed aquaculture present significant opportunities to aid their recovery. For example, while oyster reefs worldwide have declined by over 85% in the past two centuries, oysters produced through aquaculture may enhance water quality and provide habitat for fish and invertebrates, among other benefits (Figure 1). Knowledge of where bivalve and seaweed aquaculture might provide the greatest ecosystem health enhancement (e.g., in areas of known nutrient pollution and/or degraded habitat) can inform global-scale prioritization of aquaculture development.

Within a Geographic Information System (GIS) framework, we conducted a global assessment of where bivalve and seaweed aquaculture could be expanded to aid in ecosystem health recovery. Specifically, we integrated spatial data on the global extent of eutrophication, estuarine habitat degradation, overfishing, ocean acidification, and other relevant factors to identify priority marine ecoregions for “restorative” bivalve and seaweed aquaculture. The outcome of our assessment provides a global perspective on the magnitude of the opportunity for bivalve and seaweed aquaculture expansion to aid the recovery of coastal ecosystem health.

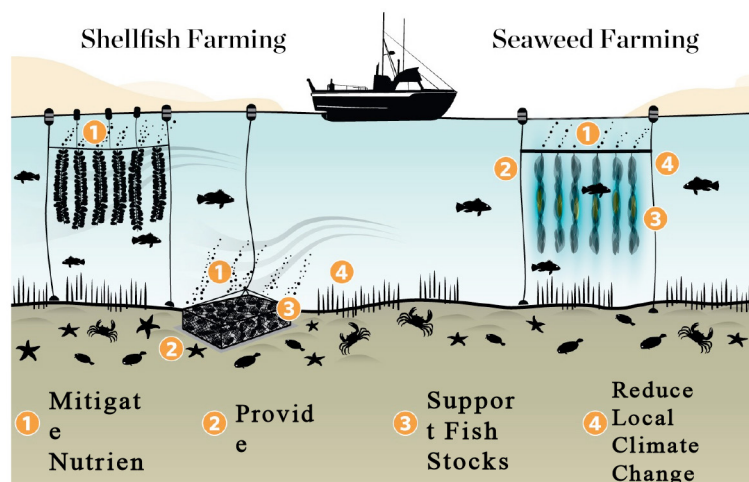


Figure 1. Potential ecosystem health benefits of shellfish & seaweed aquaculture. © The Nature Conservancy

MODELING RAPID CHILLING OF EASTERN OYSTERS (*Crassostrea virginica*)

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Eastern Oysters (*Crassostrea virginica*) are naturally occurring shellfish along the east coast of Canada and the United States and into the Gulf of Mexico. The oysters are harvested for consumption throughout the year leading to high rates of seafood-borne illnesses when the water temperatures rise and bacteria flourish. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are the leading causes of seafood-borne bacterial illness due to the consumption of raw shellfish. The frequency of illnesses increase along with the water temperature. Regulations are in place to combat the increase of bacteria creating strict time/temperature requirements for harvesting oysters in the summer months. Alternatives have arisen to increase the time from harvest to mechanical refrigeration by way of chilling the oysters using an ice-slurry. These alternatives could be more effective with more information on the thermodynamics of oysters and modeling.

The modeling of oyster thermodynamics takes into account different parameters and chilling techniques. The wide expanse of locations from Canada to the Gulf Coast give a range of temperatures and conditions the oysters can be harvest from resulting in different exposure times to the chilling requirements. This model would allow location specific requirements to adjust chilling times. Oysters are also harvested in different ways depending on the region, some harvesters dredge the area and extract hundreds of oysters at a time while others cultivate oysters in bags. The model can be adjusted depending on the volume of oysters needing to be chilled. This would allow for a more comprehensive chilling of all oysters to reduce the risk of seafood-borne bacterial illness.

This presentation encompasses the varying conditions of oyster harvesting and treatment to help prevent illness from the consumption of seafood. This work can be implemented into current practices of oyster chilling to ensure temperature requirements are met. This model can also be modified to incorporate other aquaculture products as the industry continues to grow.

EVALUATION OF A MENTORSHIP PROGRAM IN AQUACULTURE: A QUALITATIVE STUDY TO IDENTIFY HIGH SCHOOL STUDENTS' PERCEPTIONS AND EXPERIENCES

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The purpose of this exploratory case study was to identify the impact of a mentor-guided, project-based learning program through hands-on aquaculture activities as perceived by high school students. This study attempted to explore the educational experiences, perceptions, attitudes, and behaviors of secondary students' and their intrinsic desire to learn more about STEM-related fields and careers when exposed to hands-on/minds-on aquaculture projects.

Qualitative data generated included field observations of participants' activities in the mentorship program, structured face-to-face interviews with open-ended questions, focus groups, student journal reflections, and closed-ended questionnaire. Inductive data analysis methods were used to identify patterns that emerged in the data revealing secondary students' feelings, opinions, and beliefs (attitudes) and what they actually do (behaviors) as a result of their participation. The data analysis involved an iterative process of data coding, management, interpretation, and verification of data and it continued until dominant themes had been refined and isolated. The research methodology of this study was exploratory-based as well as open-ended systematic inquiry to identify participants' experiences in and perceptions of the mentorship program. Qualitative methods were likely best suited to this project, since it provides an in-depth understanding of people's experiences in a specific environment. Further, this method of inquiry allows stories to be told in context and compiles evidence drawn from several methods of data collection.

Three primary themes that emerged from the analysis include: 1) students gained self-confidence in their ability to explore and try new things, and viewed college as attainable after their experience in the program; 2) gained practical life skills such as communication, collaborative teamwork, and responsibility of their learning experience; 3) and results demonstrated that the aquaculture mentorship program enhanced students' awareness of, interest in, and motivation toward learning more about STEM areas of study and careers.

A GUIDE TO COLLABORATION AMONG DIVERSE STAKEHOLDER GROUPS TO ADDRESS PUBLIC MISPERCEPTIONS ABOUT MARINE AQUACULTURE IN THE U.S.

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Well-managed wild-capture fisheries and marine aquaculture will play an increasingly important role in our food supply for the health and wellbeing of people and the environment. Marine aquaculture can produce a healthy food source with fewer environmental impacts relative to other animal proteins. It can be produced using less freshwater and land resources and with fewer greenhouse gas emissions. Aquaculture accounts for more than half of the imports and most of it is from Asia and other countries that may not have the strong environmental standards the U.S. has in place to ensure that operations are safe and sustainable. Despite these advantages, the U.S. lags behind the rest of the world in marine aquaculture production and continues to rely heavily on seafood imports—more than 90 percent of its supply. The limited growth of marine aquaculture in the U.S. is due in large part to poor public perception that is fueled by misinformation that is often out of date or provided out of context.

Public perception plays an important role in the future of marine aquaculture development in the U.S. Even with regulatory confidence in the science and tools available to inform decisions, perception-based concerns can influence permitting decisions and hinder aquaculture development. Aquariums and science institutions have a unique opportunity to engage visitors with strategic outreach messaging to educate the public about marine aquaculture's role as a conservation tool. The Aquarium of the Pacific and its Seafood for the Future program have played a leading role in educating the public about marine aquaculture and its important role as a conservation tool, but these efforts would be much more successful with more diverse participation. Effective communications and outreach strategies to address public misperceptions about marine aquaculture will require collaboration and cohesive messaging among a diverse group of stakeholders, including: government and nongovernment organizations, scientists, formal and informal educators, and industry. Pre-competitive collaboration, where industry identifies and addresses a common issue and solutions related to the resource they all share, is a key component. The narrative about marine aquaculture also needs to shift to highlight marine aquaculture's role in the global food supply, human health, and providing ecosystem services and benefits. It is also critical that these groups collectively acknowledge and address issues, real and perceived, to garner public trust. It is possible—with collaboration—to change public perceptions about marine aquaculture in the U.S.

ALIGNING MARINE AQUACULTURE PERSPECTIVES WITH THE CURRENT STATE OF THE SCIENCE: A PANEL DISCUSSION

Kimberly Thompson*, Sebastian Belle, Halley Froehlich, Robert Jones, Michael B. Rust,
and Wojciech Wawrzynski

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Well-managed wild-capture fisheries and environmentally responsible marine aquaculture will play an increasingly important role in our food supply for the health and wellbeing of people and the environment. Marine aquaculture can produce a healthy food source with fewer environmental impacts relative to other animal proteins. It can be produced using less freshwater and land resources and with fewer greenhouse gas emissions. Aquaculture accounts for more than half of the imports and most of it is from Asia and other countries that may not have the strong environmental standards the U.S. has in place to ensure that operations are safe and sustainable. Despite these advantages, the U.S. lags behind the rest of the world in marine aquaculture production and continues to rely heavily on seafood imports—more than 90 percent of its supply. The limited growth of marine aquaculture in the U.S. is due in large part to poor public perception that is fueled by misinformation that is often out of date or provided out of context.

Public perception plays an important role in the future of marine aquaculture development in the U.S. Even with regulatory confidence in the science and tools available to inform decisions, perception-based concerns can influence permitting decisions and hinder aquaculture development. Addressing misperceptions about aquaculture will require collaboration among diverse stakeholder groups and access to the most up-to-date scientific information. This interactive panel will feature experts from industry, government, nongovernment, and academic sectors who will provide their perspectives on real and perceived perceptions about marine aquaculture and the science-based tools and resources available to address them. They will also discuss the broader context in which marine aquaculture needs to be presented in order to reframe the current narrative about the topic in order to align public perceptions with the current state of the science.

Panelists:

Sebastian Belle: Executive Director, Maine Aquaculture Association;

Michael B. Rust: Science Advisor, NOAA Office of Aquaculture;

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THE EFFECTS OF DENSITY AND ARTIFICIAL SUBSTRATE ON INTENSIVE SHRIMP *Litopenaeus vannamei* NURSERY PRODUCTION

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Intensive marine shrimp production is gaining interest at inland locations and can provide fresh, never-frozen shrimp to local metropolitan markets. A nursery phase is commonly used in shrimp production, as it allows for enhanced biosecurity and better quantification of animals while utilizing space effectively. Recirculating systems are especially useful as they can be contained indoors, allowing shrimp to be grown year-round in nearly any climate. However, it is unclear what the optimal density may be in such systems and whether extra substrate may help improve system and animal performance.

This experiment compared four treatments, dictated by differences in stocking density and artificial substrate: low density-no substrate (LD), low density-substrate (LD-S), high density-no substrate (HD), and high density-substrate (HD-S). Each treatment included four, randomly assigned 160 liter culture tanks; all systems had an external settling chamber and biofilter. The study included 53 cm (L) by 43 cm (W) pieces of high density polyethylene (HDPE) 2.5 cm mesh, which was used for treatments that included substrate. The shrimp were grown for 50 days in nursery tanks stocked with either 3,000 PL/m³ or 1,500 PL/m³ based on the treatment, with an initial weight of 4 mg. Daily parameters measured were temperature (°C), dissolved oxygen (DO), pH, and salinity (ppt). Shrimp were weighed a little over halfway through the study to determine mean individual weights. An α -value of 0.05 was used during data analysis to determine significant differences between treatments.

Final data are still pending, but significant differences were found between treatments with regard to DO, pH, and average shrimp weights (Table 1). These results suggest that stocking density and substrate addition may have significant impacts on water quality and nursery shrimp production, and that producers should consider these factors to help enhance production.

Table 1: Mean \pm SE values of DO, pH, and shrimp weight at approximately the middle of the study.

		Treatment			
		LD	LD-S	HD	HD-S
DO (mg/L)	AM	6.49 \pm 0.09 ^a	6.71 \pm 0.69 ^a	6.47 \pm 0.09 ^{ab}	6.43 \pm 0.08 ^b
	PM	6.44 \pm 0.09 ^a	6.46 \pm 0.10 ^a	6.41 \pm 0.10 ^{ab}	6.38 \pm 0.09 ^b
pH	AM	8.44 \pm 0.04 ^a	8.45 \pm 0.04 ^a	8.41 \pm 0.04 ^b	8.38 \pm 0.04 ^c
	PM	8.45 \pm 0.05 ^a	8.47 \pm 0.05 ^b	8.41 \pm 0.05 ^c	8.40 \pm 0.05 ^d
Mean Shrimp Weight (g)		0.45 \pm 0.07 ^a	0.60 \pm 0.11 ^b	0.39 \pm 0.07 ^a	0.46 \pm 0.08 ^a

OXYGEN REQUIREMENTS OF SEPARATED HYBRID CATFISH ♀ *Ictalurus punctatus* x ♂ *I. furcatus* EGGS

Les Torrans* and Brian Ott

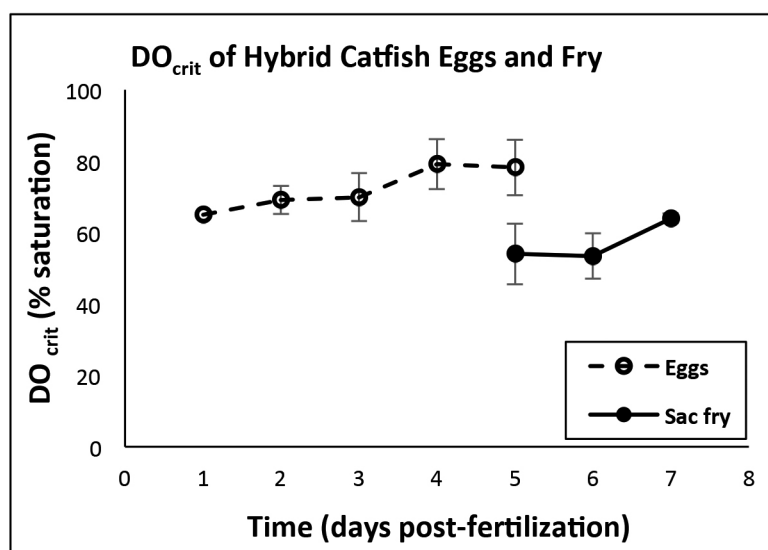
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Channel catfish *Ictalurus punctatus* egg masses require ambient water with over 95% air saturation to maintain maximum oxygen consumption as they near hatch. Since hybrid catfish eggs (channel catfish ♀ X blue catfish *I. furcatus* ♂) are often kept separated after fertilization by the addition of fuller's earth and incubated in large tube incubators, it is assumed that the critical oxygen requirement is lower. This study was conducted to determine the recommended dissolved oxygen concentration for incubation of hybrid catfish eggs.

A 300 ml Wharton BOD bottle with a Hach LED oxygen sensor was used as the respirometer. A 2" magnetic spin bar was placed in the bottle and used to mix water throughout the chamber and suspend the loose egg/sac fry sample. The bottle was placed in a water bath at 24.8°C on a stir plate and the rotational speed adjusted to the minimum required to suspend the eggs in the bottle (60-90 RPM). The exact time of fertilization was noted for each spawn as the eggs were fertilized and placed in separate McDonald jars to incubate. The age (hours post-fertilization) was noted when each egg or fry sample was tested. A 15-45 gram egg or fry sample was siphoned from each of several McDonald jars daily through the incubation period and three days post-hatch.

DO and temperature were measured and recorded every 1-5 minutes (more frequent measurements with older eggs having a higher metabolic rate) for 1-4 hours. A total of 51 measurements were made on 16 different spawns at 1-5 days post-fertilization, and a total of nine measurements were made on sac fry from five different spawns ranging in age from 0-2 days post-hatch.

The maximum DO required by hybrid catfish eggs increased throughout the incubation period, peaking at 79% air saturation during the last two days of incubation. Sac fry required approximately 55% saturation for the first two days post-hatch. Hatchery managers should maintain DO above 80% during the last two days of incubation.



CONSUMER PERCEPTION ON SEAFOOD: QUALITY EXPECTATIONS RELATED TO CHILLED/THAWED PRODUCTS

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Technological advances in food cultivation, harvesting and logistics are giving consumers greater choice with their purchases. Advances in processing, freezing and thawing are key technologies that allow seafood to be provided over longer timeframes and distances. However, there is a negative connotation associated with frozen seafood products compared to fresh products, leading to the price of frozen products remaining low. These days many seafood products sold at supermarkets, presented as fresh products, have been frozen in advance and then thawed before being placed on the shelves. Up until recently the information that these products have been frozen in advance have not been prominently displayed. Consumers are becoming more aware of this, due to regulations requiring labelling thawed products as having been previously frozen, and due to media coverage. This study aims to explore the consumer attitudes towards seafood products, explicitly salmon and cod, and compare it to other common meat types. This is done through a survey deployed in four European markets (United Kingdom, France, Germany, and Norway) exploring the perception across five categories: taste, healthiness, convenience, value for money and availability. Further, the survey examines the attitudes towards fresh and frozen products, and explore how basic information treatment may influence the perception of frozen and thawed products across the various quality categories.

EFFECT OF CONSUMPTION OF COMPLEMENTARY TRADITIONAL FOOD ON THE GROWTH AND IRON STATUS OF 6 TO 15 MONTHS OLD CAMBODIAN CHILDREN IN PREVEANG PROVINCE CAMBODIA

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With prevalent malnutrition problems largely due to food and nutrition insecurity, locally available foods need to be maximized for increased consumption. The effect of two rice-based complementary foods prepared using edible spider (Winfood CF) and small fish (Winfood-Lite) vis-à-vis two corn soya blend products (CSB+ and CSB++) was explored in this single-blind randomized intervention trial among 126 Cambodian infants aged 6 months without severe wasting (<-3 weight-for-length z-score), pitting edema, signs of vitamin A deficiency or anemia (Hb<80g/L). The samples were recruited and randomized from 7 communes in Prea Raing District, Prey Veng province, Cambodia. Intervention was daily supplementation with one of the four products for nine months: 50g/d from 6-8 months, 75g/d from 9-11 months and 125 g/d from 12-15 months of age.

Mean weight and height of infants significantly improved and remained within normal range at endline. The weight for age Z score (WAZ), length for age Z score (LAZ) and weight for height Z scores (WHZ) also increased. In contrast, there was a three-fold decrease in serum ferritin though it remained within normal range. Hemoglobin levels remained unchanged and indicated anemia. No difference in weight, length, WAZ, LAZ, WHZ, serum ferritin and hemoglobin was observed between the four groups of children indicating that the Winfoods had the same effect as the CSB products on iron status and growth. There is a weak but positive association between ferritin and weight, length and age, hemoglobin and vitamin C intake. Weight and gender were also correlated.

The study concludes that Winfood products (Winfood CF and Winfood Lite) are at par with CSB products (CSB+ and CSB++). The promotion and utilization of nutritious and locally available foods should be sustained and enhanced to increase consumption either in their natural or processed state. The conduct of further studies on the nutritional impact of the edible spider and small fish, as well as reviews of their proportion in product formulation are likewise suggested.

SELECTION TO IMPROVE DISEASE RESISTANCE IN SHRIMP

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Genetic disease resistance is very important in all aquaculture species. Without genetic resistance animals are more susceptible to pathogens and when they become infected the morbidity and mortality is higher. In most cases the genetic resistance is not perfect, i.e. the genetic resistance is less than 100%.

Genetic selection has been effective in improving the resistance to several pathogens in shrimp. Today many strains have high resistance to TSV. In TSV challenge tests the survival is typically more than 80%. Improving the resistance to TSV occurred quickly and only a few generations of selection were required to increase the TSV resistance to a high level. Resistance to WSSV and AHPND has progressed at a slower rate. The slower rate of improvement can be due a low frequency of the resistance alleles in the population and/or due to the resistance being determined by many genes with small effects. After many generations of selection by different groups strains of *Penaeus vannamei* have been developed with some resistance to WSSV and AHPND. The following table shows the survival of four different strains at the end of challenge tests designed to measure the resistance to WSSV and AHPND.

The slow rate of improvement in WSSV and AHPND resistance is following the typical pattern of improving genetic disease resistance. Generally, the improvement is made in small incremental steps with the rate of improvement depending on the design of the selection program. Programs can be designed that will improve survival between 1% and 6-7% per generation. Consequently, increasing genetic resistance of shrimp so the survival is increased from 1% to 50% may require between 7 and 50 generations of selection. In the next few years quantum leaps in genetic disease resistance may be possible by using the new genetic techniques that are on the horizon.

Survival of Four Strains at the End of WSSV and AHPND Challenge Tests				
Pathogen	Strain A	Strain B	Strain C	Strain D
WSSV	4%	23%	0%	3%
AHPND	53%	4%	32%	6%

DEVELOPMENT OF A LOW-COST NOVEL ALGAE BASED AQUACULTURE FEED PRODUCT

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Meat and dairy production will double and fish production will triple by 2050. Animal feeds play a leading role in the global food industry, and feed is the largest and most important component to ensuring safe, abundant and affordable animal proteins. Feed accounts for 40-60% of the production costs in aquaculture, so access to good quality feeds at reasonable prices is essential for success and profitability in the aquaculture industry. Most fish feeds contain a minimum level of fishmeal in order to ensure an optimal content of amino acids and other nutrients needed for fish growth and flesh quality. Global capture fisheries has been level for several decades, so alternative protein and omega-3 oil sources are needed. Microalgae sources of protein and omega-3 oil could be a tipping point for faster development of mariculture and change the role of some regions such as North America and Europe in global production.

Global Algae Innovations has developed economical, scalable, open pond microalgae production technology that will enable cost competitive production of microalgae for aquaculture feed (Figure 1). Global Algae has also isolated an algae strain that attains 71.4% protein and has an amino acid profile that compares favorably with fishmeal. Combining this strain with a high omega-3 diatom algae would provide a feed profile very similar to fishmeal, so it is likely to be a highly favorable aquaculture feed ingredient.

Global Algae Innovations' high protein algae strain and high omega-3 diatom were grown at large-scale utilizing our low-cost production technology. Data will be presented on the compositional analysis, fatty acid profile, and rainbow trout digestibility trials conducted at the USDA Agricultural Research Service. These results confirmed the potential of this new algal feed ingredient. The positive data has opened the door for longer-term production tests to generate key metrics needed to support scale-up and more extensive feed trials to validate the efficacy for various species.



Figure 1. 33 acre Kauai algae facility operated by Global Algae Innovations.

SPLIT POND DESIGN AND MANAGEMENT FOR CATFISH FARMING

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Split ponds are constructed by dividing an existing catfish pond into two unequal basins with an earthen levee. Fish are confined in the smaller basin (usually about 15-20% of total water area) while the larger basin serves as a waste-treatment lagoon. A high-volume pump circulates water between the lagoon and fish-holding basin during daylight and aerators maintain adequate dissolved oxygen in the fish-holding basin at night. Split ponds are easy to manage and allow the genetic potential of fish to be expressed by maintaining favorable environmental conditions for growth.

Practical experience indicates that the fish-holding basin should not be larger than approximately 2 acres. Oxygen management in larger basins is difficult and fish biomass may become so great that problems are encountered with harvest and marketing. Accordingly, ponds larger than 8 to 10 acres should not be used as starting points for construction.

The original split pond design was based on overcoming limitations associated with dissolved oxygen availability. Approximate aeration requirements are easy to calculate from target fish biomass and aerator oxygen transfer rates. Aeration effectiveness is enhanced in split ponds compared with traditional ponds because fish are confined to a relatively small water volume. In that small water volume, fish become the primary oxygen-consuming component rather than plankton and sediment as in traditional ponds. The amount of aeration required to meet the total oxygen demand in the fish basin of split ponds is about half that needed in traditional ponds.

Optimum daytime pumping rate has been more difficult to quantify than aeration requirement. Pumping rate in the original design was estimated by oxygen mass balance at approximately 1,200 gallons per minute (gpm) per 10,000 pounds of final fish biomass. Pumping rates in most commercial systems are less than 700 gpm per 10,000 pounds of fish. Low pumping rates cause chronically low daytime dissolved oxygen concentration near the end of the cropping cycle, which reduces fish growth rate for part of the production cycle.

In practice, fish production in split ponds is first limited by growing season length and second by practical limitations on pump size. At high fish standing crops (>20,000 pounds/acre), the required pumping rate becomes impractical and cost-prohibitive.

MORPHOMETRIC AND MOLECULAR ANALYSES OF *Tilapia guineensis* POPULATIONS FROM NIGERIAN COASTAL WATERS

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Information about the current level of diversity and genetic structure of *T. guineensis* populations in Nigeria which will be useful for fishery management, aquaculture production, stock conservation, and fish improvement through selective breeding is lacking. Morphometric and molecular techniques were used to characterize and investigate genetic diversity of *T. guineensis* populations from some Nigerian coastal waters for breeding and conservation purposes. Samples were collected from six coastal states in Nigeria.

Thirteen morphometric variables were measured for determination of phenotypic variation while ten microsatellite markers were utilized for genetic diversity assessment. Results of morphometric analysis showed that *T. guineensis* populations can be differentiated into only two distinct groups. The results revealed low variability among the populations of *T. guineensis* from the coastal locations studied. This implied that *T. guineensis* populations from Nigerian coastal waters are morphologically different. Iwoama and Brass (Bayelsa state) populations were the most varied among all the studied populations (Figure 1). Furthermore, When compared to other locations, fish in Iwoama and Brass had the highest mean weight of 0.29 ± 0.006 kg and 0.27 ± 0.004 kg with mean total length of 0.24 ± 0.002 m and 0.23 ± 0.001 m. Molecular studies showed that Buguma in Rivers state, Badagry in Lagos state and Brass in Bayelsa state populations had the highest genetic diversity as was revealed by heterozygosity and shannon indices. Clustering using SSR data gave four major clusters reflecting some level of genetic variability (Figure 2). Rivers, Lagos and Bayelsa states show greater genetic and morphological divergence and are therefore considered suitable areas for sourcing *T. guineensis* for fish improvement in Nigeria.

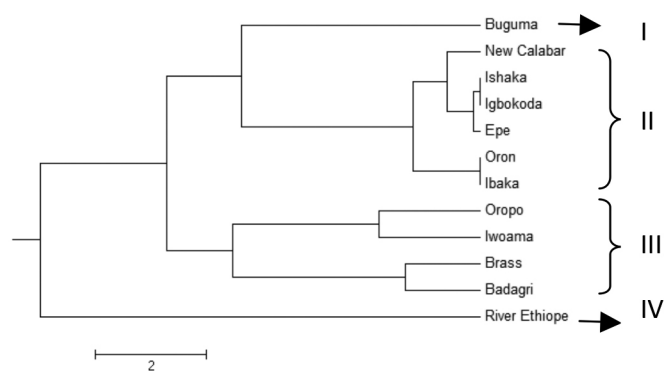


Figure 2. UPGMA Dendrogram Showing the Genetic Relationships among 12 Populations Based on Nei's Genetic Distance.

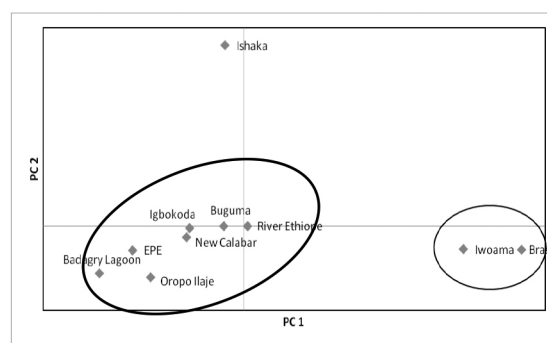


Figure 1. Principal component analysis of morphometric data based on location distribution of samples.

GENETIC VARIATIONS STUDIES OF WILD AND CULTURE CICHLID POPULATIONS (*Sarotherodon melanotheron*) IN LAGOS STATE, NIGERIA

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Sarotherodon melanotheron (Ruppell, 1852) is a typical estuarine species which can be found in abundance in most of the lagoons and estuaries of West Africa which has been a major support to lagoon fishery. It can live and reproduce in a wide range of salinities. They have adapted to diverse habitats: permanent and temporary rivers, large equatorial lakes, tropical and subtropical rivers, open and closed estuaries, lagoons, swampy lakes, deep lakes and coastal brackish lakes (Trewavas, 1983). In this habitat, other species more traditionally used in tilapia culture are either not locally available or do not tolerate the prevailing saline conditions. *Sarotherodon melanotheron*, species is not well known for aquaculture purposes. Therefore, this study is carried out to establish the genetic characterization for the delineation of this Cichlid specie as an important specie not only for aquaculture and hatchery operations, but also for capture fisheries management.

A total of forty (40) samples were collected; 10 samples each of farm-raised (Akinsateru and NIOMR farms, Lagos) and wild (Lagos and Badagry Lagoon, Lagos). Random amplified polymerase DNA (RAPD-PCR) analysis was carried out using Operon primers (OPC 04, 05, 10, OPR 02 and OPI 05). Of the 5 primers, only 4 primers showed both stable amplification and polymorphism except on some samples having low reproducibility.

Figures 1. Shows the similarity coefficients of some of the individuals (SW6, SW7, SW13 and SW12) samples from wild to be 100 %. The similarity from wild and culture were up to 82% and 88% respectively. Such genetic similarity in populations of a species from both wild and cultured has previously been observed for some tilapia species (Kusemiju, 2010).

This study therefore provides information on getting pure strains brood-stock from this natural water body, maintaining pedigree information of the brood-stock to avoid mating of close relatives and also needed for genetic information for effective decision toward managing the fish populations, for genetic manipulation in aquaculture; policy making, management and conservation.

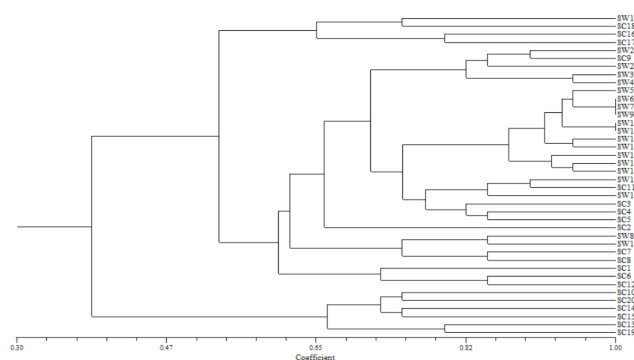


Figure 1: Dendrogram depicting the degree of relationship among *Sarotherodon melanotheron* from wild and cultured samples.

SW1 - SW10 = *S. melanotheron* from the Lagos Lagoon.

SW11 -SW20 = *S. melanotheron* from the Badagry Lagoon.

SC1 - SC10 = *S. melanotheron* from the Akinsateru farm.

SC11 - SC20 = *S. melanotheron* from the NIOMR farm

NITRATE REMOVAL IN MARINE RECIRCULATING SYSTEMS

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Discharge of organic matter, dissolved inorganic nitrogen and phosphorus is a major cause of environmental pollution by recirculating aquaculture systems (RAS). While ammonia removal through incorporation of nitrifying biofilters is well established in RAS, active removal of nitrate, the end product of nitrification, is less common. Hence, in most RAS, nitrate levels are mainly dictated by the system's water exchange rate and to some extent by passive nitrate removal processes in the different system components.

Mainly due to stricter environmental regulations, an increased number of RAS are operated with incorporation of denitrification reactors in which nitrate is biological reduced to elemental nitrogen gas. Fixed film reactors, in which heterotrophic denitrification is fuelled by addition of external carbon sources, are most often used for this purpose. Alternatively, uneaten feed and fish faeces may be used as endogenous carbon and energy sources for the denitrifying organisms.

This latter strategy was used in a zero discharge system which produces marine fish with no pollutant discharge and minimal use of valuable fresh, makeup water. In this particular RAS, denitrifying activity takes place in a digestion basin which is fed with organic-rich effluents from the fish basins. In the digestion basin, denitrification is part of an array of interactive anoxic/anaerobic biogeochemical processes which collectively cause an effective reduction of carbon, nitrogen, phosphorus and sulphide in the treatment water. Nitrate reduction in these basins was found to take place by either one of the following processes: heterotrophic denitrification, autotrophic denitrification on sulfide or dissimilatory nitrate reduction to ammonia (DNRA). The relative contribution of each of these processes was found to depend on the carbon, nitrogen and sulfide concentrations in the various parts of the basins. At ample concentrations of available organic matter, heterotrophic denitrification was the dominant nitrate removal process. DNRA was pronounced at relatively high levels of available carbon and low levels of nitrate (high C/N ratios) or at high sulphide concentrations. Autotrophic denitrification, with sulfide as electron donor, was evident both in absence and presence of heterotrophic denitrification. Sulfide, accumulating in the sludge as a result of sulphate reduction and desulfurization during organic matter decomposition, influenced the relative contribution of the various nitrate removal processes. Results presented are illustrative for the complex biogeochemical processes underlying nitrate removal in organic-rich, marine systems.

INCREASING SURVIVAL RATES IN WHITE LEG (*L. vannamei*) SHRIMP CULTURE AFFECTED BY *Vibrio harveyi* AND *Vibrio parahaemolyticus* CAUSING EMS, WITH ALGAE-INTERSPACED CLAY AND COPPER

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Moving towards sustainable aquaculture, prevention of disease is becoming more important. However, high stocking densities create conditions that become stressful for both the pond environment and the animals grown in them. With the higher demands needed to produce under high stocking densities conditions are also becoming right for bacterial growth and potential disease outbreaks. Once the pathogenic bacteria is present in the production environment it is difficult to get rid of it. There are various strategies where disease problems are being dealt with. In line with the increasing awareness for sustainability, antibacterial products need to be applied in a way that it will not easily be over used or misused nor will it create resistance in the bacterial populations due to selective pressures.

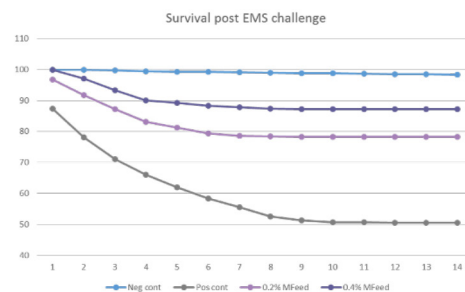
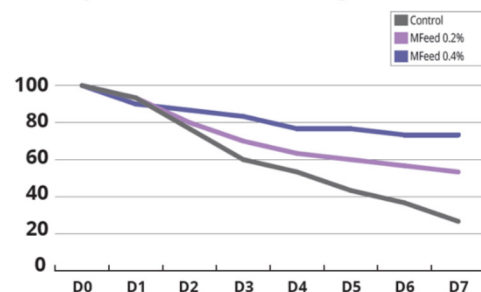
Olmix has specialized in working with algal extracts and montmorillonite clay. Supported by scientific research Olmix has developed a unique technology where an algal-interspaced montmorillonite is associated with copper in order to potentiate the electrostatic activity of clay and the antibacterial properties of copper. This unique complex results in a synergistic action against pathogenic bacteria and contributes to improve the animals gut health. This application was tested in two universities under disease challenge with two common pathogens in *L. vannamei* shrimp culture.

The first trial was carried out at Kasetsart University, in Thailand, where 365 shrimp were randomly distributed into three treatments: a control group, a group received 0.2% MFeed in their feed, and a group receiving 0.4% MFeed in their feed. After a challenge test with *Vibrio harveyi* there was a dose dependent increase in survival among the different groups. The group receiving 0.4% MFeed showing the highest increase in survival followed by the group receiving 0.2% MFeed in their diets.

A following trial was conducted at Can Tho University in Vietnam, where PL5 *L. vannamei* shrimp were raised in 500L tanks at a density of 50 pcs/L. The PLs were randomly divided into 4 groups; a negative and positive control group, a group received 0.2% MFeed in their feed, and a group receiving 0.4% MFeed in their feed. When the PLs reached stage PL25 they were submitted to an immersion challenge test with *Vibrio parahaemolyticus* causing EMS disease. Results show a dose dependent increase in survival among the different groups. The group receiving 0.4% MFeed, showing a nearly 90% survival rate, followed by the group receiving 0.2% MFeed in their diets.

These scientific results support the ability of MFeed as an efficient and sustainable strategy on management of EMS in white leg shrimp.

→ Shrimp survival after the challenge

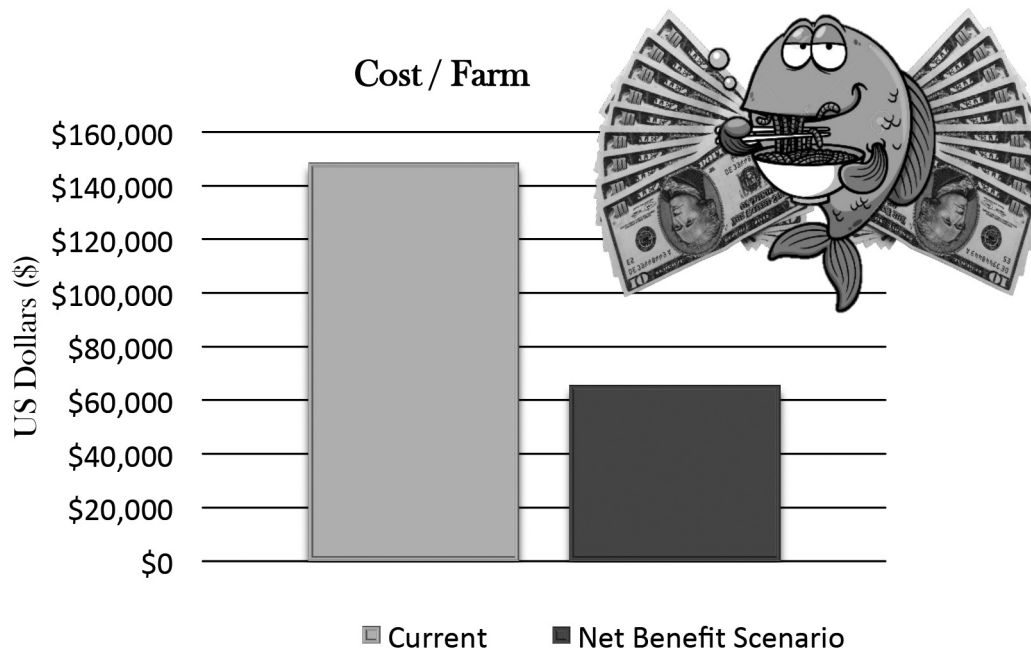


IMPROVED PROFITABILITY THROUGH BETTER ANIMAL HEALTH? POTENTIAL IMPACTS OF A UNIFORM CODE FOR COMMERCIAL AQUATIC ANIMAL HEALTH

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The diversity and growth of aquaculture, in terms of volume, number of species, and production systems, bring with them many challenges for establishing effective animal health policies. Producers of U.S. baitfish and sportfish, sold and shipped live, must contend with widely varying certification and testing requirements to sell their products. Baseline farm level costs were obtained through a survey of commercial producers in 13 states, which captured 74% of domestic baitfish and sportfish production volume. The baitfish and sportfish industry spends an estimated \$12 million per year on regulatory compliance. Fish health regulations comprised 16% of the total cost of regulations identified. To assess the potential impacts of a non-regulatory and voluntary uniform code for aquatic animal health, a total of eight scenarios were modeled. Results demonstrated that a uniform code could result in an estimated savings of \$6,606,963 to the industry; assuming wide adoption of the third-party surveillance and testing program. Individual cost savings per farm were an average savings of \$81,175. Cost savings of this magnitude are a strong incentive for the baitfish and sportfish industry to support a uniform code in addition to the benefits of shifting aquatic animal health policy to a risk-based epidemiological approach.



THE EFFECTS OF REGULATIONS ON U.S. TROUT PRODUCERS: COSTS AND IMPACTS

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A survey to assess the costs and impacts of regulations affecting trout aquaculture in the U.S. was conducted starting in January of 2017. The study was designed and conducted as a census, with the top 17 trout production states included in the survey. Data were collected through telephone and in-person visits with trout producers across the country. While thorough analysis of the data is still underway, there are summary findings that can be shared regarding the costs and impacts affecting producers. Approximately one third of respondents identified “regulations” as the #1 challenge to their business; with the study identifying over 300 different permits and regulations across the various states. On average trout producers had to maintain 7 different permits and licenses every year for their business to remain compliant. About 30% of respondents indicated that discharge regulations were the most challenging for their farm; with about 40% of producers reporting having made changes to their business to be compliant with discharge regulations. Interstate shipping and associated fish health testing requirements were not far behind, as the second most challenging regulations for respondents. It should be noted, that many producers made specific mention of wanting to protect the natural environment and understanding the need for there to be some measure of testing for fish health and water quality. However, the demand for frequent testing, reporting, and costly changes in management or infrastructure that would not benefit the environment or the health of the fish were also often expressed.



THE COSTS OF REGULATORY COMPLIANCE ON WEST COAST SHELLFISH FARMS: EFFECTS AND IMPACTS ON PRODUCERS

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Through the end of 2016, a survey to assess the costs and impacts of regulations affecting shellfish aquaculture on the west coast was conducted. While producers widely acknowledged the importance of regulations, specifically with regards to environmental protection and food safety; many also reported indirect costs of regulatory compliance in their business. Preliminary results from the study indicate that there are several economic effects that were observed during interviews and from data analysis that are worthy of note. These included significant impacts to business development and planning resulting from permit and licensing delays, with 25% of respondents reporting to wait a year or more for a permit to be approved or processed; some delays lasting up to 20 years. Cash flow interruptions from missed planting or harvest windows; with small scale producers being particularly vulnerable to cash flow interruptions. Increased legal fees and administrative costs, and lost sales or missed opportunities for expansion and diversification; 40% of respondents indicated they were unable to expand their operations, despite increased demand for shellfish. In addition to these impacts, some producers also reported lost investment capital due to regulatory uncertainty, and the labor challenges of ensuring regulatory compliance as a small business.

Washington

Oregon

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ENHANCED GROWTH AND FEED EFFICIENCY AND REDUCED SERUM GLUCOSE AND HDL-CHOLESTEROL IN NILE TILAPIA *Oreochromis niloticus* FED DIETS SUPPLEMENTED WITH *Sargassum fulvellum*

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A feeding trial using 225 juvenile Nile tilapia *Oreochromis niloticus* (1.89 ± 0.62 g) fed five iso-lipidic (8.9%) and iso-nitrogenous (38.3%) diets with *Sargassum fulvellum* meal (SFM) level of 0% for both positive (PC) and negative control (NC), and incremental levels of 3% (C3), 6% (C6) and 9% (C9) was conducted for 8 weeks. The main objective of the study was to evaluate *S. fulvellum* as a low level feed supplement for *O. niloticus* juveniles. Results showed improved growth performance in fish fed diets supplemented with SFM, with significantly highest performance ($P < 0.05$) at C6. Feed utilization efficiency increased in juveniles fed diets supplemented with SFM. However, there were no significant differences ($P > 0.05$) found in the serum uric acid, triglyceride, creatinine, cholesterol, glutamic-pyruvic transaminase, and glutamic oxaloacetic transaminase. On the other hand, serum glucose and high density lipoprotein cholesterol values were significantly reduced ($P < 0.05$) in fish fed diets supplemented with SFM. Carcass crude fat and protein values were also significantly affected ($P < 0.05$). HSI values of fish significantly increased ($P < 0.05$) with supplementation, while VSI values of fish fed diet with SFM supplementation were not significantly different ($P > 0.05$) from fish fed PC and NC. Moreover, condition factor of juveniles increased with supplementation. Vacuolar degeneration due to lipid accumulation was evident in the livers of fish fed all treatments. Overall, SFM (low level supplementation) is shown to be a potential aquafeed ingredient for juvenile tilapia.

ENTERRA FEED: SUSTAINABLE INSECT-BASED FEED INGREDIENTS IN AQUACULTURE DIETS

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Enterra is a sustainable insect farming operation near Vancouver, BC, Canada that grows black soldier fly larvae (*Hermetia illucens*) as a renewable source of nutrients for animals and plants. The larvae are reared on pre-consumer recycled food collected from local farms, food processors and grocery stores. After harvesting, the larvae are processed into high value protein and oil products as sustainable substitutes for costly and resource-intensive ingredients. Enterra is a world leader in commercial scale rearing of Black Soldier Fly Larvae and the first to receive federal regulatory approval to use insects in animal feed in North America. The use of Black Soldier Fly Larvae products (Whole Dried Larvae, Meal and Oil) in aquaculture will be discussed, focusing on FDA and CFIA regulations, growth and digestibility trials, and benefits to feed manufacturers and finfish producers.

BIOECONOMIC ANALYSIS OF THE ANNUAL INTENSIVE PRODUCTION OF WHITE SHRIMP (*Litopenaeus vannamei*) IN A PHOTO-HETEROTROPHIC SYSTEM WITH MINIMAL SEAWATER-ADDITION IN THE SEMIARID REGION OF MEXICO

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In Northwest Mexico, shrimp farms have been using semi-intensive systems based on water exchange over the last 20 years. The industry faces severe challenges due to the presence of diseases (WSSV, EMS), increases in production costs and lack of environmental sustainability. CIBNOR has been working with producers in Baja California Sur, a state located in the desert belt of Northwest Mexico where, since 2010, it has been possible to obtain 2 cycles/year with annual yields of 20 tons/ha, using 30 hp/ha of aeration and 25% water exchange. Water exchange increases costs and biosecurity risks, so the objective of this study was to determine the level of variability on water quality, annual yields and production costs in two photoheterotrophic cultivation cycles (spring-summer, summer-fall) and to determine economic viability. We used six 1,000 m² PVC lined ponds at Biohelis®, the Innovation and Technology Park operated by CIBNOR, with 24 h aeration, that was increased, depending on O₂ demand, from 20 to 40 hp/ha. Seawater was incorporated in weekly pulses, for an equivalent of less than 2%/day, to maintain water level, so there was no water exchange. A commercial pelleted ration with 35% CP was fed 2 times/day. Spring-summer yields of *L. vannamei* after 105 days in the intensive photo-heterotrophic hypersaline system consistently reached $13,400 \pm 233$ kg/ha/cycle with a mean final weight of 13.33 ± 0.35 g (Figure 1B). Yields for the summer-fall cycle reached $17,900 \pm 530$ kg/ha, in 90 days. Mean final shrimp weight was 18.60 ± 1.07 g (Figure 1A). A stochastic model was fitted to the growth, mortality and food conversion curves. Production costs were incorporated and sale prices were established from a model adjusted to historic prices for the mean size produced. Economic variables and financial indicators for each cycle are presented. Implications for the consolidation of shrimp culture in semiarid areas, based on innovative science-based technologies, are discussed.

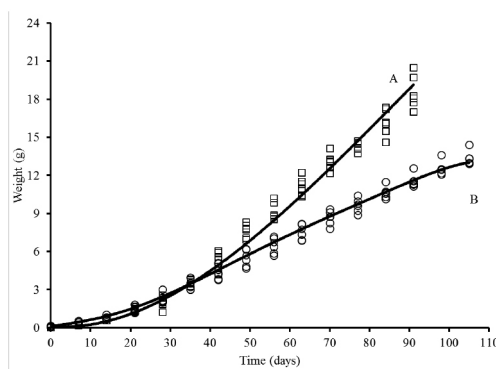


Figure 1. Weight increase through time for white shrimp cultured in a photoheterotrophic system. (A) Summer-Fall; (B) Spring-Summer.

PRODUCTION PERFORMANCE OF *Cherax quadricarinatus* FAMILIES FROM A NUCLEUS WITH INCREASED GENETIC VARIABILITY IN MEXICO

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Commercial culture of *Cherax quadricarinatus* in Mexico is considered an alternative to diversify aquaculture production. The species was introduced to Tamaulipas, Mexico in 1996, and batches of juveniles and breeders were distributed to different farms countrywide. Several semi-intensive culture systems are used for the production of the species in states such as Tamaulipas, Michoacán and Baja California Sur. However, present trends in aquaculture require better efficiency and more environmentally friendly intensive technologies. During 20 years of multidisciplinary research by CIBNOR, significant technological advances have been achieved. Technology transfers to farmers have allowed commercial production to increase from 2,500 kg/Ha in 9-month cycles, obtained in Tamaulipas in 1996, to 3,500 kg/Ha in 6-month cycles, in 1998, in Guayaquil, Ecuador, using 5%/day water exchange. In 2006, yields in Baja California Sur, were 3,500 kg/ha in 6-month cycles, without water exchange, increasing after the implementation of an intensive photo-heterotrophic system, to 5,000 kg/Ha in 6-month cycles, by 2008. However, the common practice at most farms, of restocking breeding ponds directly from grow out ponds, has generated a growing concern about the potential reduction in harvest size, deterioration of physiological health and decline in reproductive performance, due to inbreeding over time. Thus, the development and characterization of a nucleus with a wide genetic pool has become a priority for the incipient industry.

The objective of this study was to determine the level of genetic variability and differentiation of three divergent populations of *C. quadricarinatus*, and to evaluate the performance of families from parental and reciprocal crossings of these groups. We sourced and quarantined RedClaw from commercial farms in Tamaulipas, Michoacán and Baja California Sur, before selection for DNA extraction and rPCR amplification. Twenty specific microsatellites were used to determine allelic frequency. By pairing one male and one female, we established 80 families in 1,500 l tanks, with water at constant temperature ($26 \pm 2^\circ\text{C}$) and continuous aeration. Breeders were fed daily at 5 pm with a 35% CP pelleted feed. When eggs were identified in the female pleopods, the male was removed. After juvenile release, the females were removed to avoid cannibalism. After 45 days, the young from each family were weighed, counted, and cultivated in the 1,500 l tanks for 120 days. Final mean weight and survival were determined and a regression model for weight increase for each family was obtained. Results and the implications of the genetic selection program for the consolidation of the industry in Mexico are discussed.

TOFU-TOLERANT MARICULTURE: GENOMICS-ASSISTED BREEDING OF A HIGH QUALITY MARINE FINFISH FOR ENHANCED PERFORMANCE ON SUSTAINABLE, SCALABLE, SOY-BASED FEEDS

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Reliance on fishmeal and fish oil is a significant constraint to the future expansion of finfish aquaculture to meet global needs. As U.S. marine fish farming expands, genetic improvements could increase economic performance and decrease ecological footprint.

Thus far, no extensive selective breeding has been accomplished with *Seriola* or similar high-quality marine fish. However, as the U.S. marine finfish aquaculture industry transitions from a niche role to providing a significant portion of the nation's seafood supply, genetic improvement of farmed strains to increase economic performance and decrease ecological footprint will be of critical importance to the scalability of the industry and attracting the capital investment required to expand.

Kampachi Farms, in partnership with the Center for Aquaculture Technologies, are connecting SNP markers with traits we have identified as important to commercial *Seriola* culture. Results suggest that markers may exist for predicting growth performance on soy-based diets. Markers for performance on standard fishmeal-based diets, sex, fillet thickness and tissue lipid-content are currently being analyzed. Additional trials will examine marker associations with broodstock fecundity, deformities, and ectoparasite resistance. These results can be immediately applied to commercial culture of Cabo Kampachi™ at our operations under development in La Paz, in Baja California, and – with appropriate biosecurity precautions – globally.

THE ARPA-E MARINER PROGRAM AND THE OPPORTUNITIES FOR OFF-SHORE CULTIVATION OF MACROALGAE (SEAWEED)

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The Advanced Research Project Agency Energy (ARPA-E) recently launched the MARINER (MAcroalgae Research Inspiring Novel Energy Resources) funding program focusing on the development of advanced technologies for large-scale cultivation of macroalgae in the ocean. This effort has been motivated by a desire to leverage the United States' extensive ocean resources for the sustainable production of large quantities of biomass for fuels and chemicals, while not competing with farm land and freshwater needs of terrestrial agriculture. The program covers various technologies tailored to a diverse range of geographies. A particular emphasis is placed on scalability, maximizing renewable energy return, as well as ensuring optimal nutrient supply from either anthropogenic (eutrophication zones) or naturally occurring sources. As part of this program, ARPA-E is working closely with the National Oceanic and Atmospheric Administration (NOAA) to develop a marine spatial atlas for US waters, which can help identify appropriate locations for seaweed farms and assist in the permitting process. This presentation will provide an overview of the specific program goals and a preview of the scope of individual projects. In addition, the presentation will outline potential stepping stones for the growth of a U.S. based macroalgae industry as well as opportunities to engage with the MARINER program.

AQUACULTURE OF AFRICAN LUNGFISH (*Protopterus aethiopicus*) IN UGANDA: CAPTIVE BREEDING AND LARVAL REARING

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The African lungfish (*Protopterus aethiopicus*) supports many communities in Uganda, and has aquaculture potential. It's an air-breathing fish that can withstand stressful water quality conditions in the wild. Fish farmers can access from natural environments, which is not environmentally sustainable. This study reveals the genetic diversity of *P. aethiopicus* collected from Lakes Wamala, Kyoga, Nawampasa, Bisina, Edward and George, which guide its aquaculture and biodiversity. Lungfish fertilized eggs can hatch at a range of 24-32C but optimally at 27C. Hatchability in captivity is 21.7 ± 7.2 % (SD) while its mean fecundity (wild brood) = 1922.41 ± 1227.6 . Salt and temperature improves hatchability. Larvae fed on decapsulated *Artemia* sp. and microdiet (35-57% Crude Protein) indicate a fish can be raised on artificial commercial diets. Developing low-cost sustainable breeding techniques will contribute to improve nutrition and livelihoods of vulnerable communities.

AFRICAN LUNGFISH (*Protopterus aethiopicus*) IN UGANDA: CAPTIVE BREEDING AND LARVAL REARING

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National Fisheries Resources Research Institute, Uganda.

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The African lungfish (*Protopterus aethiopicus*) supports many communities in Uganda, and has aquaculture potential in the East African region. It's an air-breathing fish that withstands stressful water quality conditions in the wild. Fish farmers access seed from natural environments, which is not environmentally and economically sustainable. This study uncovers the genetic diversity of *P. aethiopicus* collected from Lakes Wamala, Kyoga, Nawampasa, Bisina, Edward and George, and a total of putative 1,437 SNPs are generated to inform its future breeding programs. Captive breeding trails reveal fertilized lungfish eggs hatch at a range of 24-32C but optimally at 27C. Hatchability in captivity is 21.7 ± 7.2 % (SD) while the mean fecundity of wild lungfish brood = 1922.41 ± 1227.6 . A combination of salt (0.5g/L) and temperature enhance its hatchability. Larvae fed on decapsulated *Artemia sp.* and microdiet (35-57% Crude Protein) at 18-20 DAH improves growth and survival rates. This study generates information that guides the domestication of African lungfish in the region, and will improve nutrition and livelihoods of vulnerable communities.

EFFECTS OF VARYING LEVELS OF BREWER'S GRAINS AND AMINO ACID SUPPLEMENTATION ON GROWTH PERFORMANCE OF JUVENILE NILE TILAPIA *Oreochromis niloticus*

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Tilapia are currently the second most popular farmed fish globally behind only carp. The success of tilapia as a farmed food-fish is based partly on their ability to efficiently utilize plant products as feed lending to both economical and sustainable production. The incorporation of locally available by-products has the potential to reduce feed and production costs. Currently, there are over 4,000 breweries in the United States. Brewer's grains (BG) are the solid residue left after the processing of cereal grains for the production of beer. BG are a concentrate of protein and fiber remaining after most of the sugar has been extracted from the mash. Tilapia have been shown to be unique in their ability to digest fibrous plant materials and are often fed agricultural and industrial by-products in other parts of the world.

An eight-week feeding trial was conducted to evaluate the effects of adding increasing levels of BG into diets fed to Nile tilapia (*Oreochromis niloticus*). Ten juvenile tilapia (average weight 6.6 g) were stocked into each of sixteen 38 L aquaria with four aquaria per dietary treatment. Fish were fed three times daily one of four experimental diets containing either 0% BG (Control), 15% BG, 30% BG or 30% BG with added crystalline amino acids (AA; 30% BGwAA). In the 30% BGwAA treatment 0.35% Methionine and 0.35% Lysine were added to the formulation. The Control diet was modeled after a traditional commercial tilapia diet. All diets contained 8% fish meal and were formulated to be isocaloric and isonitrogenous. Added BG replaced conventional soy bean meal and wheat flour in the formulations.

Water quality variables remained acceptable for tilapia production throughout the trial. After eight weeks, there was no significant difference ($P>0.05$) in survival among treatments (Table 1). The average weight (g) and specific growth rate of fish fed the Control and the 15% BG were statistically similar although they were both greater ($P<0.05$) than those for fish fed diets 30% BG (Table 1). Feed conversion ratio (FCR) was more efficient for fish fed the Control diet than for fish fed both 30% BG and 30% BGwAA. FCR for fish fed the 15% BG was better than for fish fed 30% BGwAA but was not different from the other diets (Table 1). Consumption was reduced in fish fed diets containing BG compared to the Control diet (Table 1). Based on these data, juvenile tilapia fed diets containing 15% BG resulted in acceptable growth and FCR while fish fed diets containing 30% BG resulted in a growth reduction and increased FCR.

TABLE 1. Survival, average weight, SGR, FCR and consumption of juvenile tilapia fed diets containing different levels of Brewer's Grains (BG). Means in a row with different letters were significantly different ($P<0.05$).

	Control	15% BG	30% BG	30% BGwAA
Survival (%)	97.5±5.0a	95.0±5.8a	92.5±9.6a	95.0±5.8a
Average weight (g)	56.5±2.5a	53.6±4.1a	48.7±1.8b	43.3±3.4c
SGR (g/d)	0.90±0.05a	0.84±0.08a	0.76±0.03b	0.65±0.06c
FCR	1.14±0.11a	1.32±0.50ab	1.41±0.15bc	1.54±0.17c
Consumption (g)	584.5±19.2a	551.3±19.7b	540.6±11.8b	528.0±7.3b

FKCC PRODUCT DISBURSEMENT I: TRAINED AQUACULTURE TECHNICIANS

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In 2010, Florida Keys Community College (FKCC) received a 5-year Advanced Technology Education grant from the National Science Foundation to establish a Tropical Ornamental Mariculture Technician (TOMT) Certificate Program. The primary goal of the program is to develop technician-level marine scientists with skills for the emerging 21st century aquaculture industry. The program focuses on marine ornamental aquaculture for the following reasons: (1) the facility requirements are less than those focused on food producing organisms, (2) aquaculture technology for several marine ornamental species are well developed (e.g., clownfish) and excellent for training purposes, (3) the tremendous potential for undergraduate research to develop aquaculture techniques for new marine ornamental species, (4) the potential to transfer technology to the marine aquaculture industry, and ultimately (5) to reduce the demand for wild caught marine ornamental species for the aquarium trade.

The certificate is a 30-credit hour program composed of the following courses: Introduction to Marine Biology, Survey of Mariculture, Introduction to Business, Mariculture Systems and Design, Aquaculture Best Management Practices, Nutrition of Mariculture Species, Disease and Parasites in Marine Aquaculture, Tropical Ornamental Mariculture, and an applied internship. Students gain hands-on husbandry skills using the campus's indoor recirculating and outdoor flow-through systems, which are stocked with marine species. Knowledge gained during courses is applied in the laboratory during spawning and larval rearing.

The TOMT certificate program has generated 12 graduates since 2013. In addition, the program provides five additional elective courses for students enrolled in the Associate of Science Marine Environmental Technology degree program. Students who have completed the program and/or taken TOMT electives have successfully acquired jobs in the marine ornamental aquaculture and aquarium industries (Table 1).

Tropical marine aquaculture is increasingly used for conservation efforts focused on coral reef species and coral restoration projects. Increasing environmental concerns over global degradation of many tropical marine coral reef ecosystems, and specifically Florida Keys coral reef ecosystems, necessitates the need for immediate education of qualified tropical marine aquaculture technicians. The TOMT certificate is designed to develop marine ornamental entrepreneurs and provide technician-level marine aquaculture skills that will help fill the aquaculture jobs of the future while helping supply the demand for oceanic resources from a cultured environment and not the ocean.

TABLE 1. Job Placement Post-TOMT Classes

FKCC Science Laboratory Technician
Key West Aquarium (Aquarist)
Marathon Pet Center
Petco. (Sr. Aquatics Specialist)
Proaquatix
SeaWorld (Diver, Aquarium)
Small Business-Owner (Fish Culturist)
Transferred to Four-Year Educational Institution

HETEROTROPHIC VS MIXED BIOFLOC SYSTEMS: IMPACTS ON USE OF WATER, SUSPENDED SOLIDS PRODUCTION AND ZOOTECHNICAL PERFORMANCE OF *Litopenaeus vannamei*

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The Biofloc Technology System (BFT) is characterized by the use of bacteria to remove nitrogenous compounds from the water, guaranteeing better water quality and little or no water renewal. Two major groups of bacteria are involved in the removal of nitrogen in this system: heterotrophic bacteria, through the incorporation of inorganic Nitrogen and organic Carbon into their biomass, and autotrophic bacteria, which perform nitrification through the oxidation of ammonia to nitrite and then nitrate. In this way, different techniques can be used for the formation and maintenance of the bioflocs, depending on which group of bacteria is wanted to be the dominant. The objective of this study was to analyze the effect of bioflocs formation techniques and their effects on the zootechnical performance of *L. vannamei*, on the use of water and on the production of suspended solids.

A 60-day trial was conducted at the FURG's Marine Aquaculture Station. Shrimp juveniles ($7.05 \text{ g} \pm 1.37$) were stored in 150 liter tanks at storage density of $300 / \text{m}^3$. Four treatments ($n = 3$) were tested using different floc formation techniques: no supplemental organic fertilization, fertilization according to the nominal ammonia reading (mixed heterotrophic/chemoautotrophic) and daily fertilization according to the estimated ammonia produced (heterotrophic). The temperature, salinity, dissolved Oxygen, pH, ammonia, nitrite, nitrate, alkalinity and total suspended solids (TSS) of the water were monitored. Water renewal was done every time ammonia, nitrite and SST levels exceed predetermined limits. The results were analyzed by ANOVA one-way and Tukey's test ($\alpha = 0.05$).

The water quality parameters were influenced by the treatments ($P < 0.05$), with differences in the concentrations of ammonia, nitrite, nitrate, pH, alkalinity and SST. The heterotrophic treatment had the lowest nitrate value, due to the predominance of heterotrophic bacteria, as well as higher pH and alkalinity levels, since these bacteria do not use inorganic carbon. There were significant differences in the zootechnical parameters, being the highest final weight and productivity, as well lower FCR, found in the mixed treatment. There were no significant differences in survival. The total volume of water used presented lower values in the mixed treatment, while solids removed was almost four times higher in heterotrophic treatment compared to the others. These results showed the importance of adopting a mixed heterotrophic/chemoautotrophic biofloc system to optimize water use and decrease solids production.

Acknowledgments: CNPq, CAPES, FAPERGS, Guabi, Trevisan, INVE, Aquatec e All-Aqua.

RECENT PROGRESS ON HATCHERY PRODUCTION OF BLACK SEA BASS JUVENILES TO SUPPORT FINFISH MARICULTURE GROWOUT INDUSTRY DEVELOPMENT IN THE EASTERN US

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A critical variable cost in the development of a commercial marine finfish aquaculture industry in the US is the availability and price of fingerlings that can be grown to a marketable size in land-based or offshore aquaculture systems. A reliable and affordable source of fingerlings alleviates the technical and financial burdens of a startup farmer to grow and market marine fish. To support the development of the finfish mariculture industry in NC and the southeastern US, the NC Biotechnology Center has supported the establishment of a pilot commercial marine fish hatchery at the UNCW Center for Marine Science Aquaculture Facility (Wrightsville Beach, NC). Goals are to scale up hatchery technologies for marine finfish, including the black sea bass *Centropristis striata*, evaluate economics of fingerling production, conduct research to reduce fingerling production costs, supply fingerlings to commercial mariculture companies on the US east coast for their pilot growout projects, and provide experiential opportunities for students and a business model to stakeholders.

Based on the operation of UNCW's pilot hatchery, an earlier economic analysis of a hypothetical commercial scale black sea bass hatchery posited that fingerling prices may be effectively and practically lowered by maximizing safe nursery tank stocking densities (no. fish per unit of rearing capacity) and shortening the duration of a rearing cycle (number of days per crop) to a transport-ready fingerling stage. Research currently underway at UNCW supported by the NC Sea Grant has revealed that stocking of post-metamorphic stage black sea bass (~0.54 g and 47 days post-hatching) in nursery tanks at relatively high densities of 4.5 to 6.5 fish/L does not adversely affect survival, growth variation, and FCR, yielding robust, transport-ready fingerlings (~1.6 g mean weight) as early as 60 days post-hatching (see Carroll et al., these proceedings). These results of recent biological research, as well as engineering and cost data from the operation of the pilot hatchery, will be used to inform updated economics analyses of hypothetical commercial scale black sea bass hatchery operations comparing the financial performances of alternative facilities that produce fingerlings of different ages and sizes. By providing a source of affordable fingerlings, UNCW's pilot hatchery is enabling new farmers to establish growout technology and develop markets and is a foundational resource of finfish mariculture business and industry development, outreach, and education in NC and the eastern US.

LIPIDOMICS DERIVED TISSUE PROFILES OF JUVENILE RED DRUM, *Sciaenops ocellatus*, FED SOY PROTEIN AND SOY OIL BASED FEEDS

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A nine-week feeding trial was conducted with juvenile red drum (200 g avg. starting weight) fed diets containing 36 g 100g⁻¹ soybean meal or soy protein concentrate and 0, 25, or 50% soy oil replacement of fish oil. Performance on these diets was compared to two fishmeal-based diets, one with all lipid provided by fish oil and one with 50% soy oil replacement of fish oil. Fish were maintained in a recirculating aquaculture system and fed to satiation daily.

At the initiation and conclusion of the trial, multiple tissues were collected including liver, adipose, plasma, kidney, heart, muscle, and brain. Tissue samples were snap frozen in liquid nitrogen and stored at -80 °C until processing. Tissues were homogenized using a cryomill to prevent thawing and extracted via standard methods for utilization in a liquid chromatograph/mass spectrometer. Tissue samples along with control and reference materials were analyzed via high resolution tandem mass spectrometry to provide both quantitative values for specific lipid species and semi-quantitative comparisons of lipid classes across the entire lipidome. Fatty acid profiles were also determined on fillet tissue samples via industry-standard gas chromatography protocols.

This presentation will highlight the differences in critical lipid species observed in various tissues (ex: Figure 1, liver and brain combined control materials) due to dietary protein and lipid source/concentration and the differences observed in fillet muscle profiles from the two profiling techniques employed.

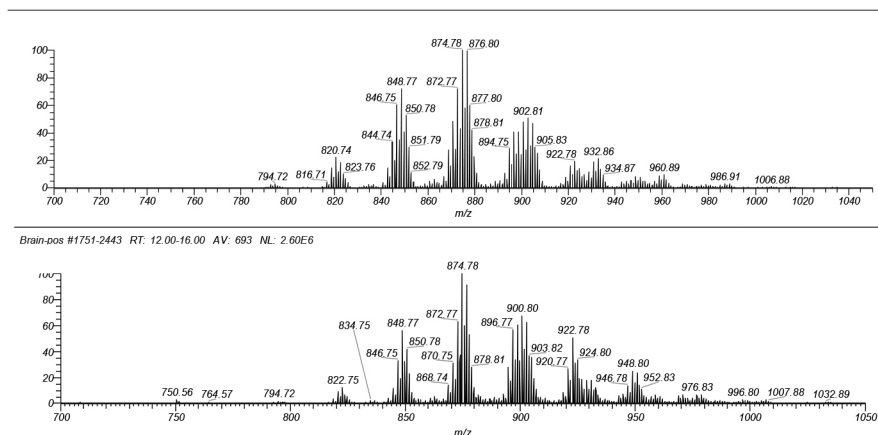


Figure 1. Liver (top) and brain (bottom) control material triglyceride profiles as derived by mass spectrometry on a Fusion Lumos Orbitrap.

USE OF PHA COLUMNS TO CONTROL NITRITE PEAKS DURING ACCLIMATION OF MARINE SHRIMP PRODUCTION

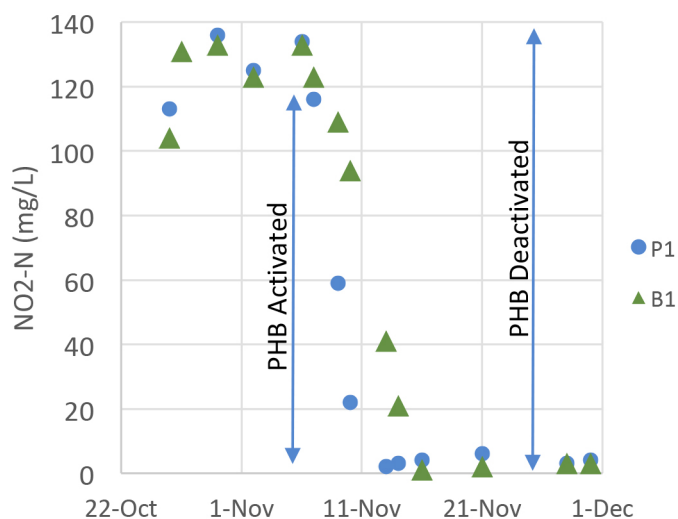
Lisa Weaver, EI*, Dr. Timothy Pfeiffer, Ph.D., Dr. Ronald Malone, Ph.D., PE

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AST Filters was awarded a USDA SBIR grant to investigate and modify the stability of inland shrimp production biofloc systems. Two configurations were involved in this research. Configuration 1 reflects current shrimp biofloc design practice, utilizing a simple clarifier for solids control. A PolyGeyser floating bead bioclarifier served as a fixed film operation for Configuration 2. Each configuration was conducted in 75 gallon glass aquarium tanks, with shrimp stocked at a rate of 300 PL/m² to simulate intensive growout of shrimp farming. During the course of this grant, the first replicates of both configurations (Tank B1 and Tank P1) experienced acclimation issues aggravated by analytical error. As a reference, in shrimp systems, it is recommended to maintain ammonia (TAN) below 3 mg/L and nitrite at 5-25 mg/L. In our systems, we experienced spikes in nitrite of 134 mg/L and 133 mg/L in the PolyGeyser and biofloc tanks, respectively. This resulted in both systems losing over three quarters of the shrimp stock. Once these higher nitrite concentrations were discovered, actions were taken to recover the remaining shrimp and stabilize the systems. This resulted in utilizing a column filled with Polyhydroxybutyrate (PHB), a bioplastic, as a quick-fix solution to diminish nitrite accumulation in the systems.

PHB acts as an energy and carbon source. It comes in a pellet form, and can degrade either aerobically or anaerobically. As it degrades it is able to serve as a feed source for the bacterial populations and promote growth. In our application, flow was restricted in the column to promote anaerobic conditions, in which more efficient nitrogen assimilation can occur. In 7 days, nitrite decreased from peak to 2 mg/L in Tank P1 and for Tank B1, nitrite decreased from peak to 1 mg/L in 10 days. The tanks were then able to be restocked up to the desired density and proceed with the study. After roughly two weeks of running online, the external PHA columns were turned off. The tanks have so far shown no sign in nitrite instability.

Figure I. Activation of PHB Column during Peak Nitrite



TAG-BASED RNA-Seq AS A TOOL TO EXAMINE GENE EXPRESSION DIFFERENCES IN RED DRUM, *Sciaenops ocellatus*, EGGS THAT DO AND DO NOT 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 different 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 pattern is similar to a condition seen in terrestrial vertebrates and referred to in humans as Metabolic Programming. Due to constraints in sourcing large amounts of highly purified DHA with which to amend the broodstock diets, DHA levels are manipulated by using combinations of fresh and frozen feeds along with formulated pellets. This means that though the target DHA levels are reached, there are differences in other nutrients including other fatty acids. Despite these differences, the metabolic programming effect has persisted across several years and multiple brood groups. In 2016 however, there a brood group produced eggs that did not demonstrate the DHA programming effect. The diet used to produce these differences was substantially different from a diet used at the same time on a different brood group which did show the expected programming.

In order to better understand the mechanisms behind this metabolic programming in red drum, we collected batches of eggs containing high and low DHA from both brood groups along with 21-day post hatch larvae raised from these eggs to examine differences in gene expression. This was done through whole transcriptome sequencing using RNA Sequencing (RNA-Seq). To keep costs down, we used a type of tag-based RNA-Seq (Tag-Seq) that allowed us to pool multiple samples per lane. Reading of the sequences was performed on an Illumina HiSeq 2500 v4 using a single cell RapidRead. Results of a comparison of the two sets of eggs and larvae (with and without programming) will be discussed.

SEX REVERSAL OF RAINBOW TROUT BY IMMERSION OF FRY IN 17-ALPHA METHYLTESTOSTERONE

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17-alpha methyltestosterone (MT) is currently used to sex reverse genetic female rainbow trout into phenotypic males, commonly referred to as neomales. Neomales are primarily generated to propagate all-female lines. The MT is most commonly administered orally, fed during the first 6-9 weeks after swim-up. Oral administration is highly effective and uses little MT, but effluent water from the culture system may contain residual MT. Furthermore, most of the neomales do not release milt due to malformed sperm ducts, thus necessitating the removal of the testes to obtain sperm. Immersion of fry in a bath containing MT is also possible; however, reported treatment regimens have been less effective than oral administration. Interest in making it easier to contain the MT used during sex reversal procedures, and interest in avoiding use of steroids as feed additives, has renewed interest in the use of immersion for neomale production.

We have found that immersion of fry from an all-female line in 400 ug MT/L for 2 hours in a static bath at 1 week post-hatching and then once weekly for up to 9 weeks, starting at swim-up, is highly effective and results in >95% neomales. Furthermore, limited results suggest that up to half of the neomales are functional in that they can release milt.

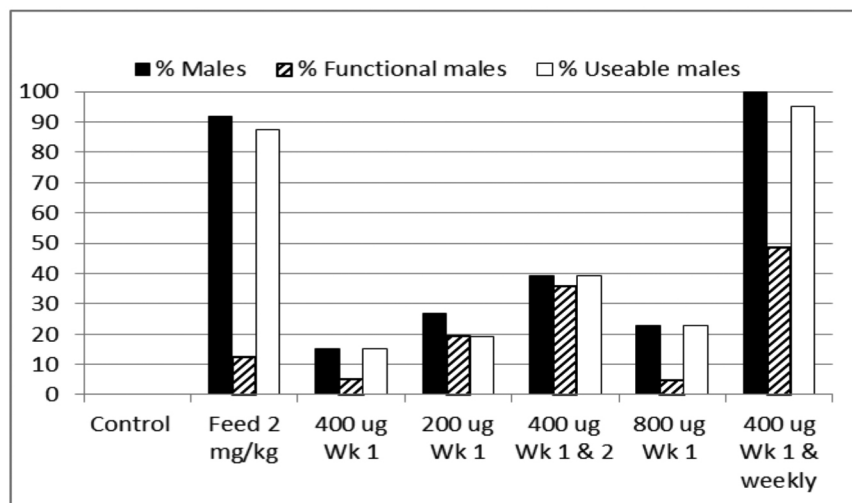


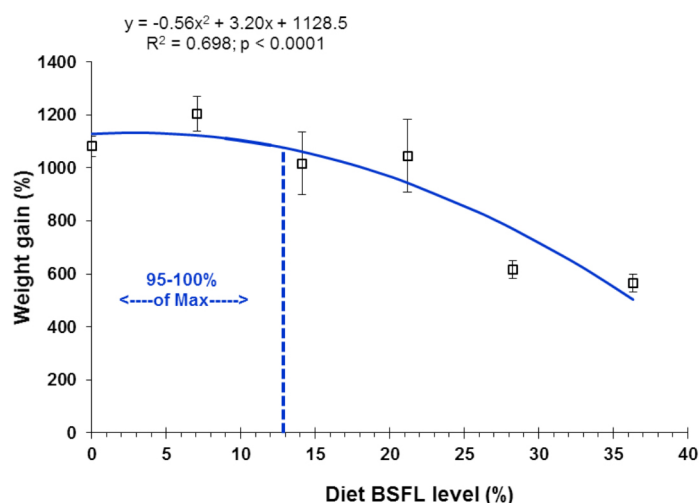
Figure 1. Effect of immersion of fry in concentrations of MT ranging from 200 -800 ug/L at week 1 post-hatching, or week 1 and week 2, or week 1 and then weekly for 9 weeks; compared with fry fed MT or without hormone treatment. N = 19-60 individuals per treatment.

EVALUATION OF BLACK SOLDIER FLY *Hermetia illucens* LARVAE MEAL AS PARTIAL OR TOTAL REPLACEMENT OF MARINE FISH MEAL IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Black soldier fly larvae (BSFL) meal, produced from the larvae of *Hermetia illucens*, has shown promise as a fish meal (FM) replacement in diets for rainbow trout, catfish and tilapia, but has not been examined as an alternative protein source in shrimp diets. Six isonitrogenous (35% crude protein, as fed) and isoenergetic (16.7 KJ available energy g⁻¹ of diet) diets containing graded levels of BSFL as replacements for protein from menhaden FM were fed to juvenile (1.24 g ± 0.01; mean ± SE) Pacific white shrimp, *Litopenaeus vannamei*. Diet 1 (the control) was formulated similar to a commercial shrimp diet containing 25% menhaden FM and 23% soybean meal. Diets 2-6 were formulated as a dose-response series that progressively replaced protein from menhaden FM with BSFL meal at inclusion rates of 7%, 14%, 21%, 28%, and 36% of diet; this equated to progressively replacing 16.5 % of dietary protein provided by menhaden FM. Diets were fed to juvenile shrimp stocked into eighteen 110-L saltwater aquaria (three replicates per dietary treatment) at a density of 15 shrimp per aquarium (50/m²) for 63 d. Nonlinear and spline regression analysis of responses indicated that the maximum level of BSFL meal inclusion varied significantly with the response being modeled. Generally, without modification of the ingredient or replacement diet nutrient profiles, 95% to 100% of most growth responses, i.e., shrimp final weight, weight gain, specific growth rate, and food conversion, could be obtained if replacement of FM by BSFL meal was limited to less than 25% of the diet, depending on performance measure. Similarly, 95% or greater of maximum whole-body protein and lipid content could be achieved when BSFL inclusion was restricted to less than 29% and 15%, respectively. Comparison of amino acid profiles in the test diets with recent requirement estimates for limiting amino acids in BSFL meal also suggest future strategies for increasing dietary substitution of FM with BSFL.



MOLECULAR AND TRANSCRIPTIONAL CHARACTERIZATION OF TWO C1Q/TNF-RELATED PROTEINS (CTRPS) FROM RED-LIP MULLET (*Liza haematocheilus*)

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C1q is a pattern recognition protein mainly involve in complement pathway. It is the key crosslinker between innate immunity and adaptive immunity. C1q has a hexadimeric structure which contain N terminal collagen-like region and C-terminal globular C1q (gC1q) domain. The gC1q is a ligand recognition domain which induce the conformational changes in collagen region while binding C1q to IgG or IgM containing immune complexes. These phenomena eventually lead to activation of complement pathway. The gC1q is also can be found in various non-complement proteins and it has structural and evolutionary relationship with tumor necrosis factor (TNF) thus form the C1q and TNF superfamily comprise with verity of proteins. In this study, CTRP4-like and 6 from red-lip mullet were investigated for molecular and transcriptional characteristics to get better understanding about their expression patterns in normal conditions and against bacterial and PAMP stimuli which might be helpful to explore functional behaviors of these proteins. ORF of ChCTRP4-like coding a protein sequence with 375 amino acids with 41.86 kDa molecular weight and theoretical isoelectric point (pI) is 9.33. The theoretical pI, molecular mass and amino acids of encoded protein sequence by ORF of ChCTRP6 are 8.55, 27.17 kDa and 242 amino acids respectively. ChCTRP4-like shares highest identity (80.3%) and similarity (86.4%) with *Acanthochromis polyacanthus* while having lowest identity (42.9%) and similarity (56.1%) with *Mus musculus*. ChCTRP6 shares highest identity (88.0%) and similarity (93.4%) with *Labrus bergylta* while having lowest identity (47.8%) and similarity (63.6%) with *Homo sapiens*. According to the multiple sequence alignment ChCTRP4-like contain two C1q domains between 57-184 and 222-362 amino acid residues. Further, it does not contain signal peptide and N- linked glycosylation site can be found at ²⁶³NKSS²⁶⁶. ChCTRP6 only one C1q domain between 110-237 amino acids and it has cleavage site between 17-18 amino acids but no N-linked glycosylation site. According to the tissue specific mRNA distribution, both ChCTRPs highly express in blood. According to the immune challenge in blood, *ChCTRP4-like* and *ChCTRP6* show significant transcript expression against all the stimuli; LPS, Poly:IC and *L. gaevieae* . Highest expression of *ChCTRP4-like* can be observed against *L. gaevieae* and highest expression of *ChCTRP6* can be observed against poly:IC. According to the results obtained, both ChCTRP4-like and ChCTRP6 show immune responses towards bacterial and PAMP stimuli. These ChCTRPs are reported to contain hormonal function and some structural functions. CTRP4-like was identified but not characterized, and, hence, its physiological function remains unknown. ChCTRP6 is reported to involve in inhibiting alternative complement pathway activation. Based on results revealed from the study, we can conclude that both ChCTRP4-like and ChCTRP6 are immunologically important genes.

PHYSICAL CHARACTERISTICS OF SINKING AND FLOATING EXTRUDED AND EXPANSION-STEAM PELLETED FEEDS AND THEIR EFFECTS ON WATER QUALITY AND GROWTH OF RAINBOW TROUT IN A COMMERCIAL SETTING

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To determine the effects of feed pellet processing (extrusion and expansion-steam pelleting) and on feed physico-chemical characteristics, fecal stability, water quality, and growth performance in rainbow trout, three types of trout feed pellets (compressed sinking, extruded sinking, and extruded floating) were prepared, analyzed, and fed to juvenile rainbow trout (initial weight = 285.8 ± 15.2 g) at three feed rates for 124 days. Results showed that all feeds had similar chemical composition and water absorption curves with soaking time, but extruded feeds had a significantly higher degree of starch gelatinization than compressed feed, which led to extruded feeds having much higher water stability, fecal durability, and lower P discharge. Extruded floating feed produced better growth and feed conversion in rainbow trout than the two sinking feeds. Feed rate also contributed to better growth, but the improvements were not seen beyond feed rate 2. The present study is the first to show that the extrusion process not only produces pellets having better quality than the expansion-steam pelleting method but also improves fecal size and durability in water, and therefore, use of extruded feeds has the potential to improve waste collection and removal and reduce contribution to pollution in effluent.

APPROACH TO AQUATIC ANIMAL HEALTH MANAGEMENT IN OFFSHORE AQUACULTURE SETTINGS

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In 2016, NOAA Fisheries published a final rule to implement a regional permitting program to manage the development of aquaculture in federal waters of the Gulf of Mexico. The program was proposed by the Gulf of Mexico Fishery Management Council through a regional Fishery Management Plan developed under the Magnuson-Stevens Fishery Conservation and Management Act. The final rule authorizes **NOAA Fisheries** to issue permits to grow finfish species such as red drum, cobia, and Almaco jack in federal waters in the Gulf of Mexico for an initial period of 10 years. The permit process includes comprehensive safeguards to ensure healthy oceans and coasts including aquatic animal health management. The final rule requires that the permit applicant address several important elements of aquatic animal health, including: contracting with an aquatic animal health expert, certifying healthy animals prior to stocking, and timely reporting (within 24 hours) and responding to pathogens of concern as directed by NOAA Fisheries and USDA Animal and Plant Health Inspection Service (APHIS). NOAA Fisheries is developing web-based tools for submission of health certificates and disease reporting to help facilitate these activities.

In addition, NOAA Fisheries is collaborating with APHIS Veterinary Services to develop optional guidance for permit applicants to assist them with management of aquatic animal health issues. A key element is to involve a veterinarian who can assist with assessing the risks of disease and design an appropriate aquatic animal health management plan. The veterinarian should be licensed in the U.S., accredited by APHIS, and have a valid veterinary-client-patient relationship with the permit holder/facility. These credentials are required for health certification for movement of fish and for other veterinary activities (e.g., use of drugs under Veterinary Feed Directive, extra-label use of veterinary pharmaceuticals). The aquatic animal health plan should address several critical topics such as training and communication, biosecurity measures, disease detection and mitigation, biomonitoring and surveillance, disease reporting, disease investigation and cleaning and disinfection. These elements are in-line with the USDA APHIS-National Aquaculture Association Commercial Aquaculture Health Program Standards (CAHPS) thus giving the permit holders/facilities the opportunity to being recognized as a CAHPS facility. The overall approach presented here in managing aquatic animal health in offshore aquaculture endeavors is likely to serve as a template for other U.S. offshore regions.

THE PAST, PRESENT AND FUTURE OF ALABAMA'S INLAND SHRIMP INDUSTRY

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Starting in 1999, two Alabama catfish producers stocked Pacific white shrimp in ponds filled with naturally-occurring low salinity aquifer water (4-6 ppt). Despite the low survival rate, the shrimp grew to a marketable size (16-25 count). In 2001, Greene Prairie Aquafarm, Alabama's largest shrimp farm, pioneered large scale inland commercial production. Using the water amendments, muriate of potash and sulfate of potash magnesia, the area farms greatly increased survival rates. For the past eighteen years the industry has not expanded greatly in acreage or in the number of producers. Currently Alabama acreage is less than 200 acres divided among five farms with a combined total annual production of less than 500,000 pounds. Recently, innovative advances have surfaced including the use of split ponds, the winter culture of rainbow trout, automatic feeders, in-situ bioassay limnocorrals, and the polyculture potential of striped mullet. Auburn University continues to provide critical water quality testing, nutrition research, and extension support. In the spring of 2017, a study was conducted using different sources of postlarvae and new biostimulants in an effort to increase overall survival rates which have for unknown definitive reasons, decreased and now threaten the economic sustainability of the industry. A farm in central Alabama, isolated from the rest of the industry in west Alabama, resumed production in 2017 and produced an average yield of 4908 pounds per acre, with a 61.6% survival, and a gross FCR of 1.19. The farm participated in a two year result demonstration with the Alabama Cooperative Extension System.

RESULT DEMONSTRATION: USING AN IN-SITU BIOASSAY SYSTEM TO DETERMINE A WATER AMENDMENT APPLICATION RATE FOR IMPROVING SURVIVAL AND PRODUCTION ON AN INLAND SHRIMP FARM IN LOWNDES COUNTY, ALABAMA

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An inland shrimp producer in central Alabama has grown Pacific white shrimp, *Litopenaeus vannamei*, for the past several years, utilizing an aquifer that typically yields 7-8 ppt salinity. Previous attempts have consistently yielded poor results for survival and overall production. Research and field trials on other Alabama inland shrimp farms indicate the absolute necessity to amend well water with potassium and magnesium to obtain good survival and growth. Given that this farm in Lowndes County is one hundred miles distant from other more productive inland shrimp farms, the authors recognized the necessity of performing an in-situ bioassay to determine the best amendment rates for improving survival. Nine limnocorrals and twelve limnocorrals were placed in two established ponds in 2016. The limnocorrals had a pond mud interface, were continuously aerated, stocked and fed identically for forty days. There were four triplicate treatments in one pond system—10 ppt artificial salt mix, untreated pond water, KMG at 375 mg/l and muriate of potash at 135 mg/l. In the second pond, the artificial salt mix treatment was absent. Statistically, given that some of the replicates were failures, it appeared the KMG treatment may have outperformed the muriate of potash treatment in terms of survival.

In 2017, both ponds were treated with 375 mg per liter (1000 lbs per acre-foot) of KMG. Potassium and magnesium concentrations following KMG treatment increased from 18 ppm to 56 ppm and 62 to 113 ppm, respectively. Sodium concentrations in both ponds ranged from 2505 to 2588 mg/l. One month prior to stocking, ponds were treated with HTH (pool chlorine) at 10 mg per liter for eradication of aquatic insects and fish. Pond production results in the fall of 2018 were 4908 pounds per acre, a net feed conversion of 1.19 with an estimated survival of 62%. However, it is quite possible the pool chlorine treatment prior to post larval stocking may have had a contributing positive impact on survival.

GULF AQUAMAPPER: INTERACTIVE GIS MAPPING TOOL VISUALIZES DATA TO HELP COASTAL MANAGERS AND INDUSTRY SITE OFFSHORE AQUACULTURE IN THE GULF OF MEXICO

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NOAA's National Ocean Service recently launched the Gulf AquaMapper to aid coastal managers and industry in siting aquaculture operations in the Gulf of Mexico. The Gulf AquaMapper makes it easy to turn complex data sets into easily understood visualizations of the coastal ocean environment. Screening and siting analyses are crucial for ensuring sustainable aquaculture development. Improper siting can lead to inefficient operations, conflict among ocean users, and even catastrophic losses in crops and investment. Site selection for aquaculture in the Gulf of Mexico is complex due to multiple fisheries, a large oil and gas industry, a high number of ecologically important habitats, many cultural resources, high frequency of extreme storm events, and multiple factors affecting water quality, such as a seasonal hypoxic zone and regularly occurring harmful algae blooms. The first step for identifying potential development areas is to map existing infrastructure, regulatory boundaries, the oceanographic, biogeochemical, and socio-economic profiles. The Gulf AquaMapper provides a unique visual experience through the online map viewer, which consolidates information from surveys and scientific expeditions, monitoring reports, environmental models, and map data from resource agencies. The Gulf AquaMapper allows visualization of a large number of data types such as bathymetric data, ocean currents and sea state, regulated areas, sensitive biological habitats, economic activity (shipping and navigation, energy production), environmental quality (dissolved oxygen, chlorophyll-a), and political boundaries and maritime jurisdictions. The Gulf AquaMapper provides an interactive digital platform for viewing and processing layers of data. Users can make timely and confident planning and siting decisions by easily analyzing and relating complex geospatial data sets.

SELECTIVE BREEDING FOR ENHANCED SPECIFIC DISEASE RESISTANCE: PRACTICAL CONSIDERATIONS AND POTENTIAL APPLICATIONS

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Endemic infectious diseases constitute a considerable economic burden in aquaculture due to direct losses as well as indirect impacts on growth, animal welfare and transportation restriction. Host genetic variation in susceptibility to specific pathogens is present in most animal populations, especially aquatic animals due to outbred origin and short domestication history. Recent progress in high-throughput animal health phenotyping combined with quantitative genetic analysis has demonstrated the feasibility of improving disease resistance through family-based selective breeding, and more recently, through genomic selection. However, there are only a few examples of successful application of this control strategy in aquaculture and most stocks remain unselected. Salmonid fish are uniquely suitable for selective breeding as reproduction can be controlled, pedigrees can be tracked, large numbers of offspring are generated from each family, and embryo development can be temperature manipulated to synchronize hatching, thus disease resistance phenotyping can be performed using animals with similar body weight. Herein, we review progress and associated logistics of our efforts toward breeding for specific disease resistance, using as an example, results generated from the National Center for Cool and Cold Water Aquaculture selective breeding program. In addition, we discuss benefits and limitations of selective breeding and how it needs to be embedded within an integrated approach for controlling disease on-farm.

UNIQUE AQUACULTURE RESEARCH AND DEMONSTRATION FACILITY EDUCATES THE FUTURE, BEGINNING, AND ESTABLISHED BUSINESSES THROUGH INTERACTIVE TRAINING OPPORTUNITIES

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Although the aquaculture industry is dramatically increasing, the workforce needed to uphold the industries are in high demand. 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, demonstrating various aquaculture systems including pond, recirculating aquaculture, raceway, larval and incubation systems raising a variety of both cool and cold water fish at all life stages. Through a variety of commercially applied research projects, the facility is strongly partnered with private, state, federal and tribal hatcheries, businesses and organizations to advance sustainable aquaculture in Wisconsin and the Midwest. Many businesses and over 450 Wisconsin jobs have been created or sustained with the assistance from UWSP-NADF. Since 2006, the facility has brought in over \$4 million in grant funding to advance Wisconsin aquaculture and aquaponic businesses. UWSP NADF outreaches to educate not only established aquaculture businesses, but also those in the beginning stages as well as the future workforce through a variety of training opportunities.

To prepare for a next generation workforce, UW-Stevens Point is the only university in Wisconsin to offer an aquaculture minor and the first in the nation to offer semester-long aquaponics course and professional aquaponics certificate through UWSP Aquaponics Innovation Center (UWSP AIC) and private aquaponics business partner, Nelson & Pade, Inc. These courses also have internship opportunities to work alongside trained staff at both the UWSP NADF and UWSP AIC to learn best management practices and techniques, various species, life stages and systems. UWSP NADF also employs limited term aquaculture technician positions. Both technicians and interns receive a world class training opportunity at unique facilities to gain hands on experience, perform applied research projects, and work alongside various project partners.

To educate beginning businesses, UWSP NADF offers workshops and events to assist in early stages of development. These workshops include online video modules on various topics, online manuals, and hands on experience at the facility. Some recent examples include a Lake Herring Manual, Pond Culture Workshop, Intensive Walleye Production Video and Manual and Aquaculture and Aquaponics Boot Camp.

To outreach to established businesses, UWSP NADF offers interactive tours, cross training for various business partners including business incubation projects, and extensive resources. Technical assistance is provided nearly on a daily basis for established businesses. Training alongside UWSP NADF staff is also offered to business partners to learn more about techniques, systems, management practices and technologies to help move the industry forward.

A PATHWAY FOR SHELLFISH SANITATION COMPLIANCE IN FEDERAL WATERS OFFSHORE

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The National Shellfish Sanitation Program (NSSP) is the cooperative state-federal-industry program for the sanitary control of shellfish to ensure that shellfish produced in accordance with these guidelines will be safe and sanitary. The Interstate Shellfish Sanitation Conference (ISSC) is the organization consisting of agencies from shellfish producing and receiving States, the U.S. Food and Drug Administration (FDA), the shellfish industry, the National Oceanographic and Atmospheric Administration (NOAA), and the Environmental Protection Agency (EPA). The ISSC provides the formal structure wherein State regulatory authorities, with FDA concurrence, can establish updated guidelines and procedures regarding the sanitary control of the shellfish industry, which are published in the NSSP Guide for the Control of Molluscan Shellfish. The NSSP Guide does not explicitly cover requirements for the sanitary control of shellfish harvested from federal waters. The lack of an NSSP pathway for this activity has impeded the harvest of shellfish in federal waters to date, and potentially impacts expansion of shellfish aquaculture in federal waters, a NOAA priority. While microbiological and marine biotoxin testing and tagging requirements are established in the NSSP for shellfish products destined for interstate commerce, there was no established framework for requirements associated specifically for growing and harvesting cultured shellfish in federal waters.

In response to a previous request by industry to harvest shellfish from federal waters where toxic algae were known to be a risk, FDA collaborated with NOAA, the shellfish industry, and the States where the product was intended to be landed to find a solution that would allow industry access to valuable surf clam and ocean quahog resources with processes in place to protect public health. A Memorandum of Understanding (MOU) was established to clarify the roles and responsibilities of all parties to ensure the shellfish were harvested in a manner consistent with NSSP requirements for product harvested in State waters. The most recent request to harvest shellfish from federal waters posed another challenge – it was related to aquaculture (i.e., an offshore mussel farm). While some of the considerations employed by FDA during the previous case to classify growing areas and control for marine biotoxins were applicable to the proposed aquaculture activity in federal waters, there were several other NSSP requirements that FDA did not have the legal authority or means to address (e.g., permitting and inspections for aquaculture). Thus, the NOAA Seafood Inspection Program (SIP) offered assistance and worked with FDA to develop a pathway that industry could use to comply with the necessary permitting and tagging requirements.

Bacillus*-FERMENTED VEGETABLE PROTEINS AS FISHMEAL REPLACERS IN JUVENILE WHITELEG SHRIMP *Litopaeneus vannamei

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An 8-week feeding trial was conducted to investigate vegetable proteins with and without *Bacillus*-fermentation process as the dietary fish meal replacers in juvenile whiteleg shrimp, *Litopaeneus vannamei*. Eight isonitrogenous and isoenergetic diets were formulated: A 30% fish meal based diet (CON) and seven other diets replacing 30% of fishmeal from the CON diet with fermented soybean meal + *Bacillus* spp (STL), sterilized STL (STD), fermented soybean meal + corn gluten meal + *Bacillus* spp (ATL), sterilized ATL (ATD), fermented soy protein concentrate + *Bacillus* spp (FSPC) and fermented soybean meal + commercial probiotics (DB), and soy protein concentrate (SPC). Shrimp averaging 0.5 ± 0.01 g (mean \pm SD) were randomly distributed into 24 aquaria as groups of 40 shrimp/tank and fed the experimental diets in triplicate. At the end of the experimental period, weight gain and specific growth rate of shrimp fed STL and ATL diets were significantly higher than those of fish fed CON and DB diets ($P < 0.05$). There was no significant difference among fish fed STL, FSPC, ATL, SPC, STD and ATD diets. We will discuss on the advantages of bacillus-fermented vegetable proteins when the other results are summarized.

PREDICTION AND GENETIC PARAMETERS OF FILLET TRAITS IN THREE NILE TILAPIA (*Oreochromis niloticus*) STRAINS BASED ON THEIR BODY MEASUREMENTS

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The aim of this study was to develop non-invasive methods to predict fillet traits, i.e. fillet weight and fillet yield, in Nile tilapia (*Oreochromis niloticus*) using their body measurements, by recording 958 male fish from 81 full-sib families. A simple linear regression model utilising body measurements on live fish was first established by forward selection procedures, and then cross-validated, 5-fold, to estimate the prediction power of the model. The best final prediction models for the two fillet traits both had two variables; body weight and body thickness for fillet weight, and body thickness and head thickness for fillet yield. The fillet weight model explained 94% of the observed variance, with a 4.0g root mean standard error prediction (RMSEP) and 0.02g prediction bias, while only 21% of the observed variance for fillet yield was explained by the model, with a 2.3% RMSEP and only 0.002% prediction bias. The estimates of heritability for fillet weight (0.17) and predicted fillet weight (0.19) were moderate, and had a high genetic correlation (0.94). The estimate of heritability for actual fillet yield was low (0.09) whereas it was higher for predicted fillet yield (0.26), but the two traits had a relatively low genetic correlation (0.50). In conclusion, selection for faster growth of *Oreochromis niloticus* is likely to result in a significant correlated response in fillet weight, whereas it is likely to only give a minor response for fillet yield.

AQUACULTURE EXTENSION SPECIALIST: MANAGING RESPONSIBILITIES AND EXPECTATIONS

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New Aquaculture Extension Specialists/Specialized Agents and their university administrators may lack extension experience, particularly at the county level. A newly hired specialist may discover disagreement in program priorities between their academic institution and their off campus extension community. Currently, extension methods are taught during college classes by some land grant institutions. Still, differences may remain between departmental and county extension personnel regarding aquaculture extension program responsibilities and expected outcomes. Conflicting expectations originate from poorly outlined specialist's responsibilities and programming priorities. This situation results from inadequate communication and agreement between the university department and off campus extension administration.

This disagreement in priorities may easily be explained. While land grant institutions emphasize applied agricultural research, extension and education, the vast majority of the funds go to research. Accordingly, researchers pursue projects that fit their training, institutional capabilities and those likely to be funded. Given this situation, research personnel consider the extension specialist's primary purpose is to teach/transfer the departments applied research to the aquaculture industry and the public. Conversely, the Cooperative Extension Service is a grassroots organization that receives their programming objectives from the public via county extension councils. Agricultural and Natural Resources (ANR) Agents share these needs with Aquaculture Extension Specialists/Agents. Specialists play a supporting role by providing them technical information. Academic and extension communities may develop different programming priorities and expectations. To the academic department, aquaculture responsibilities would mean all subject matter pertaining to their research: the farming, processing and marketing of fish, invertebrates and plants. The role of the extension specialist is much more generalized according to the extension community and fits the needs of their clientele. These needs would include but are not limited to: aquaculture, farm and recreational pond management, aquatic plant identification and control, fee fishing, water quality for a variety of purposes, wildlife damage control, and watershed management. Few ANR Agents or other Extension Specialists have aquatic environment training which creates demand for the skills of a fisheries biologist or an aquaculture specialist.

Before establishing an Aquaculture Extension Specialist/Specialized Agent position, it is recommended that the university aquaculture department and the extension administration establish and understand the positions responsibilities at the state, or regional level. This would create a clearer understanding of the position's responsibilities, related programming objectives and expected outcomes.

TECHNICAL EFFICIENCY OF CHINA AQUACULTURE: THE CASE OF LARGE YELLOW CROAKER FARMING

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In view of limited fishery resources, aquaculture has played an important role in world seafood supply and has a huge potential for contributing to world food nutrition and security in the future. According to world Food and Agricultural Organization statistics, China alone contributes to more than 60% of global aquaculture production volume. Therefore, it is crucial to investigate the current technical efficiency of China aquaculture and thus to further understand the possible potential for China to continue providing fish to meet world demand for seafood.

Large yellow croaker is one of the top three farmed finfish in China. Farmed large yellow croakers are sold both to domestic and overseas markets. Therefore, the case study of large yellow croaker could well present the situation of China aquaculture in general. In the study, we used the data collected from 348 farmers in main farming sites of large yellow croaker located along the coastline of southeastern China. A stochastic frontier function model was applied to estimate the efficiency of China large yellow croaker farming and to identify the factors affecting the efficiency.

The estimated mean efficiency rate is 0.829. It suggests China has some margin to enlarge aquaculture production by increasing productivity, but not so much. To gain efficiency, technology training, industry clustering, industry organizations, which help to coordinate farming activities between the traditional small farmers are crucially important. Fishmeal is the main contributor for growing output. Negative sign of medicine indicates fishing diseases decreases output. These results suggest that condensed farming using large amount of fishmeal has consequently increased frequency of occurrences of fish diseases. The margin to increase production efficiency is moderate. At the same time, fish farming is competing with other industries for fishing sites. Therefore, it is uncertain about the contribution of China aquaculture to world sustainable food supply in the future.

RESPONSES OF JUVENILE LARGEMOUTH BASS *Micropterus salmoides* TO DIETARY EPA AND DHA-RICH OILS

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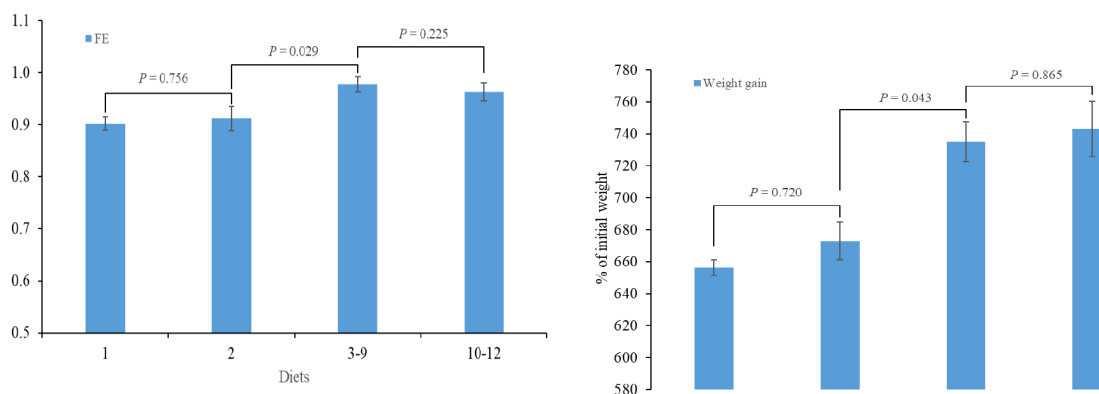
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In recent years, the aquaculture industry has faced a rapid change in the raw materials used in feed formulations with a departure from marine-based to terrestrial plant-based nutrient sources that do not supply the n-3 HUFAS eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). The indispensability of these n-3 HUFAS in the diet of Largemouth bass (LMB) remains unclear. Therefore, the objective of this study was to evaluate the responses of LMB to dietary EPA and DHA-rich oils.

A 10-week feeding trial was conducted using twelve isonitrogenous and isolipidic diets, and different levels (0, 1 and 2%) and ratios (20:80, 30:70, 40:60, 50:50, 60:40, 70:30, and 80:20%) of EPA and DHA. An n-3 fatty acid depleted diet (1) containing 0.27% n-3 fatty acids and 0.05% EPA+DHA, and a linolenic acid (LNA) rich (4.35%) diet (2) were formulated using coconut oil and linseed oil, respectively. Six additional diets were formulated to contain 1% EPA+DHA at the abovementioned ratios using EPA-rich a DHA-rich fish oils. A final set of 3 diets were formulated to contain 2% EPA+DHA at 20:80, 50:50, and 80:20% ratios.

At the end of the feeding trial, the production performance of LMB was unaffected by the various EPA: DHA ratios. However, contrast analyses on performance parameter data showed that: i) LMB fed diets with 1 or 2% EPA+DHA displayed higher ($P < 0.05$) weight gain and feed efficiency (FE) than those fed the LNA-rich and EPA/DHA-free diet (2), suggesting LMB has a limited ability to synthesize EPA and DHA from LNA to meet physiological needs for maximal growth and FE; and ii) the lack of differences ($P > 0.05$) between LMB in the n-3 fatty acid depleted and LNA-rich groups indicated a low dietary requirement for LNA. Whole-body fatty acid composition data will be presented.

Our current results suggest LMB requires around 1% n-3 HUFAS in the diet for maximum growth and FE. If the current results are supported by whole-body fatty acid composition data, additional investigations to determine the quantitative requirement are warranted.



β -1,3 GLUCAN DERIVED FROM *Euglena gracilis* AND ALGAMUNE™ ENHANCES INNATE IMMUNE RESPONSES OF RED DRUM (*Sciaenops ocellatus* L.)

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Biological modifiers such as β -glucans have been proven effective tools to enhance the innate immune responses of fish, therefore reducing susceptibility to diseases and mortality. Commercial sources of this polysaccharide are becoming increasingly more available. Algamune™ is a commercial additive produced from *Euglena gracilis*, as a source of a linear β -1,3-glucan. In order to evaluate the immunomodulatory effects of this β -glucan product, the present study assessed the innate immune parameters of red drum (*Sciaenops ocellatus*) exposed to Algamune™ *ex vivo* and *in vivo*. Isolated kidney phagocytes were incubated with graded concentrations (0, 0.2, 0.4, 0.8, 1.6 and 3.2 mg L⁻¹) of dried *Euglena gracilis* (Algamune™) as well as purified Paramylon (linear β -1,3 glucan). Increased bactericidal activity against *Streptococcus iniae*, and production of intracellular O₂⁻ anion superoxide were stimulated by both β -glucan sources. A reduced activity of extracellular anion superoxide was observed by the phagocytes incubated with Algamune™.

After corroborating the effectiveness of the glucan source *ex vivo*, a feeding trial was conducted using red drum juveniles (~26.6 g initial weight). Fish were fed diets with graded levels of Algamune™ (0, 100, 200, 400 and 800 mg kg⁻¹) twice daily for 21 days. No significant differences were detected regarding production performance parameters. At the end of the feeding trial, blood, intestinal contents, and kidney tissues were sampled. Intestinal microbiota from fecal material was analyzed through denaturing gradient gel electrophoresis (DGGE) and found to be similar among all treatments. No significant differences were detected for oxidative radical production from whole blood and isolated phagocytes, or for plasma lysozyme activity (Table 1). However, the total hemolytic activity of red drum plasma was increased in fish fed 100 and 200 mg kg⁻¹ of dietary Algamune™ when compared to fish fed the basal diet. Based on results from both *ex vivo* and *in vivo* trials, β -glucan from Algamune™ was demonstrated to have immunostimulatory effects on red drum.

Table 1: Immunological responses of red drum fed diets with graded inclusion of Algamune™ for 21 days.

Algamune™ (mg kg ⁻¹)	0	100	200	400	800
NBT mg ml ⁻¹ of blood	6.4	6.44	6.38	6.41	6.58
ECSA (nmol O ₂ ⁻ ml ⁻¹)	0.66	0.36	0.46	0.82	0.41
ICSA (Abs. 620 nm)	0.082	0.079	0.084	0.078	0.081
Lysozyme activity (units ml ⁻¹)	166	230	359	335	354
Total hemolytic activity (%)	54.0 ^B	73.0 ^A	70.0 ^A	54.6 ^B	66.4 ^{AB}

IMMUNOMODULATORY EFFECTS OF LINEAR β -1,3-GLUCAN PARAMYLON AND BRANCHED β -1,3/1,6-GLUCAN ZYMOSAN AS FEED ADDITIVES AND AS ADJUVANTS FOR HYBRID STRIPED BASS (*Morone chrysops* \times *M. saxatilis*)

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Beta glucans are biological modifiers that can activate the animal's innate immune system, enhancing the primary defense lines prior to a pathogenic insult. The molecular structure of beta glucan can play a role triggering distinct immunological responses when interacting with different cell membrane receptors. The objective of this study was to assess the immune responses of hybrid striped bass (HSB) when exposed to Paramylon, a linear β -1,3-glucan derived from *Euglena gracilis*, and Zymosan a branched β -1,3/1,6-glucan derived from the traditional *Saccharomyces cerevisiae*. Two separate trials evaluated the efficacy of these immunostimulants when they were delivered orally as a feed additive or injected intraperitoneally (i.p.) as an adjuvant.

For the feeding trial, five diets were formulated to contain Paramylon (50 or 100 mg kg⁻¹), Zymosan (50 or 100 mg kg⁻¹) and no supplementation serving as a basal diet. Each treatment was assigned to four replicate groups of 15 juvenile HSB averaging ~15.4 g. Blood and head kidney were sampled during an intermediate sampling at week 4 and at the final sampling at week 8. The results showed that no differences were detected for oxidative radical production from whole blood, total plasma protein, lysozyme, hemolytic and antiprotease activity. However, an increased intracellular superoxide anion production of isolated phagocytes was observed when compared to the basal group (Figure 1).

A second trial was conducted by injecting i.p. a solution from each glucan source and phosphate buffered saline serving as a control. For each treatment, 10 fish (~330 g) were injected with approximately 10 mg of β -glucan kg⁻¹ of body weight. Seven days after the administration, blood was collected from the caudal vasculature, and immunological profiles from whole blood and plasma were assessed. All parameters were significantly affected ($P > 0.05$) by the treatments (Table 1). The results of this study indicate that dietary and injected Paramylon and Zymosan can modulate the immunological responses of HSB in different fashions.

Table 1: Immunological responses of HSB injected with different sources of β -glucan

	Control	Zymosan	Paramylon
NBT (Absorbance at 545 nm)	0.682 ^B	0.733 ^A	0.701 ^B
Hemolytic activity (%)	79.6 ^B	87.2 ^{AB}	95.0 ^A
Antiprotease activity (%)	70.5 ^B	77.2 ^A	73.9 ^{AB}
Lysozyme activity (units ml ⁻¹)	1249 ^A	1163 ^B	1368 ^A
Total protein (mg ml ⁻¹)	92.4 ^A	80.4 ^B	92.5 ^A
Total immunoglobulin (mg ml ⁻¹)	49.1 ^{AB}	45.7 ^B	52.0 ^A

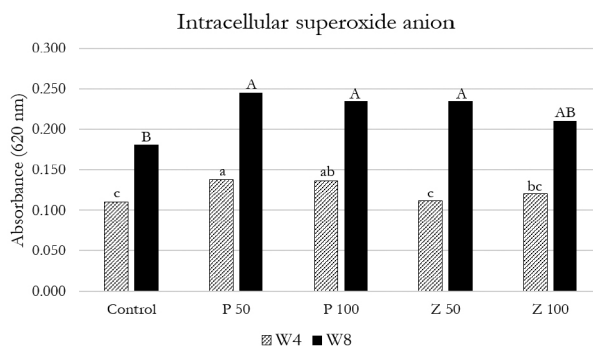


Figure 1: Intracellular superoxide anion production by isolated HSB phagocytes from fish fed different sources of β -glucan at week 4 and 8

BIOSECURITY IN AQUACULTURE

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Good health management and disease prevention are critical to the economic success of any aquaculture business. Despite this, many aquaculture facility owners build a facility and develop a business plan without incorporating a detailed health and disease management plan that incorporates solid biosecurity principles. The facility management team should include a consulting or in-house aquaculture veterinarian and/or other health professional who can help develop the plan.

Biosecurity practices reduce livestock stressors and susceptibility to disease. Over the past few decades, several important disease outbreaks in both U.S. aquaculture (e.g., spring viremia of carp, koi herpesvirus disease) and wild fish populations (viral hemorrhagic septicemia type IVb) have increased the U.S. industry's awareness of the importance of biosecurity. Determining specific points in production when pathogens (disease-causing parasites, bacteria, viruses and fungi) can enter a population or when disease outbreaks are more likely to occur will help producers develop effective and logical preventative measures. Good biosecurity minimizes the fishes' exposure and susceptibility to pathogens, reduces economic losses from mortalities and treatments, and helps prevent mandatory depopulation and other drastic measures.

Three major focus areas of biosecurity are: a) animal management; b) pathogen management; and c) people management. A number of factors should be considered when determining risk from a specific infectious disease: a) species, immune status, condition, life stage, and strain susceptibility; b) major environmental factors such as water quality, water chemistry, and husbandry practices; c) characteristics of the pathogen, such as biology and life cycle, potential reservoirs (carriers, other animals), survival on inanimate objects (equipment), options for legal treatment(s), regulatory status (exotic vs. endemic disease, reportability, and federal, state and local laws); and d) management and staff understanding of biosecurity principles and compliance with biosecurity protocols. The "five-finger" method (K. Hartman) can be used to remember where pathogens can come from: a) animals; b) water; c) feed/food; d) vectors (i.e., animals/people); and e) fomites (e.g., environment, equipment, vehicles, boats).

A good biosecurity plan should be written (or digital), easily accessible, should flow logically and be easily understood. Though not a comprehensive list, the plan should include: health team members, roles, and contact information; a layout and flow of the facility; identification of, and methods to minimize the risk of infectious disease and pathogen introduction and spread within or from a facility; quarantine and isolation protocols; cleaning and disinfection protocols; clear guidance on actions to be taken in the event of a disease outbreak and in order to return to business as usual; communication and record keeping; training programs for staff; and a mechanism to ensure regular review of the plan itself by management and employees.

PERFORMANCE OF SOY PROTEIN AND SOY OIL UTILIZED IN FEEDS FOR JUVENILE RED DRUM *Sciaenops ocellatus*

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Two nine-week feeding trials were conducted with juvenile red drum to assess their performance on feeds utilizing high levels of soy protein and soy oil as fishmeal and fish oil replacements. During the first trial, fish were fed soy based diets included either 36 g 100g⁻¹ solvent extracted soybean meal (SBM) or soy protein concentrate (SPC) and graded levels (0%, 25%, or 50%) of soy oil replacing fish oil. These feeds were compared to two fishmeal based feeds, one which contained a 50% replacement of fish oil with soy oil (Table 1). Based solely on performance metrics (growth rate, weight gain, and feed conversion ratio), the highest performing dietary formulation was selected as the base formulation for the second feeding trial.

The second feeding trial introduced a graded level of soy protein inclusion to examine possible soy protein and soy oil interactions on overall performance. A fishmeal based diet with fish oil as the lipid sources was used as a reference diet again and a natural diet of cut fish, squid, and shrimp was introduced as one treatment to assess maximum growth potential within the system.

Performance results from both feeding trials will be discussed as well as proximate compositions of fillet and whole body tissues from each trials. Table 1. Basic protein and lipid sources utilized in two feeding trials to evaluate soy protein and lipid effects on juvenile red drum.

Table 1. Basic protein and lipid sources utilized in two feeding trials to evaluate soy protein and lipid effects on juvenile red drum.

Trial #1			Trial #2	
Primary Protein Source	Fish Oil	Soy Oil	Dietary Treatment	Soy Protein Inclusion (% soy ingredient)
Fishmeal	100%	0%	Fishmeal with 100% Fish Oil	0%
Fishmeal	50%	50%	SBM 50% FISH OIL/50% SOY OIL	125%
SBM	100%	0%	SBM 50% FISH OIL/50% SOY OIL	100%
SBM	75%	25%	SBM 50% FISH OIL/50% SOY OIL	75%
SBM	50%	50%	SBM 50% FISH OIL/50% SOY OIL	50%
SPC	100%	0%	SBM 50% FISH OIL/50% SOY OIL	25%
SPC	75%	25%	SBM 50% FISH OIL/50% SOY OIL	0%
SPC	50%	50%	Fish/Squid/Shrimp "natural" diet	0%

DOES PUBLIC OWNERSHIP OF EQUITY AFFECT PROFITABILITY? THE CASE OF SALMON INDUSTRY

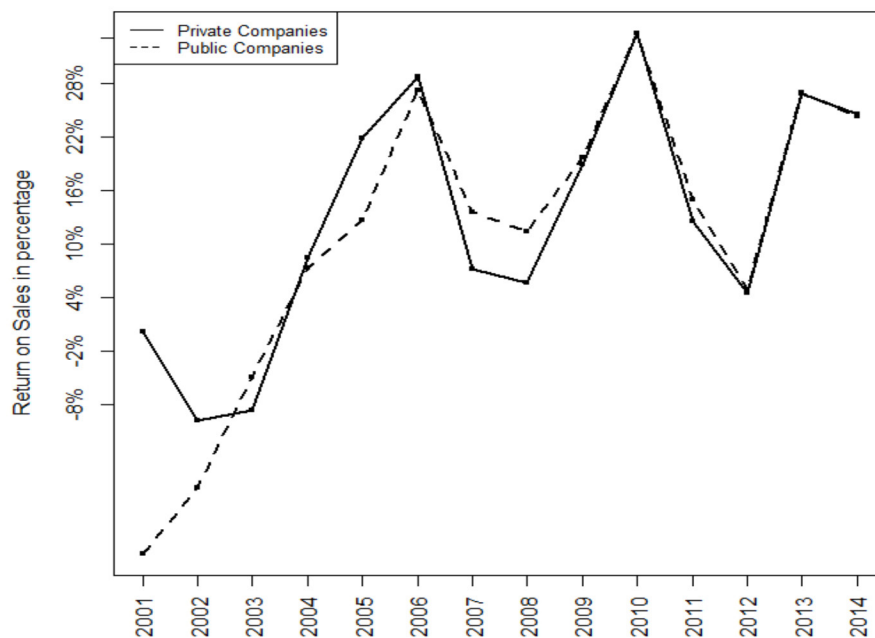
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In this paper we evaluated how equity ownership affects profitability of the Norwegian salmon farming companies. The increasing consolidation and globalization of salmon industry have stimulated the investment in this industry and consequently the publicly traded securities. This process was specially evidenced in 2007, which was the first operating year of the biggest producer, Marine Harvest, after its huge expansion through merge and acquisition. In addition another two salmon producers, Salmar and Grieg Seafood, went public in 2007. The public ownership of equity may improve corporate governance and productivity, which further contribute to profitability. We estimated two models, one for all companies with the sample period 2006-2014 and a dummy variable set for private companies, and the other for the public companies with the sample period 2001-2014 and a dummy variable set for the sub-period 2001-2006. The profitability is represented by Return on Sales (Figure). Following the literature on profitability, the control variables include operating leverage, financial leverage, and liquidity ratio. As the public companies have become large after 2007 and are relatively larger than the private companies through time, the firm size variables are incorporated in the models. The empirical results show that, holding other factors constant, the private companies have a 5.22% lower profitability than the public companies. For the public companies as a whole, the huge expansion after 2007 has not enhanced their profitability.

Fig. ROS of the Norwegian Private and Public Companies



CATABOLISM OF BRANCHED-CHAIN AMINO ACIDS IN TISSUES OF HYBRID STRIPED BASS (*Morone chrysops* x *M. Saxatilis*)

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Glutamate in diets is extensively degraded by the small intestine of fish. However, it is among the most abundant amino acids in the body and is likely synthesized by various tissues of fish. The present study was conducted with hybrid striped bass (HSB) to test the hypothesis that leucine (LEU), isoleucine (ILE) and valine (VAL) are degraded in these tissues to generate glutamate. Slices of 10 tissues (Table 1) were obtained from juvenile HSB and incubated at 26°C for 2 h in oxygenated (95% O₂/5% CO₂) Krebs-Henseleit bicarbonate buffer (pH 7.4, 5 mM D-glucose) containing for 2 mM [1-¹⁴C]leucine, [1-¹⁴C]isoleucine, or [1-¹⁴C]valine. Production of ¹⁴CO₂ and each branched-chain ketoacids (BCKAs) was determined with the use of our established methods. Furthermore, activities of branched-chain amino acid (BCAA) transaminase and BCKA dehydrogenase in HSB tissues were determined to provide bases for understanding BCAA catabolism.

All the fish tissues actively transaminate leucine, isoleucine and valine with α -ketoglutarate to form glutamate and BCKAs, with the highest rate (nmol/g tissue tissue) in the kidney. Data on the activities of BCAA transaminase and BCKA dehydrogenase are summarized in Table 1. The highest activity of BCAA transaminase was observed in the heart ($P < 0.05$), whereas the highest activity of BCKA dehydrogenase in the heart. In HSB, rates of glutamate synthesis from BCAAs may vary greatly with tissues and further catabolism of BCKAs may involve interorgan cooperation to provide ATP, glucose and lipids.

Table 1 The activities of Branched-chain amino acid aminotransferase activity (BCAAT) and Branched-chain keto acid dehydrogenase activity (BCKAD) in 10 tissues from hybrid striped bass

	Branched-chain amino acid aminotransferase activity (BCAAT)			Branched-chain keto acid dehydrogenase activity (BCKAD)	
	LEU (nmol/mg tissue/20min)	ILE (nmol/mg tissue/20min)	VAL (nmol/mg tissue/20min)	—Phosphatase (pmol/mg protein/15min)	+Phosphatase (pmol/mg protein/15min)
Muscle	7.40±0.69 ^e	7.49±0.30 ^e	7.60±0.68 ^g	474 ±51 ^{fg}	594 ±68 ^d
Liver	9.65±0.73 ^e	9.63±0.83 ^e	10.15±0.75 ^f	735 ±62 ^{bc}	1075 ±121 ^b
Kidney	44.2±2.8 ^b	45.1±3.9 ^b	52.9±3.3 ^b	1084 ±88 ^a	1502 ±83 ^a
Intestine	17.5±1.7 ^d	18.7±1.6 ^d	17.8±1.2 ^e	558 ±25 ^{def}	674 ±33 ^{cd}
Stomach	29.7±1.9 ^c	30.1±1.5 ^c	31.24±1.03 ^c	700 ±49 ^{bcd}	1112 ±96 ^b
Heart	86.0±6.3 ^a	108.1±10.1 ^a	105.9±9.0 ^a	685 ±34 ^{bcd}	1133 ±91 ^b
Brain	26.4±1.5 ^{cd}	30.5±1.4 ^c	29.6±1.4 ^{cd}	600 ±35 ^{cde}	775 ±54 ^c
Gill	21.5±0.9 ^d	25.0±1.4 ^c	22.7±1.3 ^{de}	824 ±58 ^b	1095 ±73 ^b
Spleen	20.1±1.4 ^d	23.6±1.1 ^c	18.3±1.4 ^e	404 ±34 ^g	533 ±31 ^d
Gallbladder	2.03±0.45 ^f	2.28±0.37 ^f	1.90±0.26 ^h	589 ±57 ^{cdef}	684 ±44 ^{cd}

ADDENDUM

REVISITING HATCHERY BIOSECURITY AND RISK MANAGEMENT

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Emerging shrimp pathogens are increasingly prevalent in hatcheries worldwide, resulting in increased health risks for the shrimp aquaculture industry. PL health and quality are critical to hatchery profitability. Many hatcheries are rigorously screening for diseases and tightening their biosecurity protocols to ensure the health of their postlarvae. The prevention of stocking diseased postlarvae into growout ponds is critical to achieving good survivals. For decades, viral diseases have been the dominant disease threat to the industry. Since the early days of Baculovirus penaeii hatchery mortalities and IHHNV related stunting, most hatcheries have focused on exclusion, and the use of specific pathogen free (SPF) breeding stocks to control disease. More recently, breeding programs have focused on developing disease resistant genetic lines. However, the emergence and spread of virulent bacterial disease coupled with stubborn microsporidian infections have made disease management much more complicated.

Acute Hepatopancreatic Necrosis Disease (APHND) spread quickly through the Asian shrimp industry before the causative agent was finally diagnosed. EMS was found to be caused by a virulent strain of *Vibrio parahaemolyticus* carrying plasmids with genes encoding for powerful toxins causing necrosis of the hepatopancreas. In Ecuador, the disease has become a serious problem in hatcheries causing rapid and severe mortalities in young PL. While vibriosis has always been a major cause of mortalities in hatcheries, the emergence of new more virulent strains makes the control of bacterial pathogens more important than ever. *Enterocytozoon hepatopenaei* (EHP) causes a disease characterized by slow growth and increased susceptibility to infections. This microsporidian is an intracellular parasite that produces spores which are extremely difficult to eradicate, leading to their buildup in the culture environment if not controlled.

Refocusing hatchery biosecurity entails continual risk assessment and risk management based on the most up to date information on pathogens and control strategies. Hazard analysis and critical control point (HACCP) methods. Using HACCP methods, mechanisms of transmission and amplification can be identified and controlled to eliminate excludable pathogens and to prevent the buildup of non-excludable bacterial pathogens, both in the hatchery and at the farm. Treatment of incoming water and management of water systems and effluents can minimize introduction and buildup of bacteria and spores in the hatchery. Broodstock can be another significant vector of disease, necessitating quarantine and rigorous disease testing prior to their introduction into maturation production systems. Fresh and live maturation feeds have also been shown to be important vectors for introducing pathogens into hatcheries. Many hatcheries are finding that the use of probiotics and partial replacement of live and fresh feeds with high quality prepared diets with disease free fresh feed supplements can significantly reduce the loading of pathogens in their maturation and larval rearing systems, leading to more consistent larval health. The future of shrimp farming will require continued scientific advancement in pathology, genetics, innovative nutrition and hatchery management.

SHELLFISH AQUAMAPPERS: A NATIONAL ANALYSIS OF DATA, ARCHITECTURAL DESIGN, AND UTILITY

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Marine aquaculture in the United States contributes to the nation's seafood supply, provides coastal habitat and species restoration opportunities, and contributes to the economic vitality of coastal communities. Transparent spatial data analyses and advanced visualizations within a marine spatial planning framework can reduce user conflicts and streamline permitting. Currently, the majority (two-thirds by value) of the United States' marine aquaculture production is comprised of bivalve mollusks (oysters, clams, mussels) in estuarine and coastal ocean areas. As the aquaculture industry footprint increases in the U.S., so too does the potential for conflict for space. To inform opportunity and siting of shellfish aquaculture in state waters, many states have developed shellfish aquaculture online map viewers (AquaMappers) aimed at informing speculation of new aquaculture areas or expansion of existing areas. These AquaMappers are being used by industry and coastal managers to identify potential conflicts before aquaculture is sited. In this study, we analyzed all of the existing shellfish AquaMappers in the U.S. to identify the most common data layers, the functionalities of each viewer, architectural design approaches, and size (value) of the shellfish industry relative to complexity of the viewer. This information is being used by industry and coastal managers to inform development of new shellfish aquaculture map viewers and to revise and update existing viewers.

GENETIC EFFECTS AND COMBINING ABILITY FOR OVERSIZED AND UNDERSIZED CHANNEL CATFISH *Ictalurus punctatus* FEMALE X BLUE CATFISH *I. furcatus* MALE HYBRID CATFISH

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Oversized hybrid catfish (channel catfish, *Ictalurus punctatus*, female X blue catfish, *I. furcatus*, male) have become a significant problem at harvest for food-sized fish in the catfish industry. Conversely, hybrid catfish fingerling producers complain concerning undersized fingerlings. The population distribution of hybrids from F1 crossbred channel catfish dams was more variable than that from pure-strain dams with the greatest percentage of large fingerlings that would likely grow into oversized food fish from these crossbred dams. The same phenomenon was observed for hybrid progeny from F1 blue catfish crossbred sires. The F1 crossbred sires and dams were also more likely to produce undersized hybrid progeny. This initial study indicates that the use of F1 crossbred blue catfish males or F1 crossbred channel catfish females greatly increases variation in fingerling hybrid progeny. There are multiple ways to analyze size variability. If alternative measures of size variability were used such as the coefficient of variation, vagaries of the calculation will result in the opposite, but incorrect conclusion regarding variability of the hybrid progeny from crossbred brood stock because of the large standard deviation.

When the combining abilities were calculated from pooled full-sib and half-sib families across all genetic groups, there was no significant specific or general combining ability for the extremely large hybrid fingerlings, indicating that selection for combining ability in the parent species would likely not alleviate the oversized hybrid food-fish problem. However, there was significant dam general combining ability for slightly larger hybrid fingerlings and undersized fingerlings, indicating selection of the appropriate dam would likely increase uniformity in hybrid fingerlings, positively impacting the hybrid fingerling industry, which could also lead to greater uniformity of hybrids in the food-fish industry. Significant combining abilities existed for all of the other economically relevant traits of hybrid catfish.

EFFECT OF *Nigella sativa* AND CIPROFLOXACIN ON GENE EXPRESSION IN BLOOD DURING GRAM-NEGATIVE BACTERIAL INFECTION IN *Oreochromis niloticus*

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Aquaculture are facing several problems of water quality and associated diseases. A close look at the immune system in the blood during bacterial infection allows us to properly control the pathogens. The present study aimed to investigate the effect of both *Nigella sativa* oil (NSO) and Ciprofloxacin (Cip) through evaluating their therapeutic and immunity properties on expression of hepcidin, IL-1 β and CYP1A genes and some blood parameters against bacterial infection of *Oreochromis niloticus*. A healthy *O. niloticus* fingerlings were equally divided into 13 experimental groups (T₁₋₁₃) and each group contained sixteen which were stocked with duplicates. T₁₋₅ were served as controls, T₆₋₉ were infected by *Aeromonas hydrophila* (Ah) and T₁₀₋₁₃ were infected by *Pseudomonas fluorescens* (Ps). The suggested treatments were distributed among them.

The results showed that hepcidin and IL-1 β transcripts was up-regulated with bacterial infection. However, Cip and NSO treatments were presented a significant up-regulation in hepcidin transcript during *A. hydrophila* infection and IL-1 β gene expression was a significant up-regulated by Cip with bacterial infection compared to NSO (Fig 1). CYP1A gene expression was down-regulated by Cip and NSO with infection than controls (Fig 1). On the other hand, T₁₀₋₁₃ groups showed a significant increase (P<0.05) in total protein, albumin and globulin levels compared to the uninfected control group (T₁). Also, the total protein, albumin and globulin levels were a significantly increased (P<0.05) by the treatment with ciprofloxacin in infected groups (Table 1). In conclusion, *Nigella sativa* was decreased IL-1 β and CYP1A gene expression. Both of Ciprofloxacin and *Nigella sativa* were involved in the defense against bacterial infection where the effect varies depending on the bacterial infection.

TABLE 1. Total Protein, Albumin and Globulin levels in blood

Groups	TP g/dl	Alb g/dl	GLu g/dl
T1 (Control (C))	3.76±0.18 ^{DE}	2.64±0.27 ^{AB}	1.12±0.07 ^E
T2 (Corn (Cr))	3.34±0.15 ^{EF}	2.03±0.28 ^{EF}	1.32±0.17 ^{ED}
T3 (NSO)	4.0±0.16 ^D	2.51±0.03 ^{CD}	1.49±0.11 ^{ED}
T4 (Cip-Cr)	4.86±0.18 ^C	2.72±0.16 ^{ABC}	2.14±0.15 ^C
T5 (Cip-NSO)	3.73±0.19 ^{DE}	2.14±0.06 ^{DEF}	1.58±0.16 ^{DE}
T6 (Ah)	3.77±0.31 ^{DE}	2.86±0.27 ^{AB}	1.73±0.05 ^{CD}
T7 (Ah-NSO)	3.16±0.11 ^F	1.16±0.12 ^G	1.66±0.29 ^{CDE}
T8 (Ah-Cip-Cr)	5.3±0.08 ^{ABC}	2.04±0.04 ^{EF}	3.44±0.26 ^A
T9 (Ah-Cip-NSO)	5.62±0.26 ^{AB}	2.50±0.09 ^{BCD}	3.1±0.18 ^{AB}
T10 (Ps)	5.70±0.14 ^{AB}	2.39±0.09 ^{BCD}	3.31±0.09 ^A
T11 (Ps-NSO)	5.75±0.08 ^{AB}	2.22±0.08 ^{CDE}	3.52±0.09 ^A
T12 (Ps-Cip-Cr)	5.8±0.06 ^A	3.11±0.11 ^A	2.69±0.12 ^B
T13 (Ps-CIP-NSO)	5.24±0.07 ^{BC}	1.71±0.21 ^F	3.53±0.26 ^A

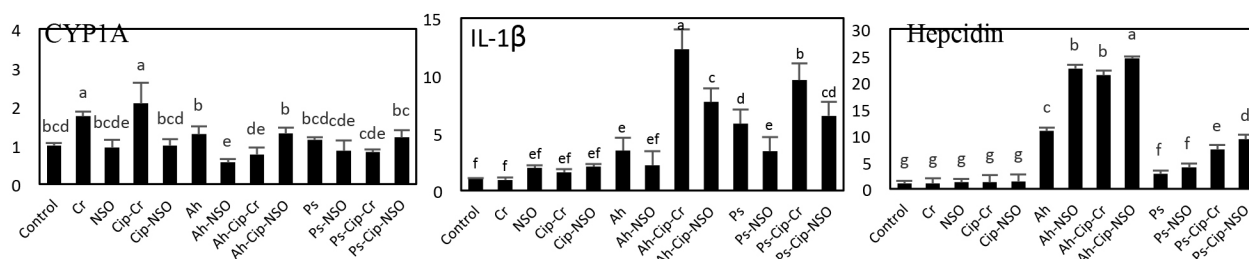


FIGURE 1. Fold change of hepcidin, IL-1 β and CYP1A genes in blood of *O. niloticus* with untreated and treated of *Nigella sativa* oil (NSO) and Ciprofloxacin (Cip) during infection of *A. hydrophila* and *P. fluorescens*. Different letters above the bars indicate significant difference (Duncan test, P < 0.05).

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 weight of 0.03lbs 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 Pepsoygen™). 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 Pepsoygen™ diet, but the Pepsoygen™ diet was higher than the other soy diets. The FCR was lowest (best) in the fish fed the fish meal or Pepsoygen™ diets and highest in the Fermented Soy 2 diet. The FCR of the fish fed the fish meal or Pepsoygen™ diets did not differ. The alternative complement activity and hepatosomatic index were higher in fish fed the Pepsoygen™ 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 Pepsoygen™ diet worked well in two key areas. While growth was higher in the fish fed the fish meal diet, Pepsoygen™ appears to be a promising alternative protein source.

THE REGULATION OF PHYSIOLOGY AND IMMUNOLOGY BY TYROSINE HYDROXYLASE ON *Litopenaeus vannamei*

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Tyrosine hydroxylase (TH) belongs to the biopterin-dependent aromatic amino acid hydroxylase enzyme family, and it represents the first and rate-limiting step in the synthesis of catecholamines that are required for physiological and immune process in invertebrates and vertebrates. A short alpha helix domain, a catalytic core, a regulatory domain, a phosphorylation site and two potential N-linked glycosylation sites included the cloned *Litopenaeus vannamei* TH (LvTH). LvTH expression was abundant in gill and haemocytes by quantitative real-time PCR. The TH mRNA expression and activity in both haemocytes and brain, and glucose level in haemolymph significantly increased in pathogen challenged and in hypothermal stressed shrimp. In addition, the immune responses and resistance were observed in TH silenced shrimp. In conclusion, the cloned LvTH in our current study is a neural TH appears to be involved in the physiological and immune responses of whiteleg shrimp, *L. vannamei* suffering stressful and infective stimulation.

DIETARY OMEGA-3 FATTY ACIDS TAILORED TO PROTECT AGAINST THREAT OF HYPOTHERMAL FISH AND SHELLFISH MORTALITY

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Episodes of high fish and shellfish mortality are often preceded by atmospheric cold waves which cause water temperatures to plummet within a short period of time (Figures 1 and 2). Documented incidences of fishkills of this nature, commonly known as hypothermic mortality, number in the hundreds over the past few decades. Sadly, many recent investigations into high fish and shellfish mortality that have followed plummeting water temperature are *not* attributed to hypothermia; especially when the temperature decrease is bracketed within what would qualitatively be considered “warm water,” above 12° (Figure 3). There is evidence to suggest that farmed fish maintained in the natural environment may also be vulnerable to hypothermic mortality.

The author has identified 2 dietary fatty acids, EPA and DHA, that, when administered selectively and in proper proportion prior to anticipated cold fronts, may serve as a powerful safeguard against hypothermal mortality. Several examples of these dietary attributes associated with variable hypothermic mortality will be presented.

A PILOT AQUACULTURE AND STOCK ENHANCEMENT PROGRAM FOR THE RED SNAPPER IN MISSISSIPPI

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The red snapper *Lutjanus campechanus* is a major commercial and recreational fishery in the Southeast United states. Harvests by recreational fisheries in federal waters are severely restricted as part of the on-going rebuilding plan for wild stocks. Aquaculture could contribute to supply the food market for this species and also to increase recruitment of harvest-size red snapper on reef habitats in a stock enhancement program. A pilot stocking program was initiated in Mississippi in collaboration between the University of Southern Mississippi and the Mississippi Department of Marine Resources (MDMR) in the late 1990s. Objectives of the project include developing the production of juveniles in intensive systems and evaluating the potential for stocking hatchery-reared red snapper on artificial reefs deployed in Mississippi coastal waters by the artificial reef bureau of MDMR. The production of juveniles is currently limited by the unpredictable spawning activity of captive broodstock, the frequent occurrence of unfertile spawns, and the low survival rates through the larval phase. Egg production currently relies on the induction of ovulation and spermiation in wild-caught mature brooders using chorionic gonadotropin which leads to variable egg quality and viability of hatched larvae to first feeding. To date, the culture of red snapper larvae has been successful when copepod nauplii were provided as an initial feed. Survival rates through the larval culture phase remain relatively low (<10% in most trials). Current research focuses on evaluating feeding protocols incorporating the results of larval nutrition studies to improve survival and expanding the production capacity for copepod live feeds. Tagged hatchery juveniles released on artificial fish are monitored through trapping. A pilot acoustic tagging study on a near-shore reef in the Mississippi sound showed promising results with retention of some of the released specimens exceeding five months. A genetic program is in development and aims to assist with the spatial management of releases through studies of population structure and local adaptation of regional populations and the monitoring of releases through genetic tagging.