

APA 18

ASIAN-PACIFIC AQUACULTURE 2018

Innovation For Aquaculture Sustainability and Food Safety

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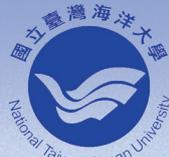
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Welcome to Asian Pacific Aquaculture 2018

Dear delegates and guests,

On behalf of the Asia-Pacific Chapter of the World Aquaculture Society, I would like to extend to you a warm welcome to #APA18. For our annual meeting in Taipei this year, we have decided to join forces with the National Taiwan Ocean University, selecting “Innovation for Aquaculture Sustainability and Food Safety” as our conference title. Collaborating with NTOU was an obvious choice given their importance in the Taiwanese education landscape; also, the timing is perfectly in line with increased WAS activities and visibility in Taiwan. We expect a large number of new members from a Chinese speaking background – not only from Taiwan, but also from all around Asia-Pacific.

The conference title has been chosen with care by the Steering Committee, and it succinctly reflects both the needs and the changes already taking place in our industry. I firmly believe that innovation will drive most of the upcoming changes not only in our industry but also in education programs in the decades to come. Do we have any choice but to embrace new ways of working to adapt to a rapidly changing world? Do we have any choice but to integrate new technologies and adjust our business models to adhere to increasingly scientifically based and stringent regulations? We are constrained by limited natural resources, yet we must produce more to feed the ever-expanding global population. Our industry must support the transformation of food production systems such that hunger can be eradicated globally as preconized by the UN Sustainable Development Goals. Our farms must ensure that ecosystems are protected to allow long-term prosperity of aquatic ecosystems. Our private and public stakeholders must ensure that the products we grow are of high quality and are tested rigorously to safeguard the consumer’s health.

Our conference focus might be on sustainability and food safety, but advancements in other fields of aquaculture will not be neglected – for example breeding, feed formulation, and species specific technical presentations. I am convinced we will have an outstanding conference, and I would take this opportunity to express my utmost appreciation to our conference and program managers who have been working hard to ensure a well-organised event. I am also confident that the tradeshow will once again be a tremendous success for all companies showcasing their new products to the industry.

There is no doubt that technology adoption and integration will be the key to sustainability and progress of our industry. This conference will serve as a platform for like-minded professionals to interact, exchange valuable ideas and gain perspective of the multifaceted challenges our industry is facing.

My final words will go out to our future members of the World Aquaculture Society. Enjoy the excellent Taiwanese hospitality and take your time to learn more about us. Together with the board, we look forward to seeing you join our team and supporting the development of aquaculture in the Asia-Pacific region!

I wish you a fruitful and successful conference in Taiwan!

Dr. Ching-Fong Chang & Dr. Guillaume Drillet
Co-Chairs

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APA 18
Asia Pacific Aquaculture

ABSTRACTS

DIETARY SUPPLEMENTATION OF LOCAL MEDICINAL HERBS FOR HYBRID GROUPEL *Epinephelus fuscoguttatus* X *E. lanceolatus* JUVENILE

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Use of herbs in aquaculture is getting popular nowadays. Malaysia is rich in many traditional herbs in which its full potential has not been fully discovered yet. Therefore, present study was conducted to screen some of the local medicinal herbs and introducing them in the feed for hybrid grouper juvenile.

Methanolic extracts of four fresh local medicinal herbs, betel (*Piper betle*), turmeric (*Curcuma longa*) and “tuhau” (*Etilingera coccinea*) were screened for antioxidant and antimicrobial activities *in-vitro*. Antioxidant activity was tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity while disc diffusion for antibacterial and fungus block dipping for antifungal activity. Four *Vibrio* species were used for antibacterial activity while *Fusarium moniliforme* was used for antifungal activity. Results from the screening showed that betel extract provided best results for all tested activities followed by turmeric and tuhau.

Thus, a feeding trial was then conducted to investigate the effects of herbs supplementation in the diets on the growth, feed utilization and apparent digestibility coefficient of hybrid grouper juvenile for 8 weeks. Fish with initial body weight of 8.91 ± 0.02 g were fed with experimental diets supplemented with 0.5% herbs which are D-betel, D-turmeric, D-tuhau. Experimental diet without supplementation of herbs was served as control. Fish were fed until apparent satiation level twice a day. Results showed highest growth in fish fed D-turmeric, followed by D-tuhau while fish fed D-betel showed the lowest growth. However, the growth of fish fed D-turmeric and D-tuhau supplementation has no significant difference than the fish fed with the control diet ($p > 0.05$) (Table 1). Better feed utilization was also observed in fish fed D-turmeric and D-tuhau compared to D-betel. Supplementation of turmeric and tuhau improved the fish's body condition and body composition however there were no significant difference than the control ($p > 0.05$). The apparent digestibility coefficient (ADC) of protein and lipid of fish fed D-turmeric and D-tuhau were significantly higher than the control ($p < 0.05$). Meanwhile, fish fed D-betel showed lower ADC of protein and lipid suggests that presence of betel perhaps its active compounds or inclusion level may interrupt the nutrient digestion and absorption of the fish. Overall, the supplementation of turmeric and tuhau gave positive effects in terms of growth, feed utilization and apparent digestibility coefficient to the fish in all tested parameters.

TABLE 1: Growth performance of hybrid grouper juveniles fed with experimental diets for 8 weeks

	Dietary treatments			
	Control	D-betel	D-turmeric	D-tuhau
Final BW (g)	59.88 ± 8.93 ^{ab}	46.21 ± 4.52 ^b	64.69 ± 7.54 ^a	61.70 ± 7.35 ^a
WG (%)	572.28 ± 97.56 ^a	417.63 ± 49.86 ^b	626.63 ± 86.44 ^a	593.69 ± 81.80 ^a
SGR (%)	3.39 ± 0.26 ^a	2.93 ± 0.17 ^b	3.53 ± 0.22 ^a	3.45 ± 0.20 ^a
Survival (%)	91.67 ± 2.89 ^a	83.33 ± 7.64 ^b	88.33 ± 2.89 ^{ab}	95.00 ± 0.00 ^a

DIETARY INFLUENCE OF YEAST AS A SOURCE OF NUCLEOTIDES ON TILAPIA *Oreochromis niloticus*

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The objective of this study was to evaluate the effect of yeast as nucleotide source on growth performance, oxidative stress, immune response, expression of some immune related genes and mortality of Tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila* and *Lacococcus gravaeie*.

A total of 270 *O. niloticus* (50.7±0.8 g of BW) were distributed in a completely randomized design, with 3 treatments: Control; 0.2% of *Saccharomyces cerevisiae* as a source of nucleotides (YNU - Hilyses®, ICC Brazil); 0.2% of YNU, with 90 fish each treatment (three replicate/tanks). The fishes were fed with 3% of its total biomass during 3 months; the acclimation period was 2 weeks. The growth parameters measured were body weight (BW, g/ind.), body weight gain (BWG, g/ind.) and feed conversion ratio (FCR) at 1 and 2 months. Also the clinicopathological, oxidant parameters, relative quantitative PCR of immune gene expression, phagocytic activity (%) and index, lysozyme activity (µg/mL) were evaluated at 2 months. After 2 months, the fishes were challenged against Gram positive bacteria *L. gravaeie* and Gram negative *A. hydrophila* and the mortality rates were observed during 1 week. The data were analyzed by Tukey test (p<0.05) using SPSS version 21.

YNU supplementation improved the growth performance in relation the control group (P<0.05). Similarly, YNU in both levels increased catalase and G-reductase activities, and expression of immune related genes parameters (P<0.05). Also, the phagocytic and lysozyme activity were increased (P<0.05). After the challenge with the both bacteria's, mortality decreased (P<0.05) in both groups with YNU (Table 1).

In general, the inclusions of 0.2 and 0.4% of YNU have proven to improve the growth performance of Nile tilapia, boost the immune response and increase the resistance against diseases.

Table 1. Growth performance, oxidative stress, expression of immune related genes, innate immunological and mortality parameters of tilapia.

Parameters	Control	0.2% YNU	0.4% YNU
BW (g) 2 months	97.86 ^a ±1.18	123.6 ^b ±2.54	130.5 ^b ±4.08
BWG (g)	48.1 ^a ±1.96	71.3 ^b ±2.14	80.5 ^b ±2.04
FCR	2.1 ^a ±0.26	1.65 ^b ±0.11	1.5 ^b ±0.07
Catalase	268.73 ^a ±43.8	354.87 ^b ±39.6	402.27 ^c ±25.4
G-reductase	142.7 ^a ±3.5	160.76 ^a ±2.34	269.93 ^b ±20.6
IL1-β	0 ^b	0.6 ^b ±1.9	4 ^a ±2.2
TNF-α	0 ^b	3.07 ^a ±.2	4.28 ^a ±0.3
Phagocytic activity (%)	57 ^b	66 ^a	68 ^a
Phagocytic index	1.8 ^b	2.3 ^a	2.1 ^a
Lysozyme activity (µg/mL)	435.8 ^b	466.1 ^a	481 ^a
Mortality (%) <i>L. gravaeie</i>	90 ^b	30 ^a	10 ^a
Mortality (%) <i>A. hydrophila</i>	100 ^c	40 ^b	0 ^a

*Different letters in the same row indicate differences by Tukey test (P<0.05).

DIETARY INFLUENCE OF YEAST CELL WALL ON THE GROWTH PERFORMANCE, OXIDATIVE STRESS PARAMETERS AND IMMUNITY OF CULTURED *Oreochromis niloticus*

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To evaluate the effect of dietary supplement (Immunowall®) on growth performance, clinicopathological parameters, oxidative stress, expression of some immune related genes, innate immunity and mortality of Tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila* and *Lacococcus gravaeie*.

A total of 270 *O. niloticus* (50.7±0.8 g of BW) were distributed in a completely randomized design with 3 treatments: Control; 0.1% of *Saccharomyces cerevisiae* yeast cell wall (YCW- ImmunoWall®, ICC Brazil); 0.2% of YCW, with 90 fish each treatment (three replicate/tanks). The body weight (BW, g/ind.), body weight gain (BWG, g/ind.) and feed conversion ratio (FCR) at 1 and 2 months were measured. Besides, the clinicopathological, oxidant parameters, relative quantitative PCR of immune gene expression, phagocytic activity (%) and index, lysozyme activity (µg/mL) were evaluated at 2 months. After 2 months, the fishes were challenged against *L. gravaeie* and *A. hydrophila* and the mortality rates were observed during 1 week. The data were analyzed by Tukey test (p≤0.05) using SPSS version 21.

There was no significant difference (P>0.05) among treatments for performance results. However, YCW improved clinicopathological results as well as, PCR expression of IL1-β, phagocytic and lysozyme activities (P<0.05). There was the reduced of G-redutase enzymatic activities and 0.2% of YCW supplementation increased catalase enzyme. After the challenge with the both bacteria's, mortality decreased (P<0.05) in both groups with YCW.

The inclusions of 0.1 and 0.2% of YCW were able to improve clinicopathological response and innate immunity. YCW supplementation decreased oxidative enzymes activity and mortality rates when the fishes were challenged.

Table 1. Performance, clinicopathological results, oxidative stress, expression of immune related genes, innate immunity and mortality parameters of tilapia.

Parameters	Control	0.1% YCW	0.2% YCW
BW (g) 2 months	94.86	118.5	120.8
BWG (g)	48.1	67.8	70.4
FCR	2.1	1.73	1.66
WBCs	130 ^a ±18.0	201 ^b ±17.4	156 ^a ± 4.4
GPT	19 ^a ± 1.1	23 ^a ± 0.9	31 ^b ± 1.2
GOT	52 ^a ± 3.2	54 ^a ± 2.3	74 ^b ± 2.3
TP	2.23 ^a ±0.08	2.97 ^b ± 0.08	2.27 ^a ± 0.08
Globulin	1.34 ^a ±0.05	2.08 ^b ± 0.03	1.27 ^a ± 0.08
Catalase	588.7 ^a ±42.0	402.3 ^b ±25.4	618.3 ^a ±60.7
G-redutase	269.3 ^a ±20.6	142.0 ^c ±3.5	192.9 ^b ±21.2
IL1-β	0 ^b	7.5 ^b ± 1.1	16 ^a ± 1.3
Phagocytic activity (%)	57 ^b	61 ^a	70 ^a
Phagocytic index	1.8 ^b	1.75 ^b	2.5 ^a
Lysozyme activity (µg/mL)	435.8 ^b	450.95 ^a	464.3 ^a
Mortality (%) <i>L. gravaeie</i>	90 ^b	50 ^a	60 ^a
Mortality (%) <i>A. hydrophila</i>	100 ^b	40 ^a	50 ^a

^{ab}Means with different letters in the same row differ significantly by Tukey test (P<0.05). WBCs: white blood cells. GPT: glutamate pyruvate transaminase. GOT: Glutamate oxalacetate transaminase. TP: Total protein.

AN INSIGHT INTO THE AQUACULTURE SECTOR MANAGEMENT IN NIGERIA

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Aquaculture is no doubt a characteristic homogenous to coastal states and have over the years become a vital aspect in planning towards sustainability and economic development of coastal states in particular. Nigeria being a country with a wealth of resources (biotic and abiotic) within its inland, coastal and offshore regions has not only drawn utility from these resources but have also had a fair amount of daunting challenges arising from a questions on how, why, where and who is to take responsibility for effective management of this resources as these challenges overlap different institutions in the country. This study aims to know the key aquaculture management institutions in Nigeria for the past five (5) years, their operational framework, their jurisdiction of authority, administrative challenges, administrative successes, lags in the management of the this sector and answer the question of why effective sustainable management should be adopted especially for the aquaculture sector which has shown remarkable potentials in recent times.

PREVALENCE, RISK FACTORS AND TRANSMISSION OF VIRAL NERVOUS NECROSIS IN A HATCHERY PRODUCING HYBRID GROUPEL FRY

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Grouper is one of the high value and demanded cultured marine fish especially in the Southeast Asia countries. However, the supply of groupers from aquaculture is often limited due to betanodavirus. This study investigates the prevalence, risk factors and transmission of viral nervous necrosis (VNN) in a hatchery producing hybrid grouper (♀ giant grouper *Epinephelus lanceolatus* x ♂ tiger grouper *E. fuscoguttatus*) fry.

The experiment was conducted in a marine fish hatchery located in Terengganu, Malaysia. The eggs and sperm of giant grouper (GG) and tiger grouper (TG) brood stocks, respectively, were taken for VNN detection using PCR. After fertilization, the fry were observed for VNN status at 5, 10, 20, 30, 40, 60, 90 and 120 days post hatching, while the water quality during the study period were recorded. The detected VNN were proceed for molecular characterization.

The prevalence of VNN in brood stocks throughout 12 months of sampling showed low detection of VNN. VNN only detected in April 2016 with a percentage of 50% for GG and 33.33% for TG brood stocks. However, there were correlations between the presence of VNN in GG, TG and hybrid grouper (HG) juveniles with water quality parameters. Moreover, iron and ammonia consistently showed significant relationship with the presence of VNN in GG, TG and HG. VNN transmission study and molecular characterization analysis suggested possible horizontal and vertical transmission of VNN between the groupers in this hatchery (Table 1). This study provides significant information for control and prevention of VNN to farmers in this country that producing HG fry at hatchery level.

TABLE 1: Vertical transmission of VNN from broodstocks to juveniles at hatchery level.

Days post hatched	GG+ x TG+			GG+ x TG-			GG- x TG-		
	Length (cm)	Weight (kg)	VNN status (%)	Length (cm)	Weight (kg)	VNN status (%)	Length (cm)	Weight (kg)	VNN status (%)
GG broodstock	140.0	70.00	+	140.0	70.00	+	150.0	80.00	-
TG broodstock	46.0	2.07	+	48.0	2.13	-	73.0	5.54	-
5	2.8	0.00015	+(100)	2.7	0.00011	+(100)	2.8	0.00018	-
10	2.9	0.00039	+(100)	2.9	0.00035	+(60)	3.0	0.00047	-
20	3.9	0.00163	+(80)	4.1	0.00157	+(100)	4.3	0.00169	-
30	5.8	0.00374	+(100)	5.6	0.00369	+(80)	6.0	0.00407	-
40	7.7	0.01623	+(80)	7.2	0.01154	+(80)	7.8	0.01702	-
60	8.9	0.06111	+(90)	8.3	0.05641	+(80)	9.0	0.06985	-
90	11.1	0.13211	+(100)	10.4	0.12000	+(100)	11.5	0.15143	-
120	12.6	0.25000	+(90)	11.9	0.22000	+(100)	12.9	0.28900	-

GG: Giant grouper; TG: Tiger grouper; +: positive to VNN; -: negative to VNN; x: breed between.

GROWTH RATE OF *Kappaphycus striatum* var *cottonii* AT DIFFERENT POSITIONS OF THE CULTIVATION LINES TO THE DIRECTION OF WATER CURRENT

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The study was conducted to determine the effects of different positions in cultivation lines towards the direction of water current on growth rate of *Kappaphycus striatum* var. *cottonii* for a duration of 45 days at 04°30'936"N and 119°22'068"E of Bungin Pondohan, Sitangkai, Tawi-Tawi. The experiment has three treatments; the parallel (T1), perpendicular (T2) and diagonal (T3) positions towards the direction of water current with 5 replicates in each treatment following the randomized complete block design (RCBD).

Results showed that higher mean weight of 473 g and growth increment of 8.3 g/day were obtained from diagonal positions (T3), although not significantly different from mean weights and growth increments parallel (T1) and perpendicular (T2) of 431 g and 7.4 g/day, and 408 g and 6.8 g/day, respectively. However, results showed statistically similar growth rates of *Kappaphycus striatum* var *cottonii* among all treatments. Thus, the positions of cultivation lines with respect to the direction of the current have no significant effect on the growth of the said seaweed species. Hence, the treatments used in this study are viable in growing *Kappaphycus striatum* var. *cottonii* in the selected culture site.

Table 1. Periodic mean weight (MW) and growth increment of *Kappaphycus striatum* seeds during the 45 days of culture period.

Treatment	Initial (g)	15 days		30 days		45 days		Final growth Increment (g)
		MW (g)	GI (g)	MW (g)	GI (g)	MW (g)	GI (g)	
(T1) Parallel	100	144.5	44.5	329	184.5	431	102	331
(T2) Perpendicular	100	134	34	299	165	408	109	303
(T3) Diagonal	100	152	52	365	210	473	108	373

Table 2. Growth Increment and Net production of *Kappaphycus striatum* seeds after 45 days of culture period

Treatment	Mean Weight		Mean growth Increment (g)	Relative growth increment/day	
	Initial (g)	Final (g)		(g)	(%)
(T1) Parallel	100	431	331	7.4	30.21
(T2) Perpendicular	100	408	308	6.8	32.47
(T3) Diagonal	100	473	373	8.3	26.81

Table 3. Analysis of variance (ANOVA) of Randomized Complete Block Design

Source of variation	Degree of Freedom (DF)	Sum of Square (SS)	Mean Square (MS)	Computed F	Tabular F	
					5%	1%
Replicates	4	19189.17	4797.292	1.10383	3.84	7.01
Treatment	2	11590	5795	1.333397	4.46	8.66
Experiment Error	8	34768.33	4346.042			
total	14	65547.5				

CV=20.16%

ns = not significance

SUITABILITY OF ARTIFICIAL SEAWATER AND BRINE SOLUTION AS GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* LARVAE CULTURE MEDIA

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The giant freshwater prawn, *Macrobrachium rosenbergii* is a highly valued delicious food and commands very good demand in both domestic and export market. The culture peculiarity of prawn is that though they grow, mature, fertilize, even hatch but their larvae neither can survive nor grow up to post-larval stage without brackish water. Unfortunately, because of the continuing increase in the transportation cost for saline water and prawn post larvae, increased production is becoming less feasible. Furthermore, lower survival rate of larvae can occur due to stress from long hours of transportation, hence the growing need for local hatcheries. However, these hatcheries still need to transport concentrated seawater from the source, which still commands high transportation cost and in turn makes it difficult for local hatcheries to continue larval production. Therefore, it is necessary to find an alternative source of saline water to lower the cost of operation. Thus, the use of artificial sea water and brine solution constitutes a cornerstone in prawn culture. Such systems can reduce the costs of using seawater, allowing the establishment of hatcheries in areas distant from the coast, where land is less expensive and large consumer markets are located. To address this problem, the use of commercial artificial and brine solution has been investigated in this study.

This experiment was conducted for a period of 42 days to evaluate the suitability of artificial seawater and brine as giant freshwater prawn larvae culture media in 2015. Prawn larvae were reared under three treatments, each treatment had three replications. The treatments were: T₁- brine solution, T₂-artificial sea water-1 (complex formula) and T₃-artificial sea water-2 (simple formula). The cost of 12 ppt brine water was 12 US\$/m³, artificial seawater-1 was 95 US\$/m³ and artificial seawater-2 was 71 US\$/m³. There were no significant differences (p>0.05) among the water quality parameters during the study period. The survival rate of prawn larvae was 56.45%, 55.63% and 55.78% for T₁, T₂ and T₃ respectively. There were no significant differences (P>0.05) among the survival rates and metamorphosis time of different treatments. Significantly lower (P>0.05) production cost (1000PL) was observed in T₁ (US\$ 8.97) followed by T₂ (US\$ 13.42) and T₃ (US\$ 11.53). The results of this study revealed that brine solution is the best larvae culture media for an inland hatchery if the location of the hatchery not so far from the brine source. Where the brine solution is not available or the price of brine is higher than artificial seawater due to the long transportation, artificial seawater can be used for prawn PL production. In this case simple formula of artificial seawater is more suitable than complex formula due to low cost, well defined and easily replicated by farmers.

Treatment	Days of metamorphosis		Average survival rate (%)	Production Cost/1000 PL (USD)
	Start	Complete		
T ₁ (Brine)	21	33	56.45± 1.80 ^a	8.97±0.23 ^a
T ₂ (ASW-1)	22	32	55.63± 1.34 ^a	13.42±0.32 ^c
T ₃ (ASW-2)	22	33	55.78 ±3.45 ^a	11.53±0.29 ^b

NURSERY CULTURE OF SEA CUCUMBER *Holothuria scabra* IN SEA-BASED FLOATING HAPA BAG NETS: EFFECTS OF DENSITY AND REARING DURATION

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Global sea cucumber populations are declining because of overfishing. Hatchery production of sea cucumbers, particularly of the tropical sandfish *Holothuria scabra* have been progressing in the past decade but the corresponding improvements in nursery and grow-out technology have been slow. Various nursery systems for rearing of sandfish has been developed and modified through the years to optimize growth performance and survivorship. Sea-based nurseries, particularly the floating hapa nursery systems have shown great production potential and practical applications especially in the Philippines and the West Pacific regions. To evaluate rearing performance of floating hapa bag net for juvenile sandfish, various experiments were conducted by SEAFDEC/AQD in Iloilo and Guimaras, central Philippines. Performance indicators such as growth, survival, and optimal stocking densities were assessed using 40-45 d old post-metamorphic juveniles (~5mm, ~0.03g) produced in the hatchery. Floating hapa bag nets were made from fine-mesh (1 mm) PE net fabricated to a dimension of 1×2×1 m (Length×Width×Depth) and fitted on floating PVC pipes to be suspended from the water surface (Fig. 1). Experiments were conducted at the Igang Marine Station (IMS) of SEAFDEC/AQD located in Guimaras Island, central Philippines.

The general trend in survival and growth of sandfish juveniles with different initial stocking densities after a 30-d culture duration is presented in Table 1. During a good season with no heavy rains, high survival (>80%) and good growth (>2 g) can be achieved at densities of <700 individuals per hapa without supplemental feeding. Sandfish only fed on naturally accumulating algal biofilm on the nets. Results indicated that growth and survival is significantly influenced by stocking density, where average growth rates increase as density decreases. However, other variables such as food composition and abundance, seasonality and competition/predation are among the challenges that must be considered for future studies. With an understanding of these site-specific factors, nursery rearing can be programmed to optimize production potential of sandfish juveniles in sea-based floating hapas.



FIG 1. Sandfish floating nursery at SEAFDEC/AQD Philippines

TABLE 1. Survival and growth of sandfish juveniles in floating hapas with different stocking densities after 1 mo rearing

Stocking density (Individuals/hapa)	Survival / Individuals	Ave Weight
250	95% / 237	3.2 g
500	90% / 450	2.3 g
700	85% / 595	1.8 g
1000	75% / 750	1.6 g
2000	68% /1360	0.8 g

ADVERSE EFFECTS OF STORMS ON GROWTH AND SURVIVAL OF JUVENILE TIGER SHRIMP *Penaeus monodon* IN A MANGROVE SILVO-AQUACULTURE PEN

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Thousands of hectares of global mangrove forests were lost in the last few decades. In Southeast Asia, conversion of intertidal mangrove areas into earthen aquaculture ponds is among the major causes of this decline. Particularly in central Philippines, >90% of mangroves gave way to ponds during the boom of the tiger shrimp culture industry in the 1980s. However, shrimp diseases eventually caused many of those ponds to become unprofitable and abandoned. A number of abandoned ponds have naturally been recolonized by mangroves and the concept of silvo-aquaculture, silvo-fisheries or aqua-silviculture (an integrated mangroves and aquaculture system) was promoted in the Philippines in order to support mangrove rehabilitation while providing alternative livelihood to communities through mangrove-friendly aquaculture of important seafood.

The silvo-aquaculture of tiger shrimp *Penaeus monodon* was tested successfully for nursery rearing in a 1,000 m² pen within a 2-ha recovering mangrove area (pond:mangrove ratio of 1:20) in Aklan, central Philippines. Unfortunately, during some culture runs in 2014, storm events occurred with prolonged rain that drastically affected growth and survival of shrimps (Fig. 1). Losses in terms of growth potential of shrimps ranged from 63-78%. In one culture run, the projected average weight of 0.81 g with 42% survival after 30 d was disrupted by a 3-day storm, leaving only with 0.30 g with 13% survival at 30 d. However, an advantage of a silvo-aquaculture pen is that water is naturally exchanged by the tide which mitigates extended stratification of the water column, affording good recovery for the remaining shrimps. These results has implications in managing future culture runs, assessing potential losses, and in planning for a more resilient system for silvo-aquaculture in the light of climate change.

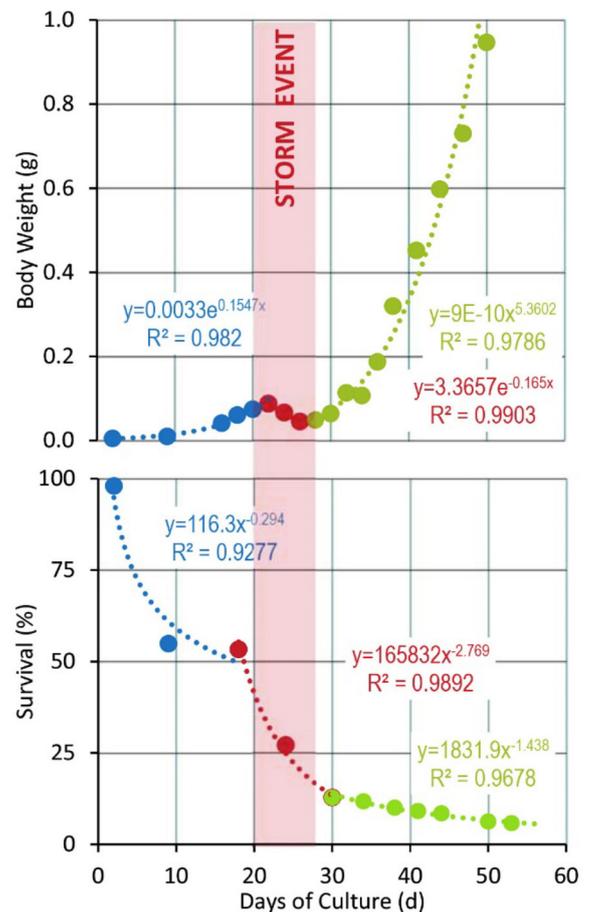


Fig. 1. Body weight (g) and survival (%) of juvenile tiger shrimps in a silvo-aquaculture pen before, during, and after a storm event.

POTENCIAL OF CLIMBING PERCH, *Anabas testudineus* CULTURE AT COASTAL ZONE

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A study on the effect of water salinity ranging from 0-30 ppt on hatching success of climbing perch, *Anabas testudineus* was conducted in a 15-liter glass aquarium (water volume 10 liters) containing 500 eggs for various level of water salinity. Fertilization rate at 0, 5, 10, 15, 20, 25 and 30 ppt were 76.67, 61.33, 77.00, 47.33, 0, 0 and 0 %, respectively. The fertilization rate at 0-10 ppt water salinity was significantly ($P<0.05$) higher than that at 15, 20, 25 and 30 ppt water salinity. Hatching rate at 0, 5, 10 and 15 ppt were 91.33, 87.90, 86.20 and 77.49 %, respectively. The hatching rate at 0-10 ppt water salinity was significantly ($P<0.05$) higher than that at 15 ppt water salinity. The times of hatching out at water salinity of 0-15 ppt were between 1,255-1,300 minutes.

The survival tolerance of climbing perch larvae in different water salinity (0-30 ppt) within 24 hour were then studied using a 50-liter glass aquarium (water volume 30 liters) containing 50 larvae at each level of water salinity. All treatments were done in triplicate. The survival rate of fish larvae in the 0, 5, 10, 11, 12, 13, 14, 15, 20, 25 and 30 ppt water salinity were 100, 100, 100, 83.3, 83.3, 73.3, 70, 70, 0, 0 and 0 %, respectively. The survival rate at 0-12 ppt water salinity was not significantly ($P>0.05$). The gain rate of total body length at 8 weeks at water salinity ranging from 0, 5, 10 and 12 ppt were 1.33, 1.41, 1.36, 1.43 and 1.63 cm, respectively. The gain rate of total body length at 0-12 ppt water salinity was not significantly ($P>0.05$). Water salinity in the range of 0-12 ppt was suitable for climbing perch culture.

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.

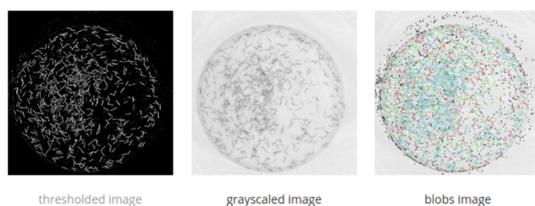


Table 1. Counting accuracy of Giant tiger prawn post-larvae using artificial intelligence.

Mean (%)	Standard deviation	N
97.6	2.1	723

ANALYSIS EFFECTIVITY OF MORODEMAK FISH AUCTION MARKET USING INSTITUTIONAL ECONOMY APPROACH

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Previous research of auction market only focused on explaining quality and availability of the infrastructure and neglected the institutional perspective. This paper aim to analyze if the Auction Market at TPI Morodemak, Demak, Central Java has been working effectively based on act No. 23 2014 – by using the institutional economy approach which focus on agency theory, transaction cost, and law enforcement. This research is qualitative descriptive research. The Data has been collected by using structured investigation and deep interview with a random sampling. The subject of interview consists of 5 fishers, 6 bidders, an auction officer, and also the governance agent (Fisheries and Maritime Ministry of Demak and Central Java). The data's been tabulated by using the triangulation method. The result will be shown on the table below:

The table has shown the incompatibility of auction market process to the rule. It can be seen through the registration and payment process. The incompatibility might happen because of the lack of law-enforcement supervision. It also might be supported by the existence of incoherent regulation done by either the district government or the province-government. Hence, it created the situation of ineffective auction market process.

Table 1. Triangulation Result

No	Indicators	Fishers	Bidders	TPI
1.	Auction Recruitment	All Fisher has to pay the retribution fee with difference value.	All bidder are allowed to join in auction without any registration needed but they have to save certain money at first. They have to pay the retribution fee with different value	The outsider-bidder has to save certain amount of money before they join the auction. Neither will be registration needed nor the administrative stuff. Bidders has to pay 2% retribution fees if they win.
2.	Payment	Fisher will get the money after one week to three months of payment	.Some bidder pay full of charge directly after winning the auction. Some will pay a half of payment and will be paying it a week later. Others will pay it by credit system	All bidders has to pay full of charge directly after the auction market has begun.
3.	Price	Fishers has no power of making the reservation price. That's all been considered inside of the market. Either the ceiling price or the floor price been decided based on the stock of the fish itself.	Bidders has no power of making the reservation price. That's all been considered inside of the market. Either the ceiling price or the floor price been decided based on the stock of the fish itself.	That's all been considered inside of the market. Either the ceiling price or the floor price been decided based on the stock of the fish itself.

SOCIAL MONITORING IN SEAFOOD SUPPLY CHAINS

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Introduction

Environmental issues are widely addressed in current governance through standards, certification schemes and guides of best practices. But social issues are trickier to assess, measure and monitor. To foster improvement in social, labor and human rights compliance within the seafood supply chain, social benchmarking should be developed to fit aquaculture farms and boats’ needs. This means that relevant indicators must be identified and accordingly, new tools for social monitoring would be designed.

The Social Expert Group (SMEG) is a group of experts in human rights, human-trafficking, child labor, fisheries and aquaculture sustainability from credible organizations and companies in the Asia-Pacific region. These experts have developed a set of metrics divided into 12 categories (see figure 1).

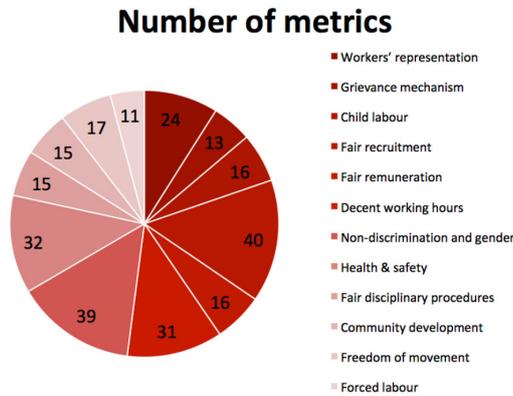


Figure 1. 12 categories of metrics and the metric distribution.

Verifik8 and Social Monitoring

Verifik8 is a web-application where producers can input data on their farm and practices. Environmental data is asked from the farm, as is social data. The social data is related to the workers on the farm, fair recruitment and non-discrimination, child labor etc. But Verifik8 also crosschecks this data with other external data streams, such as worker voice tools, governmental databases or data from NGOs or other organizations. These external data streams provide information that can also be used in algorithms to quantify and assess the social metrics. Through the regular data input and updates of farmers, and with the help of external data streams, Verifik8 can be part of an innovative solution to the lack of social measuring and monitoring in seafood supply chains.

Social Metrics and Algorithms

It is not always easy to quantify and measure social metrics. This is why algorithms are needed to make sure metrics can be assessed and quantified. Algorithms make use of different types of information to get to one conclusion.

For example, for metrics in child labor, it is important to first know the size of the farm. In small-scale farms, the household often runs the business, and children are common on the farm. If there are children on the farm, then it is also important to know how many of these children help out on the farm. As long as the children go to school on a regular basis, it is not considered child labor. But in larger-scale farms, there should not be any children.

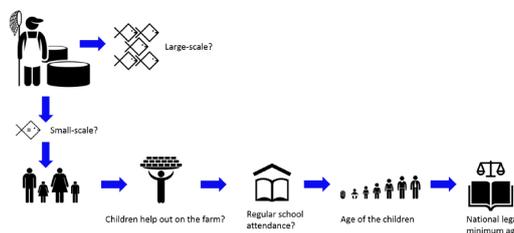


Figure 2. Example of algorithm for child labor.

(Continued on next page)

Another example is on the metrics for non-discrimination. To know whether any worker has been discriminated based on parameters such as national origin, legal status, gender etc. in terms of payment (compensation), information on the parameters mentioned above about the workers is needed. An upload of the working contract is required as well. By comparing the worker profile and the working contract of one worker with the ones of another worker, possible differences in payments for similar work can be noticed. To make sure workers are also paid either the legal minimum wage or above, their contracts are compared to their payment slips received (if applicable) and this is in turn compared to the legal national minimum wage. These algorithms enable Verifik8 to spot the red flags in social practices on a farm.

A lot of information is gathered from the farm profile if more environmental-related information is needed, and from the worker profile if more social information related to workers is required. By combining information from both environmental and social sides, Verifik8's analytics provide an integrated approach for data collection, measurement, monitoring, and verification.



REARING PERFORMANCE OF NILE TILAPIA FINGERLINGS *Oreochromis niloticus* AND PANGASIOUS *Pangasius hypophthalmus*, PRODUCTIVITY OF LETTUCE *Lactuca sativa* AND WATER QUALITY IN RAFT-AQUAPONIC SYSTEM AT VARYING TILAPIA FINGERLINGS STOCKING DENSITIES

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This study evaluated the rearing performance of Nile tilapia fingerlings (*Oreochromis niloticus*) and Pangasius (*Pangasius hypophthalmus*), productivity of lettuce *Lactuca sativa* and water quality in raft- aquaponic system at varying stocking densities. Different stocking densities of tilapia (2.97 g \pm 0.08) were small (80 pcs), medium (100 pcs) and large (120 pcs) were placed in fish tank and 10 Pangasius and 20 lettuce were cultured in each hydroponic bed. The rearing experiment was carried out for 77 days. Fish sampling was conducted every 14 days while initial and final weight and length of lettuce were recorded. Furthermore, sampling of water quality parameters was conducted every 72 hours.

Different stocking densities had no significant effects on rearing performance of tilapia fingerlings and Pangasius, productivity of lettuce and water quality in raft-aquaponic system. The recorded final weight of tilapia fingerlings, Pangasius and lettuce ranged from 12 g to 14 g, 8 g to 9 g and 4 g to 11 g, respectively. In addition, the recorded Total Ammonia-Nitrogen level ranged from -0.15 to 0.06 mg/L in fish tank and from -0.07 to -0.01 mg/L in hydroponic bed, while Phosphorous level ranged from 0.41 to 0.43 mg/L in tank and from 0.45 to 0.62 in hydroponic bed. Optimum level of pH, % saturation and temperature were also recorded.

This implies that the different stocking densities in the study had no effect on the rearing performance of Nile tilapia fingerlings and Pangasius, productivity of lettuce and water quality in raft- aquaponic system. Prolonging of rearing period up to marketable size is recommended to further asses the productivity of raft-aquaponic system using tilapia fingerlings.

OXIDATIVE STRESS IN LARVAE OF JAPANESE PEARL OYSTER, *Pinctada fucata martensii*, CAUSED BY ICHTHYOTOXIC HARMFUL ALGAE, *Chattonella marina* and *Karenia mikimotoi*

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Several species of harmful algal blooms cause mass mortalities of fish in aquaculture farms, including the cosmopolitan ichthyotoxic dinoflagellate, *Karenia mikimotoi*, and the raphidophyte, *Chattonella marina*. Several aspects of the toxinology of these harmful algae remain intractable due to the complexity of the toxicity mechanism underlying the fish- and shellfish-kills. In addition, several harmful algal species have been recently shown to affect the reproduction of shellfish at very low cell densities, such as species of the cosmopolitan genus, *Alexandrium*.

Under laboratory conditions, the gonads of adult Japanese pearl oyster, *Pinctada fucata martensii*, were stripped of spermatozoa and oocytes, and artificial fertilization was carried out. Three larval stages were reared and used for experimental exposure to *Karenia mikimotoi* and *Chattonella marina* in mono-specific and multi-specific experimental frames. The activities of the larvae were monitored regularly during the exposure, and samples were taken at different exposure times, immediately preserved at -80°C until analyses of the oxidative enzymes.

Both the activity and the antioxidant enzymes in larvae were modulated following exposures to *K. mikimotoi* and *C. marina*. Allelopathic relationships were found, and *Karenia mikimotoi* exhibited the highest effects in mixture on all three larval stages. The results of the current study, along with recent findings, stress the effects of harmful algal species on the reproduction of bivalve molluscs and the importance of monitoring harmful algal blooms in coastal areas and shellfish aquaculture farms.

ENVIRONMENTAL DNA (eDNA) AS A NOVEL FORENSIC TECHNIQUE TO DETECT PATHOGENS IN AQUACULTURE SYSTEMS

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Food security will be one of the biggest challenges for humanity as the global population nears 9 billion. Aquaculture, as the fastest growing agribusiness in the world (annual production of 73.8 million tonnes and sale value of US\$160.2 billion), is shaping to be a major supplier of humanity's future animal protein needs. However, 40% of current aquaculture production is lost due to diseases. The risk of economic loss due to disease outbreaks can be catastrophic and constrains the continued growth and profitability of the sector.

DNA-based detection technologies linked to environmental data have the ability to accelerate the response of farmers to diseases. It can do this by providing farmers with an understanding about the number of pathogens and how their population dynamics correlate with extrinsic environmental and management drivers. One promising technique as a front-line detection method is environmental DNA (eDNA), which identifies genetic material discharged in the environment (water or sediment) by macro and microorganisms. This technique has great sensitivity and it can be used even when there is no visible presence of the target organism during sampling. Through the association of eDNA with farm environmental parameters it is possible to predict fish mortalities and advise the best approaches for disease management before outbreaks occur.

Ciliate protozoans are considered economically important parasites affecting many aquaculture species. Specifically, *Chilodonella* spp. are ciliates that can cause losses of 50–95% in fish stocks without early warnings. *Chilodonella hexasticha* was used as a model to investigate the potential use of environmental DNA (qPCR assay based on SSU-rDNA gene) and associated water quality data to predict protozoan parasite outbreaks in a freshwater barramundi farm from Australia. Using eDNA methodology, we were able to detect the abundance of our target species in as little as 15 mL of water collected from ponds within a commercial barramundi farm, over 10 months. Increased *C. hexasticha* eDNA levels were found to be highly correlated with occurrence of later fish mortality events ($r = 0.402$; $P < 0.001$) and also with size of fish ($r = -0.189$; $P < 0.05$). Furthermore, there were significantly more fish mortalities observed during the warmer, wetter monsoonal season compared to the cooler, dry season (1280 vs. 135 mortalities, respectively; $P < 0.05$).

This study shows that the incorporation of eDNA for parasite surveillance associated with local environmental data can predict disease outbreaks, allowing fish farmers to adopt preventive strategies to boost fish production (e.g. water treatment, improvement of oxygen levels, stock of juveniles in ponds after rainy season, etc.) and avoid economic losses.

CLIMATE CHANGE ATTITUDE INVENTORY OF THE COASTAL FISHERFOLKS IN THE COASTAL AREAS OF PALANAN, ISABELA, PHILIPPINES

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The study investigates the climate change attitude inventory of the coastal fisherfolks in the coastal areas of Palanan, Isabela, Philippines. A total of 380 respondents have participated in the study from the coastal areas of Palanan, Isabela which includes barangay San Isidro, Maligaya, Culasi and Dialawyo. The instrument used was adapted from a standardized questionnaire.

Data were analyzed using ANOVA, and Pearson correlation. Statistical Package for the Social Sciences (SPSS) aided the computation of the statistical parameters. Analyses of data revealed a good agreement among the fisherfolks regarding their perceptions and attitude inventory about climate change though they are uncertain as to the real cause of the phenomenon. Fisherfolks exhibited a high positive attitude towards climate change in terms of concern, optimism and sense of responsibility. Noteworthy is the relatively low mean score for commitment since they are not really clear on the cause of the phenomenon and thus, they have no idea as to how they can be of help.

Even though, there were significant differences on their perceptions and attitude inventory when grouped according to their demographic locations, age, gender, and source of information. No significant difference was found in terms of religious affiliations.

Indeed, there were significant relationships between respondents' perceptions and attitude inventory of the coastal fisherfolks in the coastal areas of Palanan, Isabela towards climate change.

ARID LAND AQUAPONIC SYSTEM OF TILAPIA (*Oreochromis niloticus*) CULTIVATION AND CHERRY TOMATO PRODUCTION; BIOCHEMICAL NUTRITIVE CHARACTERISTICS OF SYSTEM AND YIELD

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Aquaponic especially in Arid Zones would be the healthiest and most efficient food production system. It uses less than 90 percent of water than that used in convention crop production, crops grow faster plus no pesticide, herbicide nor fertilizers are used in aquaponics. Three aquaponic units each contain a 400 m² greenhouse with plantation area 120 m² in four turfs (each .2*24* .4 m³ covered with perforated Styrofoam sheet), two circular fish tanks with 7.7m² fish tanks connected to water treatment units include swirl separator for mechanical filtration followed by two biological filter tanks with plastic media then a CO² stripping unit. Water moves from fish tanks to the water treatment system and plantation raceways by gravity and return to fish tanks using a 3Hp water tanks. The system was aerated by 5Hp air blower. Fish *Oreochromis niloticus* tanks were stocked with 100 fish m³, 120 fish m³ and 140 fish m³ in each green house with an average weight of 5 gm. Nile tilapia were fed to satiation three times a day a floating commercial tilapia diet. Raceways were planted at a rate of 3 cherry tomato *Lycopersicon esculentum* seeds per square meter. All aquaponics systems production characteristics namely water, feed, electricity consumption, as well as fish, cherry tomato and sludge production were monitored. Environmental factors namely light intensity, temperature, water quality parameters and microbiological evaluation were monitored. During six months' experimental duration, the system produced seven tons of Cherry tomato (average 15g each tomatoes) and 1.8 tons of fish (average 400g / fish) per greenhouse. During the period of experiment the fish effluent water contain mineral concentration were tested. Also, the produced tomato fruits nutritive proximate compositions were estimated.

Keywords; arid land, aquaponics, tilapia, cherry tomato

STUDY ON THE IMPACT VIUSID-AQUA AS ANTIVIRAL AND IMMUNE MODULATOR FOR SUSTAINABLE HEALTH MANAGEMENT AND SEED PRODUCTION OF PRAWN (*Macrobrachium rosenbergii*) IN HATCHERY CAPTIVITY IN BANGLADESH

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Influences of the Viusid-aqua, an antiviral and immune modulator product for aquaculture of Catalysis, Spain were tested on hatchery phase of freshwater prawn (*Macrobrachium rosenbergii*) to specifically find out its impact on water quality, gravid prawns' rearing, larval survival and growth and on PL transportation were tested. The study was to find out a sustainable practice of prawn hatchery management applying the antiviral and immune stimulant in the system to combat any viral disease and/or to help prevent viral and bacterial disease occurrences resulting improved production of prawn PL in hatcheries. Newly added rearing water of trial larvae rearing tanks (LRTs) was treated @10.00 ppm with liquid product followed by a regular treatment @3.00 ppm for old water of hatchery tanks including broodstock tanks and LRTs. Viusid-aqua powder was used in the artemia hatching tanks @ 15.00 ppm and in the custard feed for larval stages @ 05.00 ppm. Viusid-aqua was not used in the control LRTs. PL transportation water was treated with Viusid-aqua @10 ppm.

Findings of Viusid-aqua based trials were compared with a control group of treatments without Viusid in favor of the objectives of the study. Water pH, DO, NH₃, NO₃ and NO₂ level were found to be comparatively more stable and more favorable in VIUSID based trials than that of control tanks. Reduced larval cycle span (22 days instead of 28 days, 5 larval stages ahead in 35 DoCs), higher survival rate (17%), increase PL production (25%; 51,000 pcs/tank of 2.5 MT or 20,400/MT; 20.4PL/liter), increased body length (17.6%), increased body weight (15.0%), more growth rate (15.0%), earlier completion of larval conversion to PL (11 DoCs earlier in trial tanks) revealed much better efficiency and sustainability of using Viusid-aqua in prawn hatchery phase of fresh water prawn. No occurrence of any viral disease, better appetite and feeding efficiency of berried prawns resulting better performances of hatchlings in larval phase in hatchery indicated improved efficiency of the product in preventing diseases and immune stimulation. Reduction of transport mortality of PL during distant transportation is indicative of positive influence of Viusid-aqua reducing transport-stresses of prawn PL.

PROPHYLACTIC PROPERTIES OF BIOFLOC OR NILE TILAPIA-CONDITIONED WATER AGAINST *Vibrio parahaemolyticus* INFECTION OF *Penaeus vannamei* (WHITELEG SHRIMP)

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AHPND or acute hepatopancreatic necrosis disease, caused by isolates of *Vibrio parahaemolyticus* (VpAHPND) that carry a plasmid encoding two Pir-like toxins, is listed by the OIE as a key bacterial threat to commercial whiteleg shrimp (*Penaeus vannamei*) production. Host infection results in progressive degeneration and destruction of the hepatopancreas, and since 2009 this disease has caused economic losses in Asia amounting to billions of US dollars. However, recent studies have shown that biofloc technology may help to reduce the magnitude of shrimp mortalities. Therefore, the present study aimed to advance our understanding of possible protective properties conferred to whiteleg shrimp by laboratory cultures of biofloc or Nile tilapia-conditioned greenwater when reared and subsequently challenged with VpAHPND. Groups of shrimp were challenged with VpAHPND in four concentrations of biofloc (0%, 25%, 50%, 100%) cultured in 15 ppt seawater, and shrimp cultured in >50% biofloc (14-day culture, ca. 0.5 g dry organic matter L⁻¹) had significantly lower mortality at (37% and 27% over a 96 h post-infection period versus 60% (0% biofloc) and 59% (25% biofloc). The second trial explored shrimp density (1, 3 or 5 shrimp (Shr); av. 0.36 g) when challenged with VpAHPND in either 400 mL of 50% or 100% biofloc. The mortality followed expectations with the level of mortality in the 50% biofloc being higher than that in the 100% biofloc (mortality in 50% biofloc: 1 Shr - 80% mortality, 3 Shr - 73%; 5 Shr - 78%; versus mortality in 100% biofloc: 1 Shr - 80%, 3 Shr - 53%, 5 Shr - 50%). There was, however, only a single significant difference between the concentrations of biofloc and shrimp stocked at 5 shrimp per vessel in each (p=0.0013). In the third trial, the mortality of shrimp reared and challenged in three different salinities of either clear (CW) seawater (5, 10 and 15 ppt) or in three salinities of Nile tilapia-conditioned greenwater (GW) were compared. The percentage mortality by 96 h post-infection was CW10ppt (94%) > CW15ppt (94%) > GW15ppt (87%) > CW5ppt (67%) > GW10ppt (27%) > GW5ppt (20%). The results indicate that biofloc, Nile-tilapia conditioned greenwater and low salinity may offer protection for whiteleg shrimp against VpAHPND challenge, and this new knowledge could be factored into AHPND disease management programmes.

GENETIC TOOLS AND THE FUTURE OF BREEDING IN SHRIMP

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Pacific white shrimp (*Penaeus vannamei*) is the most important shrimp species in the aquaculture industry and accounts 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 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 (e.g. disease resistance) prevents candidates from returning and contributing to the breeding nucleus. Genetic tools can be used to increase the accuracy and intensity of selection, leading to increased genetic gain. Tools needed include tools to assess parentage, relatedness, and diversity among cultured populations. 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 >99%, was able to provide measures of diversity, inbreeding and population structure within and among stocks. This is a flexible panel, and SNPs linked to traits can be easily incorporated for marker assisted selection. More than 40,000 shrimp have been screened with this SNP panel, with an overall assignment rate of 99.1%. Also for breeding, genomic selection is a recent approach developed for agricultural breeding programs to increase the accuracy and intensity of selection, accelerate the genetic gain per generation, all while allowing for the control of genetic structure on a whole-genome level. It relies on the estimation of genomic similarity between phenotyped animals and future broodstock to predict breeding values, rather than sib-ship relationships. It is a powerful tool for many reasons: 1) it allows for increases in selection accuracy; 2) it allows for selection of breeding candidates that are more likely to perform well; 3) it allows for the control of inbreeding (relatedness) in whole-genome wide fashion; 4) it allows improved selection based on phenotypes that cannot be measured on the breeding candidates and without depending solely on family information. This last point can have a great impact on shrimp breeding, as it allows the accurate incorporation of genetic data from culture ponds without increasing biosecurity risks. Application of genomic selection is economically feasible when combining a cost-effective method for scanning the genome of broodstock for SNP markers and genomic imputation. We have pioneered successful methods for aquaculture using both genotyping by sequencing (GBS) and SNP chips for genomic scores for broodstock with an inexpensive approach using smaller SNP panels and imputation for test animals. We have demonstrated that with such a system, the economic costs for incorporating genomic selection to accelerate shrimp breeding programs are no longer barriers to implementation, and the power of genomics can be engaged to accelerate genetic gains in shrimp breeding.

EFFECT OF INULIN AND FRUCTOOLIGOSACCHARIDES ON IMMUNE RESPONSE OF STRIPED CATFISH (*Pangasianodon hypophthalmus*)

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This study aimed to evaluate the effects of inulin and fructooligosaccharides (FOS) on immune system of striped catfish (*Pangasianodon hypophthalmus*). Experiment was randomly designed with 5 treatments (control; 0,5% Inulin; 1% Inulin; 0,5% FOS and 1% FOS) and triplications for each treatment. After 28 days of supplemented inulin and FOS, fish was collected for immune evaluation and also challenged for with bacteria (*Edwardsiella ictaluri*). The mortality of fish was recorded every day for 14 days. Several immune parameters including the total erythrocyte cells, leucocyte cells, each type leucocyte, lysozyme and complement activity were analyzed for evaluation of fish immune response. Results showed that hematology parameters, lysozyme and complement activity in inulin and FOS supplemented treatments were higher than those of control treatment after 28 days. Treatment of 1% inulin showed that the total of leukocyte, monocyte, neutrophil, lymphocyte, lysozyme and complement activity were significantly increased with control treatment ($p < 0,05$) after 28 days. After challenge with *E. ictaluri*, the mortality of supplemented treatments were lower than control treatment; treatments 1% Inulin had the lower mortality 42,67% and significant difference when compared to control. These results indicated that administration 1% Inulin can stimulate non-specific immune reponse and protect striped catfish from bacterial damage.

IMPROVING SHRIMP FEED PERFORMANCE WITH KRILL MEAL

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Krill meal is prepared from an aqueous extract of ground whole Antarctic krill (*Euphausia superba*), which is harvested in the Southern Ocean. The meal contains about 60 percent protein with a nutritionally well-balanced amino acid profile and 25% lipids that are high in phospholipids, omega-3 fatty acids and astaxanthin esters. Moreover, krill meal is rich in chitin from the exoskeleton and soluble compounds like trimethylamine oxide, free amino acids and nucleotides that may all act as feeding stimulants. Feeding stimulation of the whiteleg shrimp, *L.vannamei* by partial replacement of fish meal by krill meal in diets leads to increased growth by extending feed intake. Several studies have investigated the effects of krill meal feed inclusion on penaeid shrimp performance. How krill meal acts as feeding stimulant, growth accelerator and cost saver will be summarized in this presentation.

BARCODED PYROSEQUENCING OF THE BACTERIAL COMMUNITY DISTRIBUTION IN DIFFERENT SEGMENTS OF THE GASTROINTESTINAL TRACT OF WILD CAUGHT *Penaeus monodon*

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Bacterial community associated with the gastrointestinal (GI) tract of aquaculture animals can play important roles in health, nutrition, and disease. Crustacean digestive GI tract, compared to that of vertebrates, have unique structures and surfaces that may contribute to differences in the bacterial communities. The objective of this study was to examine the bacterial composition and distribution in different segments along the gastrointestinal tract and the digesta of wild-caught adult *Penaeus monodon* using Automated Ribosomal Intergenic Spacer Analysis (ARISA), real time quantitative PCR, and barcoded pyrosequencing of 16S rRNA genes.

The results showed that bacterial communities showed some differences along the GI tract segments, particularly the hindgut ($P < 0.001$). Bacterial community of the digesta was also significantly different ($P < 0.05$) from that of the digestive tract (all segments). One hundred and sixty bacterial genera in nine phyla were represented in the GI tract of adult *P. monodon*. *Proteobacteria* comprised over 80% of reads abundance of the bacterial community in most segments of the shrimp GI tract, except in the hindgut where it was less dominant. The knowledge about the distribution of bacteria could be useful in understanding interaction of commensal bacteria and pathogens in different segments and developing better targeted probiotic to shape the GI tract bacteria of shrimp.

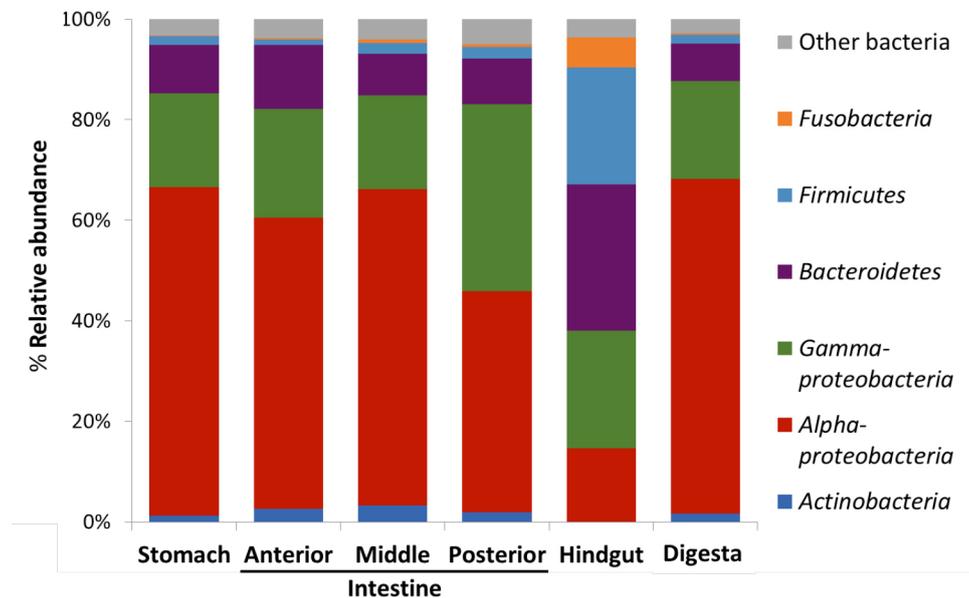


Figure 1. Relative abundance of important bacteria groups in different segment of the GI tract and the digesta of *P. monodon* (pool N = 10 shrimp) based on barcoded-pyrosequencing of V3-V4 16S rRNA genes.

SALINITY EFFECTS ON STRATEGIES OF CARBOHYDRATE METABOLISM IN MILKFISH LIVERS UNDER LOW-TEMPERATURE STRESS

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The milkfish (*Chanos chanos*) is an important aquaculture species in Southeast Asia, they are tropical species and intolerant of water temperature below 12°C. In winter, the cold snap leading to high mortality and huge economic loss has been reported for several decades in Taiwan. The euryhaline milkfish can survive in fresh water (FW) and seawater (SW) environment, whereas the fishermen prefer to culture in FW due to better feed conversion rate. Acclimation of milkfish to SW or FW induces a variety of physiological responses, including changes in their tolerance to low-temperature stress. SW milkfish was found to exhibit better hypothermal tolerance than FW milkfish in the previous study. Under cold stress, reduction of appetite led to loss of energy source in organisms, and hepatic glycogen plays a crucial role in providing energy supply for the whole body. In carbohydrate metabolism, the ATP production via aerobic metabolism was important for detox function of the liver. Our studies revealed that SW milkfish under cold stress (18°C) have the ability to keep consistent energy supply. On the other hand, when the FW milkfish were under cold stress, the hepatic glycogen degradation and gluconeogenesis occurred to maintain the constancy of blood glucose levels and ATP production was reduced due to down-regulation of aerobic metabolism. In addition, the energy flow of TCA cycle inclined to amino acid biosynthesis. Salinity together with low-temperature challenge may lead to a more stressful condition that affected strategies of carbohydrate metabolism.

INFLUENCE OF NOVEL PROTEIN KINASE C ON IMMUNITY IN HEMOCYTE OF *Litopenaeus vannamei*

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Protein kinase C (PKC) was originally identified as a serine/threonine protein kinase, which was classified into three major groups, classical PKC (cPKC), novel PKC (nPKC), and atypical PKC (aPKC) forms. We successfully cloned a novel protein kinase C in hemocyte of *Litopenaeus vannamei*, designated as Lv-nPKC. The full-length cDNA of Lv-nPKC was 2548 bp in length, and an open reading frame (ORF) of 2234 bp encoding 744 amino acids. The protein domain features of Lv-nPKC included the Ca²⁺-independent translocation of the enzyme, two tandem domains that bind to phorbol ester (PS)/diacylglycerol (DAG) signature, and a conserved region of PKC/AGC kinase C-terminal domain. In this case, we investigated the effects of lipopolysaccharide (LPS) *in vitro* and *Vibrio alginolyticus* *in vivo* on hemocyte, respectively, with quantitative real-time PCR assay, and the Lv-nPKC expression in both tests significantly increased. In further, the investigation on their potential function was conducted by gene silencing, and the results revealed that the Lv-nPKC expression in hemocytes of *L. vannamei* received double strand (ds) dsLv-nPKC for 1 day was silenced, and the significant decreased respiratory bursts and prophenoloxidase activity were observed. Thus, Lv-nPKC might play crucial roles in the immunity of shrimp.

PRODUCTION OF HYBRID GROUPER (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) USING CRYOPRESERVED SEMEN TRANSPORTED BY DRY ICE

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Hybrid groupers are popular cultured fish for the Asian marine finfish aquaculture industry. The shortage of *E. lanceolatus* semen is a constraint for the production of hybrid grouper fry. Cryopreserved semen were transported by packaging in a Styrofoam box with dry ice as preservation medium. Transportation of cryopreserved semen from the cryopreservation centre to production hatcheries was by commercial courier service provider. The performance of cryopreserved semen transported using dry ice was tested in artificial insemination of hybrid grouper based on the fertilization rate, hatching rate and abnormalities of the produced larvae. Five treatments were employed in the experiment: exposure of the cryopreserved semen inside a sealed Styrofoam box loaded with dry ice for 24 hours (Treatment 1), 48 hours (Treatment 3) and, 72 hours (Treatment 5) and immersion of the cryopreserved semen into liquid nitrogen after exposure to dry ice for 24 hours (Treatment 2) and 48 hours (Treatment 4). The two controls were fresh semen (negative control) and cryopreserved semen in liquid nitrogen (positive control). There was no significant difference ($P > 0.05$) on the fertilization rate for all controls and treatments. For hatching rate, there was no significant difference ($P > 0.05$) among two controls and Treatment 1 and Treatment 2. There was also no significant difference ($P > 0.05$) among the positive control, treatment 1, treatment 2 and treatment 3. There was significant difference among treatment 1, treatment 3 and treatment 5. Hatching rate of Treatment 4 was significantly lower compared to other treatments except to Treatment 5. There was no significant difference ($P > 0.05$) in the larval abnormalities among the controls and treatments. The conclusion of the study was clear that transported cryopreserved semen can be used effectively in artificial insemination of hybrid grouper.

TRANSCRIPTOME ANALYSIS OF HYBRID TILAPIA (*Oreochromis* spp.) WITH STREPTOCOCCUS AGALACTIAE INFECTION IDENTIFIES TOLL-LIKE RECEPTOR PATHWAY-MEDIATED INDUCTION OF NADPH OXIDASE COMPLEX AND PISCIDINS AS PRIMARY IMMUNE-RELATED RESPONSES

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Streptococcus agalactiae infection is one of the most significant bacterial diseases in tilapia aquaculture. Identification of immune-related genes associated with *Streptococcus agalactiae* infection may provide a basis for breeding selection or therapeutics to augment disease resistance. Therefore, we utilized transcriptome profiling to study the host response in tilapia following *Streptococcus agalactiae* infection. Based on GO and KEGG enrichment analyses, we found that differentially expressed genes are widely involved in immune-related pathways, including the induction of antimicrobial peptides. Moreover, the main components of two immune-related pathways (Toll-like receptor signaling and leukocyte transendothelial migration) and four environmental information processing pathways (TNF, PI3K-Akt, Jak-STAT and MAPK) were identified. Finally, a time-course expression profile for several of the identified transcripts including tilapia piscidin 3 (TP3), tilapia piscidin 4 (TP4), TLR2, TLR5, MyD88, TRAF6, p38, and interleukin components was performed by qRT-PCR. Collectively, these results provide a starting point to study molecular mechanisms of tilapia immune response to *Streptococcus agalactiae* infection and may be applied as a basis for developing disease resistant strains by breeding selection.

MICROBIAL WATER QUALITY AND ANTIMICROBIAL RESISTANCE IN AQUACULTURE FARMS IN SINGAPORE

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Microbial water quality can jeopardize the health and safety of food produced by aquaculture farms. Antibiotic resistant bacteria (ARB), pathogens, and antibiotic resistance genes (ARGs) present in water and sediments of aquaculture farms provide a possible route of transmission from fish culture to humans either through consumption or secondary contact. Microbial water quality and antimicrobial resistance determinants were determined in three fish farms and transect sites approximately 100 m away from the farms.

No significant differences were found between concentrations of indicator organisms (*E. coli*: 123.6 – 1638.0 MPN/100 mL, Enterococci: 98.7 – 816.4 MPN/100 mL, *Pseudomonas aeruginosa*: 1.0 – 1968.3 MPN/100 mL) of fish farms and transect sites in the Johor Straits and Singapore Straits however, multidrug resistant *E. coli* were isolated only from the surface waters of sites in Johor Straits on Tryptone Bile X-Glucuronide (TBX) media supplemented with either extended-spectrum beta-lactam (ESBL) or carbapenem (KPC) antibiotics. All *E. coli* strains isolated were susceptible to amikacin, and resistant to ampicillin and cefazolin. A total of 81.8 % of the *E. coli* strains were ESBL producers and isolates exhibited resistance to at least 3 of the 16 antibiotics tested. The detection of multidrug resistant (MDR) *E. coli* in the Johor Straits suggests sources coming from terrestrial runoffs flanking the waterway. Toxigenic *Vibrio* species were not detected in surface water or sediment samples.

The relative abundance of *sull*, *qnrA* and *intI1* genes were higher in sediments than surface waters (5 magnitudes) although there is no reported usage of antimicrobials in the fish farms sampled. Among the surface water samples, higher concentrations (10^{-1} - 10^1) of beta-lactamases (*bla_{SHV}*, *bla_{OXA}*, *bla_{CTX-M}*) were detected in the transect sites, and the two carbapenem resistance genes *bla_{NDM}* and *bla_{KPC}* (10^{-6} - 10^{-5}) were low in the surface waters of both fish farms and transects. This study concludes that toxigenic *Vibrio* species do not appear to be an immediate threat to aquaculture operations however the high relative concentrations of antimicrobial risk determinants (e.g. beta-lactamase resistance genes, MDR *E. coli*) compromises water quality and highlights a potential transmission chain from environmental waters, to animal carriers and humans.

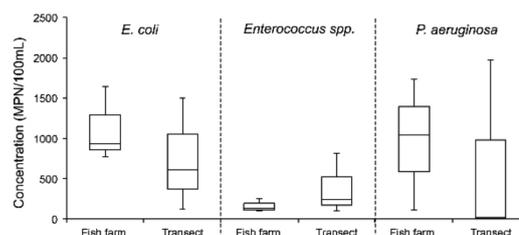


FIGURE 1. Boxplot comparing the range values for *E. coli*, *Enterococcus* spp. and *P. aeruginosa* in surface waters of Fish farms (n=3) and transect sites (n=3).

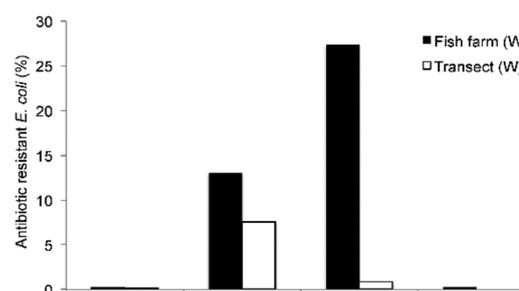


FIGURE 2. Percentage of antibiotic resistant *E. coli* isolated from Fish farms and transect sites. Abbreviation of antibiotics is as follows; AMK- Amikacin, COT-Cotrimoxazole, ESBL-extended spectrum beta lactams, KPC- carbapenems.

FLUCTUATIONS OF CELLULAR CALCIUM LEVELS IN *Litopenaeus vannamei* Y-ORGAN CELLS DURING A NATURAL MOLTING CYCLE

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Molt-inhibiting hormone (MIH) suppresses the production of ecdysteroids in Y-organs via cGMP and/or cAMP. Studies from different lines suggest that phosphodiesterase I (PDE1), a calcium/ calmodulin-dependent PDE isoform, may play a critical role in controlling cyclic nucleotides levels to mediate the production of ecdysteroids. As a first step to investigate the role of Ca^{2+} signaling in ecdysteroids synthesis in *L. vannamei*, free Ca^{2+} levels in Y-organ cells were measured during a natural molting cycle. Results showed that Ca^{2+} levels were low during postmolt (A/B), intermolt (C), and early premolt stages (D1); however it showed a remarkably increase in the middle premolt (D2) stage. This data provide the first evidence suggesting that calcium signaling may promote ecdysteroids production in *L. vannamei*.

Y-organs from animals at different molting stages (A/B, C, D1 and D2) were dissected, rinsed with shrimp saline and then transferred into 0.25% collagenase solutions (2 Y-organs/ 0.5ml) for 60- min incubation at 30°C. Cell solutions were filtered through 40 μ m nylon mesh, centrifuged at 100g for 10min to collect disassociated Y-organ cells, washed once and then re-suspended and incubated for 30 min in shrimp saline containing Fluo-4 AM (10 M) and Pluronic F-127 (2%). After incubation, Y-organ cells were collected by brief centrifugation (100g), washed twice, and then re-suspended in 50 μ l shrimp saline. 10- μ l cell solutions were used for image taken. The fluorescent signal from Y-organ cells was visualized using a Leica confocal microscope fitted with a high-energy argon laser at 488 nm; image analyses were carried out using MetaMorph 7.6 software.

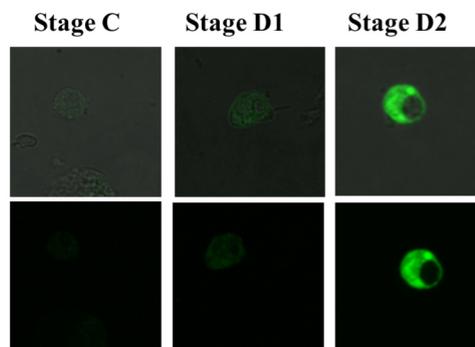


Fig.1. Representative Y-organ cell images at different molting stages.

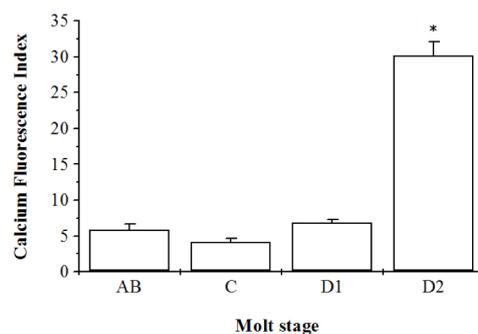


Fig.2. Mean Ca^{2+} -specific fluorescence in Y-organ cells in a molting cycle.

A STUDY OF FUCOIDAN EXTRACTION FROM TAIWAN LOCAL *Sargassum* SPP.

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Sargassum spp. are widely distributed throughout Taiwan coast waters, and they possess a polysaccharide with many bioactivities, named fucoidan. Previous studies show that fucoidan is water-soluble, and its main components are fucose and sulphate groups. The bioactivities of fucoidan are associated with its structure, monosaccharide composition, the content and the location of sulphate groups. The primary aim of this study was establishment of a database component in Taiwan local algae. This investigation was analysis monosaccharide composition from Taiwan local *Sargassum fusiforme* and *Sargassum ilicifolium*, with an optimization extraction method. After optimization extraction, the fucoidan was acid hydrolyzed and pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP). In addition, high performance liquid chromatography (HPLC) with UV-Vis detector was used to analysis monosaccharide composition, such as mannose, glucose, galactose, xylose, and fucose. The HPLC analysis was performed on a Hypersil BDS-C₁₈ column with acetonitrile-water 23:77 (v/v) containing 0.05 M phosphoric acid as mobile phase at a flow rate of 1.0 ml/min and the method provided excellent linearity ($r^2 = 0.9999$) for all analytes. The detective wavelength was set at 245 nm and column temperature was room temperature. Finally, this study build the monosaccharide composition and percentage of sulphate groups with two kinds of Taiwan local *Sargassum* spp. successful. In the present study, the monosaccharide composition and percentage of sulphate groups was obtained from Taiwan local *Sargassum*.

ANALYSIS OF CELL DEATH PROFILE INFECTED WITH IRIDOVIRUS ISKNV STRAIN IN TARGETED TISSUES SPLEEN AND HEAD-KIDNEY OF GIANT GROUPE

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Iridovirus is highly infectious to several marine fish and increasingly threatens aquaculture in Taiwan. The iridovirus ISKNV strain was isolated from infected the giant grouper (*Epinephelus lanceolatus*) in south Taiwan aquaculture area. In the present, we examined the molecular cell death pathways from infected tissues.

First the ISKNV strain infected giant grouper with different time point incubations from day 1 to day 5. We found that ISKNV induces a lot of mortality and that damaged major targeted tissues such as spleen or head kidney, which are also enlarged and darken. Furthermore, we checked the viral expression level in targeted tissues by qRT-PCR approach. In the results of the viral major capsid protein (MCP) gene and DNA dependent DNA polymerase (DdDp) gene expression level also received a lot of increasing fold-change either in spleen (200-folds change) or in head kidney (500-folds change).

Then, cell death assays by using H&E staining or TUNEL approaches in tissue sections. Both assays also received the consistent results that apoptotic cell or post-apoptotic cell also strongly staining by HE staining and TUNEL analysis in spleen and head-kidney in ISKNV-infected tissues. Third, in the cell death signaling analysis, we found that Fas gene could be upregulated about 100-folds (head-kidney) and 50-folds (spleen).

Furthermore, at caspase-8 expression level assay, received up to 10-folds change in head-kidney. Taken together our results suggest that ISKNV strain can infect giant grouper very well via a lot of viral replication. Then, Fas-mediated death signaling was triggered by ISKNV.

This finding also provides new insight into iridovirus pathogenesis.

STUDY ON MINIMAL INHIBITION CONCENTRATION (MIC) AND MINIMAL BACTERICIDAL CONCENTRATION (MBC) OF PHENYLACTIC ACID AND NISIN FROM LACTIC ACID BACTERIA TO AGAINST *Vibrio parahaemolyticus*

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The aim of this study is to investigate the minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) against *Vibrio parahaemolyticus* BCRC12959. The MIC of phenyllactic acid (PLA) and EDTA against *V. parahaemolyticus* (10^6 CFU/mL) are 2.5 mg/mL and 146 mg/mL (0.5 mM), while MBC values are 5 mg/mL and 11.68 mg/mL (40 mM), respectively. As pH value of reacting solution was adjusted to 6, different dosages of PLA showed higher MIC and MBC as compared to unadjusted. Nisin is not affected to its antibacterial activity to *V. parahaemolyticus* (Table 1). In the combination of PLA, nisin, or EDTA, the results of MIC and MBC against *V. parahaemolyticus* showed that 50 mg/mL nisin with 146 mg/mL (0.5 mM) EDTA exhibited better inhibition effect (Table 2). Concentration ranged from 400 mg/mL to 1.5625 mg/mL of freeze extracellular substance (FES) from PLA producing strains *Lactobacillus plantarum* K8, KP3, KP4, and *Lb. paracasei* subsp. *paracasei* DP2 and nisin producing strain *Lactococcus lactis* BCRC10791 were tested there inhibition effect on *V. parahaemolyticus*, respectively. Strains K8, DP2, and BCRC10791 showed lower MIC (12.5 mg/mL) and MBC (25 mg/mL) to *V. parahaemolyticus* (10^6 CFU/mL) (Table 3). In the combination of LAB FES, MIC (mg/mL) and MBC (mg/mL) of K8 + DP2, 10791 + DP2, and 10791 + K8 are 6.25 + 6.25 / 12.5 + 25, 6.25 + 6.25 / 12.5 + 25, and 6.25 + 6.25 / 12.5 + 25 exhibited the stronger inhibition activity to *V. parahaemolyticus* BCRC12959 (Table 4).

Table 1. MIC and MBC of PLA, nisin and EDTA against to *V. parahaemolyticus* BCRC12959 with and without pH correction

	MIC	MIC (pH 6 ± 0.1)	MBC	MBC (pH 6 ± 0.1)
Phenyllactic acid (mg/mL)	2.5	> 10.0	5.0	> 20.0
Nisin (mg/mL)	-	-	-	-
EDTA (mM)	0.5	0.5	40.0	40.0

Table 3. MIC and MBC of extracellular substance from PLA producing isolated LAB *Lactobacillus plantarum* K8, KP3, KP4, *Lb. paracasei* subsp. *paracasei* DP2 and nisin producing isolated LAB *Lactococcus lactis* BCRC10791 against to *V. parahaemolyticus* BCRC12959

	MIC (mg/mL)	MBC (mg/mL)
<i>Lactobacillus plantarum</i> K8	12.5	25.0
<i>Lactobacillus plantarum</i> KP3	100.0	200.0
<i>Lactobacillus plantarum</i> KP4	25.0	50.0
<i>Lb. paracasei</i> subsp. <i>paracasei</i> DP2	12.5	25.0
<i>Lactococcus lactis</i> BCRC10791	12.5	25.0

Table 2. MIC and MBC of different concentration PLA, nisin and EDTA against to *V. parahaemolyticus* BCRC12959

	MIC	MBC
Nisin + EDTA	50.00 µg/mL Nisin + 0.50 mM EDTA	100.00 µg/mL Nisin + 1.00 mM EDTA
PLA + EDTA	1.25 mg/mL PLA + 0.50 mM EDTA	5.00 mg/mL PLA + 0.25 mM EDTA

Table 4. MIC and MBC of different concentration extracellular substance from PLA producing isolated LAB *Lactobacillus plantarum* K8, KP3, KP4, *Lb. paracasei* subsp. *paracasei* DP2 and nisin producing isolated LAB *Lactococcus lactis* BCRC10791 against to *V. parahaemolyticus* BCRC12959

	MIC	MBC
KP3 + KP4	25.00 mg/mL + 12.50 mg/mL	25.00 mg/mL + 6.25 mg/mL
K8 + KP4	6.25 mg/mL + 25.00 mg/mL	6.25 mg/mL + 50.00 mg/mL and 12.00 mg/mL + 25.00 mg/mL
K8 + DP2	6.25 mg/mL + 6.25 mg/mL	12.50 mg/mL + 25.00 mg/mL and 25.00 mg/mL + 12.50 mg/mL
10791 + DP2	6.25 mg/mL + 6.25 mg/mL and 3.125 mg/mL + 12.50 mg/mL	12.50 mg/mL + 25.00 mg/mL and 25.00 mg/mL + 12.50 mg/mL
10791 + K8	6.25 mg/mL + 6.25 mg/mL and 3.125 mg/mL + 12.50 mg/mL	12.50 mg/mL + 25.00 mg/mL and 25.00 mg/mL + 12.50 mg/mL

RECOVERY OF COMPONENTS OF SHRIMP SHELL WASTE FERMENTATION BY CHITINOLYTIC, PROTEOLYTIC AND LACTIC ACID BACTERIA ISOLATED FROM *Litopenaeus vannamei*

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Shrimp shell waste (SSW), about fifty thousand tons annually in the world, is an excellent source for proteins, calcium, carotenoids and bioactive substances. In this study, 135 strains of chitinolytic bacteria, 52 strains of proteolytic bacteria, and 29 strains of lactic acid bacteria (LAB) were isolated from *Litopenaeus vannamei*, white shrimp. The chitinolytic strain of LV1111, proteolytic strain of LV2122 and LAB strain of LV1204 were screened out as the tested strains for the further evaluation of the single- and mix-cultured fermentation efficacy on SSW utilization. The free amino nitrogen (FAN) content in the fermented SSW by LV1111, LV2122 and LV1204 strains were 113.94, 141.24 and 126.44 mg/100 mL, respectively; while the FAN was greatly increased to 568.28 mg/100 mL using the combination fermentation of mixed cultures. The mineral content in the fermented SSW by using mixed-cultures was 19.85 mg/mL, which was 1.2-64.0 times of those in single-cultured fermented product. The total phenol content in the mixed cultured product was 78.57 μ g gallic acid equivalents (GAE)/mL, which was much higher than that (eg. 6.98 mg GAE/mL) obtained by single-cultured product. In conclusion, the mix-cultured fermentation technique could more efficiently recover the protein and minerals from SSW, and much higher total phenol content was obtained, compared to the single culture fermentation. With good quality of protein and bioactive substances, the mixed-cultured fermented SSW product shows a potential as a valuable supplement in the feed for fishes and crustaceans.

TABLE 1. Mineral content, free amino nitrogen content and total phenol content of 10% shrimp shell waste fermented by single-cultured and mix-cultured at 37°C for 7 days.

	Mineral content (mg/mL)	Free amino nitrogen (mg/100 mL)	Total phenol content (μ g gallic acid equivalents /mL)
Control	0.41 \pm 0.27	37.51 \pm 5.00	6.98 \pm 3.30
Single-cultured fermentation			
LV1111	0.31 \pm 0.03	113.94 \pm 5.00	83.24 \pm 19.34
LV2122	0.70 \pm 0.12	141.24 \pm 10.21	169.22 \pm 43.40
LV1204	16.34 \pm 1.36	126.44 \pm 2.16	1716.88 \pm 24.94
Mix-cultured fermentation			
LV1111+2122+1204	19.85 \pm 1.96	568.28 \pm 11.01	78.57 \pm 14.52

TO ESTABLISH OF A ZEBRAFISH XENOGRAFT MODEL OF HUMAN METASTATIC BREAST CANCER FOR EVALUATING THE THERAPEUTIC EFFICACY OF TILAPIA PISCIDIN 4 (TP4)

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TP4 is a cationic antimicrobial peptide (CAP) derived from Nile tilapia (*Oreochromis niloticus*). Previous studies have shown that breast cancer cells are sensitive to some CAPs treatment and TP4 has been shown to trigger the death of some cancer cells in vitro; however, whether TP4 is toxic to breast cancer has not yet been evaluated. Here, we use three breast cancer cells with different molecular profiling (MDA-MB-231/MDA-MB-453/MCF-7) to show that TP4 selectively toxic to these breast cancer cells but not to normal control cells (M10/HDF). Transcriptome analysis in combined with Western blot studies of TP4-treated metastatic MDA-MB-231 revealed induction of a transcription factor gene family but not observed in TP4 treated HDF cell. Overexpression of one of the gene family member by transiently transfection in breast cancer cells caused a dramatic increase in cell death. We further generated a metastatic MDA-MB-231 xenograft in zebrafish through microinjection at 48hpf and observed a high risk of neonatal deaths. High-content imaging revealed that the death of fish possibly caused by cancer cell metastasis, leading to the blood vessel disruption. Using a dose of 3 μ g/mL of TP4 which is less toxic to neonatal zebrafish prolonged survival of MDA-MB-231 xenografts in fish. Whole-mount staining was used to demonstrate that TP4 effectively decreased the area of transplanted tumor, possibly through induction of the transcription factor gene family protein. This study indicates that TP4 may possess therapeutic potential for use in metastatic breast cancer treatment.

BRAIN 5-HT AND SERUM CORTISOL INTERACT TO REGULATE CANNIBALISTIC BEHAVIOR IN ORANGE-SPOTTED GROUPEL

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Aurantiochytrium strain BL10 is rich in DHA. Previous research has shown that adding 2% of dry BL10 to increase the DHA content of feed for orange-spotted grouper does not benefit growth but significantly reduce size discrepancies between individuals and thereby decrease the chance of cannibalism. To understand whether BL10 supplement can really reduce the cannibalism rates of orange-spotted grouper, and how the BL10 supplements affect cannibalistic behavior, we produced four isonitrogenous and isolipidic experimental diets dosed with different weight percentages (0, 2, 4, 6%) BL10 meal and fed it to orange-spotted grouper fingerlings for two weeks. During the feeding period, cannibalism in the experiment groups were observed. After the feeding period, we analyzed the brain 5-hydroxytryptamine (5-HT) level and serum cortisol levels in the experiment groups and used regression analysis to examine the relationships among these biochemical variants and cannibalism rates. The results indicate that 2~6% BL10 supplements, particularly 2% BL10 supplements, could significantly reduce the cannibalism rates of orange-spotted grouper (Fig. 1). Multiple linear regression demonstrated that cannibalism is affected by serum cortisol and brain 5-HT levels together. The relationship among the three can be expressed using the following equation: cannibalism rate (%) = 1.0465 * [Cortisol] (ng/mL) + 0.4128 * [5-HT] (ng/g wet weight) - 0.0107 * [Cortisol] * [5-HT] - 33.4320 (Fig. 2), indicating that cannibalism is influenced not only by brain 5-HT and serum cortisol per se but also by the interaction effects between them.

QUICK AND SIMPLE DETECTION OF TILAPIA LAKE VIRUS BY A FULLY AUTOMATED SAMPLE-TO-RESULT PCR SYSTEM

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Tilapia lake virus (TiLV), a novel orthomyxo-like virus, is an emerging virus associated with significant mortalities in farmed tilapia. Since the first discovery of the virus in Israel in 2014, cases have been reported in several countries such as Columbia, Ecuador, Egypt and Thailand. The virus represents a huge risk to the global tilapia industry and may cause catastrophic economic losses. Although nested reverse transcription-polymerase chain reaction (RT-PCR) has been accepted as an important tool for detecting TiLV RNA, its application has been limited to laboratory settings due to requirement of well-trained person and complex processes. Sensitive and easy tools for TiLV detection would allow timely identification of pathogen at points of need.

The POCKIT™ Central Nucleic Acid Analyzer (POCKIT™ Central, GeneReach) is a benchtop sample-in-answer-out solution that provides easy qualitative results. It integrates magnetic bead-based nucleic acids extraction and fluorescence-based insulated isothermal PCR amplification/detection technology to offer a walk-away nucleic acid detection protocol, minimizing the needs in human resource and the risks of human errors with affordable reagents/consumables. The POCKIT™ Central system could be useful for timely detection of TiLV in tilapia at various point-of-need settings to facilitate disease management and minimize disease spread.

In this study, we evaluated the performance of a commercial TiLV RT-PCR assay on the POCKIT™ Central system. The TiLV RT-PCR has a limit of detection LoD95% of 12 genome equivalents, determined with a 10-fold serial dilution of a plasmid DNA containing the target sequence. Performance of the POCKIT™ Central system to detect TiLV RNA in tilapia was compared to a nested RT-PCR (Tsofack *et al.*, 2017) with 80 brain and liver samples of tilapia. The TiLV RT-PCR had great agreement (>95%) in sensitivity and specificity with the reference PCR method.

Available in a convenient lyophilized and single-dose format, the TiLV RT-PCR on the sample-in-answer-out POCKIT™ Central system can serve as a point-of-need bio-detection tool to aid diagnosis and/or screening of TiLV infection at laboratory, pond side, and customs where qualified human resources for molecular diagnosis are limited.

GLUCOCORTICOID/GLUCOCORTICOID RECEPTORS (GRs) PATHWAY OF GROUPEL INVOLVED IN MODULATING IMMUNE RESPONSES DURING NODAVIRUS INFECTION

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Mammalian glucocorticoid signaling is one of the important regulators of a wide range of adaptive physiological responses. We, for the first time, report the complete sequencing of glucocorticoid receptor (GR) cDNA from orange-spotted grouper. Despite the difference in the lengths of the GR transcripts, all of the GR molecules consist of three domains: (i) the N-terminal transactivation domain (ii) the central, DNA binding domain, is highly conserved among nuclear receptors (iii) the C-terminal is the site of hormone binding, and also serves as a binding site for heat shock proteins. The grouper GR gene was differentially expressed *in vivo* and contributed differently to high-level expression of GR in brain. Real-time RT-PCR analysis demonstrated an increase level of tumor necrosis factor (TNF), HSP90 and GR in nodavirus-infected grouper compared with healthy grouper during 48 hours post-infection. Overexpression of GR-EGFP results in higher transcriptional activity level of TNF promoter. We used to fluorescence resonance energy transfer (FRET) experiment conclude that cortisol exerts their influences by binding to the grouper GR and dissociating with HSP90, capable of regulating several genes. These findings have implications that biological effects of glucocorticoid/GR involve cross-talk with inflammatory and immune responses during nodavirus infection.

SPECIES IDENTIFICATION AND GROWTH OF THE GOLDEN OYSTER IN TAIWAN

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Taixi is one of the main oyster farming regions in Taiwan. To increase the commercial value of cultured oyster, the Mariculture Research Center of Fisheries Research Institute has conducted breeding programs on golden oyster years ago. In this study, the difference between golden oyster and local cultured oyster were investigated through species identification and growth comparison. The research was divided into (i) species identification of golden oyster, (ii) estimation on wild golden oyster population, and (iii) comparing the growth and survival between golden oyster and general cultured oyster. Based on the result of COI gene sequence, the golden oyster is the same species as general cultured oyster, i.e., *Crassostrea angulata*. The percentages of golden oyster among native populations of Taixi, Qigu, and Kinmen were insignificantly different ($p > 0.05$), i.e., $9.7 \pm 5.2\%$, $9.5 \pm 5.4\%$, $8.4 \pm 5.8\%$, respectively. The survival rate between golden and general oysters was similar ($>90\%$). The mean size and weight of golden oyster were smaller than the general one, and cultural conditions also affected oyster growth. Sampling bias through small stocks of bred parent and offspring are not ruled out for the inferior growth in the golden oyster.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF HSP60S FROM TWO MARINE PATHOGENS

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In the recent years, vaccine was proved as a safe and effective strategy to control the disease in aquaculture. In the development of vaccines, identifying a good antigenic target is important. By using immunoproteomic, heat shock protein 60 (HSP60) was recognized as highly immunogenic proteins in *Vibrio campbellii* and *Photobacterium damseale subsp. Piscisida*. The HSP60s of these two marine bacteria share 90% amino acid sequence similarity, and enzyme-linked immunosorbent assay (ELISA) analysis showed that anti-*Vc*HSP60 grouper antiserum was able to recognize both *Vc*HSP60 and *Pd*HSP60. However, we observed that anti-*Pd*HSP60 grouper antiserum could well recognize *Pd*HSP60 but poorly for *Vc*HSP60. It is interesting to identify the specific epitopes and investigate the selective immunogenic mechanisms of these highly conserved HSP60s. Here, we studied the multimeric state of HSP60s by gel filtration and small-angle X-ray scattering (SAXS). The results indicated that they both formed a large complex composed of 14 subunits. In addition, crystal structure of *Pd*Hsp60 and *Vc*Hsp60 were also determined at 3.7Å and 4.0Å, respectively. Both models showed the highly similar tertiary structure arranged in a double-stacked ring. Nevertheless, different surface charge distribution between these two HSP60s were observed, and the variance in the local surface charge may affect the immunogenic properties of HSP60s. Further investigation on the biochemical characteristics of HSP60s would be helpful to develop the fish vaccines against marine bacteria pathogen.

COMPARISONS OF AEROBIC RESPIRATION METABOLISM IN HEPATIC MITOCHONDRIAL BETWEEN FRESH WATR- AND SEAWATER-ACCLIMATED INDIAN MEDAKA (*Oryzias dancena*) UNDER LOW TEMPERATURE

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Temperature is considered a critical abiotic factor and important regulator of biological processes in all kinds of organism. The requirement for energy will change when animals were exposed to low-temperature environments. This study revealed the effects of low temperature (18°C) on the aerobic respiratory enzymes of mitochondria and mitochondrial biogenesis genes in fresh water (FW)- and seawater (SW)-acclimated medaka. In the mammalian model, mitochondrial biogenesis is defined as the growth and division of pre-existing mitochondria. Voltage-dependent anion-selective channel (VDAC) abundance can be defined as the changes in mitochondrial outer membrane and expressions of aerobic respiratory enzymes represented volume of mitochondrial contents. Our results showed that in both FW and SW groups, relative abundance of cytochrome c oxidase subunit I and IV related to mitochondrial electron transport chain (ETC) increased significantly after 24 hrs and continued the increasing trend in 168 hrs under low temperature. Being a rate-limiting enzyme in the tricarboxylic acid (TCA) cycle, the citrate synthase (CS) was not significantly different in the 18°C-exposed FW group, but increased significantly after 48 hrs 18°C-exposure in the SW group. In both FW and SW groups, VDAC expression was not significantly different under low temperature. In summary, mitochondrial outer membrane area of livers of FW and SW medaka was not changed under low temperature, but the protein expression of aerobic respiratory enzymes was increased to provide the energy required under low-temperature exposure.

STUDY ON THE EFFECTS OF DIFFERENT pH VALUES IN PRODUCTION OF BIOBUTANOL BY *Clostridium* sp. FROM *Kappaphycus* sp.

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The aim of this study is to investigate the effects of different pH values in production of biobutanol by *Clostridium* sp. from *Kappaphycus* sp. 5% (w/v) *Kappaphycus* sp. powder in 100 mL distilled water was treated with hot acid hydrolysis. Followed by hydrolysis of cellulases and 2.5% multiple crude enzymes from strains *Pseudomonas vesicularis* MA103 and *Aeromonas salmonicida* MAEF108. The pH value of *Kappaphycus* sp. hydrolysate is 5.44, which contained reducing sugar 30.23 ± 1.37 g and total sugar 54.15 ± 13.30 g. The changes of pH value, *Clostridium* count, and absorbance at 600 nm of strains *C. acetobutylicum* BCRC10639-ST-BT and *C. beijerinckii* BCRC17950-ST-BT are 4.87 and 5.22 (144 h), 2.0×10^8 and 4.9×10^9 (log cfu/mL) (24h), and 1.54 and 0.68 (24h), respectively (Table 1 & 2). Inoculating 10% strains *C. acetobutylicum* BCRC10639-ST(salt tolerant)-BT(butanol tolerant) (10^8 CFU/mL) and *C. beijerinckii* BCRC17950-ST-BT (10^8 CFU/mL) into *Kappaphycus* sp. hydrolysate with pH 5.5, 6.0, and 7.0 for acetone-butanol-ethanol (ABE) fermentation at 37°C. The butanol yield of fermentation solution with pH 5.5, 6.0, and 7.0 are 0.028%, 0.026%, and 0.027%, respectively, after fermentation 24 hr.

Table 1. Changes of pH value, titratable acid, TDS, salinity, total sugar and reducing sugar conc., *Clostridium* count and absorbance 600 nm during the *C. acetobutylicum* BCRC10639-ST-BT growth

Fermentation	pH value	Titratable acid	TDS (%)	Salinity (%)	Residual total sugar conc. (g/100 mL)	Residual reducing sugar conc. (g/100 mL)	<i>Clostridium</i> count (log cfu/mL)	Absorbance at 600 nm
Blank	6.52	0.4	1.027	35	11.57	6.40	0	0.40
24 h	4.95	1.00	1.025	33	4.56	1.05	2.0×10^8	1.54
48 h	4.90	1.00	1.025	32	2.70	0.59	1.1×10^{10}	2.17
72 h	4.78	1.10	1.025	33	3.95	0.91	1.7×10^7	1.34
96 h	4.87	1.40	1.025	33	3.80	0.70	5.2×10^7	1.69
120 h	4.89	1.30	1.025	32	3.13	0.63	2.2×10^8	1.81
144 h	4.87	1.00	1.024	31	5.13	0.69	1.7×10^6	2.11

Table 2. Changes of pH value, titratable acid, TDS, salinity, total sugar and reducing sugar conc., *Clostridium* count and absorbance 600 nm during the *C. beijerinckii* BCRC17950-ST-BT growth

Fermentation	pH value	Titratable acid	TDS (%)	Salinity (%)	Residual total sugar conc. (g/100 mL)	Residual reducing sugar conc. (g/100 mL)	<i>Clostridium</i> count (log cfu/mL)	Absorbance at 600 nm
Blank	6.91	0.40	1.027	35	9.20	4.02	0	0.40
24 h	5.11	0.70	1.026	35	2.43	0.76	4×10^9	0.68
48 h	5.21	0.70	1.026	35	2.24	0.74	1.5×10^9	1.48
72 h	5.21	0.90	1.026	35	2.05	0.75	8.0×10^6	1.40
96 h	5.27	0.70	1.026	35	2.71	0.71	3.2×10^6	1.32
120 h	5.24	0.80	1.027	35	2.03	0.67	1.6×10^6	1.33
144 h	5.22	0.70	1.026	35	2.54	0.72	7.5×10^4	1.32

COMPARISON OF CALCIUM BALANCING STRATEGIES DURING HYPOTHERMIC ACCLIMATION OF TILAPIA (*Oreochromis mossambicus*) AND GOLDFISH (*Carassius auratus*)

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We would like to submit our manuscript entitled “Comparison of calcium balancing strategies during hypothermic acclimation of tilapia (*Oreochromis mossambicus*) and goldfish (*Carassius auratus*)” by Tsung-Yu Han, Han Chuan Tsai, Wei-Fan Chen, Jay-Ron Lee and Pung-Pung Hwang, Fu-I Lu as a research report to *Frontiers in Zoology*.

Most teleosts are poikilotherms, meaning that their body temperature will fluctuate depending on the environment. Different fishes respond to cold stress by controlling whole body ion fluxes, but how this occurs is largely unknown. Here, we compare physiological and biochemical parameters related to Ca²⁺ absorption during cold acclimation of freshwater goldfish and tilapia (representative models of eurythermic and stenothermic species, respectively). From this analysis, we made several important discoveries:

(1) Compared to goldfish, tilapia showed a greater decrease in whole-body Ca²⁺ influx and Ca²⁺ content after acute cold exposure.

(2) Goldfish recovered from decreased whole-body Ca²⁺ flux by increasing influx rate after cold acclimation. However, this compensation was not detected in tilapia.

(3) The cold acclimation-associated increase in goldfish Ca²⁺ influx rate is evidenced by increased V_{max} and decreased K_m. Increased expression of PMCA and elevated Na⁺/K⁺-ATPase activity may contribute to this adaptation to cold.

The present study investigates the regulation of Ca²⁺ uptake at levels ranging from whole body physiology to molecular mechanisms. As such, we were able to discover key differences in cold acclimation strategies of teleost species. Therefore, we think that this study will be of broad interest to the readers of *Frontiers in Zoology*.

ENVIRONMENTAL SALINITY EFFECTS ON OLIGOPEPTIDE ABSORBING MECHANISMS IN INTESTINES OF THE SPOTTED GREEN PUFFER *Dichotomyctere nigroviridis*

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The intestine is the main organ for absorption of organic nutrients. Intestinal epithelia carry out the absorption through microvilli distributing over apical membrane of epithelial cells to enhance the ability of absorption by increasing the functional surfaces. Oligopeptide is the crucial form for protein absorption in fish. Oligopeptide absorption in intestines is accomplished by co-transport with proton gradient. The proton gradient in mammals is established by active pumping sodium into plasma through sodium- potassium pump (NKA), driving sodium-hydrogen exchanger (NHE) to secrete proton, and then co-transporting the oligopeptides into cytoplasm by peptide transporter 1 (PepT1). In many teleosts, vacuolar-type hydrogen-ATPase (VHA) is also thought to be an important active transporting pump in proton secretion. The purpose of this study is to investigate the changes in PepT1 expression and proton gradient pumping proteins related to oligopeptide absorption in the intestines of the euryhaline spotted green puffer (*Dichotomyctere nigroviridis*) when acclimated to fresh water, brackish water or seawater. Our results revealed that there were significant differences in gene and protein expression of oligopeptide absorbing mechanisms in the spotted green puffers. For protein expression, NKA, VHA, NHE, PepT1, and villin, the marker of microvilli, were significantly higher in intestines of freshwater and brackish water puffer. NKA activity showed significantly higher values in both freshwater and seawater groups rather than brackish water group. In summary, the oligopeptide absorbing mechanisms tend to be enhanced in freshwater environment, which may further reflects that the oligopeptide absorption of puffer is affected by environmental salinity.

PROGRANULIN A PROMOTES HEPATOCYTE PROLIFERATION BY MEDIATING HGF/C-MET SIGNALING IN ZEBRAFISH LIVER REGENERATION AFTER PARTIAL HEPATECTOMY

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Physiologic liver growth not only occurs in embryonic development, but also in the liver regeneration process that was activated after liver injury. According to our previous study, it was indicated that progranulin could modulate c-met, the receptor of hepatocyte growth factor, signaling to regulate liver outgrowth during development. However, the regulatory role of progranulin and the molecular mechanism involving in liver regeneration are still elusive. Therefore, the aim of this study attempts to clarify the regulatory role of progranulin that involves in liver regeneration. In this research, the liver regeneration model was established by partial hepatectomy in liver-specific fluorescent adult zebrafish. The result of Q-PCR showed that gene expression of progranulin, cell cycle and proliferation related genes were induced after partial hepatectomy. To study the function of progranulin A in liver regeneration, both loss-of-function *grnA* morphant and gain-of-function liver-specific *grnA* overexpressed transgenic zebrafish were analyzed after partial hepatectomy, respectively. In the *grnA* morphant, both HGFs and met expression were suppressed during liver regeneration. It leads to weak recovery with delayed cell cycle related gene and suppressed cell proliferation. Further, we combined the results of DNA microarray and previous studies, and showed that *Ets1* may participate in transcription of c-met. On the other side, the liver-specific *grnA* overexpressed transgenic zebrafish showed a faster recovery than wild-type zebrafish after partial hepatectomy. In conclusion, *grnA* mediates HGF/c-met signaling is required for hepatocyte proliferation during liver regeneration.

The zebrafish that was pretreated with PBS recover to the original liver size in 7 days (**Fig.1**), as it studied in zebrafish without any treatment. However, the zebrafish that was pretreated with vivo-*grnA*-Morpholino couldn't recover to original size until 7 days (**Fig.1**). The *grnA* morphant showed a weak recovery with less cell proliferation compared with control group.

The result showed that gene expression of hepatocyte growth factor receptor, c-met, which encodes a receptor tyrosine kinase, is known to be critical for hepatocyte proliferation in cell cycle G1/S in liver regeneration, cell cycle and cell proliferation related gene in *grnA* morphant was down-regulated remarkably after 36 hours after partial hepatectomy (**Fig.2**). In conclusion, we demonstrated that zebrafish *grnA* could participate in liver regeneration process through HGF/met signaling to modulate cell proliferation and cell cycle after partial hepatectomy.

Fig.1

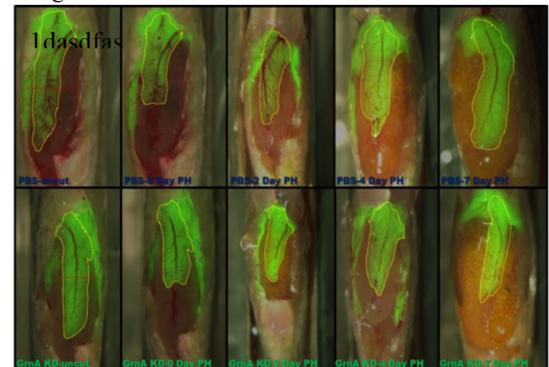
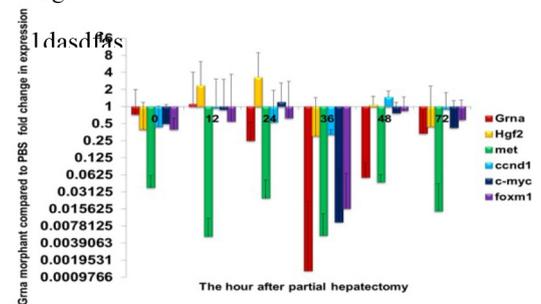


Fig.2



THE 3C STRATEGY : ANTIBIOTIC FREE HEALTH MANAGEMENT PRACTICES FOR SHRIMP FARMING IN THAILAND

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The “3C” health management program, making use of Clean seed, Clean water and Clean pond bottom, has been introduced in Thailand to help shrimp farmers to cope with an increasing number of important shrimp diseases i.e. WSSV, AHPND, EMS, WFS, EHP and ATM. These shrimp diseases are devastating and causing important economic losses to Thai shrimp farmers due to crop loss and/or increased production costs following low survival and growth retardation. Disease progression patterns vary. WSSV can whip out a complete farm in less than a week if the viral carriers/vectors such as crab are present in the farm system. On the other hand, AHPND/EMS and WFS/EHP exhibit medium to slow disease progression. Oral infection, cannibalism and cohabitation continue to be the most potential mode of infection for these diseases. The 3C strategy combined with completed Biosecurity system which comprised of a set of physical, chemical and biological precaution measures, aims at a sustainable shrimp production without the use of antibiotic.

Clean seed is the first priority in 3C which requires a very strict implementation to avoid the losses caused by contaminated seed. Only post larvae will be stocked have to pass through the strict diseases screening, QC control and properly stress test. An extension of the nursery phase at the grow out farms for around one month before transfer to the grow out pond is recommended. Typical nurseries consist of round HDPE tanks of up to 150-200 m³ with an adequate aeration, water exchange, central drain and waste removal system.

Clean water aims at reducing water contamination by precipitation solids in suspension (TSS) and dissolved organic carbon (DOC)/dissolved organic nitrogen (DON) which function as nutrients for pathogenic bacteria causing AHPND and serve as substrate for fixation of EHP spores. Adequate pond design is important, including sufficient reservoir ponds to hold the treated water, ready to use (RTU) ponds to keep clean and treated water in quantities sufficient for the entire crop cycle (70/30 reservoir/culture pond being the ideal ratio).

Clean pond and bottom concept is focused on removing as much as possible the accumulated waste in the pond, including sludge, feces, uneaten feed, death shrimp, exoskeletons and died-off plankton etc. This is achieved by using a shrimp toilet with central drain and sludge pumping machine, installed with an effective aeration position to concentrate the suspended waste in the center of the pond. The sludge is regularly removed and kept in a closed system sludge pond prior to sludge treatment and discharge.

Making shrimp to be strong by avoiding stressful conditions by farming at optimal densities with stable water quality parameters throughout the crop cycle will help promote the shrimp’s immune responses to combat diseases. Furthermore, promote shrimp health by suitable immunostimulant ,mineral and vitamin ,probiotic and functional feeds containing broad spectrum health promotors etc. will support the zero antibiotics regimen. The successful of the 3C and biosecurity concepts will depend on a good understanding of the practical implementation by shrimp farmers.

THE RELEVANCE OF MITOCHONDRIAL LINEAGES OF TAIWANESE CULTURED GREY MULLET *Mugil cephalus* TO COMMERCIAL PRODUCTS OF ROE

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Mugil cephalus is an important aquaculture species in Taiwan with highly valuable roe. In order to obtain its roe, mullet fry from various Taiwan estuaries are raised in aquaculture ponds until maturity. However, not all female mullets have developed ovaries. Therefore, we have attempted to use DNA profiling to aid selection of mullet fry for aquaculture. A large proportion of North coast mullet and some West coast mullet were identified as cytochrome oxidase subunit I (COI) mitochondrial lineage 1. COI mitochondrial lineage 2 was dominant in the west and east coast estuaries, whereas COI mitochondrial lineage 3 was fewest and only was present in Chang-hua county (middle west of Taiwan). The gonadosomatic index (GSI) of lineage 1 individuals ranged from 0 to 5 no matter where the mullet fry were captured. The GSI of both the west and east coast lineage 2 individuals ranged from 0 to over 15, but the GSI of lineage 2 of the I-lan (north east of Taiwan) population was generally lower than that of western populations. These findings suggest that a genetic difference whereby west coast lineage 2 mullet yield heavier roe even though the body size of lineage 1 individual is larger than that of lineage 2. Thus, lineage 2 individuals with their normal GSI distribution are the most economically viable. The application of the rapid screening of mitochondrial lineages is expected to help aquaculture farmers cultivate lineage 2 fry for roe production rather than lineage 1.

DEVELOPMENT OF NUCLEIC ACID IMMUNE STIMULANTS FOR AQUACULTURED FINFISH AND SHRIMP

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Infectious diseases are a major cause to the devastating economic loss in the finfish and shrimp aquaculture operations. An effective strategy against infectious diseases is to enhance the host immunity against the pathogens. CpG oligodeoxynucleotides (ODNs) are potent immune stimulants functioning via activating the Toll-like receptor 9 signaling pathway. Synthetic unmethylated CpG oligodeoxynucleotides (ODNs) have been widely used in mammals to stimulate innate immune response or to serve as vaccine adjuvant. We have developed a series of CpG ODNs for aquacultured finfish and shrimp. In both finfish and shrimp, these compounds can promote the functionality of immune cells, such as phagocytosis, respiratory burst and the synthesis of cytokines and antimicrobial substances. For fish, these compounds can also be used as adjuvant in combination with various forms of anti-viral vaccines to promote the efficacy of the vaccines. In addition, these nucleic acid stimulants can be delivered into the animals via the route of injection, or ingestion of feed additive, demonstrating the potential application of the compounds in commercial feed additives and vaccines.

EXPERIMENTAL ASSESSMENT OF THE EFFECTS OF SALINITIES ON GROWTH PERFORMANCE, FEED INTAKE AND PHYSIOLOGICAL STRESS RESPONSES IN ASIAN SEABASS (*Lates calcarifer*)

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Salinity is one of the environmental factors that strongly affects cellular and organismal physiological functions of teleost. For Asian seabass (*Lates calcarifer*), a worldwide aquaculture species, study on the effects of ambient salinities on growth performance, feed intake and physiological stress responses would be helpful to depict an appropriate range of salinity for aquaculture operation. Therefore, a 4-week experiment with four salinity treatments that are fresh water, 10, 20 and 30‰ was conducted. After long-term exposure, specific growth rate (SGR), total feed intake, feed conversion ratio (FCR), oxygen consumption rate, plasma glucose, liver glycogen, heat shock protein 70 and osmoregulatory responses at organismal and molecular levels will be studied in juveniles Asian seabass. The findings of the present study would be able to provide the science-based information to suggest the proper salinity range to maintain optimal growth rate and alleviate osmotic stress for culturing Asian seabass (*Lates calcarifer*).

CAN CRUSTACEANS SYNTHESIS LONG-CHAIN POLYUNSATURATED FATTY ACIDS? RECENT EVIDENCE FROM MOLECULAR CHARACTERISATION OF BRACHYURAN ELONGASES

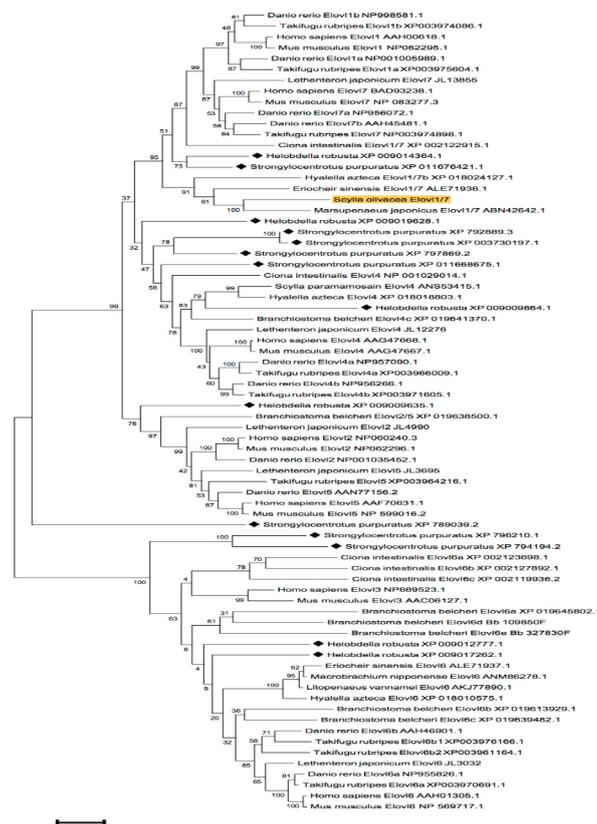
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Long-chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) are critical for energy, membrane integrity and gene regulation. Multi-cellular organisms obtain LC-PUFA from diet or through bioconversion from shorter chain PUFA. While considerable progress has been made in understanding LC-PUFA biosynthesis in a large number of aquaculture teleost species, there is a paucity of data from crustaceans. Insights from molecular cloning of enzymes critical for biosynthesis of LC-PUFA from different species are needed to correlate with fatty acid nutritional studies.

We describe here the cloning and functional characterisation of elongases (Elovl) from *Scylla olivacea*, an important commercial mud crab species in the South China Sea and Indo-Pacific region. One of the clones (Elovl 1/7-like), showed the capacity to elongate monounsaturated fatty acids *in vitro*, albeit at lower activities. Phylogenetic tree also indicates the presence of four major groups of Elovl families in crustaceans, which are further divided into two well supported clades. These molecular evidences will be discussed in relation to the role of brachyurans' success in occupying diversified terrestrial and aquatic habitats. The functional activities of *S. olivacea* Elovl's will also be highlighted together with recent orthologs from other crab species to postulate the LC-PUFA biosynthesis pathway in crustaceans. The significance of the putative pathway to improvement of crustacean fatty acid nutrition for farming will also be discussed.

Figure 1: Phylogenetic tree of *Scylla olivacea* Elovl1/7-like elongase.



CHARACTERIZATION OF MUCOSAL IMMUNITY RESPONSE THROUGH IMMUNOGLOBULIN T (IGT) IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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The orange-spotted grouper is a commercial marine fish that is widely farmed in Asia. However, the nervous necrosis virus (NNV) classified to nodavirus threatens larvae and juveniles groupers with high levels of mortality. When virus infects at surfaces such as the mucosa, the mucosal immune defense mechanisms will elicit immune regulation. However, mucosal immunity is known less so far in orange-spotted grouper. To investigate the adaptive immune system in the mucosal-associated lymphoid tissues (MALTs), the main immunoglobulin (Ig), IgT, is cloned for observing locally immunological responses.

Heavy chains of *osgIgT* was first cloned from orange-spotted groupers. The cDNA for *osgIgT* is provided with an open reading frame of 1,641 nucleotides encoded polypeptides 547 amino acid and a predicted molecular weight of 61 kDa. Compared with other vertebrates, *osgIgT* has the highly conserved heavy chain domains and intra-chain S-S bonds are similarly location. Using qPCR to measure the expression of *OsgIgT* and *osgIgM* transcript in groupers. Fish larvae were exposed under NNV treatment, the level of *osgIgT* significantly increased in MALTs, highlighting the importance of *osgIgT* in mucosal immune response. In conclusion, the result provides that a molecular characterization of *osgIgT* which points out its possible role playing in mucosal immunity of orange-spotted grouper.

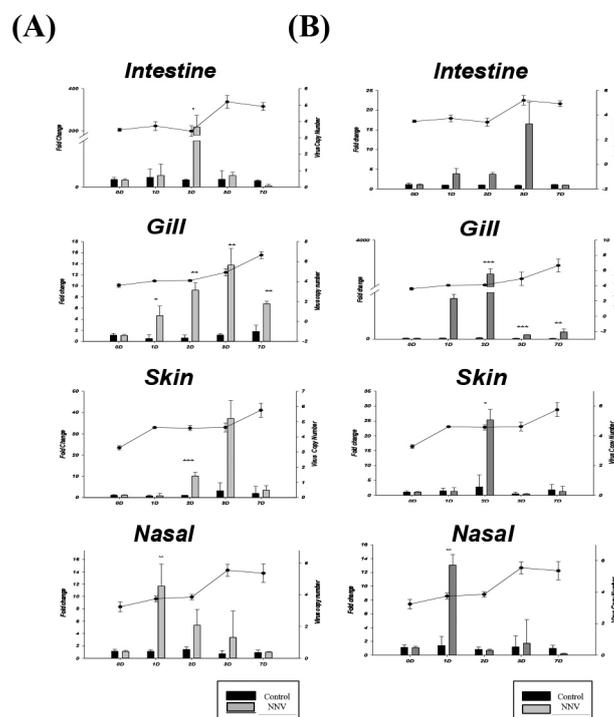


FIGURE 1. Effect of NNV challenge on expression of (A) *osgIgT* and (B) *osgIgM* in main mucosal-related organs.

THE EFFECTS OF DIETARY FISH MEAL REPLACED WITH DIFFERENT DIETARY PROTEIN SOURCES ON THE GROWTH PERFORMANCES AND BODY COMPOSITION OF ROCKY PORGY *Oplegnathus fasciatus*

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A feeding experiment was conducted to study the effects of dietary different protein sources on the growth, muscle composition of rock porgy, *Oplegnathus fasciatus*. Four kinds of supplemental protein source, Peruvian fish meal (PFM), Taiwan fish meal (TFM), poultry meal (PM) and meat and bone meal (MBM) were tested in experiment diet. All experiment diets were design isonitrogenous (crude protein 45%) and isolipidic (crude lipid 10%). Each diet was randomly allocated to triplicate groups of fish in aquarium (60×30×45 cm), and each aquarium was stocked with 10 fish (initial average weight of 10.85 g). Fish were fed three daily (09:00, 15:00 and 21:00) to apparent satiation for six weeks and cleaning aquarium every 2 weeks interval. Fish were maintained in a recirculation seawater system and water temperature and salinity were kept from 24 to 26 C and 33 to 35 ppt, respectively. The rock porgy fed diets containing MBM had the worst weight gain. The rock porgy fed diets containing TFM had the highest weight gain among treatments. There was no significantly different in survival among treatment groups and survival ranged from 90 to 100%. Feed conversion ratio (FCR) of rock porgy fed diets containing TFM was lower than that of rock porgy fed other treatment diets. The hepatosomatic index (HSI) of rock porgy fed diets containing PMF was lower than that of rock porgy fed other treatment diets. The specific growth rate (SGR) of rock porgy fed diets containing TFM was highest, and significantly higher than that of rock porgy fed BMB treatment. In this study, diet protein source was TFM had highest weight gain and showed no adverse effect on growth performance of rock porgy. Therefore, it can be suggested that TFM is the optimum protein source for rock porgy diet.

DEVELOPMENT OF A HIGH DENSITY, CONTINUOUS ROTIFER PRODUCTION SYSTEM USING WATER CHANGE TO CONTROL AMMONIA

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The bottleneck of ammonia control in the high density rotifer production is solved by using a novel self-cleaning rotating drum water changer to perform fast and safe water changes. Two culture trials were conducted with *B. plicatilis* stocked in a 100 L glass aquarium tank. The rotifers were fed with concentrated freshwater chlorella *Chlorella vulgaris* in 12 hour intervals. Water was changed 1 or 2 times before feeding to control ammonia concentration to below 10 ppm, each time changing 60% of the culture water in 9 minutes. In Trial 1, the rotifer densities reached 3,000 rotifers/ml in 5 days, maintained for 13 days, then reached 6,000 rotifers/ml and maintained for 6 days. In Trial 2, the rotifer densities reached 6,000 rotifers/ml in 8 days and maintained for 7 days (Fig. 1). A daily harvest of 20% of the culture volume when the rotifer density reached 6,000 rotifers/ml results in an average harvesting rates of 131 and 133 million rotifers/day, respectively, which are sufficient for a general size grouper hatchery in Taiwan. The results demonstrated that a simple, water change-based culture system that used no recirculation equipment or chemical compound to treat the water can reliably produce rotifers at high densities on a daily basis while minimizing food loss.

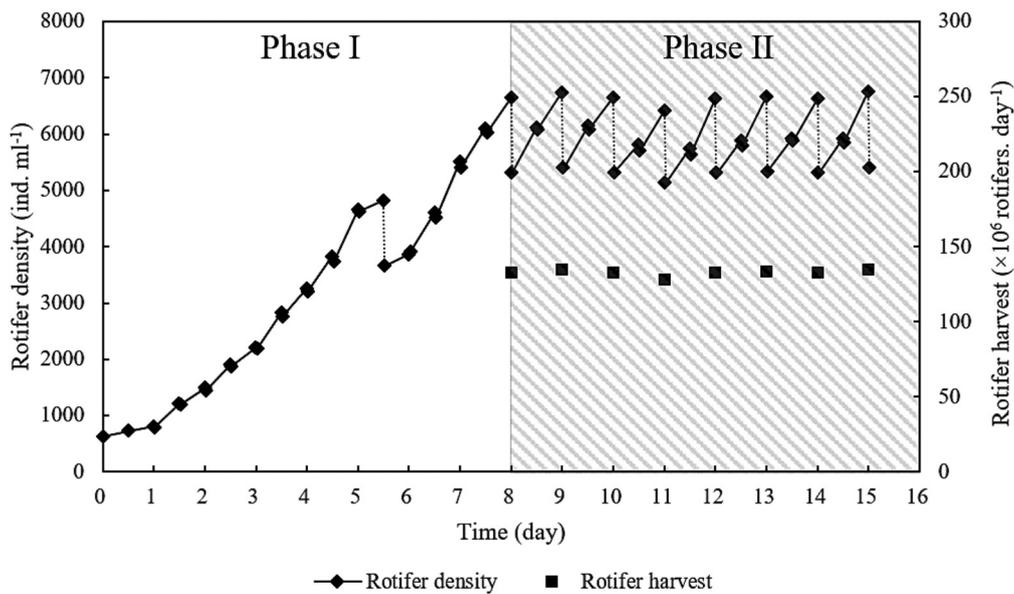


Fig. 1. Results of rotifer density and harvest in Trial 2.

DETECTION KITS DESIGNED FOR DISEASES IN AQUACULTURE

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Aquaculture has emerged as one of the most promising enterprise due to the increasing demand of animal-based proteins with quality and food security. The major problem in aquaculture industry is the occurrence of infectious bacterial diseases, like acute heptaopancreatic necrosis (AHPND) and edwardsiellosis. AHPND was officially reported in China, the disease signs associated with mortality typically appear within 20 to 30d after stocking ponds with post-larvae. *Edwardsiella tarda* is the causative agent of edwardsiellosis that causes serious economic losses in the global aquaculture industry, especially in cultured eel. Therefore, quick and easy detection kits are critical for the disease diagnosis and control.

In our study, a lateral flow assay (LFA) combined with colloidal gold and antibodies was developed to detect AHPND/diarrhea and edwardsiellosis. The antigen of AHPND is a fragment of ToxA sequence from a gene database which was reported in previous studies. The antigen of diarrhea used in this study is a fragment of TLH which is a major toxin of *Vibrio parahaemolyticus* (ATCC-17802™) to result in diarrhea of human being. The minimum limitations of LFA sensitivity for ToxA and TLH were 1.0×10^4 colony forming units (CFU) and 1.0×10^6 CFU (figure 1), on the other hand, the minimum limitations of rapid immune colloidal gold strip sensitivity for *E. tarda* were 1.2×10^5 CFU (figure 2), separately. In the field trials, the results are also consistent with Real-time PCR.

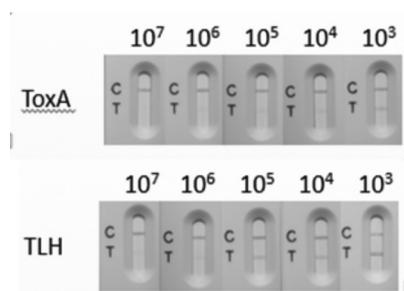


Figure 1. Sensitivity assay of ToxA and TLH competing colloidal gold rapid

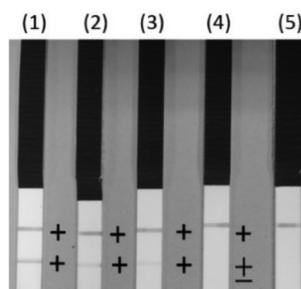


Figure 2. Sensitivity assay of *E. tarda* colloidal gold rapid diagnosis kit. The *V. parahaemolyticus* suspension was serially diluted at 10-fold from 1.2×10^8 CFU/ml to 1.2×10^4 CFU/ml in buffer and was tested by using the strips.

DIFFERENTIAL EXPRESSION AND LOCALIZATION OF ROMKS IN GILLS AND KIDNEYS OF SEAWATER- AND FRESH WATER-ACCLIMATED EURYHALINE MILKFISH, *Chanos chanos*

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Potassium is a major monovalent cation in vertebrate animals and plays an important role in cellular function and homeostasis. Renal outer medullary potassium channel (ROMK, also called KCNJ1 or Kir1.1), is a crucial protein in potassium regulation and is responsible for ion reabsorption and potassium excretion in mammalian kidneys. Recently, ROMK was identified in apical membrane of ionocytes in the zebrafish, tilapia, and Japanese medaka and was suggested to be involved in potassium secretion. In this study, we aimed to investigate differential expression of ROMK in gills and kidneys of the milkfish acclimated to different salinity environments and hope to illustrate the osmoregulatory role of ROMK in milkfish. We first found partial sequences of ROMKa and ROMKb from the transcriptome database of milkfish. Tissue distribution analysis revealed that both *kcnj1a* (the gene name of ROMKa) and *kcnj1b* (the gene name of ROMKb) were widely expressed in detected organs. The expression of branchial *kcnj1a* was more abundant than *kcnj1b* and increased after transferred to SW in 12 hr. The expression of renal *kcnj1a* in the FW-group was significantly higher than in the SW-group and renal *kcnj1b* expressed in both SW- and FW-groups. Furthermore, the protein abundance of ROMKs in gills and kidneys was higher in the SW-group than the FW-group. By whole-mount immunostaining, we first found that ROMKs were colocalized with NKA in the basolateral membrane of ionocytes and renal epidermal cells. Salinity-dependent expression and localization of ionocytes in gills suggested the ionoregulatory role of ROMK for hyperosmotic acclimation in gills and kidneys of the milkfish. Further studies will deeply investigate the functions of ROMKs in osmoregulation of the euryhaline milkfish.

COMPARISON OF GROWTH PERFORMANCE OF OLIVE FLOUNDER *Paralichthys olivaceus* USING PROBIOTICS, PREBIOTICS AND TAURINE AS FEED ADDITIVES

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As aquaculture develops rapidly, its production is also increasing, leading to the higher need for higher production as well as method for disease prevention is becoming highly demanding. Feed additive is considered as a promising way for cost reduction, growth efficiency, and immunity enhancement. Microbiological feed additives include probiotics and prebiotics. Probiotics are used as a way to improve the health and immune system of fish and also increase their growth performance. Prebiotics provide a nutrient for long-term maintenance of the probiotics, and this reinforced probiotics help to inhibit harmful bacteria and enhance the growth fish. Besides, Taurine is also an essential element of fish by supporting fish growth. It is also an important factor to control inflammation and the immune system. Thus, this study aimed to compare the growth performance of olive flounder by feeding with probiotics, prebiotics, and taurine.

There were four different treatment of feeds were used in this study including treatment one (control diet), treatment 2 (taurine diet, 1%) treatment 3 (probiotic diet using 10^8 CFU/g of *Lactococcus lactis* supsp. *lactis* strain WFLU12), and treatment 4 (prebiotic diet, 1% Fructooligosaccharide (FOS) and 10^7 CFU/g of *Lactococcus lactis* supsp. *lactis* strain WFLU12). The fish were fed 2-3% of the total body weight per day. Body weight and total length were measured at the beginning and at 4 weeks and then every 2 weeks. Survival rate was measured at the end of the experiment.

The result showed that treatment 3 with probiotics had the highest value in growth rate and survival rate. The second highest growth rate after feeding trial was treatment 2, but fish in this treatment showed significant difference in size within group. However, growth rate was lowest in treatment supplemented with prebiotics fed section, so the result for prebiotics supplementing would be confirmed through re-experiments in the coming time.

SEAWEED SOLUTION AND CARBON ZERO SEAWEED TOWN (CØST) WITH ANAMAM

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It is the time to go beyond sustainable development to achieve low carbon green growth. We propose to build a net zero carbon emission community with seaweed-based mitigation and adaptation measures. Here we declared the initiation of the Wando Carbon Zero Seaweed Town Project (hereafter CØST) during the 2nd Wando Seaweed Expo (2017) at the Wando Province, the Home of Seaweeds in Korea. As the Paris Agreement adopted the Nationally Determined Contributions (NDC), which gives opinions of each country with a bottom-up goal setting method and a policy progress report every five years, a new additional paradigm in the NDC business in the ocean. Under the Wando CØST we could manage a carbon neutral community by maximizing the ecosystem services provided by the Blue Carbon and Seaweed Aquaculture Beds (SABs), and with other renewable ocean energy options as solutions of the NDC option for all seaweed-loving countries. Seaweed Solution and CØST provide sustainable mitigation and adaptation measures in the context of climate change and create the new green jobs and revenues. The Asian Network of Using Algae as Mitigation and Adaptation Measure (ANAMAM) would promote the international coalition.

A FIELD-DEPLOYABLE PCR SYSTEM FOR RAPID AND SENSITIVE DETECTION OF EMERGING SHRIMP HEMOCYTE IRIDESCENT VIRUS

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Shrimp hemocyte iridescent virus (SHIV) infection in *Litopenaeus vannamei* grow-out leads to symptoms including slight loss of color, empty stomach and guts, soft shells, slightly reddish bodies and black legs, and massive die-offs. In China, the disease was first reported in Zhejiang in 2017 and has spread south to Fujian and Guangdong with potential to reach Hainan soon, posing serious threats to the aquaculture industry. Polymerase chain reaction (PCR) for pathogen detection is a powerful tool in bio-security to improve overall shrimp production. The field-deployable PCR system, POCKIT™ (GeneReach), with easy manual or automatic nucleic acid extraction methods, generates simple qualitative results from sample within one hour, providing a cost-effective and user-friendly tool for timely pathogen detection at points of need. Various PCR assays on this system are available for important shrimp pathogens, including SHIV) and covert mortality nodavirus (CMNV).

Here we evaluated the performance of the SHIV RT-PCR/POCKIT™ system. Sensitivity comparison study using 10-fold serial dilutions ($10^1 - 10^9$ folds) of a SHIV-positive sample showed that the index RT-PCR and a reference nested RT-PCR (Qiu *et al.*, 2017) had comparable sensitivity, with detection endpoints at 10^5 and 10^4 dilution, respectively. The index assay did not react with the non-targeted shrimp pathogens, including *Vibrio parahaemolyticus* (acute hepatopancreatic necrosis disease/ mortality syndrome), white spot syndrome virus, infectious hypodermal and hematopoietic necrosis virus and *Enterocytozoon hepatopenaei*. Performance of the RT-PCR/POCKIT™ system to detect SHIV in shrimp was compared to the nested RT-PCR with 28 PL samples. Nucleic acids extracted by TIANamp Marine Animals DNA Kit (Tiangen Biotech) were tested side by side by the two RT-PCR methods. Nineteen and nine samples were positive and negative with both RT-PCR/POCKIT™ and nested RT-PCR methods (Table 1). The excellent agreement (100%) found between the two methods suggests that the field-deployable RT-PCR system had performance comparable to that of the laboratory method.

Taken together, this field-deployable RT-PCR/POCKIT™ system can be useful to allow timely SHIV detection in the aquaculture industry, facilitating shrimp disease management and control.

TABLE 1. SHIV detection in PL: Comparison between the nested RT-PCR and RT-PCR/POCKIT™ assays

		SHIV Nested PCR		
		Positive	Negative	Total
IQ plus SHIV Kit	Positive	19	0	19
	Negative	0	9	9
	Total	19	9	28

OPTIMISING LIVE FEED ENRICHMENT FOR LARVICULTURE

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The supply of sufficient numbers of high quality fingerlings is a constraint to the commercial production of several marine finfish species. Providing larvae of some fish species with a diet that meets their nutritional requirements and includes essential dietary components is a challenge when working with traditional rotifer and *Artemia* live feeds. Without appropriate nutrition, larvae may have low survival rates, issues with swimbladder inflation, manifest abnormal behaviour, develop skeletal abnormalities, or experience long-term impacts on digestive function. Along with water quality and the tank environment, high quality diets are a critical contributor to the efficient production of high quality fingerlings.

Different approaches are employed to identify the optimal diets for larvae. As a benchmark, most formulations begin with the biochemical composition either of eggs from wild broodstock or of the natural diet of larvae, that is largely comprised of copepods. Enrichments are formulated for rotifers and *Artemia* that may contain graded levels of a nutrient to be assessed or a combination of different nutrients. Most studies measure the content of the nutrient being investigated in the live feed immediately after enrichment, then in the larvae at different ages, and analyse the effect of the nutrient levels with larval performance. Yet, the actual nutrient content of the enriched live feeds will change over time in a larviculture tank as the nutrients are metabolised.

This study will provide an example of the change in lipid and vitamin profile over time when live feeds are transferred to clearwater or greenwater (containing microalgae) larviculture systems. If live feeds are added more than once each day, and uneaten live feeds are not removed from the larviculture tank, there will be a mixture of recently enriched and 'old' (poorly enriched) prey items. The actual level of nutrient that the larvae receive will be an average of the content of the live feeds ingested over time.

This change in nutrient levels has implications for applying research results to optimise enrichments for commercial larviculture. The actual nutrient level or enrichment composition determined as optimal in an experiment is highly dependent on the larviculture operating parameters (e.g. density of greenwater, microalgae species, flow rate, temperature, larval density, prey density). The nutrient content in larval tissue can be measured to explore causal relationships with performance. However, the nutrient contents of the enrichment and the live feeds should be interpreted cautiously. Translation of optimal nutrient levels from experiment data requires validation in commercial hatcheries where the operating parameters are often substantially different to those in research facilities.

EARLY MORTALITY SYNDROME, NO MORE: PROBIOTIC FROM TILAPIA GREEN WATER, *Pseudomonas luteola*, INHIBITS GUT COLONIZATION, PREVENTS *Vibrio parahaemolyticus* INFECTION AND ENHANCED GROWTH PERFORMANCE OF *Penaeus monodon*

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The significant decline in global shrimp production due to Early Mortality Syndrome disease outbreak has caused panic among shrimp growers and becomes a pressing concern requiring a rapid, practical and applicable solution. In an attempt to elucidate the *Vibrio* inhibitory activity of Tilapia green water, we have isolated *Pseudomonas luteola*, exhibiting potent secreted antibiotic effects against *Vibrio parahaemolyticus*. In this report, we tested *P. luteola* pathogenicity against *P. monodon* and its efficacy to protect the shrimp against *Vibrio parahaemolyticus* infection. To test for pathogenicity, shrimp were exposed to increasing levels of *P. luteola* () and mortality recorded daily for two weeks. To test the efficacy of this probiotic against *V. parahaemolyticus* infection. Two groups of post larvae were tested with one group received diets supplemented with *P. luteola* at 10^9 cfu/g and the other group received no probiotic. Following a month of feeding, the shrimp were subjected to *V. parahaemolyticus* infection challenge test. Our results indicate that *P. luteola* is not pathogenic to shrimp, no mortalities were observed in all treatment groups. The feeding trial indicates that shrimp in the treated group exhibited higher survival and improved growth performance than the control group. The improved growth in the treated group is associated with a low gut content of *V. parahaemolyticus* indicating the active exclusion of this pathogen in the gut. The infection challenge test with pathogenic *V. parahaemolyticus* administered via oral route indicates that the group receiving the probiotic has significantly higher survival than the control group. Lower counts of *V. parahaemolyticus* in the gut of the probiotic treated group were also recorded. Collectively our results indicate that application of probiotic *P. luteola* is an effective, practical and applicable means to prevent *V. parahaemolyticus* infection in *P. monodon* culture.

BIOTECHNOLOGY-ENHANCED COPRA MEAL (PECM™) AS A PROTEIN SOURCE IN THE DIET OF JUVENILE PACIFIC WHITE SHRIMP, *Penaeus vannamei*

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Declining fish meal supply, the rising cost and the erratic availability of feed grade soybean, due to intense usage competition among the animal growing industries, are issues threatening the economic viability of aquaculture that raises global concerns. Biotechnology, specifically solid state fermentation, may provide a solution by bio-converting agricultural biomass to a high-protein, highly digestible feed material. However, the practical applicability of this technology to the feed industry, remains not fully realized. In the present work, we evaluated the nutritional and feed value of high protein fermented copra meal (PECM™) to replace soybean in the diet of juvenile *Penaeus vannamei*. Four experimental diets containing graded levels of PECM™ replacing 0 (%), 25 (%), 50 (%) and 75 (%) by weight of soybean meal were formulated and fed to four triplicate groups of juvenile *Penaeus vannamei* for 60 days. Our analyses showed that PECM™ contained 40.90 (%) crude protein with 3.54 (%) lipid and 36.83 (%) of carbohydrates. Apparent digestibility coefficient (ADMD) of 82.97 (%) was determined for the dry matter. We found that this feed ingredient has an essential amino acid index (EAAI) of 97 and an amino acid chemical score index (ACSI) of 79 with tryptophan as the most limiting amino acid. Replacement of soybean meal with PECM™ at all levels did not affect the overall growth performance but a declining trend is observed at the highest inclusion level. Survival, feed conversion and feed intake were similar in all dietary treatments. No significant effects on protein retention were observed but lipid retention exhibited declining trend with significant decrease observed at the highest replacement level. Collectively these results suggest that fermented copra meal could replace 75 (%) of soybean meal in the diet of *P. vannamei* without affecting survival, feed conversion and overall growth performance. This work provides evidence that the use of biotechnology-produced feed material (fermented copra meal) is feasible, practical and offers a sustainable approach to meet the growing demand of feed protein sources for *P. vannamei* aquaculture.

RELATIONSHIPS BETWEEN ENVIRONMENTAL FACTORS AND ABUNDANCE OF *Vibrio parahaemolyticus* IN THE INTENSIVE TANK-BASED CULTURE OF *Penaeus vannamei*

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Disease outbreaks caused by some species of bacteria belonging to Vibrionaceae strains are prevalent in shrimp aquaculture. Recently, a strain of this *Vibrio parahaemolyticus* was identified as the causative agent of early mortality syndrome or AHPND which devastated shrimp farms in many parts of Asia. *V. parahaemolyticus* was identified to be one of the dominant *Vibrio* species frequently isolated in aquaculture farms. In the present study, different water quality indicators in the intensive tank-based culture of *Penaeus vannamei* were monitored and their relationship with the occurrence of *V. parahaemolyticus* was evaluated. Three shrimp culture techniques were evaluated, i.e. clear water, tilapia green water, and biofloc technology. In all culture techniques, the abundance of *V. parahaemolyticus* were found to be negatively correlated with dissolved oxygen and plankton count and positively with concentrations of nitrite, phosphorus, and settleable solids. Changes in temperature, total ammonia nitrogen, carbohydrate concentration, alkalinity and pH of the water did not have significant relationship with the *Vibrio* sp. density.

PROTEIN-ENRICHED COPRA MEAL (PECM) AS A PARTIAL REPLACEMENT OF SOYBEAN MEAL IN THE DIET OF WHITE LEG SHRIMP, *Penaeus vannamei* AND MILKFISH, *Chanos chanos*

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Copra meal is a byproduct of the coconut oil industry that has a low feed value due to its low protein content, high fiber, gritty appearance and coarse texture. A breakthrough technology was developed, which involves solid state fermentation and utilizes food grade fungi to enhance the protein of carbohydrate rich biomass. This process has enriched copra meal protein from 12% to 45% protein, comparable to that of the soybean meal. The present study was conducted to evaluate the potential of protein enriched copra meal as partial replacement of soybean in the diet of white leg shrimp and milkfish

A 60-day laboratory growth trial was conducted using thirty post larvae *P. vannamei* weighing 0.03 ± 0.003 g, and 0.04 ± 0.00 g milkfish larvae randomly stocked in a 50-L plastic aquaria equipped with aeration in a closed recirculating system. Animals were fed with respective formulated diets containing different levels of PECM; Diet 1 (control) 0%, Diet 2 25%, Diet 3 50%, Diet 4 75% soybean replacement. Thereafter, the best concentration was tested against a commercially available shrimp feeds and milkfish feeds in a large scale growth out system.

Results showed that PECM inclusion did not affect the survival rate of shrimps at all dietary inclusion levels, lowest final abw was recorded in Diet 4 (75% soybean replacement) for both species (Table 1 & 2). Results on a large scale outdoor trial using 50% soybean replacement against commercially available feed showed no significant difference on the final abw, feed utilization and production for both species (Table 3 & 4). These suggests the PECM could replace 50% of soybean meal thereby reducing the dependency in soybean meal and reduction in feed cost.

TABLE 1. Growth performance of *P. vannamei* fed with increasing levels of PECM

Indicators	PECM inclusion level			
	Diet1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet 4 (75%)
FABW (g)	0.96	1.23	0.91	0.87
SGR	5.70	5.73	5.64	5.37
Survival (%)	72.22	82.22	81.11	83.34
FCR	2.02	1.83	1.82	1.70

TABLE 2. Growth performance of *Chanos chanos* fed with increasing levels of PECM

Indicators	PECM inclusion level			
	Diet1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet 4 (75%)
FABW (g)	0.13	0.16	0.22	0.12
SGR	3.0	3.35	4.11	2.74
Survival (%)	35.83	30.83	50.73	40.0
FCR	3.11	3.18	1.98	2.8

TABLE 3. Growth performance of *P. vannamei* fed with commercial diet and formulated feed with PECM after 80 days culture in a 70 m³ concrete tanks

Indicator	Treatment	
	Commercial Feed	50% PECM
FABW (g)	22.09	21.67
Pdtn (ton/ha)	3.77	3.60
SGR	2.89	2.71
% Recovery	85.41	83.70
FCR	1.59	1.80

TABLE 4. Growth performance of *Chanos chanos* fed with commercial diet and formulated feed with PECM after 100 days culture in a 500 m² pond

Indicator	Treatment	
	Commercial Feed	50% PECM
FABW (g)	378.29	452.29
Pdtn (ton/ha)	2.55	2.98
SGR	2.18	2.36
% Recovery	95.29	94.29
FCR	1.82	1.53

OPTIMUM DOSE AND APPLICATION FREQUENCY OF DIETARY PEPTIDOGLYCAN IS VITAL TO PROTECT JUVENILE *Penaeus vannamei* AGAINST EARLY MORTALITY SYNDROME-CAUSING *Vibrio parahaemolyticus* AND WHITE SPOT SYNDROME VIRUS INFECTIONS

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Vibrio parahaemolyticus, causative agent of Early Mortality Syndrome (EMS) and the White Spot Syndrome Virus are two dreaded infectious agents linked to severe economic losses in the global industrial shrimp production. The pressing needs to develop a solution to these problems has led us to evaluate the optimum dose and frequency of peptidoglycan (PGN) supplementation to enhance the resistance of *Penaeus vannamei* to WSSV and *V. parahaemolyticus* infections. Shrimp were fed diets containing increasing supplementation levels of peptidoglycan (0.1%, 0.2% and 0.4% of diet) given at two feeding frequencies (daily and once every three days) for 35 days. Following the feeding trial, shrimp were subjected to *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV) infection challenge. The immune responses associated with the treatments including total hemocyte count, Phenoloxidase, respiratory burst and serum antibacterial activities were quantified. Our results indicate that immune indices including serum antibacterial activity and survival after challenge with *V. parahaemolyticus* and WSSV are influenced with the interaction of Peptidoglycan dose and application frequency. Shrimp fed on diets containing 0.2(%) PGN supplemented once every three days had higher survival upon challenged with *V. parahaemolyticus* and WSSV ($P < 0.05$). The significant enhancement of resistance against these infectious agents was supported with elevated immune indices i.e. total haemocytes count, phenoloxidase activity, hemocyte superoxide anion production and serum antibacterial activity. Collectively, our findings indicate that dietary supplementation of PGN at 0.2% fed to shrimp once every three days could effectively enhance the immune system of *P. vannamei* and enhance their resistance against *V. parahaemolyticus* and WSSV infections. This dietary supplementation is a practical approach to lower the risk of EMS and WSSV infection in *P. vannamei* aquaculture.

WAVE POWERED AIRLIFT PUMP

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The invention (patent pending) relates to the field of devices aimed to harness wave energy (WEC) especially for artificial upwelling, forced downwelling, production of compressed air.

In its basic form, the pump consists of a hydro-pneumatic machine, driven by wave energy, characterised by the fact that it has no moving mechanical parts, and is made up of only two structural components: an hollow body, which is open at the bottom to the sea and partially immersed in sea water, and a tube, both joined together to form a single body.

The shape of the hollow body is like a mushroom whose cap and stem are hollow; the stem is open at both ends and the lower part of its surface is crossed by holes; the tube is external and coaxial to the stem and is joined to it so as to form a single body.

This shape of the hollow body and the type of connection to the tube allows the pump to operate simultaneously as an air compressor (OWC) on the cap side, and as an airlift on the stem side.

The pump can be implemented in four versions, each of which provides different variants and methods of implementation:

1. firstly, for the artificial upwelling of cold, deep ocean water;
2. secondly, for the lifting and transfer of these waters to the place of use (above all, fish farming plants), even if kilometres away;
3. thirdly, for the forced downwelling of surface sea water;
4. fourthly, for the forced downwelling of surface water, its oxygenation, and the simultaneous production of compressed air.

The transfer of the deep water or the downwelling of the raised surface water (as for pump versions indicated in points 2 and 3 above), is obtained by making the water raised by the airlift flow into the upper inlet of another pipe, internal or adjoined to the airlift; the downwelling of raised surface water, oxygenation, and the simultaneous production of compressed air (as for the pump version indicated in point 4), is obtained by installing a venturi tube on the upper end of the pipe, whose restricted section is connected to the external atmosphere, so that it also operates like a hydraulic air compressor (trompe).

Furthermore, by combining one or more pumps for the upwelling of cold, deep water, with one or more pumps for the downwelling of the warm surface water, the system can be used in an OTEC plant to supply the cold and the warm water required for the operation of the same, thus allowing to use, without increased costs, in addition to the mechanical energy of the waves, for the purposes indicated in points 1 to 4, the thermal one of the marine water treated in the process.

YNSECT PRODUCTION FOR THE AQUACULTURE SECTOR: GROWTH PERFORMANCES

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In 2015, Ynsect demonstrated the high quality and performance of its blockbuster product TMPTM (*Tenebrio molitor* defatted protein meal) in juvenile rainbow trouts in comparison with a super prime fish meal 70 LT (+34% weight gain and -15% FCR after 90 days). Unpublished trials (for confidentiality reasons) on poultry and mice also showed significant impact on growth, well-being and the health of these animals.

The company aims to diversify its market targets in many species and different regions. The white leg shrimp (*L. vannamei*) is one of the main species in aquaculture, of which total production amounts to 3.7 million tons per year (FAO 2014) and where the largest producers are China, Thailand, Vietnam and Indonesia. Ynsect launched a new trial with Kasetsart University (Bangkok, Thailand) on juvenile shrimps in 2016. The control diet contains 25% fish meal (FM) and a total of five different diets with increasing rates of inclusion of TMPTM as a replacement for the FM, which were designed with iso-nutritive contents. No significant difference was assessed in the palatability test between the diets. The T5 diet (100% FM replacement by TMPTM) increased weight gain by 21% and final body weight by 12.4% after 8 weeks of feeding, but the best results were found for the 10.3% TMPTM inclusion in the diet (50% FM replacement): an increase by 33.7% in weight gain and by 24% in final body weight after 8 weeks of feeding. The FCR decreased significantly by up to 25%. The apparent digestibility of proteins and lipids was above 97.4%.

IMMUNO-STIMULANT PROPERTIES

A challenge test was performed with a frequent pathogen in aquaculture (*Vibrio parahaemolyticus*), responsible for the well-known Early Mortality Syndrome (EMS). After 10 days, the survival rate reached 90% in the diet with 50% FM replacement by TMPTM compared to 56.7% in the control diet. Mortality could be observed directly from 5% TMPTM inclusion. The mortality was divided by up to 4, which is due to the patented bacteriostatic effect of TMPTM and the constant increase of the phenol oxidase activity (up to +400% in the diet with 100% FM replacement by TMPTM). Since the shrimp does not have an acquired immune system, this immuno-stimulant property is very promising.



Figure 1 : Evolution of the weight gain over time

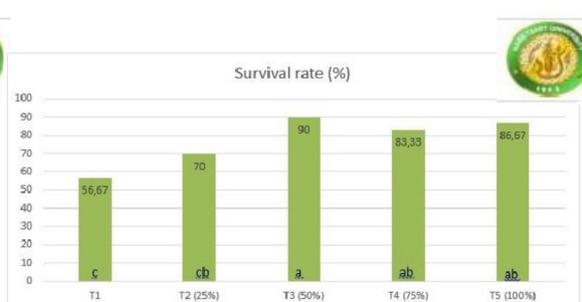


Figure 2 : Survival rate after 10 days after injection of the pathogen

ECOLOGICAL & BIOLOGICAL CONDITIONS OF MARICULTURE PARK IN SUAL, PANGASINAN IN CONNECTION WITH MILKFISH MORTALITIES IN CAGES

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Sual mariculture park is one of the major cage culture production sites of milkfish (*Chanos chanos*) in the Philippines. In the recent years, milkfish mortalities had become a normal incidence in the area. This study was conducted to determine the connection between these fish kill incidences with the current ecological conditions of mariculture park. Physiological (temperature, total dissolved solids, ocean current), and chemical (pH, dissolved oxygen, salinity, nitrite, phosphate, ammonia, organic and inorganic matters) parameters were examined in five sampling stations within the area. Also, fish hematological data, as well as microbial load (Total and Fecal Coliform levels) was examined to see the current status of the fish cultured in the site.

Results of the water and soil quality assessment revealed that the data obtained are already within the limits of the ideal range with an indication of deterioration particularly on the dissolved oxygen, pH, ammonia, organic and inorganic matter. Hematological analyses revealed no difference on white and red blood cells counts compared to control fish taken from ponds. However, fish in cages were observed to have lower hematocrit ($P=0.008$) and hemoglobin ($P=0.008$). Analysis also revealed a high correlation of soil inorganic matter to the lymphocytes and a correlation between ammonia and lymphocytes. Furthermore, microbial profile was found to be within the prescribed standard.

Evaluation of the current ecological conditions of Sual's Mariculture Park suggests that the area is becomingly loaded with high organic matters as a result of current aquaculture practices and activities that generally affect the environment and the fish being cultured. It is highly recommended to address the pressing issues on balancing the protection of the environment with aquaculture activities to reduce fish mortalities for sustainable aquaculture operation.

Parameters	Sampling Sites					Mean
	Site 1	Site 2	Site 3	Site 4	Site 5	
I. Water						
Water pH	9.57±0.21 ^a	9.17±0.11 ^b	9.14±0.08 ^b	9.14±0.12 ^b	8.94±0.09 ^c	9.19**
Temperature	30.08±0.16 ^b	29.95±0.14 ^b	29.97±0.13 ^b	30.16±0.49 ^b	30.64±0.84 ^a	30.16*
DO	7.01±4.17 ^a	4.21±1.51 ^b	4.64±1.77 ^{ab}	5.64±2.18 ^{ab}	3.78±2.70 ^b	5.06
TDS	29.37 ±1.25	29.50±0.99	29.35±1.09	29.42±1.85	29.73±0.73	29.47
Salinity	30.28±3.20	31.72±1.26	31.52±1.42	28.98±7.02	31.56±1.14	30.81
Nitrite	0.02±0.01 ^a	0.01±0.01 ^{ab}	0.01±0.00 ^{ab}	0.01±0.01 ^b	0.01±0.01 ^{ab}	0.01*
Phosphate	0.49±0.19	0.41±0.17	0.45±0.12	0.36±0.12	0.47±0.09	0.43
Ammonia	0.01±0.02	0.01±0.01	0.02±0.02	0.00±0.00	0.01±0.02	0.01
II. Soil						
Soil pH	7.11±0.22 ^a	7.87±0.30 ^{ab}	8.06±0.22 ^{ab}	8.91±0.86 ^b	8.05 ±0.04 ^{ab}	8.01**
Organic matter	12.51±2.84 ^a	20.27±6.43 ^b	7.96±1.72 ^a	5.51±0.52 ^a	8.62±3.35 ^a	10.31**
Inorganic matter	27.65±9.71 ^a	29.33 ±8.95 ^a	13.01±1.70 ^a	10.64±0.67 ^a	37.03 ±16.00 ^a	23.12*

PHYTOGENIC FEED ADDITIVE Anta®Ox FlavoSyn IMPROVES HARVEST VOLUMES OF RED NILE TILAPIA *Oreochromis Niloticus* DUE TO HIGHER SURVIVAL

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Improving the survival of red Nile tilapia, *Oreochromis Niloticus*, measured by mortality, is an important factor to maximize production. Over the last 6 years, Dr. Eckel Animal Nutrition has conducted several trials in order to test different combinations, of additives used in aqua feed, to find an optimal combination with effects to improve aquaculture production. Here, the feed additive manufacturer uses its own test facility for fish and crustaceans in Germany. The goal of this study was to determine the effect of the phytogenic feed additive Anta®Ox FlavoSyn, on performance and survival rate, in high standard healthy environments.

A 49 days feeding trial was conducted in our in-house research facility in Niederzissen, Germany. 8 tanks (45 liter water volume, 0.18m² water surface per tank) of the recirculation system were stocked with *O. Niloticus* with mean body weight of 0.39g (SD=0.03) at a density of 50 fish/tank (277 fish/m², 0.43 kg/m³). Water temperature was maintained at 27°C ±1°C. Application of the product was tested against a negative control diet and each diet had 4 replicates. The inclusion level of the additive mix was 2 kg/t. The additive mix was added on top of the negative control diet powder mix. Afterwards the mash was pelleted. Final body weight and survival rate were recorded at the end of the trial and the feed conversion ratio (FCR) and average weekly gain were calculated.

After 49 days, fish grew to a mean body weight of 2.15g (SD=0.14). The product group achieved a significant better total harvest than the negative control diet (Figure 1). Overall survival was higher than 90% in both groups. However, the product group reached 94%, 2% higher than the control (Figure 2). The FCR was not significantly different in the control group compared to treatment. We can conclude from this trial that the treatment with the phytogenic additive Anta®Ox FlavoSyn improves total harvest of tilapia also in high standard conditions.

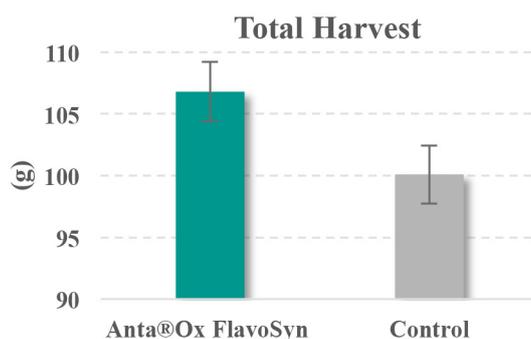


Figure 1 Final total harvest of Red Nile Tilapia after 49 days

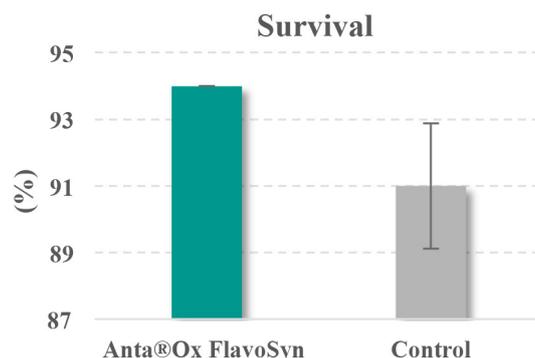


Figure 2 Survival rate at the end of the trial

MORPHO-BEHAVIORAL AND HEMATO-IMMUNOLOGICAL RESPONSES OF VACCINATED PROACTIVE AND REACTIVE NILE TILAPIA (*Oreochromis niloticus*) SUBJECTED TO HANDLING STRESS

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The study was conducted to evaluate morpho-behavioral and hemato-immunological responses of vaccinated proactive and reactive Nile tilapia subjected to handling stress and determined which stress-coping styles had better responses. Proactive individuals are usually described as the bold and aggressive ones in response to stress while reactive are characterized as shy and subordinate individuals. FaST strain of Nile tilapia were screened according to stress-coping style whether proactive and reactive using eye color pattern (ECP) values. Post-vaccination of formalin-killed *Aeromonas hydrophila* and application of handling stress was administered prior to the commencement of the study. Result in terms of morpho-behavioral responses revealed that proactive individuals had better ECP than reactive individuals. Eye darkening that signifies higher degree of stress was significantly higher among reactive individuals than proactive individuals. In terms of ventilation rate (VR), comparable result was recorded through time. Hemato-immunological responses suggest that the immunological function of WBC and lymphocytes was activated even handling stress was present as it appeared to have increasing trend and obtained comparable values among stressed and unstressed fish. RBC components seems to be affected by the increasing level of WBC and tend to decreased as time progressed. Immune response through hemaagglutination test revealed that all treatments had positive agglutination. However, proactive individuals had better immunity in *Aeromonas hydrophila* than reactive individuals as significantly higher agglutination titer was notable among proactive individuals. The study concluded that vaccinated proactive Nile tilapia performed better as a stress-coping style in response to handling stress and had better immune stimulation as tested with *Aeromonas hydrophila*.

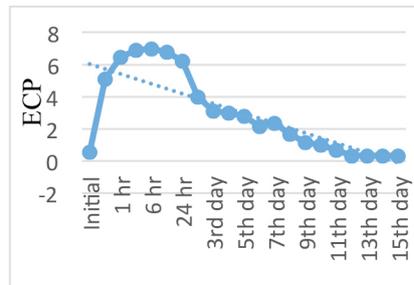
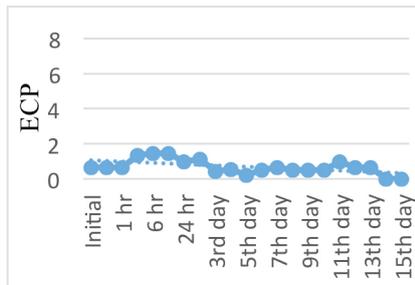


Figure 1. ECP trend of proactive individuals as time progressed

Figure 2. ECP trend of reactive individuals as time progressed

Figure 3. Comparison of agglutination titer between proactive and reactive

EFFECTS OF VARYING LEVELS OF HORSERADISH (*Moringa oleifera*) LEAF MEAL ON THE GROWTH AND SURVIVAL OF RED NILE TILAPIA (*Oreochromis niloticus* L.)

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Reduction of feed cost is one of the challenge that aquaculture production faces nowadays as most of expenses are allotted for feed provision among cultured species. It is considerably one of the effective way to attained better production outcome is to reduce the level or substitute feed ingredients that are usually expensive to those ingredients that are cheaper without neglecting its effectiveness as source of nutrition. The study was conducted to evaluate the effectiveness of incorporating *Moringa oleifera* leaf meal on the growth performance of red Nile tilapia. It also aimed to evaluate varying levels of *Moringa oleifera* leaf meal and identify what level of incorporation will provide best result in terms growth and survival of red Nile tilapia. The treatments evaluated were: Treatment I – control diet (0% *Moringa oleifera* leaf meal); Treatment II - diet with 10% *Moringa oleifera* leaf meal; Treatment III - diet with 15% *Moringa oleifera* leaf meal and Treatment IV – diet with 20% *Moringa oleifera* leaf meal. Result of proximate analysis revealed that experimental diets contain an acceptable levels of crude protein ranging from 29.84% to 31.63%. Result of the study showed that *Moringa oleifera* leaf meal had comparable growth and survival with the fish fed with control diet. The fish fed with experimental diet of 20% *Moringa oleifera* performed better than the fish fed with diets of 10% and 15% *Moringa oleifera* leaf meal. The study concluded that utilization of up to 20% *Moringa oleifera* level of incorporation on the diet of red Nile tilapia can provide acceptable growth performance.

Parameters	Treatment I	Treatment II	Treatment III	Treatment IV
Final weight (g)	23.12 ^a (±1.196)	17.71 ^a (±1.609)	18.94 ^a (±1.617)	21.83 ^a (±1.867)
Body weight gain (g)	19.23 ^a (±1.303)	13.98 ^a (±1.72)	15.04 ^a (±1.513)	18.08 ^a (±1.074)
Specific growth rate (%)	1.82 ^a (±0.087)	1.58 ^a (±0.128)	1.6 ^a (±0.057)	1.79 ^a (±0.048)
Absolute growth rate (g/day)	0.2 ^a (±0.023)	0.14 ^a (±0.03)	0.15 ^a (±0.027)	0.19 ^a (±0.019)
Survival rate (%)	83.33 ^a (±14.240)	88.33 ^a (±1.667)	91.67 ^a (±4.410)	86.67 ^a (±1.667)

Growth performance (± standard deviation) of red tilapia after 97 days of rearing

KEY POINTS OF CONTROLLING THE FISH BODY COLORALATION

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Fish body colour is very important for ornamental fish and other commercial fish species. But recently the body colour changes have been more frequently observed in many farmed fish species. The colouration of the marine fish, Japanese flounder *Paralichthys olivaceus*, red snappers *Lutianus spp* and large mouth sea bass *Micropterus salmoides* may change to white or black from natural body colour. Some scaly freshwater fish, black carp, grass carp, common carp and black tilapia often show abnormal white, black or yellow colouration. The biggest worry for freshwater catfish farmers is a banana colouration in *Clarias fuscus* and *Pelteobagrus fulvidraco* and totally white or yellow colouration in *Ictalurus punctatus*, *Ameiurus nebulosus*, *Silurus asotus* and *Leiocassis longirostris*. For the fish fillet processing factories, it is an issue to get tilapia or pangasius fellet with red, pink or yellow color. Fish with abnormal skin or fillet colouration may fetch lower market prices, reducing profit margins of farmers.

This presentation discusses the physiological basis for fish colour change and the reasons leading to changes in fish body colouration, summarizes some key efficient ways to control fish body colour through nutrition.

THE USE OF MICROBIAL PREPARATIONS TO SUPPORT CULTURES OF COPEPODS FOR USE AS LIVE FEEDS

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Copepods have nutritional and behavioural characteristics which makes them an excellent type of live prey for fish larvae with a wide range of mouth gape sizes. The calanoid *Acartia tonsa* has been used successfully for increasing survival of many species including cold water fish such as cod as well as tropical water fish such as Pompano. The harpacticoid *Tisbrintra* sp has also been used successfully in the breeding of milkfish and while harpacticoids are considered difficult to use because of their benthic drawler behaviour, feeding techniques have been developed to circumvent this issue.

Over a set of experiments, we have used heat inactivated bacteria to improve the vital rates of *Acartia tonsa* cultured on fresh phytoplankton with success. The growth rates, egg production rates and hatching rates were increased using this approach and therefore opens future opportunity to increase the production of eggs from copepod culture systems. These eggs can be stored and used for later use. We also applied preparation of probiotics (Live bacteria) to evaluate the possibility of increasing the productivity of harpacticoids (*Tisbrintra* sp.) in culture when fed live phytoplankton. The approach was also successful but the overall productivity was still limited by the obligatory cohort successions in cultures. Separation of life stages could be implemented to provide optimal production of this species.

Overall, the results from both these experiments support that the use of microbial preparation (live or inactivated) is a way to boost copepod cultures and therefore support the inclusion of such live feeds in commercial larval rearing.

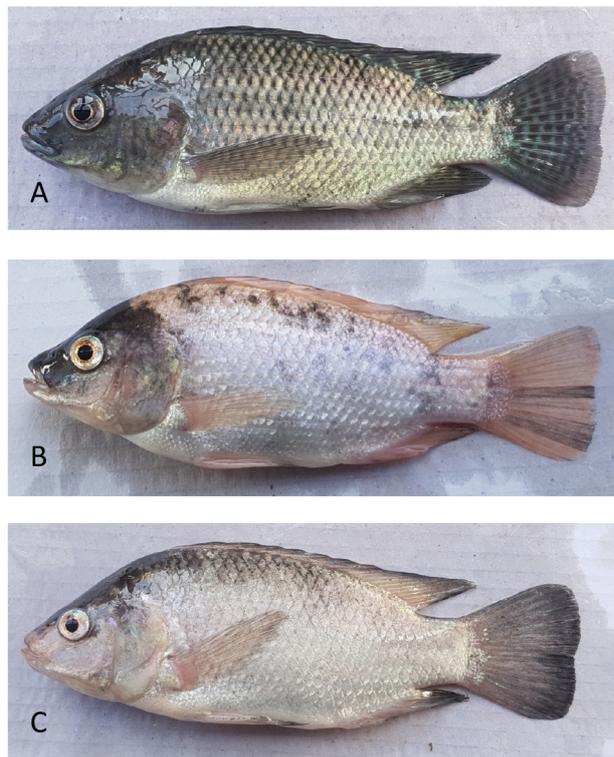
COMPLETE AQUACULTURE OF NEW SALINITY-TOLERANT TILAPIA STAIN

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It is believed that tilapia developed from a marine teleost ancestry. The salt-tolerance limits of different tilapia species vary considerably, and there are even individual differences in each species. In order to enhance the salt-tolerance with the best breeding effect of tilapia, the rapid-growing red tilapia TsRn strain and the marine tilapia TFS strain from *Oreochromis spilurus* were used for reciprocal cross experiments. (TFS♀×TsRn♂) F₁ could be successfully produced fry with TFS (TFS♀×TFS♂), and the salinity tolerance of F₁ is equivalent to TFS. The body color variability of F₁ (TFS♀×TsRn♂) has age differences: 1-month-old fries could be divided into two groups by the body color, black and red; In 1- to 2- month-old fries, the red group was divided into red with block black and brown; 3-month-old black and red with a block black had not changed, but the body color of brown group turned into red background with black point at 3-month-old and all three body color types were fixed. Overall F₁ body color was divided into three groups: black, red with block black and red with black points, and the ratio is $7.26 \pm 3.89\%$, $47.92 \pm 6.59\%$ and $44.82 \pm 7.30\%$, separately. The results showed that the inheritance of salt-tolerance was decisively influenced by TFS, and the inheritance of body color was influenced by parents.

Fig.1 4 month-old individuals of TFS and F₁ (TFS♀×TsRn♂). The body color of TFS was black (A), and F₁ (B and C) was red with different levels of black spots. The color of sire (TsRn) is red and dam (TFS) is black.



THE PROTECTIVE ROLE OF SOME MARINE EXTRACTS AGAINST XENOBIOTICS USING ZEBRAFISH (*Danio rerio*) EMBRYOS

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Bioassays play a basic role in different aspects of scientific approaches. Meanwhile, bioassays with zebrafish - an established model organism in different research area s- are increasingly being utilized in effect direct analysis. Aiming to contribute for the optimal application of zebrafish bioassays in effect direct analysis.

Over the recent decades, zebrafish bioassays have guided effect direct analysis of natural products and environmental samples. A vast majority of studies performed bioassays with embryos and early larvae, which allowed small-scale experimental setups. Furthermore, biotesting applied zebrafish methods in both screening phase as well as for further investigations. The present study was realized to investigate the protective role of extracts of some marine algae against the chlorinated paraffin toxicity and its metabolites.

Confirming that, the action of chlorinated paraffin on zebrafish (*Danio rerio*) embryos were assayed. Embryos were subjected to different doses of chlorinated paraffins to study the effect of such class of chemicals on the fish embryogenesis during the first 120 hours of fertilization. General morphological abnormalities and teratogenicity score were analyzed.

EFFECTS OF DIETARY PROTEIN HYDROLYSATE SUPPLEMENTATION ON GROWTH PERFORMANCE, FEED UTILIZATION, DIGESTIBILITY, INTESTINAL MORPHOLOGY, INNATE IMMUNITY AND DISEASE RESISTANCE OF RYED SEA BREAM (*pagrus major*)

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The effects of inclusion of protein hydrolysate products were evaluated in diets for red seabream (*Pagrus major*). A FM-based diet was regarded as a high FM diet (HFM) and a diet containing soy protein concentrate (SPC) as a substitute for FM at replacement level of 44.5% was considered as low-FM diet (LFM). Five other experimental diets (ED1, ED2, ED3, ED4 and ED5) were prepared by dietary inclusion of 5% hydrolysate products (krill meal, tilapia hydrolysate powder, tilapia hydrolysate liquid, shrimp hydrolysate powder and shrimp hydrolysate liquid, respectively) to the LFM diet in exchange of FM.

Triplicate group of fish (8.5g) were fed one of the experimental diets twice a day until satiation for 15 weeks. At the end of the feeding trial, fish fed HFM, ED1, ED4 and ED5 diets obtained significantly higher FBW and WG compared to fish fed LFM diet. FCR was significantly declined in HFM, ED4 and ED5 groups compared to others and significantly higher PER was observed in ED5 group. Significantly higher dry matter and protein digestibility were observed in HFM and all the hydrolysates supplemented diet groups compared to those of LFM group. Hemoglobin level, SOD activity and NBT activity of fish fed HFM ED1, ED2, ED3, ED4 and ED5 diets were significantly increased than the LFM group. Significantly higher intestinal diameter was observed in fish fed ED5 diet than that in fish fed LFM diet. Significantly lower expression levels of liver IGF-1 mRNA was observed by LFM diet. After challenge with *Edwardsiella tarda*, higher survival rates were observed in fish group fed ED4 and ED5 diets and the lowest survival rates were observed by LFM and ED1 diet groups. The findings of this study show that the protein hydrolysates can be used as a supplements or FM replacer in diets for red seabream. Among the hydrolysates, shrimp hydrolysate liquid form was the best.

Table 1. Growth performance and feed utilization of red sea bream fed the experimental diets for 15 weeks

	Experimental diets						
	HFM	LFM	ED1	ED2	ED3	ED4	ED5
Initial body weight (g)	8.48±0.06	8.53±0.06	8.45±0.07	8.47±0.08	8.40±0.09	8.53±0.12	8.38±0.04
Final body weight (g)	65±2.55 ^{cd}	52.9±1.68 ^{ab}	63.2±3.51 ^{cd}	58.1±1.56 ^{abc}	59.9±0.62 ^{bcd}	67.3±1.69 ^d	76.3±4.79 ^e
Weight gain (%)	666±29.4 ^{cd}	520±15.6 ^{ab}	649±47.9 ^{cd}	587±14.1 ^{abc}	613±11.1 ^{bcd}	689±30 ^d	810±60.7 ^e
Feed intake	91.3±3.94	93.1±0.91	97.6±5.48	90.3±1.87	93.5±8.48	95.1±6.46	103±6.22
Feed conversion ratio	1.60±0.08 ^a	2.01±0.05 ^b	1.74±0.14 ^{ab}	1.78±0.09 ^{ab}	1.79±0.15 ^{ab}	1.61±0.10 ^a	1.51±0.05 ^a
Protein efficiency ratio	1.33±0.07 ^a	1.06±0.03 ^a	1.19±0.09 ^{ab}	1.17±0.06 ^{ab}	1.17±0.09 ^{ab}	1.31±0.09 ^a	1.39±0.05 ^b
Survival (%)	90.0±5.77	84.4±1.92	84.4±5.09	90.0±3.33	88.9±5.09	88.9±7.70	90.0±3.33

EFFECTS OF LIGHT SOURCES AND PHOTOPERIODS ON GROWTH OF DUCKWEED *Landoltia punctata* AND ITS WATER QUALITY

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Duckweed has been considered a good source of dietary plant protein for fish due to its high nutritive value, up to 40% crude protein; fast growing, 8 days a generation; easy to harvest, overflow collection and other favorable production features. To have expected output, programmable production and to control its quality, it has been culture indoors. Therefore, this experiment was to find out the optimal combination of light source and photoperiod of duckweed *Landoltia punctata*.

A factorial arrangement of nine (9) treatments, three (3) light sources (T5, LW and LB) x three (3) photoperiods (L:D-12:12, 16:8 and 24:00) were assigned to 27 experimental units. Each experimental container (14.5 cm x 10.5 x 7.5 cm) filled with 900 ml water and inoculated with 0.2 g duckweed. Light intensity of all treatments set to 110 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD. Duckweeds was cultured until 16 days. Monitoring duckweed growth and water quality parameters was at 4-d interval.

The results showed that overall SGR of duckweeds reached about 13%/day for 16 days' culture. Both light source and photoperiod had no effect on SGR and on water quality. However, their interaction had significant ($p \leq 0.05$) effects on duckweed's growth and also water quality. An empirical model involving NO_3^- , TAN and PO_4^{3-} was successfully constructed with high R^2 (0.9945). Nitrate was the most important one in influencing weight gain about more than 75 %. Practical applications of this model can be used in any aquatic plants reared in controlled environment to identify which nutrients is the most sensitive for growth

Empirical model determined from the stepwise linear multiple regression:

Weight Increment (WI) = 0.01108 N^2 + 0.02031 TN + 0.31660 NO_3^- - 0.97552 TAN + 0.03529 T^2 + 0.25656 P^2 ($R^2 = 0.9944$)

Table 1. The main effect of various light sources, photoperiods and their interaction on the specific growth rate (\pm standard error) of *Landoltia punctata* at 16 days culture (n=3).

Parameters	Specific growth rate (%/day)
Light source (L)	
LED white (LW)	13.0 \pm 0.14 ^a
Fluorescent white (T5)	13.0 \pm 0.14 ^a
LED Blue light (LB)	13.0 \pm 0.14 ^a
Photoperiod ¹ (P)	
12:12	13.1 \pm 0.12 ^a
16:08	12.9 \pm 0.11 ^a
24:00	12.8 \pm 0.16 ^a
Interaction	
L \times P	0.0083

ALTERNATIVE TO CONTROL VIBRIO LOAD AND AHPND/EMS IN SHRIMP FARMING

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One of the main threats for shrimp farmers are pathogens, bacteria and specially *vibrios* spp. which are ubiquitous can be a real nightmare. Fluctuation on their concentration varies with salinity, temperature, density, water management, neighbouring conditions, feed, etc. Many factors have to be consider to maintain the best environment and conditions in the production and some are external which are impossible to control. However, one of the most important action is to be ready to prevent any coming risk instead of trying of cure when mortalities or slow growth is seen. Phytobiotics or phytochemicals can modulate gut flora reducing vibrio load in gut and hepatopancreas, therefore improving health and zootechnical parameters. Phytobiotics are plant-derived products used in animal feeding, compounds found botanicals, essential oils and organic acids which together can act as immunoboosters, antioxidants, prebiotics, growth enhancers and antimicrobial.

Several experiments done with a phytobiotic made with organic acids, plants and essential oils in Kasetsart University showed positive results in growth, survival, vibrio counts in intestine and when making a challenge with virulent *Vibrio parahaemolyticus* causing AHPND/EMS to Specific Pathogen Free Pacific white shrimp. This *vibrio* was added into each tank at the dose of 10^5 CFU/ml.

After 7 days challenged trial, shrimp in the group fed with Phytobiotic showed the highest survival rate and the lowest total number of *Vibrio* spp, *Vibrio* green colony and *Vibrio* yellow colony. Both treatments showed significant differences (Table 1).

Prevention, through gut health, is a key factor that has to be taken into deep consideration to control disease outbreaks and reduce the use of chemicals or antibiotics. If using phytobiotics at early stage (PL10) farmer start combating and preventing from later *vibrios* outbreaks, such as AHPND/EMS. The inclusion of the phytobiotic in the diet has a clear effect in the total *vibrio* spp (green and yellow colonies) counts, reducing its number progressively along the days of culture. This has a direct relation with survival rate in the production.

Table 1. The average survival rate and total number of *Vibrio* spp. (green and yellow colony) ($\times 10^5$ CFU/g) after challenging with *V. parahaemolyticus* causing EMS for 7 days.

Treatment	Survival rate	Total number of <i>Vibrio</i> spp.		
		Total ($\times 10^5$ CFU/g)	Yellow colony ($\times 10^2$ CFU/g)	Green colony ($\times 10^5$ CFU/g)
Control	67.78 \pm 1.92 ^a	1.06 \pm 0.05 ^a	5.67 \pm 0.58 ^a	1.05 \pm 0.05 ^a
Phytobiotic 3	84.44 \pm 1.92 ^b	0.37 \pm 0.13 ^b	2.67 \pm 0.58 ^b	0.37 \pm 0.13 ^b

ALL-FEMALE SHRIMP CULTURE – PERFORMANCE DATA IN A COMMERCIAL SETTING

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Mono-sex culture is arguably the next big revolution of almost every farmed animal. Breeders of hens, cattle, swine, turkey and fish, are all racing to develop mono-sex technologies. In Shrimp culture this is not different. In most shrimp species, freshwater or marine, all-female presents a superior strategy over the traditionally used mixed culture, enabling superior performance of individuals and more importantly, affecting the overall performance of the population.

In most shrimp species, there is a clear sexual dimorphism: growing both sexes together inhibits the growth potential of females, enables undesired social interaction and reproduction related behavior, and suppresses growth and feed conversion. Territorial and dominant behavior of the males in some species, limits intensification of culture conditions, while adding labor costs for grading and selective harvest. Thus, a revolutionary technology enabling all-female culture was called for.

As of 2017, such a technology is finally commercially available. Developed by biotechnology company Enzootic, it taps into the natural sex differentiation mechanism of shrimp and harnesses their unique sexual plasticity to realize the goal of all-female culture without the use of hormones, chemicals or genetic modification. This novel technology was published in peer-reviewed scientific journals and is protected by several patent applications.

The technology, which can be tailored to almost any decapod crustacean, was first applied to freshwater shrimp *M. rosenbergii*, where social-reproductive morphotypes of the dominant males affect the efficient and intensive farming of this highly desired shrimp. Commercial production of all-female *M. rosenbergii* culture is now a reality based on unique super-female broodstock supplied by Enzootic, which will produce all-female progeny when mated with any male.

A large-scale field study performed in collaboration with Ben Gurion University in Israel, demonstrated the performance superiority of all-female in every parameter tested. When compared to mixed population under traditional stocking densities, we've observed up to 36%(!) increase in the total crop with outstanding uniformity and average weight of 40 grams (Fig 1). When higher stocking densities were tested, the socially passive all-female populations, presented clear advantages in intensified conditions, opening the way to a modern, more efficient industry, with dramatically improved economics.

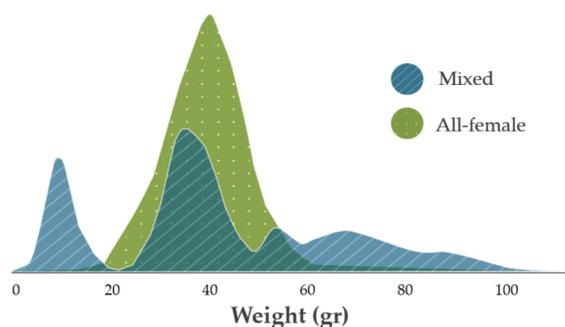


Figure 1. The unifying effect of culturing all-female compared to mixed populations, as demonstrated in freshwater shrimp

MOLECULAR INSIGHTS INTO IMMUNE RESPONSE OF ALLOGRAFT INFLAMMATORY FACTOR 1 FROM MULLET *Liza haematocheilus*

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Allograft inflammatory factor 1 (AIF-1) plays a pivotal role in innate immune regulation upon pathogen infection, tissue injuries and other infectious exogenous agents. In the present study, *in silico* analysis, tissue specific transcription, and immune modulatory transcription of mullet, *Liza haematocheilus* AIF-1 (MuAIF-1) were documented. Putative open reading frame encoded 147 amino acids with 17 kDa polypeptide and a *pI* of 5.2. The 5' un-translated region was 63 bp where 3' un-translated region extended 330 bp. Characteristic EF-hand calcium-binding domain profile was observed at ⁴³QKLEMFKKKYMFDLNDKGDIDMMGLKRMLEKLGLAK⁷⁹. Moreover, several Ca²⁺ binding sites were observed at ⁵⁶Asp, ⁵⁸Asn, ⁶⁰Lys, ⁶⁷Gly, ⁹²Ala, ⁹⁴Gly, ⁹⁶Ser, and ¹⁰⁴Asp. It shared highest sequence identity (90%) with *Poecilia mexicana* AIF-1 like followed by *Poecilia formosa* AIF-1 like (90%) orthologs. Multiple sequence alignment revealed the conservation of functionally active EF-hand calcium-binding domain and Ca²⁺ binding sites among the other AIF-1 counterparts. Phylogenetic analysis revealed the common ancestral origin of MuAIF-1 where cladded within the fish AIF-1 group. Healthy mullets showed prominent transcription of *MuAIF-1* in spleen with ubiquitous gene expression in all the other examined tissues including, gill, intestine, stomach, head kidney, muscle, heart, kidney, brain, blood, skin, and liver. *Lactococcus garvieae* is a well-known Gram positive bacterial pathogen to mullet, where its infection increases the *MuAIF-1* transcription in spleen after 48 h post injection. Moreover, lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (poly I:C) injection showed significant up-regulation of *MuAIF-1* transcripts but lower than to the *L. garvieae* infection. Hence, present outcomes illustrate that the MuAIF-1 is belonging to the teleostean AIF-1. Potential immune responses upon bacterial and viral challenges suggest that *MuAIF-1* could increase the innate immune responses and crucial member of mullet immune defense system.

FATE OF [³H]-DEOXYNIVALENOL IN RAINBOW TROUT (*Oncorhynchus mykiss*) JUVENILES: TISSUE DISTRIBUTION AND EXCRETION

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Deoxynivalenol (DON), a *Fusarium* mycotoxin, is one of the most prevalent mycotoxins found in aquafeeds (Gonçalves *et al.* 2016, Gonçalves *et al.* 2017). The toxicokinetics of DON are rarely studied in aquatic species, hence the lack of development of detoxifying methodologies for DON. The present study used juvenile rainbow trout (*Oncorhynchus mykiss*) with a mean initial body weight of 7.72 ± 1.42 g in order to evaluate the pharmacokinetic behaviour and the metabolism of radiolabelled DON (³H]-DON). Six trout per sampling point (1 h, 3 h, 6 h, 12 h and 24 h) were tube-fed (Costas 2011) with four pellets containing 133 ng of [³H]-DON, or four pellets containing 165.75 ng of unlabelled-DON. The metabolic fate of the [³H]-DON was assessed in a Tri-Carb 2910TR low activity liquid scintillation analyser (Perkin Elmer, USA). The excreted amount and metabolism of DON was analysed by LC-MS/MS as described by Streit *et al.*, (2013). [³H]-DON was detected in the fish liver one hour after tube-feeding, indicating a rapid absorption of DON. In the first hour, [³H]-DON achieved its maximum in the gastro-intestinal tract (GIT) (20.56 ± 8.30 ng [³H]-DON), however, 6.19 ± 0.83 ng of [³H]-DON was also detected in the water at this sampling point. The fast excretion of [³H]-DON (above the average gastric emptying time for trout) might be related to its high water solubility and consequent excretion with the liquid phase of digestion. Moreover, the concentration of [³H]-DON in the GIT is stable during the first six hours, which is a considerable amount of time that DON is present in GIT, increasing the potential for damage and absorption at this level. Peak excretion occurred between 6h and 12h where the excretion of [³H]-DON increased 2.4-fold. Data suggests that an effective DON detoxifying method requires a period of action lower than six hours to reduce DON toxicity and/or its bioavailability. No DON metabolism was observed in the present trial.

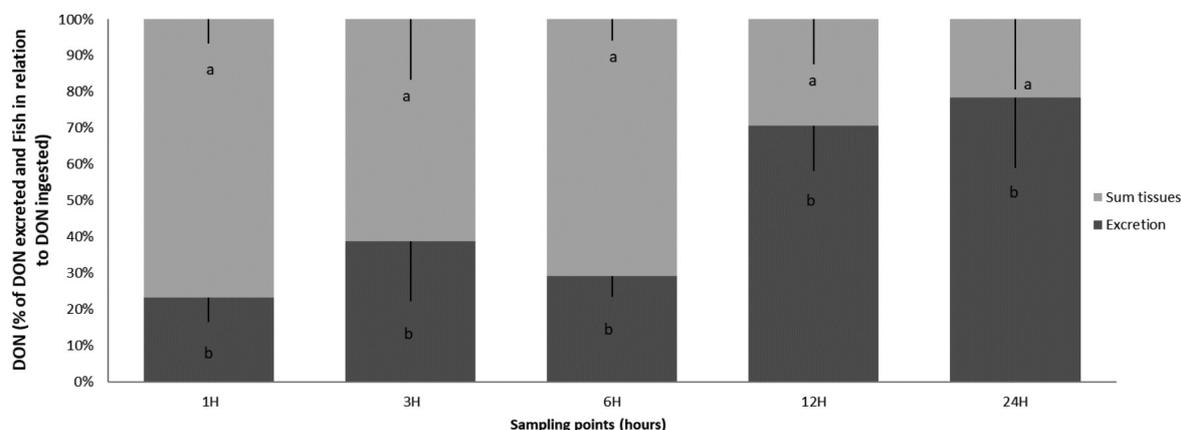


Figure 1: DON distribution in fish tissues (sum of total DON determined in samples from the gastro-intestinal tract, liver, muscle, kidney and skin) and excretion into water at 0h (except muscle and skin), 3h, 6h, 12h and 24h after tube-feeding a meal labelled with [³H]-DON. Proportion (%) of DON absorbed (fish) and excreted (water) in relation to the total quantity of DON determined in tissues and water. Values are mean \pm S.D. of DON in total weight of all sampled tissue and in freshwater. Different letters indicate statistical differences ($p < 0.05$, one-way ANOVA) of DON in fish and freshwater.

IMPACT OF DEOXYNIVALENOL ON RAINBOW TROUT: GROWTH PERFORMANCE, DIGESTIBILITY, KEY GENE EXPRESSION REGULATION AND METABOLISATION

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The impact of deoxynivalenol (DON) on rainbow trout, *Onchorynchus mykiss*, is mainly characterised by impaired growth performance and reduced feed intake, usually with the total absence of any visible clinical signs. Despite the high concentrations of DON in the present study (up to $11,412 \pm 1,141 \mu\text{g kg}^{-1}$), no clinical signs (except anorexia at the higher DON dosage) were observed, which confirms the difficulties of diagnosing DON ingestion (Figure 1). Compared to the control group, the proteolytic enzyme activities (pepsin, trypsin and chymotrypsin) in trout were altered by DON ingestion. However, it was not clear if the observed impact on digestive enzymes was due to the direct action of DON, or a consequence of the lower feed intake determined for DON-treated animals. The impact of DON on the abundance of specific measured mRNA transcripts was unexpected with higher expression levels for insulin-like growth factors, *igf1* and *igf2*, which are directly related to elevated insulin levels in plasma. This can also in part be influenced by the trypsin activity and by neuropeptide y (*npv*), given its higher mRNA expression levels. The apparent digestibility of dry matter, protein and energy was not affected by dietary levels of DON, however, nutrient retention, protein, fat and energy retention were significantly affected in animals fed DON. Adenylate cyclase-activating polypeptide (*PACAP*) expression seems to play an important role in controlling feed intake in DON fed trout. In the present study, we have shown for the first time that DON is metabolised to DON-3-sulfate in trout. DON-3-sulfate is much less toxic than DON, which helps to explain the lack of clinical signs in fish fed DON. Being a novel metabolite identified in trout makes it a potential biomarker of DON exposure. Suppression of appetite due to DON contamination in feeds might be a defence mechanism in order to decrease the exposure of the animal to DON, therefore reducing the potential negative impacts of DON.



Figure 1: Rainbow trout's visual differences in growth between the three dietary treatments (Control, DON 5 [5 mg kg^{-1}] and DON 11 [11 mg kg^{-1}], down to top). Fish from the best performing treatment (CTRL, down) showed a 9.8-fold increase of initial body weight. No clinical signs were observed, except the accentuated anorexia in DON 11 (top).

MYCOTOXINS IN SOUTHEAST ASIAN AQUACULTURE: PLANT-BASED MEALS AND FINISHED FEEDS

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The awareness of mycotoxin-related issues in aquaculture industry has grown as manufacturers and producers realize the importance of mycotoxins and their potential to impact production. The objective of the present work was to analyze the risk of mycotoxin contamination in conventional plant ingredients used for aquafeeds. Analysis of the by-products of these commodities, which are starting to be used in aquafeeds due to their better price and availability, was also conducted. Particular attention was also given to local non-conventional meals and aquaculture by-products. The aim was to analyze 18 mycotoxins in each sample in order to understand the occurrence of masked and alternative metabolites of mycotoxins, as well as the most commonly occurring mycotoxins already analyzed in past works. Additionally, finished feeds for fish and shrimp were also analyzed. Due to the increasing globalization of trade, and the incorporation of imported raw materials into aquafeeds, the mycotoxin contamination of locally produced commodities was compared to the same imported commodities. The current presentation will summarize the results of one year of sampling (January 2016 – December 2016, in which 175 samples of different plant protein sources, aquaculture/fishery by-products and finished aquaculture feeds were analyzed. Samples were tested for: aflatoxins (AF; AFB₁, AFB₂, AFG₁ and AFG₂); zearalenone (ZEA); type B trichothecenes (deoxynivalenol (DON); Nivalenol (NIV); 3-acetyldeoxynivalenol (3-AcDON); 15-acetyldeoxynivalenol (15-AcDON) and fusarenon X-glucoside (FUX); fumonisins (FB; FB₁, FB₂ and FB₃); type A trichothecenes (T-2; HT-2; diacetoxyscirpenol (DAS) and neosolaniol (NEO)) and ochratoxin A (OTA). All samples were sourced in Southeast Asia. Sourced commodities were divided into: maize products, soya products, rice products, wheat products, non-conventional products and aquaculture/fishery by-products. Maize product samples included: maize grains (M), maize bran (MB), corn gluten meal (CGM), ground maize (GM) and maize dust (MD). Soya product samples included: full fat-soy (FFS), soybean meal (SBM) and soybean cake (SBC). Rice product samples included: rice bran (RB), broken rice (BR) and polished rice (PR). Wheat product samples included: wheat (W), wheat bran (WB), wheat flour (WF) and distiller's dried grains with solubles (DDGS). Non-conventional/local product samples included: alfalfa (AL), cassava (CA), groundnut cake (GNC), rapeseed (RP), sesame (S) and sunflower cake (SFC). Aquaculture by-products included sun-dried fish (DF) and shrimp head meal (SHM). Within the analyzed samples, 4% were free of detectable mycotoxins. Of the 96% of samples with mycotoxins detected, the majority (88%) were contaminated with more than one mycotoxin. Mycotoxin occurrences in fish and shrimp feeds in the present samples were higher than reported in the previous studies for the same region. In samples of shrimp feed with DON detected, the mean average DON level was 882 µg/kg with a maximum level of 2,287 µg/kg. These values are within the reported sensitivity levels of various aquaculture species, for example white leg shrimp (*Litopenaeus vannamei*).

DEVELOPMENT OF HEPCIDIN FAMILY GENES ASSOCIATED MICROSATELLITES FOR MARKER-ASSISTED SELECTION OF DISEASE-RESISTANT TILAPIA

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Streptococcus and tilapia lake virus (TiLV) are most critical pathogens causing significant economic loss of tilapia aquaculture in various countries. The hepatic antimicrobial peptides hepcidin/HAMPs in teleost have been reported to defend against various bacterial pathogens and viruses. According to newly released genome assembly of Nile tilapia (*Oreochromis niloticus*), we found that gene amplification of *hepcidin* to *hepcidin* gene family composed of 18 *hepcidin* genes including 7 *HAMP1* genes, 1 *HAMP2* gene, 1 *HAMP3* gene, 3 *HAMP4* genes in LG11, and 5 *HAMP1*, 1 *HAMP4* genes in two separate contigs, contig825 and contig1099. Four kinds of Nile tilapia *HAMP* genes encoding 22-a.a. *HAMP1*, 26-a.a. *HAMP2*, 19-a.a. *HAMP3* and 22-a.a. *HAMP4* mature peptides were differentially activated not only in the liver but also in head kidney, spleen and gill of Nile tilapia NTOU NT1 strain treated by IP injection with virulent *Streptococcus iniae* 89353 in the dose of LD₅₀ (1.5×10⁵ CFU/g BW). Identification of DNA markers associated with disease-resistant genes may facilitate the breeding selection for disease resistance of tilapia. Hence the study aimed to investigate the association of genotype of microsatellites associated with *hepcidin* genes and disease resistance of Nile tilapia NT1 strain which is more resistant to *Streptococcus iniae*. We discovered 17 hepcidin-related SSRs and twelve polymorphic microsatellites were used for genotyping of 276 tilapia fish samples (95 and 90, and 91 tilapia fish of NT1, NT2 and their hybrid populations) to examine the associations between their genotypes and disease resistance. We found that three alleles in *HAMP1* genes-associated microsatellites SSR7, SSR9 and SSR16 have the significant difference in allele frequency between survival and dead groups in NT1 strain infected by *S. iniae* in the dose of LD50. These *hepcidin* genes associated SSRs could be developed as potential DNA markers for marker-assisted selection of new disease-resistant commercial tilapia for sustainable tilapia aquaculture.

REARING PERFORMANCE AND HAEMATOLOGICAL PARAMETERS OF NILE TILAPIA *Oreochromis niloticus* FED DIET WITH FERMENTED CHLORELLA *Chlorella* sp. FOLLOWING *Aeromonas hydrophila* INFECTION

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The study evaluated the effect of fermented *Chlorella* sp. (FC) on the rearing performance and haematological profile of Nile tilapia *Oreochromis niloticus* following *Aeromonas hydrophila* infection.

The 180 fish were assigned to 12 glass aquaria following complete randomized design where each treatment was triplicated with 15 fish per aquarium. The fish were fed with 50 g Kg⁻¹, (FC50), 100 g Kg⁻¹ (FC100) or 150 g Kg⁻¹ (FC150) FC – supplemented diet for 8 weeks while a practical diet without any FC served as the control (C). Growth and survival were monitored periodically. Blood sampling was conducted after rearing and one day after *A. hydrophila* infection to evaluate the haematological parameters.

Results revealed an increase in the growth performance of fish. The fish fed with FC50 had the highest WG and SGR among treatments. Conversely, no significant difference on haematological profile and survival was found after rearing. However, fish fed with FC100 had the most stable haematological profile and highest survival among treatments after *A. hydrophila* infection. Overall, FC improves the growth performance of fish and its hematological profile and survival under *A. hydrophila* infection.

INTER-SPECIES COMPATIBILITY OF THE MICROBIAL FLORA OF THREE TROUT SPECIES ON THE SAME CULTURE CONDITION

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Importance of bacterial and zoonotic diseases are day by day increasing owing to its risks and effects. For this reason, careful evaluation, examination and monitoring of foodstuffs in terms of aquaculture and human health are important. The microbial flora of processed and fresh aquatic organisms should be carefully examined in the evaluating stage. Also the bacterial flora of the aquatic organisms is determined by the environment in which it lives. Different species can give different responses to the environment. According to, this the objective of the study was to evaluate, examine, research and monitoring the condition of the microbial flora for three trout species on the same culture condition.

In this study, compared of bacterial flora of three trout species, which are rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta forma fario*) on the same trout farm and same condition.

30 fish for each species were collected after harvesting for sample. Microbiological samples collected from different organs of fish.

Totally 33 different isolates were obtained from fish. 10 isolates from the rainbow trout, 11 isolates from brook trout and 12 isolates from brown trout were isolated.

At the results of identification of isolates, 5 different bacteria were found from these 33 isolates, which names are *Pseudomonas aeruginosa*, *Ochrobactrum anthropi*, *Mannheimia haemolytica*, *Vibrio fluvialis* and *Stenotrophomonas maltophilia*.

All of isolated these are opportunistic pathogen to different organisms, which are *V.fluvialis* for fish, *P.aeruginosa* for both fish and human, *M.haemolytica* for ruminants, *O.anthropi* and *S.maltophilia* for human. These results shown that, microbial flora of different fish species can effect from each other and environment on the same culture condition. For this reason, monitoring of fish farm and inter-species compatibility are important subject for aquaculture.

Table 1. Water parameters of farm

Parameters	Results
Permanganate index (mg L ⁻¹)	<0.5
Hardness (mg CaCO ₃ L ⁻¹)	20
NO ₃ ⁻ (mg L ⁻¹)	2.77
NO ₂ ⁻ (mg L ⁻¹)	<0.002
NH ₃ (mg L ⁻¹)	<0.02
P (mg L ⁻¹)	<0.016
TSS (mg L ⁻¹)	0.5
pH	6.9
O ₂ (mg L ⁻¹)	8.8
Temperature (°C)	7.2

Table 2. Total bacterial count of fish

Organs	cfu mL ⁻¹		
	Rainbow	Brook	Brown
Gill (G)	2x10 ³	2x10 ⁵	4x10 ³
Kidney (K)	4x10 ³	7x10 ⁴	1x10 ⁵
Intestine (I)	3x10 ³	1x10 ⁴	2x10 ⁵

Table 3. Identification results of bacteria

Bacteria	Rainbow			Brook			Brown		
	G	K	I	G	K	I	G	K	I
<i>P.aeruginosa</i>	+			+	+	+	+	+	+
<i>O.anthropi</i>	+	+	+	+	+		+	+	+
<i>M.haemolytica</i>	+	+							+
<i>V. fluvialis</i>							+		
<i>S. maltophilia</i>			+						

THE JOINT STATEMENT OF THE INTERNATIONAL SYMPOSIUM ON THE DISCOVERY OF FORMOSA LANDLOCKED SALMON'S 100 ANNIVERSARY

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Conservation of Critically Endangered Formosa landlocked salmon (*Oncorhynchus formosanus*) requires the development and implementation of a national conservation Program of Action. The objectives and schedule of the changes in the number of Critically Endangered species must be clearly defined in the Program of Action, for example, to prevent decreases in the number of species experiencing sustained decline (stop bleeding) within 3 to 5 years, to promote the steady increase in the number of rebounding species within 6-15 years, and the need to reach a substantial increase in the population number and remove listings of species as "Critically Endangered" as the ultimate goal within 20-30 years. It is necessary to have sufficient scientific understanding of the dilemmas and threats faced by endangered species, and to develop a task-oriented and feasible program of action based on this information.

Overall, this group formulated the following management actions.

1. Most critically, there is urgent need for independent assessment of the conservation status of Formosa landlocked salmon under the direction of IUCN. It is very important for the IUCN to reassess the species on the IUCN Red List. These include the estimation of population size and genetic diversity, and survey the possible causes and the prevalence of fish with morphological abnormalities.
2. Formosa landlocked salmon is at the southern range of masu salmon distribution. Basic biological and ecological information relating to the growth, feeding, and behaviour of Formosa landlocked salmon needs to be collected for effective conservation.
3. The interactions of Formosa landlocked salmon and *Onychostoma barbatulum* needs to be investigated, to determine if there are negative impacts, and to guide any future removal of check dams.
4. The practices of the hatchery, relating to genetic variation in Formosa landlocked salmon and the culturing of any other species need to be investigated.
5. Need scientific evaluation on the order of priority for dam removal (e.g., PVA, simulation). Demographic parameters (e.g., survival rate) would differ between the reaches. If survival rate is higher in the upstream, removing upstream dam will result in increased population viability of the whole population.
6. Attendees at the meeting encouraged efforts to establish independent populations (*ex situ* preservation) in Taiwan to reduce the risk of extinction of the species. Restocking habitats had better form a metapopulation structure to promote better connectivity between habitats.

DOMESTICATION AND SELECTION OF BROODSTOCK FOR SEED PRODUCTION OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*) IN THE MEKONG DELTA, VIETNAM

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Giant freshwater prawn (*Macrobrachium rosenbergii*) is an important species for culture in the Mekong Delta, Vietnam. Currently, there is 15,270 ha of prawn culture in the region with total production of 5,306 tons, of which the coastal provinces cover for 90.1% of total culture area and 64.8% of total production. A total of 59 prawn hatcheries is currently in operation, that yearly produce 266 millions postlarvae for the farming in the region. However, more works on improving quality of prawn seeds for farming are needed.

This study aimed to evaluate domestication and seed selection of giant freshwater prawn in order to improve quality of prawn seed for grow-out in Vietnam. The 3-year study included (i) generally evaluating genetic and morphology characteristics of 4 sources of prawns in 4 areas of Vietnam with 785 samples, (ii) comparing spawning and growing performances from larvae to juveniles of 4 sources with totally 120 families, (iii) through studies of (i) and (ii), prawn broodstocks from Dong Nai province were selected as the best one for evaluating spawning and growing performance through three generations, and (iv) commercial culture of F_2 and F_3 seeds of Dong Nai source compared to normal seeds.

In domestication and seed selection of Dong Nai prawn source, a total of 100 largest pairs of prawns selected from grow-out phase were cultured in 1-m² hapas (1 pair/hapa) to become broodstocks. Each berried broodstock was then used for egg hatching and larval culture in modified static green water system with separated batches. The largest postlarvae (PL₁₅) (10% of population) from the larval rearing (F_1) of each batch were selected for nursing in a happa (150 inds/m²) to reach PL₄₅, and then to commercial sizes (15 inds/m²) and then to broodstocks (1 pair/hapa). About 10% of the largest prawns from each stage were selected for the following stage. The largest broodstocks were then used for F_2 and then F_3 generations with similar methods. Results indicated that fecundity of prawn broodstocks of F_2 were strongly increased at 109% compared to those of F_0 and 68% compared to F_1 . PL₁₅ body length (BL) of F_3 were improved 18.95% compared to those of F_1 and 3.67 compared to F_2 . Body weight of prawns of F_3 after 4 months of grow-out were improved 15.28% compared to F_1 and 6.96% compared to F_2 . Yield of F_3 prawn in commercial culture (10 prawns/m²) were increased 19% compared to those of F_2 and 56.3% compared to those of local prawn seeds. Net-income of prawn farming of F_3 was improved 15.9% compared to those of F_2 and 273% of the local seeds. The findings indicated that domestication and seed selection of giant freshwater prawn are very promising for further application to improve broodstock and seed quality for prawn farming.

APPLICATION OF PROBIOTICS AGAINST ACUTE HEPATOPANCREATIC NECROSIS DISEASE IN SHRIMP THROUGH GENOMIC ANALYSIS OF VIBRIO SPP.

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Acute hepatopancreatic necrosis disease

Acute hepatopancreatic necrosis disease (AHPND) is a bacteria disease that causes substantial mortalities at maximum 100% in farmed penaeid shrimp. This disease was first seen in China in 2009, and subsequent outbreaks have been reported in South-Eastern Asian countries and Latin American countries. The causative agents of AHPND are *Vibrio* bacteria, predominately *V. parahaemolyticus*, and these AHPND-bacteria secrete PirA- and PirB-like binary toxin that causes deterioration in the hepatopancreas of infected shrimp. The genes responsible for the production of this toxin are located in a large plasmid residing within the bacterial cells.

Genomic analysis

We analyzed the plasmid sequence from the whole genome sequences of AHPND-*V. parahaemolyticus* isolates and identified the region that exhibited a clear geographical variation: a 4243-bp Tn3-like transposon. The Tn3-like transposon was only found in the isolates from some Latin American countries, but not in South-Eastern Asian countries. In addition, we developed PCR methods to characterize AHPND-*V. parahaemolyticus* isolates as either Latin American- or South Eastern Asian-type based on the presence of the Tn3-like transposon. The genetic variation can serve as markers useful in determining the origins of new outbreaks, which is necessary for keeping recent management strategies and subsequently preventing the spread of the disease.

Use of probiotics

Considering the lack of feasible remedies reported for AHPND, development of effective treatment methods is necessary. We isolated 3 *Bacillus* spp. from sea water and the stomachs of shrimp, and these isolates were examined for antimicrobial activity test against AHPND. Bacterial growths of AHPND-*V. parahaemolyticus* isolates were effectively inhibited by isolated *Bacillus* spp. Furthermore, in the AHPND challenge experiments, shrimp survival rates increased at 34% after administration of *Bacillus* spp. Based on these results, isolated *Bacillus* spp. can be considered as a potential prophylactic candidate against AHPND.

OPTIMIZING WATER TRANSPARENCY AND PHYTOPLANKTON BLOOMS IN SHRIMP PONDS

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Water quality plays an important role in the productivity of shrimp culture. Maintaining the water transparency at desirable levels (25-35 cm) is crucial to enhance the survival and growth of shrimp. Transparency refers to the penetration of light through water, which indicates the concentration of suspended solids. Suspended solids which make water turbid in shrimp culture systems include plankton blooms, flocculated organic matter, chemical precipitates, and sediment stirred up from the bottom of the pond. Presence of plankton blooms affect the sediment quality leading to release of toxic gases like ammonia in to the pond water. Application of zeolites and water exchange are routine practices in shrimp culture to control the turbidity. In the present study, application of natural silicates and plant extracts for the control of turbidity in shrimp ponds is discussed. Various natural zeolites were evaluated and shortlisted based on their cation exchange capacity (CEC). Plant extracts were evaluated for the control of ammonia levels and plankton blooms in the water. A formulation containing the selected zeolites and plant extracts was developed and evaluated for application in shrimp culture systems. Safety studies were conducted by incubating juvenile white shrimps (*Penaeus vannamei*) in water containing 10x dosage of the formulation for 72 h. There was no mortality, morbidity or indications of stress observed in the shrimps during the study period.

Trials were conducted in commercial shrimp farms in three different locations in India to evaluate the efficacy of the formulation in field conditions. The formulation was compared with other commercial products in adjacent ponds with comparable culture conditions, and an untreated pond with the same water source was maintained as control. Products were applied by broadcasting in to culture ponds and the water transparency was measured before and after application using secchi disk reading. Water transparency measured 4 h, 24 h and 48 h after application of the new formulation showed improvement (by 6-10 cm) as compared to the control and other commercial brands and the turbidity was observed to reduce further after second application (48 h) to the desired levels. Level of plankton blooms was also observed to significantly reduce with application of the formulation, as measured by plankton counts. The same trend was observed in three trial locations. There were no visible adverse effects observed in the shrimps after application. The dosage of the formulation was finalized at 10kg/ acre, and two consecutive applications at an interval of 2 days were finalized to reduce turbidity based on the field trials.

ANTIOXIDANT PROPERTIES AND INSTRUMENTAL QUALITY ATTRIBUTES OF SPRAY DRIED VISCERAL PROTEIN HYDROLYSATE PREPARED BY ENZYMATIC AND CHEMICAL METHODS

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Antioxidant properties of spray dried visceral protein hydrolysate from *Pangasius* viscera hydrolyzed with enzymes i.e. papain, pepsin, hydrochloric acid and sodium hydroxide were investigated. From the results, it was observed that fish viscera protein hydrolysate (FVPH) had good antioxidant properties. Among the different antioxidant activities (DPPH, ABTS and reducing assays), pepsin fish visceral protein hydrolysate had indicated strongest antioxidant activity at higher concentration. Amino acid profiling of protein hydrolysate in the present investigation revealed that Gly and Thr are the most abundant amino acids which can work as hydrogen donor for antioxidant activity of hydrolysate. Nevertheless, highest quantity of Glu was found in pepsin fish visceral protein hydrolysate. According to the results of FTIR spectroscopy, not much difference was observed among the treatments. Results of scanning electron microscopy indicated that the spray dried sample of fish visceral protein hydrolysate treated with pepsin had spherical beads like structure. Among the different treatments, the visceral protein hydrolysate prepared with pepsin had better quality. Therefore it can be concluded from the present investigation that pepsin has potential for the preparation of good quality fish visceral protein hydrolysate from *Pangasius* viscera with better antioxidant properties.

WSSV TRIGGERS GLUTAMINOLYSIS AND REDUCTIVE GLUTAMINE METABOLISM AT THE VIRAL GENOME REPLICATION STAGE

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White spot syndrome virus (WSSV) can cause huge global economic losses, providing the impetus to determine its pathogenesis. We reported that WSSV induced a Warburg effect and glutaminolysis during viral genome replication (12 hpi). There are two pathways of glutamine metabolism: an α -KGDH-mediated oxidative pathway and an IDH1 or 2-mediated reductive pathway. In the present study, gene expression of both α -KGDH and IDH1 were increased during WSSV infection, with increased enzyme activity of reductive IDHs and oxidative α -KGDH. Suppression of α -KGDH, IDH1 and IDH2 by dsRNA silencing suggested that all of these enzymes were important for WSSV replication. Moreover, using [U-¹³C] and [1-¹³C]glutamine as tracer, both oxidative and reductive glutamine metabolic flux were significantly increased during WSSV genome replication (12 hpi). Based on *in vitro* studies, glutamine was essential for viability of WSSV-infected cells. In conclusion, at genome replication (12 hpi), gene expressions and enzyme activities of related enzymes were induced and there were increased amounts and production rates of metabolites to replenish the TCA cycle to support WSSV replication.

EXPRESSION PROFILES OF THE p38 MAPK SIGNALING PATHWAY FROM CHINESE SHRIMP IN RESPONSE TO VIRAL AND BACTERIAL INFECTIONS

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Fenneropenaeus chinensis (*F. chinensis*) is one of the most commercially important farmed aquatic species in China. However, frequent outbreaks of viral and bacterial diseases have posed a significant challenge to the shrimp culture industry, resulting in drastically decreased production and catastrophic economic losses. Compared with mammals and insects, information on the molecular mechanisms involved in the p38 MAPK signaling pathway in response to viral and bacterial infections in *F. chinensis* is limited. This study explored the potential role of the p38 MAPK signaling pathway in *F. chinensis* immunity by investigating the expression of four genes in the hemocytes and gills of shrimp after infection with white spot syndrome virus (WSSV), *V. anguillarum* and *S. aureus*. The results may provide important information on *FcMKK3*, *FcMKK4*, *Fcp38* and *FcATF-2* defense against viral and bacterial infections and will improve our understanding of the role of p38 MAPK signaling pathway in the innate immunity response of *F. chinensis*.

The expression levels of the four genes were apparently modulated in hemocytes and gills when shrimp were stimulated by WSSV or bacteria, particularly at 3–24 h after infection. MKK3, p38 and ATF-2 were most sensitive to *V. anguillarum* (Gram-negative bacteria), followed by WSSV and *S. aureus* (Gram-positive bacteria), while MKK4 gene was most sensitive to *S. aureus*, followed by WSSV and *V. anguillarum*.

The expression levels of *FcMKK3* and *FcATF-2* decreased after *Fcp38* was silenced by dsRNA at all time points, and the lowest expression levels of two genes were at 6 h. However, significant differences were not observed at 12, 24 and 48 h, which showed that silencing *Fcp38* could down regulate the transcription of *FcMKK3* and *FcATF-2*

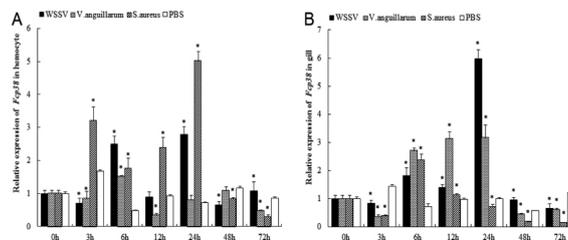


Fig.1 Expression profile of *Fcp38* in hemocytes (A) and gills (B) in *F. chinensis* after challenge with WSSV, *V. anguillarum* and *S. aureus*

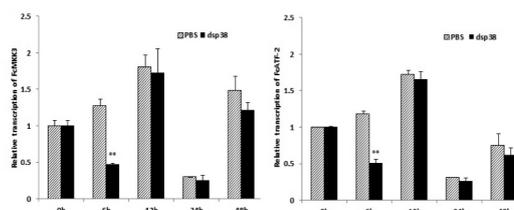


Fig.2 Relative transcription levels of *FcMKK3* and *FcATF-2* injected with dsRNA for *Fcp38*

STUDY ON PREBIOTIC EFFECTS OF *Ulva lactuca* MIXED OLIGOSACCHARIDES

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The purpose of this study is to investigate the prebiotic effects of *Ulva lactuca* mixed oligosaccharides (UMO_{5k}) on the growth of bifidobacteria and *Clostridium perfringens*. *Ulva lactuca* polysaccharides were hydrolyzed with enzymes induced from strains MA103 and MAEF108 and collected by ultrafiltration system. Inoculated 1% activated *Bifidobacterium adolescentis* BCRC14608, *Bif. bifidum* BCRC14615, and *Cl. perfringens* BCRC13019 which added 1% UMO_{5k} in the basal medium and Inulin (positive control) or no carbon source added (negative control). The *Bifidobacterium* strains and *Cl. perfringens* were cultured alone and in combinations as *Bif. adolescentis* + *Cl. perfringens* and *Bif. bifidum* + *Cl. perfringens* for 24 h at 37°C. The results showed the absorbance (OD_{600nm}) and pH value of *Bif. adolescentis* and *Bif. bifidum* are 1.88 to 2.88 and from the initial 6.10 to the range 4.87-4.90 (Table 1). In combinations, the results showed growth of *Bif. adolescentis* and *Bif. bifidum* increased from 6.41 to 8.29 log CFU/mL and 6.39 to 7.89 log CFU/mL, respectively. Besides, the growth for *Cl. perfringens* was decreased from 5.18 to 1.11 log and 1.00 log CFU/mL (Figure 2). These findings indicate the growth of bifidobacteria increased after adding 1% UMO_{5k} when co-cultured with *Cl. perfringens*.

Fig. 1. Growth of *Bifidobacterium adolescentis* BCRC14608, *Bif. bifidum* BCRC14615, and *Clostridium perfringens* BCRC13019 when mono-culture in basal medium containing UMO_{5k} as carbon source.

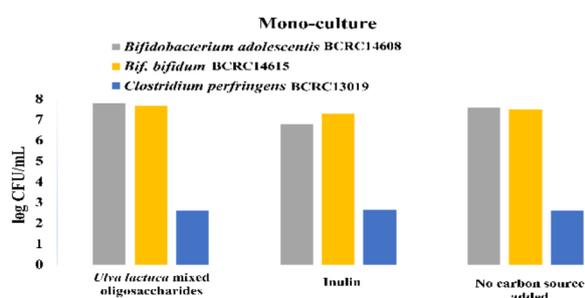
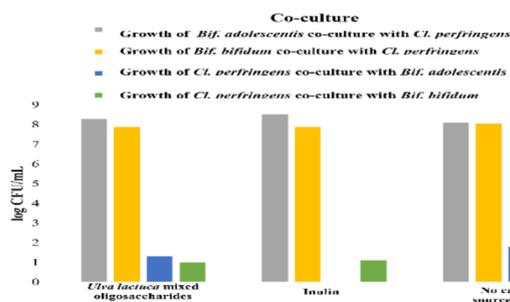


Fig. 2. Growth of *Bifidobacterium adolescentis* BCRC 14608, *Bif. bifidum* BCRC 14615, and *Clostridium perfringens* BCRC 13019 when co-culture in basal medium containing UMO_{5k} as carbon source.



NILE TILAPIA HEPATIC ANTIMICROBIAL PEPTIDE HAMP GENES ENCODING FOUR HEPCIDIN PEPTIDES WERE DIFFERENTIALLY ACTIVATED IN RESPONSE TO *Streptococcus iniae* INFECTION

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Four Nile tilapia hepatic antimicrobial peptide *hepcidin/HAMP* genes, encoding 87-a.a. HAMP1, 90-a.a. HAMP2, 83-a.a. HAMP3 and 133-a.a. HAMP4 were identified from Taiwan Nile tilapia NT1 strain infected by virulent *Streptococcus iniae* 89353. The secreted mature peptide sequences of 22-a.a. HAMP1 and 26-a.a. HAMP2 of Nile tilapia with four disulfide bonds are identical to previously identified strong HAMP TH1-5 and TH2-3 of Mozambique tilapia, respectively. The 19-a.a. HAMP3 is a novel HAMP composed of only three disulfide bonds and 22-a.a. mature HAMP4 is a new HAMP1-like HAMP with four disulfide bonds. Four Nile tilapia *HAMP* genes were differentially activated not only in the liver but also in head kidney, spleen and gill of NT1 Nile tilapia treated by IP injection with *S. iniae* in dose of LD₅₀ (1.5x10⁵ CFU/g BW) to contribute for defense against *S. iniae*. From the new Nile tilapia genome assembly ASM185804v2 (GCA_001858045.2), we found 12 *hepcidin* genes, including 7 *hamp1*, 1 *hamp2*, 1 *hamp3*, and 3 *hamp4* genes in LG11, and 6 *hepcidin* genes in two unplaced contigs, contig825 and contig1099. Among them, *hamp1* and *hamp4* genes were highly amplified to 12 and 4 genes, respectively. We propose that tilapia *hepcidin* gene amplification during evolution may contribute to disease-resistance of tilapia.

STUDY ON HARMFUL EFFECTS OF LIGHT-EMITTING DIODES ON OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Light is one of the important factors that affects to fish physiological responses, consisting of their growth and maturation (Semensen *et al.*, 2000). In the recent year, the use of blue light (400-500 nm) as a mono-therapy is gaining increasing attention due to its potential antimicrobial activity (Roh *et al.*, 2017). Besides, previous studies have shown that green light is considered as promising light therapy to promote wound healing (Cheon *et al.*, 2010). However, there is little information on the effect of these specific wavelength after exposing on fish. Thus, this study was conducted to evaluate the harmful effects of blue and green light using short wavelength of 405 and 520 nm of light emitting diodes (LEDs) on growth performance and stress response in olive flounder.

Fish (31.3±3.98g) were randomly divided into 3 groups (15 fish/group) were exposed to ambient light (control), 405 nm and 520 nm with light intensities reach to the fish at 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. Fish were cultured at 20±1°C, and fed by 2% of body weight with the photoperiod of 12 light: 12 dark. 70% water was exchanged every day for 8 weeks. Initial fish number and body weight were measured for growth performance parameters and head kidney of 5 randomly fish were collected for gene expression.

Results here show that blue light and green light illumination did not induce significantly expression of HSP 70 and SOD, caspase-3 and catalase in head kidney in all groups. Surprisingly, after 8 weeks of exposing light treatment, 520 nm showed significant higher in final length gain and final weight gain compared to 405 nm and control. The highest value of length gain, weight gain, specific weight gain, feed intake as well as the lowest value of feed conversion ration was found in 520 nm group, followed by 405 nm and control. This result indicates that there were no harmful effects in olive flounder exposed to blue light with high intensity for 8 weeks. This will be useful for future studies on application of blue light in aquaculture.

TABLE 1. Initial body length (Cm)- IBL, Final body length (Cm)- FBL, Length gain (cm)-LG, Initial body weight (g)- IBW, Final body weight (g)- FB, weight gain(g)-WG, specific growth rate (%/day)-SGR, feed intake (g/fish)-FI and Feed conversion ratio-FCR of olive flounder after 8 weeks of exposing LED. Means in the column with different letters were significantly different (P<0.05)

	control	520 nm	405 nm
IBL	15.2±0.8	14.9±0.6	14.9±0.7
FBL	18.8±1.2 ^a	20.1±0.8 ^b	19.3±0.9 ^{ab}
LG	23.4	34.8	29.3
IBW	32.3±4.39	30.6±3.83	31.1±3.78
FBW	60.4±10.73 ^a	72.1±7.74 ^b	67.8±11.75 ^{ab}
WG	87.1	135.8	118.2
SGR	1.12	1.53	1.39
FI	0.9	1.2	1.1
FCR	5.78	4.02	4.63

FARM APPLICATION OF A FUNCTIONAL FEED ADDITIVE : AN ESSENTIAL TOOL IN THE HEALTH MANAGEMENT OF WHITE FECES SYNDROME IN ASIAN SHRIMP FARMING

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White Feces Syndrome (WFS) is the cause of significant crop failures of white shrimp in Indonesia, Thailand, Vietnam and India. Although the causative agent is not yet clearly identified, WFS is easily diagnosed by the presence of white strings in the surface of the water and edges of the pond. Affected shrimp exhibit a white hepatopancreas and gut, which results in mortality and persisting stunted growth.

The inclusion of natural products with bactericidal and quorum quenching activities is an important factor in current bio-security protocols to prevent the horizontal spread of opportunistic bacterial pathogens such as *Vibrio spp*, *Photobacterium spp*, *Flavobacterium spp*, *Tenacibaculum spp*. Furthermore, a healthy gut microbiota enhances the overall health status and immune defences, which may explain the positive effect of gut modulators on reducing the impact of certain endo as well as ectoparasites on productivity in fish and shrimp.

Three field studies reported in this compilation;? explored the preventive and/or curative effect against WFS of a health promoting, functional feed additive with broad spectrum activity (SANACORE® GM, Nutriad), added to the feed either at the farm by top-dressing or at the feed factory, or a combination of both strategies. Field trials were executed in close collaboration with shrimp producers following standard operational procedures in Malaysia, India and Indonesia.

METAGENOMIC ANALYSES OF GROUPEL (*Epinephelus coioides*) GUT MICROBIOTA IN RESPONSE TO RHAMNAN SULFATE FROM THE GREEN LAVER

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With the development of NGS techniques, alterations in intestinal microbiota composition are associated with several chronic conditions, including the onset of diseases. This is also crucial immunologic, hormonal and metabolic homeostasis of the host. In the present study, the gut microbiota composition of orange-spotted grouper (*Epinephelus coioides*) by feeding two concentrations of rhamnan sulphate (RS, from green laver) diets were compared with normal diet using 16S rRNA gene pyrosequencing. Genus *Photobactrium*, *Acinetobacter*, *Anoxybacillus*, *Vibrio* and *Donghicola* were detected as the predominant genus regard of RS and time course. According to the functional metagenomics predictions that ATP binding cassette (ABC) transporters and secretion system in bacteria significantly changed in 7~14 days after feeding with RS feeds. ABC transporters are recognized for their ability to modulate absorption, distribution, metabolism, secretion, toxicity of xenobiotic, and play important role in tissue defense. In summary, our data supported the interrelationship between diet, microbiota, and health status of grouper.

EFFECTS OF *Mugil cephalus*, *Oreochromis niloticus* POLYCULTURED WITH *Litopenaeus vannamei* ON IMMUNE RESPONSE AND DISEASE RESISTANCE OF WHITE SHRIMP CHALLENGED WITH *Vibrio alginolyticus*

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Polyculture systems have been considered as, an effective strategy to minimize waste from culturing systems (Muangkeow, et al., 2007), a potential alternative farming system that decrease organic pollution and prevent shrimp disease in shrimp culture. Some studies have investigated that shrimp polyculture with fish could be a simple and efficient way to solve shrimp culture problems, especially, low water quality, diseases and improve shrimp's survival (Luong, et al., 2014; Martínez-Porchas, et al., 2010). The aim of this study was to verify whether polyculture systems have an impact on the shrimp immune system function.

After rearing for 75 days in composite tanks (5 m³), shrimp from polycultured treatments: (1) shrimp+tilapia (fish was kept in cages), (2) shrimp+gray mullet (fish kept in cages), (3) shrimp+gray mullet (fish was stocked free in tanks) were subjected to a challenge test. Shrimp from monoculture were also selected to perform positive and negative challenge test. The experiment was conducted in fifteen aquaria (40 L) in triplicates. Shrimp were stocked at 20 shrimps aquarium⁻¹ and challenged with *Vibrio alginolyticus* by injection into the ventral sinus of the cephalothorax of each shrimp, with a dose of 2.1 x 10⁵ CFU shrimp⁻¹. Shrimp were fed two times a day using 35% protein pellet during challenge with a feeding rate of 2-3 % on the biomass.

After challenge, the levels of PO and respiratory burst (RB) activity in the shrimp of all polyculture treatments were not higher than that of the challenged shrimp from the control (monoculture) treatment (p>0.05). The survival of the challenged shrimp from the shrimp+gray mullet (stocked free in tanks) seemed to be higher as compared to the other polyculture treatments and challenged shrimp from the positive control treatment. However, there were no significant differences between them. Overall, this study demonstrated that the application of polyculture systems does not contribute to the robustness of cultured shrimp by immunostimulation.

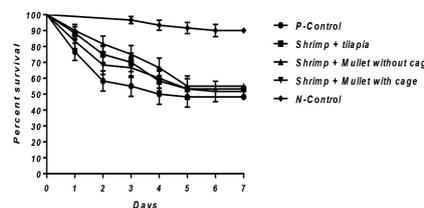


Fig. 1 Survival rate of the shrimp challenged with *V. alginolyticus*

Table 1. Immune parameters of white shrimp before and after challenged with *Vibrio alginolyticus*. Values within the same column marked with a different superscript are significantly different (P < 0.05).

Treatment	Pre-challenge			Post-challenge		
	THC (x 10 ⁶ cells mL ⁻¹)	PO (OD ₄₉₀ 100 μL ⁻¹)	RB (OD ₆₃₀ 10 μL ⁻¹)	THC (x 10 ⁶ cells mL ⁻¹)	PO (OD ₄₉₀ 100 μL ⁻¹)	RB (OD ₆₃₀ 10 μL ⁻¹)
Positive control	11.60 ^a	0.144 ^a	0.243 ^a	8.50 ^a	0.098 ^a	0.158 ^a
Shrimp and tilapia with cage	13.40 ^a	0.161 ^a	0.297 ^a	9.50 ^a	0.096 ^a	0.246 ^a
Shrimp and mullet without cage	14.90 ^b	0.205 ^a	0.402 ^a	10.10 ^a	0.117 ^a	0.320 ^a
Shrimp and mullet with cage	12.20 ^a	0.175 ^a	0.315 ^a	9.30 ^a	0.103 ^a	0.256 ^a
Negative control	--	--	--	11.30 ^a	0.146 ^a	0.257 ^a

THC: total haemocyte count; PO: phenoloxidase activity; RB: respiratory burst.

ADVANCED IN THE MOLECULAR PATHOGENESIS OF IRIDOVIRUS: CELL DEATH AND IMMUNE RESPONSE

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So far main virus, iridovirus, can endanger grouper aquaculture in Taiwan. Infected fishes by megalocytivirus belonging to family Iridoviridae appear organomegaly and cause severely damaged in aquatic industry, so understand the mechanism pathogenesis of viral infection and finding prevention methods to solve this problem is a big issue. In this present, the infectious spleen and kidney necrosis virus (ISKNV) induces host cell death pathways and immune responses were examined *in vitro* and *in vivo*.

In vitro system, the GF-1 cells as a host cell, we found that ISKNV can induce host cell death via ROS/Bax/Bak signaling pathway in GF-1 cells. Then, in downstream of the mitochondrial membrane potential (MMP) loss, and activate caspase family such as caspase-8, caspase-9 and caspase-3 were examined.

Dramatically we found that ISKNV-induced host cell death was blocked by antioxidant treatment and rescued host cells. Then, *in vivo* system, ISKNV can infect and induced host death from young gain grouper (about at 3.5 to 4 inches). In the targeted tissues such as spleen and head-kidney we found that can induce Fas/caspase-8-mediated cell death pathway and host cell death by TUNEL assay. On the other hand, the innate immune response was induced by ISKNV infection that IL-1 β and IFN- λ .

Taken together our results suggest that megalocytivirus infection can induce host cell death via Fas/caspase-8 and induces innate immune response such as inflammatory cytokines IL-1 and TFN- in target tissues. This finding may provide new insight into iridovirus molecular pathogenesis.

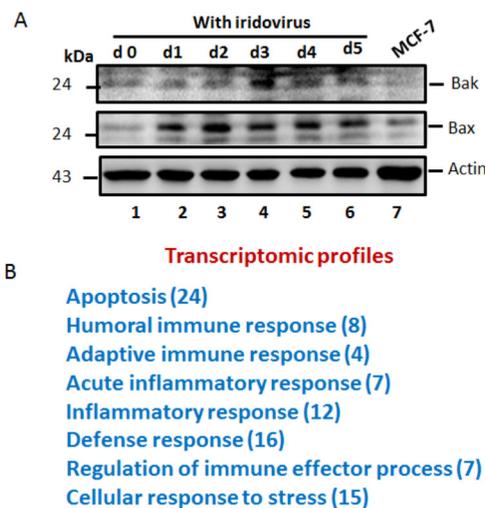


Fig. 1. Identification of iridovirus induces cell death via Bax/Bak-mediated signaling pathway in GF-1 cells (*in vitro*) (A) and apoptosis signaling *in vivo* (B).

THERMAL STRESS AFFECTS SECONDARY STRESS RESPONSES TO ACUTE SUBLETHAL AMMONIA EXPOSURE IN JUVENILE TRA CATFISH (*Pangasianodon hypophthalmus*)

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Global warming is predicted to rise a further 1-6°C in the coming decades. Alterations in ambient temperature indeed influence physiological and biochemical reactions in ectotherms. In addition, elevation of waterborne ammonia usually occurs in intensive aquaculture operation which is an environmental stress and limiting factor for aquaculture. Scientific information on physiological stress responses to acute sublethal ammonia exposure under global warming scenario, however, is limited for economically important aquaculture species. In this study, juveniles tra catfish (*Pangasianodon hypophthalmus*) were acclimated to 27 and 33°C as current and global warming scenarios, respectively. Subsequently, tra catfish were exposed to two sublethal concentrations of total ammonia nitrogen (TAN) (i.e., 0 and 6 mg/l) for 96 hrs. Physiological status was evaluated by measuring secondary stress responses, including serum osmolality, sodium, chloride, glucose, muscle water content, liver glycogen content as well as protein amounts of heat shock protein 70 (HSP70). Thermal and sublethal ammonia stresses nominated in this study are environmentally realistic relevant. Therefore, the results of the present study would be able to provide science-based information for water quality management of ammonia for tra catfish aquaculture operation to abate the ammonia-induced stress under the scenario of climate change.

THE EFFECTS OF REPLACEMENT OF FISH MEAL PROTEIN WITH POULTRY BY-PRODUCT MEAL SUPPLEMENTED WITH FISH SILAGE AND HYDROLYZED FISH PROTEIN ON THE GROWTH PERFORMANCE OF ASIAN SEABASS *Lates calcarifer*

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A feeding trial was conducted to investigate the effects of replacement of dietary fishmeal with a combination of poultry by-product meal (PBM), fish silage (FS) and hydrolyzed fish protein (HFP) on the growth performances of Asian seabass *Lates calcarifer*. Six isonitrogenous and isolipidic diets (crude protein 45% and crude lipid 12%) were formulated. Five diets to replace 0% (CON), 25% (FM25), 50% (FM50), 75% (FM75) and 100% (FM100) fishmeal protein by combination of PBM, FS, and HFP. One additional diet POM was designed as only PBM to replaced 100% FM protein. Each diet was randomly assigned to triplicate tanks with ten fish (initial average body weight 1.72 g) per tank. Fish were fed three times daily for 4 weeks. At the end of the feeding trial, a decreasing trend in the final weight, weight gain and SGR were noted with increasing dietary PBM inclusion. The final weight and weight gain of fish fed CON diet had no significantly different from those of fish fed FM 25 and FM50. Meanwhile, fish fed diet FM75, FM100 and POM had significantly decreased final weight, weight gain compared with fish fed CON diet. Fish fed diet CON, FM25, FM50 and FM75 had no significantly different in SGR. Fish fed diet FM100 and POM had significantly lower SGR then fish fed CON diet. The combination of PBM, FS and HFP could replace dietary fishmeal protein for *L. calcarifer* up to 50% with no adverse effects.

GLUCOCORTICOID RECEPTOR 2 MEDIATES GLUCOCORTICOID PATHWAY ON CROSS-TALK BETWEEN INFLAMMATORY AND INNATE IMMUNE RESPONSES DURING NODAVIRUS INFECTION

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In Taiwan, orange-spotted grouper (*Epinephelus coioides*) is one of the commercial aquaculture species. The corticosteroid in fish is considered as an important transducer under the stress condition and decreases the congenital immunity of fish. However, stress often influence the growth and immunity of the grouper. As corticosteroid signaling in mammalian is an important regulators of a wide range of adaptive physiologic reaction, research the regulatory mechanism of corticosteroid pathway is necessary.

The glucocorticoid receptor 2 in glucocorticoid pathway involve in modulating inflammation cytokine in stress condition. The transcription level of tumor necrosis factor 1 (TNF1), heat shock protein 90 AB (HSP90AB) and glucocorticoid receptor (GR) compared with healthy one show the increasing expression level under nodavirus infection at 48 hours post-infection in grouper by real-time PCR analysis. Overexpression of GR-EGFP results in higher transcriptional activity level of TNF1 promoter. The study represents the first analysis of a coordinated GR activity regulates the NF- κ B pathway and the control of inflammatory mediators during nervous necrosis virus infection.

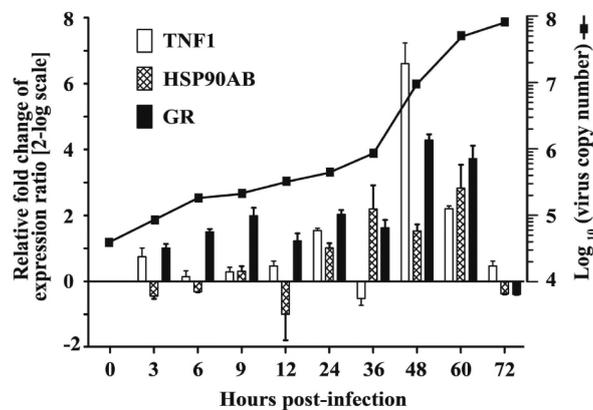


Fig 1. TNF1, HSP90AB, and GR genes expression in the juvenile grouper following nodavirus. The TNF1 (open bars), HSP90AB (gridded bars) and GR (closed bars) defined the gene expression, respectively. The ef-1 alpha gene was used as an internal control to normalize the cDNA template and to do real time PCR calculations. The continuous line shows the virus copy number of nodavirus.

EFFECT OF STORAGE DENSITY AND LIGHT INTENSITY ON THE GROWTH OF *Tetraselmis* sp. DS3

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Tetraselmis sp. DS3 is a heat-tolerant microalgae. The present study was undertaken to evaluate its potential in aquaculture. The influence of storage density and light intensity on algal growth were examined. For the storage duration experiment, two different densities (4 and 9 million cells/ mL) of *Tetraselmis* sp. DS3 were stored in a refrigerator (2 - 4°C) for 1 - 4 weeks. Then, the stored algae were cultured under the temperature of 24.5 - 25.5 °C and illumination of 10000 lx for 5 days. In the second experiment, the effect of different illumination (5000 lx, 10000 lx and 15000 lx) on the growth of *Tetraselmis* sp. DS3 were tested. The algae were cultured under the temperature of 25.7 - 26.5°C for 3 days. In the first experiment, *t*-test was applied to determine the differences between treatments. As for the second experiment, one-way ANOVA with Duncan's Multiple Range Test were performed.

In results, the first experiment shows in day 1 and day 2, there were significant difference between treatments (day 1: $p=0.02$; day 2: $p=0.00$). The density of 4 million cells/mL (day 1: 2.76 ± 0.45 ; day 2: 3.60 ± 0.33 million cells/mL) grew better than the other treatment (day 1: 1.81 ± 0.60 ; day 2: 2.09 ± 0.42 million cells/mL). However, from day 3 to day 5, there was no significant difference. In the second experiment, on day 2, there were significant differences between treatments ($p = 0.036$), 10000 lx treatment (5.80 ± 1.70 million cells/mL) grew better than 15000 lx treatment (2.64 ± 0.05 million cells/mL). On other days, there was no significant difference between treatments.

SOURCE IDENTIFICATION OF THREE MAJOR SPARIDAE FISHES BY USING BAYESIAN CLUSTERING METHOD FOR STOCK ENHANCEMENT IN TAIWAN

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Sparidae fishes, including black seabream (*Acanthopagrus schlegelii*), yellow fin seabream (*Acanthopagrus latus*), and Silver seabream (*Rhabdosargus sarba*), are important species for the stock enhancement in Taiwan. To develop the genetic management strategy for the stock enhancement, 4 to 9 microsatellite markers were used to distinguish the fish from hatchery or wild groups. No or little genetic differences between the hatchery and wild samples were found, and no useful single microsatellite markers can use for source identification. Therefore, Bayesian clustering method based on the STRUCTURE software simulation analysis was further used in this study. Given the prior information and compared with that of the wild samples, the accuracy of the re-analysis of the three species of seabream was 98-100%. These results show that when the prior genetic information has been established, the source identification of these three Sparidae fishes for the stock enhancement is possible.

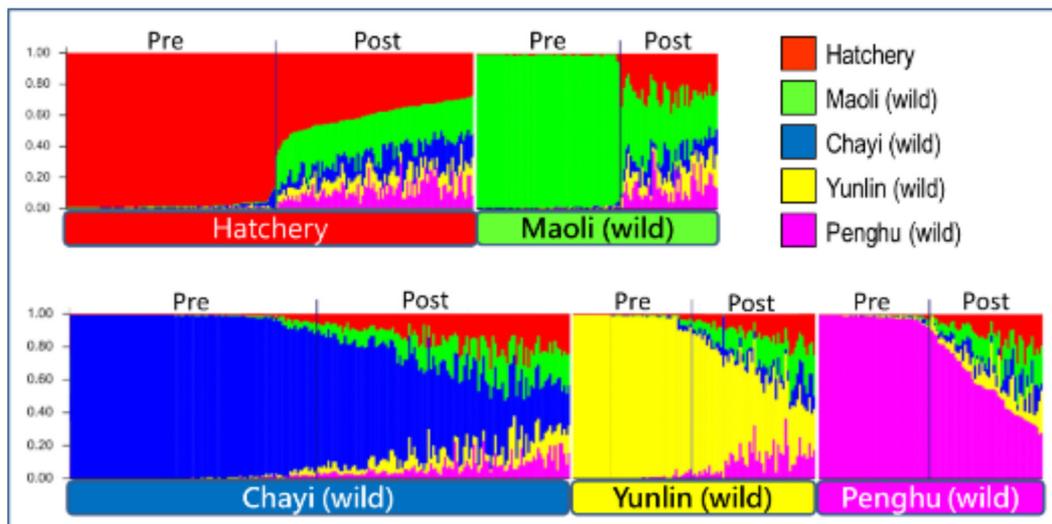


Fig 1. STRUCTURE software simulation analysis for five populations.

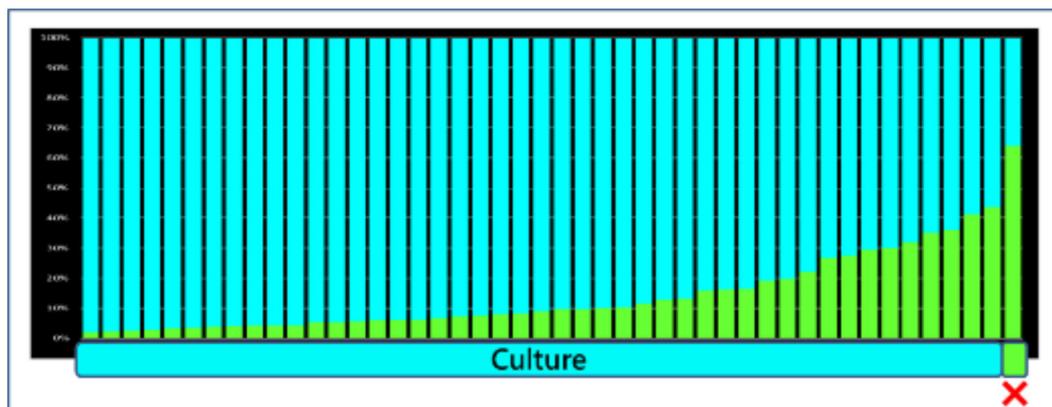


Fig 2. STRUCTURE software simulation analysis for two populations. The accuracy of the re-analysis was 98%.

GAMETE MATURATION IN ZEBRAFISH REQUIRES m⁶A RNA MODIFICATION CATALYZED BY METTL3 METHYLTRANSFERASE

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N⁶-methyladenosine (m⁶A), catalyzed by Mettl3 methyltransferase, is a highly conserved epigenetic modification in eukaryotic mRNA. Previous studies have implicated m⁶A modification in multiple biological processes, but the *in vivo* function of m⁶A has been difficult to study, because *mettl3* mutants are embryonic lethal in both mammals and plants. In this study, we have used transcription activator-like effector nucleases and generated viable zygotic *mettl3* mutant, *Zmettl3^{mm}*, in zebrafish. We find that the oocytes in *Zmettl3^{mm}* adult females are stalled in early development and the ratio of full grown stage (FG) follicles is significantly lower than that of wild type. Human chorionic gonadotropin-induced ovarian germinal vesicle breakdown *in vitro* and the numbers of eggs ovulated *in vivo* are both decreased as well, while the defects of oocyte maturation can be rescued by sex hormone *in vitro* and *in vivo*. In *Zmettl3^{mm}* adult males, we find defects in sperm maturation and sperm motility is significantly reduced. Further study shows that 11-ketotestosterone (11-KT) and 17 β -estradiol (E2) levels are significantly decreased in *Zmettl3^{mm}*, and defective gamete maturation is accompanied by decreased overall m⁶A modification levels and disrupted expression of genes critical for sex hormone synthesis and gonadotropin signaling in *Zmettl3^{mm}*. Thus, our study provides the first *in vivo* evidence that loss of Mettl3 leads to failed gamete maturation and significantly reduced fertility in zebrafish. Mettl3 and m⁶A modifications are essential for optimal reproduction in vertebrates.

TRANSCRIPTOMIC ANALYSES REVEAL DIFFERENTIAL ACTIVATION OF GENETIC INFORMATION PROCESSING PATHWAYS IN GILL STRUCTURE PLASTICITY OF 18°C- AND 15°C-EXPOSED INDIAN MEDAKA, *Oryzias dancena*

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Ambient temperature is an important factor that affects aquatic ectotherms. Fish is an ectotherm which changes its body temperature with the environment. In response to temperature changes, modulations of the endocrine secretion, corticosteroid response, osmoregulation, immune-response change, and metabolism may occur in fish. To evaluate the physiological status of the organisms under low temperature, relative changes of temperature coefficient (Q₁₀) in biological reactions is a reliable parameter. The lowest-tolerant temperature of Indian medaka in seawater (SW) was reported to be 15 °C. Thus, transcriptome of the gills of medaka treated at 28 °C, 18 °C, and 15°C were analyzed. It was hypothesized that cold acclimation may induce gill remodeling to change available gill epithelial surface area. Such tremendous change depends on cell proliferation and may induce a complex network of interacting proteins and enzymes required for DNA replication. Generally, DNA replication follows a multistep enzymatic pathway. According to the functional annotation in the category of genetic information processing in KEGG database, gene expression data for functional enrichment were analyzed. Comparison between 18°C- and 15°C-exposed groups, the numbers of differentially expressed genes were much higher in gills of 15°C-exposed FW Indian medaka. The pathway ko03010, ko03030, ko03040, and ko03050 related to gill structure plasticity were enriched in the gills of 15°C-exposed FW medaka. Among them, 47.86% of genes in the pathway ko03010, 67.74% of genes in the pathway ko03030, 57.66% of genes in the pathway ko03040, and 89.47% of genes in the pathway ko03050 were upregulated. Meanwhile, slight differences were found between the numbers of differentially expressed genes between 18°C- and 15°C-exposed gills of the SW Indian medaka. In summary, the induction of genetic information processing may be linked to higher plasticity of gill structure.

EFFECTS OF *Corbicula fluminea* EXTRACTS AND EPA, DHA ON NME1-REGULATED CANCER PROGRESSION IN HUMAN HEAD AND NECK CARCINOMA SAS CELLS

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Introduction:

Head and neck squamous cell carcinoma (HNSCC) own early metastasis and poor response to chemo-therapy, led the overall 5-years survival is less than 50%. Our previous studies revealed that NME1 (NME/NM23 nucleoside diphosphate kinase 1; Nm23-H1) is metastatic suppressor of HNSCC; low expression of NME1 in HNSCC primary tumor causes chemoresistance, cancer relapse and tumor metastasis. This study aimed to investigate the therapeutic potential of the *Corbicula fluminea* hydroxylysate (CFH), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) on HNSCC, and to investigate the anti-cancer mechanisms.

Methods:

The CFH was obtained by hot water-extracted muscles of freshwater clam, and following hydrolyzed the soluble and insoluble parts by Prozyme 6®. The LCFH (<5 kDa of CFH) was collected by ultrafiltration. Besides, lipid-soluble components of *Corbicula fluminea* contained 1.86% of EPA and 1.42% of DHA. Stable clones of NME1-knockdown (SAS_{shRNA^{Nm23}}) and knockdown control (SAS_{shRNA}) cells were derived from an oral squamous cell carcinoma SAS cell line. The chemosensitivity of SAS cells were observed by trypan blue exclusion assay and cell cycle analysis. The change of cyclins were examined by Western blotting. Cell mobility was measured by transwell migration assay. Cancer cells stemness were observed by sphere formation and aldehyde dehydrogenase (ALDH) activity. Statistical comparison of data between treatments was performed using one-way analysis of variance, followed by Student's t-test.

Results:

Co-treatment of cisplatin with CFH, LCFH (50, 100 mg/ml) or EPA, DHA (3~6 µM) increased chemosensitivity on SAS_{shRNA^{Nm23}} cells. Treatment with CFH and LCFH significantly reduced cell migration in SAS_{shRNA^{Nm23}}. Treatment with EPA and DHA increased cell population of Sphase and G₂M phase, as well as elevated expression levels of cyclin B and cyclin dependent kinase 2 (CDK2). Additionally, EPA and DHA inhibited SAS_{shRNA^{Nm23}} cells sphere formation and decreased ALDH activity in SAS_{shRNA} and SAS_{shRNA^{Nm23}} cells. *Corbicula fluminea* hydroxylysate and EPA, DHA regulate chemosensitivity of HNSCC cells which are potent cancer therapeutic agents.

IMPROVED OMEGA3 POLYUNSATURATED FATTY ACIDS BIOSYNTHESIS AND STUDY COLD TOLERANCE MECHANISM IN TILAPIA BY TRANSGENESIS

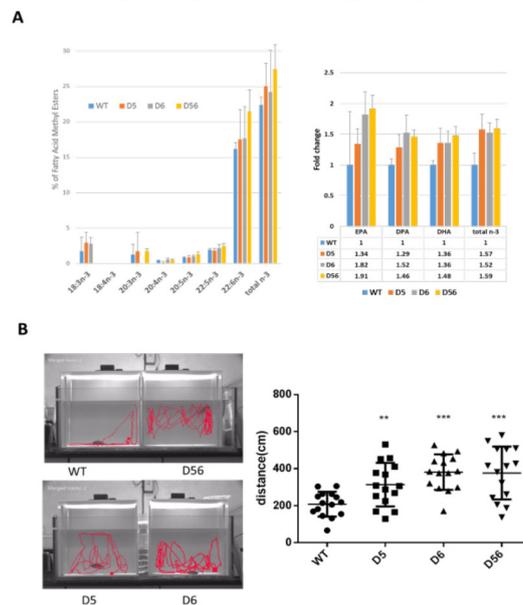
Shin-Jie Haung *, Cheng-Wei Lin, Hui-Chin Huang, Jen-Leih Wu

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Tilapia is an important farmed fish for export and domestic consumption in Taiwan. However, as a tropical fish species, tilapia exposure to cold temperature usually causes mass mortality and severe economic loss. Omega-3 polyunsaturated fatty acids (PUFAs) are a group of essential fatty acids, which can provide anti-cold ability when increased EPA and DHA content. However, the related molecular and physiological mechanisms under cold stress in tilapia remain unclear.

In this work, we investigated the effects of the single and double enzyme combinations on accumulating the high value omega-3 PUFA synthesis by liver specific promoter using the conventional Delta 6-pathway. Until now, double enzyme complex on omega-3 PUFA synthesis has not been thoroughly studied. Here, two single *Fadsd5*, *Fadsd6* and one double transgenic tilapia expressing in both muscle and liver were established to augment the endogenous fatty acid biosynthetic pathway and study cold tolerance ability. The D56 double transgenic tilapia increased the EPA and DHA content to 1.92- and 1.48-fold, respectively. Our findings revealed that combinations of conventional delta-6 omega-3 PUFA critical enzymes cause's alterations in omega 3 PUFA level and improve survival rates under cold stress. In order to gain a deeper understanding the potential molecular mechanisms under cold stress, the transcriptome sequencing of the liver samples from transgenic tilapia were conducted here.

Figure 1 The level of n3 PUFAs and cold tolerance ability are significantly increased in transgenic tilapia



EFFECT OF POLYPHOSPHATES ON LIPID PEROXIDATION ACTIVITY OF FISH SARCOPLASMIC RETICULUM

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The effects of tripolyphosphates (TPP) on lipid peroxidation activity of an iron-stimulated NADH-dependent sarcoplasmic reticular (SR) lipid oxidation system in hybrid tilapia were investigated. Production of thiobarbituric acid-reactive substances (TBARS) was used to measure the activity of lipid oxidation in this system. The system preferred NADH as the reducing agent. The results showed that lipid oxidation activities of SR for control without TPP increased when reaction time increased at 6 °C. Under the same experimental conditions, the lipid oxidation activities of SR containing TPP also increased, but the values were higher than control ($p < 0.05$). The reason for this phenomenon requires further investigation.

GENOMICS ORGANIZATION OF DOWN SYNDROME CELL ADHESION MOLECULE (Dscam) IN TIGER SHRIMP (*Penaeus monodon*)

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Down syndrome cell adhesion molecule (Dscam) is active against pathways in many pathogens, due to its alternative adaptive immunity in invertebrates. Dscam consists of a cytoplasmic tail (associated with signal transduction) and an extracellular region (may be the crucial mechanism for pathogen recognition), both of which are hypervariable. The mechanism of action of Dscam against pathogens is similar to that of antibodies produced by vertebrates. Over past 4 years, we used PacBio and Illumina to elucidate the backbone of Dscam genomics in *P. monodon* (*PmDscam*) and filled the gap with Sanger sequencing. The total genome is ~250 kbp. We analyzed the extracellular region including N-terminal Ig2, N-terminal Ig3 and the entire Ig7 domain and cytoplasmic tail; these had diverse features in *Daphnia* and many other kinds of invertebrates. In addition, we organized the phylogenetic tree including *Daphnia pulex*, *Daphnia magna*, *Drosophila melanogaster* and *Penaeus monodon* by comparing their variable structures and differences of cytoplasmic tail. We concluded that *PmDscam* may be more complex than previously reported.

GROWTH PERFORMANCE, FEED INTAKE AND PHYSIOLOGICAL STRESS RESPONSES IN JUVENILE SABAH GIANT GROUPEL CHRONICALLY EXPOSED TO SUBLETHAL AMMONIA LEVELS

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In intensive farming practice or recirculating aquaculture system, the waterborne ammonia is a crucial limiting factor for aquaculture since it readily accumulates to the level which affects the physiological performance of cultured animals. In this study, juvenile Sabah giant grouper were exposed to different sublethal levels of total ammonia-nitrogen (TAN) for 21 days. The impacts of sublethal ammonia on organismal and cellular stress responses, growth performance, feed intake and osmoregulation were examined. The results showed that no significant difference was detected in plasma glucose while plasma cortisol evidently increased at 20.56 mg/L TAN. Elevation of protein abundance of hepatic heat shock protein 70 (HSP70) was observed in fish exposed to 10.26 and 20.52 mg/L TAN. Moreover, the growth rate in length (GRL), weight (GRW), specific growth rate (SGR) and hepatosomatic index (HSI) significantly decreased at 10.26 and 20.52 mg/L TAN. In addition, rise in the rate of residual feeds and feed conversion ratio (FCR) were found from 10.26 mg/L to 20.52 mg/L TAN and at 20.52 mg/L TAN, respectively. Furthermore, gill Na⁺/K⁺-ATPase (NKA) activity up-regulated in 20.52 mg/L TAN exposed grouper concomitantly with decrease of plasma osmolality and chloride concentration. It was suggested that chronic exposure to 10.26 mg/L to 20.52 mg/L TAN would be physiologically stressful for Sabah giant grouper which led to influence on growth performance and feed intake. The results of the present study would be able to provide the science-based information for waterborne ammonia management in aquaculture system of Sabah giant grouper.

TAN	GRL	GRW	SGR	Rate of total feed intake	FCR	Rate of residual feeds	Condition factor
(mg/L)	(%)	(%)	(%/day)	(%)		(%)	
0	10.95 ± 1.61 ^a	53 ± 6.37 ^a	2.68 ± 0.15 ^a	93.14 ± 1.45 ^a	0.7 ± 0.03 ^a	7.24 ± 1.19 ^a	1.5 ± 0.02 ^a
2.56	10.75 ± 0.9 ^a	50.08 ± 3.92 ^a	2.66 ± 0.07 ^a	93.36 ± 1.33 ^a	0.73 ± 0.01 ^a	6.84 ± 1.14 ^a	1.54 ± 0.03 ^a
5.13	8.53 ± 0.61 ^a	44.9 ± 4.19 ^{ab}	2.19 ± 0.12 ^b	83.3 ± 1.74 ^{ab}	0.89 ± 0.03 ^b	15.78 ± 1.8 ^b	1.55 ± 0.03 ^a
10.26	7.62 ± 0.8 ^{ab}	32.41 ± 4.73 ^b	1.75 ± 0.14 ^c	72.81 ± 3.85 ^b	0.87 ± 0.02 ^b	26.73 ± 3.21 ^c	1.48 ± 0.03 ^a
20.52	4.29 ± 0.58 ^b	10.82 ± 3.41 ^c	0.72 ± 0.21 ^d	39.05 ± 6.91 ^c	1.36 ± 0.1 ^c	56.89 ± 5.9 ^d	1.2 ± 0.08 ^b

PRODUCTION OF ULVAN OLIGOSACCHARIDES BY CRUDE ENZYMES OF MARINE BACTERIA

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This study is aim to hydrolyze ulvans by marine bacteria induced-enzymes for producing ulvan oligosaccharides. Ulvans were gained from *Ulva* sp. that treated by hot- water, then collected by ultrafiltration system (> 30 kDa), and precipitated in 95% ethanol. The yield, sulfate content, and soluble total phenols of ulvans are 15.43%, 21.29%, and 0.11%, respectively (Table 1). Adding 0.3% ulvans in modified marine broth media could induce marine bacteria *Pseudomonas vesicularis* MA103 and *Aeromonas salmonicida* MAEF108 to produce ulvan- degrading enzyme at 26°C (Fig. 1). After ulvans were hydrolyzed by crude enzymes solution from MA103 and MAEF108, using an ultrafiltration system (Membrane: 3 kDa) differentiated ulvans hydrolysate to acquire ulvan oligosaccharides (UOS_{3k}). The yield, sulfate content, and soluble total phenols of UOS_{3k} are 2.78%, 33.99%, and 0.73%,

respectively (Table 1). HPLC chromatogram of ulvans (Column: SB-804) showed molecular weights (MW) of ulvans are 769.4 kDa and 136.9 kDa (Fig. not shown). The relative ratio of fractions in ulvans were about 53.2% and 9.1% based on total peak area. Further, HPLC chromatogram of UOS_{3k} (Column: GS-320) indicated MW of UOS_{3k} are 24.5 kDa, 6.4 kDa, and 825 Da (Fig. 2).

Table 1. The yield, sulfate, and soluble total phenols of ulvans and UOS_{3k} from *Ulva* sp. powder (n = 3)

	Ulvans	UOS _{3k}
Yield (%)	15.43	2.78
Sulfate (%)	21.29	33.99
Total phenols (%)	0.11	0.73

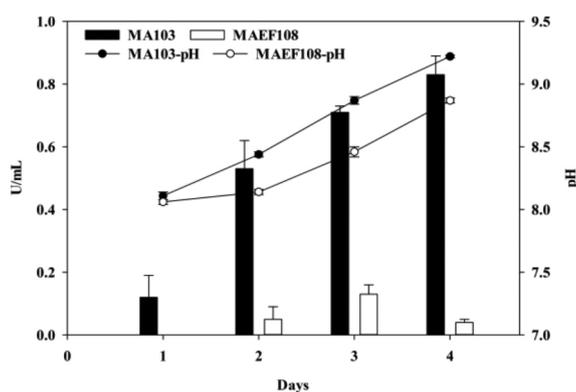


Fig. 1. Changes of activity and pH of endo-ulvan lyase induced by MA103 and MAEF108 (n = 3).

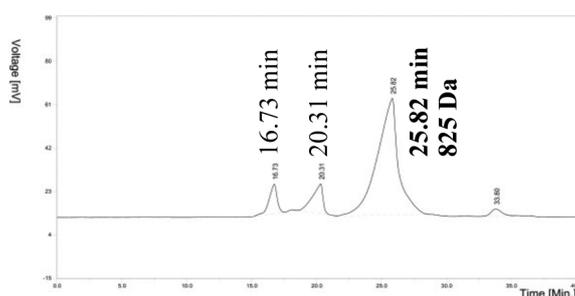


Fig. 2. HPLC chromatogram of UOS_{3k}.

IDENTIFICATION AND FUNCTIONAL ANALYSES OF ZEBRAFISH *mucin* GENE FAMILY

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Mucins are large molecular weight glycoproteins and grouped into two types of mucins including transmembrane mucin and gel-forming mucin. Gel-forming mucins are the major component of mucus layer and gastrointestinal mucus layers acts as first line of defense against bacteria. However, mucin members and their function in zebrafish are still unclear. Here, we identified 14 mucin genes in zebrafish. They include gel-forming mucins (*muc2.1*, *intestinal mucin-like*, *muc5.1*, *muc5.2*, *muc5.3*, *muc5f* and *muc6-like*) and transmembrane mucins (*muc1-like*, *muc3a*, *muc4*, *muc13a*, *muc13b*, *muc17* and *muc18*). Transcripts of *muc5.2* and *muc13a* were observed in the epidermis from 24 to 96 hours post fertilization (hpf). *muc5.3* was expressed in the pharynx at 96 hpf while *muc5.1* was expressed in both epidermis and pharynx. Expression of *intestinal mucin-like* and *muc2.1* was detected in the intestine from 72 hpf and 96 hpf. *muc13b* was expressed in the pronephric duct from 24 to 96 hpf and in the intestine from 72 to 96 hpf. Transcript of *muc6-like* was detected in the olfactory bulb and anus from 24 to 96 hpf. *muc18* was expressed in the lateral line primodium and brain region from 24 hpf. After 72 hpf, *muc18* was also expressed in the semicircular canals. Furthermore, we knocked down *muc2.1* expression with specific morpholino oligonucleotide. The presence of mainly immature goblet cells in the intestine was identified in *muc2.1* morphants as compared to wild type embryos at 104 hpf and 192 hpf by Alcian Blue staining. This result is consistent with the appearance of small and condensed goblet cells in the colon observed in *Muc2* deficient mice. We also treated *muc2.1* morphants and wildtype embryos with or without dextran sodium sulfate (DSS), a colitis-inducing agent and compared expressions of different inflammatory cytokines. We discovered that *muc2.1* deficiency did not result in up-regulation of inflammatory cytokines as that detected in *Muc2* deficient mice. Together our study demonstrates the important function of *Muc2.1* in intestinal barrier function.

WATER ACIDIFICATION: A LIMITING FACTOR ON LARVAL SETTLEMENT OF DONKEY'S EAR ABALONE *Haliotis asinina* L.

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A study was conducted to determine the effects of varying pH levels on the settlement of veliger larvae of donkey's ear abalone, *Haliotis asinina* in a recirculating culture condition at Tawi-Tawi Multi-species Hatchery in Barangay Lato-Lato, Bongao, Tawi-Tawi for the duration of three months and eight days, from July 21 to October 29, 2013 with four pH values as treatment factors: 1) pH 7.6-8.0 as control, 2) pH 7.1-7.5 3) pH 6.6-7.0 and 4) 6.6-6.5 using a complete randomized design (CRD).

The calculated mean of settled larvae population in different pH levels revealed that control has the highest mean at 265.17. The mean for treatment 1 (T1) was 84.50. For treatment 2 (T2), the mean was 40.83 and for treatment 3 (T3), 30.50. Differences between means of replicate analysis at 0.05 level of significance were analyzed using the Tukey's (HSD) test and the analysis of variance (ANOVA). These revealed that control was significantly different from the three treatments, but T1, T2 and T3 were not significantly different from each other. The pH reduction affected settlement in these three treatments.

The frequency and abundance of settlement, and the survival rate declined as pH reduced or water acidity increased. Deformities and mortality increased as water acidity increased. Mass mortality and severe dissolution were observed in high water acidity levels. This study revealed that water acidification is detrimental to settlement of the *Haliotis asinina* or donkey's ear abalone larvae.

Table 2. Calculated Mean of Treatments

TRIAL	REPLICATE	CONTROL	TREATMENT 1	TREATMENT 2	TREATMENT 3
1	R ₁	120	144	65	58
2	R ₁	272	53	27	16
3	R ₁	344	75	18	6
1	R ₂	135	106	78	60
2	R ₂	362	75	47	38
3	R ₂	358	54	10	5
Mean		265.17	84.50	40.83	30.50

Table 5. Relative frequency (%), Relative abundance (%) and Survival rate (%)

Treatment	Relative frequency (%)	Relative abundance (%)	Survival rate (%)
Control	56.25	3.30	2.10
T1	48.96	1.06	0.27
T2	41.00	0.51	0.00
T3	36.46	0.38	0.00

Table 6. Percentage of Deformities in Settled Larvae

Treatment	Unshelled	Minute Size and not well-developed	Crashed	Dissolved	Empty Shell
Control	-	-	10%	-	42%
T1	12%	10%	15%	33%	30%
T2	31%	20%	-	49%	-
T3	13%	-	-	87%	-

GROWTH RATES OF *Kappaphycus striatum* AND *Kappaphycus alvarezii* USING RAFT METHOD

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The study was conducted to investigate the growth rate of seaweeds using raft method in coastal waters of Pababag, Bongao, Tawi-Tawi with a culture duration of 45 days. There were two species of seaweeds used, the *Kappaphycus striatum* (T1) and *Kappaphycus alvarezii* (T2) with three replicates in each treatment.

Results showed that higher mean weight of 338.75 g and growth increment of 5.3g/day were obtained from *Kappaphycus alvarezii* (T2), although not significantly different from mean weight of 322.5 g and growth rate of 4.9g/day obtained from *Kappaphycus striatum* (T1). Higher production of 1911 g/m² was also obtained from the same species *Kappaphycus alvarezii*. However no significant difference was observed between the two treatments. The difference in growth and production of *Kappaphycus alvarezii* was observed to be related to morphological aspects of the thalli. *Kappaphycus alvarezii* had more branches and many spines compared to *Kappaphycus striatum*. These results showed that farming of *Kappaphycus alvarezii* or *Kappaphycus striatum* using raft method are profitable and economically viable.

Table 1. T-test analysis of mean weight in grams of *K. striatum* and *K. alvarezii* after 45 days culture period.

Variables	n	Mean	Variance	SD	Computed t	Tabular t $\alpha=0.05$
T1- <i>K. striatum</i>	3	322.50	207.81	14.42	1.56 ^{ns}	2.13
T2- <i>K. alvarezii</i>	3	338.75	118.75	10.9		

ns = not significant

Table 2. T-test analysis of mean weight gain in grams of *K. striatum* and *K. alvarezii* after 45 days culture period.

Variables	n	Mean	Variance	SD	Computed t	Tabular t $\alpha=0.05$
T1- <i>K. striatum</i>	3	222.50	207.82	14.42	1.56 ^{ns}	2.13
T2- <i>K. alvarezii</i>	3	238.75	118.75	10.9		

ns = not significant

Table 3. Mean weight, mean weight gain and daily weight gain in grams of *K. striatum* and *K. alvarezii* after 45 days culture period.

Variables	n	Mean Weight	Mean Weight Gain	Daily Weight Gain
T1- <i>K. striatum</i>	3	322.50	222.50	4.94
T2- <i>K. alvarezii</i>	3	338.75	238.75	5.31

TILAPIA CULTURE, AN EMERGING TREND IN FISH FARMING IN PUNJAB, PAKISTAN

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Tilapia culture has shown excellent progress in aquaculture sector in Punjab, Pakistan in last six years. Three important steps were taken for the development of this. The fish farmers training programs were arranged. The establishment of first tilapia hatchery in the country made it possible for the availability of monosex tilapia seed locally. The manufacturing of floating tilapia feed through extruder technology has also contributed in the development of this sector.

The monosex culture of tilapia (*Oreochromis niloticus*) is practiced on semi-intensive aquaculture system. Both locally produced and imported (from Thailand) monosex tilapia seed is stocked in earthen ponds (stocking density vary from 6250-10000 fry/ha). The fish is reared for 7-8 months (April onwards) and harvested in the start of winter. There are reports of fish mortality in ponds due to delayed harvesting. The fish is fed on high quality floating tilapia feed (30% protein). The fish yield is also variable from 4400-7000kg/ha. This is due to the level of fish farm management, technology adopted and input administrated by the farmers.

The monosex tilapia has established its position in local market and is high in demand too. The price of fresh fish depends on supply and demand as well as the size of the fish. This year, 600-700g fish had good price (Pak. Rupees. 220-230/kg, approximately US\$ 2.0/kg). However, last year the fish price of fish was almost half than this year price.

The polyculture of monosex tilapia with major carp like: *Labeo rohita* is also practiced by some fish farmers. These two fishes have shown good results and yield has been excellent (average weight 900g and 3100g for monosex tilapia and *L. rohita* respectively).

Although, monosex tilapia culture has shown emerging trend in Pakistan and is growing fast. Yet fish farmers raise certain issues such as: low cost fish feed, fry survival, fish health management, fish processing, marketing and maintaining quality standard. The aspects of tilapia culture in the province of Punjab, Pakistan are discussed.

ASSESSMENT OF THE INHIBITORY EFFECT OF SELECTED MEDICINAL PLANTS AGAINST *Aeromonas sobria* IN NILE TILAPIA (*Oreochromis niloticus* L.)

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In vitro experiment was used to examine the inhibitory effect of selected medicinal against *A. sobria*. In the In vivo experiment Nile tilapia was exposed at two concentrations of acacia, annatto and mango to evaluate the inhibitory effect against *Aeromonas sobria*. Results in in vitro experiment show that Zone of inhibition in acacia against the bacterium was only recorded in 100% extract concentration only. Extracts of annatto and mango in all concentration levels (25%-100%) showed zone of inhibition against the bacterium having the widest and narrowest diameter in 100% and 25% concentration, respectively. Using the highest bacterial suspension (10⁻⁷), extract of annatto and mango showed significant and wider zone of inhibition as compared to mango across extract concentrations.

In vivo experiment showed that disease symptoms such as red spots, haemorrhages and lesions have occurred in the challenged tilapia as early as Day 3. As compared to the initial TWBC, the study found out that there was apparent increase in TWBC count right after the appearance of the symptoms (three days after the challenge test). Significant increase in TWBC was only recorded in Treatment VII After 6 weeks prior to leaf extraction administration, there was decreased in the final TWBC of the experimental fish. Even not statistically significant, T5 (3% annatto) and T7 (3% mango) were effective in reducing the final TWBC count (T5 = reduced by 17.49 /mm³; T7 = reduced by 23.02/mm³) prior to infection. Four types of WBC were identified namely, monocytes, neutrophils, lymphocytes and basophil. Initial differential count revealed that percent composition of WBC was dominated by neutrophils, followed by lymphocytes and lastly monocytes.

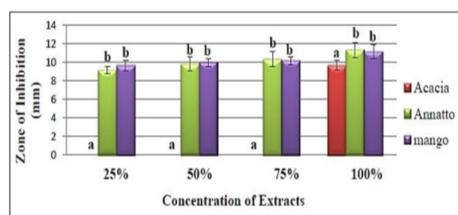


Figure 1a. Inhibitory zone of the three plant extracts against 10-5CFU/mL *Aeromonas sobria* (Different letter was significant at $p < 0.05$).

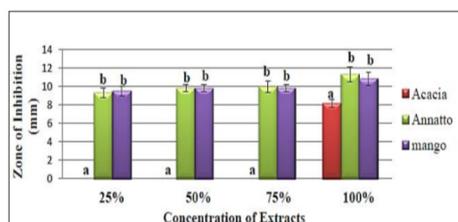


Figure 2a. Inhibitory zone of the three plant extracts against 10-6CFU/mL *Aeromonas sobria* (Different letter was significant at $p < 0.05$).

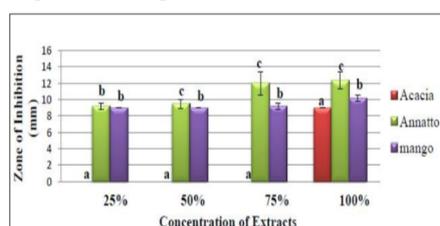


Figure 2c. Inhibitory zone of the three plant extracts against 10-7CFU/mL *Aeromonas sobria* (Different letter was significant at $p < 0.05$).

EFFECT OF A NOVEL FEED ADDITIVE ON PERFORMANCE AND HEALTH INDICATORS DURING NATURAL THERMAL FLUCTUATIONS IN GILTHEAD SEA BREAM *Sparus aurata* CAGE CULTURE

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In the Mediterranean basin on-growing and fattening of gilthead sea bream (*Sparus aurata*) occurs in sea cages. However, a significant disadvantage of sea cage culture in temperate climates is that fish are exposed to low sea water temperatures during winter which negatively affects growth and fish physiology, mainly by the fattiness condition (reviewed in Ibarz et al., 2010). The induced cold leads to fast mobilization of perivisceral fat, a rise in liver lipid content, and a decrease in muscle lipid content. The impact of the seasonal temperature variations can be alleviated by the use of seasonal feed formulations to enhance liver condition and metabolism throughout temperature adaptations. The aim of these study was to evaluate the potential of a novel feed additive based on natural compounds with metabolic and liver protectant properties in winter preparation and recovery in seabream. A feeding trial was run under production conditions and aimed to provide empirical data of the effects of the feed additive on the fish performance and a series of indicators for the health status in gilthead sea bream.

The trial was performed in a sea cage farm in Spain from November to May following standard operational protocols. Two groups of gilthead sea bream (5 cages per treatment) were fed a commercial diet (49%CP, 16% CL) without or with the additive, Control and Treatment, respectively. Fish were sampled in November, February and May (25 fishes per condition) collecting epidermal mucus, blood, plasma and liver.

Based on the sampled fish, Treatment group showed higher SGR in the different sampling points, i.e. during the cold period in February, and during recovery in May. Plasma markers reflected seasonal fluctuations but were not affected by the diet. With regards to liver metabolism, Treatment fish diminished the use of amino acids as source of energy (measured by hepatic transaminases), decreased lipogenesis (measured by G6PDH enzyme) and showed a higher redox capacity (measured by glutathione turn-over) during the cold period (February). During temperature recovery, liver metabolism did not significantly differ between diets, but lower visceral fat deposits were found in the treatment group. Furthermore, mucus metabolites (glucose/protein ratio) indicated Treatment fish were less affected by stress compared to Control fish. Data obtained from a broad set of plasma, liver and mucus markers, during natural seasonal thermal fluctuation, appointed to a better condition of seabream fed the additive during the cold season.

EFFECTS OF SEAWATER SALINITY ON GROWTH PERFORMANCE OF JUVENILE THREAD-SAIL FILEFISH *Stephanolepis cirrhifer* AND APPLICATION OF RECYCLED AQUACULTURE SYSTEM

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The thread-sail filefish (*Stephanolepis cirrhifer*), a popular aquaculture species in Japan, is thought to have various characteristics suitable for a recycled aquaculture system (RAS); however, the seawater salinity appropriate for rearing has not been clarified. In this study, using triplicated experiments, we investigated the effects of various seawater salinities on the growth performance, stress tolerance, starvation tolerance, etc., of juvenile fish, and examined their growth performance in an RAS.

Experiment I: Ten fish (average body weight 1.52 g) were kept in 200 L tanks with salinities of 34, 26, 18, and 10 psu, and reared for 21 d to assess growth performance. Net-handling stress tolerance was compared among salinities. Experiment II: Ten fish juvenile (average body weight 2.7 g) were housed in a 200 L tanks with salinities of 10, 13, 16 and 19 psu, and raised for 21 d to assess growth performance and plasma osmotic pressure. Experiment III: Juveniles (average body weight 14.4 g) reared at 13 psu were individually stocked in 30 L tanks of 34, 13, and 10 psu, and changes in weight gain without feeding were examined after 1, 3, 5, and 7 d. The water content of whole body (WCB) were measured after 7 d. Experiment IV: Ten juveniles (average body weight 1.9 g) were placed in 200 L RAS tanks with salinities of 10, 13 and 34 psu, and reared to assess growth performance.

Experiment I: Weight gains at 18 and 10 psu were markedly higher than at 34 psu. Feed efficiency and condition factor tended to be higher at 10 psu than at other salinities. Net-handling tolerance tended to be higher at low salinities than at 34 psu. Experiment II: Survival rate did not differ significantly, though weight gain and feed efficiency at 10 and 13 psu were markedly superior to those at 19 psu. The total length at 13 psu was significantly higher than that at 10 psu, and conversely, the condition factor was markedly higher at 10 psu than at 13 psu. The plasma osmotic pressure at 19 to 13 psu was lower than that of their seawater, and was not significantly different among salinities; however, the plasma osmotic pressure at 10 psu was the lowest measured, though it was higher than that in 10 psu seawater. Experiment III: The weight gain at 10 psu increased at 1 d, then significantly increased compared to that at other salinities. In addition, the WCB at 10 psu tended to be higher than that at 34 psu. Experiment IV: The growth of juveniles at 13 psu was the largest measured, and was significantly higher than that at 34 psu.

These results suggest that 13 psu seawater is best for rearing juvenile Thread-sail filefish; 10 psu seawater showed high levels of the condition factor, WCB, and plasma osmotic pressure compared to 10 psu seawater, indicating that rearing water is likely to flow into the body. The effect of 13 psu seawater was also confirmed in the RAS system.

MUSHROOMS ON AQUACULTURE: WILD TYPE MUSHROOMS AGAINST FISH PATHOGENS *Streptococcus iniae* AND *Pseudomonas aeruginosa*

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Wood-rotting mushrooms were collected inside the premises of Isabela State University to evaluate its antimicrobial activities against fish pathogens, *Streptococcus iniae* and *Pseudomonas aeruginosa*. These fish pathogens were most common among Nile Tilapia (*Oreochromis niloticus*) and were also zoonotic with infections in humans associated with the handling and preparation of infected fish. Nowadays, there have been numerous reports stating the current condition of aquaculture in the Southeast Asia as affected by these pathogens resulting to low yields in tilapia farming. This study was conducted to assess the capability of four (4) wild mushroom ethanol extracts in the control of *S. iniae* and *P. aeruginosa*.

Four mushrooms were collected and subjected for mycelial production for the preparation of the ethanol extracts including *Ganoderma lucidum*, *Auricularia auricula*, *Polyporus sanguineous* and *Trametes hirsuta*. Five hundred grams (500g) of air-dried mycelial mats were pulverized and soaked on a 95% ethanol for 48 hours prior filtration. Filtrates were subjected to rotary evaporator at 300 rpm for three hours until a thick residue remained. These were stored on a lab refrigerator until further use.

Ten (10) ml of sterile nutrient broth were aseptically inoculated with bacterial cultures of *S. iniae* and *P. aeruginosa* incubated at 37°C for 12 hours. This was used as inoculum swabbed in a previously prepared sterile Mueller-Hinton agar plates. Meanwhile, 6-mm of paper disc were seeded with mushroom ethanol extracts. Plates were then incubated at 37°C for 24 hours. Zones of inhibition around the disc were recorded.

Results revealed that *G. lucidum* ethanol extracts has the highest zone of inhibition against *S. iniae* (28.65mm) which is not significantly different at 5% level of significance compared to *A. auricula*. On the other hand, *G. lucidum* elucidated the highest zone of inhibition against *P.aeruginosa* compared to other treatments. However, it was observed that *P.sanguineous* (19.31mm) ethanol extracts has low resistance against *S.iniae*, while *T. hirsuta* (19.46mm) has a low resistance against *P.aeruginosa* compared to other treatments (Table 1).

Table 1. Zones of inhibition of four mushroom ethanol extracts against fish pathogens after 24 hours of incubation

Mushroom ethanol extracts	Zone of inhibition (mm)	
	<i>S. iniae</i>	<i>P. aeruginosa</i>
<i>G. lucidum</i>	28.65 ^b	26.76 ^b
<i>A. auricula</i>	27.93 ^b	23.87 ^c
<i>P. sanguineous</i>	19.31 ^c	22.34 ^d
<i>T. hirsuta</i>	20.44 ^c	19.46 ^e
Streptomycin (+)	32.68 ^a	34.45 ^a

Note: superscript with the same letters are not significantly different at 5% level of significance.

CHANGES OF DIGESTIVE ENZYMES ACTIVITY WITH DIFFERENT DIETS IN *Rutilus frisii kutum*

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The ontogenesis and specific activities of digestive enzyme (pepsin, protease, trypsin, chymotrypsin, amylase and lipase) in four diets (starved, egg yolk, *Artemia* nauplii, egg yolk and *Artemia* nauplii) were investigated in *Rutilus frisii Kutum* from the start of exogenous feeding (3 day after hatching, DAH) to the juvenile stage at 30 DAH.

Protease activity increased with growth after 21 DAH. Specific pepsin content peaked between 5-7 DAH and then decreased in all treatments.

No significant differences in total chymotrypsin activity were observed between larvae fed on *Artemia* and egg. After the first feeding, specific activity of amylase and lipase is increased and reach a peak at 11 DAH. The total lipase activity generally increased with the larval development. Generally the total amylase activity among fed larvae decreased with time till 16-24 DAH after which the activity increased. These findings suggested that Caspian kutum larvae should be able to ingest, digest and absorb food particles within 50- 100 μ m from 3 DAH onwards. The functional alimentary tract of kutum larvae was completed by the 30 DAH. A combination diet of *Artemia* and egg yolk was the best for the culture of kutum during early life stages. Our results suggest that Kutum is capable of digesting protein, lipid and carbohydrates at early stages of growth.

Specific activity of most digestive enzymes exhibited a sharp increase with co-feeding started from 9-13 DAH.

Further studies on the larval feed development should be conducted to improve the production and quality of kutum fry.

EXPRESSION OF *KISS/KISSR* IN THE JAPANESE EEL, *Anguilla japonica*

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The eel is a catadromous basal teleost, which exhibits a complex life cycle with spawning in the ocean and growing up in continental waters. Silvering is a prepubertal metamorphosis preparing the eel to the oceanic reproductive migration. A moderate gonad development occurs during this metamorphosis from the sedentary yellow stage to the migratory silver stage. However, before the oceanic reproductive migration or under captive condition, the eels have immature gonads due to a lack of gonadotropin synthesis and release. Kisspeptin system is known plays an important role in the onset of puberty in mammals by activating the GnRH neurons and inducing GnRH release. The aims of the present study were to decipher the expression patterns of *Kiss/ KissRs* in brain-pituitary-gonadal (BPG) axis at different ovarian developmental stages in wild female Japanese eels, the effects of sex steroids and salmon pituitary homogenates (SPH) on *Kiss/KissRs* expression levels in BPG axis of female Japanese eels. In the brain, *KissR-1* mRNA-expressing cells are observed in the ventral zone of periventricular hypothalamus and habenula; *KissR-2* mRNA-expressing cells are observed in the periventricular pretectal nucleus, Hv and nucleus of the lateral recess; *KissR-3* mRNA-expressing cells are exclusively observed in the habenula. The GSI of the wild female eels were in the range of 0.18-2.3 %, corresponding to yellow, pre-silver and silver stages. The *Kiss1*, *Kiss2*, *KissRs*, *mGnRH* and *GnRHRs* transcripts did not change in the brain and pituitary of wild female eels suggest that the Kiss/GnRH system is not significantly activated during silvering. In the SPH-treated eels, our previous study has showed that GSI, serum estradiol-17 β (E2) and testosterone concentrations, brain mGnRH transcripts, pituitary *LH-b* transcripts were significantly increased. In the present study, we found *Kiss2* transcripts in the brain, *KissR-1* transcripts in the brain, pituitary and ovary, *GnRHR-2* transcripts in the pituitary were significantly increased in the SPH-treated eels. *KissR-2* transcripts in the ovary were also significantly increased but pituitary *KissR-2* transcripts were significantly decreased in the SPH-treated eels. No increase in GSI was observed after treatment with E2 or T in female eels. E2 significantly increased brain *KissR-1* transcripts and pituitary *LH-b* transcripts in female eels. Our results could explain why eels need chronic treatment with exogenous hormones to stimulate the gonadal development. This study also revealed that *KissR-1* and *GnRHR-2* may involve in reproduction regulation in eels.

REVIEW: APPLICATION OF BIO-HYDROLYZED SOYA PROTEIN IN AQUAFEED

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High demands for the rapid development of global aquaculture and limited supply cause high price for fish meal (FM). Therefore, searching suitable alternative protein ingredients to replace FM has become a most important issue. Soybean meal (SBM) is a most widely used plant ingredient in aquafeed, but the use still limited by presence of anti-nutritional factor. However, fermented soybean meal (FSBM) is the application of microorganism fermentation, in addition to reduce the anti-nutritional factors and improve the nutritional value, its functional like enhancing of aquatic animal immune response and disease resistance had also been gradually concerned in recent years. The aim of this study was to review a series trial which investigate the effect of dietary Bio-hydrolyzed soya protein (BHSP) on growth, immune response and gut health in aquatic animal.

3 trials regarding the effect of dietary BHSP on growth performance in grouper, white shrimp and Asian seabass were conducted by Prof. Win Ton Cheng from aquaculture department of National Pingtung University of Science and Technology (NPUST), Taiwan. The results of these three trials showed the growth were not significantly different compared between FM diet without plant ingredient and those fed diet with BHSP replaced FM.

The trials investigated the effect of dietary BHSP on feed digestibility in grouper and white shrimp were conducted by associate Prof. Yu Hung Lin (NPUST). These two studies indicated that BHSP could improve the adverse effect caused by the use of SBM in aquatic animal (FIGURE 1).

At the latest established journal, the study comparison of dietary commercial/fermented soybean meal on non-specific immune response in white shrimp, Lin and Mei (2017) demonstrated that non-specific immune response of white shrimp significantly reduced when dietary FM replaced by 30% SBM. Finally, BHSP prepared using *Lactobacillus* spp. can improve these negative effects (TABLE 1).

FIGURE 1. Protein digestibility of grouper and white shrimp fed different diets for 3 and 4 weeks respectively.

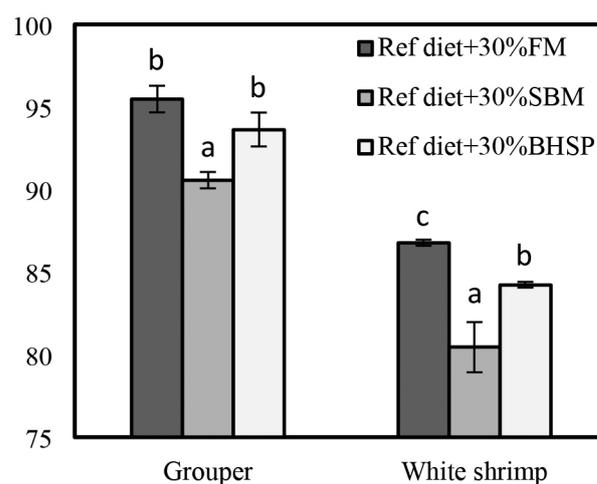


TABLE 1. Immune parameters of white shrimp fed different diets for 12 weeks.

	Control	SBM	BHSP
THC	3.68 ± 0.25 ^c	2.10 ± 0.13 ^a	2.87 ± 0.19 ^b
HC	1.88 ± 0.14 ^c	1.28 ± 0.09 ^a	1.57 ± 0.16 ^b
SGC	1.23 ± 0.10 ^c	0.65 ± 0.05 ^a	0.93 ± 0.09 ^b
GC	0.57 ± 0.01 ^c	0.18 ± 0.05 ^a	0.38 ± 0.07 ^b
PO	1.49 ± 0.21 ^b	1.09 ± 0.02 ^a	1.45 ± 0.06 ^b

EFFECT OF PURE NATURAL OREGANO ESSENTIAL OIL AS FEED ADDITIVE ON MORTALITY CONTROL IN JUVENILE OF SHRIMP *Litopenaeus vannamei* IN COMMERCIAL RACEWAYS IN ECUADOR

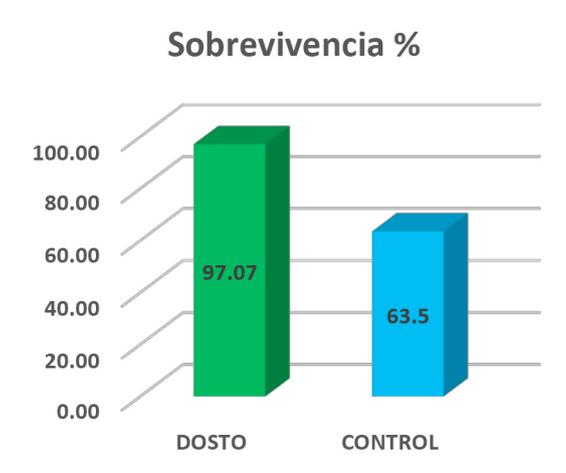
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Shrimp aquaculture production technology in Ecuador includes the use of Raceways and nursery ponds as starting productions units after stock the grow out ponds. Post larvae comes from the commercial hatcheries and maintained in this units for 8 to 15 days in average, depending if they are concrete tanks commonly called Raceways or earthen ponds called nursery. Using this units allows the farm managers have a better control of diseases, bacterial load in gut, improve nutritional profile and clear the population from weak post larvae that may come from the hatcheries.

Field trial was conducted in a commercial Raceways operation to incorporate pure natural Essential Oil to a feeding protocol that already uses other alternatives as functional additives. Challenging commercial conditions, it was expected a disease outbreak during the production time. Tanks were randomly assigned to receive the DOSTO OREGANO treatment 0.2 ml/Kg of feed daily during all the cycle and control group with functional feed with probiotics and organic acids. Final survival was measured and compared to control group, ROI was estimated based on Juvenile commercial price in Ecuador for a shrimp farm, and commercial cost of DOSTO OREGANO Pure Natural Essential Oil.

After 8 days of production, bacterial disease causes high mortalities in control group, but significant difference was noticed in treatment group were up to 97.07% of survival was measured vs 63.5% on control group. ROI was totally out of measurement due to high value of juveniles vs cost of DOSTO Pure Oregano Oil.



ENHANCING CAROTENOIDS EFFICACY IN AQUAFEED

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Carotenoids are lipid soluble pigments that are responsible for skin color of fish and shrimp. The yellow, orange and red hues found in fish skin and shrimp result from carotenoid pigments, both carotene and xanthophylls. Not only important for color, carotenoids are also vital nutrients for healthy growth, metabolism, light absorption, reproduction and fertility, and for their functions as antioxidant and vitamins. Carotenoids cannot be synthesized by most animals including aquatic species and must be ingested with the diet. Carotenoids accumulate in the integument and other tissues. Different species metabolize carotenoids differently. Fancy carp can convert lutein, zeaxanthin to astaxanthin and goldfish are highly efficient at converting lutein and β -carotene to astaxanthin. Shrimp has the ability to convert carotenoids like lutein, β -carotene, canthaxanthin to astaxanthin and accumulate astaxanthin in carotenoproteins which are stable complexes. These complexes in which carotenoids are bound to high-density lipoproteins give a blue to green color to live crustacean. Upon cooking, astaxanthin is released from the protein complex and generates a red-orange color. Because aquatic animal cannot synthesize astaxanthin, they acquire it from external sources such as microalgae, yeast, salmon fishmeal, trout fishmeal, krill, shrimp meal, crayfish, crustaceans, feathers of some birds or from supplements in the feed. Astaxanthin can be accumulated better than xanthophylls (oxygenated carotenoids) and possesses high antioxidant activity. In aquaculture, many factors such as temperature, light intensity, salinity, and animal stress affect carotenoid stability, hence have an impact on fish skin and/or shrimp color. To enhance carotenoids efficacy, supplementary feed additives or combination of carotenoids are recommended in aquafeed.

IS SHRIMP FARMING LIKE GAMBLING? A BEHAVIOURAL ANALYSIS OF FARMERS' DECISIONS

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Shrimp farming is considered a “risky business” and often compared to gambling for farmers. It is associated with a diverse range of risks, including volatile markets, climate variability, and production risks. As a response to risk, farmers deploy different risk management strategies such as, initiating contract farming, stocking pathogen-free post larvae, using diverse pro-biotic and/or water treatments or implementing a sequential stocking and harvesting technique.

To understand the underlying reasons explaining farmer’s behaviour, we explored, using multivariate statistics, the relations between perception of different sources of risk, confidence in mitigating risk and adoption of risk management strategies. We performed the analysis on dataset collected from 250 Vietnamese shrimp farmers in the Mekong Delta operating extensive, semi-intensive or intensive farms (Figure 1).

Results indicate that different farm types are associated with different patterns of risk management strategies and that farm types predict different levels of perceived risk severity and confidence in risk mitigation. Mediation analysis shows that the perception of market risk is a key predictor of risk management strategies deployed by farmers. The level of confidence in selecting the right inputs to manage the pond is a predictor of knowledge-based management strategies, while confidence in controlling disease predict market-based strategies.

Our results highlight the absence of efficient market risk mitigation measures available to farmers and underline the lack of regulation or absence of specific value chain organisation to mitigate this type of risk. Using a behavioural approach provides new insights into how farmers approach risk and make management decision for their farms. It highlights differences in risk perception, and in responses to risk across farm types. We show that farmers, unlike actual gamblers, build their choices on the evaluation of different sources of risk, their confidence in their ability to mitigate risk and the implementation of diverse management strategies.

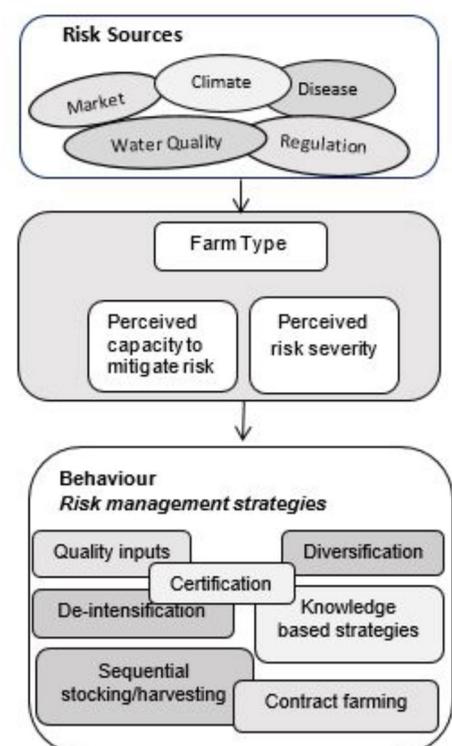


FIGURE 1: Relationship between risk sources, farm type, risk attitude and adoption of risk management strategies

THE PRODUCTION OF COMET-TAILED GOLDFISH (*Ciprinus auratus*) AND SEVERAL TYPE AQUATIC PLANTS IN AQUAPONICS SYSTEM

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Aquaponics is the combination of aquaculture and hydroponics together in one integrated system. In aquaculture, excretions from fish the animals accumulate in the water and increasing ammonia (NH₃), nitrites (NO₂) and nitrates (NO₃). In an aquaponic system, the ammonia, nitrites and nitrates are utilized by the plants as nutrients, and the water is then recirculated back to the aquaculture system. Plants that are common as vegetable reported success in aquaponics, such as cucumbers, shallots, tomatoes, lettuce, chiles, capsicum, red salad onions and snow peas. This study aims to determine the type of aquatic plants to reduce ammonia and to find out water quality for comet-tailed goldfish (*Ciprinus auratus*).

The plants are reared in glass container filled with 20 liters of ground water and aerated. Each tank were contained of 20 fish. The researchs were designed in Completely Randomized Design (CRD), 4 treatments and 3 replications. The treatments were different types of aquatic plant, which were (1) Control (2) Echinodorus sp (3) Anubias sp and (4) Bacopa sp. Fish used were *Ciprinus auratus* (total length 2.79 ± 0.25 cm and weight 0.24 ± 0.083 g). The results showed that the using of aquatic plants improves the water quality by reducing the ammonia (NH₃), nitrites (NO₂) and nitrates (NO₃) in media, especially by reducing the ammonia (NH₃) content. However the treatments were no effect on fish growth and survival rate of fish ($P > 0,05$)

TABLE 1 water quality

Parameters	Treatments			
	<i>Echinodorus</i> sp	<i>Anubias</i> sp	<i>Bacopa</i> sp	control
temperature (oC)	26,6-27,9	25,9-27,6	25,9-27,6	25,9-28,4
pH	5-7	5-7	4,71-5,7	5-7
DO (mg/L)	4,86-6,27	4,86-6,27	5,78	4,86-6,7
Hardness (mg/L)	33,71-122,18	33,71-124,09	33,71-127,8	33,71-188,19
Ammonia (mg/L)	0,001-0,411	0,001-0,409	0,001-0,415	0,001-0,630
Nitrite (mg/L)	0,028-0,127	0,028-0,112	0,028-0,180	0,028-0,127
Nitrate (mg/L)	0,77-0,175	0,077-0,181	0,077-0,189	0,77-0,153

TABLE 2. Growth and survival rate of comet-tailed goldfish (*Ciprinus auratus*) after 40 days reared.

Treatments	Fish			
	Weight (g)	Total length (cm)	Standard length (cm)	Sintasan (%)
<i>Echinodorus</i> sp	5.21±0,1 ^a	7.5±0,15 ^a	4.97±0,02 ^a	98,33±2,89
<i>Anubias</i> sp	4.79±0,32 ^b	7.06±0,56 ^a	4.75±0,42 ^b	98,33±2,89
<i>Bacopa</i> sp	5.35±0,42 ^b	7,44±0,16 ^a	4,95±0,10 ^b	100±0
control	4.75±0,25 ^b	7.19±0,16 ^a	4.64±0,23 ^b	95±5

EVALUATION OF RAW SHELLED OYSTERS BY JAPANESE CONSUMERS

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The purpose of this research is to elucidate the evaluation by Japanese consumers of shelled oysters produced in Miyagi Prefecture destined for raw consumption. In stores in Japan, raw oysters that meet certain sanitary conditions are sold for raw consumption. Raw oysters that do not meet these conditions are, on the other hand, sold for cooking. Miyagi Prefecture is one of the many oyster farming areas in Japan that produces oysters also for raw consumption. The prefecture, in terms of policy, regards oyster farming as highly important. However, producing and selling these shellfish under the prefecture's strategy for oyster farming has not been validated. Clarifying what value consumers place on these products is therefore warranted.

We applied the contingent valuation method in our analysis and collected data from Internet research in October 2017. The subjects of the research were the consumers who lived in the main areas where Miyagi Prefecture oysters were consumed. A grouped-data regression model was used to estimate consumers' willingness to pay (WTP) for Miyagi Prefecture oysters.

The research finds that consumers attributed an approximately 11.6% higher value (average WTP) to Miyagi Prefecture shelled oysters destined for raw consumption than to the same-origin oysters destined for cooking. Further, it provides insight into the characteristics of these consumers (Table 1).

TABLE 1. Result of Grouped-Data Regression Model

Variable	Explanation	Coefficient		Standard error	Mean
<i>FREQ</i>	Frequency of shellfish purchases	0.1343	***	0.0252	2.3371
<i>PRICE</i>	Placing highest importance on price	-0.2101	**	0.0952	0.1758
<i>FRESH</i>	Placing highest importance on freshness	0.2235	***	0.0741	0.3642
<i>RAW</i>	Placing highest importance on "for raw consumption"	0.3655	**	0.1594	0.0443
<i>LOCAL</i>	Purchasing food that contributes to the revitalization of the area and region	0.0885	**	0.0354	3.1375
<i>INCOME</i>	Income	0.0003	***	0.0001	546.8275
<i>CONST</i>	Constant term	0.9070	***	0.1352	
σ	Standard deviation	1.1952	***	0.0299	
Sample size		1,513			
Maximum log-likelihood		-2,873.8410			
Average WTP		11.6345%			

Note: ** and *** denote statistical significance at the 5% and 1% levels, respectively.

ANTIMICROBIAL ACTIVITY OF TWO NATURAL PRODUCTS AGAINST GROUPER IRIDOVIRUS AND BACTERIA

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Outbreaks of viral and bacterial diseases along with high mortalities have threatened the aquaculture for years. To date, disease problems still impact the development of industry continuously. In order to seek an eco-friendly disease prevention strategy, the use of dietary supplements derived from plants has accelerated in recent years. Herein, the antiviral activity and bactericidal effect of two natural products, Rhamnan sulphate (RS) from Green laver (*Monostroma latissimum*) and Oregano essential oil from *Origanum vulgare* against important fish pathogens will analyze respectively. Groupers (Family *Serranidae*) are viewed as one of the important maricultured fish species in Asia-Pacific region. Therefore, grouper will use as a model fish in this study. First, the cytotoxicity effect of these two products was determined on grouper kidney (GK) and grouper fin (GF-1) cells, and appropriate concentration range was selected for following experiments. Subsequently, the antiviral activity of RS and Oregano against grouper viruses was confirmed by MTT tests and inhibition of GIV replication. However, antibacterial effect against *Streptococcus agalactiae*, *S. iniae*, *Vibrio brasiliensis* and *V. parahaemolyticus* varied of these two products. After feeding natural extracts for 14 days and challenged by GIV, the relative survival rate increased to 70% in groups with feeding natural products that indicated these two natural products show good antiviral effect on the grouper.

CONTINUOUS AND PULSE-FEEDING APPLICATION OF A MULTI-SPECIES PROBIOTIC IN WHITELEG SHRIMP *Litopenaeus vannamei*

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We compared continuous application and three different alternating application protocols of a multi-species probiotic product at 3g/kg inclusion. Whiteleg shrimp ($1.1 \pm 0.01\text{g}$) were stocked in a RAS tank system at a density of 15 shrimp/100L and fed with a commercial diet (Uni President V991, 40% crude protein) according to body weight for 12 weeks.

T1: control feed

T2: continuous 3g/kg AquaStar® Growout (Biomin GmbH, Austria)

T3: 1 week 3g/kg AquaStar® Growout- 1 week control feed

T4: Two weeks 3g/kg AquaStar® Growout - two weeks control feed

T5: Three weeks 3g/kg AquaStar® Growout - one week control feed

Irrespective of application form, the multi-species probiotic led to significantly faster growth and a better feed conversion ratio. Hematological profiles demonstrate that the probiotic product indeed was able to stimulate the immune response in shrimp. The extent of the response was dependent on the application regime. After the feeding trial the shrimp were challenged intramuscularly with *V. parahaemolyticus*. Probiotic treated shrimp had a higher capacity to mount a respiratory burst response after infection. Probiotic treatment appeared to increase shrimp survival after IM injection of *V. parahaemolyticus*. The tested multi-species probiotic product was demonstrated to have growth-promoting effects in shrimp through an improvement of feed conversion ratio and an apparent activation of the shrimp's innate immunity.

Table 1: Growth performance and hematological profile of shrimp fed according to different application protocols of a multi-strain probiotic at 3 g/kg feed.

Parameter	Unit	T1	T2	T3	T4	T5	SEM
Weight	g	12.6 ^a	14.9 ^b	14.8 ^b	14.9 ^b	15.5 ^b	0.10
SGR	%	2.9 ^a	3.1 ^b	3.1 ^b	3.1 ^b	3.1 ^b	0.53
FCR		2.1 ^a	1.6 ^b	1.7 ^{ab}	1.7 ^{ab}	1.6 ^b	0.29
Survival	%	76.2	78.1	76.2	75.2	76.2	1.56
THC	log cells/ml	6.8	6.8	6.8	6.9	7.0	0.07
HC	%	76.1 ^{ab}	84.0 ^a	70.1 ^b	76.6 ^{ab}	72.1 ^b	1.36
SGC	%	14.3 ^a	10.1 ^b	15.8 ^a	12.2 ^{ab}	14.2 ^a	0.62
GC	%	9.6 ^{ab}	5.9 ^a	14.1 ^b	11.1 ^b	13.8 ^b	0.95
PO	U/mg protein	0.06 ^a	0.07 ^{ab}	0.06 ^a	0.09 ^b	0.18 ^b	0.019
RB	ΔOD_{630}	0.08 ^a	0.07 ^b	0.08 ^{ab}	0.07 ^b	0.17 ^{ab}	0.020

SGR, specific growth rate; FCR, feed conversion ratio; THC, total hemocyte count; HC, hyaline cells; SGC, semigranular cells; GC, granular cells; PO, phenoloxidase; RB, respiratory burst; superscript letters indicate significant differences ($P < 0.05$) a row using ANOVA or Kruskal-Wallis test with subsequent adhoc comparisons.

TRANSCRIPTOME ANALYSIS OF HEAD KIDNEY FROM BLACK ROCKFISH (*Sebastes schlegeli* Hilgendorf) INFECTED WITH *Aeromonas salmonicida* subsp. *masoucida* RFAS1

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Black rockfish, *Sebastes schlegeli* Hilgendorf, is one of main species in Korean aquaculture. During winter of 2008 and spring of 2009 in a black rockfish (*Sebastes schlegeli* Hilgendorf) aquaculture farm in Korea, we isolated and identified *Aeromonas salmonicida* subsp. *masoucida* RFAS1 from diseased fish showing ulcer lesions on their body. In this study, we conducted transcriptome analysis of head kidney to understand physiological responses of fish infected with atypical *A. salmonicida*. Black rockfish (average B.W.=19g, n=100/group) were intraperitoneally injected with low (7.7×10^5 CFU/fish; L-group) and high (3.4×10^7 CFU/fish; H-group) concentration of RFAS1. While L-group showed no recovered bacteria except for light infection only at an initial stage, H-group showed heavy infection with bacterial counts above 10^6 CFU/mg of head kidney from dpc3. Mortality in each group were 5% and 30% over 7 days, respectively. For transcriptome analysis, head kidney samples (n=3/group) were selected from each group at dpc 1 and 5 based on proportion of leukocytes through flow cytometry; control-dpc0 (0C), low-dpc1 (1L), low-dpc5 (5L), high-dpc1 (1H) and high-dpc5 (5H) (Figure 1). From iso-seq (PacBio) data, 57,637 unigenes were produced and used as reference and thus about 79% of reads generated from Illumina Hiseq 2500 were mapped on those unigenes. Differential expressed gene (DEG) analysis (q-value <0.05) in 1L, 5L, 1H and 5H were performed against 0C, and resulted in 210, 327, 598 and 1758 genes, respectively. Based on gene ontology (GO) and KEGG pathway materials, the expression of genes associated with the immune and inflammatory responses and chemotaxis were down-regulated in 5H groups compared to other groups. In addition, we confirmed black rockfish in heavy infection were down-regulated in most immune-related genes. This transcriptome approach have provided comprehensive insights on interactions between black rockfish and atypical *A. salmonicida*.

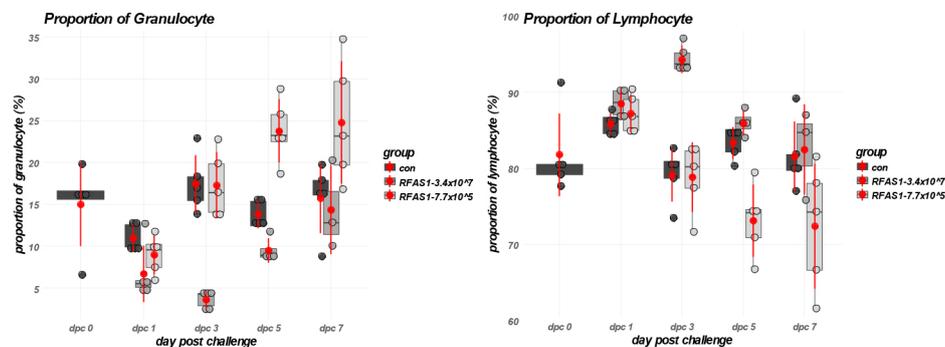


Figure 1. proportion of leukocytes in head kidney from black rockfish

FISH MEAL REPLACEMENT WITH A MIXTURE OF PLANT PROTEIN SOURCE IN THE DIETS FOR OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Fish meal is the most important source of animal protein in aquafeeds. Recently, its demand has increased but the supply is seriously limited. Therefore, this study was conducted to examine a mixture of plant protein sources as fish meal substitute in diets for the olive flounder.

The basal diet was formulated to contain 65% fish meal. Other four diets were formulated replacing fish meal by 25%, 30%, 35% and 40% with a mixture of soybean meal, wheat gluten and soy protein concentrate. Three synthetic amino acids (lysine, threonine and methionine) were added to meet their requirements. Experimental fish (mean BW: 96.8±0.2g) were distributed into 425L tanks at a density of 25 fish per tank with three replicates per dietary treatment. Fish were fed one of the experimental diets to apparent satiation for 15 weeks.

At the end of feeding trial, weight gain, specific growth rate and feed intake were numerically increased in fish fed FM25 and FM30 compared to those of fish fed the control and FM40 diets (Table 1). These results show that there would not be a problem in replacing fish meal with the mixture of plant protein sources up to 40% in diets for growing olive flounder.

Table 1. Growth performance, feed utilization and survival of growing olive flounder fed the experimental diets for 15 weeks.

	Diets				
	Control	FM25	FM30	FM35	FM40
Weight gain (%)	319±34	340±28	350±10	330±26	318±17
Specific growth rate (%)	1.15±0.11	1.22±0.08	1.25±0.03	1.19±0.08	1.15±0.06
Feed intake (g/fish)	155±17	164±21	172±5	157±22	143±8
Feed conversion ratio	0.73±0.07	0.71±0.03	0.71±0.70	0.70±0.06	0.68±0.05
Protein efficiency ratio	2.34±0.21	2.35±0.10	2.34±0.06	2.36±0.19	2.48±0.17
Survival (%)	80.0±8.0	84.0±6.0	82.0±6.0	84.0±2.3	84.0±8.6

COMPARATIVE GENOME ANALYSIS AND RE-IDENTIFICATION OF *Edwardsiella* SPECIES

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Edwardsiella tarda has been regarded as a notorious fish pathogen affecting diverse fish species around the world. Recent studies showed that *E. tarda* was phylogenetically re-classified as *E. anguillarum*, *E. piscicida* and *E. tarda* based on average nucleotide identity. In this study, we performed comparative genome analysis using hitherto sequenced genomes of strains belonging to the genus *Edwardsiella*. Thirty five hitherto sequenced genomes consisted of total of 10,002 protein-encoding genes, only 14.1% of the genes (1,443) were core genes, and the remaining 85.9% were dispensable and singleton genes within the genus. Pan development plot analysis showed that *E. piscicida* and *E. anguillarum* had open pan-genome, indicating that *Edwardsiella* spp. are adaptable to a variety of environments, and it is expected that the genes shared by species may be related to adaptation ability and species specificity. In addition, we attempted to re-identify 52 Korean isolates belonging to the genus *Edwardsiella* using our developed specific primers. As a result, 41 and 11 isolates were identified as *E. piscicida* and *E. anguillarum*, respectively. Serotyping were also performed with anti-sera that were prepared in rabbit with *E. tarda* ATCC15947, *E. piscicida* ETW41 and *E. anguillarum* EET61. Strains of *E. piscicida* and *E. anguillarum* were serologically grouped well, but *E. tarda* strains exhibited very low agglutination with all 3 anti-sera. This study shows that 3 *Edwardsiella* species were clearly distinguished from others, and most strains that were known as *E. tarda* were re-identified as *E. piscicida* or *E. anguillarum*. This result would provide very valuable information on species-specific control measures against Edwardsiellosis.

STUDY ON INTESTINAL GIANT CYSTIC DISEASE OF COMMON CARP CAUSED BY *Thelohanellus kitauei* IN VIETNAM

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Common carp is major cultural species in Northern of Vietnam. Disease is a big problem lead to lose economic for fish farmers. Major disease is giant cystic in the intestine caused by *Myxobolus* sp. This paper provides information about disease characters based on survey 212 fish farm households with 257 earthen ponds in four districts in Haiduong province of Vietnam. Fish diseased samples were collected for clinical diagnosis, stained samples used for morphology, measure and analysis. The results showed that major fish culture system in Northern Vietnam was intergrated system (99.61%), where Common carp was raised with rating from 15.63-32.10% of total fish stocking. The prevalence was 31.91% of fish ponds. The ponds which were not disinfected could be infected 4.28 times compared to disinfected ponds. Infected fish often grow slowly, black color, swelling abdomen and intestine which contain liquid and white spores (up to 92 spores) with the size of 2.65x2.04 cm (Max 5.3x3.7 cm). *Myxobolus* sp. was identified as *Thelohanellus kitauei*. This disease caused a lot of damages for fish farms due to slowly grow, high FCR, low market table fish and high mortality.

STOCKING MATERIAL PRODUCTION OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* IN RUSSIA

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Giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1876) is one of the crustacean species cultivated in Russia and is non-specific for this area. The first experiences of *M. rosenbergii* cultivation in closed recycling water systems (CRWS) in Russia conducted in 1993 - 1995 years. The brood stock was originally formed on prawn imported from Vietnam and Japan. First experimental prawn farms appeared in the 2000s. Methods of *M. rosenbergii* cultivation in warm waters, in ponds in southern regions of Russia and in recirculating aquaculture systems were developed. Features of the Russian climate do not allow cultivating this tropical species all year round in open reservoirs, as occurs in its native area. In Russia was another successful cultivation scheme. Broodstock during the cold period contained in closed recycling water system maintain the water temperature of 26-28° C. By the end of the embryonic period, berried females are transferred to hatchery tanks. The salinity of water is 12-14 ‰. After hatching the females are returned to the broodstock holding system, and the larvae are rearing in CRWS units until transforming into the post-larval stage. After metamorphose of 80% of larvae into postlarvae the individes are transported into rearing tanks with freshwater. The stocking material rearing there until the temperature in the open waters reaches a sufficient level. The giant freshwater prawns get a marketable size to the beginning of autumn.

For the most effective cultivation of prawn stocking material, knowledge of the characteristics of its biology and physiology is necessary. The scientists of the mariculture of invertebrates laboratory (VNIRO, Moscow) investigated for many years the nutrition of larvae, postlarvae and juveniles of giant freshwater prawn, and its aspects associated with molting and maintenance in CRWS (tab. 1).

To date, we examined daily food needs of larvae and juveniles of giant freshwater prawn, special aspects of nutrition associated with molting have been studied. Feeding schemes for all stages of cultivation of *M. rosenbergii*, recommendations for farms have been developed. Feeding schemes for periods of prawn cultivation, recommendations for prawn farms have been developed. In addition, a number of experiments was conducted for optimize a maintenance of prawns with the aim of improving the survival and stocking material quality.

TABLE 1. The scheme of differential feeding of *M. rosenbergii* larvae in VNIRO (Moscow)

Stage of development	Number of nauplii Artemia sp./ind	Value of egg mixture, mg/ind.	Particle size of egg mixture,	Feeding regime	
				Artemia sp./times per day	Egg mixture
II	50	-	-	4	-
III-IV	100	0,5-2,5	300-500	3	1
VI-VIII	150	2,5-4,5	500-700	2	2
IX-postlarvae	200	4,5-12,0	900-1200	3	2

BILE ACID AND BILE ACID TRANSPORTERS ARE INVOLVED PATHOGENESIS OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE IN WHITE SHRIMP *Litopenaeus vannamei*

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Although AHPND, caused by *V. parahaemolyticus* is an important threat to the shrimp industry, particularly in Asia, its pathogenesis is not well characterized. The objective was to explore the role of bile acid transporters in *Litopenaeus vannamei* stomach during *V. parahaemolyticus* infection. Transcriptome profile data and mRNA expression of bile acid transporters in stomach and hepatopancreas were analyzed, followed by determination of bile acid concentrations during development of AHPND. Based on transcriptome profiling and gene expression, in AHPND infected shrimps the bile acid transporters were upregulated and downregulated in the stomach and hepatopancreas, respectively. These results were further validated by bile acid concentrations that were higher in stomach of AHPND-infected shrimp compared to control. Additionally, bile acid concentrations were correlated with high copy numbers of pVA plasmid and PirAB^{vp} toxins. Based on *in-vitro* assays for biofilm formation, bile acids enhanced biofilm formation of *V. parahaemolyticus* compared to the controls. In addition, based on western blot analysis of PirAB^{vp} toxins, there was increased release of toxins into the supernatant in the presence of bile acids. Furthermore, *in-vivo* silencing of the bile acid transporter ASBT reduced copy numbers of pVA plasmid and PirAB^{vp} toxin, reduced mortality and decreased amounts of bile acids in the stomach of AHPND-infected shrimp. Taken together, we concluded that bile acid transporters and bile acids in shrimp stomach have important roles in AHPND pathogenesis.

CLIMATE CHANGE AND HUMAN INTERVENTION HAS CHANGED THE FISHERY STATUS OF RIVER GANGA

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Streams and rivers are an important part of the environment. The character of landscapes away from the coasts is provided primarily by rivers. Their status had deteriorated seriously in the past. Due to climate change and human intervention through water engineering works over the last few decades, resulted the river Ganga considerably modified or artificial. This river is also polluted by contaminants and nutrients from industry, private households and agriculture.

The river Ganga supports fishery resources and contributes significant economic benefits to the riparian communities and the national economy. Changing hydrology, apart from deteriorating environmental conditions, has been to a large extent responsible for change in the fishery scenario in the river. This change has also affected the income levels of riparian fishers. The river Ganga, despite the proclamations and heart beating, is gradually dying. Fish stocks have declined considerably and embankment construction and waterways development continue to threaten its endangered aquatic species. Now the river Ganga is a great place for sand removal practices. Indian Government trying to regain the life of Ganga with project likes “Namami Gange”.



Pic: Sand removal practices in Ganga (Near Patna, Bihar, India)

TRANSCRIPTOME ANALYSIS OF ANTIGEN PROCESSING AND PRESENTATION RELATED GENE EXPRESSION PATTERN DURING DIFFERENT METAMORPHOSIS STAGES ON ORANGE-SPOTTED GROUPEL (*Epinephelus coioides*)

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The aquaculture of grouper makes high economic profit in Taiwan. It was constructed by divided works cooperation according to the size stage of fish. Each stage of fish will be raise in different farm. However, the larval stages of grouper doesn't have a complete immune system, so the hatchery farm usually gets high mortality rate even up to 90% when disease outbreaks. Adaptive immunity is an important resistance mechanism, which protects organism from pathogens infection. To induce specific immune response, antigen has to be processed into peptides and presented to T cell by MHC. In other words, antigen processing and presentation play an important role in the adaptive immunity. In this study, transcriptome analysis was aimed to antigen processing and presentation related gene expression pattern during different metamorphosis stages on orange-spotted grouper by next-generation sequencing (NGS). The result showed there are several significant differential expression genes including MHC Class II beta, AEP, β 2M, CD74, MHC class I alpha, NFY β , TAP1, TAP2 and TAPBP. These antigen processing and presentation related gene expression patterns have also been found relative to the maturation process of immune organs by Real-time PCR.

POTENTIAL APPLICATION OF ALGAL-BACTERIAL CONSORTIUM AS PROBIOTIC FOR COMBATING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN SHRIMP

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Picochlorum is a common marine microalga widely used in mariculture. Previous research has shown that adding *Picochlorum* strain S1b to the rearing tanks of grouper larvae can significantly reduce *Vibrio* density in the water. The anti-*Vibrio* activity of S1b has been shown to originate from its interactions with three types of marine bacteria: *Muricauda*, *Labrenzia*, and *Arenibacter*. Thus, the objective of this current study was to leverage this algal-bacterial consortium to develop a probiotic which can reduce the occurrence of acute hepatopancreatic necrosis disease (AHPND) in shrimp. For this, we first created alginate beads by coating a co-culture of S1b and the three marine bacteria with alginate. We then fed these alginate beads to shrimp or poured the co-culture directly into the rearing tanks and observed whether either of the two approaches was effective in protecting *Litopenaeus vannamei* from AHPND. Specifically, *L. vannamei* were fed alginate beads that equaled 0.8% of their body weight for 10 days and then challenged with the AHPND pathogen *Vibrio parahaemolyticus* strain 5HP. We found that the alginate beads provided protection after a 10 day feeding period (relative percentage survival [RPS] = 18.1%) (Fig. 1). In contrast, pouring the algal-bacterial co-culture directly into the rearing tank for a two-week soaking treatment revealed that applying a smaller dose of the S1b co-culture (10mL/3L tank water) every day offered shrimp adequate protection against the AHPND pathogen (RPS=33.2%). However, increasing the dose 5 and 10 fold reduced the protection effect to 7.6% and -3.2%, respectively (Fig. 2). Therefore, pouring the low-concentration algal-bacterial co-culture into the rearing tank provided better protection against AHPND. This treatment method is easy to execute and has a low cost, making it a fairly feasible approach.

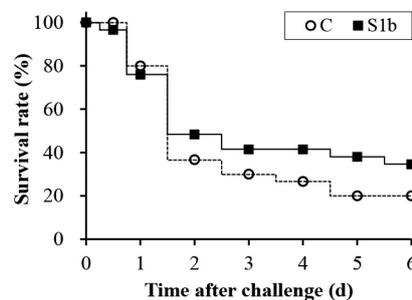


Fig. 1 Survival rates of *L. vannamei* that were fed either blank alginate beads (○C) or alginate beads containing algal-bacteria consortium (■S1b) after being challenged with *V. parahaemolyticus*.

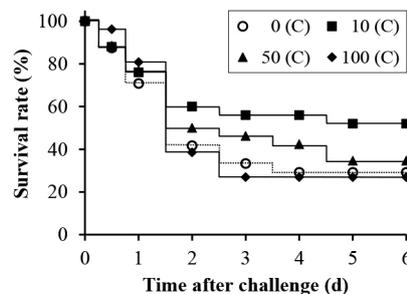


Fig. 2 Survival rates of *L. vannamei* that were soaked in different amounts of algal-bacteria consortium (0, 10, 50, 100 mL added to each tank daily for two weeks) after being challenged with *V. parahaemolyticus*.

PRODUCTION PERFORMANCE AND TECHNICAL FEASIBILITY OF TILAPIA-SHRIMP POLY CULTURE IN AN IMPROVED TRADITIONAL SHRIMP FARM IN THE POKKALI FIELDS OF KERALA, INDIA

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Aquaculture is one of the fastest developing growth sectors in the world. The successful entrepreneurship of aqua farming relies on the production of aquatic animals in the cost effective, social and environmental friendly approach. Polyculture of shrimp with filter feeding fishes and molluscs increase the species diversity of the ecosystem in the ponds and maximise the use of space, improve the utilization of the organic materials. The polyculture of shrimp and tilapia increase the production of shrimp, with tilapia production as a secondary benefit. The current study analyses the production performance and technical feasibility of polyculture in two brackish water prawn filtration ponds at Chellanam Village, Ernakulum District, Kerala with an extent of 7.78 acres and 10 acres respectively. Both were lying between 9^o58' North Latitude and 76^o16' East Longitude. Growth of *Penaeus monodon* in prawn filtration pond showed the maximum size during the month of January, it was attained an average size of 100 gm (10 count) in pond 1 and 68 gm (15 count) in pond2. The growth of Tilapia in prawn filtration pond showed maximum size of 200 gm in pond2 and 130 gm in pond1. However, tilapia can be added in prawn filtration ponds as the secondary species could help the shrimp to utilize tilapia feeding waste and natural foods and to achieve extra economic returns. The present experiment indicated that adding tilapia in to prawn filtration pond is technically feasible, however more research is needed to optimize the tilapia-shrimp polyculture system.

INTRODUCTION

- Traditional system of brackish water aquaculture practiced in the seasonal fields and the perennial fields lying adjacent to the coastal and back water areas of Kerala has a very long history.
- Shrimp, which earns huge revenue through export, is an important aquaculture commodity in India.
- In a polyculture system, tilapia and shrimp can utilize different niches in the culture environment.

MATERIALS AND METHODS

- The Study was carried out in to brackish water prawn filtration ponds at Chellanam Village, Ernakulam District, Kerala with an extent of 7.78 acres and 10 acres respectively. Both were lying between 9^o58' North Latitude and 76^o16' East Longitude.

RESULTS AND DISCUSSION

- The shrimp production from pond 1 and pond 2 were 680 Kg and 586 Kg and the price realized were Rs. 4,21,600 and Rs. 3,63,320 respectively.
- Polyculture of *Penaeus monodon* with Tilapia gives the shrimp farmer an additional yield of 1611 kg of Tilapia per crop with a sale value of Rs. 1,74,650 from pond 1 and 2510 kg of Tilapia per crop with a sale value of Rs.2,72,150 from pond 2.
- The growth of Tilapia in prawn filtration pond showed maximum size of 200 gm in pond 2 and 130 gm in pond 1.
- Water temperature and pH ranged from 26.0 to 35.70C and from 6.0 to 9.7 throughout the experimental period in both the ponds, respectively. DO concentrations at dawn tended to be lower towards the end of the experimental period in pond 1, while DO concentration showed increased trend in pond 2. Salinity ranged from 7.6 to 23.6 ppt throughout culture period and at the end of culture period the maximum salinity 23.6 ppt in pond 1 was recorded. Phosphate and Nitrate concentration ranged from 0.64 to 3.56 mg/l and from 1.12 to 1.53 mg/l were recorded in pond 1 and pond 2 respectively.

(Continued on next page)



Fig : 1 Study Area, Pond 1



Fig : 2 Study Area, Pond 2

- Methods include primary data collection through Key Informant Interviews (Kumar 1989). In-depth interview was done with a brackish water shrimp farmer in Chellanam area.
- Species used for culture : *Penaeus monodon* and *Oreochromis mossambicus*
- Stocking mode : Continuous stocking (Shrimp) and auto stocking from wild (Tilapia)
- Stocking date of pond 1 : 5th September and 25th November 2016
- Stocking date of pond 2 : 2nd October and 27th December 2016
- Stocking density of shrimp in pond 1 : 1,50,000 with a PL size of 18 and 1,00,000 with a PL size of 16
- Stocking density of shrimp in pond 2 : 1,75,000 with a PL size of 18 and 1,00,000 with a PL size of 16
- Purchase of shrimp seed from : Kodungallur shrimp hatchery (Aqua plus)
- Culture period of pond 1 : 1st September to March 31st 2016-17
- Culture period of pond 2 : 1st October to April 15th 2016-17
- Feed used : Farm made feed (mixture of cooked wheat bran , ground nut oil cake and dried fish especially Sardine)
- Size of the ponds : 7.78 acres and 10 acres
- Harvesting method : Partial and complete harvesting



Fig : 3 Harvested Shrimp, *Penaeus monodon*



Fig : 4 Sorting of harvested shrimp



Fig : 5 Harvesting of shrimp through gillnet operation



Fig : 6 Harvesting of Tilapia through gillnet operation



Fig : 7 Harvested Tilapia collected in hapa



Fig : 8 Harvesting of Tilapia through cast net operation

THE EFFECT OF TEMPERATURE ON IMMUNE RESPONSE OF INTERFERON REGULATORY FACTOR 4 (IRF-4) IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Climate changes affecting and challenging fisheries and aquaculture industries by causing sea temperature unstable, acidification, CO₂ accumulation and etc., these factors influences marine environment by reduce the productivity and increase the incidence of aquatic diseases outbreak. One of the high mortality aquatic viruses, nervous necrosis virus (NNV) has been previously demonstrated the higher temperature on the ability of NNV infection by promotes virus proliferation and transmission was assessed as a possible evidence for seasonal incidence of viral nervous necrosis disease (VNN). Upon viral infection, IFN-mediated antiviral pathways are activated which cooperative initiate and regulates by a transcription factors of the interferon regulatory factor (IRF) family. Among, IRF-4 has been reported as a negative transcription factor in immune response, additionally play a vital transcription partner regulates the thermogenic expression to maintain cold tolerance. Hence, IRF-4 may be an important factor correlates between viral-induce immune response and temperature modulation.

In this study, the role of IRF-4 in antiviral immune response and the effect of temperature on the expression pattern of IRF-4 in NNV-infected orange-spotted grouper were investigated. The transcript of *osgIRF4* was constitutive highly expression in immune organs such as head kidney and spleen, as well as was responsive to viral mimic poly I:C stimulation and NNV infection. These imply that IRF4 may play a crucial role in viral induced-immune responses. When fish larvae were exposed to low (20°C) and high (36°C) temperature, the expression of *osgIRF4* elevated drastically which support its thermogenic role. Moreover, the expression of virus coat protein was significantly higher in GF-1 cells with overexpression of *osgIRF4*. When GF-1 cells exposed to high temperature (36°C), *osgIRF4* promoter was induced at 12 h and 24 h, whereas low temperature suppressed the *osgIRF4* promoter activity at 24 h Hence, IRF-4 may be one of a key thermogenic regulator involved in NNV infection.

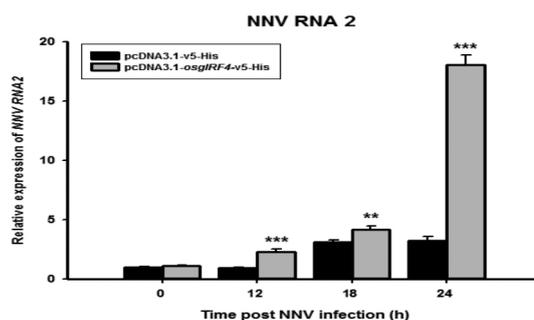


FIGURE 1. The effect of *osgIRF4* on NNV infection in GF-1 cells. Expression was measured by real-time PCR and normalized to β -actin.

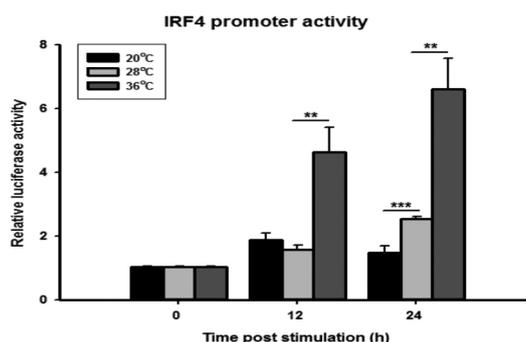


FIGURE 2. The activity of *osgIRF4* promoter in response to temperature changes. The luciferase activities were assessed at 12 and 24 h post transfection under 20°C, 28°C and 36°C

THE ROLE OF SIRT4 IN WHITE SPOT SYNDROME VIRUS (WSSV) PATHOGENESIS

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White spot syndrome virus (WSSV) causes dramatic economic losses in shrimp aquaculture. This virus induces the Warburg effect and glutamate-driven anaplerosis during viral genome replication. In cancer cells, SIRT4 functions as a tumor suppressor and inhibits activity of the GDH gene, which is involved in glutaminolysis. In the present study, we identified the *LvSIRT4* gene in *Litopenaeus vannamei*. Although *LvSIRT4* had the same characteristic SIR2 domain compared to the SIRT4 gene in other species, phylogenetic analysis suggests that *LvSIRT4* is similar to the gene invertebrates such as *Drosophila melanogaster* and *Anopheles gambiae* but distinctly different from the gene in vertebrates. Furthermore, the *LvSIRT4* gene was highly expressed in the muscle, gill and pleopod. In addition, *LvSIRT4* mRNA was significantly increased at the late stage of WSSV infection. To further confirm the function of *LvSIRT4* in WSSV replication, it will be silenced with dsRNA. In addition, a recombinant plasmid that contains the open reading frame of SIRT4 and a WSSV ie1 promoter will be used to determine effects of gene overexpression. Determining the role of SIRT4 during WSSV infection may be valuable for identifying WSSV-resistant shrimp.

MODULATION OF NF- κ B/I κ B α COMPLEX BY TEMPERATURE EFFECT IN ORANGE-SPOTTED GROUPER

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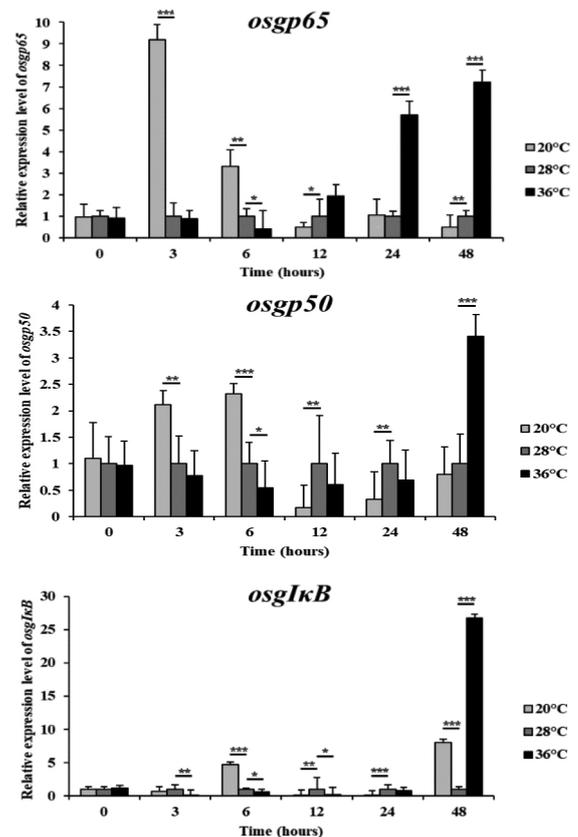
Epinephelus coioides, orange-spotted grouper is one of the most important and highly valued cultured fish and is farmed in countries such as Japan and Taiwan. The continuously increasing demands for orange-spotted grouper drives the effort of farmers to breed fishes with higher growth rates. As fishes are always in constant interaction with their environment through the gills and skin, therefore they are very susceptible to the environment stress, such as temperature. Hence, the study was conducted on modulation of the NF- κ B/I κ B pathway in response to different environmental stress in the orange-spotted grouper.

According to previous studies, pathogens invasion induces the degradation of inhibitor of kappa B α (I κ B α) and activates the transcription factor κ B (NF- κ B) that regulates pro-inflammatory cytokine genes. Under different environmental stress, it is possible that grouper might activate this mechanism.

With luciferase assay, TNF α promoter Δ κ B site inactivation was seen. With NNV treatment, TNF α promoter activity was found to be higher than TNF α promoter Δ κ B site, manifesting that NNV may activate the NF- κ B/I κ B α complex.

With different temperatures, 20°C, 28°C and 36°C, modulation of NF- κ B/I κ B α response was studied by cloning p65 and I κ B α , components of NF- κ B/I κ B α complex and groupers were challenged with nervous necrosis virus (NNV).

In a conclusion, upregulation of I κ B α by NF- κ B controls excessive inflammation under different environmental stress



Expression of (A) *osgp65*, (B) *osgp50* and (C) *osgIkB* at 20°C, 28°C and 36°C and on day-0 was set as 1 for comparisons. (*p<0.05, **p<0.01, ***p<0.001)

ASSESSMENT OF THE NUTRITIONAL VALUE OF FEED INGREDIENTS FOR FEED DEVELOPMENT FOR MALABAR GROUPER, *Epinephelus malabaricus*

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Considerable progress has been made towards the development of a manufactured feed for Malabar grouper, *Epinephelus malabaricus*. However, there is little data on the nutritional value of common feed ingredients suitable for use in Malabar grouper pelleted feeds. The evaluation of the digestible protein and energy of feed ingredients is critical to the cost-effective formulation of such modern pelleted aquaculture diets. Therefore in this study we aimed to assess the digestible protein and energy value of a range of feed ingredients widely available in the central region of Vietnam for use in feed formulations for Malabar grouper.

A reference diet of 570 g/kg DM protein, 125 g/kg DM fat (21 MJ/kg DM gross energy) was formulated and also used as the basis of the other test diets in the experiment. Each test ingredient was combined with a reference diet component in a 30:70 ratio. A total of ten test ingredients were studied, these included; Peruvian fishmeal, KienGiang fishmeal, DaNang fishmeal, shrimp (Acetes) meal, krill meal, squid meal, meatbone meal, poultry by-product meal, Indian soybean meal and wheat gluten. Feeds were fed for seven days to tanks of 10 groupers per tank before faecal collection commenced. Apparent digestibility coefficients were measured *in vivo* by using a flow through modified Guelph faecal collection system with filtered aerated sea water. Faeces were collected over a six-week period and pooled within tanks and faecal samples were kept frozen prior to analysis. For analysis the faeces were dried and both faecal and feed samples ground and analysed for chromium, nitrogen, lipid, ash and dry matter. Digestibilities of each of the diets were calculated and the digestibility coefficients of each diet and the composition of the diets and ingredients used to calculate the digestibility of each test ingredient. The results of this study show that Malabar grouper can digest protein and energy from a wide range of feed ingredients. These data could be used to better define nutrient and energy requirements and to formulate nutritionally-efficient, cost-effective feeds for this species.

Table 1. Composition and digestibility of key feed ingredients for Malabar grouper

	Ingredient Specifications (all values g/kg DM)						Ingredient Digestibility	
	DM (g/kg)	Protein	Lipid	Ash	CHO	Energy (MJ/kg)	Protein ADC	Energy ADC
Peruvian fishmeal	900	650	90	157	103	20.7	97.5 ^e	92.0 ^d
KienGiang fishmeal	904	650	70	180	100	19.8	95.3 ^{de}	92.7 ^d
DaNang fishmeal	932	625	80	218	77	19.2	92.6 ^c	88.6 ^c
Shrimp (Acetes) meal	931	639	40	152	169	19.6	94.9 ^d	89.1 ^c
Antarctic Krill meal	929	580	180	130	110	22.7	95.6 ^{de}	85.0 ^b
Vietnamese Squid meal	927	580	119	85	196	22.3	89.7 ^b	82.4 ^a
Meatbone meal	946	480	100	336	84	16.7	86.6 ^a	82.5 ^a
Poultry by-product meal	900	600	140	80	180	22.8	84.7 ^a	84.0 ^{ab}
Indian soybean meal	880	480	30	63	427	19.9	91.0 ^{bc}	87.7 ^c
Wheat gluten	932	700	10	8	282	21.8	91.5 ^{bc}	85.3 ^b

DM : Dry matter, ADC: Apparent Digestibility Coefficient, CHO: Carbohydrate

SPERM CRYOPRESERVATION OF TIGER GROUPER *Epinephelus fuscoguttatus*

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Tiger grouper (*Epinephelus fuscoguttatus*) is a protogynous hermaphroditic species. Fish smaller than 37 cm total length (TL) are females and fish with TL longer than 45 cm are males (www.fishbase.org). Due to the large size, the source of male broodstock is limited; thus, collecting semen for seed production is not always possible. Cryopreservation of tiger grouper semen would reduce the number of males needed, minimize handling stress through less frequent stripping, facilitate artificial propagation when ova are available, promote genetic and breeding studies, and enhance seed production on a commercial scale. However, a protocol for sperm cryopreservation of tiger grouper, an important candidate for marine aquaculture of Vietnam, have not been developed yet. The goal of this study was, therefore, to develop a protocol for sperm cryopreservation of tiger grouper. The specific objectives in this study were to find the best extender, cryoprotectant, dilution ratio, cryopreserved volume and freezing method for sperm cryopreservation of tiger grouper. An effective protocol was established by comparing different cryodiluents created by mixing various cryoprotectants (DMA, DMSO, methanol, glycerol, ethylene glycol at the concentration of 5, 10, 15 or 20%) and extenders (ELRS3, ELS3, ES1-3, LG-ASP2; 150 mM NaCl, 300 mM Glucose, DGS1, DGS2, MPRS). The different dilution ratios (1:1, 1:3, 1:6 or 1:9), freezing methods (direct submersed in liquid nitrogen (-196°C), placed strawed sperm to -80°C for 5 minutes and then plunged in liquid nitrogen, placed strawed sperm to -20°C for 5 minutes, -80°C for 5 minutes and then immersed in liquid nitrogen) and cryopreserved volumes (0.25 ml, 0.5 ml, 2.0 ml) were also used in an effective protocol. The motility (MOT), straight-linear velocity (VSL) and fertility rates of post-thawed sperm were comparable to the fresh sperm. The results indicated that at ratio of 1:3 in cryodiluent contained ES1-3 as extender supplement 15% DMSO as cryoprotectant and cryopreserved with the second freezing method at the cryopreserved volume as 0.25 ml, reached the best MOT and VSL of post-thawed sperm. As a result, the fertilization rate and hatching rate of the post-thawed sperm cryopreserved for 1 week, 1 month, or 1 year in liquid nitrogen ($51.87 \pm 3.94\%$ and $36.57 \pm 4.74\%$, $48.20 \pm 3.37\%$ and $35.77 \pm 6.69\%$, or $47.53 \pm 3.89\%$ and $34.49 \pm 6.08\%$) were similar to that of fresh sperm ($52.73 \pm 4.40\%$ and $38.86 \pm 5.80\%$). In conclusion, using cryodiluent contained the ES1-3 as extender and 15% DMSO as cryoprotectant to semen at the ratio of 1:3 (v/v) in the freezing method as two steps (-80°C for 5 minutes and -196°C) at the cryopreserved volume as 0.25 ml is an effective protocol for cryopreservation, especially hatching success of egg fertilized by post-thawed sperm of tiger grouper.

EFFECT OF A MULTI-STRAINS YEAST-BASED FUNCTIONAL ADDITIVE ON EHP-CHALLENGED JUVENILE WHITE SHRIMP *Litopenaeus vannamei*

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Enterocytozoon hepatopenaei (EHP) is an intracellular parasite targeting the shrimp hepatopancreas (HP) and gut epithelial lining causing stunted growth. It generates severe losses across Asia either directly or in association with other pathogens. Lallemand Animal Nutrition has developed a multi-strains yeast-based additive (YANG) presenting contrasted microbe-associated molecular patterns for broad immune-modulation at low inclusion level.

The trial lasted 34-day (14-day pre-challenge, 10-day EHP challenge by cohabitation; 10-day post challenge period) using juvenile whiteleg shrimp (BW = 0.61 ± 0.01 g) stocked within 120L self-contained test units allocated to 3 treatments (9 replicate units/ treatment; 30 shrimp / unit): A negative control (basal diet; non-challenged); a positive control (basal diet; EHP-challenged) and a supplemented diet (YANG at 800 g/T; EHP-challenged). The EHP-load in the HP was measured by qPCR at day 0, 5 and 10 post-challenge (GLM; post-hoc Wald test).

Survival averaged 81 ± 2 % across groups with no significant differences between the negative and positive controls and no diet effect.

The EHP-challenge had a significant negative effect on end-point BW which was, on average, 27 % lower in the positive compared to the negative control. YANG significantly improved mean BW compared to the positive control (+7.9 %) via a lower prevalence of shrimp severely compromised in weight (Fig; 7 % and 21 % of shrimp with a BW below 1.6 g in the YANG and positive control respectively).

Accordingly, mean EHP-load was reduced in the YANG compared to positive control at all time-points (Table a). This reduction was significant at day 5 post-challenge (-63.8 %) when the mean infection load peaked in all groups. Accordingly at day 5, the proportion of shrimp with a high EHP-load was 15% lower, and with low EHP-load 11% higher, in the YANG group compared to the challenged control (Table b).

The trial further validated the EHP-challenge model by cohabitation and confirmed that the disease causes stunted growth but not mortality in isolation.

YANG applied preventively and over an EHP challenge period can contribute to reducing the severity of the EHP-outbreak, related loss of growth and occurrence of ‘runt’ shrimp. In practice, preserving crop-size uniformity has strong benefits by facilitating subsequent stock and pond management while preserving the crop duration and stock potential value.

Figure: Cumulative body-weight distribution at the end of the trial

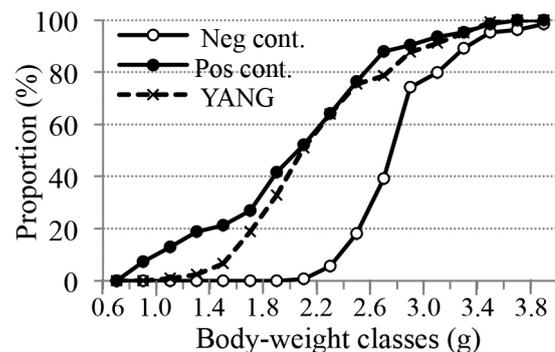


Table: Mean EHP-load & proportion of shrimp per EHP-load class at day-5 post-challenge

	Pos control	YANG	
a) Mean EHP-load (million EHP / g of HP)			
Day 0	554±281	311±160	-44%
Day 5	1,267±432 ^a	459±89 ^b	-64%
Day 10	34±14	26±14	-23%
b) Rate of shrimp per EHP-load class (Day 5)			
Low	7.4 %	18.5 %	+11%
Medium	59.3 %	63.0 %	+7%
High	33.3 %	18.5 %	-15%

Low < 10; *Medium*: 10 to 1,000; *High* > 1,000 million copies of EHP / g of HP

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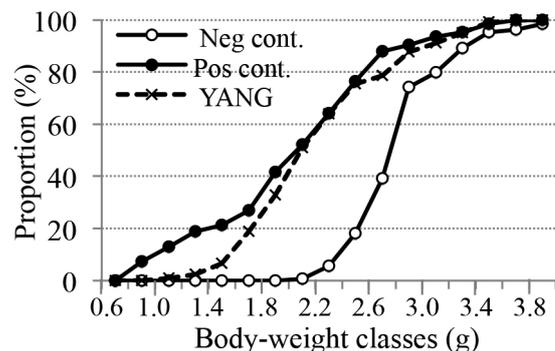


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SOME CRITICAL STEPS TO THE SUCCESSFUL DEVELOPMENT AND DEPLOYMENT OF AN (INTEGRATED) BIOREMEDIATION STRATEGY IN POND AQUACULTURE

Eric Leclercq^{a*}, Stéphane Ralite^a, Mathieu Castex^a

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An evident target for a viable shrimp grow-out business is to maximize crop output while minimizing the risk of severe losses. The desire to safely intensify the crop can only be fulfilled by exploiting and expanding the capacity of the pond to carry a healthy biomass to harvest.

Applying a successful bioremediation strategy can vastly sustain the pond carrying capacity by actively channeling the pond ecosystem dynamics, helping to maintain a healthy soil and a positive microbial environment in particular. Bioremediation can be powerful but is also a complex technology requiring informed management attuned to local conditions and targets.

Based on *in-vitro*, pilot-scale and field trials, the aim of this presentation is to highlight some key factors of success in the selection and deployment of an effective bioremediation strategy.

A critical preliminary step is the selection of a bioremediation product category(-ies) best-suited to the farm needs. To do so, a strong understanding of the key microbial processes at play within the pond is paramount to identify the (integrated) strategy most appropriate to support the targeted microbial processes. The product itself must be carefully selected based on its documented environmental tolerance and functionalities. For example, heterotrophic bacteria for organic matter degradation should grow under variable environmental conditions (e.g. salinity) as well as presenting a complete enzymatic profile (Fig 1) to efficiently degrade complex organic wastes under varying local conditions. Importantly, bioremediation bacteria can also be selected for their ability to inhibit selected potentially harmful bacteria (Fig 2).

Based on practical experience, some key principles for testing and deploying a bioremediation strategy will be presented. Care must be taken to closely control other limiting factors (e.g. oxygen) to prevent them outweighing any potential benefits of bioremediation. The survey strategy should identify a coherent panel of environmental parameters to be measured accurately and consistently at meaningful intervals above and beyond routine farm practices for meaningful diagnostic. Some guidelines for assessing stock performance and comparing (or not) different ponds data will also be provided.

The microbial dynamics are central to pond aquaculture and the establishment of a successful bioremediation strategy requires dedicated management. The presentation provides an overview of some basic but important principles to follow in that aim.

Fig 1: Enzymatic profile

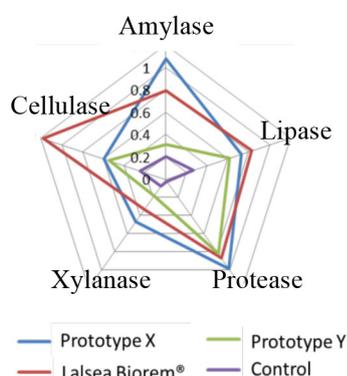


Fig 2: Inhibition of pathogen growth (*Vibrio parahaemolyticus*)

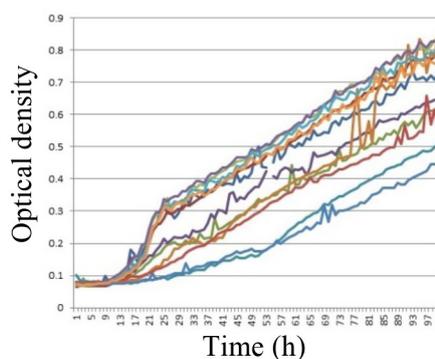
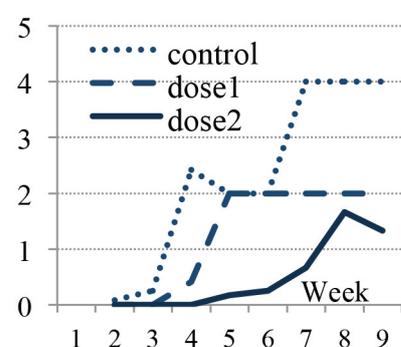


Fig 3: Nitrite concentration (mg/L)



EFFECT OF SELECTED FUNCTIONAL HEALTH FEED ON WHITE SHRIMP *Litopenaeus vannamei* CHALLENGED WITH A NOVEL INFECTION MODEL FOR WHITE FECES SYNDROM

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^a Lallemand SAS, 19 rue des Briquetiers, 31700, Blagnac, FRANCE

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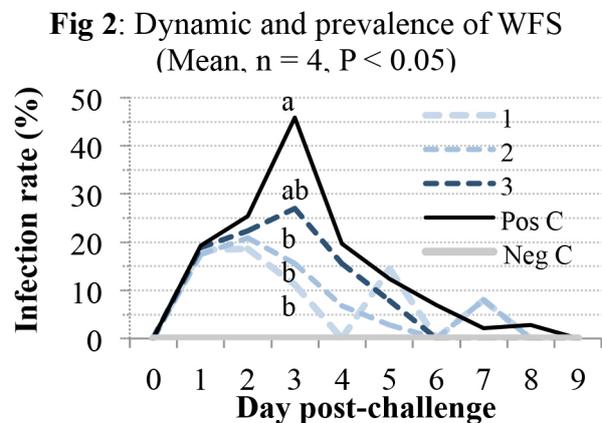
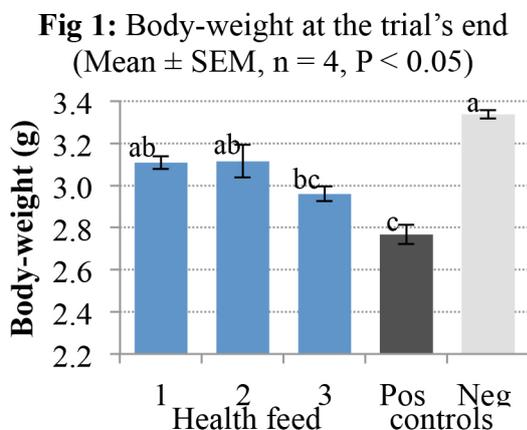
White Feces Syndrom (WFS) causes significant economic losses for shrimp farmers across Asia. The syndrome originates from the sloughing of the hepatopancreatic epithelial cells aggregating into vermiform bodies within the hepatopancrea (HP) and mid-gut before being discharged in the form of floating, white fecal strings in severe cases (Sriurairatana et al., 2014). Recent works have confirmed the infectious nature of the disease, validated the Koch's postulated for the disease and developed a standardized *per os* challenge methods simulating the natural infection route (Tran et al., 2017). The aim of the trial was to assess the potential of selected functional health feed at mitigating the severity and impact of WFS.

The trial lasted 26 days (14 days pre-challenge, 2 days *per os* challenge; 10 days post challenge period) using 120L self-contained test units with 8 replicate units per experimental groups (30 juvenile whiteleg shrimp / unit). Functional health compounds were incorporated into a basal diet and tested against a positive (challenged) and a negative control (not challenged) fed the non-supplemented diet. Experimental groups were hand-fed at 5% biomass daily with 4 meals / day over the trial's duration, except over the challenge period. Routine water quality parameters were monitored daily (Temp = 27.5°C).

Results will present the effect of the infectious-challenge and the mitigating effect of the test diet on daily feed intake (appetite); feed and growth performance over the trial's duration. The dynamic and prevalence of the disease propagation will be described by the daily assessment of specimens showing WFS post-challenge (infection rate). Finally, histopathological data on specimens sampled 2 days post-challenge will be provided.

A preliminary trial under the same challenge model showed a significantly lower loss of body-weight when fed the health diet (Fig 1; Health diet (2): -6.7%; Positive control: -17.1% compared to the negative control) associated with a significantly lower prevalence of the WFS at the peak of infection (Fig 2; day 3 post-challenge) and a quicker resolution of the disease.

Using a novel challenge method; these trials highlights the possibility to mitigate the severity and impact of WFS by the preventive application of selected functional health feed.



EVALUATION OF THE EFFICACY OF *Pediococcus acidilactici* AS PROBIOTIC SUPPLEMENTS TO PROTECT *Caridina logemanni* AGAINST A COMMON PATHOGEN *Aeromonas hydrophila*

Po-Tsang, Lee*, Kuan-Xuan, Chen, Kuo-Kau, Lee, and Ping-Chung, Liu

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The study aims to investigate the bacterial diseases in ornamental shrimp, *Caridina logemanni*, which occurred during the process of culture, transportation or environmental change, and an attempt was made to provide *Pediococcus acidilactici* orally as probiotic to enhance the immunity of the host and to reduce the risk of infection by a common pathogenic bacterium, *Aeromonas hydrophila*.

The shrimps used in the study were obtained from different breeders all over Taiwan and bacteria were isolated from diseased shrimps during the process of culture or after transportation. A strain of *A. hydrophila*, was identified using several biochemical tests, 16S rRNA sequencing and histological sections, and the *pathogenicity* of the bacterium was evaluated by immersion challenge test. Two strains of lactic acid bacteria, *P. acidilactici*, were isolated from pickled foods with their identity confirmed by the aforementioned methods, and the potential of these bacteria as *probiotics* was evaluated by an *in vitro* co-culture test with *A. hydrophila* or as feed supplement for shrimps.

Histological *sections* revealed that the foregut, midgut gland and midgut of shrimps infected by *A. hydrophila* exhibiting bacteria aggregation, a large amount of necrotic epithelial cells and different degree of inflammatory response. The results of *in vitro* co-culture test and supplement of lactic acid bacteria in feed showed that the isolates *P. acidilactici* (strain La0707) had better bacteriostasis ability than *P. acidilactici* (strain Lp0709).

According to the results of the study, it is suggested not to use the frozen tubifex as food due to the abundance of *A. hydrophila*. Moreover, addition of *P. acidilactici* in artificial feed can significantly improve the health status of shrimp and reduce the risk of infection that may break out due to stress.

DIFFERENTIAL EFFECTS OF INORGANIC ARSENIC ON *Sarcodia suieae*

Po-Yi Lee, Tsung-Meng Wu

Department of Aquaculture, National Pingtung University of Science and Technology

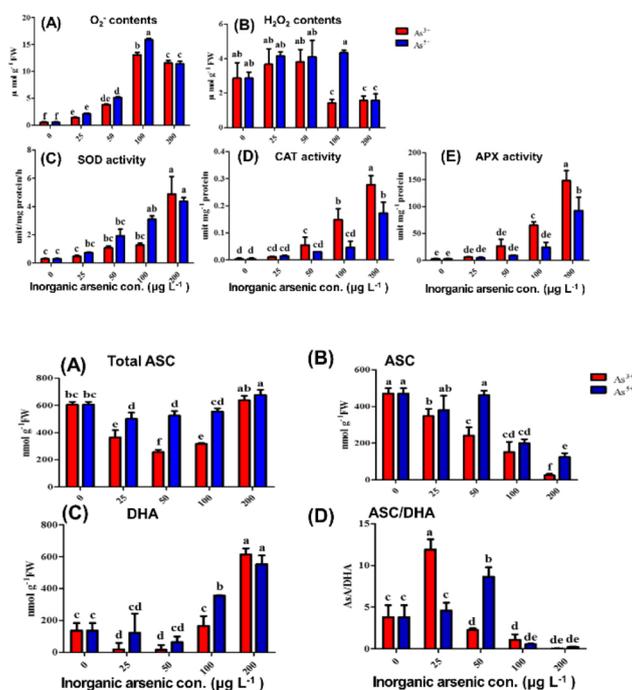
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In natural waters of arsenite (As^{3+}) and arsenate (As^{5+}) are common forms of inorganic arsenic (iAs). Algae can accumulate arsenic concentrations higher than the surrounding water nearly 1000 times. In order to investigate the effect of iAs toxicity to macroalgae, *Sarcodia suieae* was treated with various concentrations of As^{3+} and As^{5+} for 5 days. According to the results, the growth rate was significantly inhibited under 200 $\mu\text{g L}^{-1}$ iAs treatment. The superoxide radical and MDA content were increased with the increase of iAs concentration. Thus, iAs could induced oxidative stress in *S. suieae*. Total chlorophyll content dramatically decreased with the increase of iAs concentration, but that was significantly higher in As^{5+} treatment than As^{3+} treatment. Meanwhile, the ascorbate content in *S. suieae* under As^{5+} treatment was significantly higher than that in As^{3+} treatment. It suggested that *S. suieae* might enhance the tolerance to As^{5+} by increasing the ascorbate content. In addition, under As^{3+} treatment, *S. suieae* could alleviate the oxidative stress by increasing the activity of antioxidant enzymes such as APX and CAT. In conclusion, *S. suieae* might use different strategies to cope with the iAs-induced oxidative stress.

Results

Superoxide radical (A), Hydrogen peroxide radical (B), SOD activity (C), CAT activity (D), APX activity (E) of replicates that treated with various concentrations of As^{3+} and As^{5+} for 5 days. Each bar represents the mean value from 3 replicates with the standard error. Data (Mean \pm SE) with different letters significantly differ ($p < 0.05$) among treatments.

Total ASC(A), ASC (B), DHA (C), ASC/DHA (D) of replicates that treated with various concentrations of As^{3+} and As^{5+} for 5 days. Each bar represents the mean value from 3 replicates with the standard error. Data (Mean \pm SE) with different letters significantly differ ($p < 0.05$) among treatments.



TILAPIA DISEASE MANAGEMENT – CONTROL AND PROTECT YOUR FISH WITH GOOD FARM HUSBANDRY PRACTICES AND VACCINES

Lee Yeng Sheng

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Aquaculture is a booming business, where intensive fish farming has been implemented to supply fish protein for the world's growing population. Among the various fish species farmed globally, tilapia is one of the most economically important species where farming takes place across three continents: Asia, Africa, and South America.

Tilapia, often thought of as a hardy fish that can withstand various environmental stresses, is already succumbing to diseases under high intensity farming. It is essential to understand the spectrum of diseases that affects tilapia aquaculture, in order to implement strategies to mitigate the diseases. Parasites, bacteria and viruses are always present at various operational stages of a tilapia farm. It is crucial to know what diseases are present in your farm, how the disease impacts your farm's operations, and what tools are available to help you manage the diseases.

As a rule of thumb, sustainable aquaculture always begins with good planning and effective biosecurity management practices. Good sanitary and husbandry processes to keep your fish clean and healthy are cornerstone to every farm's success or failure. Although vaccines have been regarded as the gold standard to prevent diseases, vaccines are not available against all tilapia diseases based on current science.

Effective Streptococcal vaccines are available in the market with proven economic benefits to mitigate Streptococcal risks. However when dealing with parasites, other bacteria and certain viruses, comprehensive biosecurity measures are required to control the influx and outbreak of the diseases.

In this presentation, we will take a look at the diseases in tilapia, at what stage the diseases affects the farm operations, and what solutions can we implement to mitigate the risks.

OMEGA-3 POLYUNSATURATED FATTY ACID CAN LOWER INFLAMMATOIN CAUSED BY *Streptococcus agalactiae* IN TILAPIA

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The ω -3 polyunsaturated fatty acid (ω -3 PUFA) were shown to have a significant effect on inhibiting the inflammatory reaction, but the molecular mechanism is still unknown in teleost. The previous study proved that after injected by *Streptococcus agalactiae* for 12 hours the inflammation index was reduced and induced the antioxidant capacity in ω -3 PUFA enrich diet tilapia (*Oreochromis niloticus*). The transcriptomic analysis showed that the expression on *nlrc3* (NLR family, CARD domain containing 3), which was found out to have the effect of down-regulating the inflammatory reaction, was up-regulated in ω -3 PUFA diet tilapia after injection. However, the pathway of how ω -3 PUFA is up-regulating *nlrc3* is still unknown. In this study, we use $\Delta 5$, $\Delta 6$ desaturase transgenic tilapia as a model to prove how this pathway work. We inject the tilapia by *Streptococcus agalactiae* for 12 hours, extract total RNA of the intestine, liver, and brain, and analyze the expression of inflammation index, antioxidant capacity and *nlrc3* through RT-PCR. We find that after injection of *Streptococcus agalactiae*, the expression of inflammatory genes was decreased and the expression of *nlrc3* was increased in $\Delta 5$, $\Delta 6$ desaturase transgenic tilapia. Therefore, our study shows that the inflammatory reaction was inhibited and the expression of *nlrc3* was up-regulated in $\Delta 5$, $\Delta 6$ desaturase transgenic tilapia. Also, other studies have shown that there is a correlation between the gut microbiome and anti-inflammatory. In the future, we will study the gut microbiome of $\Delta 5$, $\Delta 6$ desaturase transgenic tilapia to analyze this pathway more clearly.

TRANSCRIPTOME ANALYSIS OF *Streptococcus parauberis* IN THE SERUM OF OLIVE FLOUNDER *Paralichthys olivaceus*

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Streptococcus parauberis is a gram-positive, alpha-hemolytic bacterial fish pathogen causing massive economic losses in aquaculture industry. There are steady studies regarding on its pathogenicity, serotype and antibiotic resistance. However, little is known about mechanism of infection in host. Therefore, this study attempted to understand survival strategies of this pathogen in host by analyzing gene expression pattern through high-throughput sequencing methods.

Briefly, *S. parauberis* strain SPOF3K originally isolated from diseased olive flounder was suspended in the serum of olive flounder and BHIB (+1%NaCl). Total RNA was extracted from bacterial culture sampled at 1, 2 and 4 h after suspension, and RNA-sequencing was performed with Illumina hiseq 2500 platform. Differential gene expression (DEG) was analyzed by comparing normalized read counts of broth- and serum-cultured samples at each sampling time point. Finally, quantitative reverse transcription-PCR (qRT-PCR) was conducted to validate the expression levels of RNA sequencing for several selected genes.

As a result, 31.74%, 28.56% and 26.37% of genes, and 35.63%, 24.44% and 26.99% of genes were significantly up- and down-regulated at 1, 2, 4_hr, respectively (FDR<0.0001). Notably, 35 virulence-related genes in all samples suspended in the serum were found to be up-regulated, indicating they might be involved in a very crucial part in immune evasion, adhesion to host cells, invasion and dispersion. Genes in defense mechanism category from COG (Clusters of orthologous groups of proteins) analysis were over-represented at 2 and 4_hr, while the genes were under-represented at 1_hr. Finally, qRT-PCR showed corresponding expression result with RNA-sequencing. In conclusion, this study provides a comprehensive view of global gene expression changes in a serum environment, and also shows critical virulence related genes in *S. parauberis*.

MOLECULAR EFFECTS OF POLYCHAETES ON TESTIS MATURATION OF MALE DOMESTICATED BLACK TIGER SHRIMP *Penaeus monodon* USING MICROARRAY TECHNIQUE

Rungnapa Leelatanawit*, Umaporn Uawisetwathana, Amornpan Klanchui, Juthatip Kudej, Suwanchai Phomklad, Somjai Wongtripop, Pikul Jiravanichpaisal, Nitsara Karoonuthaisiri

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Polychaetes are commonly used to induce reproductive maturation in shrimp due to its high levels of protein, lipid, polyunsaturated fatty acids (PUFAs), and other hormonally active compounds. Previously, it was found that polychaetes provide benefits to growth, survival, and sperm performance in male domesticated broodstock *P. monodon*. To reveal molecular mechanism of how polychaetes enhanced reproductive maturation in the male black tiger shrimp, in this current work, transcriptomic profiles of male reproductive organs (testes and vas deferens) between polychaete-fed and commercial pellet-fed male brooders were compared using cDNA microarray. The overall profiles were distinguishingly different between the two feed groups as well as between testes (TT) and vas deferens (VD) (Figure 1).

Gene expression profiles of TT and VD were subsequently analyzed to identify affected genes/pathways related to testis maturation (Figure 2). The Significance Analysis of Microarrays showed that 48 transcripts of TT and 114 transcripts of VD were significantly up-regulated in polychaete-fed shrimp, whereas none of transcripts was down-regulated in polychaete-fed shrimp. Additionally, eleven differentially expressed genes were selected from microarray results and validated for their expression levels using quantitative real-time PCR. Of those, six genes (*HNRPUL1* and *GCP4* in TT, *MAT2B*, *CDC16*, and *CSN5* in VD, and *SLD5* in both organs) were found to exhibit significantly higher expression levels in polychaete-fed shrimp than those in commercial pellet-fed shrimp. Subsequently, the differentially expressed genes were mapped into pathways important to spermatogenesis, such as DNA replication, cell cycle regulation, microtubule nucleation, protein refolding and apoptosis.

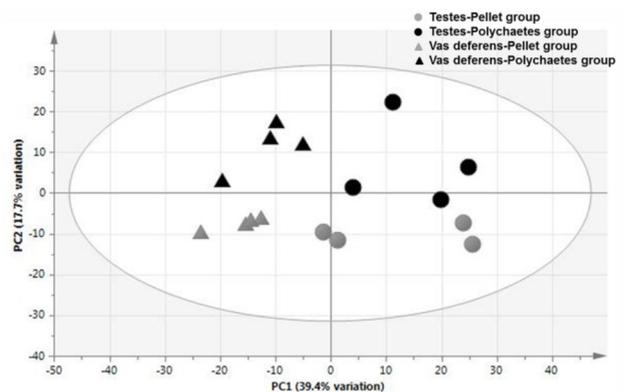


Figure 1 Principal component analysis (PCA) of overall gene expression profiles from testes (TT) and vas deferens (VD) after feeding with commercial pellet and polychaetes for 1 month.

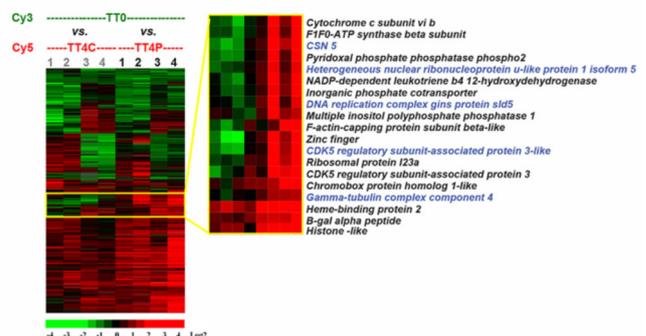


Figure 2 Gene expression profiles of testes from commercial pellet-fed shrimp (TT4C) and polychaete-fed shrimp (TT4P) at week 4 compared to those of shrimp at Week 0 (TT0).

INTERRELATIONSHIP OF PHOTOPERIOD ON GROWTH PERFORMANCE AND REPRODUCTION OF GUPPY (*Poecilia reticulata*)

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Photoperiod is an important environmental factor modulating biological functional of fish such as feeding strategy, locomotion activity, reproduction and metabolism. Long photoperiod have been used to improve somatic growth and feeding efficiency, but may delay sexual maturation in some species. Naturally, photoperiod vary depending on seasonal cycle either at temperate or tropical region. With these knowledge as background, this study was aimed to investigate the effect of different photoperiod regimes on the growth and reproductive performances of guppy (*Poecilia reticulata*). We hypothesized that long period of light or darkness condition might effectuate hyperactive reaction or promote resting mode. This may result more energy being mobilized for basal metabolism need and feeding deprivation which can depress growth performances as a consequence. In order to address this hypothesis, an experiment was set with four different photoperiod regimes, (i) 6 hours light versus 18 hours dark (6L:18D); (ii) 12L:12D; (iii) 18L:6D; and (iv) 24L:0D) by using aquarium full spectrum light system for two months. Each photoperiod regime consisted of triplication with 10 fishes each. Feeding rate was set at 6% daily. As expected, long darkness period was found to negatively impact the overall growth and reproductive performances of guppy. Similar effect was also noticed for fishes which received only 6L. In conclusion, medium lightness and darkness condition provided a positively effect on growth and reproductive performance. Accordingly, this experiment recapitulate that appropriate light and darkness cycle is important to provide sufficient active and resting mode to ensure optimal fitness status for growth and reproduction of guppy.

IDENTIFICATION AND EXPRESSION ANALYSIS OF 19 CC CHEMOKINE GENES IN ORANGE-SPOTTED GROUPEP *Epinephelus coioides*

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Chemokines are a large, diverse family of cytokines with chemoattractant activities, and they induce immune cell chemotaxis under both inflammatory and normal physiological conditions. After migration, the recruited cells can then perform their immune functions or are subjected to further regulation and differentiation. As a result, chemokines are considered important to both innate and adaptive immunities. Chemokines are characterized by the presence of four conserved cysteine residues and are divided into four subfamilies based on the arrangement of the two N terminal cysteines: CXC, CC, CX3 and C classes. Of the four classes, CC chemokines comprise the largest family. Analysis of CC chemokine genes in various fish species reveals that the number of CC chemokine genes and their tissue expression patterns vary greatly. Iridoviruses are important viral pathogens that seriously affect the grouper aquaculture industry in Taiwan. Therefore, developing the strategies that prevent, control and even cure these viral pathogens has been the focus of related research.

To unveil how the immune-related genes respond to iridovirus infection in grouper, we had used next generation sequencing technology to perform transcriptome analysis in the spleen of *Epinephelus coioides* infected with grouper iridovirus (GIV). The results showed that after GIV infection, a vast array of immune-related gene exhibited changed expression, and, of these changed genes, several CC chemokine genes were included. Further analysis showed that the spleen transcriptome contained 19 CC chemokine genes, and this research focused on the study of these CC chemokines to investigate their importance to the immunity of *E. coioides*. We firstly characterized the complete coding sequence of each CC chemokine gene, then investigated their gene expression pattern in various tissues, and finally determined the effect of GIV, poly I:C and LPS on the expression of these genes in the spleen.

FISH DISEASE IN HONG KONG AQUACULTURE: INSIGHTS ON THE DIAGNOSIS AND PREVENTIVE MEASURES ON FISH HEALTH MANAGEMENT

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The aquaculture industry in Hong Kong mainly involves grow-out culture of marine (e.g. grouper and pompano) and freshwater (e.g. jade perch, grey mullet and tilapia) fishes using cages and ponds, respectively. The majority of fish farms employ traditional husbandry practices with high stocking densities. Under such circumstance, implementation of good biosecurity measures can be challenging. The Agriculture, Fisheries and Conservation Department has started to study on fish diseases since 1994. The department provides technical advice and assistance to local fish farmers on fish disease diagnosis, prevention and control.

For freshwater fish culture, disease outbreaks are usually related to inadequate water exchange, high stocking density and seasonal influence. Common pathogens include *Aeromonas* spp., *Streptococcus* spp., *Trichodina* spp., *Centrocestus* spp., and *Ichthyophthirius multifiliis*. For marine fish culture, *Dactylogyrus* spp., *Cryptocaryon irritans* and *Trichodina* spp. seriously affected the fry and fingerling of most species during the early stages of their introduction to floating net-cages. Secondary bacterial infections with *Vibrio* spp. are not uncommon. Megalocytivirus (RSIV and ISKNV) and Viral Nervous Necrosis Virus (VNN) were observed in both marine and freshwater fishes. Saprolegniasis caused by *Saprolegnia* spp. was the only fungal disease observed. Non-infectious diseases (fatty liver disease, gas bubble disease and swim bladder stress syndrome) also reported occasionally.

To serve the Hong Kong aquaculture industry better, an active Fish Health Inspection (FHI) surveillance was implemented by the department with three objectives: (1) to facilitate early detection of fish diseases and outbreaks on local fish farms; (2) to identify potential latent or emerging clinical infections; and (3) to implement the primary health strategies for controlling the spread of fish disease and minimizing fish loss.

In order to provide technical advice, recommended treatment and preventive measure at an early stage to minimize the spread of disease, the department is equipped with Vitek System, bacterial identification by Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI TOP) and viral screening by pocket PCR kits (IQ PLUS) with a view to shorten the laboratory turnover time as far as possible.

THE GENETIC DIVERSITY OF THE SINIPERCIDS

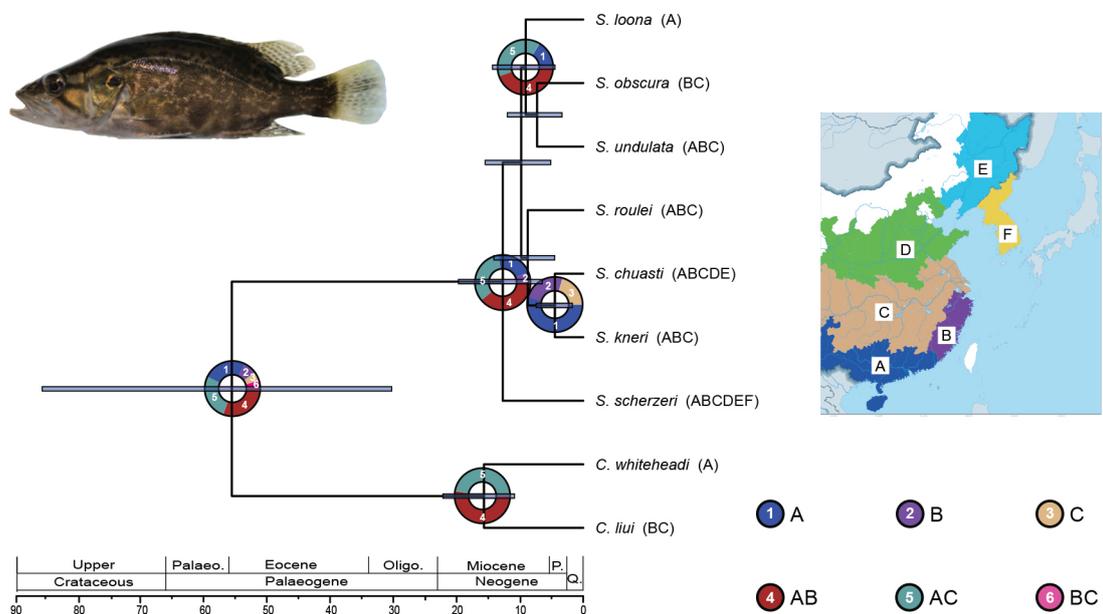
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The sinipercids are endemic freshwater fishes of East Asia, distributed in China, Vietnam, Japan, Russia and Korea Peninsula, with most species found in China. There are 9 to 12 species according to different researchers. The validity of some sinipercid species and their phylogenetic relationship are still controversial. The sinipercids include many economically important species, with delicious and tender meet and have a well-established aquaculture industry. Different species may have different growth performance. For example, *Siniperca chuatsi* grows faster than *S. kneri*. There are also significant variations between populations within species. For example, *S. scherzeri* in Fujian and Guangxi are different, and *S. chuatsi* of northern China are different from that of southern China. Therefore, elucidating evolution history of the sinipercid fishes and evaluating their genetic diversity are important to better conserve and utilize genetic resource of the sinipercids.

In this study, we collected sequence data from 16,943 nuclear loci of 33 individuals of 9 species through gene capture and Illumina sequencing. Bayesian factor delimitation support that *S. loona* and *C. liui* are valid species. Maximum likelihood analysis indicates that *S. scherzeri* is the basal Siniperca. *S. chuatsi* and *S. kneri* are sister taxa and together they are grouped with *S. roulei*. *Siniperca loona*, *S. obscura* and *S. undulata* form a clade.

We also identified outlier loci and loci that were not affected by genetic introgression from *S. kneri* to *S. chuatsi*. Those results illustrated that useful genetic information could be mined through comparing genome-scale data at both inter-specific and intra-specific level.



RESEARCH PROGRESS IN THE INNATE IMMUNITY OF PENAEID SHRIMP AND ITS POTENTIAL APPLICATION IN AQUACULTURE

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Shrimp aquaculture plays very important roles for providing high-quality proteins to people. However, with the development of aquaculture, disease problem become the major obstacles to hinder the sustainable development of shrimp culture industry. Understanding the immunity of shrimp is the basis for disease control. Shrimp belongs to invertebrate which has only innate immunity. In the last two decades, researchers have paid more attention on the innate immunity of shrimp, and a series progress have been made. In our group, EST sequencing, cDNA microarray, RNA seq. and proteomics approach were used to screen the genes related to bacterial and viral infection and the functions of the related genes were analyzed. Based on the accumulating data obtained in our group and other researcher in China, we summarized the outlines of humoral immunity and cellular immunity of shrimp. On the basis of these works, we specifically focused on the functions of immune effectors and developing genetic markers related to disease resistance. These information will not only enrich the knowledge of immunity in crustacean, but also provide instructions to disease control and genetic breeding of shrimp.

COLD TOLERANCE GENE-ASSOCIATED MARKERS DISCOVERY FROM THE TRANSCRIPTOME DATABASE BY NEXT GENERATION SEQUENCING IN NILE TILAPIA (*Oreochromis niloticus*)

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Tilapia is one of the major aquaculture species in Taiwan. The tropical fishes are sensitive to cold, and thus leading to mass mortality of fish when cold current arrived. The genetics and brood stock management strategies of molecular marker-assisted selection and breeding are the most important key tools to promote improved varieties of seed in the tilapia species. The purpose of this study was to develop molecular markers of cold tolerance related genes by using the digital gene expression analysis of next-generation transcriptome sequencing in Nile tilapia.

We constructed and sequenced cDNA libraries from eight closely related Nile tilapia tissues of brain, gill, liver and muscle in cold-tolerance (CT) and cold-sensitivity (CS) groups. Of these, a total of 38,377 SSRs and 65,527 SNPs were identified, cold tolerance-related gene sequences were established out of 5 functionality SSR loci and 37 SNP markers were selected from 25 genes after filtered. To develop cold tolerance-related markers, we use artificial control of environmental conditions to simulate the cold current model for testing different tilapia population (Wild, Farm 1, Farm 2). 12 of 37 SNPs are located on exon region and 7 of them have amino acid change. 5 microsatellite markers (Table 1) showed polymorphism in different population and marker CL5902_1 has correlation with cold tolerance in wild population ($q=0.004$).

FIGURE 1. Cooling experiment results

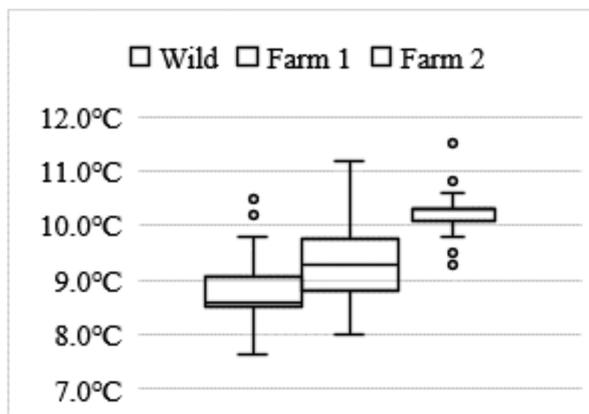


TABLE 1. Correlation between cold tolerance and SSR markers

ρ -value	Wild	Farm 1	Farm 2
UNH916	0.409	0.551	0.857
UNH999	0.072	0.878	0.872
CL1876_16	0.537	N/A	N/A
CL5902_1	0.004*	0.451	N/A
CL9541_2	0.456	0.767	0.817

MIR-145 MEDIATES ZEBRAFISH HEPATIC OUTGROWTH THROUGH PROGRANULIN A SIGNALING

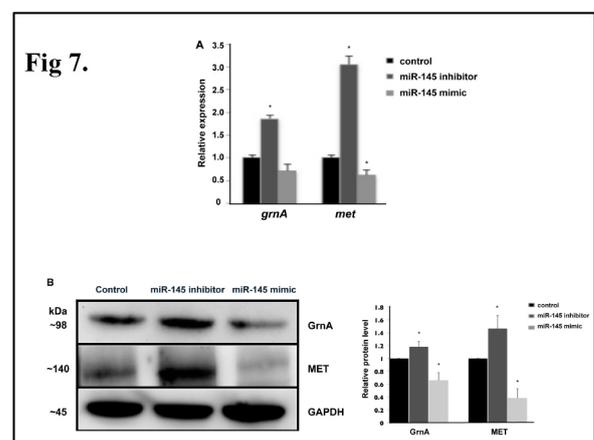
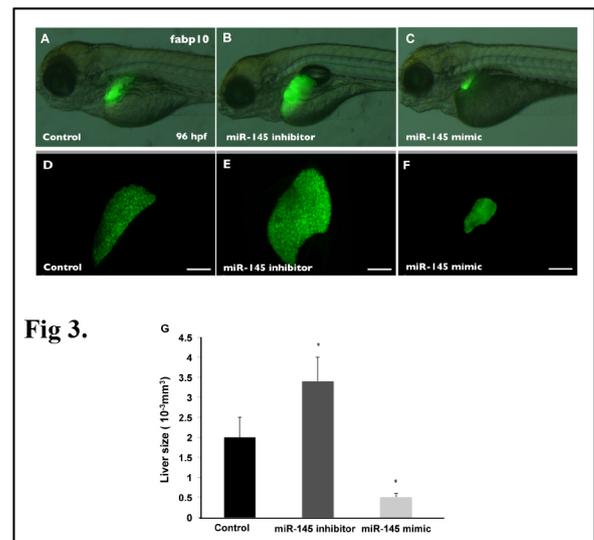
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MicroRNAs (miRs) are mRNA-regulatory molecules that fine-tune gene expression and modulate both processes of development and tumorigenesis. Our previous studies identified progranulin A (GrnA) as a growth factor which induces zebrafish hepatic outgrowth through MET signaling. We also found that miR-145 is one of potential fine-tuning regulators of GrnA involved in embryonic hepatic outgrowth. The low level of miR-145 seen in hepatocarcinogenesis has been shown to promote pathological liver growth. However, little is known about the regulatory mechanism of miR-145 in embryonic liver development. In this study, we demonstrate a significant decrease in miR-145 expression during hepatogenesis. We modulate miR-145 expression in zebrafish embryos by injection with a miR-145 mimic or a miR-145 hairpin inhibitor. Altered embryonic liver outgrowth is observed in response to miR-145 expression modulation. We also confirm a critical role of miR-145 in hepatic outgrowth by using whole-mount in situ hybridization. Loss of miR-145 expression in embryos results in hepatic cell proliferation, and vice versa. Furthermore, we demonstrate that GrnA is a target of miR-145 and GrnA-induced MET signaling is also regulated by miR-145 as determined by luciferase reporter assay and gene expression analysis, respectively. In addition, co-injection of GrnA mRNA with miR-145 mimic or MO-GrnA with miR-145 inhibitor restores the liver defects caused by dysregulation of miR-145 expression. In conclusion, our findings suggest an important role of miR-145 in regulating GrnA-dependent hepatic outgrowth in zebrafish embryonic development.

After miR-145 inhibitor injection, miR-145 inhibition significantly increased the size of liver expressing EGFP at 4 dpf (Fig 3B). In contrast, embryos with overexpression of miR-145 exhibited smaller EGFP-expressing liver compared to that seen in control-injected liver (Fig 3A and 3C). The liver size was determined by measuring the volume of EGFP-expressing livers using three-dimensional confocal images (Fig 3D-F and 3G). These results indicate that miR-145 expression level negatively affects liver size at 4 dpf in zebrafish.

We determine whether endogenous GrnA expression is affected by miR-145. Control mimic, miR-145 inhibitor and miR-145 mimic were transfected into ZFL cells to assess the activation of GrnA and MET signaling using qPCR (Fig 7A) and Western blotting (Fig 7B). These data suggest that miR-145 modulates GrnA expression through binding to GrnA mRNA in zebrafish liver.



EFFECT OF DIETARY SMALL MOLECAULAR NITROGEN COMPOUNDS ON GROWTH, FEED UTILIZATION AND EXPRESSION OF GROWTH RELATED GENES OF JUVENILE TURBOT (*Scophthalmus maximus* L.) FED WITH HIGH PLANT PROTEIN

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A 10-week experiment was conducted to investigate the effects of dietary small molecular nitrogen compounds on growth performance, body composition, intestinal morphology and growth related genes of juvenile turbot [(initial weight of 9.46 ± 0.01 g)] fed with high plant protein. There were 6 isonitrogenous and isolipidic diets were formulated. A 30% fish meal positive (FM), a negative control (SB) with soybean meal in exchange of 30% fish meal protein and four SB diets were produced added 1% taurine (SBT), 0.6% hydroxyproline (SBH), 0.03% nucleotide mix (SBN), 0.39% carnosine (SBC). Fish fed SB diet obtained the significantly lower specific growth rate (SGR), protein efficiency ratio (PER) and protein productive value (PPV) than the control group ($P < 0.05$), and there was no significant difference with those in SBN group and SBC group ($P > 0.05$). Compared with fish fed SB group, there exist significantly increase of SGR in fish fed SBT diet and feeding rate (FI) in fish fed SBT, SBH, SBN and SBC diet ($P < 0.05$) as well as PER and PPV in fish fed SBT and SBH diet ($P < 0.05$), and no significant difference was observed with those in the control group ($P > 0.05$). Fish fed diet SBT, SBH, SBN, SBC had significantly higher total amino acid and total essential amino acid than those in SB group and control group ($P < 0.05$). Both of SBT group and SBN group indicated significantly lower glutamic oxalacetic transaminase (GOT) activities ($P < 0.05$) and SBH group showed the lowest glutamic-pyruvic transaminase (GPT) activities in serum compared with SB diet ($P < 0.05$), while it showed the opposite trend in liver. Amylase activity in intestines of fish fed SBH diet and trypsin activities in pyloric caecum of fish fed SBT and SBH diet showed significantly higher than those of fish fed SB diet ($P < 0.05$). Meanwhile, the intestinal morphology analysis showed all of the mid- intestine fold height in SBT and SBC group, enterocyte height in SBH and SBC group, and microvillus height in SBT, SBH and SBN group increased significantly than those in fish fed diet SB ($P < 0.05$). The results also showed that in addition to slightly higher IGF-1 mRNA relative expression of fish fed diet SBN ($P < 0.05$), dietary diets supplementation with small molecular nitrogen compounds had significantly higher IGF-1, NPY, PepT1 mRNA relative expression than those in fish fed diet SB ($P < 0.05$). In conclusion, these results indicated that dietary small molecular nitrogen compounds supplementation with soybean in exchange of 30% fish meal significantly enhanced the feeding intake and intestinal morphology as well as up-regulated IGF-1, NPY, PepT1 mRNA relative expression of juvenile turbot. Furthermore, dietary 1% taurine supplementation obtained the best growth performance of juvenile turbot.

PHYCOERYTHRINS PURIFICATION AND CHARACTERISTIC IN *Acrochaetium sp.*

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The coastal area of Taiwan is known for its rich seaweed resources and diversity. Each alga have photosynthetic pigments with bioactive substances that can be obtained by various extraction strategies. *Acrosiaceae sp.*, is a filamentous marine red algae in Taiwan based from morphological characterization and genetic identification. This species has the ability to grow in wide temperature ranges (15-25°C) and have a crude protein content as high as 31%. In the present study, this algae was isolated and purified for Phycoerythrin (PE) extraction. PE was subjected to light-emitting property and was obtained by gel filtration after being freeze-dried and extracted. The results in this study, following the above mentioned method, showed that *Acrosiaceae sp.* had yielded 93.6% PE with phycoerythrin purity (PI) of 4.31%. The fluorescence intensity used in the protein purification was linear with the content and had similar fluorescence characteristics as the commercial phycoerythrin standard. The freeze-dried extraction technique on *Acrosiaceae sp.* were efficient to obtained PE compounds and the presence of which in the alga is useful for many biotechnological applications in food science, pharmaceuticals, nutraceuticals and analytical processes and can compromise PE preparation from other marine rhodophyta in the future.

WARMING CONDITION INDUCED HIGH LIVING COST AND DEPRESSED SPAWNING PERFORMANCES OF AIR BREATHING FISH

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Climate warming threatening fish population by intensifying environment deterioration that affects their feeding, reproductive performance, growth and survival. Growing evidences show that tropical species are sensitive to climate warming because they have a narrow thermal limit, where they are living closer to their maximum thermal capacity compared with species from higher latitudes that experience seasonal thermal fluctuation. Many researches have been carried out to investigate the impact of climate change on important aquaculture species especially water-breathing fishes, which are more sensitive. Adversely, air-breathing fishes are assumed to be more tolerant to climate warming. Thereby we hypothesized that, air-breathing fishes can compromise oxygen intake atmospherically when dissolved oxygen in water is limited. However, osmorepiration can be compromised, but under such high living cost may result in insufficient energy for reproduction. Therefore, this study investigates the impact of high environmental temperature on physiological responses and spawning performance of pearl gourami. Physiological responses was assessed by using respirometry assay, while spawning performance was investigated via selective pairing natural spawning method. Fishes were gradually adapted to 34°C from 28°C within two weeks prior to respirometry assay and spawning performance were monitored for four months. As expected, high metabolic oxygen intake was recorded. Moreover, our results also showed that 34°C depressed feeding, mating and spawning performances as compared to 28°C. Nest bubbles were small in size and scattered that resulted in low hatching and survival rate. With all of these information obtained highlighted that climate warming highly impair overall feeding, reproduction performance and basal metabolism responses.

SALINE-TOLERANT MOZAMBIQUE TILAPIA GENETIC SELECTION FOR IMPROVE GROWTH RATE

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Diminishing supplies of freshwater globally will adversely impact high intensity tilapia culture, therefore there is a need to develop saline tolerant tilapia strain. We have been conducting genetic selection for growth performance in high salinity condition on Mozambique tilapia (*Oreochromis mossambicus*) with the aim to develop a tilapia strain that perform well in brackish and seawater. After three generations of selection the average body weight (ABW) at 3 mph increased from 21.5g (F₂) to 33.9g (F₄), achieving more than 10% improvement for each generation. A set of nine microsatellite genotyping panel in a single multiplex PCR was also developed to monitor genetic diversity and for parentage assignment. The selection is still ongoing with the long-term goal of producing a commercial viable saline-tolerant tilapia line.

DIETARY pH PREFERENCE IN JUVENILE MARBLE GOBY (*Oxyeleotris marmorata*) AND THE POSSIBILITY OF WEANING THEM WITH ACIDIFIED DIETS

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Marble goby *Oxyeleotris marmorata* is a popular freshwater fish species cultured mainly in the Southeast Asian region. However, fish mass mortality usually occurred in the hatchery during the early juvenile stage when the weaning process take place. In our previous study to identify species-specific feeding stimulant from organic acids for this fish, we discovered that *O. marmorata* showed high ingestion ratio of the acidic agar gel pellets. This finding suggested that dietary pH may play an important role in the food selection of *O. marmorata*. Therefore, the present study was aimed to identify the preferred dietary pH of *O. marmorata* juveniles (Exp. 1), and to determine the possibility of weaning them with acidified diets (Exp. 2).

In Exp.1, behavioral test was conducted on the *O. marmorata* juveniles (50 individuals or replicates; total length 6.6–7.0 cm) using agar gel pellets. Eight pH levels of agar gel pellets were prepared (pH 2.4, 3.0, 3.2, 3.4, 4.1, 4.9, 5.1, and the pure agar gel pellet without pH modification—pH 5.9). The ingestion ratio for each pH treatment of agar gel pellet was calculated, and the binary data (ingested or rejected) was analyzed using binomial test. Negative relationship was found between the ingestion ratio and pH level of the agar gel pellets. The highest ingestion ratio was found in the agar gel pellet with pH 2.4 (ingestion ratio, 94%), following by pH 3 (85%), pH 3.2 (3% agar gel powder; 65%), pH 3.2 (2% agar gel powder; 58%), pH 3.4 (24%), pH 4.1 (6%), pH 4.9 (6%), pH 5.1 (6%), and pH 5.9 (2%). The ingestion ratio of pH 2.4 agar gel pellet was significantly higher ($P < 0.05$) than that in the other treatments except that of pH 3.0. These results confirmed the taste preference of *O. marmorata* for acidic foods, and the most preferred was the one with pH 2.4–3.0.

In Exp. 2, a 20-day feeding trial was conducted. Five fish meal-based diets were prepared: 4 of them were acidified diets (AD) pH 5.3, 4.3, 3.2, and 2.5 by hydrochloric acid (HCl), and the control diet was at pH 6. Each of these dietary treatments was fed to 10 individuals of wild-caught *O. marmorata* juveniles (BL 7.7 \square 9.3 cm) in separate aquarium (each fish represented a replicate), and the daily ingestion ratio (IR) was calculated (total number of the ingested pellet/ total number of the given pellet – 5). This data was analyzed using the Kruskal – Wallis test and the Conover-Inman post-hoc test. At the end of the feeding trial, the fish within each dietary treatment were categorized into the “Good feeding performance” or “Poor feeding performance” group according to the highest IR (Poor = 0.0, 0.2 or 0.4; Good = 0.6, 0.8 or 1.0) that they ever achieved, and the data was analyzed using Chi-square test. Along the feeding trial, *O. marmorata* juveniles showed clear increment in the IR of AD 2.5 (the highest IR among all dietary treatments at most of the time), AD 3.2 and AD 4.3. The control diet and AD 5.3 were almost totally rejected by the fish. Although there was no significant difference ($P > 0.05$) among the IR of all diets at day 9, the IR of AD 2.5 (0.46) and AD 3.2 (0.36) was significantly higher ($P < 0.05$) than those of AD 5.3 (0.1) and the control diet (0.02). In addition, 70% of fish fed AD 2.5 achieved the “good feeding performance”, and this was the best result among all treatments (AD 3.2, 60%; AD 4.3 50%; AD 5.3 and control, 10%). These results suggested that acidified diets can be used to wean *O. marmorata* juveniles, and the most recommended pH level was 2.5.

EFFECT OF DIFFERENT SIZES AND STOCKING DENSITIES ON PRODUCTIVITY AND WATER QUALITY OF GET EXCEL NILE TILAPIA *Oreochromis sp.*/ PANGASIOUS *Pangasius hypophthalmus*- LETTUCE *Lactuca sativa* RAFT-AQUAPONIC SYSTEM

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The study evaluated the productivity and water quality of GET Excel Nile Tilapia (*Oreochromis sp.*)/ Pangasius (*Pangasius Hypophthalmus*)-Lettuce (*Lactuca sativa*) raft-aquaponic system. Large and small fish were used in this study.

Two blocks of experimental units were consisted of two fish sizes: large (2000 grams biomass) and small (low, medium and high density which consisted of 80, 100 and 120 1-g tilapia, respectively). Each hydroponic bed was consisted of 10 pangasius and 20 lettuce. The rearing experiment was carried out for 77 days. Fish sampling was conducted every 14 days while initial and final weight and length of lettuce were recorded. Furthermore, sampling of water quality parameters was conducted every 72 hours.

Small fish group exhibited the highest percentage of weight gain (%), specific growth rate, and feed conversion ratio as compared to that of the large fish. Growth performance of Pangasius in the hydroponic bed was also comparable among all treatments. Plants in large fish showed better growth and yield performance than other treatments. Acceptable TAN and PO₄ levels were detected in large fish which exhibited higher production of plant. Overall, stocking density displays a major role in the aquaponic system, as it corresponds to a better water quality which may contribute to better growth performance of both fish and plants. Prolonging of rearing period from 1-g tilapia up to marketable size is recommended to further asses the productivity of raft- aquaponic system using tilapia fingerlings.

DECREASED EXPRESSION OF SPARC BY IMMUNO-INHIBITION ENHANCES CELL PROLIFERATION IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Groupers are economically valuable fish species of marine aquaculture in Asia, and the optimal growth performance of fish is one of the keys for successful aquaculture industry. However, due to limited methodologies for *in vivo* investigation of protein function in commercial fish, the molecular mechanisms of cell growth and physiological properties were not fully exploited. Therefore, the immuno-inhibition technology was developed, which the specific antibody were induced for inhibition of target protein function in grouper by feeding with recombinant protein containing epitopes of target protein. Here we want to investigate the function of SPARC (secreted protein acidic and rich in cysteine) in orange-spotted grouper using the immuno-inhibition technology, since some researches indicated SPARC could regulate the interactions between cells and extracellular matrix and then restrict adipocyte differentiation.

The results showed the antibody against SPARC increased in the serum and SPARC protein expression were suppressed in muscle tissue of the SPARC-inhibited (SPARC-i) grouper. The tissue section analysis of meat showed that fat distribution area was larger, and the notable weight gain and larger muscle cells were found in SPARC-i grouper. Besides, the increased expression level of myogenic regulatory factors (*myoD*, *myogenin*, *mrf4* and *mrf5*) in muscle tissue isolated from SPARC-i grouper, indicating the muscle differentiation was promoted by inhibition of SPARC. We proposed that SPARC may interact with myostatin to regulate downstream smads signaling pathway involved in muscle cell growth. The transcripts of smads (*smad2*, *smad3*, *smad4* and *smad7*) in orange-spotted grouper were identified. The RNA expression levels of smads in muscle tissue were analyzed and showed the expression of *smad7* increased in SPARC-i grouper. On the basis of these results, the inhibition of SPARC could promote muscle cell growth and increase the adipocyte size and fat content in grouper. Furthermore, the immuno-inhibited model would be applied to commercial fish for *in vivo* biological processes and physiological function studies.

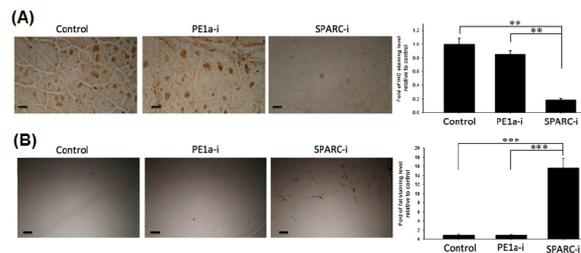


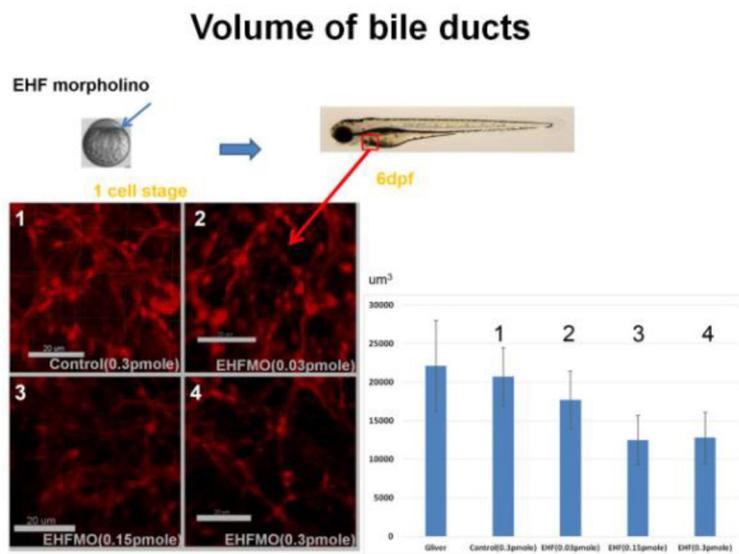
FIGURE 1. (A) Less SPARC protein expressed in the muscle of SPARC-i grouper using IHC analysis. (B) More fat existed in the muscle of SPARC-i grouper using oil red O staining.

THE TRANSCRIPTION FACTOR EHF regulates the BILE DUCT DEVELOPMENT AND INTRAHEPATIC CHOLANGIOCARCINOMA TUMORIGENESIS IN human and ZEBRAFISH

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Intrahepatic cholangiocarcinoma (ICC), a highly malignant and second most common liver cancer worldwide, is still difficult to diagnose and cure until today. In our previous publication, we found that TGF- β signaling was activated in the hepatitis virus induced ICC in Zebrafish. In this study, we found that TGF- β may promote the bile duct development and tumorigenesis of ICC through regulating the transcription factor EHF. Knockdown of EHF interrupts the bile duct development, while EHF overexpression increases the proliferation of two human ICC cell lines HuCCT1 and HepaRG. These results indicated that TGF- β may promote the bile duct development and intrahepatic cholangiocarcinoma tumorigenesis through regulating EHF.

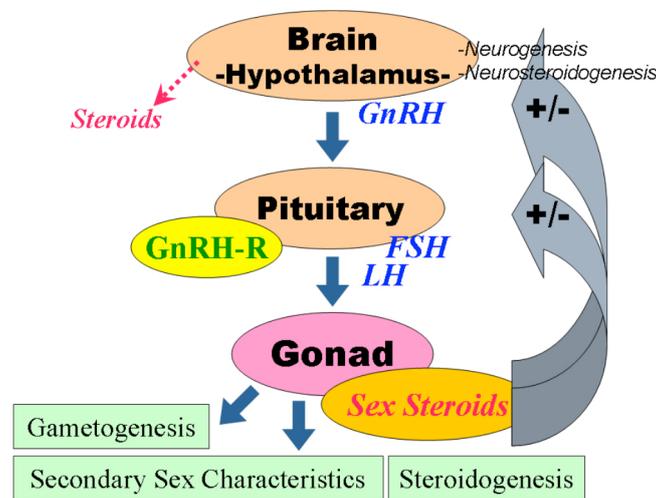


GONADOTROPIN RELEASING HORMONE AND GONADOTROPIN VARIATIONS DURING THE GONADAL DIFFERENTIATION IN PROTANDROUS BLACK PORGY *Acanthopagrus schlegelii*

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Hypothalamus-Pituitary-Gonad axis (HPG axis) is the key regulator of sex development and reproduction in vertebrates, including teleost. The presents study analyzed the regulatory factors involved in the HPG axis during the time of gonadal differentiation in protandrous black porgy. The transcript levels of *gnrh-r1*, *gtha*, *fsh β* , *lh β* in pituitary, and *sbgnrh*, *cgnrh-II*, *sgnrh*, *cyp19a1b* in brain were analyzed during sex differentiation and testicular development by real-time PCR. During sex differentiation, *gnrh-r1* (in pituitary), *sbgnrh* (in forebrain [FB] and hypothalamus [HY]), and *cyp19a1b* (in FB and HY) significantly increased, but *gtha*, *fsh β* , and *lh β* in pituitary had no significant difference. In testicular development and close to spawning season, the gene profiles of *gnrh-r1*, *gtha*, *fsh β* , *lh β* in pituitary and *sbgnrh*□*cgnrh-II*□*cyp19a1b* in brain show significant increase pattern. To investigate the E2 effect on transcript levels during sex differentiation, undifferentiated fish were fed with E2. The treatment of E2 upregulated gene expression of *gnrh-r1*, *fsh β* , *lh β* , but had no effect on *gtha* in pituitary. E2 treatment had no increment effect on brain *sbgnrh*. These results indicate that all three forms of GnRH involve in gonadal differentiation, while *sbgnrh* may be the most relevant form in the regulation of the first spawning season. The gonadotropin subunits may involve in the regulation of spawning season, but may not in the period of sex differentiation. The transcripts expression of brain *sbgnrh* and pituitary *gnrh-r1* may involve in reproduction regulation in first spawning.



IDENTIFICATION OF BETANODAVIRUS INDUCES mTOR/ULK1-MEDIATED AUTOPHAGY SIGNALING PATHWAY IN GROUPEL FISH CELL

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Red-spotted grouper nervous necrosis virus (RGNNV), is a kind of betanodavirus, which infects grouper mainly. Betanodavirus belongs to *Nodaviridae* family, which cause viral nervous necrosis leading large mortality of larve and juvenile grouper in a short time. It may cause economic loss. In our studies we found that grouper be infected could lead the reactive oxygen species (ROS) level.(Figure 1).Autophagy is a high conserve metabolic mechanism of cells that maintains a constant intracellular stability that can be used to recover old and damaged organelles, to provide energy for the production of new organelles by synthetic metabolism or as a raw material for cell assembly. In addition, when the cells face stress or pathogen invasion can induce to occur.

In our pervious study, we found that betanodavirus (RGNNV strain) infection can induce the autophagy in grouper fin cell line (GF-1) system. Then, we found that betanodavirus can activate mTOR – mediated autophagy signaling pathway. So in this present, we further ask how about the upstream and downstream signaling in mTOR pathway. In the result, we found that reactive oxygen species (ROS; H_2O_2 , O_2^-) involved in the upstream of mTOR signaling, which also blocked by antioxidants. Then, in the downstream of mTOR, we also found that ULK1 protein for triggering autophagy process also activated by mTOR activation. Additionally, mTOR/ULK1 signaling dramatically inhibited by ROS signaling, which blocked by antioxidant treatment. Taken together our results, we found that ROS signaling can regulate the mTOR activation via phosphorylation on serine 2448. Then, mTOR signaling further can regulate ULK1 activation on serine 757 in the downstream for autophagy process, which also correlated to control host survival and viral replication (Figure 2).This finding may provide new insight into betanodavirus pathogenesis.

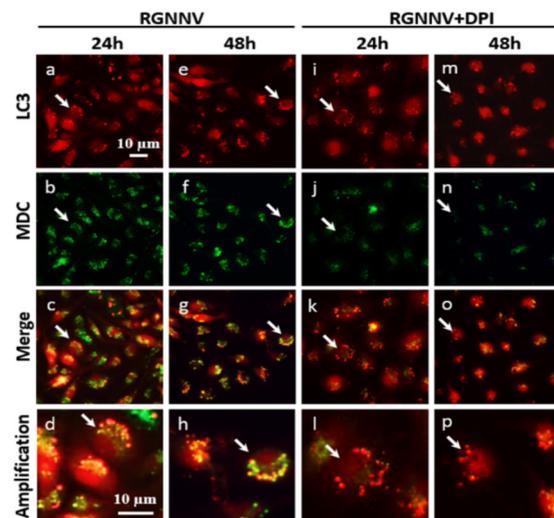


Figure 1.Reduction of oxidative stress can reduce autophagy.

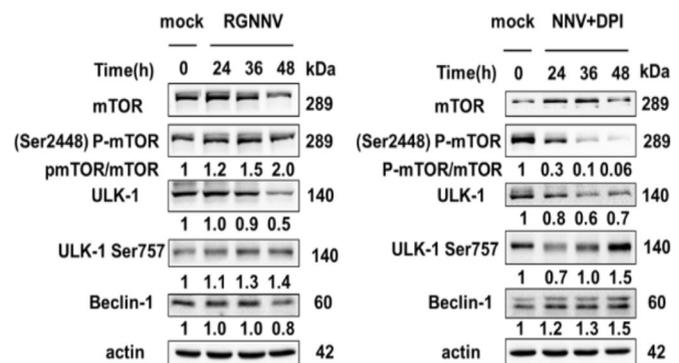


Figure 2.Western blot showing that mTOR can regulate ULK-1 on Ser757 during the autophagy process.

PERIPLASMIC SECRETION AND OSMOTIC SHOCK EXTRACTION OF FUNCTIONAL GROUPEY TYPE I INTERFERON FROM *Epinephelus lanceolatus*

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Interferon (IFN) plays crucial roles in innate immune responses against viral infections. Here, we transformed the IFN gene which was from the *Epinephelus lanceolatus* into *Escherichia coli* expression system, and characterized the best expressing condition of recombinant IFN protein. We compared the concentration of the proteins extracted by ultrasound or osmotic shock. We used pET-26b vector which contained specific signal peptide sequence PelB and *E. coli* C43 as expression host. The best expression condition was induction 12 hour in 24°C. We compared protein activity between two purify methods. In Mx gene expression, osmotic shock group was 5 folds higher Mx expression than ultrasound group. We confirmed that using osmotic shock method obtain a higher activity of proteins than using ultrasound method.

Epinephelus is one of the largest genera of groupers in the family Serranidae. However, a variety of grouper fish species are endangered because of overfishing and poor management of coral reef fisheries, encouraging the rapid development of the grouper aquaculture industry. In recent years, there have been many reports regarding the grouper viral infections with red-spotted grouper nervous necrosis virus (RGNNV), a two-single-stranded RNA piscine nodavirus, which resulted in high mortality rates (80%–100%) of affected larvae, juveniles and some adults of groupers. An approach to reduce RGNNV has been reported by introducing interferons (IFNs) to fish, and it has been reported to prevent viral infection in vivo and in vitro. IFNs are secretory cytokines in vertebrates that are involved in inhibition of virus replication and modulation of immune responses.

Table 1. Purification of recombinant IFN.

Purification steps ^a	Total proteins(mg) ^a	Yield (%) ^a
Crude ^a	13.5 ^a	100 ^a
Osmotic shock ^a	2.7 ^a	20% ^a
Affinity chromatography ^a	2 ^a	15% ^a

^aThe crude enzymes were from 1 L *E. coli* cells overexpressing IFN at 24°C for 12 h.

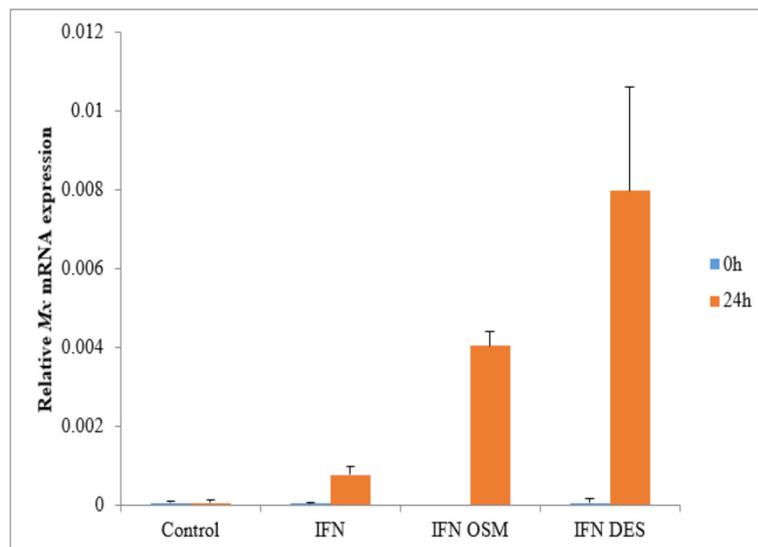


Figure 1. The relative mRNA expression level of IFN in different purification methods. The relative mRNA expression level of IFN extracted by ultrasound, Osmotic shock, or desalted process.

ESTIMATION OF DIETARY NIACIN REQUIREMENTS OF JUVENILE GROUPEL, *Epinephelus malabaricus*

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The study was aimed to evaluate the optimal dietary niacin requirements of juvenile grouper, *Epinephelus malabaricus*. Niacin was supplemented into a basal diet at 0, 2, 5, 10, 20, 50, 100 and 200 mg/kg diet, providing actual thiamin concentration of 0, 2.69, 5.56, 9.97, 20.69, 50.28, 104.33 and 204.62 mg/kg diet, respectively. Total of 8 experimental diets were each fed to triplicate groups of juvenile grouper (initial weight: 11.58 ± 0.61 g) in a recirculation rearing system for 8 weeks. Grouper fed diets with 20.69 and 50.28 mg/kg diet had higher weight gain than other dietary treatments. Hepatosomatic index was higher in fish fed the diet with 20.69 mg/kg diet than that in fish fed the niacin-free control diet. Hepatic nicotinamide adenine dinucleotide (NAD) concentration increased by increasing dietary niacin supplementation levels ($Y=0.20X-0.048$, $r=0.98$). Hepatic hexokinase activity was the highest in fish fed the diet with 20.69 mg/kg diet, followed by fish fed diets with 9.97 and ≥ 50.28 mg/kg diet, and the lowest in fish fed diets with ≤ 5.56 mg/kg diet. Analysis of weight gain and hepatic hexokinase activity by broken-line regression indicated that the optimum dietary niacin requirement of juvenile grouper is about 15 mg/kg diet.

ESTIMATION OF DIETARY THIAMIN REQUIREMENTS OF JUVENILE GROUPEL, *Epinephelus malabaricus*

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The study was aimed to evaluate the optimal dietary vitamin B₁ (thiamin) requirements of juvenile grouper, *Epinephelus malabaricus*. Thiamin was supplemented into a basal diet at 0, 0.5, 1, 2, 3, 6, 10 and 20 mg/kg diet, providing actual thiamin concentration of 0, 0.67, 0.86, 2.31, 2.66, 5.05, 11.50 and 14.47 mg/kg diet, respectively. Total of 8 experimental diets were each fed to triplicate groups of juvenile grouper (initial weight: 4.09 ± 0.08 g) in a recirculation rearing system for 8 weeks. Fish fed the thiamin-free control diet showed lower ($P<0.05$) weight gain, feed efficiency, protein efficiency ratio and survival than other dietary treatments. Plasma lactic acid and whole blood pyruvate concentrations were similar in all dietary treatments. Fish fed the control diet had the highest hepatic thiamin pyrophosphate effect, followed by fish fed the diet with 0.67 mg/kg diet, and the lowest in fish fed diets with ≥ 0.86 mg/kg diet. Fish fed diets with ≥ 0.67 mg/kg diet had higher blood thiamin concentration but lower transketolase activity than fish fed the control diet. Analysis of weight gain and blood thiamin concentration and blood transketolase activity by broken-line regression indicated that the optimum dietary thiamin requirement of juvenile grouper is 0.7-1.0 mg/kg diet.

DIETARY VITAMIN K REQUIREMENTS OF JUVENILE GROUPER, *Epinephelus malabaricus*

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The study was aimed to evaluate the optimal dietary vitamin K requirements of juvenile grouper, *Epinephelus malabaricus*. Menadione sodium bisulfite was supplemented into a basal diet at 0, 1, 2, 5, 8, 12, 15 and 20 mg/kg diet, providing actual vitamin K concentration of 0, 0.98, 2.31, 5.31, 8.66, 12.05, 14.50 and 20.47 mg/kg diet, respectively. Total of 8 experimental diets were each fed to triplicate groups of juvenile grouper (initial weight: 9.95 ± 0.05 g) in a recirculation rearing system for 8 weeks. Weight gain, feed efficiency and survival of grouper were not affected by the dietary treatments in this 8-weeks feeding trial. Fish fed the diet with 8.66 mg/kg diet had higher red blood cell count than fish fed the control diet. Total plasma prothrombin concentration was the highest in fish fed diets with ≥ 8.66 mg/kg diet, followed by fish fed the diet with 5.31 mg/kg diet, then fish fed diets with 2.31-5.31 mg/kg diet, and the lowest in fish fed the vitamin K-free control diet. Hepatic menaquinone-4 (MK-4) and whole body calcium concentrations increased by increasing the dietary vitamin K supplementation levels. Intestinal MK-4 concentration was similar in all dietary treatments. Analysis of total plasma prothrombin concentration by broken-line regression indicated that the optimum dietary niacin requirement of juvenile grouper is about 15 mg/kg diet.

ESTIMATION OF DIETARY RIBOFLAVIN REQUIREMENTS OF JUVENILE GROUPER, *Epinephelus malabaricus*

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The study was aimed to evaluate the optimal dietary riboflavin requirements of juvenile grouper, *Epinephelus malabaricus*. Riboflavin was supplemented into a basal diet at 0, 2, 4, 6, 8, 12 and 20 mg/kg diet, providing actual riboflavin concentration of 1.09, 3.25, 4.62, 6.63, 8.57, 12.46 and 20.32 mg/kg diet, respectively. Total of 8 experimental diets were each fed to triplicate groups of juvenile grouper (initial weight: 7.65 ± 0.08 g) in a recirculation rearing system for 8 weeks. Weight gain, feed efficiency and protein efficiency ratio were higher in fish fed diets with ≥ 3.25 mg/kg diet than those in fish fed the diet with 1.09 mg/kg diet. Fish fed diets with ≥ 8.57 mg/kg diet had higher red blood cell count and hematocrit value than fish fed the diet with 1.09 mg/kg diet. Hepatic D-amino acid oxidase activity was the highest in fish fed the diet with 8.57 mg/kg diet, followed by fish fed diets with 3.25-6.63 and ≥ 12.46 mg/kg diet, and the lowest in fish fed the diet with 1.09 mg/kg diet. Riboflavin concentration in liver was the highest in fish fed diets with ≥ 6.63 mg/kg diet, and followed by fish fed diets with 3.25-4.62 mg/kg diet, and the lowest in fish fed the diet with 1.09 mg/kg diet. Analysis of weight gain and hepatic riboflavin concentration by broken-line regression indicated that the optimum dietary riboflavin requirement of juvenile grouper is 4-5 mg/kg diet.

DIETARY TAURINE SUPPLEMENTATION IN DIET ENHANCES GROWTH AND NUTRIENT DIGESTIBILITY OF GROUPEL, *Epinephelus lanceolatus* FED A DIET WITH SOYBEAN MEAL

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The study was to evaluate the effects of dietary taurine supplementation on growth, tissue taurine concentration and nutrient digestibility of grouper, *Epinephelus lanceolatus*, fed a diet with soybean meal. Basal diet containing soybean meal (replacing 40% fish meal protein) was supplemented with 0.1, 0.3, 0.5 and 1 g/kg diet of taurine. The basal diet without taurine supplementation was used as control. All fish meal diet was also included for comparison. Total of six experimental diets were each fed to triplicate groups of grouper (initial wt: 14.42 ± 0.07 g) in a recirculating rearing system for 8 wks. Weight gain was the highest in fish fed the all fish meal diet, followed by fish fed the diet with 1 g/kg taurine, and the lowest in fish fed diet with 0 and 0.1 g/kg taurine. Fish fed the all fish meal diet and 1 g/kg taurine-diet had the highest muscle taurine concentration, followed by 0.5 g/kg taurine group, and the lowest in ≤0.3 g/kg taurine groups. All the taurine supplemented groups (0.1-1 g/kg taurine) had higher apparent dry matter and protein digestibility than the fish fed the control diet and all fish meal diet. Results indicated that 1 g/kg taurine supplementation in diet can improve growth and nutrient digestibility of juvenile grouper fed a diet with high levels of soybean meal.

EFFECTS OF DIETARY *Lactobacillus* FERMENTED SOYBEAN MEAL AND LACTIC ACID SUPPLEMENTATION ON GROWTH, NUTRIENT DIGESTIBILITY AND INTESTINAL MORPHOLOGY OF GROUPEL

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The study evaluated the effects of dietary two lactate sources, sodium lactate and *Lactobacillus* fermented soybean meal, on the growth, nutrient digestibility and intestinal histology of the giant grouper *Epinephelus lanceolatus* that was fed a diet containing soybean meal. The diet that included with soybean meal, which replaced 40% fish meal protein, was used as the control. One percent lactate from the two sources was added to the control diet to form the two experimental diets. Also, the all fish meal diet without lactate was also included for comparison. A total of four experimental diets were each fed to three groups of giant grouper (initial wt: 68.57 ± 0.28 g) in a recirculating rearing system for 8 weeks. After the growth trial, the nutrient digestibility by the fish was determined by using 0.5% chromic oxide as an inert indicator. Weight gain, feed efficiency, survival and whole body proximate composition were not affected ($P > 0.05$) by the dietary treatments. Fish fed the diet with 1% lactate from fermented soybean meal showed lower visceral fat content than fish fed the all fish meal diet. Fish fed diets with 1% lactate from the two sources showed similar dry matter and lipid digestibility to the fish fed the all fish meal diet. The fish fed the control diet showed disordered lamina epithelialis and extended lamina propria comparing to fish fed the all fish meal diet. Fish fed diets with 1% lactate from the both source improved the negative effects for intestinal histology. The results indicated that nutrient digestibility and intestinal histology were negatively affected by feeding the diet containing high level of soybean meal for giant grouper. Lactate supplemented in diet from both sodium lactate and fermented soybean meal can improve improved the negative effects for the fish.

NUTRITIONAL CONCEPT IN PLANT-BASED AQUAFEED: A BRIEF REVIEW FOR CHOLESTEROL AND BILE SALT

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Fish meal is an ideal protein source for fish and shrimp. It is an important issue to find adequate alternative ingredients for fish meal due to the limited global supply of fish meal. Soybean meal is the most widely used plant ingredient for aquafeed industry. However, the nutritional imbalance or inhibitors in soybean may influence lipid nutrition for fish and shrimp. Our recent study indicated that giant grouper can synthesize cholesterol *de novo* to meet their cholesterol requirement when the fish fed a diet with high soybean meal. It is not necessary to supplement cholesterol in diet for giant grouper even the diet containing high level of soybean meal. Unlike fish, it is necessary to supplement cholesterol in diet due to the absence of cholesterol in soybean meal and lack of *de novo* cholesterol-synthesis ability for shrimp. Cholesterol is essential in cell membrane components and molting functions for shrimp. Our study indicated that dietary fish meal replaced by soybean meal depressed cholesterol concentration in hemolymph and hepatopancreas as well as molting relative gene expression in shrimp. The soybean meal-based diet supplemented with cholesterol can improve the hypocholesterolemia effect and molting relative gene expression. However, cholesterol is the most expensive ingredient in shrimp feed, it is worthy to find an alternate to cholesterol. Bile salt which has similar chemical structure to cholesterol is a potential cholesterol replacer for shrimp. The previous work demonstrated that bile salt can partially replace cholesterol in diet to maintain shrimp growth and molting functions.

GONADAL CELLS TRANSDIFFERENTIATION IN ESTROGEN-INDUCED ECTOPIC OOCYTES IN PROTANDROUS BLACK PORGY, *Acanthopagrus schlegelii*

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Protandrous black porgy is a hermaphroditic teleost with a digonic gonad. In our previous study, the estradiol (E2) treatment would inhibit testis growth and ectopic oocytes were observed in the regenerated testis after E2 withdrawal in E2-treated fish. In this study, we used black porgy as a model fish to investigate how ectopic oocytes could survive in the wrong environment (testis). According to the IHC results of Dmrt1 (Sertoli cell marker) and Cyp19a1a (follicle cell marker), we found oocyte-surrounded cells were altered from Sertoli cells to follicle cells. In this study, our data showed ectopic oocytes were observed after E2 administration withdrawals. In addition, these ectopic oocytes were broadly distributed in the regenerated testis. Thus, our data demonstrated that ectopic oocytes are differentiated in a low E2 environment.

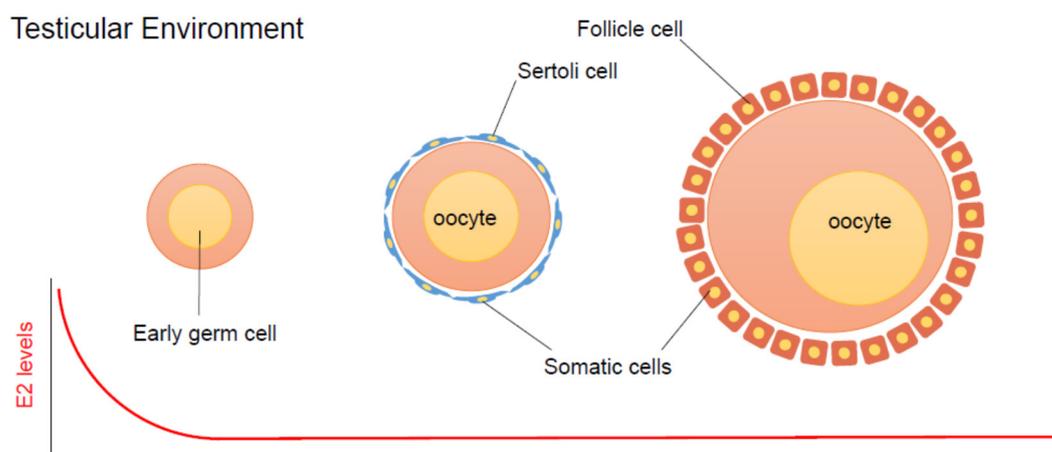


Figure 1. The alteration of oocyte-surrounded cells from Sertoli cells to follicle cells and E2 level in testis.

HIGH-RESOLUTION LINKAGE MAPPING AIDED BY GENOME SURVEY AND TRANSCRIPTOME SEQUENCING IN *Portunus trituberculatus*: APPLICATIONS IN GROWTH-RELATED QTL AND GENE IDENTIFICATION

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A high-resolution genetic linkage map is an essential tool for decoding genetics and genomics in non-model organisms. In this study, linkage mapping was constructed for *Portunus trituberculatus* using specific-length amplified fragment sequencing (SLAF-seq). A high-resolution genetic linkage map with 10,963 markers was obtained, as far as we know, this has never been achieved in any other crustacean. The linkage map covered 98.85% of the whole genome with a mean marker interval of 0.51 cM.

A genome survey and transcriptome sequencing enabled 2,378 explicit annotated markers to be anchored to the map. Quantitative trait locus (QTL) mapping revealed 12 growth-related QTLs with a high mean *PVE* value of 23.7. Nine genes identified from the growth-related QTL region were considered important growth-related candidate genes. In particular, RE1-silencing transcription factor and RNA-directed DNA polymerase genes encoded nonsynonymous amino acids, which suggests a potential influence in growth regulation.

We have demonstrated that high-resolution linkage mapping aided by genome survey and transcriptome sequencing could serve as an important platform for QTL mapping and the identification of trait-related genes.

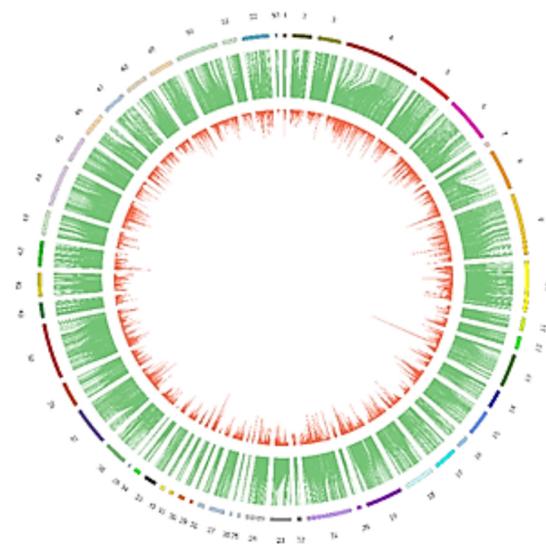


Fig Integration of linkage map, genomic scaffolds and transcripts. Outer ring, the linkage group; Intermediate ring, contigs or scaffolds of genome assembly aligned with markers from the linkage map; Inner ring, transcript sequences of transcriptome aligned with markers of the linkage map.

THE CRUCIAN CARP DERIVED FROM FEMALE COMMON CARP × MALE BLUNT SNOUT BREAM

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Biologists generally believe that hybridization would be a catalyst not only for speciation but also for major evolutionary innovations. It may accelerate speciation via adaptive introgression or cause near-instantaneous speciation. The near-instantaneous hybrid speciation accompanied by rapid genomic changes which may lead to new beneficial phenotypes and selection for fertility and ecological traits.

Here, we report the spontaneous occurrence of a new crucian carp-like homodiploid fish ($2n=100$) that originated from the interspecific hybridization of female common carp (*Cyprinus carpio*, *Cyprininae*, $2n=100$) × male blunt snout bream (*Megalobrama amblycephala*, *Cultrinae*, $2n=48$). The crucian carp-like homodiploid species exhibited morphological, physiological (reproductive traits), and genetic alterations at the mitochondrial genome, transcriptome, FISH, genomic DNA, and genome levels and included the presence of chimaeras, deletions, insertions, and mutations. The phenotype and genotype of this new crucian carp-like homodiploid fish were found to be very similar to those of the existing diploid species (diploid crucian carp; *Carassius auratus*). The analyses on the gene expression and phylogenetic trees also support this conclusion. The results provide evidence of the existence of a possible route through which the distant hybridization of female common carp × male blunt snout bream can generate crucian carp.

The findings of this study provide novel insights into chromosomal evolution in vertebrates. In addition, dramatic genomic alterations may be valuable for genetic breeding and aquaculture applications.

DEVELOPMENT AND SENSORY EVALUATION OF SEAFOOD PRODUCTS FOR SENIORS

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Aquatic product provides protein and functional ingredients that offer health benefits for seniors. However, our previous semi-quantitative food frequency questionnaire revealed that average daily seafood intake amount was less than 15 gram per senior. Therefore, the objective of this research is to develop seafood products for seniors based on the principle of DASH (Dietary Approaches to Stop Hypertension) diet. Milkfish (*Chanos chanos*) and tilapia (*Oreochromis mossambicus*) sturgeon are rich in functional nutrients, such as collagen, minerals and unsaturated fatty acids. In this study, milkfish, tilapia meat and sturgeon bone were used to develop fish dumpling, bone stock and herbal cuisine for senior. The food proximate content and composition of amino acids of all products were measured. Sensory evaluation of thirty-seven senior participants rated their level of preference of products on 9-point scales. . All products are non-cholesterol, high protein and minerals-rich. Amino acids profiles demonstrated that all products contained functional amino acids, such as arginine, taurine and carnosine. The results of sensory evaluation show that the fishy intensity of products were in low levels, and the preference of products were in high levels. In summary, this study create critical aquatic food processing technology for further commercial production, promote senior aquatic foods and efficiently raise utilization and values of aquatic resources.

MOLECULAR INSIGHTS, EXPRESSION ANALYSIS AND RADICAL SCAVENGING ACTIVITY OF NOVEL PROTEIN THIOREDOXIN DOMAIN CONTAINING PROTEIN 17 FROM *Hippocampus abdominalis*

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Thioredoxin is an intracellular protein ubiquitously found in all kingdoms of living organisms. It possesses a redox active thiol di-sulfide CXXC motif with two cysteines. Thioredoxin protects the proteins and cellular organelles from the oxidative aggregation and inactivation, protect the cells from environmental stresses like reactive oxygen species (ROS), arsenate and peroxynitrite and apoptosis via programmed cell death. The big-belly seahorse (*Hippocampus abdominalis*) is an important seahorse species in oriental medicine, ability to suppress neuroinflammatory responses and collagen release. Also, it exhibits antitumor, anti-aging properties. In this study, novel thioredoxin domain-containing protein 17 (TXNDC17) from the big-belly seahorse were characterized. The open reading frame (ORF) of TXNDC17 369 bp and comprises 123 amino acids. The estimated molecular mass and the predicted isoelectric point (pI) of TXNDC17 was 14.1 kDa and 5.04 respectively. According to the pairwise alignment of amino acids with other TXNDC17 orthologs, 99.2% of highest identity was revealed with *Hippocampus comes*. The phylogenetic tree showed TXNDC17 were positioned within the teleost group.

Spatial expression profile of TXNDC17 under normal physiological conditions was observed in 14 different tissues. The highest mRNA expression level of TXNDC17 was detected in muscle (12.81-fold) and the lowest expression observed in spleen (Figure 1). TXNDC17 normally exhibit a broad range of tissue distribution due to the essentiality of maintaining cellular redox homeostasis. Highest TXNDC17 availability usually occurred in the organs with the high antioxidant requirement.

According to the radical scavenging assay, figure 2 shows the percentage of DPPH radical scavenging capacity of TXNDC17 with ascorbic acid as a reference compound. A concentration-dependent relationship was found in the radical scavenging activity of TXNDC17. The protein showed maximum 319.76 % inhibition of DPPH radicals within the concentration range. Further, TXNDC17 showed IC₅₀ value at the concentration of 25.73 $\mu\text{g}/\text{mL}$. TXNDC17 from the *Hippocampus abdominalis* is assessing for the first time in fish by this study and provides better knowledge about the TXNDC17 gene and their functional domain insights to the fish and aquaculture industry.

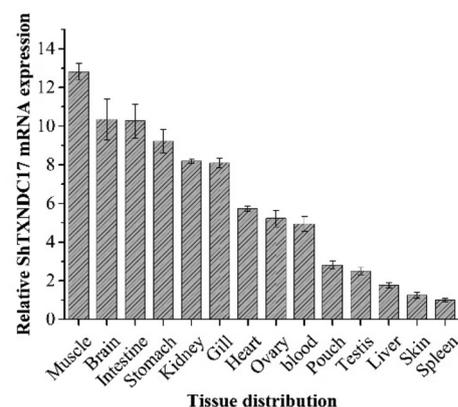


Figure 1: Relative mRNA expression of TXNDC17 in healthy seahorses.

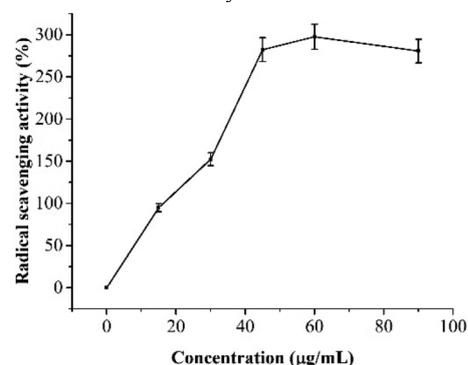


Figure 2: DPPH radical scavenging activity of seahorse TXNDC17 to different concentrations (0-90 $\mu\text{g}/\text{mL}$).

PREPARATION AND IDENTIFICATION OF A MULTIFUNCTIONAL DIATOMITE SILICA NANOPARTICLE

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Diatomite is a natural porous silica material of deposition. It is considered as a novel potential drug delivery carrier because its unique properties of porous and good biocompatibility. In this study, diatomite will be converted into silica nanoparticles by physical methods. In the present study, the diatomite silica nanoparticle (DSN) was used as the main material. Target molecule (aptamer and folic acid) were modified on the surface of DSN. Target properties were added to the vector. In order to make the drug with controlled release property, the surface of DSN is modified form of disulfide bonds on the drug, the drug into the target location will be released. This property can reduce the side effects caused by drugs. The experiment is divided into three parts. The first part is the synthesis of materials: using physical grinding method, the large particles of diatomite were broken into small particles of nanoparticles, and the use of acid treatment and calcination method, cleaning the impurities contained in the nanoparticles. The second part is to modify the target molecules on the surface of nanoparticles, so that the nanoparticles become the target drug carrier. The third part is bonding the drug molecules on the carrier, so that the whole carrier not only has a specific target property, but also has the ability to treat cancer. In this study, the drug carrier for physical and chemical properties study, and in vitro test.

PRODUCTION OF WSD-RESISTANT SHRIMP – AN INTERVENTION THAT HELPS SHRIMP IN THE ARMS RACE AGAINST WSSV

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WSSV is the causative agent of white spot disease. The virus, which has a double-stranded circular DNA genome of over 300 kbp, deploys a range of anti-host defense strategies, that make the disease very difficult to control. In our previous studies, we have looked at some of the strategies the virus uses to overcome the host's defenses, and from the shrimp's point of view, our findings were discovering – generally speaking, it's always a losing battle for any shrimp infected with WSSV. People might think that to control WSD, all you'd need to do is monitor and survey for the presence of the virus, and then quarantine, or otherwise keep separate, the virus and its as yet uninfected shrimp host. To some extent, this is indeed helpful. However, if you look at the history of this disease, you can see that, despite our best efforts, since its emergence ~25 years ago in East Asia, WSD has spread to become an almost global pandemic. This suggests that we need to look beyond diagnosis and quarantine. Our laboratory is always seeking new knowledge to prevent or minimize the effects of WSSV on shrimp, and our findings in this area are much more encouraging. One intervention strategy currently being explored by us is the production of WSD-resistant shrimp. We have already identified founder families of shrimp that are resistant to WSSV infection, and these families have already been bred to the 2nd generation by full sibling cross. Our results have shown that the WSD-resistant trait can be passed on and also that we can increase the proportion of resistant shrimp in subsequent generations. Building on this achievement, the next step will be to develop shrimp lines that are both disease resistant and fast growing – and ready for actual use in the shrimp industry.

KEY POINTS OF COMMERCIAL FEED FORMULATION FOR ASIAN SEA BASS IN CHINA

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Volume of Chinese aqua feed has been stable while total value of aqua feed continues increasing because of high value species. Thus, premium and functional aqua feed will be the new engine for Chinese aqua feed industry. It can be predicted that more and more high value species will be cultured and consumed in China. Asian Sea bass, or barramundi, *Lates calcarifer*, is known as Golden-Eye Sea bass in China. Because of its special golden color of eye and tasty flavor, it becomes more and more popular in China. In the past 30 years, cultured model and nutrient requirement of Asian Sea bass have been researched. Utilization of this fish for different raw materials has been discussed as well. Plenty of significant results can be referred in aim to develop feed for Asian Sea bass.

However, there are still lots of factors should be considered when design a commercial feed for Asian Sea bass in China. China has its long coastline while climate is quite different from north to south, that results to different cultured models for even one single species. It is suggested that positioning of feed product should take the first place to consider. After the clear positioning, selection of raw materials is the foundation for formulation. Due to the higher price and unsustainability, the value of traditional fish meal should be re-evaluated. New protein sources from China locally and other parts of the world can be adopted. How to evaluate a new material is a key point for design an aqua feed. When it comes to formulation, nutrient profile is not the only thing should be focused on. Beside from basic nutrient requirement, special function is another mission of high value aqua feed. Different functional additives such as *macleaya cordata*, peptides and yeast products can improve culture profit and be environment friendly.

In this report, presenter will share experience of positioning for high value aqua feed in China, evaluation of several new raw materials and ideas for formulation for Asian Sea bass.

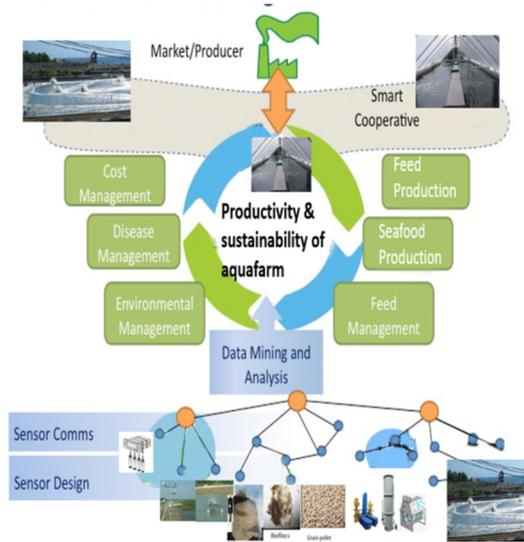
SMART AND PRECISE AQUACULTURE SYSTEM FOR INCREASING PRODUCTIVITY AND SUSTAINABLE PRODUCTION OF AQUACULTURE

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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

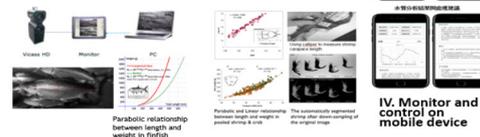
I. Online Environment monitoring and automation system



II. On-line aquatic disease diagnosis system



III. Online growth performance monitoring system



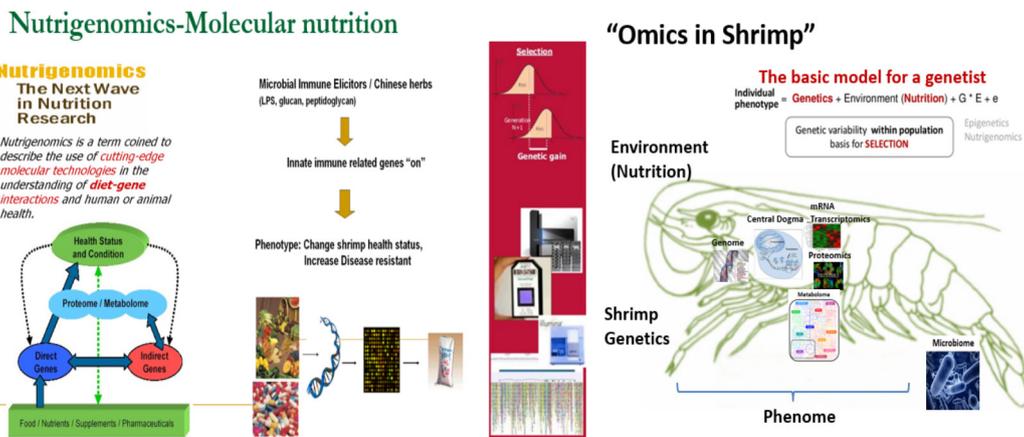
IV. Monitor and control on mobile device

MOLECULAR NUTRITION AND NUTRIGENOMICS FOR THE DEVELOPMENT OF FUNCTIONAL FEEDS OF AQUACULTURE

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From the molecular nutrition and nutrigenomics point of view, nutrients are feeding signals, which are detected by the cellular system of sensors and which influence the expression of genes and proteins and in consequence, the production of metabolites. Therefore, today, it is generally accepted that feeding components have a substantial impact in the expression of related genes, as well as in the welfare of reared aquatic animals. Many feeds are now incorporated to health management and termed functional feeds, which are believed to improve fish growth, health status, reduce disease outbreaks and/or improve post-infection recovery. The aim of present study is to use new omics technologies to investigate how foods and/or bioactive compounds affect genes and how individual differences in genetic makeup affect the ways in which aquatic animals respond to nutrients with regard to health status and growth performance, and using these transcriptomics data for development of novel functional aqua-feeds. In present presentation, we have used transcriptomics technology for development of functional feeds which include specific (micro) ingredients targeting specific functions or product characteristics, such as attractants, immunostimulants, maturation stimulants and growth promoters which added to feeds for cultured fish and shrimp, thus bringing solutions to recurrent problems in aquaculture production cycles rather than only focusing on growth. It also demonstrated that the use of high-throughput new omics techniques allows nutritionists to identify the gene networks and molecular pathways that would not be possible when investigating only a small number of genes at any one time. There are advances being made using other technologies for deeper understanding of gene regulation including non-coding RNAs, epigenetics and metabolomics.

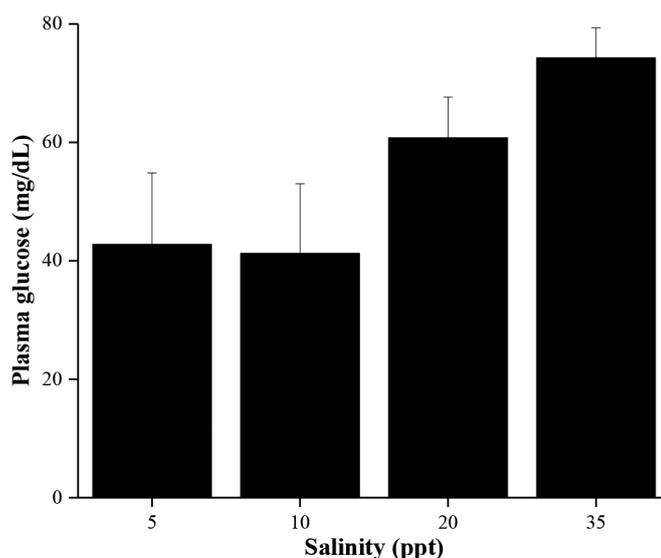


OSMOREGULATORY, METABOLIC AND CYTOPROTECTIVE RESPONSES IN JUVENILE SNUBNOSE POMPANO (*Trachinotus blochii*) ACCLIMATED TO DIFFERENT SALINITIES

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Euryhaline Snubnose pompano (*Trachinotus blochii*) is an economic aquaculture species commercially cultured in environments of various salinities. However, changes in environmental salinity would impose osmotic stress on fish. To seek a favorable salinity range for rearing snubnose pompano to mitigate salinity stress is essential for aquaculture practice. With this regard, four groups 5, 10, 20 and 35 ‰ were used to examine the physiological responses in terms of osmoregulation, glycogen metabolism and cytoprotection of pompano. In osmoregulation, plasma osmolality, Na⁺, Cl⁻, muscle water content, Na⁺/K⁺-ATPase activity and protein abundance in gills and kidney will be detect. On the other hand, this study is going to study oxygen consumption rate, plasma glucose and lactate as well as glycogen content and protein expression of glycogen phosphorylase in gills, liver and kidney. In cytoprotection, protein abundance of heat shock protein 70 in gills, liver and kidney will be necessary. According to the results from above experiments, a favorable salinity range which is less energy consumption to cope with osmotic stress would be suggested for a better environmental condition for snubnose pompano.



NEW STRATEGIES FOR NERVOUS NECROSIS VIRUS RESISTANCE IN GROUPEL INDUSTRY

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Nervous Necrosis Virus (NNV) is an aquatic virus that can infect more than 30 species marine fish including the grouper, which is a valuable fish species in Taiwan. NNV causes up to 90-100% mortality in the aquaculture industry and that results in great economic loss in Taiwan grouper culture industry. In this study, we proposed a total solution by using biotechnology to control the virus infection.

In present, the anti-NNV strategies such as inactivated vaccines, subunit vaccines and recombinant vaccines which rely on adaptive immunity can only be applied to 30-days-post-hatching grouper larvae. However, the NNV acute infection is before adaptive immunity development. Using the feed additives could suppress the replication of virus effectively. By the large scale test, we also tried to feed the grouper juveniles the feed additives in NNV outbreak farm. The survival rate also significantly increased from 10% to 50-95%. These techniques were already developed and suitable for grouper industry in the future.

HYPOTHERMAL EFFECTS ON GLUCOSE TRANSPORTER 1 (GLUT1) EXPRESSION AND LOCALIZATION IN GILLS OF SEAWATER- AND FRESH WATER-ACCLIMATED MILKFISH, *Chanos chanos*

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Glucose is known to be transported through facilitative glucose transporters (GLUTs) in cells and is an essential energy resource for living creatures. The glucose transporter proteins play an important role in glucose metabolism. In this study, *Ccglut1*, the glucose transporter 1 gene of milkfish (*Chanos chanos*) was successfully cloned. *Ccglut1* was predominantly expressed in the brain and less expressed in the gill, heart, intestine, and kidney. Lower levels of *Ccglut1* were also found in the muscle and liver. The CcGLUT1 protein expression as well as the other glucose metabolism-related indicators (i.e., hexokinase activity, glucose contents, and glycogen contents) in gills of seawater (SW)- and fresh water (FW)-acclimated milkfish under hypothermal stress (18°C) were determined. In addition, effects of hypothermal stress on CcGLUT1 expression in gills of SW and FW milkfish were studied. Under hypothermal stress, the CcGLUT1 protein levels were both up-regulated in the SW and FW groups. On the other hand, hexokinase activities were significantly higher in SW individuals than those in FW ones at normal (28°C) or low (18°C) temperature. Meanwhile, only SW milkfish revealed significant decrease in gill hexokinase activity at 18°C compared to those at 28°C. Higher glucose contents were detected in gills of both hypothermal SW group and FW groups, but only significantly in hypothermal SW fish. Branchial glycogen contents were significantly decreased in the SW fish but not in the FW individuals at 18°C. To sum up, hypothermal stress induced branchial CcGLUT1 protein expression as well as glucose levels. The results indicated that milkfish gills might uptake more glucose upon hypothermal challenge. Results of the other glucose metabolism-related indicators suggested that SW milkfish might use glycogen storage to cope with hypothermal stress.

THE STABILITY TESTS OF THE PHYCOBILIPROTEINS FROM *Porphyra dentata*

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Phycobiliproteins (PBPs) are water-soluble proteins present in cyanobacteria, red algae and cryptomonads that capture light energy which passed on to chlorophylls during photosynthesis. The PBPs were easily decayed in response to physical and chemical parameters, such as temperature, pH and light. In this study, the stability of the PBPs crude extracts from red algae *Porphyra dentata* was studied in various temperatures, pH and lights. Besides, addition of alcohol, benzoic acid, L-ascorbic acid, citric acid and sucrose into the PBP crude extracts was evaluated for the stability of the PBPs. The recovery yield of phycoerythrin (PE) in the phycobiliproteins extraction [5% (w/v) biomass loadings] was 1.12 mg/g biomass. The PE was stable in fluorescence at 4–40°C for 1 h. The pH stability test indicated that the PE was stable at pH 5–9 in fluorescence. In the light exposure experiments of phycobiliproteins for 2 days, the phycobiliproteins remained 7.6%, 42.7% and 32.0% in fluorescence after the exposures of white, red and blue lights, respectively. Addition of alcohol into phycobiliproteins extracts could enhance their stability. The presence of 12% (v/v) alcohol retained the fluorescence by 81 and 16 folds as compared to the control at 4°C and 25°C. Addition of food additive benzoic acid (5 mM) could also enhance the stability of phycobiliproteins by 90 folds at 4°C for 90 days

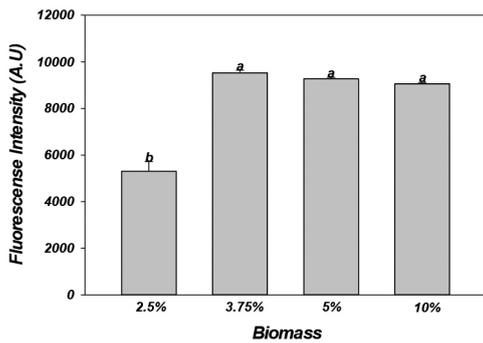


Fig. 1. The fluorescence of phycobiliprotein extract with various biomass loadings.

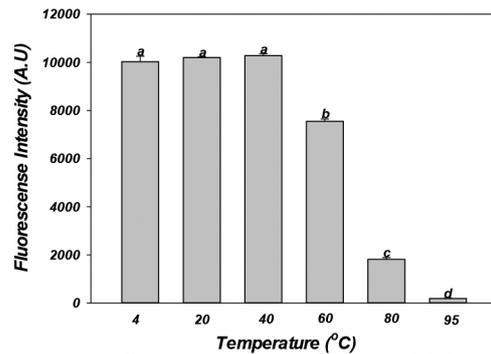


Fig. 2. The fluorescence of phycobiliprotein extract at various temperatures.

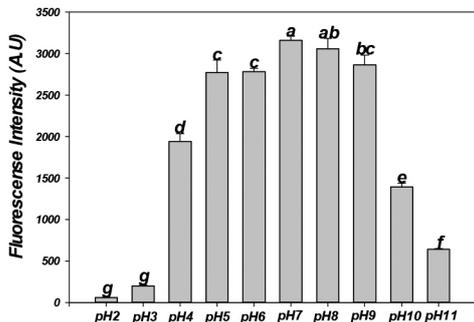


Fig. 3. The fluorescence of phycobiliprotein extract at various pH.

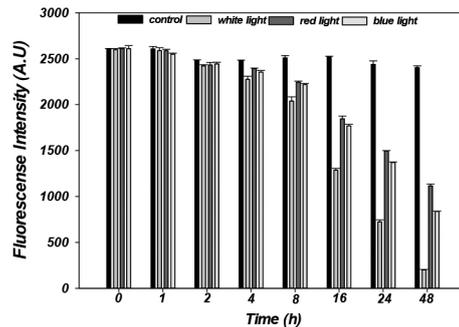


Fig. 4. The fluorescence of phycobiliprotein extract at various light exposure.

NET PRESENT VALUE ANALYSIS OF YELLOWTAIL CULTURE IN JAPAN TO BUILD BUSINESS RELATIONSHIPS WITH LOCAL FOOD-PROCESSING FIRMS

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The yellowtail culture sector in Japan is currently experiencing difficulties in ensuring income, as well as a serious weakening of economic foundation due to low fish prices or rising fish meals' costs. Under these circumstances, farmers are attempting to improve their management practices by switching their shipping destination from the market to local food-processing firms, as well as by introducing new technologies. However, there have been few studies identifying the economic conditions, such as the unit price of yellowtail at shipment and the unit cost of feed, needed to improve the management through these two measures.

Thus, by considering a yellowtail culture in Kumamoto, Japan, as a case study, this study carried out a Net Present Value (NPV) analysis to identify the economic conditions needed to build a business relationship with local food-processing firms and introducing new technologies. Specifically, the present research defined the structure of the yellowtail culture management as consisting of 3 labors and 32,000 shipped yellowtails (160,000kg of weight). The analysis was then performed assuming the transition from the current management practice to a new one, represented as follows: under the current practice, the yellowtail is shipped to the market, both Moist Pellet (MP) and Extruded Pellet (EP) are used for feeding, and the cleaning of crawl nets is outsourced; under the new practice, the yellowtail would be shipped to local food-processing firms, only EP would be used for feeding after the adoption of an EP feeding machine, and crawl nets would be self-washed via a net cleaning robot.

The results of the analysis indicated that, if the unit price of yellowtail at shipment is the same as the current unit price in the market (738 JPY/kg), the EP unit cost should be 199 JPY/kg or less. Moreover, if the price at shipment is 10% higher (812 JPY/kg) than the current unit price in the market, the EP unit cost should be 226 JPY/kg or less (Table 1).

TABLE 1. The results of the NPV analysis. Each ○ and × denotes that the investment is advantageous or not.

		EP unit cost (JPY/kg)					
		199 (100%)	204 (103%)	210 (106%)	215 (108%)	220 (111%)	226 (114%)
Unit price (JPY/kg)	738 (100%)	○	×	×	×	×	×
	753 (102%)	○	○	×	×	×	×
	768 (104%)	○	○	○	×	×	×
	782 (106%)	○	○	○	○	×	×
	797 (108%)	○	○	○	○	○	×
	812 (110%)	○	○	○	○	○	○

FISH SIZE EFFECTS ON GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) AND PANGASIOUS (*Pangasius hypophthalmus*), PRODUCTIVITY OF LETTUCE (*Lactuca sativa*), AND WATER QUALITY IN RAFT-AQUAPONIC SYSTEM

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The study determined the effect of fish size on growth performance of Nile tilapia (*Oreochromis niloticus*) and Pangasius (*Pangasius hypophthalmus*), productivity of lettuce (*Lactuca sativa*) and water quality in raft-aquaponic system.

Two different fish sizes, small (100 pcs: 2.6 g) and large (50 pcs: 31.48 g) were placed in fish tank. The 10 pangasius and 20 lettuce were cultured in each hydroponic bed. The rearing period was carried out for 77 days. Fish sampling was conducted every 14 days while initial and final weight and length sampling were recorded. Furthermore, sampling of water quality parameters was conducted every 72 hours.

Small fish exhibited higher weight gain % (436.59), specific growth rate (2.18 %) and lower feed conversion ratio (1.87) than that of large fish. No significant difference was observed in rearing performance of pangasius. However, the growth of lettuce cultured in large fish group was higher than that of cultured in small fish group. In addition, no significant difference was found on Total Ammonia-Nitrogen in fish tanks and hydroponic beds. The recorded Total Ammonia Nitrogen level in fish tanks and in hydroponic beds ranged from -0.15 to 0.28 mg/L and from -0.07 to 0.26 mg/L, respectively. No significant difference was found on Phosphorous level (0.41 to 0.79 mg/L) in fish tanks but higher Phosphorous level was found in large size fish-group in hydroponic beds. Optimum level of pH, temperature (°C) and SAT % were also recorded.

These results indicated that fish size has an effect on growth performance of fish, productivity of lettuce and water quality in tilapia/pangasius-lettuce raft-aquaponic system. Small fish has a better growth performance, while large fish contributed to the better productivity of lettuce by providing sufficient amount of nutrients needed.

MONOGLYCERIDE TECHNOLOGY AND APPLICATION IN AQUACULTURE

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Bacterial diseases are major threats to aquaculture that can cause significant losses to the industry in many countries. Acute Hepatopancreatic Necrosis Disease (AHPND, or more commonly known as Early Mortality Syndrome, EMS) in shrimp for example, has damaged some of the shrimp producing countries by billions of dollars of GDP as a result of pathogenic strains of *Vibrio parahaemolyticus*. Production shifts between countries have largely helped to sustain the supply to global demand but has taken years to recover in some countries with measures to reduce the likelihood of the disease breakout. These include changes to the pond environment by farm management practice, introducing additives to water and feed to reduce the pathogenic bacterial load, or to enhance the immunity of host against diseases.

Many feed additives and technologies have been introduced through the years to address such bacterial problems in aquaculture. One of the most effective solutions being the use of formic acid in either fish or shrimp feed to directly reduce the GI tract pathogen load, or the use of Astaxanthin in shrimp aquaculture to improve the animal's immune parameters to fight against diseases.

One of the recent innovations include the use of monoglycerides of short (C1-C5) to medium chain length (C6-C12) fatty acids that combines the benefits of respective organic acid, with enhanced performance improvement. Further extending the benefits of organic acids, the composition of the organic acids is optimized by the monoglyceride technology to deliver a more specific approach towards different bacterial pathogens, reducing stress factors and thus increasing performance and yield.

In vitro trial results showed that the monoglycerides could inhibit the targeted *Vibrios spp.* and some other bacterial species, whilst not affecting the healthy bacteria (probiotics or heterotrophs). Tank trials have shown promising results for shrimp and demonstrated up to 30% improvement in survival rates in AHPND challenge studies. On-going pond trials in salmonids and shrimp and the preliminary results point to the direction of improved yield and performance, allowing a greater latitude for farm management.

CHARACTERIZATION OF STRESS-INDUCED AUTOPHAGY BY LC3 IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Groupers are one of the most remunerative aquaculture species in Asia. However, as groupers grow up, there are various stress conditions that they might face, such as nervous necrosis virus (NNV) infection, salinity changes and climate changes. Autophagy is used by organisms as a defense strategy against environmental stress. The mechanism is one of the most important intracellular pathways responsible for the degradation and recycling of organelles and proteins. Microtubule-associated protein light chain 3 (LC3) is one of the constituent of the autophagosome and utilized as a reliable marker for autophagy. However, the stress-regulating function of autophagy is still not yet known in orange-spotted groupers. In this study, we are investigating stress-induced autophagy by LC3 marker in grouper in response to different stimulations.

The full length sequence of orange-spotted grouper *LC3* (*osgLC3*) mRNA had been cloned with 1,440 bp that can be translated to 126 amino acid sequence. The immune-stimulating agents such as poly I:C or LPS treatment induced *osgLC3* in brain, eye, head kidney has significantly increased. Moreover, in grouper larvae, the expression of *osgLC3* under NNV infection in brain, eye and head kidney has significantly increased than other tissues. Besides, expression of *osgLC3* in low salinity higher than others concentration. In conclusion, based on the results of the interaction between autophagy and stress condition confirmed by *osgLC3*, the findings provide that autophagy might play an important stress-regulating role in orange-spotted grouper.

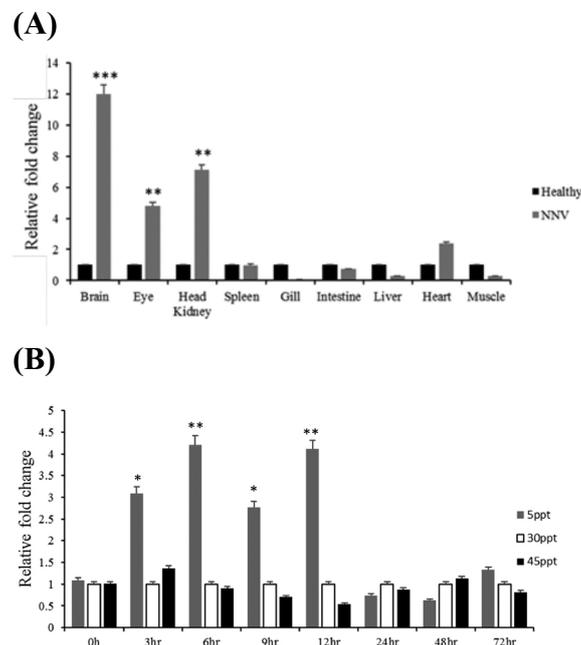


FIGURE 1. (A) Effect of naturally NNV infected grouper larvae on *osgLC3* in various tissues. (B) Effect of salinity changes on *osgLC3* in orange-spotted grouper.

THE USE OF SUBSTRATES FOR AQUACULTURE SPECIES' DOMAIN IMPROVEMENT AND BIOFILTRATION

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Placement of substrate surface area into aquaculture production containment is an emerging technology applied to cages, ponds and Recirculating Aquaculture Systems (RAS) with varied climatic conditions and culture species. Substrates are being employed to improve utilization of pond volumes, health and growth rate, and to decrease impact of territorial hierarchies, alone and, in parallel with other emerging technologies. Living plants and synthetic and organic materials have been used as physical substrates in a variety of configurations. Substrate use is most prominent with prawn aquaculture but has been applied with various fish species. Biofloc technology demonstrates promising productivity improvements but coincident substrate use shows a synergistic effect. Integration of biofilter with substrate within production containment has shown measurable beneficial effects through nutritional utilization of biofilter generated biomass. There are different motivations for substrate employment with various species resulting from specific characteristics, such as ability to graze on biomass or, preference for vertical or horizontal surfaces. This review examines synergistic and other effects in studies across materials, processes, conditions and species. Limitations are recognized in measurement of the contributions from a wide range of variables arising from different substrates, species and operating systems and conditions. The analysis is vital to understanding of the commonality and distinctions in utilization of substrates and to validate fundamental principles for their use. New frontiers will be predicted for aquaculture production where substrates are beneficially employed in hatchery, nursery and grow out environments across a widening range of species. This study will clarify research directions to achieve design for maximum productivity with substrate utilization and, to simultaneously improve sustainability across the applicable range of species, systems and processes.

COMPENSATORY GROWTH IN SCALLOPS *Nodipecten nodosus* FARMED IN TROPICAL REGION (CARAGUATATUBA, SP, BRAZIL)

Helcio Luis Almeida Marques*, Cíntia Fernanda Garcia, Márcia Santos Nunes Galvão, José Luiz Alves e José Donizete Oliveira Santos

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The production of scallops *Nodipecten nodosus* in Brazil is still in the beginning. Consequently, studies on aspects of culture management are necessary, in order to reduce production costs. In this sense, an important study would be the confirmation of occurrence of compensatory growth (CG), in view of to enable the storage of juveniles at high densities in the intermediate culture phase. This work aimed to assess the occurrence of CG in *N. nodosus* stocked at high densities (800, 1600 and 3200 m⁻²) during the intermediate phase and subsequently transferred to low densities (50 m⁻²) at growing phase. The experiment was carried out from November 2016 to May 2017 at the Cocanha farm of mussels and scallops (MAPEC), Caraguatatuba, Brazil. Juveniles of *N. nodosus* (18.6 ± 3.2 mm in height), were stocked in Japanese lanterns at four densities: 50 (control), 800, 1600 and 3200 juveniles m⁻² (intermediate phase) with five replicates. It was assumed that the density control (50 m⁻²) did not influence the growth and survival. Monthly, dead individuals were replaced by scallops cultured in extra lanterns (lanterns-stock), at the same density. After 104 days the lanterns were harvested, the scallops were counted and measured in height and were newly stocked at 50 m⁻² (growing phase), according to the treatments: T50, T800, T1600 and T3200 (scallops previously stocked at 50, 800, 1600 and 3200 m⁻² in the intermediate phase respectively). Initial mean heights at each treatment correspond to the height registered at the end of intermediate phase for respective density. After 148 days of growing, the scallops were harvested, counted and measured in height. The table shows that survival and height differed significantly and similarly among the densities in intermediate phase. In the growing phase survivals did not differ among treatments but heights differed significantly among T50 and the other densities. On the other hand, the increase in height along 148 days was significantly lower for the treatment T50. The results showed the occurrence of a partial CG in scallops reared at high densities in the intermediate phase when transferred to an optimum density in the growing phase. However the CG was insufficient to allow scallops to reach the same height of mollusks reared at an optimum density from the beginning of the experiment.

Table – Data (mean ± SD) of survival and height (intermediate phase) and increase in height (growing phase) according respective densities and treatments

Intermediate phase	d = 50	d = 800	d = 1600	d = 3200
Survival (%)	98.9 ± 2.5 ^a	87.3 ± 5.1 ^b	85.6 ± 4.3 ^{b,c}	74.4 ± 6.5 ^c
Height (mm)	46.4 ± 2.9 ^a	31.6 ± 1.6 ^b	28.3 ± 1.2 ^{b,c}	26.8 ± 1.3 ^c
Growing phase	T50	T800	T1600	T3200
Survival (%)	84.4 ± 12.3	68.9 ± 5.0	80.0 ± 9.3	77.8 ± 11.1
Height (mm)	74.5 ± 2.5 ^a	70.3 ± 2.5 ^{b,c}	69.2 ± 1.4 ^{b,c}	69.1 ± 2.0 ^{b,c}
Increase in height (mm)	28.1 ± 2.5 ^b	38.7 ± 2,5 ^a	40.9 ± 1.4 ^a	42.3 ± 2.0 ^a

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EFFECTIVENESS OF EMERGENCY AQUACULTURE LIVELIHOOD RECOVERY PROJECTS FROM SUPER TYPHOON YOLANDA IN THE PHILIPPINES

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Super Typhoon Yolanda (Haiyan) in November 2013 was the most severe typhoon in decades hitting the central Philippines. Government and donors implemented emergency rehabilitation projects. In the aquaculture sector, the Super Typhoon washed away the fish farming facilities in Leyte and Samar region causing estimated damages of ₱940 million including crops. The current case study compares two emergency livelihood recovery projects at Tanauan and Basey located at opposite shores of San Juanico Strait in the Leyte Gulf to learn lessons for better preparedness in future aquaculture relief operations.

The livelihood recovery projects for the two communities (Table 1) were implemented jointly by the governments of Japan and Philippines. The donor provided funds for construction of facilities for both projects. The main difference was in the applied management system of farming operation. The Tanauan management was family-based with operational funds from private investors identified by the families, while the Basey management was association-based with funds from the national BFAR and local governments (LGU), except the labor counterpart of the beneficiaries in both sites.

The two projects resulted to a distinct difference in effectiveness. Tanauan project performed better in: number of operational facilities, mean number of days started operation after construction of facilities, number of stocking/harvest, total sales, as well as mean sales per family-beneficiary (Table 2). The mean operational profit per harvest of Basey was negative implying unsustainable future operations and short-time economic benefits in the emergency recovery period. Also, the Tanauan project was highly cost-effective with much smaller total project costs. Causal analyses for these results suggested the involvement of private investors in the family-based management system as the key factor for effective and efficient implementation for quick livelihood recovery.

Table 1. Characteristics of two emergency livelihood recovery projects in aquaculture from Super Typhoon Yolanda

Community	Tanauan	Basey
Species raised	Milkfish	
Farming system	Fish pens	Fish cages
Management units	Family	Association
Funds for facility	By donor	
	₱2 mil	₱8 mil
Construction	Beneficiary	Donor
Fingerling supply	Investor	BFAR
Feed supply	Investor	LGU
Profit sharing	50%-family 50%-investor	Equal share by members

Table 2. Comparison of two emergency livelihood projects in aquaculture after two years from the Typhoon

Performance Indicators	Tanauan	Basey
Facility rehabilitated	42	40
Facility operational	40	20
Mean number of days started operation after construction	76	236
Number of stocking	69	31
Total sales	₱11.8 mil.	₱4.5 mil.
Number of family-beneficiaries	31	79
Mean sales per family-beneficiary	₱379,837	₱58,569
Mean operational profit	₱66,072	-₱8,986
Total project cost	₱2.0 mil.	₱8.2 mil.

BOILED *Jatropha curcas* SEED MEAL ENHANCES NILE TILAPIA *Oreochromis niloticus* FINGERLINGS GROWTH

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Aquaculture and livestock production was rapidly expanding to respond to the increasing demand of food. However, it is constricted due to unviability of good quality feed resources and increased in feed price due to limited source of ingredients. Most of the feed ingredients was in conflict to human consumption, like soybean. There was a need to identify alternative source of protein which is not in conflict with food security. *Jatropha curcas* seed meal [JCSM] was a candidate when it is detoxified. The study was conducted to evaluate the effects of boiled *Jatropha curcas* Seed Meal (JCSM) on growth and survival of Nile Tilapia as replacement to soybean meal. The treatments were Commercial Diet (C), *Jatropha* 150g.kg⁻¹ + Soybean 200g.kg⁻¹ (J150+S200) and *Jatropha* 300g.kg⁻¹ + Soybean 50g.kg⁻¹ (J300+S50), in 3 replicates. Tilapia fingerlings were fed twice daily at 5% body weight within 60 days of culture (DOC).

It was discovered that growth parameters of Nile tilapia fed with inclusion of 150g.kg⁻¹ and 300g.kg⁻¹ of JCSM were significantly higher than the fish fed with Commercial Diet. Tilapia fingerlings fed with JCSM diets have a better growth performance in terms of Weight Gain [WG] (P=0.02), Average Growth Rate [AGR] (P=0.023), Relative Growth [RG] (P=0.02), Relative Growth Rate [RGR] (P=0.02), Specific Growth Rate [SGR] (P=0.027) and Percent Specific Growth Rate [%SGR] (P=0.022) compared to fish fed with commercial diet.

Feed Intake [FI] of tilapia fed with J150+S200 and J300+S50 were higher than the FI of fish fed with Commercial diet (P=0.033). Feed with JCSM were more palatable than the Commercial diet.

Feed efficiency ratio [FER] of Nile tilapia fed with JCSM diets were statistically higher (P=0.05) compared to fish fed with commercial diets. Feed conversion ratio (FCR), Growth conversion ratio (GCR) and percent survival, were statistically the same in either with or without JCSM. Boiled *Jatropha curcas* seed meal is a potential feed ingredients for Nile tilapia.

Table 1. Growth parameters, feed efficiency and survival of Nile Tilapia *Oreochromis niloticus* in 60 Days of Culture fed with *Jatropha curcas* Seed Meal Replacing Soybean Meal

	Diet			P-value
	C	J150+S200	J300+S50	
WG (g)	2.240 ^b	3.462 ^a	3.179 ^a	0.020*
AGR (g)	0.0347 ^b	.0533 ^a	.0490 ^a	0.023*
RG (g)	224 ^b	346 ^a	318 ^a	0.020*
RGR (g)	3.733 ^b	5.770 ^a	5.298 ^a	0.020*
SGR (g)	0.020 ^b	0.025 ^a	0.024 ^a	0.027*
%SGR	1.948 ^b	2.488 ^a	2.381 ^a	0.022*
FI (g)	1.261 ^b	1.431 ^a	1.318 ^a	0.033*
FER (%)	178.01 ^b	241.62 ^a	241.07 ^a	0.05*
FCR	6.881 ^a	5.017 ^a	4.432 ^a	0.158 ^{ns}
GCR	1.548 ^a	1.738 ^a	1.806 ^a	0.126 ^{ns}
Survival	77.78 ^a	80.00 ^a	71.11 ^a	0.369 ^{ns}

FERMENTED PLANT JUICE KANGKONG *Ipomea aquatica* ENHANCES GROWTH PERFORMANCE, FEED EFFICIENCY AND SURVIVAL OF NILE TILAPIA *Oreochromis niloticus* FINGERLINGS

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There was a decline of fish capture. Intensification in aquaculture was a need to suffice fish consumption that causes aquaculture diseases. Treating aquaculture diseases means more chemicals, antibiotics and unapproved drugs, which is detrimental to ecosystem and human as final consumer. Intensification also increases cost of production, due to high feed cost of inefficient commercial feeds and the environment suffers. There were needs to address these issues in aquaculture. Fermented plant juice (FPJ) is now a trend in organic farming and was categorized under effective microorganism (EM) for bioremediation, bioaugmentation and probiotics. A study evaluated the growth performance, feed efficiency and survival of *Oreochromis niloticus* fed diets with fermented plant juice (FPJ) Kangkong *Ipomea aquatica*. The treatments were Commercial Diet (C), 250 ml FPJ Kangkong in 1 Kg Commercial Feed (C+250FPJ Kangkong) and 500 ml FPJ Kangkong in 1 Kg Commercial Feed (C+500FPJ Kangkong), in triplicates. The fishes were fed 5% of the body weight within 60 days of culture (DOC), given 2x a day.

The treatment with the highest growth performance, feed efficiency and survival was the 250 ml FPJ kangkong, followed by 500 ml FPJ kangkong and the control diet (Commercial Feed (C)). Weight Gain (WG), Average Growth Rate (AGR), Relative Growth (RG), Relative Growth Rate (RGR) of tilapia fed diets with FPJ were all significantly higher ($P=0.004$) than C. The Percent Specific Growth Rate (%SGR) was also higher in FPJ fed tilapia than C

The feed efficiency of tilapia fed diets with FPJ was higher than C. The Feed Efficiency Ratio (FER) was better in FPJ fed fish than C ($P=0.001$). The Feed Conversion Ratio (FCR) was significantly lower in FPJ fed tilapia than C ($P<0.001$). The Growth Conversion Ratio (GCR) was significantly higher in tilapia fed diets with FPJ than C ($P<0.001$).

The 500 ml FPJ kangkong got the lowest survival compared to the commercial diet and 250 ml FPJ kangkong. The inclusion 250 ml FPJ Kangkong in 1 kg commercial feeds improves growth performance, feed efficiency and survival of Nile tilapia.

Table 1. Growth parameters, feed efficiency and survival of Nile tilapia *Oreochromis niloticus* fed diets with fermented plant juice (FPJ) *Ipomea aquatica*

	Diet			P-value
	C	C+250 FPJ Kangkong	C+500 FPJ Kangkong	
WG (g)	1.80 ^b	2.73 ^a	2.65 ^a	0.004*
AGR (g)	0.03 ^b	0.05 ^a	0.04 ^a	0.004*
RG (g)	180.04 ^b	272.70 ^a	264.62 ^a	0.004*
RGR (g)	3.00 ^b	4.55 ^a	4.41 ^a	0.004*
%SGR	1.72 ^b	2.19 ^a	2.15 ^a	0.002*
FER (%)	0.35 ^b	0.48 ^a	0.46 ^a	0.001**
FCR	3.07 ^b	2.13 ^a	2.35 ^a	<0.001**
GCR	0.33 ^b	0.38 ^a	0.38 ^a	<0.001**
Survival	88.89 ^a	95.55 ^a	82.22 ^a	0.244 ns

NOVEL APPLICATION OF NUCLEAR TECHNIQUES FOR SEAFOOD PROVENANCE

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The global population is predicted to increase to around 8 to 11 billion by 2050. Along with the increase in global population, there will be a commensurate increase in food consumption. Of this, most will consist of an increased intake of seafood. Seafood has been suggested to be part of a nutritional diet because it is considered an essential source of omega-3 fatty acids, protein, vitamins and iodine. The importance of seafood is highlighted by the market value of seafood, which is around \$94 billion USD as compared to other meats. With seafood consumption increasing on a global scale, and with 44% of the world's fisheries being fully to heavily exploited, the only sustainable solution is the use of aquaculture to sustain consumption. However, the rapid growth of aquaculture has presented new challenges. Food safety and quality have become increasingly important world-wide concerns. This is namely because of the presence of toxins, contaminants, hormones, and antibiotics in farmed fishes which raises questions about public health and safety. Food safety and quality authentication also require meeting certification standard for international trade.

The Australian Nuclear Science and Technology Organisation (ANSTO) is leading a research project, in collaboration with UNSW and Macquarie University that focuses on the novel application of nuclear techniques in seafood provenance and quality authentication. The overarching aim is to develop a quick analytical tool for seafood provenance and authentication. We have applied stable isotope analysis (SIA) and X-ray fluorescence (XRF), and Nutrition Activation Analysis (NAA) to distinguish the geographical origin and production methods of seafood. Two important high-value seafood products (*Penaeus monodon* and *Lates calcarifer*) were collected from 7 different bio-regions in the Asia-Pacific and analysed for their isotopic values and elemental profiles. Analytical results suggest that nuclear-based techniques can effectively distinguish production methods (farm vs wild caught) and the geographic locations of *P. monodon* and *L. calcarifer* with a higher degree of accuracy (>90%) and provide insights on quality in relation to elemental abundance in the samples.

EFFECT OF ENRICHED ARTEMIA (LEACH) NAUPLII WITH FATTY ACIDS ON GROWTH PERFORMANCE OF *Penaeus vannamei* POST LARVAE

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The shrimp post larvae were fed 4 times daily starting at the onset of exogenous feeding for seven days with artemia at 100nauplii/PL/day. Triplicate groups of shrimp post larvae were offered one Control and 4 treatments in 100litres tanks : 1) newly hatched *Artemia* (Leach) nauplii (unenriched) as control and 2) Different concentration of Fatty acid (0.5%, 1%, 2% and 3%.) enriched *Artemia* (Leach) nauplii as treatments. Then all groups of shrimp post larvae were switched to the commercial diet for an additional period of 18 days. Shrimp post larvae were fed with the nursery feed of crude protein 39 % based on the weekly growth rate.

Results :

Fatty acid composition of enriched artemia shows that the EPA (4.7%) level in treatment 4 was generally high compared to treatment 3 (4.3%). The linoleic (18:2n-6) and linolenic acid (18:3n-3) levels in fatty acid emulsion oil were generally high compared to EPA and DHA in all treatments.

Similarly in the fatty acid composition of shrimp post larvae on 7th day, animals fed with enriched diet showed higher w-6 fatty acid accumulation in tissues and Σ n-3 HUFA ranged from 21.3 % to 25.07%. EPA /DHA ratios ranged from 1.297% to 2.612% .On 21st day w-6 fatty acid accumulation in tissues were same in T2 and T3. The Σ n-3 HUFA ranged from 14.38 % to 20.54%. EPA /DHA ratios ranged from 1.297% to 2.612%

Best growth performance was achieved in the shrimp postlarvae fed with 2% fatty acid emulsion and this found to have a beneficial effect in the shrimp postlarval growth and survival. The fatty acid composition of shrimp post larvae with 2 % showed better nutrient absorption levels when compared to 3 % emulsion and this reveals the optimum nutrient absorption levels of vannamei post larvae.

Zootechnical performance of the shrimp post larvae reared for 30 days in the experimental trial

Parameters	Control	Treatment-1	Treatment-2	Treatment-3	Treatment-4
Initial weight (mg/individual)	8±2.2	8±2.3	8±2.1	8±2.3	8±2.2
Final weight (mg/individual)	98±16 ^c	102±28 ^c	104±25 ^b	133±26 ^a	112±19 ^b
Survival	86.3±1.4 ^c	90.6±1.9 ^b	92.6±2.2 ^b	96±1.5 ^a	94.9±1.8 ^a
Initial Length (cm)	1±0.3	1±0.2	1±0.4	1±0.3	1±0.3
Final Length (cm)	3.89±0.17 ^{bc}	4.17±0.29 ^c	4.36±0.78 ^b	5.2±0.45 ^a	4.28±0.63 ^b
Weight gain (%)	746.21±241.25 ^{bc}	754±511.47 ^c	759±233.21 ^c	799.97±549.25 ^a	772±431.25 ^b
FCR	0.81±0.02 ^c	0.75±0.02 ^b	0.77±0.03 ^b	0.66±0.02 ^a	0.74±0.01 ^b
SGR(%/day)	9.58±0.12 ^{bc}	9.71±0.17 ^b	9.76±0.21 ^b	9.96±0.19 ^a	9.81±0.08 ^b

IMMUNE RESPONSE AND DISEASE RESISTANCE OF VANNAMEI SHRIMP FED BIOFLOC GROWN ON *INSITU* AND *EXSITU* METHODS

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This study evaluated the effect of biofloc application on immune response, water quality and production performance of *Penaeus vannamei* in *insitu* and *exsitu* biofloc fed culture ponds of 400m³ in triplicates . The shrimp seeds of 1.06±0.16 gm at a density of 40animals/m³ were stocked in Control(C) (without external carbon input) and *insitu* biofloc treatment (T1) and *exsitu* biofloc (T2) at 10 ppt. Distillery spentwash was added on biofloc treatments as the organic carbon source at a C/N ratio of 15:1. Shrimps were fed with commercial pelleted feed in C & T1 and biofloc incorporated pelleted feed in T2 for 70 days with a crude protein level of 35 % . NO₃-N L⁻¹ was significantly higher in T1, accumulating to a peak mean concentration of 216 mg NO₃-N L⁻¹ whereas it was below 24 mg NO₃-N L⁻¹ in C. Biochemical oxygen demand (BOD₅) and Total suspended solids (TSS) concentration in T1 was significantly higher compared to T2 due to increased bacterial biomass. T1 showed significant difference in Myeloperoxidase and NBT activity compared to T2. Significant difference in FCR between T1(1.23±0.01) and T2(1.31±0.02) systems revealed , biofloc could be maintained and fed easily in T1 than T2. The survival and yield did not differ significantly between the treatments and specific growth rate was significantly different in T1(5.14±0.89). The results of this study demonstrate engineering immune responses in biofloc feeding trials that can have important implications for both water quality and shrimp production in fish meal replacement in minimal-exchange culture systems.

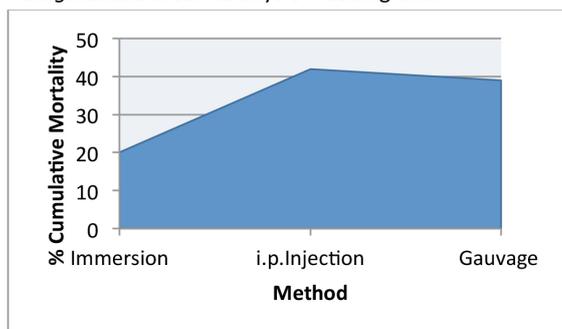
EFFECT OF DIETARY SUPPLEMENTATION OF PHYTOBIOTIC FEED ADDITIVE ON GROWTH PERFORMANCE AND RESISTANCE OF NILE TILAPIA (*Oreochromis niloticus*) TO *Streptococcus agalactiae* SEROTYPE II

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The present study was carried out to investigate the effect of a phytobiotic feed additive on growth performance, feed utilization, body composition and susceptibility of GIFT strain of Nile tilapia (*O. niloticus*) fingerlings to *Streptococcus agalactiae* Serotype II challenge. Three hundred numbers of disease free all-male Nile tilapia (50 ± 0.2 g fish⁻¹) fishes were stocked in lined pond for acclimatisation one week before starting the experimental trial. Fishes were randomly distributed in triplicate into one treatment and control group. Treatments were performed in three FRP tanks by stocking 10 fishes /litre of 500 litres capacity. These fishes were fed with commercial diet supplemented with the concentrations 5 g kg⁻¹ of phytobiotic feed additive served as treatment and control fishes fed with commercial diet without containing phytobiotic feed additives. Fishes were fed their respective diets twice a day for 6 weeks at 8% of their body weight for the first 3 weeks finally 6% for the last three weeks. The LD₅₀ for *Streptococcus agalactiae* Serotype II GIFT, was lower at 1.31×10^6 cfu/ml. At the end of feeding trial, fishes were challenged with *Streptococcus agalactiae* Serotype II (1×10^6 cells ml⁻¹) by intraperitoneal injection, immersion and gavage methods. The study results indicated that fish groups fed on phytobiotic mixture exhibited the least cumulative mortality percentage by immersion method (20%) and highest mortality (42%) by i.p. Intraperitoneal injection challenge method for over 10 days of exposure. Fish fed on with phytobiotic mixture showed significant ($p < 0.05$) improvements in its feed intake, live weight gain, specific growth rate and feed conversion ratio. The phytobiotic feed additive administration led to intensifying the respiratory burst activity, lysozyme and Glucose activity than control variants ($p < 0.05$). In conclusion, we demonstrated that the dietary supplementation of phytobiotic feed additive has improved the healthy status and resistance against *S. agalactiae* in tilapia farming.

% Cumulative mortality of GIFT Tilapia challenged with *S. agalactiae* after 42 days of feeding trial



Zootechnical Performance of GIFT Tilapia in control and treatment for the period of 42 days of feeding trial.

Parameters	Control	Treatment
Initial length (cm/individual)	13.64±0.36	14.41±0.43
Final length (cm/individual)	15.54±0.68	16.02±0.36
Initial weight (g/individual)	50.26±0.99	50.29±0.71
Final weight (g/individual)	67.32±2.01 ^a	71.98±4.96 ^b
Weight gain	16.86±2.69 ^a	21.9±4.01 ^b
FCR	2.51± 0.30 ^a	2.23 ± 0.31 ^b
SGR	1.13 ± .0107 ^a	1.28 ± 0.127 ^b

Data represented as means ± SE (n=30/group/replicate). Within rows, values with different superscripts a and b are significantly different at ($p < 0.05$)

INTERACTIVE IMPACT OF TEMPERATURE AND PH (ACIDIC AND ALKALINE) ON OSMORESPIRATION AND ENERGY MOBILIZATION OF AMMONOTELIC FRESHWATER FISH (*Leptobarbus hoevenii*)

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Ammonotelic freshwater teleosts excrete ammonia as a main nitrogenous waste product. Current warming environment combined with water pH fluctuation pose a challenge to fish to excrete endogenous ammonia efficiently. Thereby to avoid ammonia accumulation, fish need to re-strategize their excretory capability. This physiological compromising strategies performed by fishes still remain uncovered. Therefore, this study was designed to investigate the interactive impact of warming temperature and pH on physiological responses of freshwater ammonotelic fish, the Hoven's carp (*Leptobarbus hoevenii*). In order to understand how Hoven's carp strategize their physiological response, fish were subjected to different temperatures (28 °C vs. 32 °C) and pH (5, 7 and 9.5) for two weeks followed by osmorepiratory assay. Our finding showed that metabolic oxygen intake (MO_2) was significantly increased at 32°C in all conditions. Ammonia excretion (T_{amm}) was significantly affected by high environment temperature in acidic and alkaline pH compared to neutral, while reversal pattern of T_{amm} was noticed in acidic and alkaline pH at 28°C. Furthermore, Hoven's carp seem to reserve their energy when exposed to either acidic or alkaline conditions at low temperature. Contradictory, energy was significantly mobilized when fish exposed to high temperature. Nevertheless, high energy mobilization was noticed in fish exposed to acidic compared to alkaline condition. Overall, this study reveals that Hoven's carp was more sensitive toward acidic and alkaline pH in terms of osmorepiration and energy mobilization especially at high temperature. Finally, this study broadens the understanding of how Hoven's carp strategize their osmorepiration and metabolism in order to overcome impending environmental changes as a model of tropical freshwater species.

NATURAL CONCURRENT INFECTION OF *Vibrio harveyi* AND *V. alginolyticus* IN HYBRID GROUPERS (*Epinephelus polyphkadion* x *E. fuscoguttatus*)

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In September 2016, a disease outbreak resulting in high mortality of hybrid grouper fingerlings was reported at a farm located in Pulau Ketam, Malaysia. The cumulative mortality rate recorded was 29%. The moribund fish were dissected and the brains, eyes, livers, and kidneys were swabbed for bacterial isolation. The brains, livers and kidneys were then collected for histological examinations. Bacterial identification and pathogenicity study of the isolated bacteria were conducted.

The affected hybrid groupers displayed lethargy, excessive mucus production, rotten fins, haemorrhagic livers and kidneys, and enlargement of spleens. It was found that 80% (n=40) of the moribund grouper were positive with *V. harveyi* and/or *V. alginolyticus*.

In this work, experimental co-infection challenge of *V. harveyi* and *V. alginolyticus* on seabass were carried out. In triplicate, a total of 30 healthy seabass were challenged with 0.1 ml of 10⁸ CFU/ml of *V. harveyi*, 10⁸ CFU/ml of *V. alginolyticus*, and mixed culture of 10⁸ CFU/ml of *V. harveyi* and 10⁸ CFU/ml of *V. alginolyticus*, respectively by intraperitoneal injection. Fish mortality was recorded for 10 days post-challenge. All moribund seabass were dissected and the organs were swab for bacterial presence and fixed in 10% buffered formalin. Cumulative mortality observed in the tanks with *V. harveyi*, *V. alginolyticus* and co-infection tank were 100%, 60% and 100% respectively. Major diseased signs showed by infected seabass mimicked those on naturally infected fish. Moribund seabass that were exposed to both *V. harveyi* and *V. alginolyticus* exhibit severe scale drop, skin erosion and hemorrhage, rotten fins and haemorrhages in the internal organs (Figure 1). Histological examination of the diseased seabass showed congestion, haemorrhage and necrosis in vital organ.

Our results demonstrate that both *V. harveyi* and *V. alginolyticus* are pathogenic to seabass. However, concurrent infections involving *V. harveyi* and *V. alginolyticus* lead to more devastating impact to the cultured fish.

Figure 1: Scale drop, skin erosion and rotten caudal fin (arrow) of Asian seabass experimentally infected with *V. harveyi* and *V. alginolyticus*



EVALUATION OF SOYBEAN MEAL PRODUCTS WITH SUPPLEMENTATION OF PHYTASE IN THE FEEDS FOR HYBRID GROUPEL, BROWN-MARBLED GROUPEL (*Epinephelus fuscoguttatus*) X GIANT GROUPEL (*E. lanceolatus*) JUVENILE

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The study was conducted to evaluate soy protein concentrate (SPC) and defatted soybean meal (DSM) in replacement of fish meal with supplementation of phytase in the feeds for hybrid grouper (brown-marbled grouper, *Epinephelus fuscoguttatus* x giant grouper, *E. lanceolatus*).

Isoproteic (50% crude protein) and isolipidic (12% crude lipid) feeds were formulated with 30% of SPC or DSM protein replacement at five levels of phytase supplementation 0, 1000, 2000, 3000 and 4000 FTU/kg (SPC₀, SPC₁₀₀₀, SPC₂₀₀₀, SPC₃₀₀₀ and SPC₄₀₀₀; DSM₀, DSM₁₀₀₀, DSM₂₀₀₀, DSM₃₀₀₀ and DSM₄₀₀₀) and feed with 100% of fish meal protein (CON-FM1 and CON-FM2 for SPC and DSM based feed, respectively) served as control feed. Twenty fish (initial body weight (BW): 5.84 – 6.19g) were stocked in 100L fiberglass tank equipped with flow-through water system and fed twice a day to apparent satiation level for 12 weeks.

The growth of fish fed SPC based feed with or without phytase was higher than fish fed with CON-FM1 (Table 1). However, no significant ($P>0.05$) difference was observed among the SPC treatments. In addition, the survival, feed utilization and apparent digestibility of protein and lipid were not significantly ($P>0.05$) different among all the treatments.

Similar to the SPC treatments, the growth of fish fed with DSM based feed with or without phytase was higher compared to fish fed with CON-FM2 (Table 2). However, fish fed DSM based feed with or without phytase showed significantly ($P<0.05$) higher feed intake (1.63 – 1.68g fish⁻¹day⁻¹) and lower net protein utilization (NPU) (28.01 – 29.80) and apparent digestibility coefficient (ADC) protein (96.47 – 96.59%) compared to CON-FM2 (feed intake: 1.37g fish⁻¹day⁻¹, NPU: 34.03, ADC protein: 97.56%).

In general, the present study indicates that hybrid grouper can utilize well the SPC and DSM at 30% of protein replacement and phytase supplementation did not affect the fish growth, feed utilization and ADC of nutrients.

Table 1: Growth performances of hybrid grouper fed with SPC feed with or without phytase supplementation for 84 days feeding trial (Mean±SD)

Parameter	Experimental Feeds Treatment					
	CON-FM1	SPC ₀	SPC ₁₀₀₀	SPC ₂₀₀₀	SPC ₃₀₀₀	SPC ₄₀₀₀
Weight Gain (g)	1,312.09	1,768.92	1,711.75	1,521.21	1,477.11	1,415.25
(%)	±180.48	±241.41	±78.62	±299.26	±277.17	±366.39
SGR (% day ⁻¹)	3.15	3.48	3.45	3.30	3.27	3.21
	±0.16	±0.15	±0.05	±0.22	±0.22	±0.28

Table 2: Growth performances of hybrid grouper fed with DSM feed with or without phytase supplementation for 84 days feeding trial (Mean±SD)

Parameter	Experimental Feeds Treatment					
	CON-FM2	DSM ₀	DSM ₁₀₀₀	DSM ₂₀₀₀	DSM ₃₀₀₀	DSM ₄₀₀₀
Weight Gain (g)	1,821.92	2,037.70	1,987.48	2,036.12	2,049.19	2,026.89
(%)	±105.06	±86.87	±21.32	±68.64	±103.14	±100.04
SGR (% day ⁻¹)	3.52	3.60	3.57	3.60	3.58	3.58
	±0.06	±0.07	±0.07	±0.11	±0.07	±0.14

ASSESSMENT OF TRACE ELEMENTS IN *Euthynnus affinis*, *Thunnus tonggol*, and *Nemipterus furcosus* FROM EAST COAST OF PENINSULAR MALAYSIA

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Toxic elements can enter the aquatic environments through several pathways such as agricultural activities and domestic sewage. In Kuala Terengganu, offshore embankments and domestic sewage are measured as the sources of toxic elements contamination in aquatic environments. Growing activities of offshore embankment because of coastal corrosion along the beaches in Terengganu may enhance the quantity of toxic elements that flow into ocean. Hence, this study was carried out to verify the level of elements accumulation (zinc, selenium, arsenic and copper) in muscles, liver, intestine and gill of *Euthynnus affinis*, *Thunnus tonggol*, and *Nemipterus furcosus* landed at landing port Pulau Kambing, Terengganu. This study was also intended to assess the human health impacts due to the intake of toxic elements through the intake of these species. The detections of toxic elements content in fish's tissues were using Inductively Coupled Plasma-Optical Emission Spectrometry and the estimation of human health was calculated by using formula. In this study, concentrations of all four toxic elements were higher in viscera tissues (liver and intestine) than in muscle and gill. In addition, Zn has highest concentration in all tissues. Provisional Tolerable Weekly Intake (PTWI) was calculated and it was showed that concentrations of Zn, Se, As (organic) and Cu were safe for consumption while As (inorganic) form was exceeded the permitted level set by JECFA. But, currently, there are unidentified value whether content exists in organic or inorganic form of As.

EFFICACY OF THREE DISINFECTANTS ON INACTIVATION OF FISH PATHOGENIC *Streptococcus agalactiae* UNDER DIFFERENT CONDITIONS

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Streptococcosis is an important bacterial disease in fish causing severe economic losses to the aquaculture worldwide. To promote biosecurity in aquaculture and therefore prevent economic adverse impacts of bacterial infections, disinfectants have been widely used as preventive measures to reduce the risk of infectious disease transmission. To determine the efficacy of disinfectants on *Streptococcus*, five isolates of *Streptococcus agalactiae* were tested against three commercial disinfectant products with the main active ingredients being povidone iodine (Anidine 100™; AD), benzalkonium chloride (Better BKC 80™; BKC 80), and a mixture of quaternary ammonium compounds and glutaraldehyde (Chloraldehyde™; CR). Among three commercial disinfectants, CR demonstrated highest efficacy to *S. agalactiae* reduction, followed by BKC 80 and AD, respectively. The efficacy of disinfectant was decreased by higher-level soiling condition, low temperature, diluted concentrations, and short exposure time. CR and BKC 80 provided more than 5 logs inactivation at 1 min exposure at 20°C under high-level soiling conditions, and also with ten-fold-diluted concentrations at 60 min exposure time at 30°C. However, AD required 10 min exposure to effectively remove bacteria under LS conditions at 30°C. This information could assist in the selection of appropriate disinfectants and in recommendations of effective practices for disease control in fish husbandry.

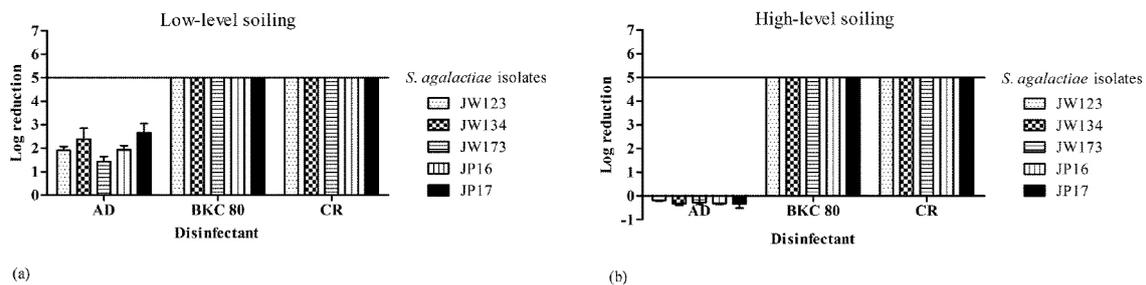


FIGURE 1 Log reduction in 5 strains of *S. agalactiae* with low-level soiling interference (LS; a), and water with high-level soiling interference (HS; b) when exposed to Anidine 100™ (AD), Better BKC 80™ (BKC 80) and Chloraldehyde™ (CR) at recommended concentrations.

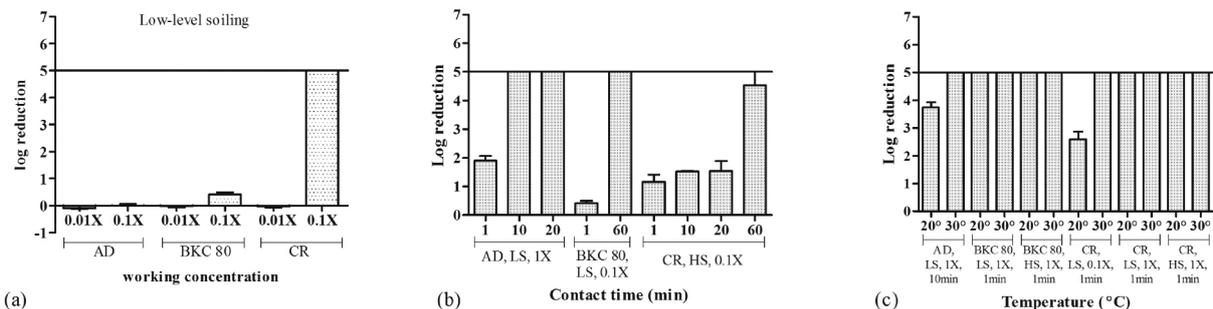


FIGURE 2 Log reduction of *S. agalactiae* under different conditions

INTEGRATIONS OF MARICULTURE IN THE COASTAL WATERS OF BANGLADESH

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Bangladesh is a thriving maritime nation. Bangladesh needs to diverse the right strategy to utilize its maritime waters in order to shape the blue economy. Although Bangladesh ranked fifth in global aquaculture but mariculture is not popularized yet. The limited availability of protected sites and the probable conflicts with other activities such as fishing, tourism and navigation are key factors likely to influence mariculture development, besides finance, expertise and government policy. The present study aims to find out the suitability of Asian seabass (*Lates calcarifer*) culture using floating net cages (locally available materials) with low-cost feed ingredients in the coastal region of Bangladesh. Considering all these criteria, the Bankkhali River Estuary, Cox's Bazar has been selected. Locally collected fingerlings were acclimatized, sorted (19.5 ± 1.03 g) and stocked at 50 indivs/m² and 45 days later it was reduced to 30 indivs/m² till harvest. Three supplementary feeds; flesh of pangush fish (*Pangasius sutchi*), mussel meat (*Perna viridis*) and flesh of chewa fish (*Trypauchen vagina*) were used with three replicates. Feeds were applied trice daily in first two months @ 8% of their total biomass and then twice daily @ 5% till harvest. After 120 days fishes were harvested (Table 1). SPSS-Post Hoc comparison indicates that pangush as feed gives the best result followed by mussel and chewa. The present study reveals that there is a good opportunity to integrate cage culture in the marine waters of Bangladesh.

TABLE 1. Final length and weight, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate of seabass fed supplementary feeds for 120 days.

Feed type	Final length, cm (Mean±SD)	Final weight, g (Mean±SD)	SGR (%/day)	FCR	Survival rate (%)
Pangush fish	25.99±1.40	238.17±34.97	2.09	3.56	94.5
Mussel meat	25.80±1.52	226.48±28.10	2.04	4.60	92.3
Chewa fish	24.30±1.41	169.18±16.27	1.80	5.38	85.2

USING CONDITION INDEX TO ASSESS THE EFFECTS OF CALCIUM SUBSTITUTION BY MARINE SNAIL SHELLS IN THE DIETS OF JUVENILE KURUMA SHRIMPS, *Marsupenaeus japonicus*

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Shells derived from marine mollusks represent over 80% of shellfish waste. Therefore, a trial was conducted to assess the efficacy of snail shells (SS) as a calcium replacement in the diets of kuruma shrimps over a period of 42 days. Evaluation of shells' ability to replace calcium was done by applying principles from previously researched condition criteria index to quantify the external characteristics of juvenile kuruma shrimps (initial body weight, 0.12 ± 0.01 g each) with relation to their growth performance. Five pellet diets were formulated to include increasing levels of snail shells at 0%, 2%, 5%, 10%, and 0% (D1, D2, D3, D4 and D5, respectively). D5 was the negative control with no snail shells and no calcium included in the diet. Each diet was randomly allocated to triplicate groups of 15 shrimps per tank.

Survival, body weight gain, specific growth rate, apparent feed efficiency ratio, individual dry weight, total body length, condition index, freshwater stress resistance and fatty acid composition were evaluated. Results indicated that shrimps fed 2% and 10% SS had significantly higher stress resistance ($P < 0.05$). Generally, dietary SS supplementation improved growth performances (such as final body weight, % weight gain, specific growth rate, apparent feed efficiency ratio), where significantly highest performance was found in shrimps fed 10%SS.

Diets with $\leq 2\%$ SS supplementation had significantly lower condition index and hepatosomatic index (D1, D2 and D5). Under the present experimental environment, using the condition index as a tool to evaluate kuruma shrimps' fitness was beneficial, however it was a poor indication of freshwater stress resistance. Instead, the ratio of n-3/n-6 in the shrimps' bodies seemed to be correlated with stress resistance. Additionally, supplementing SS in place of Ca is recommended for better growth performance.



Fig. 1. External appearance of kuruma shrimps fed the diet containing varying levels of snail shells for 42 days. A, B; Arrow is pointing at the pale hepatopancreas as was seen in shrimps fed D1 and D2. C; Arrow is pointing at the loss of limbs present in a few shrimps that experienced possible cannibalism in D5 group. D; Example of a healthy shrimp, arrows are pointing at healthy hepatopancreas and healthy gut. E; Arrow is pointing at black gills present in shrimps fed D5. F; Arrows are pointing at gills of a shrimp from D1 group, (top) shrimp with clean gills after molting, (bottom) molted exoskeleton is showing previously infected gills which recently shed.

WORLDWIDE MYCOTOXIN OCCURRENCE IN PLANT MEALS: AN OVERLOOKED RISK TO AQUACULTURE?

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The inclusion of plant-based meals in compound aquafeeds is not novel, however, when considering plant-based meals for aquafeeds it is commonly agreed that one of the negative aspects is the presence of anti-nutrients (e.g. cyanogens, saponins, tannins etc.) that are detrimental to fish/shrimp. Although there are processes to remove or inactivate many of these compounds, the same does not apply for mycotoxins which are reasonably stable to processing conditions. Over a period of one year (January 2017 – December 2017), samples of plant meals, have been analyzed within the scope of BIOMIN® mycotoxin survey program. This study focuses on corn, corn gluten meal, corn DDGS, soy bean meal, wheat, wheat, rice and rice bran. The samples were tested for aflatoxins (sum of AFB1, AFB2, AFG1 and AFG2), ochratoxin A and the *Fusarium* mycotoxins zearalenone, deoxynivalenol and other type B trichothecenes (B Trich), fumonisins (FUM, sum of FB1 and FB2), and T-2 and other type A trichothecenes (A Trich); these being the six main mycotoxin types of interest for aquaculture and agriculture). Briefly, during 2017, 18,757 samples were analysed in the whole survey with 8284 samples belonging to the aforementioned plant materials commonly used in aquaculture), comprising 31036 individual analyses of the major mycotoxins. *Fusarium* mycotoxins were the most prevalent compounds found in the samples testing positive, followed by aflatoxins. Concerning the contamination levels, some plant meals such as corn gluten meal and corn DDGS, showed high levels of contamination. The most common mycotoxins in corn DDGS included B Trich (95% of samples with a mean average of positive samples of 2,725 $\mu\text{g kg}^{-1}$ and a maximum of 14,252 $\mu\text{g kg}^{-1}$) and FUM (91%, mean 2,972 $\mu\text{g kg}^{-1}$, max 28,605 $\mu\text{g kg}^{-1}$). Corn gluten meal was also high in these *Fusarium* mycotoxins with B Trich in 89% of samples (mean 1,251 $\mu\text{g kg}^{-1}$, max 8,871 $\mu\text{g kg}^{-1}$) and FUM in 90% (mean 3,547 $\mu\text{g kg}^{-1}$, max 16,976 $\mu\text{g kg}^{-1}$). Mycotoxin co-occurrence was generally very high for all the samples analysed. On average, 89% of the total analyzed samples presented more than one mycotoxin in the same sample. The contamination levels found in the plant meals listed above as commonly used in aquaculture were high and in 74% of samples there were more than one mycotoxin detected, potentially leading to additive or synergistic effects. These results highlight the mycotoxin-related risk to growth performance and immunosuppression that can lead to significant economic impacts in the aquaculture sector.

THE EFFECTIVITY OF AROWANA PINOH (*Scleropages macrocephalus*) VITELLOGENIN PRODUCTION USING ESTRADIOL STIMULATION BY INJECTION AND ORAL

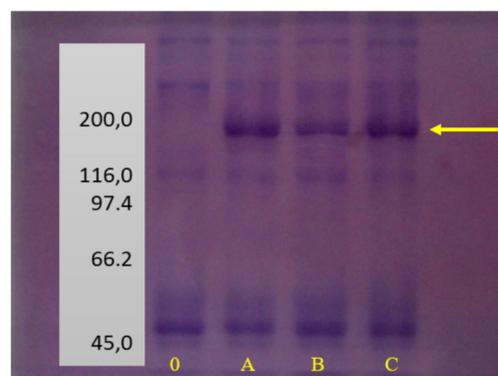
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Arowana fish (*Scleropages sp.*) are monomorphic species, those animals that physically could not be distinguished between male and female. Indonesia has five arowana species, those are Formosus (*S. formosus*), Super red (*S. legendrei*), Banjar and Pinoh (*S. macrocephalus*), Golden (*S. aureus*) and Papua (*S. jardinii*). Arowana pinoh (*Scleropages macrocephalus*) is an Indonesian native commercial ornamental fish which its spreading are was in Sumatra, Kalimantan and Papua. Vitellogenin is an egg yolk protein precursor which can be utilized for the detection of female fish sex and gonadal maturity. In order to stimulate the vitellogenin, estradiol solution contain ethanol in cocoa butter usually injected to intraperitoneal of the fishes. This way cause burn on the fish body and could affect mortality of the fish. In this study we examine to use a simple safer way to stimulate vitellogenin through oral stimulation. This study was aimed to stimulate the synthesis of Vitellogenin on arowana pinoh through Estradiol stimulation either by induction or oral.

Three arowana fishes was given Estradiol stimulation 50 mg/kg body weight, two of them through intraperitoneal induction and one through orally by accumulation in feed. The oral method was conducted by injected the estradiol solution in to small frog belly before it fed to the fish. Plasma samples were taken before (control), seven days after hormonal stimulation. Investigation using SDS-PAGE on a 7.5% Acrylamide gel showed that there was band difference on wells that contain plasma control and after treatment. Wells containing plasma after oral and induction showed a thick band that is different from the control on 180 kiloDalton of the molecular weight. These results indicated that orally Estradiol stimulation more preferred to animal welfare and also efficient for the Vitellogenin production process of arowana pinoh.



Protein expression on 7.5 % acrylamide gel. Description : 0 referred to control, A, B and C referred to fish. Arrow refers to specific protein. The number 45 – 200 refers to molecular weight (kDa)

EFFECT OF SYNTHETIC HORMONE BUSERILIN ACETATE ON BREEDING OF *Tor douronensis* (Valenciennes, 1842)

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Induced breeding of *Tor douronensis* by hormone injection was carried out from October 2012 to September 2013. The average weight of female and male brood stock were 2.74 ± 1.02 kg and 3.25 ± 1.52 kg, respectively. There were 6 treatments. Treatment 1, 2 and 3; the female were injected with 20, 30, 40 $\mu\text{g}/\text{kg}$ Buserelin acetate (BUS) + 10 mg/kg Domperidone (DOM), respectively. Treatment 4, 5 and 6 the female were injected with 40 $\mu\text{g}/\text{kg}$ BUS + distilled water, 10 mg/kg DOM + distilled water and injected with distilled water, respectively.

The result showed that the treatment 1, 2 and 3; the female fish were spawn at 50, 100 and 75%, respectively. The treatment 2 was statistically significant difference with treatment 1 ($P < 0.05$) but non significant difference with treatment 3 ($P > 0.05$). The number of eggs of treatment 1, 2 and 3 were 48.27 ± 6.68 , 89.00 ± 27.91 and 63.33 ± 23.62 eggs/kg, respectively. The fertilization rates were 73.50 ± 2.12 , 70.00 ± 8.79 and 76.66 ± 3.05 %, respectively. The average hatching rates were 90.51 ± 0.21 , 88.31 ± 5.89 and 90.88 ± 3.50 %, respectively. The survival rates were 87.44 ± 1.11 , 93.98 ± 5.66 and 94.87 ± 1.33 %, respectively and non significant ($P > 0.05$). Treatment 4, 5 and 6; the female did not spawn. The embryonic development was complete within 91 hours 30 minutes at 25 – 26 °C water temperature. Average length were 7.00 ± 0.07 mm, 3 days old larvae were 9.89 mm in length and development to most likely juvenile within 50 days, 22.82 mm in length. The result indicated that the female injected with 30 $\mu\text{g}/\text{kg}$ (BUS) + 10 mg/kg (DOM) encouraged fish to spawn was 100 % and lowest production cost.

YOLK ABSORPTION IN EMBRYONIC AND LARVAE STAGE OF *Agamyxis sp*

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Agamyxis sp known as an introduction species from Amazon River and potential to be ornamental fish in Indonesia. *Agamyxis sp*, also called by “*Spotting Talking Catfish*” or “*Spotting Raphaela Catfish*”. In nature, this species is found in various habitat types but shows a preference for slow-moving or still waters with an abundance of submerged or floating structures, among which it conceals itself during daylight hours.

Observation on yolk absorption in embryonic and larvae stage of *Agamyxis sp* was conducted in Research Institute for Ornamental Fish Culture, Depok. Egg yolk volume was calculated using formula Heming & Buddington (1988): $V = 0.1667rLH^2$ where V is yolk egg volume, H is the height and L is the length of the mass. Egg yolk absorption was calculated with formula : $A = \{(V_o - V_n) / V_o\} \times 100\%$ where A is egg yolk absorption, V_o is initial egg yolk volume and V_n is egg yolk volume in day n .

The result showed that volume of egg yolk of *Agamyxis sp* is 1.078 in day one after fertilization (Figure 1) and completely absorbed in day one after hatch (Figure 2).. Rate of yolk absorption and the efficiency of yolk utilization are important determinants of early development, growth, and survival. Larval survival is ultimately dependent on the availability of food in sufficient quantity and of adequate quality after yolk reserves are exhausted (Heming & Buddington,1988). This means that shortly after hatching, the larvae of *Agamyxis sp* should be fed. The yolk in embryonic and larvae of *Agamyxis sp* shown in Figure 3.

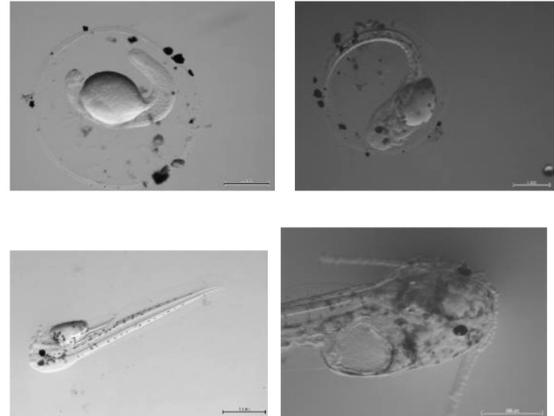


Figure 3. yolk in embryonic and larvae of *Agamyxis sp*

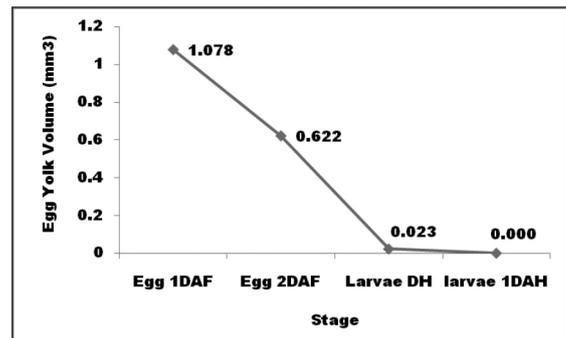


Figure 1. Egg Yolk Volume in embryonic and larvae stage of *Agamyxis s* (DAF: Day After Fertilization, DH: Day Hatching, DAH: Day After Hatch).

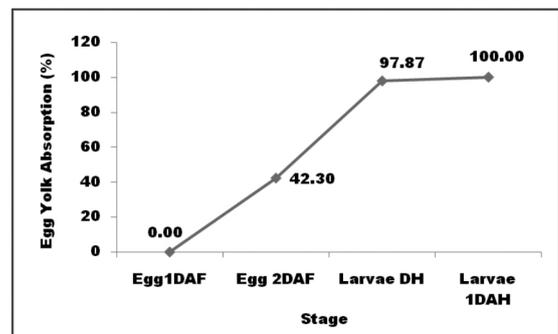


Figure 2. Egg Yolk Absorption of in embryonic and larvae stage of *Agamyxis sp* (DAF: Day After Fertilization, DH: Day Hatching, DAH: Day After Hatching).

CYCLIC CHANGES OF GAMETOGENESIS AND QUANTIFICATION OF REPRODUCTIVE OUTPUT OF HORSE CLAM, *Tresus keenae*, IN YEOSU, SOUTH KOREA

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Determining the reproductive cycle of a commercially important species is a prerequisite for a sustainable culture of a particular species. *Tresus keenae* is a commercially demanded species in Korea, Japan and China. Reproductive cycle of *T. keenae* in Yeosu, West coast of South Korea was determined by the histology and it was justified by quantifying of eggs using ELISA, Condition Index and biochemical composition of adductor muscle and siphon.

Histological examinations from October 2016 to September 2017 revealed that *T. keenae* in Yeosu has a peak spawning in October and November. First spawning in male and female clams observed on October and gametogenesis of both female and male clams started in March. Condition Index, which is generally used to characterize the physiological condition of an organism, recorded low value (16.48 ± 1.94) in November 2016 and highest value (40.38 ± 5.0) in June 2017. In the present study low value of condition index was observed during the spawning period and it clearly illustrates the relationship with the reproductive cycle of *T. keenae*.

Polyclonal antibodies were developed from the egg protein of *T. keenae* and the cross-reactivity of the antibodies with somatic tissues was removed. Western Blotting showed that the anti *T. keenae* egg protein IgG recognize several peptides with molecular weights of 244, 175, 109, 98, 62, 47, 38 kDa. Indirect Enzyme Linked Immunosorbent Assay (ELISA) was used to quantitatively measure the reproductive output of *T. keenae*. Female clam produces 131.14 million of eggs during the spawning period. Monthly mean Gonad Somatic Index (GSI) of *T. keenae* is highest in July (6.5) and lowest in January (1.0).

Monthly variations in protein and carbohydrate in the siphon and adductor muscle show a positive correlation with the oscillation of the reproductive cycle of clam. According to the present study, adductor muscle and siphon store carbohydrate in late spring and utilize for gametogenesis and spawning.

The results of the study suggest that *T. keenae* in Yeosu spawn in early winter while using the carbohydrate and protein as the energy source for gametogenesis and spawning. Reproductive output of *T. keenae*, which is difficult to measure using conventional methods, can be quantitatively measured by the indirect ELISA using rabbit anti clam egg IgG.

Table1. Monthly changes of CI (Condition Index), fecundity and GSI (Gonad somatic index). SD, Standard deviation

Year	Months	CI \pm SD	Fecundity	GSI
2016	October	20.65 \pm 4.66	131,144,117	5.8
	November	16.48 \pm 1.94	65,489,279	2.8
	December	16.95 \pm 3.67	9,869,932	1.7
2017	January	19.67 \pm 6.12	8,989,782	1.0
	February	23.59 \pm 4.21	11,954,877	1.6
	March	32.50 \pm 5.93	36,588,802	2.4
	April	36.43 \pm 9.27	29,124,666	2.1
	May	33.64 \pm 6.17	39,435,725	3.6
	June	40.38 \pm 5.0	44,419,898	6.5
	July	34.89 \pm 6.59	44,738,973	6.1
	August	25.87 \pm 5.66	34,405,515	2.0
	September	29.47 \pm 3.21	20,062,538	2.7

ASPECTS OF THE TAXONOMY OF CATFISHES (SILURIFORMES: ARIIDAE) BASED ON EXTERNAL MORPHOLOGICAL CHARACTERISTICS

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Marine ariid catfish are presently generally recognizable from other both marine and freshwater Siluriformes by several external morphological characteristics. These are namely the number of paired barbels, presence of a bony plate on their head, stiff and serrated dorsal and pectoral spines, an adipose fin located between the dorsal and caudal fins, and a deeply-forked caudal fin. However, the interspecific differences of these characters among juvenile and adolescent life stages are not as readily distinguishable. In this study, we examined 42 morphometric and 9 meristic characteristics including the ontogenetic pattern of the palatal tooth patch(es) and head shields of 15 Ariidae species (*Arius leptanotacanthus*, *A. maculatus*, *A. microcephalus*, *A. oetik*, *A. subrostratus*, *A. thalassinus*, *A. venosus*, *Cryptarius truncatus*, *Hexanematichthys sagor*, *Nemapteryx macronotacantha*, *N. nenga*, *Netuma bilineata*, *Osteogeneiosus militaris*, *Plicofollis argyroleuron*, *P. nella*). Using a combination of these characteristics, we present the analysed data for the distinguishing characters for *Cryptarius truncatus*, *Hexanematichthys sagor* and *Osteogeneiosus militaris*, the three most readily identified marine catfishes, based on external morphological characteristics. *Cryptarius truncatus* is distinguishable by its small eyes (12.6% ED/HL; n=17), eyes located nearer to the head bottom (13.7% EHB/HL) and yellowish mucus covering its body. For *Hexanematichthys sagor*, this species is distinguishable by having a short snout (7.0% SNL/HL; n=8), far distance between the eye and the operculum margin (62.2% EO/HL) and stripes on its bronze body. The distinguishable characteristics for *Osteogeneiosus militaris* are having just one pair of stiff barbel, bluish body with darker upper body, and wider interorbital distance (87.5% IB/BW; n=8).

MOLECULAR CHARACTERIZATION OF VIPERIN AND ITS EXPRESSION PATTERNS UPON IMMUNE STIMULANTS FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

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Viperin is an antiviral protein that has rapid ability to induce many type of interferon-regulated genes (ISGs). Viperin could be induced in a several of cell types by different cellular elements such as DNA and RNA viral proteins, type I, II and III IFNs, poly (I: C) and polysaccharide. Moreover Viperin has been induced by either IFN- dependent or IFN-independent pathways. In this study, Viperin of big belly seahorse (*Hippocampus abdominalis*; *HaVip*) was recognized and characterized at sequence and transcriptional levels under the immune stimulants as well as healthy animals. Viperin contains coding sequence of 1047 bp which encodes 348 amino acids in length. After homology modelling, the 3D structure of *HaVip* was obtained similar to that of the human viperin. Phylogenetic tree clearly appeared the close evolutionary relationship for vertebrate viperin counterparts, with clustering to the fish homologs. The highest tissue expression of *HaVip* was the heart then in skin tissue, while the lowest expressions were detected in spleen and liver tissues. In blood and intestine, under LPS and poly (I: C) challenges, *HaVip* expressions were significantly up regulated in all time points, but not with *S.iniae* challenge. In kidney, *HaVip* transcripts has been increased significantly upon LPS, poly (I: C) and *S.iniae* challenges respectively. According to results obtained, viperin may involve in immune defense mechanism in seahorse.

THE POTENTIAL APPLICATION OF AGRICULTURAL ORGANIC FERTILIZER TO SYNTHESIZE FRESHWATER PRAWN (*Macrobrachium rosenbergii*) REARING CULTURE TO IMPROVE GROWTH PERFORMANCES AND PROMOTE STABLE CULTURE ENVIRONMENT

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Agricultural organic fertilizer (AOF) consists of a variety of plant-derived materials from plant material waste, agricultural by-products to animal manure. Agricultural organic fertilizer is beneficial in providing additional nutrient through microbial build-up, increase organic content, inexpensive, sustainable materials and locally available. This experimental trial was conducted to investigate the potential suitable source of AOF added to the aquatic environment of *M. rosenbergii* post larvae (PL) culture system to improve growth performances (increased total length, weight gain, and condition index) and promote stable culture environment (water parameters). The experimental trial was conducted for twenty-eight days consisting of three treatments in duplicate of 30L tanks. Treatment A was control where no AOF was added, treatment B contained 0.07% of processed green waste (PGW) and treatment C contained 0.02% organic soil conditioner (OSC). The prawns were fed 8-12% of their body weight daily. Water parameters of dissolved oxygen, temperature, pH, ammonia, nitrite and nitrate concentration were monitored every five days throughout the trial. At the end of the experiment trial, treatment C (OSC) showed the highest increase of total length (1.31cm) compared to control (0.9cm) whereas the highest weight gain was observed for treatment C (OSC) 0.2g compared to treatment A (control) 0.07g. Treatments containing AOFs exhibited higher condition index compared to control. The highest survival level were obtained in the treatment B (PGW), 81.67% while treatment A (control) remain the lowest (28.33%) probably due to sudden increase of ammonia level in the final week of the trial. The variables of water quality parameters remained within the most stable range was observed for treatment B (PGW). The results from this experimental trial concludes the beneficial application of AOF in the rearing culture of freshwater prawn PL in enhancing growth and survival rate

AHPND-CAUSING *V. parahaemolyticus* ACTIVATES THE RHO SIGNALING PATHWAY IN SHRIMP

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Acute hepatopancreatic necrosis disease (AHPND) is an emerging bacterial disease caused by *Vibrio parahaemolyticus* with an additional AHPND-associated plasmid pVA1 encoding a virulent toxin (Pir^{vp}) that damages shrimp hepatopancreas. Host molecular mechanisms enabling *V. parahaemolyticus* to compromise the integrity of the stomach barrier, enabling bacteria or toxins to cross this barrier and reach the hepatopancreas, are poorly understood. Using transcriptomics and system biology methods, we identified AHPND-induced changes in the stomach of infected shrimp. Expression of 376 unique genes were differentially regulated by AHPND infection. Based on gene ontology, protein interactions and gene-to-gene correlation expression interaction analyses, these genes were greatly involved in cytoskeleton regulation by Rho GTPase. Activation of Rho disrupted epithelial structure of the stomach, facilitating transepithelial migration of pathogens and Pir^{vp} toxins to hepatopancreas. Furthermore, pre-treatment with a Rho activator enhanced the pathogenicity of AHPND-causing *V. parahaemolyticus*, whereas pre-treatment with a Rho inhibitor delayed the pathogenicity. Therefore, we inferred that AHPND pathogenesis may include regulation of the Rho pathway. In summary, our comprehensive transcriptome analysis revealed key pathways involved in AHPND pathogenesis.

THE STUDY ON MICROSPORIDIA INFECTED IN MUSCLE OF STRIPED CATFISH (*Pangasianodon hypophthalmus*)

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The study aimed to investigate the microsporidia infection in striped catfish and control methods of infected microsporidia. The total of 578 catfish samples collected in 24 catfish ponds in An Giang, Can Tho, Vinh Long provinces, including 458 fingerlings and 120 commercial striped catfish. Results showed that in fish muscle infected microsporidia, muscle tissue structure was damaged and necrosis, tissue cells began to completely lysis and there was appearance of sporophorocysts in tissue specimen. Spores were pear-shaped or ovoid appearance, and ultra-size. Inside the spore, there were one helical polar tubule which was very difficult to distinguish when observed under the microscope. Results of morphological observation combined with gene sequencing show that Microsporidia spores is *Kabatana* sp. There were some of chemicals such as formalin and ethanol (40-70%), chlorine dioxide, hydrogen peroxide, chlorine (0.1-0.7%) effectively killed microsporidia spores. However, the effective time for killing spores were highly dependent on the concentration and types of chemical. Besides, albendazole, fumagillin and TNP-470 can be able to destroy spores of microsporidia. The results of drugs inhibition on spores infected in kidney and muscle cells showed that albendazole and fumagillin, at concentration 5 µg/mL, can be applied to treat infection of spores caused by *Kabatana* sp. in fish muscle.

ADAPTATION OF EUROPEAN PROTOCOLS OF LIVE FEED PRODUCTION AND ENRICHMENT FOR SMALL SCALE IN-DOOR MARINE FISH HATCHERIES IN SOUTH-EAST ASIA

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Hatchery production of marine fishes in South-East Asian countries has quickly developed in the last two decades and contributed to the establishment of marine fish farming industry in the region. However, as most of the marine fish hatcheries in South-East Asia are of small scales with limited infrastructure investment, particularly unavailability of liquid oxygen and unstable electricity network, the application of European advanced protocols for live feed production and enrichment has not been a success. Unstable availability of well-enriched live feeds has been the bottle neck for the region's marine fish hatcheries to improve their fish fingerling quality and to sustain hatchery production business. Low survival rates and high rates of fish fingerling deformity have been observed in many hatcheries.

Since 2015, INVE Aquaculture Inc. has collaborated with local aquaculture institutions and marine fish hatcheries to adapt European advanced protocols of rotifer cultivation, Artemia hatching, and the live feed enrichment to South-East Asian infrastructure conditions using IAQ off-the-shelf products. The collaboration has developed efficient protocols for live feed production and enrichment that can be applied to South East Asian limited-infrastructure marine fish hatcheries, and the protocols' application has resulted in higher larval survival rates and robustness of the fish fingerlings in many local fish hatcheries.

This presentation describes the technical adaptations in rotifer culture, Artemia cyst hatching, and the live feed enrichments as well as results of applying the adapted protocols in marine fish hatcheries in the region, particularly in Asian seabass back-yard hatcheries in Vietnam.

PROTEIN INGREDIENTS: CONSIDERATIONS THAT IMPACT ON ANIMAL PERFORMANCES AND PROFITABILITY FOR AQUA FEED

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Unlike livestock feed, the formulation of aqua feed is highly specific and requires high quality ingredients in terms of nutritional and functional properties. However, formulation software often considers nutrient based values for low cost formulation while other non-nutrient based values, those highly impact on animal performances; are often omitted during the optimization. Protein ingredient is a key component in all animal feeds and therefore to be taken as an example in this paper for reviewing the key factors that impact on animal performances and profitability for aqua feeds.

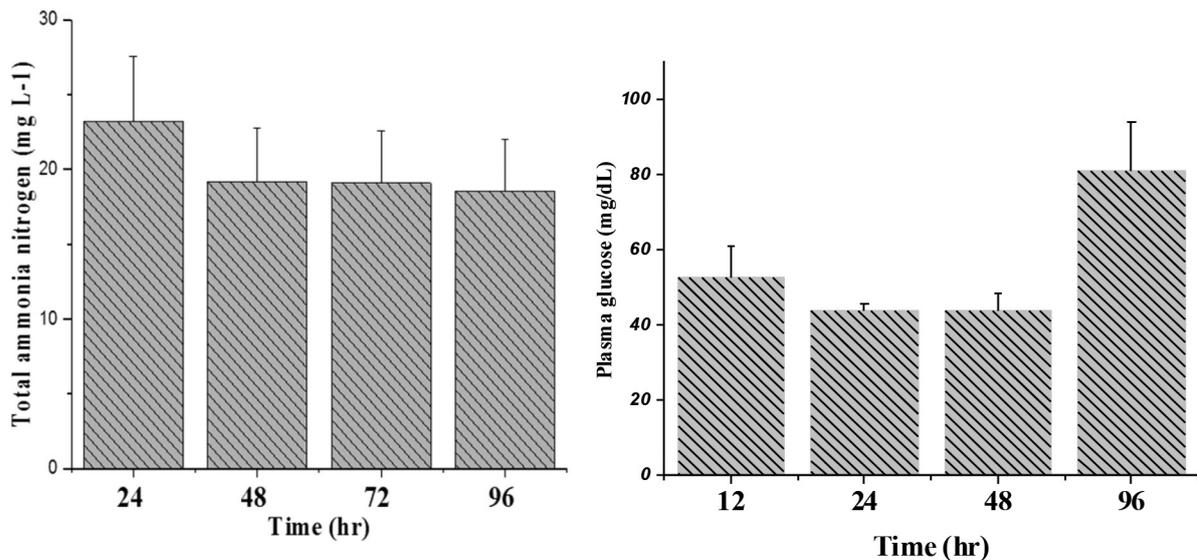
The amino acid profile and digestibility of amino acids have been nutritionally identified when considering quality of the protein ingredients. Unfortunately, the roles for amino acids in health nutrition and metabolism have sometimes ignored, such as the roles of glutamine and leucine in immunity enhancement, antioxidant defense and stress response need to be considered for protein quality. The impact of fiber content and other non-nutrient based values such as the freshness, anti-nutrient factors, contaminants and physical characters of the protein ingredients on animal performances need also to be considered when evaluating the protein ingredients for aqua feeds and therefore be reviewed in this paper.

REGULATION OF GLYCOGEN CATABOLISM IN ASIAN SEABASS (*Lates calcarifer*) UPON SUBLETHAL AMMONIA CHALLENGE

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Asian seabass (*Lates calcarifer*) is an aquaculture species with high economical value in Southeast Asia including Taiwan. Waterborne ammonia is a common stressor in intensive farming practice or recirculating aquaculture system (RAS). Elevation of waterborne ammonia induces not only sublethal stress but also even lethal effects at higher level. Accordingly, there is a need to study acute toxicity of ammonia to Asian seabass to estimate a safe level. Furthermore, to cope with environmental challenges in animals, glucose primarily stored as glycogen is a major source to fuel the activation of energy-demanding physiological stress responses. The present study measured acute toxicity of total ammonia nitrogen (TA-N) to Asian seabass in a 96-h static test. The median lethal concentration (LC50) is 23.3, 19.3, 19.1 and 18.6 mg/L at 24, 48, 72 and 96 h post-exposure. Time-course changes in plasma glucose, glycogen content and protein abundance of glycogen phosphorylase (GP) in gills, liver and muscle were then detected after exposure to 9 mg/L TA-N, a half of the 96h-LC50 to explore the energy utilization of glycogen for coping with sublethal ammonia stress.



EFFECTS OF PINE POLLEN ON SEX REVERSAL AND GROWTH OF NILE TILAPIA *Oreochromis niloticus* LARVAE

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Early maturation and prolific breeding in culture systems are the major problems with tilapia breeding that results in overcrowding, decreased growth rate and low production. Monosex culture through hormonal sex reversal is seen as one of the most effective methods used to control overpopulation and possibly produce high yield. The experiment was carried out to investigate the effects of pollen from *Pinus tabulaeformis* (PP) as possible replacement for the synthetic 17-alpha-methyltestosterone (MT) in the Nile tilapia, *Oreochromis niloticus* larvae. The experiment evaluated the effects of PP on growth, feed conversion efficiency, survival and sex inversion as compared with that of the 17a-methyltestosterone (MT). Larvae were fed 5 diets containing graded levels of PP, namely, 0 (negative control), 80, 160, 320 and 640 mg kg⁻¹ and a positive control diet containing MT. The feeding trial lasted for 8 weeks.

Results showed that after 56 days of feeding, the MT diet resulted in all the Nile tilapia converted to male (100%) but was statistically similar with those of the PP diets (81.0% to 89.0% male, $p < 0.05$). Almost half of the fish fed the control diet were male (50.5%) and almost half female (49.5%). Results of the growth trial showed that the final weight (FW) and weight gain (WG) of fish fed diets containing 0.08 g kg⁻¹ PP and 0.32 g PP kg⁻¹ were significantly higher than of those fed the MT diet ($p < 0.05$). Feed intake (FI) of all experimental fish were statistically similar ($p > 0.05$). The group fed 0.08 g PP kg⁻¹ diet exhibited significantly higher SGR than did the MT group. The MT group exhibited lower feed conversion efficiency (i.e. higher FCR) than did the control group. Those fed diets containing 0.08 and 0.32 g PP kg⁻¹ exhibited better FCR than those fed the MT diet. Survival ranged from 68% to 79% and were statistically similar in all the dietary treatments ($p > 0.05$). In conclusion, PP could replace the MT in effecting sex reversal from female to male with statistically similar results; it could also promote better growth and feed conversion efficiency at the minimum dietary level of 0.08 g PP kg⁻¹.

Table 2. Percentage of male and female Nile tilapia, *Oreochromis niloticus* at the end of 56 days feeding trial.

(g kg ⁻¹ PP)	Final % Male	Final % Female
0.00	50.0 ± 12.0 ^a	50.0 ± 12.0 ^a
0.08	81.0 ± 7.0 ^a	19.0 ± 7.0 ^b
0.16	88.0 ± 7.0 ^a	12.0 ± 7.0 ^b
0.32	89.0 ± 4.0 ^a	11.0 ± 4.0 ^b
0.64	85.0 ± 11.0 ^a	15 ± 11.0 ^b
*MT	100.0 ^a	0.0 ^b

Means in the same column sharing the same letters in superscript are not significantly different ($p < 0.05$).

SEM= standard error of the mean; Final % Male and Final % Female

*MT = 17 alpha-methyltestosterone (0.06g kg⁻¹)

Table 3. Growth performance, feed efficiency, survival and sex reversal efficiency of the Nile tilapia *Oreochromis niloticus* fed diets containing various dosage of pine pollen (PP) with a negative control (containing no PP) and positive control (methyltestosterone, MT) diets. Values given are Mean ± SEM; n=3.

(g kg ⁻¹ PP)	IW (g)	FW (g)	WG (g)	FI (g)	SGR (% day ⁻¹)	FCR	SURV
0.00	0.01	4.0 ± 0.2 ^a	3.9 ± 0.2 ^a	4.1 ± 0.1 ^a	4.3 ± 0.1 ^{ab}	1.0 ± 0.0 ^a	76.0 ± 4.0 ^a
0.08	0.01	5.9 ± 1.0 ^a	5.8 ± 1.0 ^a	6.0 ± 1.1 ^{ab}	4.6 ± 0.1 ^a	1.0 ± 0.0 ^a	77.3 ± 11.7 ^a
0.16	0.01	4.7 ± 0.5 ^{ab}	4.7 ± 0.5 ^{ab}	5.1 ± 0.5 ^{ab}	4.4 ± 0.1 ^{ab}	1.1 ± 0.0 ^{ab}	76.0 ± 3.0 ^a
0.32	0.01	4.8 ± 0.2 ^{ab}	4.7 ± 0.2 ^{ab}	4.6 ± 0.2 ^a	4.5 ± 0.1 ^{ab}	1.0 ± 0.0 ^a	78.7 ± 7.4 ^a
0.64	0.01	5.9 ± 0.4 ^a	5.8 ± 0.4 ^a	6.9 ± 1.0 ^a	4.5 ± 0.1 ^{ab}	1.2 ± 0.1 ^{ab}	69.3 ± 0.4 ^a
MT*	0.01	4.0 ± 0.2 ^a	4.0 ± 0.2 ^a	5.0 ± 0.2 ^{ab}	4.2 ± 0.1 ^a	1.3 ± 0.0 ^a	68.0 ± 3 ^a

Means in the same column sharing the same letters in superscript are not significantly different ($P < 0.05$)

SEM= standard error of the mean; IW=initial weight; FW=final weight; WG = weight gain; FI = feed intake; SGR = specific growth rate; FCR = feed conversion ratio; SURV = survival rate.

*MT = 17 alpha-methyltestosterone (0.6 g kg⁻¹)

INFLUENCE OF MANCHURIAN RED PINE *Pinus tabulaeformis* POLLEN ON DIET ATTRACTABILITY, SEX REVERSAL, GROWTH AND DIGESTIVE ENZYME ACTIVITY OF NILE TILAPIA *Oreochromis niloticus* LARVAE

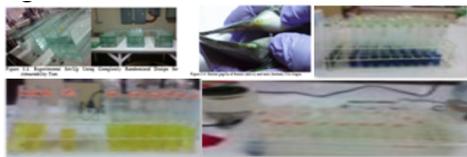
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Three experiments were carried out to investigate the effects of pollen from *Pinus tabulaeformis* (PP) as possible replacement for the synthetic 17-alpha-methyltestosterone (MT) in the Nile tilapia, *Oreochromis niloticus* larvae. In Experiment 1, attractability of PP (640mg kg⁻¹) against a control diet containing no PP was assessed. Experiment 2 evaluated the effects of PP on growth, feed conversion efficiency, survival and sex inversion as compared with that of the 17a-methyltestosterone (MT). Larvae were fed 5 diets containing graded levels of PP, namely, 0 (negative control), 80, 160, 320 and 640 mg kg⁻¹ and a positive control diet containing MT. The feeding trial lasted for 8 weeks. In Experiment 3, fish larvae were fed a control diet (no PP), a medium dose (0.32 g kg⁻¹) and a high dose (0.64 g kg⁻¹) of PP for 14 days and assayed for alpha amylase and trypsin activities.

Results showed that the diet containing PP significantly attracted the fish than did the control diet ($p < 0.05$) for the first 4 min. On the 5th to 6th min, the percentage of fish became statistically similar for both diets. Results of Experiment 2 showed that after 56 days of feeding, the MT diet resulted in all the Nile tilapia converted to male (100%) but was statistically similar with those of the PP diets (81.0% to 89.0% male, $p < 0.05$). Almost half of the fish fed the control diet were male (50.5%) and almost half female (49.5%). Results of the growth trial showed that the final weight (FW) and weight gain (WG) of fish fed diets containing 0.08 g kg⁻¹ PP and 0.32 g PP kg⁻¹ were significantly higher than of those fed the MT diet ($p < 0.05$). Feed intake (FI) of all experimental fish were statistically similar ($p > 0.05$). The group fed 0.08 g PP kg⁻¹ diet exhibited significantly higher SGR than did the MT group. The MT group exhibited lower feed conversion efficiency (i.e. higher FCR) than did the control group. Those fed diets containing 0.08 and 0.32 g PP kg⁻¹ exhibited better FCR than those fed the MT diet. Survival ranged from 68% to 79% and were statistically similar in all the dietary treatments ($p > 0.05$). After 14 days feeding trial for digestive enzyme activity, larvae has significantly similar activity of amylase in all diets but significantly higher trypsin activity on larvae fed with PP diets than the control diet. In conclusion, PP could replace the MT in effecting sex reversal from female to male with statistically similar results; it could also promote better growth and feed conversion efficiency at the minimum dietary level of 0.08 g PP kg⁻¹.



THE POTENTIAL USE OF TUBER YAM WITH PROBIOTIC FOR GONAD DEVELOPMENT OF TIGER GROUPE

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The Tuber Yam or Ubi Gadong (UG) with dietary probiotics, on the growth performances, intestinal microbiota and reproductive hormones of tiger grouper (*Epinephelus fuscoguttatus*) were assessed after 60 days feeding on supplemented diets. Molecular analysis using PCR-DGGE (denaturing gradient gel electrophoresis) approach revealed that the probiotic was able to populate the gastrointestinal tract and modulate the microbial communities. After exposition, females fed on with the supplemented diet with a isolated probiotic from digestive tract of tiger grouper presented an over-expression on gonad maturation comparing to the control group. It showed, gonad weight and Gonad somatic index (GSI) bigger than control. Histology studies on digestive tract were found significantly difference bigger on UG+probiotic rather than other control group treatment. This suggest that probiotics with UG has a potential use as probiotics supplementation in tiger grouper maturation diet for improvement of gonad development, indicating that probiotic supplementation with UG could affect female performance reproductive.

PROBIOTICS BACTERIA AS QUORUM SENSING DEGRADER IN CONTROLLING DISEASES OF CULTURED FRESHWATER FISH

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Quorum sensing is a bacterial cell-to-cell communication process that depends on their population density to produce, release, detect and respond to the small signal molecules known as autoinducers. N-acyl homoserine lactone (AHL) is widely studied of QS signals and present in many Gram-negative pathogenic bacteria which it can regulate the gene expression for pathogenicity. Interfering with quorum sensing has been suggested as anti-infective strategy against bacterial infections. In aquaculture, inappropriate farm management system can lead to the occurrence of diseases that can cause a massive loss of fish.

In this study, the potential quorum sensing degrading probiotic bacteria were isolated from cultured fish. Five isolates identified as *Bacillus oryzaecorticis* (CPi7), *Klebsiella* sp. (CPi12), *Klebsiella* sp. (CPi14), *Enterobacter* sp. (CBa5), and *Enterobacter* sp. (CBa7) were found to have strong ability in degrading AHL by *in vitro* assay. These bacteria were further studied by co-culture the isolates with pathogens at 0H, 8H, 24H and 32H. AHL inactivation assay also was done during this time interval. The results showed that the isolates could reduce the AHL production in pathogen (*Aeromonas hydrophila*) but did not affect the growth of pathogen.

These findings showed that the QS degrading isolates might be the potential QS degrading probiotic bacteria that could control the diseases against culture fishes.

MOLECULAR CLONING AND CHARACTERIZATION OF ORANGE-SPOTTED GROUPER (*Epinephelus coiodes*) TYPE-II INTERFERONS

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Grouper aquaculture is an important economic sector in Taiwan however due to large intensified aquaculture practices, the disease outbreak such as NNV can prove great disastrous leading to huge economic losses. In order to increase the grouper survival rate upon infection by deadly viruses, the study of anti-viral cytokines (Interferon system) that secreted by grouper are essential. Type-II interferons also known as $\text{IFN}\gamma$ are the key mediator for both innate immunity and adaptive immunity and have the ability to regulate the activation of macrophages leading to enhancing of the resistance toward intracellular pathogens. Previous studies have identified a second $\text{IFN}\gamma$ family member known as Interferon γ -related genes ($\text{IFN}\gamma\text{rel}$), in teleost fish as compare to their mammalian counterpart which consists of only 1 $\text{IFN}\gamma$ family member. Teleost fish $\text{IFN}\gamma$ are widely studied however the functionality differences of $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$ are still unclear.

To investigate the functionality differences of $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$, the full length of $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$ are first identified and cloned. Relative expression of $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$ in healthy orange-spotted grouper multiple tissues shows that $\text{IFN}\gamma\text{rel}$ have a surprising high expression in eye while $\text{IFN}\gamma$ shows relative high expression in gill, eye and fins. By using multiple immunostimulants to investigate their effect on $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$, found that upon viral mimic Poly I:C, $\text{IFN}\gamma\text{rel}$ shown no significant response while $\text{IFN}\gamma$ is highly stimulated. $\text{IFN}\gamma$ shown little response when treated with bacteria mimic LPS while $\text{IFN}\gamma\text{rel}$ shown significant upregulation. Both $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$ show different upregulation response upon T-lymphocyte activator PHA. This indicating that $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$ are released by T-lymphocyte and $\text{IFN}\gamma\text{rel}$ having more anti-bacteria response while $\text{IFN}\gamma$ having more anti-viral response.

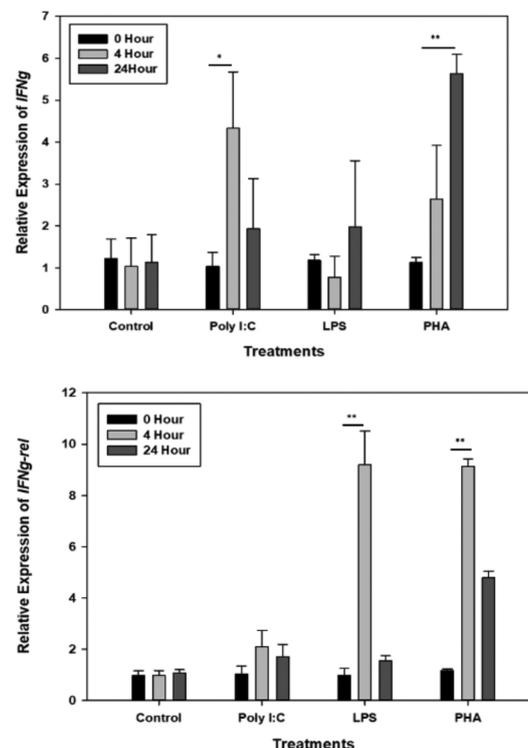


FIGURE 1: Immune stimulant response by $\text{IFN}\gamma$ and $\text{IFN}\gamma\text{rel}$ using Beta-actin as internal control.

STUDY OF THE ANTIMICROBIAL ACTIVITY OF TILAPIA PISCIDIN 3 (TP3) AND TP4 AND THEIR EFFECTS ON IMMUNE FUNCTIONS IN HYBRID TILAPIA (*Oreochromis Spp.*)

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In this study, we focused on tilapia piscidin 3 (TP3) and TP4, which are peptides derived from *Oreochromis niloticus*, and investigated their inhibition of acute bacterial infections by infecting hybrid tilapia (*Oreochromis spp.*) with *Vibrio vulnificus* and evaluating the protective effects of pre-treating, co-treating, and post-treating fish with TP3 and TP4. *In vivo* experiments showed that co-treatment with *V. vulnificus* and TP3 (20µg/fish) or TP4 (20µg/fish) achieved 95.3% and 88.9% survival rates, respectively, after seven days. When we co-injected TP3 or TP4 and *V. vulnificus* into tilapia and then rechallenged the fish with *V. vulnificus* after 28 days, the tilapia exhibited survival rates of 35.6% and 42.2%, respectively. Pre-treatment with TP3 (30µg/fish) or TP4 (20µg/fish) for 30 minutes prior to *V. vulnificus* infection resulted in high survival rates of 28.9% and 37.8%, respectively, while post-treatment with TP3 (20µg/fish or 30µg/fish) or TP4 (20µg/fish) 30 minutes after *V. vulnificus* infection yielded high survival rates of 33.3% and 48.9%. Treating fish with TP3 and TP4 under different pH and temperature conditions did not significantly increase MIC values, suggesting that temperature and the acid-base environment do not affect AMP function. In addition, the qPCR results showed that TP3 and TP4 influence the expression of immune-responsive genes, including interleukin (IL)-1 β , IL-6, and IL-8. In this study, we demonstrate that TP3 and TP4 show potential for development as drugs to combat fish bacterial infections in aquaculture.

All bacterial strains were completely eradicated by TP3 and TP4 after 60 minutes of exposure at 2 \times MIC. The SEM and TEM (Fig1) results clearly showed differences in the morphologies of *V.vulnificus* that were untreated and that were treated with TP3 or TP4. The red arrow indicates that the TP3-treated *V.vulnificus* was reduced in size, and the blue arrowhead in the TP4 panel highlights the breaks in the plasma membrane exhibited by the *V.vulnificus* cells, which were also distorted.

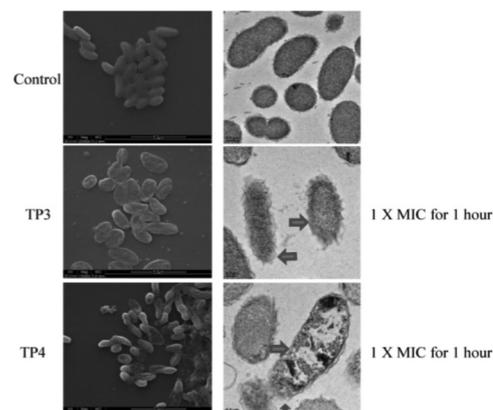


Fig1. TP3 and TP4 induce membrane permeation and cause membrane disruption in *V. vulnificus*.

DEVELOPING A NATURAL ANTIMICROBIAL SOLUTION TO EXTEND THE CHILLED STORAGE LIFE OF COOKED PRAWNS

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Background

- > The Australian domestic farmed prawn market is dominated by cooked black tiger prawns (*Penaeus monodon*) retailed in both chilled and frozen product formats
- > Chilled prawns are held at 2-4°C and have a maximum storage life of 8-10 days
- > Microbial growth is the primary cause of deterioration and quality loss. Immediately post-cooking, bacterial loads are <1,000 micro-organisms/g. This progressively increases through storage with specific spoilage organisms ultimately dominating
- > Australia has geographically long supply chains to markets and the local industry is being challenged to supply high quality prawn product with a storage life of at least 14 days
- > The study presented details work undertaken to address this challenge by developing and incorporating a natural antimicrobial solution into commercial production lines in order to reduce the initial bacterial load and retard subsequent growth

Method

- > Isolation and identification of organisms from various farmed prawn samples at point of spoilage using traditional plate enumeration and DNA sequencing
- > *In vitro* screening of isolated organisms against commercially available and customised natural food grade antimicrobials using 96-well microtitre plates
- > Optimisation of concentrations and formulations through repetitive microtitre plate screenings and pilot scale trials
- > On-farm integration into commercial production operations by incorporating optimised solution into the overnight brining step of the prawn processing

Image 1. Standard commercial production process for chilled cooked prawns



Conclusion

- > Australia's domestic prawn industry is being challenged to maintain the quality of cooked prawns through 14 days of chilled storage
- > In addressing this challenge, specific information that impacts on the storage life of chilled prawns through the supply chain was required
- > Initial microbial flora present on farmed prawns differed between farms, geographical locations and seasons. However, chilled storage caused microbial population shifts resulting in *Pseudomonas*, *Pseudoalteromonas* and *Psychrobacter* species dominating irrespective of the initial bacterial species profile
- > A natural antimicrobial solution was formulated which targeted the identified spoilage organisms and efficacy was demonstrated within commercial prawn farming operations
- > Cooked prawns treated with the natural solution showed reduced microbial growth and retained excellent sensorial quality for longer than 14 days chilled storage

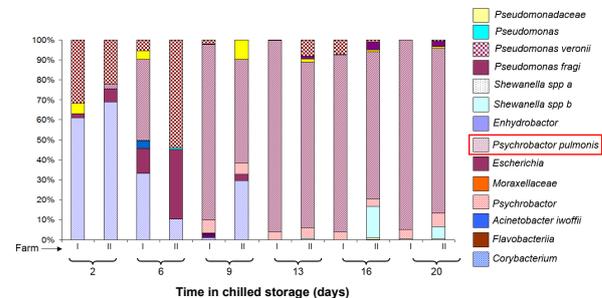
Results

Table 1. Identification of spoilage bacteria genera through plate enumeration

Prawn Farm	Gram-negative bacteria *				Gram-positive bacteria *	
	<i>Pseudoalteromonas</i>	<i>Pseudomonas</i>	<i>Psychrobacter</i>	<i>Shewanella</i>	<i>Camobacterium</i>	<i>Exiguobacterium</i>
1 a	70		25	5		
1 b	75		20	5		
2 a	90		10			
2 b	5		90			5
3	5		75		10	10
4	85	5			10	
5 a	15	30	20	35		
5 b	80		20			
6 a	80			10	10	
6 b	75			15	10	

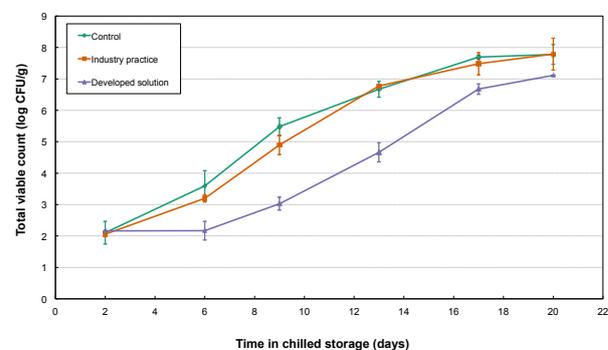
* Data presented as a percentage (%) of total bacteria present for each sampling location

Figure 1. DNA sequencing to monitor changes in microbial populations



* *Psychrobacter pulmonis* (highlighted in red) was identified as the dominant species at point of spoilage within these two farms

Figure 2. Microbial growth profile during commercial chilled storage trials



CARBOXYPEPTIDASE 1 FROM RED LIP MULLET *Liza haematocheilus*; CHARACTERIZATION AND IMMUNE RESPONSES AGAINST *Lactococcus garvieae*

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Carboxypeptidase 1 (CPN1) also known as arginine carboxypeptidase, kininase I, anaphylatoxin inactivator; is a plasma zinc metalloprotease consists with two small subunits and two large subunits. These enzymatically active subunits may protect the protein from degradation by complement system. Initially, we have characterized Mullet CPN1 using bioinformatics tools. The tissue specific mRNA expression was then analyzed using healthy mullet. An immune challenge was designed in order to determine the immune related responses of MuCPN1 against selected immune stimuli. The cDNA sequence of MuCPN1 possessed a 5' un-translated region (UTR) of 133 bp, an open reading frame (ORF) of 1347 bp and a 3' UTR of 312 bp. two domains including, PM14-Zn carboxypeptidase and C-terminal domain of M14 N/E carboxypeptidase in the MuCPN1 sequence. Moreover, two zinc-binding signature motifs were found in the MuCPN1 sequence. Zinc binding sites (H⁹¹, E⁹⁴ and H²²¹) and the other active sites (H⁹¹, E⁹⁴, R¹⁵⁶, N¹⁶¹, R¹⁶², H²²¹, G²²², N²²⁸, D²³², G²⁸⁵, Y²⁸⁹, L²⁹¹, and E³¹¹) were located among the domains and the signatures of the MuCPN1. 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 expression of MuCPN1 was observed in the spleen tissue. Highly modulated expression profiles were observed in the immune challenge analysis by MuCPN1. MuCPN1 started to up-regulate at 24 h p.i. against poly I:C in both spleen and head kidney tissues. Additionally, MuCPN1 started to up-regulate at 48 h p.i. against *Lactococcus garvieae* in both spleen and head kidney tissues. Collectively, *in silico* analysis revealed that MuCPN1 sequence is well conserved among the homologs and it shared relatively higher homology with teleosts. Additionally, the modulation of the transcription patterns of MuCPN1 against immune stimuli suggested that MuCPN1 involve with the immune responses in red lip mullet.

INNATE IMMUNE RESPONSES OF AN INFLAMMATORY CYTOKINE; INSIGHTS IN TO MOLECLULAR, STRUCTURAL AND FUNCTIONAL ASPECTS FROM ROCKFISH (*Sebastes schligelli*)

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Macrophage migration inhibitory factor (MMIF) is an important inflammatory cytokine responsible for the innate immune responses upon pathogenic stimuli. MMIF is known to regulate the macrophage function and involved in cell-mediated immunity. In the current study MMIF sequence was identified from rockfish (RfMMIF) data base and fully characterized with bioinformatics tools. The tissue specific mRNA expression and the temporal transcription pattern upon bacterial stimuli were observed *via* qPCR. Additionally, cell stimulation assay and cytokine transcripts analysis were accompanied with the recombinant RfMMIF (rRfMMIF). Moreover, oxidoreductase activity of rRfMMIF was observed through the insulin reduction assay. Putative open reading frame of RfMMIF encoded 115 amino acids (aa) with 12 kDa polypeptide and a pi of 7.4. The results of pairwise alignments discovered that 93 % of a highest identity was shared with *Oplegnathus fasciatus* MMIF sequence. Phylogenetic results were verified that RfMMIF has a close evolutionary relation with *O. fasciatus* homolog. According to the spatial distribution liver tissue exhibited the highest expressional level and followed by kidney. Significant up regulations were observed in the *RfMMIF* upon bacterial stimuli. It was spotted that cell stimulation with the rRfMMIF leads extensive inductions of other cytokines including IL 8, IL β and TNF-receptor. Time dependent, increase in turbidity in the insulin reductase assay showed nil activity at pH 7 where high activity was observed at pH 8 in the existence of rRfMMIF. *In silico* and homology analysis discovered that RfMMIF sequence is well conserved among the homologs and it shared relatively higher homology with teleosts than non teleosts. Variation of the transcription pattern upon the bacterial stimuli suggested that RfMMIF involve with the immune responses in rockfish. Enzymatic redox activity of rRfMMIF evidenced the thiol-protein oxidoreductase activity of rRfMMIF.

IMMUNE RESPONSES AND MOLECULAR CHARACTERISTICS OF B CELL LINKER PROTEIN FROM *Liza haematocheilus*

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The B cell linker protein (BLNK) encodes an adaptor or linker protein that shows a critical role in B cell development. Activation of ERK/EPHB2, MAP kinase p38, JNK and modulation of AP1 are the major functionalities of BLNK protein. Moreover, it is crucial gene in the activation of NF κ B and NFAT. Here we characterized the mullet B cell linker protein (MuBLNK) in terms of, bioinformatics, spatial mRNA expression, and temporal mRNA regulations. The open reading frame of MuBLNK consisted of 1506 bp encoded for 502 aa. Its molecular mass was 57 kDa and a theoretical isoelectric point was 7.9 pI. The 5' un-translated region was 279 bp where 3' un-translated region extended 752 bp. BLNK specific SH2 Src homology 2 (SH2) domain and SAM domain were identified in MuBLNK sequence. Two N-linked glycosylation sites were observed. MuBLNK shared higher sequence identity (76%) and similarity (85.5%) with *Stegastes partitus* BLNK ortholog. Evolutionary analysis showed that the MuBLNK was originated from common ancestor where cladded within the fish BLNK clade. Since the *BLNK* is directly involved in B cell development, the *MuBLNK* transcripts were significantly high in healthy mullet spleen, head kidney and blood. Polyinosinic:polycytidylic acid (poly I:C) is a well-known viral mimic used in immune regulation studies. Poly I:C injected mullets showed significant up-regulation of *MuBLNK* transcripts in spleen and head kidney at 6 h post injection. Moreover, *Lactococcus garvieae* is a well-known Gram positive bacterial pathogen to mullet, where its infection increases the *MuBLNK* transcription in spleen after 48 h post injection. Therefore, present results suggested that MuBLNK is a member of teleostean BLNK and important molecule in B cell development and stimulation of adaptive immune response against the pathogen infection.

DIETARY PROTEASE SIGNIFICANTLY IMPROVES FEED CONVERSION IN PACIFIC WHITE SHRIMP FED DIETS WITH GRADED LEVEL OF FISH MEAL

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An eight-week trial was conducted at the Prince of Songkla University of Thailand to assess the effects of a dietary protease complex on growth performance of Pacific white shrimp *Litopenaeus vannamei*. The trial consisted of six dietary treatments with three levels of fish meal (18%, 10% and 0%) with or without the supplementation of the protease complex.

There were significant effects of fish meal level on final body weight, weight gain and specific growth rates among the treatment. Despite no significant effects, dietary protease supplementation numerically improved the growth performance including the survival. However, dietary protease significantly reduced feed conversion at all fish meal levels.

Table: Growth performance of Pacific white shrimp fed the treatment diets

Fishmeal level	Dietary protease	Average body weight (g/shrimp)	Weight gain (WG) (%)	Specific growth rate (SGR) (%/ day)	FCR	Survival rate (%)
FM 18 %	-protease	12.0±0.79bx	415.2±34.7bx	3.03±0.13bx	1.53±0.10ay	73.3±2.7
	+protease	12.1±0.71bx	422.3±30.4bx	3.06±0.11bx	1.47±0.10ax	80.0±9.8
FM 10%	-protease	11.5±0.26bx	393.4±12.7bx	2.96±0.05bx	1.58±0.07ay	77.5±3.2
	+protease	11.8±0.63bx	409.0±27.4bx	3.01±0.01bx	1.48±0.12ax	83.3±6.1
FM 0%	-protease	10.2±0.32ax	339.9±12.8ax	2.74±0.06ax	1.61±0.04ay	82.5±1.7
	+protease	10.±0.50ax	348.3±23.3x	2.78±0.10ax	1.52±0.10ax	83.3±4.7
FM (a,b,c)		P<0.05	P<0.05	P<0.05	0.329	0.094
Enzyme (x,y,z)		0.341	0.321	0.321	P<0.05	0.060
FM × Enzyme		0.954	0.935	0.970	0.896	0.520

DISSECTING THE INFECTION MECHANISMS OF SINGAPORE IRIDOVIRUS BY SINGLE MOLECULAR DETECTION TECHNOLOGY

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Abstract: Iridoviruses are large DNA viruses which cause great economic losses in the aquaculture industry but also show significant threat to global aquatic animal biodiversity. Singapore grouper iridovirus (SGIV), the new species of genus *Ranavirus*, family *Iridoviridae*, is one of the most important viral pathogens in marine cultured fish. For the first time, the single molecule detection technology is applied in exploring the infection mechanisms of SGIV at spatial and temporal scale. The results contribute to the further study of SGIV infection mechanism, which provides an ideal model for exploring the behavior of DNA viruses in living cells, and also improve our understanding of the infection mechanism of large DNA virus. Using single particle tracking technology, we tracked the detailed process of individual SGIV particles in real time, and observed that SGIV could travel along protrusions and entered into the cells. Furthermore, using endocytic inhibitors, single particle imaging technology, immunofluorescence and western blot analysis, we found that SGIV entered host cells via the clathrin-mediated endocytic pathway but not via caveola-dependent endocytosis, and proposed for the first time that macropinocytosis was involved in iridovirus entry. In addition, by tracking the transport of intracellular virus particles, we showed the important role of actin and microtubules of SGIV. Based on atomic force microscopy (AFM), a novel Force Tracing technique was developed, and was firstly applied in study of virus infection. Conjugated with SGIV particles, the AFM tip is slowly directed toward the surface of host cell to contact the cell. Recording the deflection of AFM could directly reflect the interaction between single virus and host cell. Using the Force Tracing technique in constant force mode or in constant position mode, we found that the maximum velocity of single virus internalization in live cell membranes is about 200 nm/s; when single SGIV particle enter into host cell, the endocytosis force is about 60.8 ± 18.5 pN; the binding energy density increases with the engulfment depth.

ANTIMICROBIAL ACTIVITY OF MALE AND FEMALE SNAKEHEAD (*Channa sp.*) MUCUS AND ITS ASSOCIATED MICROBIOTA AGAINST SELECTED PATHOGENIC BACTERIA OF FRESHWATER FISHES

Marz Linnaeous L. Rabadon*, Karl Marx A. Quiazon, and Casiano H. Choresca

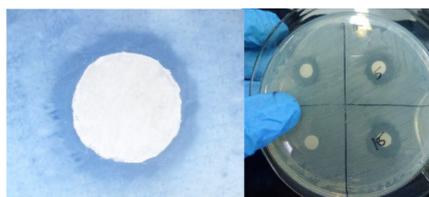
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This study focused on the *in vitro* antimicrobial activity of the skin mucus (study 1) and the associated mucus microbiota (study 2) of eight snakehead fish against freshwater pathogens as a novel approach for the repertoire of studying the mucosal immune system. Initially for study 1, the total protein content and important immune-related enzymes (i.e., lysozyme and peroxidase) of the crude mucus extracts (CMEs) were measured in respect to the sex of fish. The protein concentration of female CMEs was observed to be significantly higher ($p < 0.01$) than the male CMEs with mean values of 0.41 ± 0.18 mg mL⁻¹ and 0.37 ± 0.16 mg mL⁻¹, respectively. Significantly higher ($p < 0.01$) enzymatic activities were also observed on female CMEs compared to male CMEs with mean values of 46.25 ± 14.97 U μ L⁻¹ and 21.75 ± 11.67 U μ L⁻¹, respectively for lysozyme activity, and 1.70 ± 0.85 nmole min⁻¹ mL and 1.18 ± 0.90 nmole min⁻¹ mL, respectively for peroxidase activity. Thereafter, the antimicrobial activity of the CMEs was tested through disc-diffusion method against six freshwater fish pathogens viz., *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Micrococcus luteus*, *Enterococcus faecalis*, *Aspergillus flavus*, and *Aspergillus niger*. The antimicrobial assay against all of the six test pathogens resulted to inhibitions that were primarily exhibited by female CMEs. The sequence for the magnitude of inhibition manifested by female CMEs against the test pathogens was *E. faecalis* (13.33 ± 1.15 mm) > *M. luteus* (12.00 ± 0.00 mm) > *A. hydrophila* (11.33 ± 1.15 mm) > *P. fluorescens* (10.00 ± 0.00 mm) > *A. flavus* (10.00 ± 0.00 mm) > *A. niger* (6.67 ± 0.58 mm). On the other hand, male CMEs exhibited significantly lower ($p < 0.01$) zone of inhibitions compared to female CMEs. The sequence for the magnitude of inhibition manifested by male CMEs against the test pathogens was *E. faecalis* (12.33 ± 1.53 mm) > *A. hydrophila* (10.00 ± 0.00 mm) > *A. flavus* (10.00 ± 0.00 mm) > *M. luteus* (9.67 ± 0.58 mm).

For study 2, the resident mucus bacteria were isolated from fresh mucus and were purified thrice. The mucus bacterial isolates (MBIs) were classified through Gram classification and protease production and was further identified through 16S rRNA gene sequencing. The 16 MBIs were classified and identified to be under the genus *Bacillus*, *Acinetobacter*, *Lysinibacillus*, *Aeromonas* and *Staphylococcus*. According to their phylogeny, some MBIs were found to be closely related species. On the other hand, the antimicrobial activity of the MBIs against the six test pathogens resulted to positive inhibitions as exhibited by three *Bacillus* spp. isolates (i.e., M1_1, M2_1, and M2_2).

The protein concentrations and enzymatic activities were positively correlated with the antimicrobial activities of CMEs. Moreover, the more antagonistic female CMEs may be a potential source of antimicrobial agents. Also, some MBIs exhibited antimicrobial activities suggesting their possible antagonistic roles in the mucus. Thus, further studies for the utilization of the mucus and its microbiota may lead to novel immunotherapies.

Figure 1. Microscopic and plate view for the zones of inhibition



DNA BARCODING OF HIGH VALUED AQUATIC SPECIES IN COASTAL WATERS OF AURORA PROVINCE, PHILIPPINES

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This study was conducted to infer a phylogenetic tree by analyzing the mitochondrial DNA of high-valued species in Aurora Province.

In this study, a total of 121 high-valued species from the coastal waters of Aurora Province were barcoded using the four primer sets of CO1 gene described by Wardet al. (2005), Ivanova et al. (2007) and Folmer et al. (1994). Divergence of sequences within and between species was determined using Kimura 2-parameter distance model, and a neighbor-joining tree was accomplished using 1000 pseudo replications to increase the validity of results.

A total of 121 cytochrome *c* oxidase subunit 1 sequences were generated representing 12 orders, 38 families, 62 genera and 89 species of high valued fishes, crustaceans and molluscs collected from the coastal waters of Aurora province. The amplified sequences ranged from 621 to 710 base pairs with an average of 666 base pairs. Mean genetic distances within species, within genus, and within family was 1.69%, 13.3% and 17.9%, respectively. Phylogenetic trees have been successfully constructed using Neighbor-Joining method with Kimura-2 parameter as substitution model. DNA barcoding using the CO1 region has successfully identified the samples down to the species level with 99% success rate. It was also able to reveal possible cryptic speciation in one *Cheilinus trilobatus* and two *Nemipterus japonicas* samples with mean intraspecific distance of 2.2% and 9.2%, respectively.

Results showed that CO1 DNA barcodes are effective for rapid and accurate identification of fishes and for identifying species that need further taxonomic evaluation.

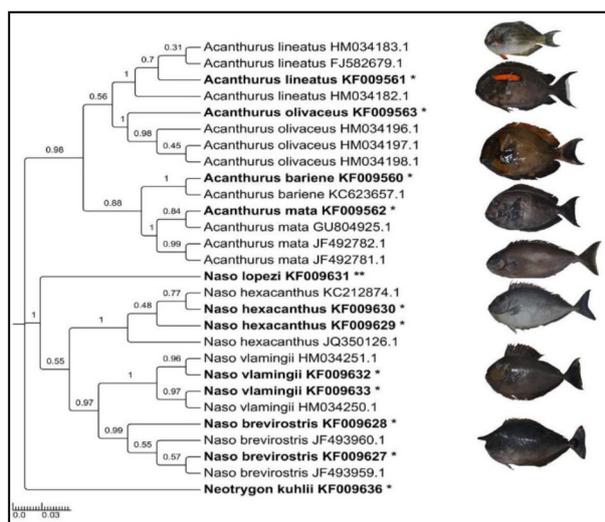


Figure 1. Rooted K2-P distance NJ tree of the family Acanthuridae

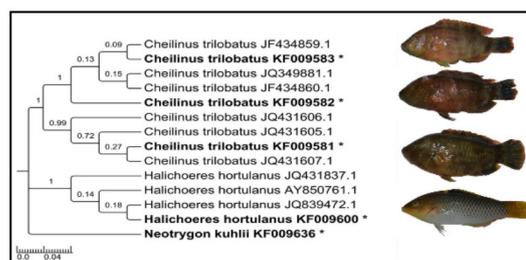


Figure 2. Rooted K2-P distance NJ tree of the family Labridae

ENERGY MOBILIZATION AND INTESTINAL MORPHOLOGY OF STARVED JAVA BARB *Barbonymus gonionotus* JUVENILES

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Starvation in fish can occur during migration, breeding season, disease infection, transportation or stressed condition. During starvation, fish metabolize their endogenous reserves in the form of protein, lipid and glycogen to meet critical energy requirements to survive. Prolonged starvation may affect fish physiological and metabolic processes, including the degeneration of vital organs such as the intestine, hence affecting digestive efficiency. It is of great importance to understand the state of recovery after a period of starvation to ensure the well-being of fish. Therefore, this study investigates the energy mobilization and intestinal morphology of starved Java barb juveniles. Fish were subjected to 3 feeding regimes; fed, fed-starved-refed and starved. Fed fish received continuous feed for 21 days. Fed-starved-refed fish were fed for one week, then starved for one week and refed for another week. Starved fish were not provided with feed for 21 days. Fish muscle and liver was sampled to determine protein, lipid, glycogen and energy content for all treatments. Fish intestine were sampled for histological analysis to determine the intestinal height and number of goblet cell. Results from this study revealed that fish utilized lipid as main energy with food availability and retained protein and glycogen. Glycogen acts as main energy while reserves protein and lipid during starvation. After refeeding, protein serves as main energy and store lipid and glycogen. Intestinal height and number of goblet cells decreased with prolonged starvation and did not show full recovery after a short refeeding phase of 7 days. In conclusion, starvation affects energy mobilization and deteriorates intestinal morphology of Java barb juveniles.

MANIPULATION OF DIFFERENT TANK COLOR FOR GROWTH OF KURUMOI RAINBOW FISH (*Melanotaenia parva*) LARVAE

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Rainbow kurumoi (*Melanotaenia parva*) is one of Indonesian endemic fish from kurumoi lake, Papua. This fish is one of the potential commodities. This study aims to find out the suitable tank color for optimizing the growth of kurumoi rainbow larvae. The treatments in this study were: A. red tank color; B. blue tank color; and C. green tank colors. The larvae were reared in a 4 L volume tank with a density of 20 indiv/L for 28 days. Larvae were feed of infusoria, rotifer, artemia and moina gradually with ad libitum method. The results showed that the larvae given the different color treatment of the tank were not significantly ($P > 0.05$) to growth and survival rate. Treatment A (red tank color) showed the highest specific growth rate of length of $2.2 \pm 0.1\%$ / day, followed by treatment B ($2.1 \pm 0.7\%$ / day) and last C ($1.8 \pm 0.7\%$ / day). For a specific weight growth rate at treatment A of $6.9 \pm 1.2\%$ / day, followed by C treatment ($6.4 \pm 0.3\%$ / day) and treatment B ($5.5 \pm 1.6\%$ / day).

Table 1. Specific Growth Rate of Kurumoi Rainbow Fish (*Melanotaenia parva*) Larvae During Treatment

Parameter	Treatment		
	A	B	C
Initial Length (cm)	4.4	4.4	4.4
Final Length (cm)	8.57	8.60	8.04
Specific Growth Rate of Length (%/day)	2.2 ± 0.1^a	2.1 ± 0.7^a	1.8 ± 0.7^a
Initial weight (g)	0.003	0.003	0.003
Final weight (g)	0.030	0.023	0.023
Specific growth rate of weight (%/day)	6.9 ± 1.2^a	5.5 ± 1.6^a	6.4 ± 0.2^a

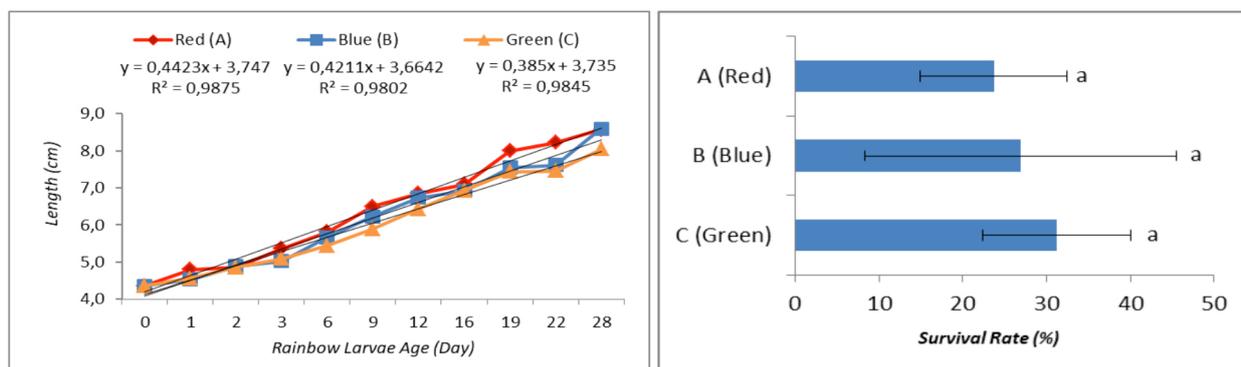


Figure 1. Growth and Survival Rate of Kurumoi Rainbow Fish (*Melanotaenia parva*)

NOT THE SAME SHRIMP: SMALL FARMERS' EXPERIENCE WITH SALTWATER SHRIMP PRODUCTION IN THAILAND AND BANGLADESH

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Amidst the decline of natural fisheries, many states in Asia have embraced the export of farmed shrimp as a development strategy. Whereas proponents of export-driven shrimp aquaculture celebrate foreign markets for shrimp as creating enhanced earning opportunities for former rice farmers, opponents decry farmed shrimp production as leading to the salinization of paddyland and the devastation of the peasantry. Yet commentators from both perspectives tend to overlook how variation in approach to aquaculture development leads to differential outcomes as is apparent in the contrast of the Thailand and Bangladesh cases. I argue that to understand Thai villagers' generally higher levels of satisfaction with shrimp farming vis-à-vis Bangladeshis, it is necessary to analyze how the factors of shrimp production – especially, land and seed – have been mobilized in each national context since in the 1980s. My argument draws on fieldwork conducted in Thailand (Nakorn Sri Thammarat) and Bangladesh (Mongla) in 2016 & 2017, interviews with farmers and stakeholders, and analysis of primary and secondary sources.

In Bangladesh, the state initially failed to safeguard the land tenure rights of smallholders, and influential persons grabbed large swaths of paddyland to cultivate Black Tiger Shrimp extensively. Although informants in my Mongla research village were initially compensated, the payments stopped; for a decade they derived no benefit from the culture. After protracted political struggle, the former paddyland was returned to its owners in 2008. Yet, by this time, villagers reported that production levels were on the decline. In 2016, farmers explained that only those with large tracts of land were able to realize a profit from their extensive cultures. Small-scale farmers described their shrimp as succumbing to “virus” – in part, a consequence of non-optimal seed and production technique, and expressed a desire to return to rice cultivation.

In Thailand, the land tenure rights of smallholders was largely safeguarded. Unlike in Bangladesh, domestic agribusiness firms invested in the production of feed and seed. Small farmers converted their rice fields to intensive cultures. When the continued viability of the industry was threatened by a disease epidemic in Black Tiger Shrimp broodstock, harvested at sea and bred in inland hatcheries, Thai agribusiness imported the domesticated Pacific White Shrimp from Hawaii which was widely adopted. Today, many former Thai rice farmers – even those whose own shrimp culture failed – regard the transition from rice to shrimp as positive, citing the money and employment it has brought to their community. Nevertheless, the continued involvement of small farmers in the industry is contingent upon access to high-quality seed (difficult because of agribusiness sales policy) and adjustment to the post AHPND environment (difficult because of land constraints preventing enhanced water treatment). Drawing from these cases, this research proposes how aquaculture can better fulfill its potential to alleviate poverty and contribute to development.

DIFFERENTIAL PHARMACOKINETICS OF FLORFENICOL IN NILE TILAPIA FOLLOWING ORAL ADMINISTRATION AT DIFFERENT WATER TEMPERATURES

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Aquaculture production of Nile tilapia has dramatically increased since 1980s. However, disease associated with bacterial infections is a major threat for the industry, and only a few drugs are available for the treatment of microbial infection in aquatic species. For effective use of antimicrobials and minimizing risk of drug residual problems, pharmacokinetic (PK) data of the drug is essential. As water temperature has profound effect on fish physiology leading to variable metabolic rates in drug elimination, the main objective of this study was to investigate the PK characteristics of florfenicol (FF) in Nile tilapia after oral administration and reared at different temperature.

Six male Nile tilapia (600-800 g) were divided into 2 groups and reared at either 28 or 32°C. FF solution was prepared by dissolving FF reference standard (Sigma-Aldrich) in N,N-dimethylformamide and adjusting the volume with 1,2-propylene glycol. The FF solution was administered via oral gavage at the dose of 15 mg/kg. The blood samples were collected at 0.25-72 h, extracted with ethyl acetate and analyzed by HPLC-UV using acetonitrile and 10 mM NaH₂PO₄-Na₂HPO₄ pH 5 (30:70) as mobile phase. PK parameters of FF were calculated by PKSolver 2.0.

The plasma concentration-time profiles of FF at both temperature levels followed a 2-compartmental model (FIGURE 1). The differential PK of FF between the two temperatures was observed (TABLE 1). The $t_{1/2\beta}$, $t_{1/2\alpha}$, $t_{1/2K_a}$, CL, V_{ss} and V_z appeared greater at the warmer water, while K_a , AUC, and T_{max} , tended to be reduced. The FF concentration declined below the detection limit after 60 h at 32°C while it could still be detected at 72 h at 28°C. The PK parameters from the present study could potentially be applied to determine the optimal dosing regimen of FF in Nile tilapia reared at different water temperature for sustainable and safer use.

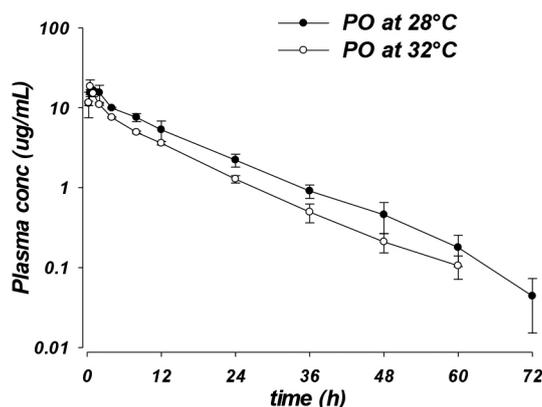


FIGURE 1. Plasma concentration-time profiles of FF 15 mg/kg oral in Nile tilapia

MITOCHONDRIAL DNA (16S rDNA) ANALYSIS OF RICE EEL *Monopterus albus* IN ISABELA, PHILIPPINES

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This study was conducted to describe the morphological characteristics of rice eel and to validate the rice eel species present in Isabela, Philippines using mitochondrial DNA (mtDNA). Rice eel was collected from the rice fields of different municipalities in the province of Isabela (Ramon, San Mateo, Mallig, Quezon and Roxas). The genomic DNA was extracted from 51 samples. The 16S rDNA of rice eel were amplified through polymerase chain reaction and the resultant products were sequenced using Big Dye® Terminator v3.1 Cycle Sequencing kit and the DNA sequences were aligned using BioEdit alignment editor v.7.

The 51 samples of rice eel were from 40 to 76 cm in length and they had a distinctive body colors which differs from light orange to brown and occasionally gold and some had yellow and black spots. Nucleotide sequences of the amplified regions of the 16S rDNA consist of 489 base pairs. Based on mtDNA analysis of rice eel, only *Monopterus albus* was identified and validated species from different municipalities of Isabela. The results also suggest that employment of mtDNA gene sequence is rather useful for identification of species and for obtaining reasonable insights into the validation of these species.

STUDY ON EFFECT OF POLLUTION ON GENOTOXIC DAMAGE IN TWO IMPORTANT FISH SPECIES

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Industrial development, expansion of urban populations and increased coverage of industrial, domestic water supply and sewerage give rise to larger quantities of municipal wastewater. Disposal of toxic sewage wastes with large volume of water could reduce biological oxygen demand to the lethal level by removing entire oxygen from the water body. Some very toxic chemicals are released into the lakes, streams and rivers e.g., compounds of mercury, Zinc, Lead and Copper etc. causing death of aquatic populations even at very low concentrations. This can cause metabolic activation giving rise to toxic metabolites in the nervous tissues. Comet and Micronucleus assays have been used to assess DNA damage in *Catla catla* and *Cirrhinus mrigala* collected from polluted areas. Heavy metals Cd, Cu, Mn, Zn, Pb, Cr, Sn and Hg were detected by atomic absorption spectrophotometry in River Chenab water. All physicochemical parameters and heavy metals were found beyond the tolerable limits. Comet assay showed significant ($p < 0.05$) DNA damage in *Catla catla* as 17.33 ± 2.42 , 11.53 ± 2.14 and 14.17% DNA in tail. Tail moment was 10.06 ± 2.71 , 3.11 ± 0.74 and 14.70 ± 1.89 , Olive moment was 8.85 ± 1.84 , 3.83 ± 0.76 and 7.11 ± 0.73 , respectively. Highly significant ($p < 0.01$) damage was reported in *Cirrhinus mrigala* as $37.29 \pm 2.51\%$, $34.96 \pm 2.53\%$ and $38.80 \pm 2.42\%$ DNA in comet tail, tail moment was 23.48 ± 3.90 , 19.78 ± 4.26 and 14.30 ± 1.82 , olive moment as 16.22 ± 2.04 , 13.83 ± 1.96 and 10.99 ± 0.90 from three different sites of the polluted area of the River Chenab. Significant ($p < 0.05$) differences were reported polluted and farmed fish but non-significant ($p > 0.05$) differences in farmed and non-polluted upstream. Micronucleus assay showed similar findings for single and double micronucleus induction as 23.20 ± 4.19 , 2.80 ± 1.07 in *Catla catla* and 44.80 ± 3.73 , 06.20 ± 0.97 /thousand cells, respectively in *Cirrhinus mrigala*. Nuclear abnormalities were found as 6.00 ± 0.84 and 09.60 ± 1.72 /thousand cells, respectively in both species. These findings infer that these novel fish DNA damage assays to detect genotoxicity, could be used as expedient toxicity screening of aquatic environments

THE POTENTIAL OF BLACK SOLDIER FLY *Hermetia illucens* PREPUPAE LARVAE AS AN ALTERNATIVE PROTEIN SOURCE IN DIETS OF POST LARVAE FRESHWATER PRAWN *Macrobrachium rosenbergii*

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Insects such as black soldier fly (*Hermetia illucens*) has potential as an alternative protein source to replace fishmeal (FM) in aquafeed as it is efficient in turning macronutrients of waste into high quality protein. Black soldier fly (BSF) larvae culture is inexpensive, sustainable to culture and harvest, can be produce locally, and induces positive growth for aquaculture animal thereby reducing both the monetary and environmental impact. A sixty-day trial was conducted to evaluate the effects of replacing fishmeal with BSF in diets for post larvae (PL) of freshwater prawns (*Macrobrachium rosenbergii*) on growth performances, survival rate and whole-body carcass composition. Three hundred and sixty *M. rosenbergii* PLs (mean initial weight 0.60 ± 0.03 g; mean initial total length of 2.07 ± 0.03 cm) were divided into three treatment groups of 25 L tanks, each with three replicates. Control treatment was fed commercial shrimp PL pellets and two experimental diets were formulated incorporating two different levels of BSF (50% and 75% replacement). The results showed that treatments containing BSF had good growth performances. Survival rates of *M. rosenbergii* PL were high for all treatment. The trial concludes that BSF can partially replace FM as an alternative source of protein in feed for *M. rosenbergii* PL.

EVALUATION OF DIFFERENT COMMERCIAL FEEDS USED IN PRODUCTION OF TILAPIA IN KUWAIT

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Fish feed quality greatly affects fish growth and meat quality. This study aimed at evaluating the effect of three commercial tilapia feeds available in Kuwait's market on growth, immunity, fatty acid profile, and economics.

Nile tilapia juveniles (47 g) were stocked in 0.43m³ tanks at 83 fish/m³ and fed daily for 183 days at a rate of 6% of body weight decreased gradually to 2.5%. The control feeds were labelled F1, F2, and F3: The same feeds were mixed with probiotics and were labelled F4, F5, and F6, respectively. The proximate composition from the manufacturer for F1 and F2 was 32% crude protein, 6% and 4% fat, 7% and 8%, ash, and 5% and 4% fibers. Although the manufacturer provided no data for F3, proximate analysis showed F3 to contain 13.4% crude protein, 4.7% fat, and 4.6% ash. Each feed represented a treatment, and each treatment was triplicated giving 18 experimental tanks. Temperature was controlled at 28 ± 2.0 °C and all fish were sampled biweekly to monitor growth and to adjust feed.

Results showed a general trend of enhanced growth through probiotic supplementation. F4 resulted in highest mean weight, specific growth rate, best feed conversion ratio, and production rate followed by F1 and F2. F3 and F6 had the lowest values.

Fish fed F4 and F5 probiotic diets showed higher gut bacterial counts and improved immunological parameters such as serum lysozyme activity, phagocytosis, and blood hemagglutination titer.

F5 resulted in higher muscle $\Sigma n-3$ than its control F2. On the other hand, F1 and F4 resulted in higher $\Sigma n-6$ fatty acids. F4 and F5 had significantly higher $\Sigma n-3/n-6$ ratio than their controls. Feed type had no significant effect on sensory and organoleptic attributes.

Economic analysis showed F1 and F4 to result in the highest profit, profit rate, Benefit-to-Cost Ratio, and the lowest cost per Kg of feed and lowest cost per ton of fish.

In conclusion, results of this study indicate that F1 and its probiotic version F4 are the best commercial feed to be used by farmers for best growth, immunity, flesh quality, and farm profitability and economics.

INTERACTIVE EFFECTS OF TEMPERATURE AND LIGHT DURING EARLY LIFE CULTURE OF SEA CUCUMBER *Isostichopus* sp. aff *badionotus*

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The knowledge on nutrition, physiology or ecology of the sea cucumber *Isostichopus* sp. aff *badionotus* is a link-tool for its culture development but many queries of its feeding and zootechnical behavior are still unknown since they have not been tested. A better understanding of these issues could provide useful information for the culture technology of the species. The purpose of this study was to identify the optimal growth temperature of juvenile *Isostichopus* sp. aff *badionotus* obtained in captivity. From a batch of cucumbers produced in the laboratory, a selection of two size groups was made. Group 1: Small size (average weight 0.045 g); Group 2: Average size (average weight 0.29 g) and three cultivation temperatures were chosen: 21 °C, 23 °C, 25 °C, in order to evaluate their behavior and growth. In turn, the two groups were subjected to two light / dark conditions (12 light hours: 12 dark hours and 0 light hours: 24 dark hours). During 96 days 360 animals were used for a total of six treatments (two replicates each): For group 1) T1: 21 °C: Light, T1.1: 21 °C: Darkness, T2: 23 °C Light, T2.1: 23 °C: Darkness, T3: 25 °C: Light and T3.1: 25 °C Darkness and for group 2) T4: 21 °C: Light, T4.1: 21 °C: Darkness, T5: 23 °C Light, T5.1.1: 23 °C: Darkness, T6: 25 °C: Light and T6.1: 25 °C. Regarding the effect of light / darkness as one of the variables that determine growth, we could be established that in size 1 there were significant differences between the conditions studied ($P \leq 0.05$). It was found that 23 °C was the optimum growth temperature for this species according to the specific growth rate, corresponding to condition T1.1, with a survival percentage of 83%. For the group (size 2), it was found that there were significant differences between the studied conditions ($P \leq 0.05$), where 25 °C resulted as the optimum growth temperature according to the specific growth rate, corresponding to the condition T6 (25 °C) with a survival percentage of 53.3%.

STUDY ON APPLICATION OF LIGHT EMITTING DIODE TO CONTROL PHYSICAL WOUND IN FISH

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Physical wound on fish is one of the biggest problems in fish farm, because it can cause to tremendously economic losses by decreasing their homeostasis as well as increasing the likelihood of infection of ectoparasite. Due to the importance of skin working as physical barrier, the strategy of promptly recovering physical wound have been important to reduce further losses through secondary infection and imbalance of homeostasis. Here, we mainly studied on the method of wound healing in olive flounder, *Paralichthys olivaceus* by applying LED technology.

Before applying the LED to teleost, the proliferation level of *Epithelioma papulosum cyprini* (EPC) cell line was verified with various light condition such as 465, 520 and 640nm with various light intensities (0, 10, 20, 40, 80 and 160 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 5d. Interestingly, 160 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of 520nm exposures and 40–80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of 640nm groups were promptly proliferated than those of control (0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Therefore, 520, 640nm were selected as potential candidates for further *in vivo* experiment. In order to verify the green and red LED efficacy, razor wound was artificially triggered and exposing lights in three times. The mortalities of 520nm exposures were significantly lower than room light group and its group has not only the highest regenerated epithelium thickness in the initial stage of recovery but also much stronger expressed of collagen fiber and connective tissue in the middle stage. Also, wound closures rate in 520nm group was significantly faster than those of control after 3 and 4 weeks post exposures. It might be considered that some immune related genes (IL-1, IL-8, TNF- α) and wound healing related gene (MMP-13) was significantly up-regulation after post green light exposure. Taken together of information (clinical signs, various histological analysis and gene expression level in wound), applying green light will be helpful to reduce the economic losses and mortality in fish farm by stimulating the wound recovery.

ASSESSMENT OF BIOTIC FAUNA IN SELECTED TRANSPLANTED MANGROVE SITES OF SANTO TOMAS, LA UNION

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The study assessed different biotic fauna (fishes, crustaceans and molluscs) found in transplanted mangrove sites of Santo Tomas, La Union (Cupang, Raois and Cabaruan).

The results showed that the three transplanted mangrove sites harbored 39 species of biotic fauna (22 species of fish, 6 crustacean species and 11 mollusc species). Cupang had the highest catch, followed by Raois and Cabaruan. Results revealed that *Mugil cephalus* was the most abundant fish species, *Thalamita crenata* was the most abundant crustacean species while *Batillaria multiformis* for mollusks. Similarity indices of fish and mollusks species are higher between Raois and Cabaruan while similarity index of crustaceans is higher between Cupang and Raois. Diversity indices of fishes and mollusks of the transplanted mangrove sites are comparable ($P>0.05$) while crustaceans is highly significant ($P<0.05$). Correlation analysis showed that water depth influenced the abundance of fishes, crustaceans and mollusks in the three mangrove sites. Chlorophyll-a was highest in Cabaruan while organic matter is the same in the three mangrove sites.

CHARACTERISTICS OF SHRIMP FARMERS PARTICIPATING IN SHRIMP CULTURE GROUP MEETINGS ON HYGIENE MANAGEMENT IN HUE PROVINCE, VIETNAM

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Shrimp is a major export in Vietnam. However, veterinary drugs have been detected at levels exceeding the residue standards in many shrimps after their arrival at export destinations. The spread of disease and veterinary drug residues have become social problems in Vietnam. An information transmission system is thus being developed in Vietnam to make shrimp farmers aware of proper hygiene management to prevent diseases in shrimps or veterinary drug residues in the following order: national and local government, district, commune, Commune Fishery Association, and groups of farmers. Therefore, the challenge to be addressed in Vietnam is how to encourage farmers to participate in the meetings for the shrimp culture groups.

This study conducted questionnaire surveys of shrimp farmers in Hue Province, Vietnam, and based on the results, the characteristics of farmers who often participated in the meetings for the shrimp culture groups were quantitatively identified.

The analytical results of an ordered logit model are shown in Figure 1. The characteristics of farmers often participating in the meeting are included in the following: considering that what was learned in meetings can be utilized in their work (*UTILIZATION*); regularly consulting public agencies or shrimp culture groups about diseases in shrimps (*CONSULT*); more workers (*LABOR*); mixed aquaculture (*MIX*); longer experience with shrimp culture (*EXPERIENCE*); more disposed shrimp in the previous year (*DISPOSE*); or farmers with no experience participating in training sessions held by public agencies (*PUBLIC*). The same characteristics mentioned in terms of relationships with others in the same commune are included in the following: having deeper relationships with other farmers in shrimp culture (*RELATIONSHIP*); more willing to advise or provide support when disease occurs in another farmer's shrimp pond (*DISEASE*); more willing to help when another farmer's shrimp pond is damaged due to floods (*FLOOD*); or less expecting of active advice or support from another farmer when disease occurs in their shrimp pond (*HELPHYOU*).

TABLE1. Parameter estimates of ordered logit model. ** and * denote that the estimate is significantly different from zero at 1% and 5% levels, respectively.

Variable	Coefficient	Standard Error
<i>UTILIZATION</i>	0.9949 **	0.3186
<i>CONSULT</i>	2.9034 **	0.5254
<i>LABOR</i>	0.3902 *	0.1830
<i>MIX</i>	1.8434 **	0.6047
<i>EXPERIENCE</i>	0.0939 **	0.0344
<i>DISPOSE</i>	0.0015 **	0.0005
<i>PUBLIC</i>	-1.1322 **	0.4295
<i>RELATIONSHIP</i>	1.2519 **	0.4324
<i>DISEASE</i>	1.6020 **	0.5571
<i>FLOOD</i>	0.5483 *	0.2637
<i>HELPHYOU</i>	-2.3141 **	0.6324
/cut1	7.1905 **	1.7511
/cut2	11.1144 **	1.9750
Number of Observations		146
Log likelihood		-96.7954

EFFECT OF COMMERCIAL FEED SUPPLEMENTS ON THE GROWTH PERFORMANCE AND IMMUNE RESPONSE OF *Litopenaeus vannamei* AGAINST *Vibrio parahaemolyticus*

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Acute hepatopancreatic necrosis disease (AHPND) is one of the most devastating diseases affecting shrimp aquaculture globally. AHPND is caused by a toxin-producing strain of *Vibrio parahaemolyticus*. Most of the commercially farmed shrimps are known to be susceptible to this infection. Several feed additives have been tested to control AHPND in shrimp farms with varying results. In this study a 42-day feeding trial was conducted to evaluate the effect of supplementing two commercial feed supplements to reduce the impacts of AHPND in whiteleg shrimp, *Litopenaeus vannamei* culture. Shrimp juveniles (3.69 ± 0.34 g) were stocked at a rate of 30 shrimps per tank containing 120 L water at 15 ppt. Experimental diets contained two levels (5 and 10 g/kg feed) of the feed supplements and three control treatments (without feed supplement and with an antibiotic feed supplement and no AHPND challenge) in triplicates. No significant differences in growth and survival were observed among the treatments after 6-weeks of feeding period. The shrimps were then challenged with a virulent isolate of *V. parahaemolyticus* (XN₈₉) at 10^5 CFU/mL. Results of the bacterial challenge showed that the survival rate was significantly different ($P > 0.005$) among the experimental treatments. The diet supplement at 5g/kg feed showed encouraging results in terms of survival rate (~60%) compared with the antibiotic control, 120 h post-challenge. Bacteriological analysis of the gut and hepatopancreas of dead and moribund shrimp in all the treatment groups showed the presence of positive green colonies. There was significantly lower ($P > 0.005$) vibrio count in haemolymph of shrimp fed the test diet supplemented at 5 g/kg of the product. The results indicate the potential of the tested product at 5 g/kg supplementation in the feed of white shrimp that can prevent the occurrence of AHPND during culture period.

ABILITY OF JUVENILE GREEN-LIPPED MUSSEL *Perna canaliculus* TO FILTER FEED ON MICROALGAE OF DIFFERENT SIZES AND TAXA

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The green-lipped mussel (*Perna canaliculus*) is the most valuable aquaculture export from New Zealand, which accounts for US\$300 million each year. However, a major impediment to the growth of the industry is a very high losses of spat shortly after they are seeded onto nursery ropes on mussel farms. These losses are thought to be due to the secondary settlement behaviour of mussels. The causes of secondary settlement behaviour in juvenile mussels are unclear but feed availability and nutritional condition of the juvenile mussels have been identified as major contributing factors. The feeding biology of the early stages of green-lipped mussels is poorly understood, but is of critical importance for the growth and retention of seed mussels. The present study was conducted to understand their filtering capabilities in order to improve feeding delivery. Improvements in feeding efficiency of juvenile mussels has the potential to overcome the intermittent shortage of seed supply in this industry.

Using flow cytometry (FCM) methods, we investigated the ability of green-lipped mussel juveniles of a range of sizes (i.e., 500 μm to 7 mm in shell length) for capturing different unicellular microalgae species to better understand their filtering capabilities. The filtering behaviour was recorded over the 60 min experimental period. The aim of this study was to determine the filtering capabilities of juvenile *P. canaliculus* of a range of sizes when provided with suspensions of microalgae of different sizes and taxa.

Distinct differences were found in the capability of mussels of different sizes for capturing the particles ($P < 0.05$) (Figure 1). Smaller sized mussels (i.e., $< 600 \mu\text{m}$) were able to capture larger species of microalgae more efficiently (i.e., 5 – 20 μm cells of diameter) ($P < 0.05$). The larger sizes of mussel were able to capture smaller size of microalgae more efficiently (i.e., $< 5 \mu\text{m}$ cells of diameter) ($P < 0.05$). This study suggested that providing microalgae of larger particle size with high quality biochemical composition might improve the efficiency of filtering and delivery of nutrition for early juveniles of *P. canaliculus* in the hatchery system.

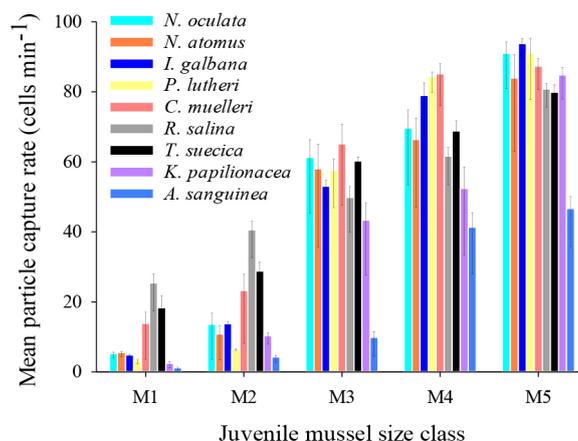


Figure 1. Mean particle capture rate for five size classes of mussels filtering of nine different species of axenic microalgae of different sizes. M₁ = 0.5 – 0.9 mm, M₂ = 1 – 1.9, M₃ = 2 – 3 mm, M₄ = 4 – 5 mm and M₅ = 6 – 7 mm.

INFLUENCE OF LOW-SALINITY POST-OPERATIVE CARE ON HEMAGGLUTINATION IN THE HEMOLYMPH OF AKOYA PEARL OYSTER *Pinctada fucata*

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Pearl deformities and blemishes can be alleviated by holding the Akoya pearl oyster *Pinctada fucata* in low-salinity seawater post-operatively, just after implanting the pearl nucleus (Atsumi et al. 2014). The effect of low-salinity post-operative care on hemagglutinating activity in the hemolymph as an innate immune response in pearl oyster has been previously investigated. It was suggested that implantation elevated hemagglutination, which was subsequently suppressed when the oysters were held post-operatively in salinities of 25 psu in an inland tank (Sano et al. 2017). In this study, we investigated transcriptions of *Pogal*, a galactose-specific lectin from the hemolymph of the pearl oyster. Lectin is an important innate immune humoral factor with hemagglutinating activity. Downregulation of *Pogal* was observed in both the operated and unoperated oysters reared in 25 psu at 8 days after implantation.

Three-year-old pearl oysters were separated into three groups. Operated and unoperated pearl oysters were reared in separate inland tanks under salinities of 25 psu, and conventionally treated operated pearl oysters were suspended from the raft in the sea as a control for 12 days. Ten oysters from each group were sampled at 4, 8 and 12 days after the salinity condition was changed. Total RNA was extracted from the hemocytes and hepatopancreas of the oysters, and cDNA was synthesized. Q-PCR was performed by specific primers to *Pogal*. Hemagglutinating activity of hemolymph was measured with sheep erythrocytes.

The transcription level of *Pogal* in hemocytes of unoperated oysters reared in 25 psu at 8 days after implantation was approximately one fourth that of the other groups, and in operated oysters reared in 25 psu at 8 days after implantation it was approximately half that of the other groups. This downregulation of *Pogal* was not observed in the hepatopancreas. The hemagglutinating activity was similar to the tendency shown in Sano et al. (2017). These results suggest that low-salinity rearing of oysters induces downregulation of *Pogal*.

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CULTURE OF PIKEPERCH (*Sander lucioperca*) JUVENILES IN INTENSIVE SYSTEM

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Sander lucioperca (Pikeperch) is one of the economies known for its high quality meat, reduced natural resources, high prices and popularity among consumers as an ideal choice for commercial production. Intensive culture for *Sander* production are in the early stages of development in Europe and Asia. The habit of artificial feeding fish is gradual (the best temperature for rearing fish that has the best conversion factor, growth rate, protein efficiency, and instantaneous growth rate is at 22-23 ° C. The appropriate light period for breeding was 16 hours of darkness and 8 hours of light. During the breeding period, the average initial weight was 2.83 ± 0.6 g, and the mean initial length was 7 ± 0.5 , to the mean final weight 43 ± 17.4 gr and the mean final length was 18.3 ± 1.9 cm. . Food conversion ratio was 1.6.

TRANSCRIPTOME ANALYSIS OF THE ACUTE PHASE RESPONSE *Penaeus vannamei* TO WHITE SPOT SYNDROME VIRUS FOLLOWING FEEDING WITH DIET CONTAINING ULVAN

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Viral diseases that result in mass mortality of *Litopenaeus vannamei* have seriously threatened the shrimp industry in the last two and a half decades. The white spot syndrome virus (WSSV) is the most important viral pathogen of shrimp farming causing large economic loss. Researchers have continuously searched for solutions that include extracts from various plants that will boost the immune system of the shrimp. Green seaweeds contain sulfated polysaccharide that is bioactive in enhancing the immune response of shrimps against degraded water environment as well as pathogens. An extract from *Ulva intestinalis* called ulvan has been proven to enhance the immune system of the white shrimp. The present study was conducted to verify at the gene level the various differentially expressed genes in the hepatopancreas that enable the immune enhancement in the Pacific white shrimp.

Two triplicate groups of Pacific white shrimp were fed the control diet (containing no ulvan) and an ulvan-containing diet for 35 days. Following feeding trial was the white spot virus challenge test for 24 h in which individual shrimps in each of the triplicate groups were injected intramuscularly with the virus at LD₅₀ of 10^{6.38} ml⁻¹ at 10 µl g⁻¹ shrimp. Total RNA was extracted for each replicate group of shrimp and were sent for RNA-seq using Illumina HiSeq 4000 platform that generated ~150 bp pair-end raw reads. Differentially expressed genes (DEGs) were identified were in the KEGG database. GO functional enrichment analyses were performed to identify the biological function of the DEGs. Results show that there were 709 DEGs between the treatment with more genes were down-regulated. Those that were down regulated were mostly involved in the anabolic metabolism while majority of those up-regulated are involved in lipid and carbohydrate metabolism.

Table 1. Transcriptomic analysis following WSSV ch.

-Gene expression – 709 DEGs (69 up-reg and 640 down-reg)
-GO enrichment
-metabolic process, cellular process, binding, catalytic activity, single organism process;
-KEGG enrichment
-carbon metabolism, biosynthesis of amino acids, glycolysis/gluconeogenesis; fructose and mannose metabolism; protein processing in endoplasmic reticulum

STUDY OF BACTERIA FROM SEAWEED AS ANTIBACTERIAL AND QUORUM SENSING DEGRADERS IN BIDONG ISLAND MALAYSIA

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Bacteria associated with seaweeds are highly diverse and rich sources of bioactive compounds. The antagonistic properties of seaweed-associated microbes from Pulau Bidong have not been explored and also very limited studies on bacteria associated with seaweed in Malaysia. In this study, we aim to identify the bacteria associated with seaweed which act as 1) antibacterial and 2) quorum sensing degraders. A total of 26 bacterial strains were isolated from six species of seaweed (*Caulerpa serrulata*, *Caulerpa peltata*, *Caulerpa racemosa*, *Lobophora variegata* and *Hypnea pannosa*) found at Bidong Island.

The agar well diffusion method was used to screen for antibacterial activity against pathogenic bacteria *Vibrio alginolyticus*. Nine strains exhibited antibacterial activity with strain LV Epi 4 significantly showed the highest antibacterial activity (16mm) against control (Chloramphenicol).

Meanwhile the enrichment of AHL degrader and AHL degradation assay were used to screen for quorum sensing degraders by using *Chromobacterium violaceum* (CV026) as the reporter strain. Bacteria associated with only brown seaweed *Lobophora variegata* showed successful screening of quorum sensing degrader, strain B4, LV Epi 2, LV Epi 4 and CS Epi 2. Among the AHL bacterial degrader tested, LV Epi 2 strain showed the strongest quorum sensing degradation activity (0cm of purple violacein).

Bacteria from seaweed that expressed anti-bacterial and anti-quorum sensing activities were identified by 16S rRNA gene sequence was identified as *Stenotrophomas pavanii* (B4), *Kocuria haloterans* (LV Epi 4), *Vibrio alginolyticus* (LV Epi 2 and CP Epi 2). Bacteria from seaweed may yield a variety of novel antimicrobial compounds useful for the development of new drugs to fight against pathogens in natural and aquaculture environments.

THE EFFECT OF PLANT EXTRACT X ON NON-SPECIFIC IMMUNE RESPONSE OF WHITE SHRIMP *Litopenaeus vannamei*

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Shrimp is regarded as an important economic culture species. However, intensive aquaculture has caused the ecological environment changes, improper medication, pathogen resistance, disease outbreaks and lead to the huge mortality of shrimp. Aquatic animal drugs are serious issues and it is very important to look for proper and safe natural compounds to replace chemicals. Recently plants with known functions are gradually used in animal husbandry and aquaculture fields to cure diseases and promote growth. It means the green-for-safety product plays an important role in global animal food production. In this study, the plant extract X (PEX) is used to verify its ability to enhance the immunity and disease resistant in shrimp. In the beginning, white shrimps were fed with the diet containing PEX (PEX diet) and measured the total haemocyte count (THC) and phenoloxidase (PO) activity. The result showed that both THC and PO activity increased in the group which ate PEX diet for 12 days. Selected immune-related genes, *Lectin*, *proPO*, *LGBP*, *Toll2* and *MyD88*, showed higher expression in the group which ate PEX diet for 7 days. It indicated that PEX can induce the immune activity in shrimp. In the next step, shrimps were fed with PEX diet and then challenged with *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV), respectively. In *V. parahaemolyticus* challenged experiment, the group which ate PEX diet for 7 days and then challenged with bacteria showed best survival rate. In WSSV challenged experiment, the group which ate PEX diet for 14 days and then challenged with virus showed best survival rate. The results in this study show that the immunity can be enhanced by oral route and shrimps treated with PEX have better pathogen resistant. It indicates that the natural PEX is an excellent candidate to develop feed additives.

DIETARY UTILIZATION OF ENZYME-TREATED FISH MEAL IN DIETS FOR JUVENILE ATLANTIC BLUEFIN TUNA *Thunnus thynnus*

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Fish meal (FM) is the main protein source in aquafeeds. However, relatively low FM utilization has been reported in tuna feeds. This study was conducted to estimate effects of dietary enzyme treated FM (EFM) as a major protein source on growth of bluefin tuna *Thunnus thynnus*.

In the first experiment (Trial 1), juvenile bluefin tuna (initial body weight 0.68 g) were randomly stocked into two experimental tanks and fed two experimental diets for 15 days. The two diets are EFM based diet (75 %) and sand lance (SL) as a raw fish feed. At the end of feeding trial, weight gain was not significantly different between fish fed EFM or SL. Feed intake was higher in fish fed SL than fish fed EFM. Feed conversion ratio was higher in fish fed SL than fish fed EFM. The survival was higher in EFM group than SL group. The pepsin and lipase activities were significantly higher in fish fed SL than fish fed EFM. The contents of omega 3 and 6 fatty acids of fish carcass were higher in fish fed EFM than fish fed SL.

In the second experiment (Trial 2), juvenile bluefin tuna were randomly stocked into two experimental tanks and fed two experimental diets for 14 days. The two diets are EFM based diet (75 %) and sardine FM based diet (FM) (75 %). At the end of feeding trial, weight gain was higher in fish fed EFM than fish fed FM. Feed intake was higher in fish fed FM than fish fed EFM. Feed conversion ratio was higher in fish fed FM than fish fed EFM. The pepsin activity was higher in fish fed EFM than fish fed FM.

This study indicates that EFM is an excellent protein source that can be used for juvenile bluefin tuna.

Table 1. Growth performance of juvenile Atlantic bluefin tuna *Thunnus thynnus* fed the experimental diets for 15 (Trial 1) and 14 days (Trial 2).

	Trial 1		Trial 2	
	SL	EFM	FM	EFM
Final mean body weight	12.7±4.70	12.2±3.75	20.1±6.50	28.5±8.02
Weight gain (%)	1760	1691	89.7	165.1
Specific growth rate (%)	19.5	19.2	4.27	6.52
Feed intake (g/fish)	17.2	8.87	33.9	26.9
Feed conversion ratio	1.44	0.77	2.85	1.21
Protein efficiency ratio	1.10	2.24	0.60	1.42
Survival (%)	46.0	56.0	73.3	86.7

DEVELOPMENT OF THE GENETIC MARKERS ASSOCIATED WITH RESISTANCE TO HERPESVIRAL HEMATOPOIETIC NECROSIS IN GOLDFISH

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Herpesviral hematopoietic necrosis (HVHN) caused by cyprinid herpesvirus 2 (CyHV-2), has affected to the commercial production of the goldfish *Carassius auratus* and gibelio carp *C. auratus gibelio*. A goldfish strain (Azumanishiki variety) resistant to CyHV-2 has been established by Saitama Fisheries Research Institute and the strain can survive in the artificially induced HVHN at over 90%. So far, the selection of broodstock has been conducted by virus challenge, but it may cause the surviving fish to become virus carrier. Therefore, in this study, we investigated genetic markers as an alternative selection method for resistant broodstock of the goldfish strain.

F1 fish between resistant (Azumanishiki) and susceptible (Kurodemekin: KD strain) strains that showed resistant to HVHN, were backcrossed with the KD strain, and the progenies (BC fish) were produced. We conducted infection experiment using the fish (n=206) of 4 crosses BC fish by cohabitation infection with the goldfish (n=20) infected with CyHV-2 Sat-1 isolate. The cumulative mortality rates in the 4 cross groups are shown in Table 1. The dead and surviving fish of a cross group ($\text{♂F1} \times \text{♀KD}$ No1: n=20 each) in the experiment and their parents were processed for genomic DNA extraction. DNA libraries of the samples by genotyping-by-sequencing (GBS) method were subjected to Illumina HiSeq 4000 sequencing. The sequence data obtained were processed in CLC Genomics Workbench to remove low quality data followed by the detection of single nucleotide polymorphisms (SNP) using the *denovo_map.pl* pipeline in Stacks. We constructed the genetic linkage map using MapDisto Genetics Software and searched SNPs associated with the resistance to HVHN by linkage analysis using R/qtl software.

We detected 229 of SNPs and mapped 208 of those in the 45 genetic linkage groups. The linkage analysis of the SNPs with fish surviving or mortality showed two SNPs significantly linked with the phenotype to HVHN with a LOD score over 15. It suggests that these SNPs are linked with the resistance to HVHN. The SNP was further tested using fish of the 4 crosses for the association with fish surviving or mortality. The results demonstrated that 95.8% and 95.0% of the surviving fish had the SNP and 95.8% and 100% of the dead fish did not have it in the $\text{♂F1} \times \text{♀KD}$ No1 and No2 fish groups, respectively. In the fish of ♀F1 No1 and No2 $\times \text{♂KD}$ groups, 80.0% and 55% of survivors had the SNP and 76.5% and 69.2% of the dead fish did not have it, respectively. The difference of these SNP ratios between ♂F1 and ♀F1 cross groups suggests that the recombination rate between the SNP and the disease resistant locus is different in female and male of goldfish. The SNP obtained in this study can be used for the marker-assisted selection of male broodstock of the resistant strain.

Table 1. Cumulative mortality rates (%) of the BC groups in the virus infection experiment

	♀KD No1	♀KD No2
♂F1	49.5	45.1
	♀F1 No1	♀F1 No2
♂KD	34.5	29.1

CHARACTERIZATION AND EXPRESSION ANALYSIS OF INTERFERON-INDUCED GTP-BINDING PROTEIN, MX GENE IN MULLET *Liza haemotocheilus*

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Mx protein is responsible for a specific antiviral state against broad spectrum of virus infections. The encoded protein is induced by type I interferons (IFN α/β) in different vertebrates. Structural analysis of mullet Mx (*MuMx*) was practiced by using different bioinformatics web tools. Pairwise alignment results revealed that protein sequence matched to *Stegastes partitus* with 83.7% sequence identity and 91.5% similarity. Results of the phylogenetic analysis of mullet Mx showed a close relationship with *Stegastes partitus*. Multiple sequence alignment showed that the Dynamamin-type guanine nucleotide-binding (G) domain in N terminal end, GTPase effector domain in C terminal end and Dynamamin-type guanine nucleotide-binding (G) signature were highly conserved among other examined Mx orthologs. Hence, results of multiple sequence alignment suggested that the antiviral function of Mx protein might be conserved among its orthologs. Quantitative real-time PCR exhibited, the highest *Mumx* mRNA expression was in blood followed by spleen, gill, head kidney, liver, kidney, and heart tissue among 12 different tissues from healthy mullet fish. Since blood is a main tissue which contains peripheral blood mononuclear cells could activate IFN production as response to engulfed foreign agents. Thus, highest mRNA expression of Mx might be observed in blood cells as a first line of defense against viruses. Blood cells, head kidney, and spleen were used for analysis of temporal expression with polyinosinic-polycytidylic acid (Poly I:C) stimulant. Highest *MuMx* expression was indicated at 6 hours post infection in both head kidney and spleen. Moreover, in blood cells, highest elevation of *MuMx* mRNA level was shown at 24 hours post-infection. Collectively, results of the present study revealed that *MuMx* act as an antiviral protein and as an immune-related substance in the mullet fish.

VIETNAM *Pangasius/Panga* RESPONSIBLE AND ENVIRONMENTAL FRIENDLY

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Vietnam *Pangasius* has been developing more than 15 years, today the aquaculture industry becomes very famous in Vietnam and the world for its responsible and environmental friendly. In particular, *Pangasius* production has been controlled by competent agencies from input materials like seed, fingerlings, feed... to growing out like disease, vaccine, environment Etc.. to processing plant with high international standard and to consumers in the international market. The Government has issued many regulations to control whole production process of *Pangasius* and make certification on quality for export.

PERFORMANCE PARAMETERS FOR FOOD PROCESSING

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Food processing is the transformation of raw ingredients, by physical or chemical means into food, or of food into other forms. Food processing combines raw food ingredients to produce marketable food products that can be easily prepared and served by the consumer. Food processing typically involves activities such as mincing and macerating, liquefaction, emulsification, and cooking (such as boiling, broiling, frying, or grilling); pickling, pasteurization, and many other kinds of preservation; and canning or other packaging.

Food processing dates back to the prehistoric ages when crude processing incorporated fermenting, sun drying, preserving with salt, and various types of cooking (such as roasting, smoking, steaming, and oven baking). Such basic food processing involved chemical enzymatic changes to the basic structure of food in its natural form, as well served to build a barrier against surface microbial activity that caused rapid decay. Salt-preservation was especially common for foods that constituted warrior and sailors' diets until the introduction of canning methods. Evidence for the existence of these methods can be found in the writings of the ancient Greek, Chaldean, Egyptian and Roman civilizations as well as archaeological evidence from Europe, North and South America and Asia. These tried and tested processing techniques remained essentially the same until the advent of the industrial revolution. Examples of ready-meals also date back to before the preindustrial revolution, and include dishes such as Cornish pasty and Haggis. Both during ancient times and today in modern society these are considered processed foods.

GOOD AGRICULTURAL PRACTICES AND FOOD SAFETY

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Good agricultural practice (GAP) are specific methods which, when applied to [agriculture](#), create food for consumers or further processing that is safe and wholesome. The [Food and Agricultural Organization of the United Nations](#) (FAO) uses good agricultural practice as a collection of principles to apply for on-farm production and post-production processes, resulting in safe and healthy [food](#) and non-food agricultural products, while taking into account economical, social and environmental [sustainability](#). “Good agricultural practices are the responsibility of everybody”. Good agricultural practices (GAPs) at the farm level involve multi-faceted efforts at ensuring that foods are safe for human consumption. Food safety has become a serious issue in our society because of pathogens and food contamination. The United States Department of Agriculture (USDA) and the United States Food and Drug Administration (FDA) are responsible for recalling contaminated foods, but because food can become contaminated at all levels of the food production system, it is essential to keep food safe through GAPs.

GAP guidelines are necessary for all agricultural operations. Self audits should be conducted on a regular basis to determine whether a farm meets individual and government GAP standards. Food safety programs should be instituted to prevent food borne illnesses and food contamination throughout the whole production process from farm to consumers.

Not instituting GAP guidelines into a farm’s operation allows contamination risks to be present. Therefore, GAP guidelines should be a priority for all farmers since they benefit both consumers and producers. GAPs can help trace contaminated foods back to the “handlers and growers” responsible for unsafe agricultural products (University of California–Davis 2008). An important aspect of each farm operation is the transportation of agricultural products because of the risk of contamination during transportation and distribution (University of California–Davis 2008).

Preventing contamination at all possible sources on the farm and during transportation and distribution is critical because of the impact on both consumers and producers. Marketing contaminated food products has very serious consequences. According to a study by the University of Maryland (2002), food borne illness is a health hazard and can cause death. It has been estimated that, each year, in developing countries, over 1,500 million children under the age of five years suffer from diarrhea and over 3 million die as a result. Costs related to food borne illness include healthcare costs, research costs to investigate and control outbreaks, lost revenues to businesses, and legal costs for related litigations.

“FOOD SECURITY THROUGH AQUACULTURE DEVELOPMENT (FISHERIES) TO STRENGTHEN THE WOMEN SPECIALLY IN RURAL AREAS”

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This abstract addresses a extremely important part of world food and nutrition security, the role and importance of Aquaculture industry (fisheries) for all specially the women in rural areas. Fish is one of the most efficient converters of feed into high quality food. Fish and fish-related products provide income and livelihoods for numerous communities across the world. Food insecurity and malnutrition arise from inequalities, including those related to gender. Gendered aspects of the role of fish in food security and nutrition include when balancing the benefits of eating fish by gestating women on foetal development and for children on childhood brain development and the risks of damage from dioxin and methylmercury contamination . In 2010, FAO and WHO (FAO/WHO, 2011) reviewed these risks and benefits and concluded that, overall, fish provide people with energy, protein and a range of other important nutrients, including the long-chain n-3 polyunsaturated fatty acids (PUFAs). With regard to maternal and childhood fish consumption, the report recognized that, in most studies, the benefits of PUFAs outweigh the risks of methylmercury to women of childbearing age and that maternal fish consumption lowers the risk of suboptimal neurodevelopment in offspring compared with the offspring of women not eating fish (FAO/WHO, 2011). Without awareness about the beneficial nature of fish as a source of good protein and micronutrients, it may not be consumed. In developing countries, an important condition to ensure this awareness is female literacy, as well as available relevant informative material. It becomes evident that aquaculture did play an important role in making food available to people and providing a source of income. The contribution is, however, dependent on the level of development or engagement in aquaculture activities in the different countries. However, there is no doubt that aquaculture impacts positively on the general economy and that sustainable engagement for countries in this activity will be beneficial in the long run.

Biography :

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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

ETHICAL ANALYSIS OF THE USE OF GM FISH: EMERGING ISSUES FOR AQUACULTURE DEVELOPMENT

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Improvements in production methods over the last two decades have resulted in aquaculture becoming a significant contributor to food production in many countries. Increased efficiency and production levels are offsetting unsustainable capture fishing practices and contributing to food security, particularly in a number of developing countries. The challenge for the rapidly growing aquaculture industry is to develop and apply technologies that ensure sustainable production methods that will reduce environmental damage, increase productivity across the sector, and respect the diverse social and cultural dimensions of fish farming that are observed globally. The aquaculture industry currently faces a number of technology trajectories, which include the option to commercially produce genetically modified (GM) fish. The use of genetic modification in aquaculture has the potential to contribute to increased food security and is claimed to be the next logical step for the industry. However, the potential use of these technologies raises a number of important ethical questions. Using an ethical framework, the Ethical Matrix, this paper explores a number of the ethical issues potentially raised by the use of GM technologies in aquaculture. Several key issues have been identified. These include aspects of distributive justice for producers; use of a precautionary approach in the management of environmental risk and food safety; and impacts on the welfare and intrinsic value of the fish. There is a need to conduct a comparative analysis of the full economic cycle of the use of GM fish in aquaculture production for developing countries. There is also a need to initiate an informed dialogue between stakeholders and strenuous efforts should be made to ensure the participation of producers and their representatives from developing nations. An additional concern is that any national licensing of the first generation of GM fish, i.e., in the USA, may initiate and frame an assessment cycle, mediated by the WTO, which could dominate the conditions under which the technology will be applied and regulated globally. Therefore, an integrated analysis of the technology development trajectories, in terms of international policy, IPR, and operational implications, as well as an analysis of a broader range of ethical concerns, is needed.

RESEARCH AND DEVELOPMENT STRATEGY FOR FISHERY TECHNOLOGY INNOVATION FOR SUSTAINABLE FISHERY RESOURCE MANAGEMENT IN NORTH-EAST ASIA

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The development of fishery technologies supports food sustainability to achieve a steady supply of fish and fishery products. However, the priorities for research and development (R&D) in fishery technologies vary by region due to differences in fish resource availability, environmental concerns, and consumer preferences for fishery products. This study examines trends in fishery technology innovations using data on patents granted as an indicator of changing R&D priorities. To clarify changes in R&D priorities, we apply a decomposition analysis framework that classifies fishery technologies into three types: harvesting, aquaculture, and new products. This study mainly focuses on China, Japan, and Korea as the major fishing countries in the north-east Asia region. The results show that the number of fishery technology patents granted increased between 1993 and 2015; in particular, the number of aquaculture patents granted has grown rapidly since 2012. However, the trend in Japan was the opposite, as the apparent priority given to aquaculture technology innovation decreased between 1993 and 2015. The trends and priority changes for fishery technology inventions vary by country and technology group. This implies that an international policy framework for fishery technology development should recognize that R&D priorities need to reflect diverse characteristics across countries and the technologies employed.

NEW INSIGHTS IN MICROBIAL MANAGEMENT TO MAKE INTENSIVE AQUACULTURE MORE SUSTAINABLE

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At the last FAO Global Conference on Aquaculture in Phuket (Thailand) in 2010 we already postulated that modern aquaculture requires a turning point: although the farming of aquatic plants and animals has evolved into a mature industry, too much of the technology is still based on empirical approaches and more knowledge-based developments are needed to make aquaculture the blue biotechnology of the future. “More microbial management for more sustainable production” was identified as a top priority.

Today we can report about significant progress that has been achieved in this field, mainly based on new insights regarding the diversity and different functions of bacteria in aquatic systems. Management of the mutual and reciprocal interactions between bacterial populations and the farmed fish/shellfish can be used to improve the viability and robustness of our aquaculture systems. This ‘join them’ approach is contradictory to the traditional ‘beat them’ strategy generally applied in microbial management in human medicine, agriculture as well as aquaculture.

Based on these new insights operational suggestions can be formulated to achieve non-selective reduction of unwanted microbes (disinfection, increased removal of organic matter,...), as well as selective enhancement of wanted microbes (K-strategist selection with mature water and RAS systems; use of pre- and probiotics). This improved microbial management technique is exemplified in the new concept of intensive shrimp farming integrated with better waste management, including co-culture with Tilapia fish and *Caulerpa* seaweed. Here, the opportunistic pathogen *Vibrio parahaemolyticus* – known causative agent of AHPND - is still present in the shrimp ponds but cannot as easily reach critical densities to express virulence. The same phenomena with regards to microbial ecological status and stability likely explain why RAS systems with over-dimensioned biofilters yield improved results in terms of survival, growth and disease resistance of fish and shrimp.

THE RESPONSE OF FLUORESCENT ORGANIC MATTER TO OZONE TREATMENT IN FRESHWATER PILOT RECIRCULATING AQUACULTURE SYSTEMS

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Water quality in intense recirculating aquaculture systems (RAS) is characterised by the accumulation of organic and inorganic compounds, potentially deteriorating the water quality. Ozone has been implemented in RAS as a continuous water treatment technology improving water quality by oxidizing organic matter and reducing bacterial abundance. The analytical efforts to detect and monitor organic matter (DOM) in aquatic systems have included absorption spectroscopy of the coloured fraction (CDOM). A part of CDOM also fluoresces (FDOM). FDOM fraction has been widely used in aquatic environments as a quantitative and qualitative measure of DOM. FDOM can be characterised by the fluorescence excitation-emission matrix (EEM) spectroscopy which can be further decomposed mathematically with methods such as parallel factor (PARAFAC) analysis to identify the independent FDOM fractions. This provides both a quantitative and qualitative measure of DOM.

Recently, it was shown that the organic matter fluorescence of RAS water is highly sensitive to ozonation and fluorescence spectroscopy could therefore be used as an indirect method to determine ozone delivery within these systems. Therefore, three ozone dosages, including a control, were injected in pilot freshwater RAS where trouts were farmed. The ozonation trial lasted eight days utilising one RAS per dosage. The test levels ranged from 52-130 mg O₃/h, equivalent to 10-25 g O₃/kg feed.

The DOM consisted of four components (Fig. 1) which differed in their fluorescence characteristics and response to ozonation. A UV wavelength fluorescent fraction (C4) typical of proteinaceous material removed by 13-20% immediately after ozone initiation. The remaining fractions that exhibited visible wavelength fluorescence, at first were unaffected but during the following days, were gradually degraded, reaching a removal of 34-66%. By the end of the experiment the fluorescence intensities of all fractions were diminished up to 60% in all applied dosages.

This study provides the first application of fluorescent EEM spectroscopy and PARAFAC analysis to determine the effect of ozone on FDOM character in aquaculture water, more specifically within continuously treated RAS. The results indicate that the response in organic matter fluorescence can be used to depict the impact of ozone dosage.

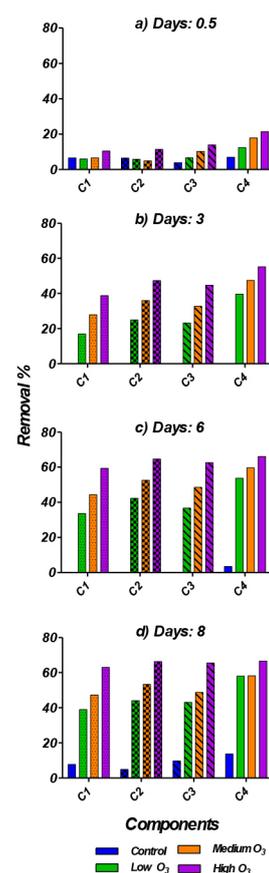


Figure 1: Effect of ozone on FDOM over time.

ACUTE TOXICITY STUDY OF AQUABOSSO™ 50 ON CREEPER SHELL SNAIL VELIGER *Cerithidea cingulate* WITHIN 48 HOURS

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Aquabosso™ 50 is molluscicide for controlling parasitic bivalve and snail infestation of the aquaculture pond soil and liners. The active ingredient in Aquabosso™ 50 is 2' 5-dichloro-4'-nitrosalicylanilide, also known as Niclosamide ethanolamine salt. It contains 50% w/w. Niclosamide, its appearance is a yellow and odor-less powder. Aquabosso™ 50 application is highly effective for eliminating adult creeper snail and horse mussle bivalve infestations especially during early stages of the culture cycle. Niclosamide activity in the pond environment has short half-life, shrimp can be stocking into Nicosamide treated pond only a 4 days after treatment.

Previous result had shown that Aquabosso™50 has an acute toxicity on creeper shell snail 0.660 ppm, over 5 days of time. A study of acute toxicity of Aquabosso™ 50 on creeper shell snail veliger was conducted under laboratory conditions by static bioassay. Snail veliger was collected and divided into 8 concentrations by logarithm method with 5 replications per each. Each replication was conducted in 1 liter beaker with 25 ppt. of marine water. Stocking density was set at 2 veligers per ml. All test units were input with marine water at 25 ppt. of salinity. After 2 days exposure, Aquabosso™ 50 concentration of 0.3848 ppm was caused 100% mortality. The median lethal concentration value is 0.3140 ppm (0.2900 - 0.3420 ppm) on the veliger which is half of lethal concentration on adult creeper shell snails.

IMPROVEMENT OF SURVIVAL RATE IN THE *Vibrio harveyi*-CHALLENGED PACIFIC WHITE SHRIMP (*Penaeus vannamei*) AFTER PRE-EXPOSURE WITH FORMALIN-KILLED BACTERIA

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Recent studies have reported some degree of specific memory in shrimp. Pre-exposure of shrimp to a specific pathogen can improve protection to the pathogen by enhancing immune capacity. The goal of our study is to develop a strategy to improve shrimp survival rate after bacterial infection by shrimp-pre-treated with formalin-killed bacteria. The effect of pre-exposed dosage and administrative frequency of the formalin-killed bacteria were investigated. Our results by the priming or pre-exposure of two doses of 10^5 formalin-killed *Vibrio harveyi* (FVh) cells/g shrimp with an interval of 6 h via intramuscular injection could reduce significant mortality (42%) in the primed shrimp, compared to non-primed shrimp (100%) after *V. harveyi* challenge. The degree of enhanced immune protection was correlated with the significantly increased of total hemocyte count (THC) and granular cell count (GC) of the primed shrimp, compared to non-primed shrimp. The specificity of the priming response using two closely related *Vibrio* bacteria (*V. vulnificus* and *V. parahaemolyticus*) were examined. Shrimp pre-exposed with formalin-killed *V. harveyi* or FVh can improve the survival rate of shrimp challenged with *V. harveyi* to be 53%. In contrast, shrimp pre-exposed with FVh and challenged with either *V. vulnificus* or *V. parahaemolyticus* resulted in 100% mortality. Taken together, our results suggest that shrimp immunity can be activated by pre-exposed to the bacterial pathogen with some degree of specificity to the type of pathogen. The knowledge from this study will lead us to design a strategic approach to control bacterial disease outbreak in shrimp industry.

SCALE DROP DISEASE INFECTION ON MARINE CAGE CULTURE OF SEA BASS, *Lates calcarifer* BLOCH IN INDONESIA

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Aquaculture is promising aquatic business of both the world and Indonesian industry. Asian Seabaass in Australia known as Barramundi, *Lates calcarifer* Bloch is high valuable fish and therefore a very active aquaculture industry in South Asian countries including Indonesia.. Movement of aquatic animals with a high market value such as Asian Seabass is necessary for development of aquaculture on both subsistence and commercial level. However, the introduction of new species increases the probability of introducing new pathogens, which can have dirconsequences on aquaculture. Since 2010, mass mortality frequently occurred in sea cage culture of Asian Seabass. The gross sign of dead fish was some scales of fish are drop and this pathogen infected to fish at size of juvenile up to adult (3.0 to 2000 gr). Sample of cultured Asian Seabass were collected from sea cages located at Batam, Lampung and Bali coastal waters, and the only Batam sample have shown very weak, lost of appetite to eat and then moribund sign. PCR technique was use to analise the diseases of moribund fish from internal tissue organs of spleen, kidney, eyes and brain and then preserved on 90% ethanol for further analyses. The PCR method was held by using two pairs of primers for Megalocytivirus, Red Sea Bream Iridovirus (RSIV) and Scale Drop Disease (SDD) primers. The result showed amplified by 643 bp DNA band visualized on 1.5% agarose moribund fish collected from Batam was confirmed positively infected by Scale Drop Disease Virus (SDD) and negative for Red Seabream Iridovirus (RSIV).

SLUDGE CHARACTERISTICS OF BIOFLOC PRODUCED IN THE *INSITU* AND *EXSITU* BIOFLOC FED GIFT TILAPIA IN INTENSIVE POND CULTURE

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GIFT strain of Nile tilapia (2.6 ± 0.2 gm) were stocked at a density of 25 animals/m³ in 300m² ponds. Two treatments such as biofloc developed by insitu (T1) and exsitu (T2) methods was used to assess the sludge characteristics in triplicates for 120 days. Animals were stocked after fertilizing the ponds to develop floc with volume of 3ml and TSS of < 50 mg L⁻¹. The blowers were operated continuously to maintain the dissolved oxygen content in the saturation level. Floc volume reached a peak level on 150th day in T1 and 180th day in T2, there was a significant difference between these two treatments. Sludge quantity and the nutrient discharge such as nitrogen and phosphorus dynamics showed significant difference between T1 and T2. Sludge volume index showed higher values from 60th day onwards in T1 and there by the loss of ignition and residue on ignition also showed similar differences between the treatments. This study confirms the typical pattern of sludge characteristics in the nitrogen and phosphorous dynamics of the insitu biofloc production method and exsitu production of biofloc could be the viable method for adapting biofloc technology with limited nutritional fluctuations.

NEED OF ENZYMES IN AQUATIC FEEDS AND NUTRITION

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Exogenous enzymes are now extensively used throughout the world as additives in animal diets. The primary purpose of enzyme application in feeds is to improve digestion. Further, aquatic animals lack certain digestive enzymes during early development or throughout their life. In the case of fishes / shrimps lacking certain enzymes, application of these enzymes results in better utilization of nutrient fractions that are digested by the enzymes. The addition of enzymes in feed can improve nutrient utilization, reducing feed cost and the excretion of nutrients into the environment.

The bacteria present in the gastrointestinal tract of fish/shrimp are potent producers of proteolytic enzymes. They may also produce cellulase moderately. The adding of live microorganisms to diets to produce enzymes is possible in specialty feed applications.

Many enzymes have been used in fish/shrimp nutrition over the past several years which includes cellulose, (β -glucanases), xylanases and associated enzymes like; phytase, proteases, lipases and galactosidases. Enzymes in the feed industry have mostly been used for culture animals to neutralize the effects of the viscous, nonstarch polysaccharides in cereals and other food grains

Addition of exogenous carbohydrates enzymes to feed increase utilization of unavailable dietary carbohydrates. High levels of non-starch polysaccharides (NSP) such as cellulose, xylanase and mannanases

The anti-nutritional activity of phytic acid can be eliminated by the addition of relevant enzymes, for example phytase.

Another important anti-nutritional factor that can be addressed with feed enzymes is non-starch polysaccharides (NSP), present in the plant materials and found to reduce the performance of animals. Since the animals lack the intestinal enzymes for the degradation of non-starch polysaccharides, the supplementation of degrading enzymes in the diet will break down these anti-nutritive factors and result in better feed utilization.

Successful and sustainable aquaculture depends on economically viable and environmental friendly feeds. Enzymes have been used in aqua feed because they are natural products of fermentation and therefore pose no threat to fish / shrimp health and pond environment. Enzymes reduces high phosphorus output problems in pond environments. Enzymes can therefore play an important role in formulating eco-friendly aquafeeds. Currently, the use of enzymes is able to reduce fishmeal inclusion by around 5 per cent in most aquafeeds. This may help to reduce the demand for fishmeal from the aquaculture sector in coming years.

THE STUDY OF COMPLETE MITOGENOME SEQUENCE OF *Cinetorhynchus reticulatus* AND PHYLOGENETIC ANALYSIS

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The reticulated hinge-beak shrimp, *Cinetorhynchus reticulatus* (Okuno, 1997), is a common marine ornamental species. According to the classification of Malacostraca, *C. reticulatus* belongs to order Decapoda and family Rhynchocinetidae. The male and female shrimp can be identified by the size of the chela (Figure 1). About the sequence of mitochondria was still unclearly. In this study, the *C. reticulatus* were collected from Penghu, Taiwan. Total genomic DNA was extracted by QIAamp DNA Mini Kit. Total 5 Gb paired-end reads were generated by NextSeq sequencer. The CLCbio genomics workbench was used for trimming of raw reads and assembling of contigs. The locations of the protein-coding genes, ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) were identified by alignment with other Decapoda mitogenome and predicted by using MITOS web server and DOGMA. We reconstructed the phylogenetic relationships of 19 Decapoda species based on *cox1* genes with Maximum Likelihood (ML) criteria by using WEGA6. Bootstrap values (1,000 replications) greater than 70 % are shown at the branch nodes (Fig. 2). The majority of nodes had support values higher than 70% and 7 were 100% supported.



Figure 1. *Cinetorhynchus reticulatus*. (A)Male. (B)Female.

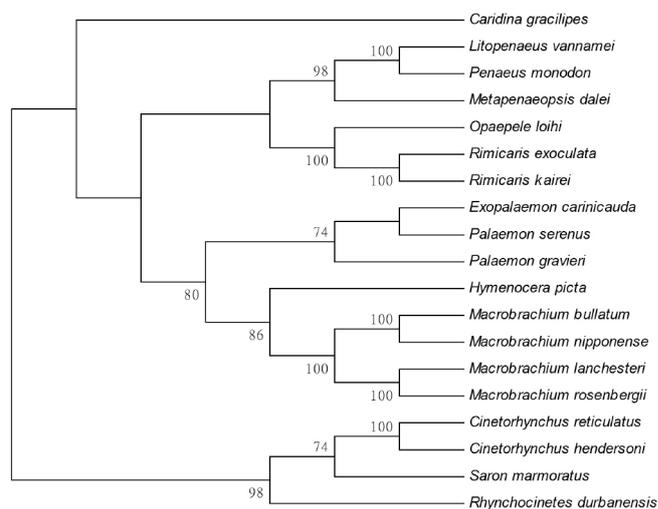


Figure 2. Phylogenetic tree of the *cox1* gene nucleotide sequence from the Decapoda.

HORIZONTAL TRANSMISSION AND NON-LETHAL SAMPLING METHOD FOR TILAPIA LAKE VIRUS IN TILAPIA

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Tilapia Lake Virus (TiLV) is an emerging infectious viral disease in tilapia. The mortality rate could reach 80-90% within 14 days since the outbreak begins. Although the virus has been reported in three continents including Asia, South America, and Africa, little information regarding the horizontal and vertical transmission of TiLV is still limited. In this study, we collected mucus and liver from tilapia with history of TiLV infection. Interestingly, TiLV-genomic RNA and infected live virus were presence in infected mucus. Within 3-7 days post infection, CPE formation was observed in E-11 cells inoculated with TiLV-infected mucus (Fig 1). Moreover, the segment 3 of TiLV was only amplified from infected mucus using reverse transcription polymerase chain reaction (RT-PCR) (Fig 2). The cohabitation challenge study suggested that susceptible fish may acquire infection within 7-10 days after expose to infected fish. In summary, our study revealed possible horizontal transmission of an emerging viral disease in tilapia. Moreover, mucus could serve as a non-lethal sample for TiLV detection. All of these information could be implemented for the control of this emerging virus. For example, strict biosecurity protocols such as removal of dead fish, quarantine of newly introduced fish will limit the morality by TiLV.

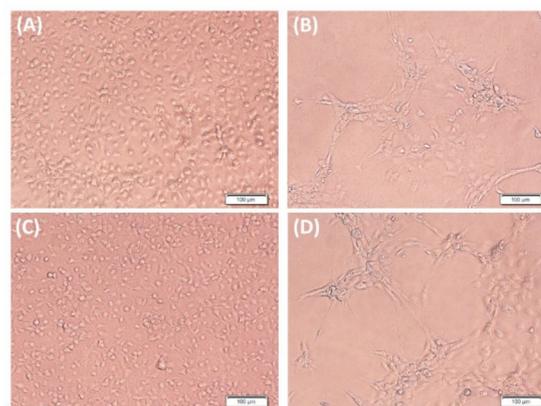


FIGURE 1. CPE formation in E-11 cells inoculated with TiLV-infected mucus. (a) normal appearance of E-11 cells and (c) cells inoculated with uninfected mucus (b) positive control (d) CPE formations were observed in E-11 cells inoculated with TiLV positive mucus; 3-7 days post infection.

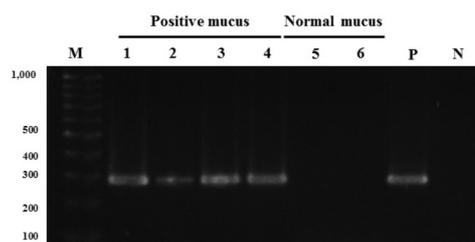


FIGURE 2. Amplification of specific PCR product in TiLV-infected mucus. Lane 1-4 RNA isolated from TiLV-infected mucus, Lane 5-6 RNA isolated from normal mucus. M = 100 bp marker, P = positive plasmid containing TiLV genomic, N = no template control.

BIOSECURITY MANagements TO CONTROL TILAPIA LAKE VIRUS AND BACTERIAL DISEASES IN TILAPIA FARMS

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Biosecurity is a standard practice that aims to limit the introduction and spread of pathogen in the production environment. For tilapia production, recent emerging of viruses and bacterial diseases such as Tilapia Lake Virus (TiLV) and *Streptococcus agalactiae* have been reported in Nile tilapia and red hybrid tilapia in different parts of the world. These emerging pathogens associate with high mortality of 80-90% within 1-2 weeks after the disease has been observed. To limit the catastrophic loss of infectious diseases, implementation of biosecurity and control measures should be applied at the farm, regional, national and international levels. Such concepts that could be employed including the screen of live fish and broodstock using PCR or real-time PCR, restricting fish movement, applying disinfectants as a standard practice, fry vaccination, and eliminating potential vectors. Figure 1 illustrates the common practice of tilapia farming in Asia with the open cages in natural water resources. Risk of pathogen entry should be identified e.g. the movement of infected fish into the farm and nearby culture area (Fig 2). Importantly, the pathogens may spread horizontal and/or vertical with infected fish. For example, a recent study by our laboratory suggested that TiLV could be detected in the mucus of moribund tilapia and that infected virus could spread through fish mucus until 12 days post infection. Therefore, removing of moribund and dead fish will reduce the risk of disease transmission and prevent the spread of pathogens in the farm and region. Overall, applying a standard of biosecurity plan and control measures at the farm and national level should limit the sources of disease outbreak.



FIGURE 1. Tilapia are produced in open environment including cage on the river and water reservoir, allowing them to expose to various pathogens.



FIGURE 2. Strict biosecurity and preventive measures such as the control of transportation, movement of live fish, prevention of potential vectors, and limitation of human activities will reduce the risk of disease introduction into the farm.

OPTIMAL DIETARY METHIONINE ON GROWTH PERFORMANCE AND FEED UTILIZATION OF FINGERING SNAKEHEAD FISH (*Channa striata*)

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Snakehead fish have long been regarded as a valuable food fish by the Asian people. It is classified as economically important fish species that has great commercial demands and the price is very expensive. It is an important of commercial aquaculture fish in Thailand, Vietnam, Philippines and Cambodia and makes up 13% of the marketable of freshwater fishes in India (Aliyu-Paiko et al., 2010). Production of snakehead fish in Thailand exceeded 4,300 metric tons in 2014 (Ministry of Agriculture and Cooperatives, 2016). While some of the feed provided to these fish, especially at the larger commercial farms, is formulated feed from commercial feed mills, many small farmers still use trash fish. From feeding trash fish, all nutrient leaching from trash fish and effect on water quality from uneatable feed. The formulated diets for further on growing of these species has also been accomplished but little information is available concerning the optimal dietary methionine of this species. Thus the present study was conducted to provide information on optimal dietary methionine for snakehead fish economically culture.

The feeding trial was investigated the optimal dietary methionine on growth performance and feed utilization of snakehead fish fingering (0.48 g per fish). The five isonitrogenous and isoenergetic diets (45% crude protein, 3.82 kcal g⁻¹, DE) were formulated to contained graded levels of methionine at 0.77 (control), 0.82, 0.92, 1.02 and 1.15 g 100 g⁻¹ dry diet. Each diet was randomly assigned to four replications of 30 fish kept in 200 liter aquaria. Feed consumption did not differ, rendering of all nutrients except methionine equal.

At four weeks, no difference was observed on growth performance and feed utilization ($P>0.05$) (weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, nitrogen retention and survival rate) when methionine was added at 0.77-1.15 g 100 g⁻¹.

The protease activity was significantly ($P<0.05$) increased up to 0.1256 ± 0.0423 and 0.1376 ± 0.0059 mU mg protein⁻¹ in fish fed diet methionine supplementation at 1.02 and 1.15 g 100⁻¹ dry diet. However, fish fed methionine supplementation diets did not affect the trypsin and chymotrypsin activity ($P>0.05$). The amino acid compositions was showed at highest level (1.41%) in fish fed diet containing methionine at level 1.15 g 100 g⁻¹ dry diet.

Non-linear regression analysis of SGR against intake methionine levels indicated that the estimated optimal dietary methionine for maximum growth of snakehead fish was 1.15 g 100 g⁻¹ of dry diet.

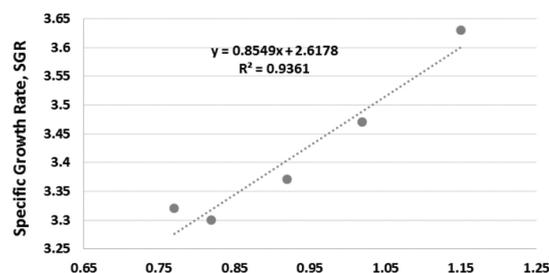


Fig.1 Non-linear regression using a 2nd order polynomial equation showed that 95% of relationship of specific growth rate (SGR) to different level of methionine

NURSERY OF MUD CRAB *Scylla olivacea* FED WITH ADULT ARTEMIA

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Rearing of mud crab crablet in nursery phase individually can produce a high survival rate. However, a factor that needs to be considered in this technique is how to optimize the growth of mud crab crablet in the limited space. One of the efforts that can be taken is to provide an optimal feed to maximize growth. A good feed for the early days of crab larvae is natural feed because it has a high nutritional quality and is easily accepted and digested by the larvae. Adult artemia is an alternative feed that is considered appropriate in the crablet phase because, in addition to having a high nutritional content, it is the appropriate size and readily available. This research is a preliminary study to determine the effect of the use of adult artemia as a feed on the growth and survival rate of *S. olivacea* crablet in nursery phase reared individually.

Four types of feed were used as a treatments with three replications each : (A) artificial feed for shrimp, (B) ebi (tiny dried shrimp), (C) trash fish, and (D) adult artemia. Crablet was reared individually in a glass of mineral water for 3 weeks. Feed was given twice a day (morning and afternoon) as much as $\pm 20\%$ of biomass. Frozen adult artemia and ebi shrimp were soaked in warm water before feeding to crablet. Molting crabs and molting interval times were also recorded.

Adult artemia treatment gave the best results for the average final weight, width and final length of carapace with 100% survival rate but not significantly different ($P > 0.05$). Natural food in the form of trash fish and adult artemia had a higher daily growth rate than the other two types of dried feed (artificial feed and ebi). Crablets fed crustaceans in the form of ebi and adult artemia have higher molting frequencies than artificial feed and trash fish with a interval between molts of 7 and 10 days respectively. This study showed that the use of natural feed in the form of adult artemia can be a good alternative choice for the nursery production of *S. olivacea* crablet as it produced the best growth overall with a higher molting frequency.

TABLE 1. Average of final body weight, daily growth rate, length dan final width of carapace, survival, and molting frequency of crablet fed the various dietary treatments

Parameter	Treatments			
	Artificial feed (\pm SD)	Ebi (Tiny dried shrimp) (\pm SD)	Trash fish (\pm SD)	Adult artemia (\pm SD)
Average of initial body weight (g)	0,033 \pm 0,005	0,023 \pm 0,005	0,020 \pm 0	0,023 \pm 0,005
Average of final body weight (g)	0,135 \pm 0,021 ^a	0,08 \pm 0,028 ^a	0,13 \pm 0,026 ^a	0,14 \pm 0,034 ^a
Absolute growth (g)	0,10 \pm 0,02	0,06 \pm 0,02	0,11 \pm 0,02	0,11 \pm 0,04
Daily growth (g/day)	0,004 \pm 0,001	0,002 \pm 0,001	0,005 \pm 0,001	0,005 \pm 0,001
Daily growth rate (%)	6,44 \pm 1,72 ^a	6,44 \pm 1,72 ^a	8,84 \pm 1,03 ^a	8,51 \pm 2,40 ^a
Survival (%)	66,66	66,6	100	100
Feed conversion ratio (FCR)	3,15	3,21	1,82	2,16
Average of initial carapace length (mm)	3,66 \pm 0,57	3,33 \pm 0,57	3,33 \pm 0,57	3 \pm 0
Average of final carapace length (mm)	6,75 \pm 0,35	5,75 \pm 0,35	6,66 \pm 0,57	7 \pm 1
Average of initial carapace width (mm)	5,66 \pm 0,57	4,66 \pm 0,57	4,66 \pm 0,57	4,66 \pm 0,57
Average of final carapace width (mm)	9 \pm 0 ^a	7,25 \pm 0,35 ^a	9,16 \pm 0,76 ^a	9,66 \pm 0,57 ^a
Molting frequency	1 \pm 0	2 \pm 1	0,66 \pm 0,57	2 \pm 0
Time between molting (days)	0 \pm 0	7 \pm 3,67	0 \pm 0	10 \pm 0,57

* Values in rows followed by the same superscript letter are not significantly different ($P > 0.05$)

PARTIAL REPLACEMENT OF FISH OIL BY PLANT SOURCE OILS IN DIET FOR HYBRID GROUPEL JUVENILE *Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*

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High dependency on marine fish oil (FO) as the major lipid source in the aqua feed has raised major concerns. Since then, many studies had been conducted using various vegetable oils (VO's) to replace FO. The expanding oil palm industry in Malaysia offers sustainable and cheaper supply of alternative lipid source than FO for the aqua feed industry. Similar with FO, palm oil is also prone to become rancid and susceptible to peroxidation thus make them unappealing to aqua feed industry. Therefore, the present study was conducted to investigate the replacement of fish oil by palm oil and other VO's (trial 1) and the effect of the oxidized palm oil in the diet (trial 2) for hybrid grouper juvenile (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*).

In trial 1, VO's used were crude palm oil (CPO), refined, bleached and deodorized, palm oil (RPO), crude palm kernel oil (CPKO), corn oil (CO), and coconut oil (COCO) in isoproteic (50%) and isolipid (12%) diet at 50% replacement of FO. The diet with only FO served as the control. The fish with initial body weight of 11.12 ± 0.07 g were stocked in 100L fiberglass tanks in triplicate groups for each treatment. The hybrid grouper juvenile were fed to apparent satiation twice daily for 12 weeks. While in trial 2, RPO was oxidized into three different levels and included in the diets at 50% replacement of FO (low oxidized, 14.73 meq/kg ; medium oxidized, 24.50 meq/kg and high oxidized, 36.10 meq/kg). Diet FO and diet RPO served as the controls. These diets were fed to triplicate groups of fish (initial weight, 9.91 ± 0.01 g) under experimental condition similar to trial 1 for 8 weeks.

The growth performance of fish fed RPO diet was highest and significantly higher ($P < 0.05$) in terms of final body weight, percentage of weight gain and specific growth rate than the fish fed CO diet and COCO diet. The feed utilization was not significant different among the treatments. The hepatosomatic index and intraperitoneal fat were also not influenced ($P \geq 0.05$) by the types of VO's. However the viscerosomatic index was significant higher in RPO diet. While in trial 2, there was no significant difference ($P \geq 0.05$) in terms of growth performance and feed utilization in all of the treatments. Fish fed the medium oxidized diet had the highest growth and lowest in the fish fed high oxidized diet. Besides, the RPO diet and low oxidized diet also showed higher growth compared with FO diet.

In general, all of the VO's used were possible to substitute 50% of FO in the hybrid grouper feed. However, the RPO diet showed the most promising growth performance and feed utilization. Besides, hybrid grouper can also accept oxidized diet less than 36.10meq/kg without reducing the growth and feed utilization.

EFFICACY OF Biotronic® PX TOP3 ON PERFORMANCE AND RESISTANCE OF NILE TILAPIA (*Oreochromis niloticus*) FROM INFECTION BY *Aeromonas hydrophila*

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Disease outbreaks are a major problem for aquaculture production and trade, as they cause economic losses for the production and trade of goods worldwide. Bacterial diseases, especially those caused by Gram-negative bacteria, are contained using disinfectants and antibiotics. However, the use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases. In addition to this, the awareness to the emergence of resistant pathogens has increased. Therefore, more environmental-friendly preventive alternatives have been investigated in the last years. Organic acids and blends of such acids have been accepted as an efficient tool to prevent and control disease proliferation in aquaculture. In this study the efficacy and the benefits of a commercially available calcium formate based product (Biotronic® PX Top3, BIOMIN, Austria) were investigated on growth performance and disease prevention in Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila*.

The experiment was conducted in 12 tanks (340 L) containing 40 pathogenic free *Oreochromis niloticus* each, in 4 replicates per treatment. The control group received a standard commercial feed, the experimental group 1 and 2 received the same feed supplemented with Biotronic® PX Top3 at an inclusion rate of 0.5 g/kg and 1 g/kg of feed. Growth parameters were recorded at week 4 and week 8 of the experiment. After 8 weeks 20 fish per tank were randomly chosen, transferred to 80 L tanks, starved for 24 hours, and then challenged by intraperitoneal injection with 7.5×10^5 CFU *Aeromonas hydrophila* per fish. Mortality was recorded daily. The challenge experiment was terminated 20 days after infection. Results are summarized in Table 1.

Statistical analysis indicated the differences in weight and FCR as statistically not significant. Differences in mortality on the contrary were found to be statistically significant for the experimental group 1 compared to control. Biotronic® PX Top3 used as feed additive for Nile tilapia resulted in higher average final weight and lower FCR. Biotronic® PX Top3 was found to significantly prevent the fish to succumb to bacterial infection.

The results obtained in this experiment clearly demonstrate that Biotronic® PX Top3 can be taken into consideration as preventive alternative to an extensive use of antibiotics, which contributes to the rise of antibiotic resistance in bacteria.

Table 1: Tilapia growth parameters and mortality rate after 8 weeks feeding and subsequent *A. hydrophila* IP challenge.

Parameter	CON	T1	T2
Body weight (g)	84.7	86.9	87.1
FCR	1.04	1.00	1.01
Mortality after challenge %	56.3	18.8	31.3

LIPID OXIDATION AND ITS IMPACT ON AQUAFEED QUALITY

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Feed quality can be described as the combination of physical and chemical properties that have a direct impact on both feed mill economics and fish farm zootechnical performance. The aim of this review is to examine the impact of lipid oxidation on feed quality. Due to their specific formulation and production processes, aquafeeds are very susceptible to oxidation. This is primarily due to the high inclusion level of fats and oils with a fatty acid profile rich in long chain polyunsaturated fatty acids (PUFAs). These are highly prone to oxidation, with DHA (C22:6) being five times more sensitive than linoleic acid (C18:2). Additionally, fish meals, the main sources of protein for aquafeeds, are especially vulnerable to oxidation due to their production process (use of high temperature) and composition. The use of other protein sources will also not reduce the risk of oxidation, as the most used alternatives are represented by rendered meals and blood meal. Rendered meals are prone to oxidation due to their production process, and blood meal contains high levels of reactive metal ions that act as a pro-oxidative compound for fats, vitamins and pigments.

Lipid oxidation has a direct effect on the nutritional properties of aquafeed. In a scientific trial, it was registered a reduction of final body weight and SGR, as well as an increase of FCR in juvenile carps fed diets containing oxidized oil (Table 1). This could be due to a reduction in metabolizable energy (ME) of oxidized feedstuffs. In literature is reported a reduction between 5 to 20% in ME in not stabilised fish meal compared to the one treated with an antioxidant (AOX). Another possible explanation is the change in the lipid profile of oxidized oils, especially the reduction of essential fatty acids (i.e. low Omega-3 in oxidised fish oils).

Good oxidative quality of aquafeeds can be achieved by using high quality and stable raw materials and by preventing the oxidation process during feed production and storage. Both targets can be achieved by including an effective AOX at the correct dose. The first criterion to select an AOX is to follow legal and market requests. The second criterion is the AOX's specific activity linked to their main features. An effective AOX needs to be easily and uniformly distributed to the substrate. Therefore, solubility of liquid formulas or particle size and distribution of active ingredients in dry formulas, represent the main features of a potential AOX. Moreover, it is recommended to prefer specific blends of active AOX with synergistic activity (BHA and Propyl Gallates heterodimers), including a chelator and with different composition depending on substrate to treat, over single ingredient AOX (Table 2).

Table 1. Effect of oxidized soybean oil (SBO) on juvenile carp performances.

	Good SBO	Oxid. SBO
Body weight g	22.8	18.6
SGR	1.54	1.16
FCR	2.63	3.16

Table 2. Antioxidant activity $(t-t_0)/(t_{BHT}-t_0)$ of BHA, BHT, their mixture and heterodimers

	Lard	Soybean oil
BHT	1.0	1.0
BHA	1.53	0.02
BHA+BHT	1.67	0.55
Heterodimer	1.66	1.47

FUNCTIONAL FEED ADDITIVES OF ENCAPSULATED BUTYRIC ACID AND MICROEMULSIFIED YELLOW CAROTENOID IMPROVE THE GROWTH, IMMUNE RESPONSE AND PIGMENTATION OF HYBRID CATFISH (*Clarias macrocephalus x Clarias gariepinus*)

BoonFei Tan*, Edwin Pei Yong Chow, Kah Heng Liong-Elke Schoeters and Widjaja Ning

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A 12-weeks feeding trial was conducted to evaluate the effects of encapsulated butyric acid and microemulsified yellow carotenoid on growth, immune parameters and their synergistic effect in pigmentation of hybrid catfish. In the experiment, the catfish was randomly divided into 12 groups of 15 fishes and then fed with four experimental diets containing 0.5kg/t of the encapsulated butyric acid, 0.7kg/t microemulsified yellow carotenoid, 0.5kg/t encapsulated butyric acid + 0.7kg/t microemulsified yellow carotenoid, or none of these supplements (control diet). The results showed that encapsulated butyric acid + microemulsified yellow carotenoid fed group gave the highest yellowness (b*) score of 18.43 in the back muscle and almost double the total carotenoid measured in the fish muscle (151.35 mg/kg) compared to the microemulsified yellow carotenoid fed group. This suggests that butyric acid in the diet had a synergistic effect on carotenoid absorption and pigmentation performance of catfish. The body weight of all treatment groups was significantly different from the control group and the catfish fed encapsulated butyric acid alone had the highest body weight gained followed by the encapsulated butyric acid + microemulsified yellow carotenoid fed group with an FCR improvement of 25 points and 13 points respectively over the control. There was no adverse effect on the immune system after feeding both butyric acid and carotenoid to the catfish and the immune parameters (number of leucocytes, erythrocyte, percent of hematocrit, haemoglobin and total protein) of encapsulated butyric acid + microemulsified yellow carotenoid fed group were improved compared with the other groups. In conclusion, encapsulated butyric acid, microemulsified yellow carotenoid and their combination have positive effects on performance and pigmentation in catfish aquaculture.

AQUATIC HEALTH MANAGEMENT IN SINGAPORE- PREPARATION FOR DISEASES PREVENTION

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Singapore Food Fish Farming Ecosystem- it has a small marine food fish farming community on both coastal and land based farms. The annual production from these farms contribute to about 4% of total seafood consume in this country, and the Authority has a target to increase food fish production to fulfil 15% of total seafood demand in Singapore.

There is a limitation of sea space and land resource in Singapore, and the encouragement to apply technology to enhance capabilities for intensifying production is important to increase productivity. Intensive farming model will in-conjunction the increased of production output that eventually lead to increase of diseases challenges.

A proper and complex aquatic health management implementation is necessary to be planned before massive production begin, to protect unnecessary production disruption due to diseases spreading and finally brings to a collapse of the farm. The process has to observe strict internal husbandry management and Area Management including, Bio-security, Water Treatment Design, Vaccination of fingerling, Acclimatisation, Husbandry and Environmental Control.

The proper aquatic health management has resulted in a successful farm in Singapore, that achieved high yield and successful up scaling program. The achievement for proper aquatic health management with vaccinated of fingerlings for tropical marine food fish species had an identical indication on the successful modelling of salmon farming.

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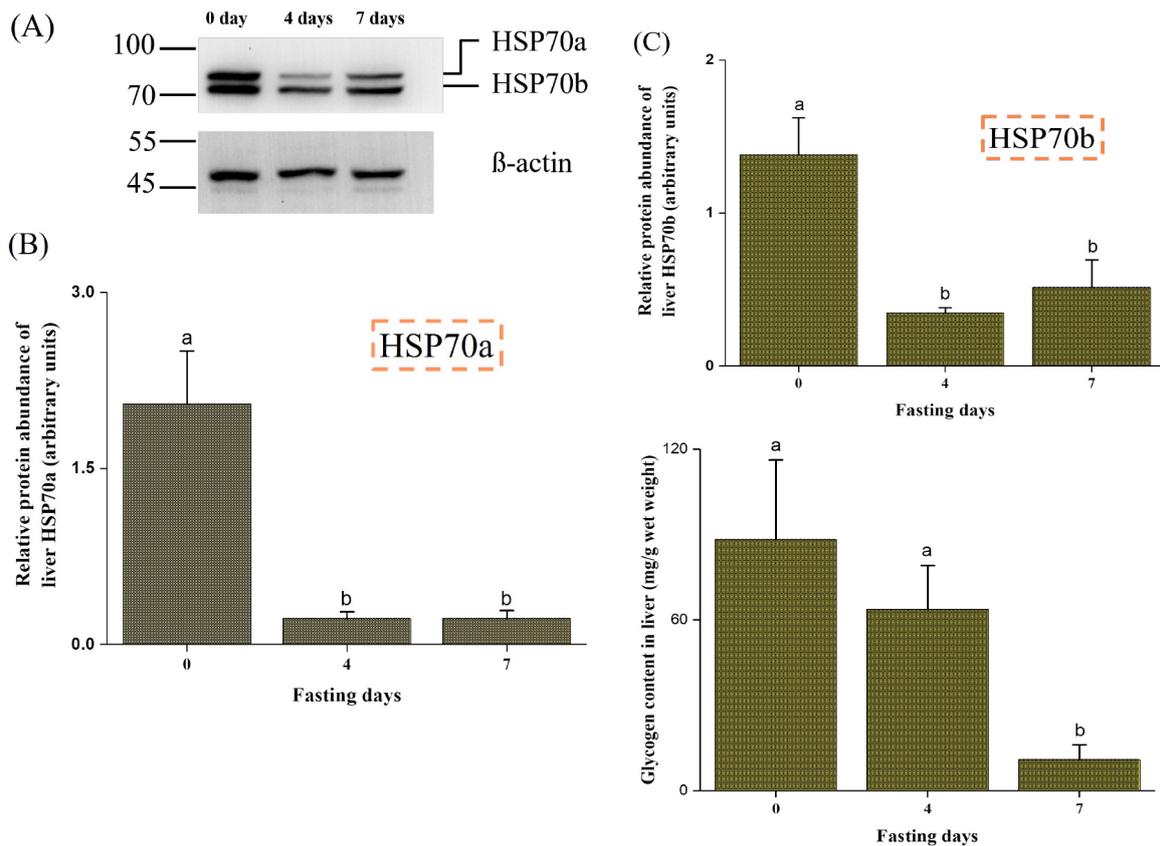
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PHYSIOLOGICAL STRESS RESPONSES IN FASTING ASIAN SEABASS (*Lates calcarifer*)

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In natural and aquaculture conditions, fasting is a common stressor which influences physiological regulation and homeostasis of fish. It is important to realize the physiological alteration in fish subjected to fasting with different duration for aquaculture practice. In this study, Asian seabass (*Lates calcarifer*) were fasted for 4 and 7 days to explore the physiological stress responses. The primary, secondary and tertiary stress responses were examined including plasma cortisol, glucose, sodium and chloride concentration, osmolality, hepatic glycogen content, heat shock protein 70 (HSP70) expression and hepatosomatic index. The preliminary results showed that glycogen content and protein abundance of HSP70 in liver significantly reduced after fasting for 4 and 7 days. However, no evident change of hepatosomatic index was observed. Taken together, the results of this work revealed that fasting stress disturbs multiple physiological regulation in Asian seabass. Indeed, there still needs further evaluation of the influences of fasting on physiological homeostasis of Asian seabass. Our study provided the information underlying the processes of physiological stress responses that may be developed as a way to estimate the effects of fasting stress in fish.



POTENTIAL APPLICATIONS OF BACTERIOPHAGES AND NATURAL PRODUCTS FOR THE CONTROL OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

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Acute hepatopancreatic necrosis disease (AHPND) is a *Vibrio* bacteria disease that has caused substantial mortalities, reaching to 100%, in farmed penaeid shrimp. This disease was first seen in China in 2009 and subsequent outbreaks occurred in Malaysia, Thailand, the Philippines, Mexico, and other Latin America countries, and in 2017, in Bangladesh and USA. Losses due to AHPND were estimated to be more than USD 1 billion per year. Therefore, the control measures to prevent catastrophic losses to the shrimp farming industry are important.

In this study, we describe the applications of bacteriophage and natural products for controlling AHPND. For bacteriophage pVP-1, its infectivity was tested in 22 strains AHPND-causing *Vibrio parahaemolyticus* (abbreviated as Vp_{AHPND}) strains from Asia and Latin America countries. This phage was able to infect 91% (20 strains) of the Vp_{AHPND} tested, and demonstrated strong bacteriolytic activity against 3 highly pathogenic strains. Furthermore, its effectiveness was evaluated in the laboratory challenge studies, shrimp treated with pVP-1 prior (prophylaxis) and after (therapy) the exposure to Vp_{AHPND} displayed significant protection, 25-50% mortalities, whereas the positive control groups (not treated with phage pVP-1, only exposed to Vp_{AHPND}) showed a 100% mortality. A second bacteriophage, pVP-2, isolated from the *Penaeus vannamei* also effectively lysed several Vp_{AHPND} and Vc_{AHPND} , and forming plaques in TSA+ plates. For the use of natural herbal extracts, approximately 140 products were screened for their inhibitory effects on PirA^{vp} secretion by an immuno-blot assay, the preliminary result showed that indirubin (part of a Chinese herb,) can inhibit the secretion of PirA^{vp}. Further studies are needed to evaluate the effectiveness of bacteriophages and natural products against AHPND in laboratory and field trials.

CHARACTERIZATIONS AND FUNCTIONAL ANALYSIS OF TYPE I INTERFERON RECEPTOR (IFNR) IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Grouper aquaculture was known as high economical species in aquaculture industry, raising interests among fish farmers. However, outbreak of diseases easily caused high death rate of grouper. In order to reduce death rate and increase grouper resistance to virus, mechanism of first line of defense against virus diseases in teleost fish through signaling of secreted cytokine (type I IFN) is studied. Previous result indicated up-regulation of gene expression level of grouper IFN and downstream Mx protein gene in response to virus infection. However, interaction of IFN to its receptor (IFNR) for signaling cascade function is unclear.

To investigate interaction between type I IFN to its receptor (IFNR), full length orange-spotted grouper IFNR1 was firstly cloned and identified. Phylogenetic analysis indicated *osgIFNR1* shared high homology similarities with other teleosts. Relative expression of *osgIFNR1* was determined using real-time qPCR in regards to interferon treatment using immune organ primary cell culture. Gene expression of *osgIFNR1* and downstream *osgMx* which act as marker was seen up-regulated in 4 hours post-stimulation indicating involvement of transcriptional level responses. We further used GF-1 cell line to observe interaction between ligand and receptor and carried out luciferase assay to analyze promoter activation to mimic signaling from ligand to downstream effector. The present study revealed effect of IFNR1 through up-regulation of gene expression level during 4 hours of interferon (IFN) treatment and up-regulation of Mx promoter expression level as activation of IFN signaling. This study contributed different insights in analysis of orange-spotted grouper IFNR role in type I interferon system.

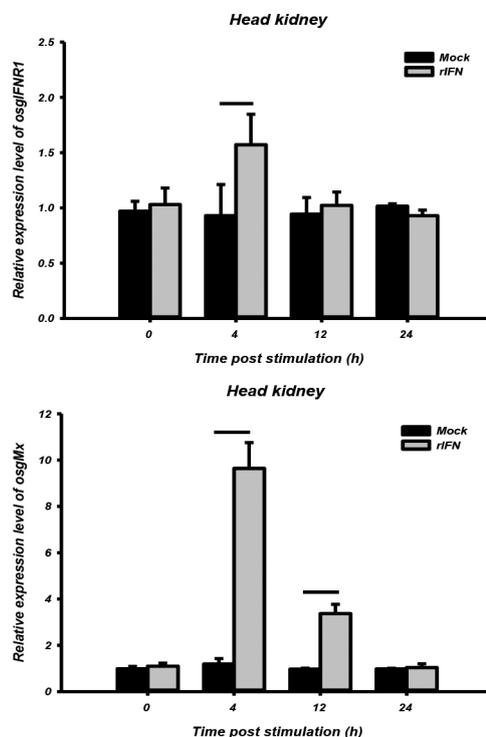


FIGURE 1. Gene expression analysis of IFNR1 and Mx in immune organ of orange-spotted grouper. Using beta-actin as internal control, up-regulated receptor and Mx expression in IFN treatment in orange-spotted grouper head kidney primary cell culture.

DEVELOPMENT OF GONAD MATURATION REAGENT BY ORAL ADMINISTRATION

Joan Tang Xiao Joe^{1*}, Abigail Elizur², Josephine Nocillado², Ming-Wei Lu¹

Department of Aquaculture, National Taiwan Ocean University

Department of Aquaculture Biotechnology, University of the Sunshine Coast

Gonad maturation was achieved out of season by the use of appropriate controlled photoperiods, temperatures, and abundant feeding. Nowadays, many research showed immature fish brought to maturity in the laboratory were spawned with suitable hormone injections and the time of spawning could be accurately predicted. Here we investigate whether oral administration is essential in order to achieve the totally same effect with injections of hormone into the fish. In our research, we utilized the luteinizing hormone from southern Bluefin tuna as a feed additive to feed the tilapia and control the temperature at the same time. After feeding the luteinizing hormone, the tilapia of sbtLH (southern Bluefin tuna Luteinizing Hormone) treatment group start to spawn and produce a numerous of larvae. We found that the gonad of sbtLH treatment group is five times mature of cold-treatment group and control group, and the expression level of cyp19a becomes highest in female tilapia. The increases of gonadosomatic index (GSI) in female following sbtLH treatment group, relative to the cold-treatment group and control group. The histological sections result showed the ovary of sbtLH group was become more mature than the other two groups. Therefore, the techniques developed of gonad maturation reagent by oral administration may have application to ornamental aquarium fish, mariculture and in more general research on marine fish reproduction.

APPLICATION OF ACIDIFIED DIETS IN THE REARING AND WEANING OF JUVENILE MARBLE GOBY *Oxyeleotris marmorata*

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Marble goby (*Oxyeleotris marmorata*) has strong preference for acidic food. This study was aimed to determine the growth performance of *O. marmorata* juveniles fed with the acidified diets (AD). Totally 2 experiments were done in this study. In Experiment I, the growth performance of *O. marmorata* fed AD were conducted through feeding trial. Five experimental diets with different pH levels manipulated by hydrochloric acid (HCl) were prepared; control (pH 6.0), AD 5.3, AD 4.3, AD 3.2, and AD 2.5. Triplicate tanks (100 L/tank) of wild-caught fish (TL~3.5cm) (19 fish/tank) were fed with each of these diets for 8 weeks. At week 4, the control, AD 5.3, and AD 4.3 were terminated due to the remarkably low total feed intake (TFI, 0–0.05 g) and weight gain (WG, -15.3 to -16.9%). Treatments AD 3.2 and AD 2.5 were continued until week 8; TFI (0.98g) of fish fed with the AD 3.2 was significantly higher ($P < 0.05$) than AD 2.5 (0.73 g). During this trial, the territorial behaviour of *O. marmorata* was noticed. Second feeding trial were done to evaluate the long term effects of feeding fish with AD for another 7 weeks. In second trial, 30 fish (30 replicates/treatment) were randomly selected from each of AD 3.2 and AD 2.5 treatment, stocked in 7 L aquaria individually. At the end of the trial, fish fed with the AD 3.2 showed significantly higher ($P < 0.05$) WG (34%) than the AD 2.5 (13%). No significant difference ($P > 0.05$) was observed in the TFI (1.22g, 1.08 g) and survival rate (96.5–100%). The WG results from the present study was very low compared to those available from literature (which were weaned and fed with the normal diets). The AD hence was not recommended for long term feeding. However, it is possible to develop a short-term weaning protocol using AD. Therefore in Experiment II, 4 protocols were designed to develop a weaning protocol for *O. marmorata* with AD (Figure 1). Ten fish (10 replicates/treatment; TL=7.7-9.3cm) stocked individually in 7 L aquaria were fed with 5 pieces of each diet followed the designed protocols for 4 to 6 weeks. At the end of experiment, the daily ingestion ratio (IR) (total number of ingested pellet/5) and percentage of fish at different IR was calculated; “Poor” (0.0-0.4) or “Good” (0.6-1.0) IR. Based on the results, none of the designed protocol could wean high number of fish to accept control diet. The IRs of fish fed with the AD 3.2 from treatment 1, 2, and 3 (“Good” IR level) increased and peaked at day-14, while the AD 4.3 (IR 0.84–0.94) in Treatment 1 from day 15 to 21. Similar findings were also observed on the percentage of successfully weaned fish. In Treatment 2, the IR (0.76 – 0.44) of fish fed mixing diets (AD 3.2: control, 3:2) was decreasing at day 15-21 after fed AD 3.2 for 2 weeks. However, the IR (0.08–0.8) for the mixing diets (AD 3.2: control, 3:2) in Treatment 4 was increasing from day 1 to 12. Apparently, weaning the fish with the mixing diets (AD 3.2: control, 3:2) as the starter would be more ideal than using the AD 3.2. Subsequently, weaning may be achieved by slowly reducing the acidified diet number.

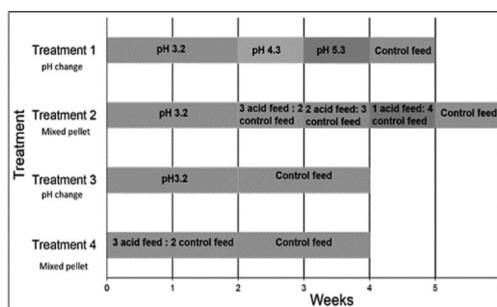


Figure 1. Weaning protocols designed in the present

REPRODUCTIVE PERFORMANCE OF PURPLE MANGROVE CRAB *Scylla tranquebarica* BROODSTOCK FED DIFFERENT MATURATION DIET

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This study was conducted to evaluate the reproductive performance of the purple mangrove crab broodstock fed with experimental diets supplemented with astaxanthin or/and DHA and natural food (NF). Four isonitrogenous and isolipids diets were prepared with supplementation of astaxanthin 500 mg/kg (FA), DHA 10 g/kg (FD), astaxanthin and DHA (FA+D) and diet without astaxanthin and DHA (Fcon). NF was comprised of trash fish (*Valamugil* sp), shrimp (*Penaeid* sp) and mangrove clam (*Polymesoda* sp). Broodstocks were purchased from local fishermen and reared in a recirculating aquaculture system for 50 weeks. Maturation performance of the broodstock was routinely monitored. Some matured female were identified and sacrificed for further examination and some were reared until spawning occurred. Colour intensity of the ovary was compared to DSM SalmoFan and Yolk Color Fan. The male was sacrificed at the end of the feeding trial to determine the sperm viability. Among all the treatments, the broodstock fed FA+D diet showed significantly higher maturation percentage, spawning rate, gonadosomatic index (GSI), oocytes diameter, ovary colour intensity (fan value), sperm viability, molting rate than broodstock fed Fcon diet ($P < 0.05$). Likewise, broodstock fed FA+D diet also resulted in significantly lower molt death syndrome (MDS) and mortality than specimen fed Fcon diet ($P < 0.05$). Broodstock fed FD and FA diets showed significantly better results than NF in term of GSI, oocytes diameter, sperm viability and zoea II survival rate ($P < 0.05$). On the other hand, the maturation, spawning, molting, MDS and mortality of broodstock fed FD, FA and NF diets were not significant differences among them. However, broodstock fed FD diet performed better than FA and NF groups. Likewise, results on the larvae showed the FA+D diet group had higher fecundity, hatching rate, total number of larvae produced and significant higher zoea II survival than Fcon group ($P < 0.05$). Overall female and male broodstock fed FA+D diet showed the best reproductive performance and larval quality, followed by FD and FA groups. The present study indicates that the supplementation of astaxanthin and DHA gave comparable or better maturation effect on the broodstock than natural food treatment. Inclusion of both astaxanthin and DHA capable to enhance reproductive performance of the purple mangrove crab broodstock.

USE OF NEXPRO™, A NOVEL PROTEIN PRODUCT FROM THE U.S. ETHANOL INDUSTRY, AS A PROTEIN SOURCE IN THE DIETS OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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The aquaculture industry continues to look for alternative protein sources that will allow sustainable growth and profitability. One such product is a novel co-product from the ethanol industry marketed as NexPro™ Protein Ingredient. NexPro is a 50% protein ingredient that is approximately 25% yeast, and 75% fermented corn products. NexPro has been tested in multiple species, including Pacific White Shrimp, Nile Tilapia, and Rainbow Trout with very positive results.

Two experiments were conducted utilizing Pacific White Shrimp, *Litopenaeus vannamei*, to evaluate the effect of feeding NexPro on shrimp growth performance and mortality. The first was a 5-week study to demonstrate the efficacy and potential range of inclusion for NexPro, while the second was a 6-week study designed to target its use in replacing higher value feed ingredients.

Four feeds were formulated with NexPro and corn starch being used to replace soybean meal in the first experiment. NexPro was included at 0, 10, 20, and 30% of the diet. A total of five 60L tanks per treatment were used, with 10-shrimp per tank. Average initial weight of the shrimp was 0.18 g. Feed was provided four times daily based on historical performance. No differences in final weight, weight gain percentage, feed conversion ratio, thermal growth coefficient, or instantaneous growth rate were observed. Survival rates were improved by adding NexPro to the diet, with survival rates of 84, 92, 94, and 94% for the 0, 10, 20, and 30% NexPro inclusions, respectively ($P < 0.05$).

In the second experiment, NexPro was included at 0, 6, 12, 18, and 24% of the diet, replacing a combination of fish meal and soybean meal, to produce iso-nitrogenous, iso-lipidic diets. Menhaden fishmeal, 12, 9, 6, 3, and 0% and soybean meal, 52.4, 50.2, 48.0, 45.8, and 43.6% of the diet were reduced with increasing inclusion of NexPro. Juvenile shrimp 0.25 g were stocked 10 per tank in 80L tanks utilizing four replicates per treatment. Feed conversion ratio for the 24% NexPro diet was poorer than for the control diet (0% NexPro; $P < .05$). No other differences were observed when compared to the control diet. Numerically the 6% and 12% NexPro diets had increased final weights and improved feed conversion ratios compared to all other treatments, but was only significant vs the 24% NexPro treatment. Survival rates were numerically ($P > 0.10$) improved with the addition of NexPro. Overall, NexPro has been shown to be acceptable for use in shrimp diets, even as a replacement for significant portions of the fishmeal in the diet.

Table 1. Growth performance of shrimp in experiment 2

Response	Control	6% Nexpro	12% NexPro	18% NexPro	24% NexPro	PSE
Final Wt, g	5.07 ^{ab}	5.37 ^a	5.13 ^a	4.60 ^{ab}	4.29 ^b	0.091
Weight gain, %	1837.7 ^{ab}	2065.7 ^a	1854.2 ^{ab}	1776.2 ^{ab}	1593.5 ^b	0.021
Survival, %	82.5 ^a	87.5 ^a	90.0 ^a	90.0 ^a	87.5 ^a	1.854
FCR	1.81 ^a	1.67 ^a	1.74 ^a	1.94 ^{ab}	2.14 ^b	0.036

THE EFFECT OF TAURINE SUPPLEMENTATION TO ALTERNATIVE DIETARY SOYBEAN CONCENTRATE USED IN FISH MEAL REPLACEMENT ENHANCES GROWTH PERFORMANCE, BODY COMPOSITION, HAEMOLYMPH PARAMETERS, IMMUNE RESPONSES AND TAURINE CONCENTRATION IN PLASMA OF JUVENILE WHITE SHRIMP (*Litopenaeus vannamei*)

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An 8-week feeding trial was conducted to evaluate the effect of taurine supplementation to alternative dietary soybean concentrate used in fish meal replacement on growth performance, body composition, haemolymph parameters, immune responses and taurine concentration in plasma of juvenile pacific white shrimp (*Litopenaeus vannamei*). A total of 600 apparently healthy of juvenile white shrimp with similar size (initial mean body weight, 1.19 ± 0.02 g) were fed triplicate with ten practical diets (39% crude protein) were formulated to contain graded levels (0, 25, 50, 75, and 100%) of fishmeal replacement by soybean concentrate with 1000 mg/kg or without any taurine added in diets. Soybean concentrate was increased in diets; methionine and lysine were increasing supplied to make the balance amino acid profile by calculated according NRC, 2011. Fish oil was increased added when level of fishmeal was decreased to maintain around 8% of lipid for all diets.

After 8-week feeding period, weight gain percentage (WG%), feed efficiency (FE), condition factor (CF) and muscle content % of white shrimp with taurine supplemented had the higher value than dietary without any taurine added while survival rate % and hepatosomatic index (HSI) showed the opposite results. Moreover, protein, lipid and ash content in whole body and muscle of white shrimp with taurine supplemented had the higher value than dietary without any taurine added. Furthermore taurine supplemented in diets could improve glutamic oxalacetic transferase (GOT) and glutamic pyruvic transferase (GPT), taurine concentration in plasma, whole body and muscle and immune responses such as total hemocyte count (THC), phenoloxidase (PO), respiratory burst (RB), total protein (TP), glutathione peroxidase (GPx) and superoxide dismutase (SOD) of white shrimp.

Base on those data, we suggested that fish meal can be replaced by soybean concentrate up to 50% with taurine added lack of any negative affected to the growth of Pacific white shrimp (*L. vannamei*)

INTRODUCING PRECONDITIONING PROCESS IN SINKING AQUA FEED PRODUCTION TO IMPROVE MILLING EFFICIENCY AND FEED MILL PROFITABILITY

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In sinking aqua feed production, pelleting is often the bottleneck and the limiting factor affecting the productivity of a feed mill. To improve the milling efficiency, apart from upgrading the milling facilities, one simple yet effective solution is introducing a preconditioning process in the pellet feed production. Preconditioning is a process implemented in the feed mixer after dry mixing. The objective of the preconditioning process is to prepare and optimize the mash feed and to achieve its target moisture level in the feed mixer for optimal pellet feed milling through conditioning, pelleting and cooling. During this process, a preconditioning solution is prepared by mixing water and a milling aid containing surface-active compounds, which is then added into a mixer to precondition the mash feed before it goes through steam conditioning.

A trial has been conducted in a feed mill in South East Asia on sinking pellet feed to evaluate the effect of preconditioning on milling efficiency, feed moisture and feed mill profitability. Two groups were evaluated in the study: Control - feed added neither with moisture nor surfactant and Treatment - Feed added with 1.5% preconditioning solution prepared by mixing water and a surfactant-based milling aid. Target feed moisture was 10.0 to 11.5%.

The trial results showed that preconditioning the feed with water and the surfactant-based milling aid improved overall milling efficiency. Improvement in throughput by 15.6% (from 6.4 to 7.4 TPH) and reduction in pellet mill energy consumption by 13.4% (16.05 to 13.90 kWh/MT) were achieved in the treatment group. The finished feed moisture in the treatment group was also closer to the target moisture level at 10.46%. With improved milling efficiency and closer to the target feed moisture, adopting a preconditioning process in the pellet feed production allowed the feed miller to enjoy a saving of USD 6.26 for every tonne of feed produced.

CHARACTERIZATION OF YELLOWFIN TUNA PEPSIN AND ITS APPLICATION FOR EXTRACTION COLLAGEN PEPTIDE FROM TILAPIA SKIN

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Collagen peptides are small bioactive peptides obtained by enzymatically hydrolysis of collagen, in other words, the breaking down of the molecular bonds between individual collagen strands to peptides. Collagen peptides are highly bioavailable. They act as building blocks, renewing bodily tissues, such as skin, bones and joints. It has been proposed that collagen peptides may act as a messenger to the cells and trigger the synthesis and reorganization of new collagen fibers, thereby supporting our tissue structure. The objective of this study was to characterize yellowfin tuna pepsin and its application for extraction collagen peptide from tilapia skin. Enzyme pepsin was extracted from stomach of yellowfin tuna with phosphate buffer (pH 7.0) at 4 °C for 3 h then mixed with 2 M acetic acid at 1:1(w/v) for 30 minute. The characterization of crude enzyme was determined. The characterization of tuna pepsin was similarly to commercial porcine pepsin. pH optimum and stability of yellowfin tuna pepsin were 2 and 2-4, respectively. Temperature optimum and stability of yellowfin tuna pepsin were 50 °C and 10 - 30 °C, respectively. This enzyme respond to calcium chloride and copper sulfate. Yellowfin tuna (3.51±0.29 unit/ml) and porcine pepsin (3.52±1.09 unit/ml) were applied for collagen peptide extraction at 55 °C for 0, 1, 2 and 3 h. Degree of hydrolysis (%DH) of tuna pepsin was similarly to commercial porcine pepsin. Both enzyme completely extracted collagen peptide within 1 h. Collagen peptide shown antioxidant properties (DPPH, ABTS and FRAPS). Yellowfin tuna pepsin can apply in food supplement production as well to commercial porcine pepsin.

USE OF BIOSURFACTANT IN DIETS FOR NILE TILAPIA *Oreochromis niloticus*: DIGESTIBILITY AND PERFORMANCE

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Fat is the main storage form of body energy and also provides fatty acids involved in several other functions in the body. The total lipids provide much of the daily energy required by the fish (15-20%). Many factors affect their use by fish, high lighting the ingredients and their proportions in the diets, the type and weight of the fish and also the processing. Whereas the digestibility of lipids is quite variable between fish and the fat is costly ingredient in a feed mill, justifying the studies to enable economically viable diets that maximize their use by fish. A commercial biosurfactant as a specific phospholipids source (Lysophospholipids) has been considered an absorption amplifier in animal nutrition, acting as a biosurfactant for fatty acids, fat-soluble vitamins, carotenoids and other dietary nutrients.

The trial was conducted in Brazil to evaluate the effectiveness of Lysophospholipids on Nile Tilapia, digestibility and performance. Diets were denominated Control (C), without addition of Lysophospholipids and Test (T), with addition of 0.1% of Lysophospholipids. The data obtained in each vessel with 15 fishes/tank were considered as a repetition.

The proximal composition of Control and Test diets are shown in the Table 1. Ethereal extract was 1% less in T vs. C treatment.

No effects ($P > 0.05$) on the productive performance of fishes were observed in the trial periods from 1 to 90 days (Table 2).

The inclusion of Lysophospholipids resulted in improvement ($P < 0.05$) the apparent digestibility coefficient (ADC) in dry matter, gross energy and crude protein, and greater availability of dietary calcium and phosphorus (Table 3).

TABLE 1. Proximal composition of experimental diets (based in natural matter).

	Control	Test
Dry matter (%)	95.19	94.48
Gross energy (kcal/kg)	4270.37	4265.64
Gross protein (%)	35.99	36.68
Ethereal extract (%)	5.46	4.46
Minerals (%)	9.01	9.60

TABLE 2 - Productive performance of Nile tilapia fed with control and test diets in a trial from 1 to 90 days. Initial Body Weight (IBW), Final Body Weight (FBW), Weight Gain (WG), Feed Conversion Rate (FCR) and Carcasses Yield (CY).

Treat	IBW	FBW	WG	FCR	CY
C	0.58	75.44	74.85	1.29	89.01
T	0.58	75.60	75.01	1.28	88.89

TABLE 3. Apparent digestibility coefficients (ADC) for dry matter (DM), gross energy (GE), crude protein (CP), ethereal extract (EE), mineral matter (MM), phosphorus (P) and calcium (Ca) for C and T diets.

TREAT.	ADC (%)						
	DM	GE	CP	EE	MM	P	Ca
C	55.77 ^b	86.02 ^b	92.53 ^b	88.47 ^a	81.27 ^b	45.51 ^b	19.07 ^b
T	65.38 ^a	88.72 ^a	94.01 ^a	89.60 ^a	84.81 ^a	55.35 ^a	29.96 ^a

PERFORMANCE OF WHITE SHRIMP *Litopenaeus vannamei* FED WITH NATURAL BIOSURFACTANT AS A SUBSTITUTE OF SOY LECITHIN

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Phospholipids have a role in the transport of shrimp hemolymph cholesterol and in the storage and mobilization of lipids in the hepatopancreas. Soybean lecithin has been used as the main source of phospholipids in commercial shrimp diets in amounts ranging from 1% to 3%. A commercial biosurfactant as a specific phospholipids source (Lysophospholipids) has been considered an absorption amplifier in animal nutrition, acting as a biosurfactant for fatty acids, fat-soluble vitamins, carotenoids and other dietary nutrients.

The objective of this study was to investigate the effect of the replacement of soy lecithin by a commercial biosurfactant on the performance of white shrimp, *Litopenaeus vannamei* (*L. vannamei*), grown in high density tanks and under controlled conditions of water quality.

The trial consisted in four treatments, using one as a control group (T-A). All feeds were formulated with similar nutritional ingredients and compositions, with only the inclusion of soy lecithin and Lysolecithin (Table 1).

The weight gain of *L. vannamei* showed no statistical difference between treatments after 70 days of culture (Table 2). Shrimp doubled body weight (123.6%) reaching 5.3 g on average in expenditure. In the studied period, there was a slow growth (0.33 g/week), but it occurred progressively for all treatments studied.

The results of the present study indicated that the inclusion of Lysolecithin in combination with soy lecithin in the feed promoted a better growth of *L. vannamei*. When Lysolecithin was used in combination with soy lecithin, no significant effects were observed when soy lecithin inclusion levels increased from 1% to 2%, meaning that Lysolecithin can replace partially soy lecithin. The use of Lysolecithin alone in diets, substituting soy lecithin, caused no negative effect on the performance of *L. vannamei*.

TABLE 1. Levels of inclusion of soy lecithin and Lysolecithin in experimental feed used in *L. vannamei* culture (kg/ton).

Treatments	Inclusion (kg/feed)		
	Lecithin	Lysolecithin	Bentonite
T-A	20.0	--	1.0
T-B	10.0	1.0	10.0
T-C	20.0	1.0	--
T-D	--	1.0	20.0

TABLE 2. Growth of *L. vannamei* fed with commercial feed containing soy lecithin, Lysolecithin and a combination of both.

Days of Culture	Treatments / Body weight (g)			
	T-A	T-B	T-C	T-D
0	2.94a	2.37b	2.57ab	2.34b
14	3.28a	3.00ab	2.80b	3.02ab
28	4.09a	3.51ab	3.40b	3.38b
42	4.80a	3.97b	4.11ab	3.80b
56	5.07a	5.09a	4.77a	4.44a
70	6.25a	5.61a	5.84a	5.65a

USE OF BIOSURFACTANT IN DIETS FOR NILE TILAPIA *Oreochromis niloticus*: DIGESTIBILITY AND PERFORMANCE

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HOLISTIC APPROACHES IN BACTERIAL DISEASE PREVENTION WITH FOCUS ON EMS/AHPND AND WHITE FECES DISEASE (WFD)

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White Feces Syndrome has been an idiopathic disease causing significant economic losses for shrimp farmers in Asia. The syndrome is characterized by transformation and sloughing of microvilli of hepatopancreatic tubule epithelial leading to accumulation of aggregated, transformed microvilli (ATM) in the tubule lumens (Sriurairatana et al., 2014), white fecal materials in the gut, and the floating feces on pond water surface. Since 2017, the ShrimpVet lab has been working on transmission models for WFD with focus on bacterial etiology. Based on initial result of these studies, several management strategies have been applied, including: algal bloom control, better feed management, probiotics application, better bio-remediation strategies, and functional diets. Those studies have proven to reduce WFD both in laboratory and grow out in pond conditions.

Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) have been characterized and determined with the pathogens since 2013. Since then, the ShrimpVet lab has made several attempts in order to reduce the impact of AHPND in productions. These includes: better hatchery, nursery, and grow out protocols. With regards to hatchery protocols, several improved practices have been applied including: PCR screening for all material (brood stock, live feed, Nauplii, and post larvae before harvest), better sanitation, better bio-remediation with focus on *Vibrio* reduction. The same sanitation, probiotics, and bio-remediation approaches have been applied in nursery and grow out practices. Several trials using “functional diets” with feed additives added in feed ingredients before extrusion showed positive result in both disease prevention and growth performance. An overall antibiotic-free farming protocol is achievable.

MODULATION OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN COBIA (*Rachycentron canadum*) FOLLOWING *Photobacterium damsela* subsp. *piscicida* INFECTION

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Photobacterium damsela subsp. *piscicida* (Pdp) is the agent of Photobacteriosis, a serious fish disease that produces an acute infection and high mortality in farmed cobia. It has been proved that regulation of pro- and anti-inflammatory cytokines play a central role in initiation of proper inflammatory responses against bacterial infection. Here we have analyzed the expression of pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6, IL-8, IL-12, IL-17 and IFN- γ) and anti-inflammatory cytokines (IL-10, IL-11 and IFN- α) in the head kidney during acute Pdp infection of cobia. Our data reveal that cytokines tested showed distinct patterns of expression. Th17-cell associated cytokine (IL-17) was in general expressed at low levels, while IFN- γ and IL-12 were coincidentally up-regulated at 3 and 48h after infection, and then decreased sharply at 96h. The inflammatory cytokines TNF- α and IL-8 almost showed a similar profile pattern of expression with a peak at 3h, while IL-1 β and IL-6 had a peak of expression at 48h after infection in response to the Pdp suggesting a switch in the inflammatory response. In contrast, Pdp infection induced the expressions of the anti-inflammatory cytokines IL-10, IL-11 and IFN- α . Together these results indicate a dominant Th1 cytokine response at initial stages of Pdp infection and show a surprising effect of Pdp infection on the dysregulation between pro- and anti-inflammatory cytokines.

SUITABLE STOCKING SNAKEHEAD (*Channa sp*) IN AN AQUAPONICS SYSTEM: ADAPTABLE AND SUSTAINABLE ECOSYSTEMS TO CLIMATE CHANGE

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Aquaponics: a great sustain combined model of fisheries and biosecurity. This model is also assumed to be green, clean and friendly to environment. Snakehead (*Channa sp*) were raised in plastic floating ponds with an area of 5 x 4 meter, 1.2 meter deep. Fish were stocked at 100 fish/m² (at weight around 5 to 7 gram/fish). Fish were randomly arranged into two experimental systems: S₁- Snakehead in aquaponics; S₀- snakehead in control ponds which were continuously aerated with about 20% daily exchange of water. Fish were fed commercial feed (30% protein) twice daily. The experimental period was 150 days. Each treatment was repeated three times. After 150 days of stocking, average weight of snakehead in S₁: 486.59±35.60 gram and in S₀: 434.63±42.75 gram. Survival ratio of snakehead in aquaponics system was 97.56% compared with only 71.40% in control ponds. Also, 1.25 feed conversation ration (FCR) of snakehead in aquaponics system was lower than 1.55 FCR of snakehead in control ponds. Water consumption in system S₁ should be more efficiency than in S₀ which need exchange 720 m³ of water (one pond). NH₃ level in S₁ were 5 times lower than in S₀, such as (0.01 – 0.03 mg/l) vs (0.05 – 0.13mg/l). It is excellent in sustainable and efficient aquaponics model of cultivation snakehead when climate changing and water resource scarce: saving water resources and reducing waste to pollute environment. This model can give a chance for people to produce fresh and safe food for their own requirements.

ISOLATION AND SELECTION OF AMMONIA AND NITRITE- METABOLIZING BACTERIA FROM BOTTOM SEDIMENT OF LOBSTER FARMING AT XUAN DAI BAY, PHU YEN PROVINCE

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This research was conducted to isolate and select bacteria capable of metabolizing ammonia and nitrite. For ammonia-metabolizing experiment, the results showed that there was a significant difference ($P < 0.05$) in the ammonia metabolism between the treatments and after 5 days of experiment, the ammonia transferring efficiency was over 90% in 3 treatments. In addition, 35 strains of ammonia-metabolizing bacteria were isolated, including five could metabolize ammonia in highest levels. These were identified as *Providencia stuartii*, *Alcaligenes faecalis*, *Massilia aurea*, *Sphingoacterium multivorum*, and *Micrococcus luteus*. For the nitrite metabolite group, after 24 hours of nitrite metabolizing, the results among treatments were statistically significantly different with the metabolizing efficiency of 95% in 10 treatments. In 16 isolates, 10 strains of the highest nitrite-metabolizing bacteria were identified by PCR and examined on BLAST. The strains identified were *Stenotrophomonas pavanii*, *Chryseobacterium gleum*, *Stenotrophomonas maltophilia*, *Delftia lacustris*, *Acinetobacter junii*.

Table 2. NH_4^+ decomposed efficiency of bacteria (%)

NH_4^+ decomposed efficiency	Treatment					
	T 01	T 02	T 03	T 04	T 05	Control
1 day	30,4	85,8	7,9	91,6	3,4	0
2 days	45,2	95,8	24,7	90,4	45,8	3
3 days	97,7	96,2	54,2	95,2	57,2	15
4 days	97,8	97,1	96,4	93,2	85,7	21,3
5 days	97,6	96,5	95,7	96,5	81,6	24,1

Table 3. Nitrite decomposed efficiency of bacteria (%)

Bacteria strains	Decomposed efficiency (%)					
	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours
C ₄ /1	15,04	30,08	63,35	85,46	92,30	93,21
C ₄ /2	12,25	26,54	60,34	85,07	91,65	95,15
CKT	16,90	34,26	64,87	89,77	94,56	96,62
TKT	27,73	35,58	74,86	89,19	93,58	95,73
C ₃ /1	9,52	19,96	56,64	83,10	90,76	95,31
C ₃ /2	11,45	26,88	57,74	83,68	92,33	93,03
T ₁	13,14	25,59	56,25	82,07	92,90	95,05
T ₂ /1	14,06	31,35	63,16	80,45	86,21	89,44
T ₂ /2	21,39	64,86	80,73	94,76	97,52	98,21
T ₃ /1	31,97	36,57	81,19	94,30	96,60	97,29
T ₃ /2	12,39	28,05	67,55	90,23	93,24	94,71
C ₁	13,73	66,54	77,01	88,65	91,44	93,53
T ₄ /1	17,96	52,52	76,17	91,41	93,91	96,18
T ₄ /2	7,93	21,46	58,30	86,05	93,68	94,62
C ₂ /1	9,80	34,64	64,95	87,06	96,86	97,99
C ₂ /2	15,53	24,67	61,90	87,94	96,16	96,16

DEGRADING ANTI-NUTRIENT FACTORS AND INCREASING BIOACTIVE COMPONENTS IN SOYBEAN MEAL USING LACTIC ACID BACTERIA ISOLATED FROM FISH INTESTINE

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The price of fish meal is greatly increased because of increasing demand and the limited supply for fish meal. The soybean meal (SBM) with the advantages of steady supply and high protein content is the best substitute for fish meal. However, soybean meal contains many anti-nutrient factors, especially the thermal stable factors of phytic acid and saponin, which can block the nutrient absorption of the organisms and cause the adverse effect on animal growth. A lactic acid bacterium, *Lactobacillus plantarum* FPS2520 isolated from fish intestine, was screened out to degrade phytate in SBM. A novel solid state fermenter was used to degrade phytate in SBM by FPS 2520. Factors of rotation (0, 1, 2 rpm) and ventilation (0, 0.5, 1.0, 1.5, 2.0 vvm) on phytate degradation were evaluated. Rotation with 2 rpm significantly increased the phytate degradation efficacy, although rotation did not affect saponin degradation, by FPS 2520 in a fermenter at 37°C for 72 h. Ventilation at 1.5 vvm did not affect bacterial growth; while it increased phytate degradation efficacy. The degradation percentages for phytate and saponin in SBM by FPS2520 were 97.60% and 68.25%, respectively, using two stages of incubation temperatures. This phytate-digesting strain could also greatly increase the isoflavone aglycoside content in the fermented SBM. The contents of daidzein and genistein (main isoflavone aglycosides) were increased by 270% and 478%, respectively after SBM fermentation.

Table 1. The glucoside and aglycon isoflavones content of the fermented soybean meal suspension by lactic acid bacteria strains FPS 2520 and different condition at 37°C during 72 hours, respectively.

Treatment	Daidzin (mg/L)%			Genistin (mg/L)%			Daidzein (mg/L)%			Genistein (mg/L)%		
	0	48	72hr	0	48	72hr	0	48	72hr	0	48	72hr
FA-S-B-4	105.1±2.0 ^a (100)	0.0±0.0 ^e (0)	0.0±0.0 ^d (6)	123.6±3.2 ^a (100)	1.7±0.0 ^e (1)	1.3±0.0 ^e (1)	4.3±0.1 ^b (100)	24.0±0.1 ^b (558)	25.8±0.7 ^b (600)	30.3±2.1 ^f (100)	323.9±0.8 ^b (1069)	336.3±10.1 ^a (1110)
FA-S-B-6	106.0±0.3 ^a (100)	1.0±0.0 ^c (1)	1.3±0.0 ^c (1)	122.0±0.5 ^a (100)	3.1±0.4 ^b (2)	0.6±0.1 ^f (0)	4.2±0.0 ^b (100)	24.1±0.3 ^b (574)	23.9±0.6 ^b (569)	30.2±0.5 ^f (100)	325.2±5.4 ^b (1077)	321.2±6.3 ^a (1064)
FA-S-B-4-60	68.2±4.9 ^c (100)	3.6±0.7 ^a (5)	4.9±0.7 ^a (7)	93.2±6.5 ^a (100)	7.2±5.6 ^a (7)	2.0±1.4 ^b (2)	7.4±0.7 ^a (100)	20.8±0.8 ^a (281)	20.0±0.8 ^c (270)	56.0±7.7 ^{ab} (100)	281.6±8.2 ^c (502)	274.4±8.1 ^d (490)
FA-S-B-4-180	65.0±2.4 ^c (100)	3.9±0.2 ^a (6)	5.9±1.9 ^a (9)	93.6±6.2 ^a (100)	7.7±4.7 ^a (7)	6.8±5.8 ^a (7)	7.3±0.2 ^a (100)	20.2±0.2 ^a (277)	20.3±0.5 ^c (278)	58.2±1.6 ^a (100)	192.0±14.2 ^d (330)	278.3±3.0 ^{cd} (478)

ASSESSMENT OF CARBON FIXATION EFFICIENCY OF THREE MICROALGAE

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Pavlova sp., *Chaetoceros muelleri* and *Thalassiosira* sp. are three commonly used dietary microalgae in aquaculture. Microalgae play an important role of carbon fixation in nature as well, and have fairly high economic value. These three microalgae have many features in common, such as microscopical cells, rapid growth rate, strong environmental adaptability and so on. By undergoing photosynthesis, transform plenty of CO₂ in the atmosphere into oxygen and organic nutrition, promoting trophic value and biomass for themselves. The present study was to estimate the CO₂ remover efficiency (RE) and elimination capacity (EC) of three microalgae after treated with different concentrations of CO₂ (0.07% (control group), 0.4% and 1.0%).

The RE and EC of the three microalgae were investigated after treated with different CO₂ concentrations (0.07% (control group), 0.4% and 1.0%). The RE (Figure 1) decreased significantly with increasing CO₂ concentrations, and the highest values were found at CO₂ concentrations of 0.07%. Nevertheless, the EC (Figure 2) was increased with increasing CO₂ concentrations. The EC of *C. muelleri* and *Thalassiosira* sp. cultured with 1% of CO₂ were higher significantly than the control group. No significant difference was found in EC and RE among three microalgae, when treated under same concentration of CO₂.

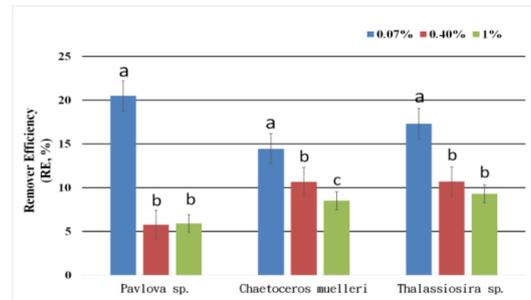


Figure 1. CO₂ remover efficiency of three microalgae with different CO₂ concentrations (0.07% (control group), 0.4% and 1.0%). Means with different letters are significant difference ($p < 0.05$) in the same species.

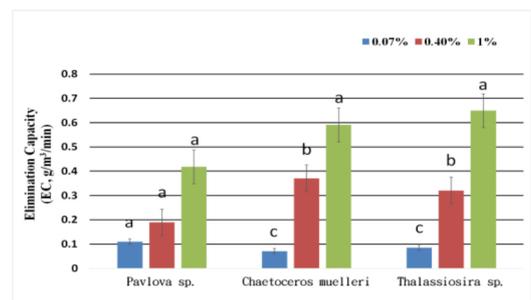


Figure 2. CO₂ elimination capacity of three microalgae with different CO₂ concentrations (0.07% (control group), 0.4% and 1.0%). Means with different letters are significant difference ($p < 0.05$) in the same species.

DEVELOP YY-SUPERMALES NILE TILAPIA BY MOLECULAR MARKER-ASSISTED SELECTION

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Nile tilapia (*Oreochromis niloticus*) is originated from Africa, it's easier to aquaculture than others species not only because of the adaptive capacity to environment but also omnivorous habit. Moreover, the aquaculture mostly give first place to male fish in the world not only can increase the total growth rate, but it also can prevent unwanted breeding before it reaches marketable size. In order to enhance the rate of all-male fish, people in the world more common to use mechanism of sex reversal (likes: feed or bath androgen) and crossbreed etc. The androgen, however, perhaps make fish have doubt of drug residue or food safety, and so on. The study will develop non-medication of natural production all-male Nile tilapia. We combine breeding, genomic chromosomes and molecular markers to speed up developing all-male strain. By using marker-assisted selection to determine the genotype between X and Y, without progeny test, which can reduce the time and space of breeding cost.

DISSECTION OF MEGALOCYTIVIRUS ISKNV INDUCED AKT/MTOR-MEDIATED AUTOPHAGY SIGNALING PATHWAY IN GF-1 CELLS

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Infectious spleen and kidney necrosis virus (ISKNV) mainly infects the spleen and kidney, leading to organs swelling and fish died, however, the relation between ISKNV and autophagy is still unclear. Autophagy is an important physiological mechanism that can degrade and recycle unwanted protein or broken organelles to regulate the intracellular sources of nutrients during development, malnutrition, or environmental stress, example for virus infection. In the present, we examined that iridovirus induced autophagy and signaling pathway related to Akt/mTOR activation. First, we found that ISKNV infection can induce autophagic process via morning with autophagy biomarkers such as increasing LC3-II ratio, and Beclin 2 degradation. Then, we found that ISKNV can induce Akt/mTOR autophagy signaling at early-middle replication stage (at 24-36 hpi) that could be blocked by Akt inhibitor (Figure 1).

Third, by treatment with Akt inhibitor, we found that can enhance autophagy process, which increase the host cell viability and further blocked the viral expression in GF-1 cell. Final, the Akt/mTOR signaling pathway can activate the downstream molecule ULK1 via phosphorylation on ULK1Ser757 and ULK1Ser555. Taken together our results suggest that ISKNV can induce autophagy process that reduces viral replication. Then, this process can activate the Akt/mTOR signaling pathway.

Figure 1. ISKNV and Akt inhibitor treatment induce autophagy.

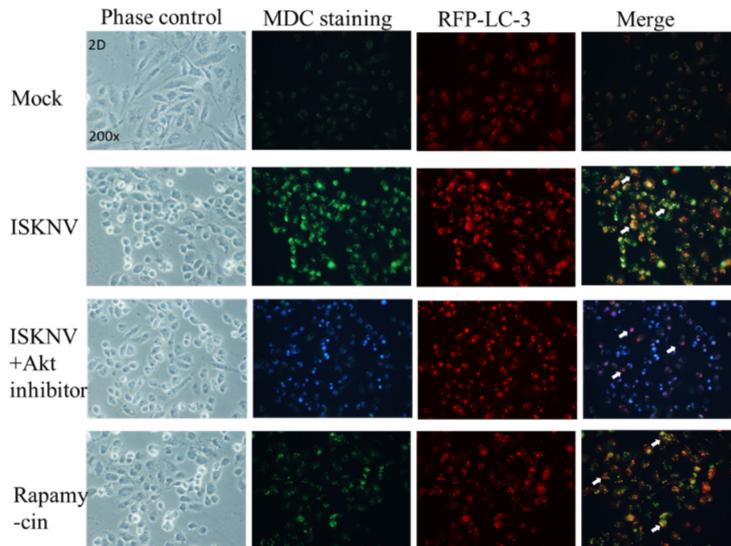
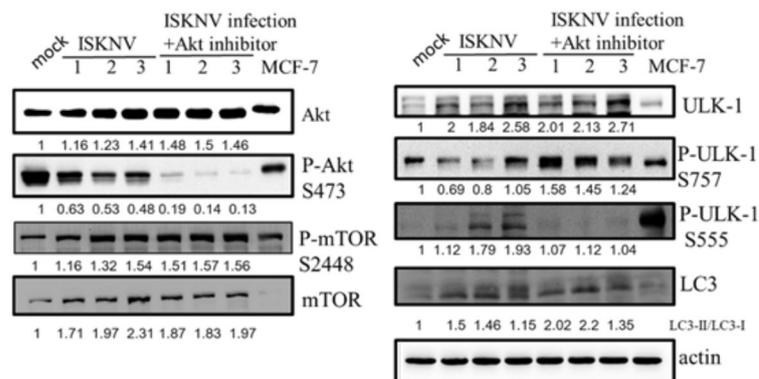


Figure 2. Identification of ISKNV-induced autophosome with ISKNV infection via autophagy markers by western blot analysis.



EFFECTS OF DIETARY CHOLESTEROL AND LECITHIN LEVELS ON GROWTH AND SURVIVAL OF JUVENILE AUSTRALIAN RED CLAW CRAYFISH *Cherax quadricarinatus*

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A 2*3 factorial experiment was conducted to evaluate the effects of dietary cholesterol and phospholipids levels on the growth, survival, tissue triglycerides, and phospholipids concentration. Basal diets supplemented with 0 or 0.5% cholesterol plus 0 or 2 or 4% phospholipids were fed to 120 individually reared shrimps with initial weight of 12.0 ± 0.23 g for 12 weeks. Survivals of all groups were higher than 89% and no difference was observed. There was no significantly difference in weight gain among all groups ($P > 0.05$) although the shrimps fed with combination of 0.5% cholesterol and 2% phospholipids have the highest WG. Other biological parameters are under investigating.

THE STUDY OF HYPOTHALAMUS GENES EXPRESSION OF THE DIFFERENT GONAD STATE IN THE PROTANDROUS BLACK PORGY, *Acanthopagrus schlegelii*

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The hypothalamus-pituitary-gonad axis controls vertebrate reproduction including fish. Black porgy had a stable maleness in first two reproductive cycles, and then half fishes sex change to female in third reproductive cycle. However, estrogen-induced female is transient and sex reversal is observed after E2 withdrawal in black porgy. This phenomenon indicated that the gender is not influenced by exogenous factors. In this study, we used transcriptome analysis to filter out genes with significant different between control fish (maleness) and testis-removal (femaleness) fish. To understand the possible role of these genes, E2 (high E2 levels) and aromatase inhibitor (AI, low E2 levels) were used to induced passive femaleness in juvenile black porgy. According to our qPCR results, testis-removal fish had lower beta-2-microglobulin-like (*b2m*) and interleukin 17N (*il17n*) expression than in control fish. These genes had no difference during gonadal differentiation in control fish. *b2m* had low expression in E2-treated fish compared with control group. *il17n* had no difference in E2-treated fish compared with control fish. According to our data, we suggest *il17n* may associated with endogenous maleness regulation.

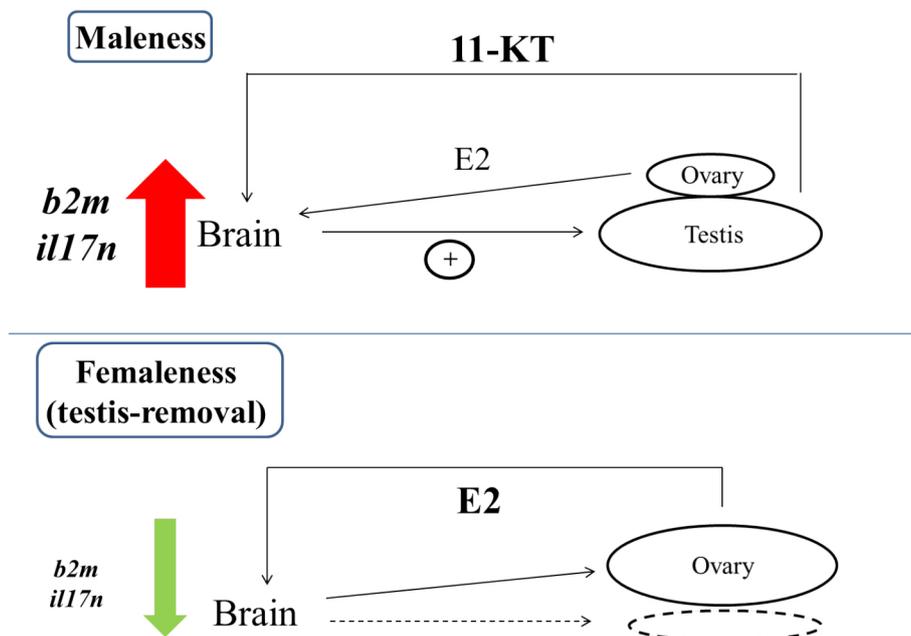


Figure 1. The expression profile of *b2m* and *il17n* in different gonad status.

COMPOSITION ANALYSIS OF NORTHEAST COAST OF TAIWANESE ALGAE AND OPTIMIZATION OF EXTRACTION CONDITION FOR LOW MOLECULAR WEIGHT POLYSACCHARIDES

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Macroalgae are abundant source of polysaccharides which demonstrate multiple bioactivities such as anti-virus, anti-coagulant and antioxidant. Low molecular weight polysaccharides (LMPs) from macroalgae demonstrate more effective and health benefit potentials than high molecular weight polysaccharides. In this study, we prepared LMPs from *Ulva fasciata* Delile (Ul), *Sargassum cristaefolium* (Sa) and *Gracilaria tenuistipitata* var. lieu (Gr) which harvested in the northeast coast of Taiwan. This study aimed to improve the yield of macroalgae LMPs which obtained by traditional high temperature (90°C, 1hr), acid (0.1N HCl) and base (0.05N NaOH), moreover high pressure (121°C, 105 kPa, 1hr) and ultrasonic-assisted (4°C, 20 mins) water solvent extraction (1:20 w/w). Subsequently, macroalgae solution were ultrafiltrated and collected within molecular weight cut-off (MWCO) less than 5 kDa then frozen-dry. The algae powder products contain about 3-5% of water, 1% of crude fat, 3-6% of crude protein, 22-37% of crude ash, and fairly 60-70% of carbohydrate. The highest yield of LMPs, 17%, was obtained by ultrasonic-assisted method. HPLC chromatograms show that the LMPs products of ultrasonic-assisted and high pressure methods were similar. Besides, base extraction and acid extraction increased purity of LMPs. In summary, ultrasonic-assisted and high pressure extractions increased crude yield of macroalgae LMPs; acid extraction and base extraction rise purity of macroalgae LMPs.

STUDY ON WATER AND SEDIMENT AGING PROCESS IN WHITE SHRIMP (*Litopenaeus vannamei*) CULTURE

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This study investigates correlations of nutrient dynamics in water and sediment of white shrimp (*Litopenaeus vannamei*) culture by analysis parameters of water and sediment qualities, and also gas productions (CH_4 , N_2O) of sediment. The stocking density of white shrimp was 103 individual/m², while no shrimp in the control group. The experiment period is 63 days and the result showed the average concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{NO}_2^-\text{-N}$ in water of the experiment group were significantly higher than the control group at day 28. The average contents of total nitrogen and total carbon in sediments were significantly higher in the experiment group than the control group at day 21. In addition, the N_2O production in sediment significantly higher than control groups at 35 days. The CH_4 was detected at day 35 in the experiment group, but not detected in the control. The results showed that the organic matter accumulated in the sediment, and the decomposing rates became lower at days 21 that leading to higher contents of organic matters in the sediment. It gradually influenced the water quality and also increased anoxic states in the sediment. In addition, positive correlations were found among the levels of $\text{NH}_4^+\text{-N}$ of water and sediment, sediment gas productions, sediment nitrogen/carbon contents, and also the growth of white shrimp.

SEAFOOD LABELING, SEAFOOD SAFETY AND ENVIRONMENTAL SUSTAINABILITY

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Taiwanese love seafood. According to the data from Food and Agriculture Organization (FAO), the annual average amount of seafood that Taiwanese consume is the fourth highest in the world, about 34kg per person. However, have you thought about where the seafood came from when it's on your dinner table? Were those fish or shrimps, or whatever else you may have, from the sea, or were they from freshwater sources, such as lakes and rivers? Were they from aquaculture or from a fishery? Were they caught in a sustainable way? Have they been raised in an environmentally friendly way? And the most important thing is, *is the seafood you are eating safe?*

Regarding the above questions, how much do you know about the seafood you eat? As the fishing skills improve, and as technology advances, and the global population increases, there are less and less fish in the oceans. Most of the fish is being threatened by overfishing, especially large predatory fishes. With the growth of the population, the demand for seafood has increased and the importance of aquaculture has also increased. However, the information that consumers have regarding their seafood is not increasing, especially in Asia. You may know the salmon on your dinner table comes from the fish farm in Alaska or Norway, but do you know if the tilapia on your plate comes from Tainan, Changhua or Vietnam? Do you know how the seafood has been farmed? With the increase in water pollution, do you still feel safe having seafood? How do you know the seafood you are having is safe?

This work will talk about the legitimacy of how seafood is labeled, as well as seafood safety and issues regarding the sustainability of our environment.

EVALUATE THE USE OF BIOFLOC TECHNOLOGY IN AQUAPONIC SYSTEM

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This study used biofloc technology in an aquaponic system and studied its effects on water quality, growth of tilapia (*Oreochromis niloticus*) and lettuce (*Lettuce sativa*). The aquaponic system consisted of fish tank, filtration tank, and hydroponic tank. Molasses was routinely added into the filtration tank of biofloc-treated aquaponic system for six week. The results indicated that levels of ammonia and nitrite in the biofloc treatment were significantly lower than the control since day 7. However, biochemical oxygen demand, turbidity, and suspended solids in the biofloc treatment were significantly higher than the control tank. No significant differences were found on growth rates, final weights, and survival rates for tilapia and lettuce between the biofloc treatment and the control. This study indicates the application of biofloc in an aquaponic system improved parts of water quality and have no effects on growth and survival of the fish and plant.

COMPLEMENT ACTIVATION LINKED TO BASIC CELLULAR PROCESS IS INVOLVED IN COLD ACCLIMATION OF TILAPIA

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Temperature is one of environmental factors that affect all physiological activities of teleost fish. Tilapia is an important aquaculture species and winter cold front often causes their large scale death. Understanding the underlined mechanisms involved in the cold acclimation of tilapia upon cold stress may be beneficial to their aquaculture. The mucosal immune system located in different compartments such as gut-associated lymphoid tissue (GALT) protects fish from the encounter of pathogen and various environmental stresses. In this study, intestinal transcriptomic profiling in juvenile was compared between wild type grown at normal temperature and wild type under cold stress or F2 cold-resistant (CR) tilapia grown at normal temperature using RNA-seq.

Common upregulated differentially expressed genes from wild type under 1 or 7 days 22°C cold treatment and F2 CR tilapia grown at normal temperature were subjected to Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes pathway analyses. Basic cellular processes such as cell cycle regulation, apoptosis regulation, response to oxidative stress, autophagy or lipid metabolism modulated by glycolysis, PI3K-AKT, AMPK, FOXO, mTOR pathways, and complement factors were the inferred genetic network involved in upregulated genes in the intestines of wild type under cold stress and F2 CR tilapia grown at normal temperature. Upregulated genes involved in PPAR signaling to modulate lipid metabolism was identified in wild type tilapia under 1 day 22°C cold treatment while increased gene expression concerned ECM-receptor interaction, insulin or calcium signaling further modulated PI3K-AKT pathway to regulate various basic cellular process was detected in F2CR tilapia grown at normal temperature.

cold stress promotes complement activation in the intestine by inducing expressions of various complement factors and proteases that in turn regulate key metabolic and signaling pathways for basic cellular process.

DEVELOPMENT OF PATERNITY TEST SYSTEM FOR *Potamotrygon* sp.

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Potamotrygon sp. is one of the earliest species of *Potamotrygon* which were introduced into Taiwan market. They are very popular due to their diverse texture and color. In recent years, *Potamotrygon* sp. with albinism has promoted the unmistakable trend, and the completed white *Potamotrygon* sp. becomes exceptionally demanded in the international market. In recent years, freshwater pearl ray with albinism has the highest market price, followed by their offspring. It is difficult to distinguish albinism offspring only based on the external body coloration. Therefore, the dispute often occurred in the process of selling. It is therefore urgent and important to develop a reliable and efficient DNA paternity test system for genotypic identification. In order to clarify the origin of pearl ray, mitochondrial D-loop sequence and nine polymorphic microsatellite loci (POMS-2, POMS-3, POMS-7, POMS-10, POMS-11, POMS-12, POMS-20, Pm-5, and Pm-17) were used in the study. Two families including W family (mother, father and one offspring) and S family (mother, father and four offspring) were collected from aquaculture farms, and their genotypes were analyzed. Moreover, a set of blind test including 5 fathers and 6 progeny were also examined. The results of mitochondrial D-loop showed that the mothers and their offspring (W1 and W3 ; S4 and S5, S6, S7, S8) have the same sequence, but the sequences of different families have 10 base pairs differences. The results of microsatellite revealed that the allele components of offspring are consistent with their parents in both W and S families; and the cumulated probability of paternity (CPP) within family members is over 99%. The blind test also successfully clarified paternity relationship. In conclusion, those techniques can clarify the origin of pearl ray and reduce the post-sale controversy.

A COMPARATIVE STUDY ON GENETIC DIVERSITY OF *Rachycentron canadum* BETWEEN AQUACULTURE AND WILD POPULATIONS BY MICROSATELLITE LOCI ANALYSIS

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The cobia, *Rachycentron canadum* is a high economic fishes owing to its fast growth and an excellent adaptability in marine cage culture. Most farms artificially propagate cobia from their own inbred offspring as the seed fish that could lead to considerably genetic problems eventually. Hence, a good genetic monitoring program can prevent the problems of inbreed due to recessive traits as well as reduce the impact of genetic decline. In the study, to understand the genetic deficiency in the aquaculture population of cobia, microsatellite loci were used to analyze the genetic diversities of the wild and aquaculture samples. All 90 specimens were provided from Tungkang Biotechnology Research Center. The wild samples revealed the highest mean number of alleles (na), effective number of alleles (ne), observed heterozygosity (H_o), and expected heterozygosity (H_E), while those of the inbred samples were the lowest. As a result, it is evident that genetic weakening occurred in aquaculture samples.

Table 1 Number (na) of alleles per locus, number of effective alleles (ne), observed heterozygosity (H_o) and expected heterozygosity (H_E) of cobia (N = 90).

Locus	na	ne	H_o	H_E
COMS-1	14	5.89	0.822	0.835
COMS-11	6	3.57	0.589	0.724
COMS-14	11	6.50	0.667	0.851
COMS-15	8	4.02	0.867	0.755
COMS-17	7	2.58	0.433	0.616
COMS-18	7	1.76	0.511	0.435
COMS-20	5	2.43	0.433	0.592
COMS-22	5	1.88	0.533	0.471
Rca 1-A 11	15	4.37	0.967	0.776
Rca 1-B12	5	2.04	0.600	0.512
Rca 1-E11	10	4.28	0.678	0.771
Mean	8.45	3.56	0.646	0.667

Table 2 Averages of na , ne , H_o , and H_E of wild and aquaculture samples from 11 microsatellite loci analyses of cobia.

	Wild	Inbreed
na	7.36 ± 3.11	5.46 ± 2.51
ne	3.88 ± 1.85	2.85 ± 1.24
H_o	0.712 ± 0.161	0.552 ± 0.234
H_E	0.695 ± 0.147	0.596 ± 0.172

THE RELATIONSHIP BETWEEN DNA METHYLATION AND *cyp19a1a* GENE EXPRESSION IN THE PROTOGYNOUS ORANGE-SPOTTED GROUPER, *Epinephelus coioides*

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Grouper is the protogynous (female-to-male sex change) teleost. Low estradiol levels (17 α -methyltestosterone, MT or aromatase inhibitor administration) were induced female-to-male sex change in grouper. However, sex reversal was observed after withdrawal chemical administration. The Cyp19a1a catalyzes the conversion of androgens to estrogens. The lower expression profiles of *cyp19a1a* gene in grouper could be an important factor in the sex change. In this study, we used orange-spotted grouper from different reproductive stages, including female, proto-male and the MT-treated-male, to investigate the relationship between DNA methylation and *cyp19a1a* gene expression. Our data showed *cyp19a1a* had lower methylation levels of promoter region and higher gene expression in ovary compared with the hypothalamus, liver and testis. Furthermore, methylation levels of *cyp19a1a* were significantly decreased in MT-induced female-to-male sex change. Thus, the DNA methylation could influence *cyp19a1a* gene expression during sex change in orange-spotted grouper.

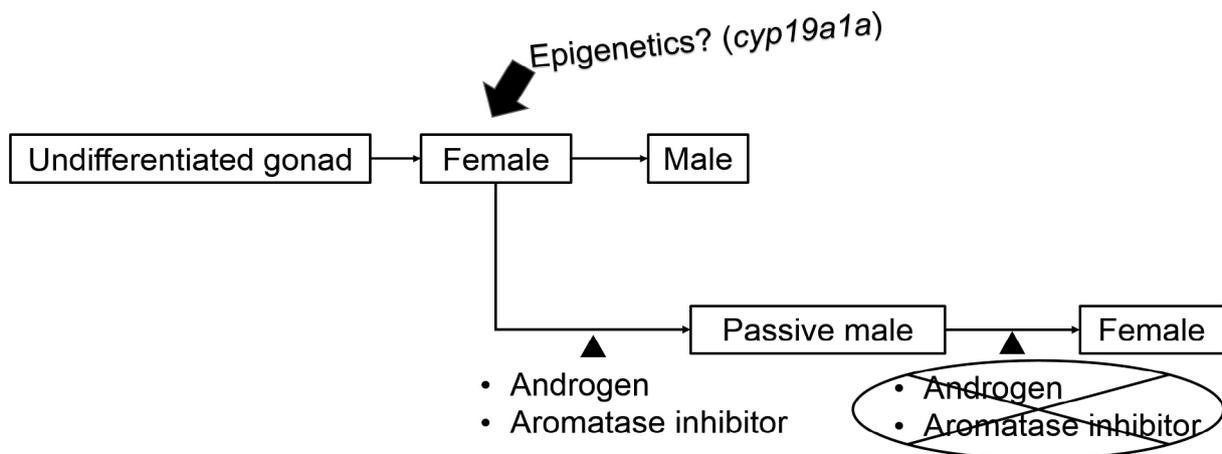


Figure 1. Epigenetics could influence *cyp19a1a* gene expression during sex change.

A DELETION AND MICROSATELLITE DNA MARKERS IN THE 3'-UTR OF *MEF2D* GENE ARE ASSOCIATED WITH GROWTH OF GIANT GROUPER (*Epinephelus lanceolatus*)

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Eleven polymorphic microsatellite DNA markers and 1 DNA deletion marker in muscle growth-related genes were applied to analyze 79 and 95 individuals in two populations (D79□D132) of giant grouper (*Epinephelus lanceolatus*) respectively, and analyzed the significance of correlation between genotype and body weight as growth phenotype. The result revealed that there are 9 and 6 DNA markers are significantly associated with body weight in the D79 and D132 populations of giant groupers, respectively. Five DNA markers including four microsatellite DNA markers, Ela348, Ela9156b, Ela8112a and Ela22361, and one DNA deletion marker Ela8112d are significant in both giant grouper populations and could be used as Type I DNA markers for marker-assisted selection of growth in giant grouper. The polymorphic microsatellite DNA Ela8112a and 51 bp deletion DNA marker Ela8112d are both in the 3'-UTR of giant grouper myocyte enhancing factor 2d (*mef2d*) gene. To further elucidate the effect of 51 bp DNA deletion in 3'-UTR of *mef2d* on gene expression, two muscle-specific expression vectors with *eAsRFP* cDNA as reporter gene linked with 3'-UTR of *mef2d* with or without 51 bp deletion were constructed, and injected into one-cell fertilized eggs of zebrafish. The result showed that red fluorescent protein expression of *eAsRFP* cDNA linked with *mef2d* 3'-UTR with 51 bp deletion was much weaker than that linked with intact 2 kb 3'-UTR. It suggests that the unique 51 bp deletion lost predicted binding site of Pbx1/Meis1 to interact with flanking MyoD in the *mef2d* 3'-UTR may reduce its expression in muscle to affect muscle growth of giant grouper and result in decrease of body weight in Ela8112d deletion heterozygotes.

EFFECTS OF I-TIAO-GUNG *Glycine tomentella* Hayata EXTRACTS TO REDUCE TRANSPORT STRESS RESPONSES ON 2 SPECIES OF ORNAMENTAL FISH

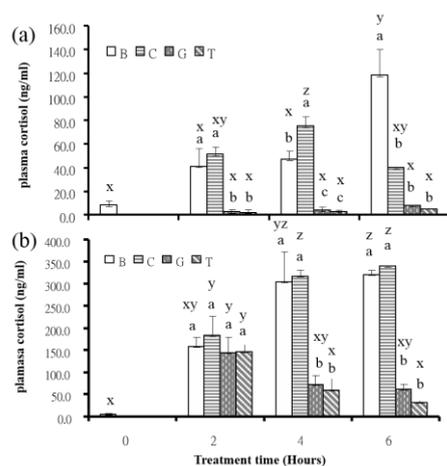
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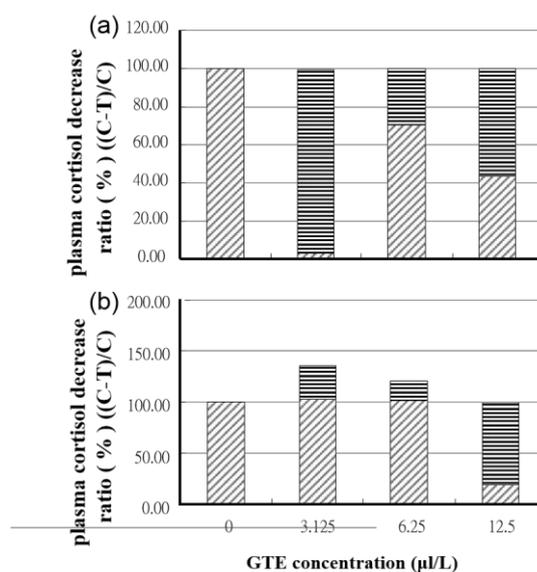
Fishkeeping market is a multibillion-dollar industry, a large number of ornamental fish is sold not only just in Taiwan but neighboring or even western countries. Reducing stress cause death of live fish transport is a economic potential consideration. In this study, we used an anti-stress function of I-Tiao-Gung extracts (GTE) treatments during transport for two ornamental fish (blood parrots and koi). It was to confirm which one is the best stress- indicator during transport.

In Experiment 1, fish were divided 4 group, stood or proceeded simulated transport, and added GTE or not. Packaged in PE bag with aeration water and oxygen, and then simulated transport for 0, 2, 4, and 6 hours. In Experiment 2, fish were divided 4 group for dose responses of GTE, 0, 3.125, 6.25, and 12.5 ppm were used for 4 hours. After these 2 experiments, to observe an anti-stress function of GTE treatments during transport on the two ornamental fish. The stress indicators included cortisol, glucose, lactate, anions, and lysozyme. In final experiment, fish were divided 2 group of GTE concentration, 0, and 12.5 ppm, and stood in the beaker 1 day without fed. Next, those proceeded simulated transport for 4 hours. Collecting the feces and water in the beakers, and the water in the bags for assay the feces weight and the NH₃-N levels in water.

In Experiment 1, the results showed that the cortisol levels was a great stress-indicator on both species (fig. 1.), and the glucose levels and plasma K⁺ was effective stress-indicator only on the blood parrots. In Experiment 2, we suggested that the best concentration of GTE for blood parrots and koi was 3.125 ppm and 12.5 ppm (fig. 2.), respectively. In the final experiment, we found the feces weight and the NH₃-N levels were non-significantly difference between the beaker mixture with GTE or not, but the GTE effective reduced the NH₃-N levels in bag water after simulated transport.



Figures 1. Comparison with the cortisol levels of (a) koi and (b) blood parrots with 4 treatments during 0-6 hours. (B) stood and didn't add GTE, (C) simulated transport and didn't add GTE, (G) stood and add GTE, (T) simulated transport and add GTE. The means in the column with different letter were significantly different. ($P < 0.05$)



Figures 2. Comparison with the decline ratio of cortisol of (a) koi and (b) blood parrots at 0-12.5 ppm GTE during 4 hours. The means in the column with different letter were significantly different. ($P < 0.05$)

TRANSCRIPTOME PROFILING ANALYSIS OF GROUPEL DURING NERVOUS NECROSIS VIRUS PERSISTENT INFECTION

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Nervous necrosis virus (NNV) infection has been considered a serious disease in farmed grouper. Particularly, the persistent infection model conducts the grouper into a carrier state that continues to spread the virus through spawning. This particular model makes disease control more difficult in the aquaculture industry. In the present study, we used RNA-Seq, a high-throughput method based on next-generation sequencing, to profile the expression of genes during the period of NNV persistent infection. We evaluated the transcriptomic changes in the brain tissue of grouper. The inactivated-NNV vaccine was used as a comparison group. Based on the differentially expressed genes, highly immune cell active signaling and surface receptor expression were triggered during persistent infection. The interferon-induced response was also highly expressed in the infected brain tissue. However, critical negative regulatory factors of T-cells, such as PD-L1 and LAG3, were up-regulated. The present transcriptome study revealed a comprehensive view of the state of NNV persistent infection and provided insights into the state of impaired NNV clearance in the grouper.

CONVERSION OF BIOWASTE CLAM SHELLS INTO WHITE-EMITTING PHOSPHORS FOR USE IN WHITE LEDS

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The increasing volume and complexity of waste associated with the modern economy poses a serious risk to ecosystems and human health. However, the remanufacturing and recycling of waste into usable products can lead to substantial resource savings. In the present study, clam shell waste was first transformed into pure and well-crystallized single-phase white light-emitting phosphor $\text{Ca}_9\text{Gd}(\text{PO}_4)_7:\text{Eu}^{2+},\text{Mn}^{2+}$ materials. The phosphor $\text{Ca}_9\text{Gd}(\text{PO}_4)_7:\text{Eu}^{2+},\text{Mn}^{2+}$ materials were synthesized by the solid-state reaction method and the carbothermic reduction process, and then characterized and analyzed by means of X-ray diffraction (XRD) and photoluminescence (PL) measurements. The structural and luminescent properties of the phosphors were investigated as well. The PL and quantum efficiency measurements showed that the luminescence properties of clam shell-based phosphors were comparable to that of the chemically derived phosphors. Moreover, white light-emitting diodes were fabricated through the integration of 380 nm chips and single-phase white light-emitting phosphors ($\text{Ca}_{0.979}\text{Eu}_{0.006}\text{Mn}_{0.015}\text{Gd}(\text{PO}_4)_7$) into a single package of a white light emitting diode (WLED) emitting a neutral white light of 5298 K with color coordinates of (0.337, 0.344).

IDENTIFICATION AND CHARACTERISTICS OF AGENT CAUSING INTERNAL WHITE SPOT DISEASE IN SNAKEHEAD FISH (*Channa striata*) IN COMMERCIAL FARM AT THE MEKONG DELTA, VIETNAM

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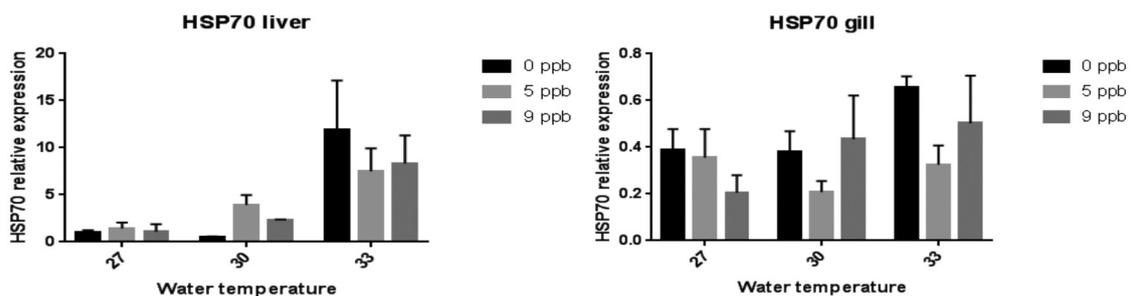
The bacterium *Aeromonas schubertii*, was isolated and identified as the aetiological agent causing internal white spot disease in snakehead fish (*Channa striata*) fingerlings, suffering from high mortality rates within commercial hatchery and commercial farms in the Mekong Delta, VietNam. Several clinical signs were observed such as: body of diseased fish are light haemorrhage and pale, specially the presence of whitish nodules in the kidney and spleen. The internal organs as liver, spleen, and kidney were swollen and haemorrhage. In many cases, in the liver, an irregular pale coloration is observed. The bacteria were isolated with tiny pale yellow colonies on tryptic soya agar after incubating at 28°C for 24 hours, negative Gram and short rod-shaped, catalase, oxidase and O/F positive, motile and fermentative. 16S rRNA gene partial sequencing were used to identify the all isolates like *Aeromonas schubertii*. An experimental injection challenge study was performed and fulfilled Kochs postulates with LD₅₀ values of 6.59x10³ and 8.12x10³ cfu per ml for isolates SCT30 and SCT95, respectively. Histological examination with two Haematoxylin & Eosin and Giemsa staining, were found numerous multifocal granulomas in internal organ tissues. Using fifteen antibiotics for susceptibility testing on twenty-five isolates that was included in the study.

ACUTE EXPOSURE TO WATERBORNE COPPER AND ELEVATED TEMPERATURE INDUCED THE CELLULAR STRESS RESPONSES IN SAILFIN MOLLY *Poecilia velifera*

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Urbanization, industrialization and increase in agriculture production have resulted in polluting numerous aquatic systems. Copper is one of the major contaminations in freshwater ecosystem. Furthermore, among the natural environmental factors, changes in water temperature can severely affect physiological regulation and biochemical reactions in ectotherms. It is therefore important to realize weather elevated temperature due to global warming would impose the harmful effects of copper on fish. *Poeciliidae* families have been employed as sentinel species in ecotoxicological studies through analysis of multiple biomarkers. In light of the background above, the aim of the present study was to investigate the associated impacts of acute exposure to environmentally relevant concentrations of waterborne copper and thermal challenge on cellular stress responses, protein quality control and antioxidative defense, in Sailfin molly (*Poecilia velifera*). Thus, protein abundance of heat shock protein 70, ubiquitinated proteins and the levels of aggregated proteins as well as enzymatic activity of superoxide dismutase, catalase and glutathione peroxidase in gills and liver of Sailfin molly were examined. In the perspective, according to the findings of this study may helpful, for regulation of the standard of the heavy metal concentration in river in order to fit to aquatic animals rather than just for human beings.



TRANSCRIPTOME ANALYSIS OF THE ACUTE PHASE RESPONSE of *Penaeus vannamei* TO WHITE SPOT SYNDROME VIRUS FOLLOWING FEEDING WITH DIET CONTAINING *Rhizoclonium riparium*

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The white shrimp industry is plagued with diseases caused by pathogens. To remedy this, a search for a plant-based immunostimulant continues to strengthen the non-specific immune system of shrimp since they do not possess an adaptive one. Pollen from the pine tree *Pinus tabulaeformis* is a good candidate as an immunostimulant and also as growth promoter which has been documented in some fish. The aim of this study was primarily to find the differentially expressed genes (DEGs) following injection of *Litopenaeus vannamei* shrimp with hot water extract of pine pollen.

Hot-water extract of pine pollen (PP) from *Pinus tabulaeformis* injected into *Penaeus vannamei* (ABW=4.18 g). Shrimp injected with 20 μ l PBS served as the control group. After 24h, hepatopancreas from the shrimp were aseptically dissected for total RNA extraction which was used for subsequent RNA-seq analysis.

Results show that administration of pine pollen (*Pinus tabulaeformis*) resulted in 57 DEGs with 28 up-regulated and 29 down-regulated genes. Up-regulated genes are involved in the immune response of the shrimp while those down-regulated are involved in biosynthesis of some nutrients.

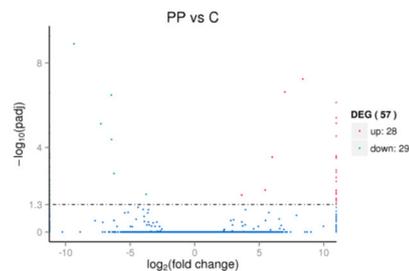


Fig. 1 Volcano plot

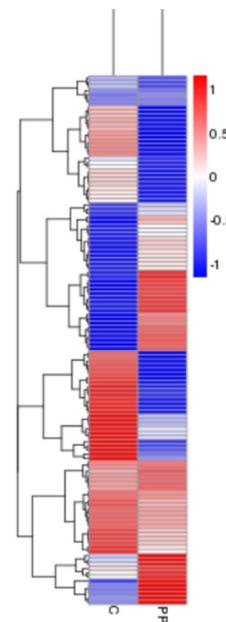


Fig. Cluster analysis of DEGs

METABOLOMICS PROFILING OF AHPND-INFECTED WHITE SHRIMP *Litopenaeus vannamei*

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Acute hepatopancreatic necrosis disease (AHPND) is a bacterial disease caused by virulent *Vibrio parahaemolyticus* (Vp) harboring a unique pVA plasmid encoding a binary toxin PirAB^{Vp}. This emerging disease has caused serious economic loss to the shrimp aquaculture industry in Asia. Although the causative agent for the disease is identified, the information about the molecular pathogenesis and host responses are still limited. To further explore AHPND pathogenesis, we applied systems biology approach to identify the significant pathway[s] in transcriptome and metabolome of AHPND-infected shrimp stomach. Firstly, we performed an immersion challenge followed by screening controls and AHPND-infected shrimp for expression of pVA plasmid and PirAB^{Vp} toxin as an infection indicator. The samples positive for infection and their corresponding controls were selected and subjected to UHPLC-QTOF-MS-based metabolomics profiling to obtain the metabolites in shrimp stomach. The differential expression of metabolites were compared to our in-house transcriptomics database of AHPND-infected shrimp stomach to obtain a first look of host responses in AHPND infection. By using this strategy, we found that several pathways were dysregulated in shrimp stomach during AHPND infection, including biosynthesis of secondary metabolites and microbial metabolism in diverse environment. This information would help in establishing a selection strategy for resistant shrimp as well as a suitable approach for biosecurity in shrimp aquaculture.

MYCOTOXIN CHALLENGE IN AQUACULTURE FEEDS

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The need for aquaculture feeds is growing rapidly and to keep growing the use of ingredients other than fish meal have to grow accordingly. Availability of fish meal, current and projected needs for aquaculture feeds and sustainability are forcing the use of alternative ingredients in aquaculture. One of the main focuses in using these alternative ingredients is making the required nutrients available for the animals to grow well. One challenge in particular that does not always receive the attention it should is the mycotoxin risk that is carried with the increasing use of plant ingredients.

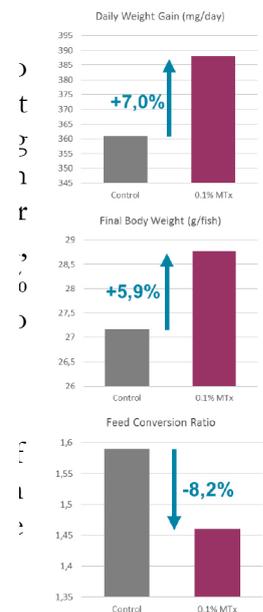
Mycotoxins are secondary metabolites produced by fungi present in grain crops, and are produced when fungi are stressed. Stress in this case can be changing temperatures, changing humidity's, harvesting, poor storage conditions, etc. These mycotoxins can be found in grain from any part of the world, present as a single mycotoxin or more commonly as multiple mycotoxins (poly-contamination) in the same batch of grain.

The effects on aquaculture are not yet fully understood, mainly because the risk of mycotoxicosis is relatively recent in aquaculture, and because on the farm level the clinical signs of mycotoxicosis are not yet easily recognized. In livestock understanding the effects of mycotoxins on the animals is much better understood and recognized, in aquaculture research is being conducted on various species and under various conditions.

Often raw materials are checked for only one or in some cases a few mycotoxins. If these remain under the "safe" levels, the raw material is automatically also "safe" to use. However a chronic subacute mycotoxicosis can influence the feed efficiency and therefore cause a negative economic impact on the farm's performance. Synergistic effects can increase the impact of single mycotoxins when they act in combination with other mycotoxins also present in the feed. Understanding the effects of not only the individual mycotoxins but also the synergistic effects mycotoxins can have, is very important.

A trial was set up in a research facility in the Mekong Delta, with two treatment groups: a control group being fed with a standard diet and a treatment group being fed the same diet with a mycotoxin binder. The diet was tested using a HPLC for 44 different mycotoxins, and 13 mycotoxins were found. 2400 fish were divided among each treatment with 400 (5,5 gram) fish per hapa and four replicates per treatment. Duration of the trial was 8 weeks. At the end of the trial, treatment groups showed a 5,9% increase in final body weight and a 7,0% increase in average daily weight gain. The food conversion ratio (FCR) also showed a decrease of 8,2%.

There is an increase in the use of ingredients that have a risk of mycotoxin contamination and these mycotoxins can have a negative impact on the farms performance. It is therefore important to understand the potential role mycotoxins play in aquaculture feeds.



EFFECT OF DIFFERENT LIGHT WAVELENGTHS ON THE BEHAVIOR AND BYSSAL ATTACHMENT OF THE PEARL OYSTERS *Pteria penguin* AND *Pinctada fucata martensii* JUVENILES

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The present study extends information on the effect of different light wavelengths on the locomotion and attachment in pearl oysters of the genus *Pteria penguin*, the most widespread cultured winged oyster for the production of valuable mabé pearls and *Pinctada fucata martensii*, the main species used for production of marine pearls, especially in China and Japan. Previously we reported that *P. penguin* juveniles undertake locomotion mostly in darkness, possibly an adaptation to reduce the risk of visual predation. Responses of pearl oysters to light have not been studied so comprehensively. For this species, light may provide information on its position in the water column and local environmental conditions.

The experiments were conducted in a darkroom, which was isolated from ambient light. The behavior assay consisted in four plastic tanks (40 x 30 x 25 cm) filled with 10 L FSW (25°C, 30 PSU). Sixteen oysters (18.8 ± 4.0 mm) were placed in each tank at 5 cm spacing and illuminated with either red, blue or white light-emitting diode (LED) lamps set approximately 50 cm above each one (350 lx). In order to separate the light, cardboard panels were placed between tanks and a tank without lamp was set as control. After 24 h, byssally attached oysters in each tank were confirmed by gently touching the juveniles with a pair of tweezers. Experiment was replicated twice under identical conditions for each oyster species. In order to measure the oyster movements, juveniles were filmed using three surveillance cameras equipped with an infrared illuminator. A total of 1440 still images were extracted from the raw video and merged using Adobe Premier CC 2014 software. Oyster movement was analyzed using the software Tracker 4.95 to estimate the distance traveled by a moving object.

The two oyster species exhibited dissimilar behavior in response to the different light colors. The mean distances traveled under white, blue, red and without light were respectively 0.2 ± 0.9 , 1.7 ± 4.7 , 0.5 ± 0.9 and 13.4 ± 18.2 cm in *P. fucata martensii* juveniles and 0.8 ± 2.0 , 4.1 ± 6.2 , 16.5 ± 12.8 and 24.4 ± 23.0 cm in *P. penguin*. The maximum distance traveled by a single *P. penguin* and *P. fucata martensii* juvenile was 66 and 70 cm respectively, and this was observed in darkness. In an overall distance-traveled comparisons *P. fucata martensii* traveled more than *P. penguin* in darkness and under red light (Tukey HSD test, $p < 0.05$). White and blue light colors were not significant between the two species. Total byssal attachment percentages in both species under darkness ranged from 60.4 to 64.1%. In general, *P. penguin* juveniles exhibited low byssal attachment percentages in all light-colored conditions (6.25-10.4%) whereas *P. fucata martensii* only in white-colored light. Percentages in *P. fucata martensii* under red and blue-colored light were 41.6 ± 7.2 and 45.8 ± 34.4 respectively. Results suggest that *P. penguin* and *P. fucata martensii* juveniles undertake locomotion mostly in darkness, possibly an adaptation to reduce the risk of visual predation. Moreover, increased byssal attachment behavior in darkness confirms the idea that juveniles select more successfully environments such as deeper and sheltered sites, which are better protected against predators and dislodgement. Byssal attachment in both species responded differently to wavelengths, suggesting the optimal light environment for byssal attachment is probably species specific in pearl oysters juveniles.

**AN IMMUNE-MODULATOR ALGAL EXTRACT TO FIGHT AGAINST DISEASES; THE
EXAMPLE OF EMS IN *L. Vannamei***

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MAINTAINING MINERAL BALANCE IN SHRIMP CULTURE SYSTEMS

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The Pacific white shrimp, *Litopenaeus vannamei* is reared in various regions in Asia including India. Though *L. vannamei* is a euryhaline crustacean, tolerant to salinities ranging from 0.5 to 40 ppt, the availability of various minerals in water in the right proportions is important to enhance productivity. The right balance of minerals helps in reducing the energy spent on maintaining osmotic equilibrium. The leading mineral products are positioned and sold for shrimp culture waters, irrespective of salinity or mineral composition of culture waters.

Objective of the study was to understand the mineral distribution across shrimp culture ponds and to develop suitable mineral supplements to increase the productivity in shrimp culture. Water samples were collected from 46 different locations including high saline and low saline culture systems. The salinity was measured using salinometer and the samples were analyzed for various minerals including sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), sulfate (SO₄), phosphorus (P), chlorine (Cl) and iron (Fe) using standard methods.

To achieve optimum performance at any given salinity, the proportion of minerals should be similar to the corresponding levels in seawater. Seawater with a salinity of 35 ppt has 0.38 ppt of potassium and hence similar optimal potassium concentration in water of 4ppt salinity should be 0.043 ppt. With this information, optimal levels of each mineral were calculated for each water sample based on its salinity and compared to the measured mineral levels in the same sample. It was found that magnesium was highly deficient (lower than the optimal requirement by 100-200 mg/L) in culture ponds above 15 ppt salinity, however the magnesium levels in low saline waters (<15 ppt) met the requirement. Potassium deficiency was found across all salinities (lower than optimal level by 33-53 mg/L). Levels of calcium and sulfate were found to be excess than required across all salinity ranges in culture waters; the reason may be due to practice of liming and gypsum application during pond preparation. Sodium and chlorine levels correlated well with salinity values in all the samples. Phosphorus and iron were found to be negligible in most of the culture waters. Based on these insights, two variants of mineral formulations were prepared for maintaining the mineral balance in low saline (<15 ppt) and high saline (>15 ppt) culture systems to achieve optimum performance in shrimp culture.

IPRS - IN POND RACEWAY SYSTEM TECHNOLOGY APPLICATION IN VIETNAM TO HELP FRESH WATER FISH FARMS TO OVERCOME PRODUCTION ISSUES AND DRAMATICALLY INCREASE YIELDS

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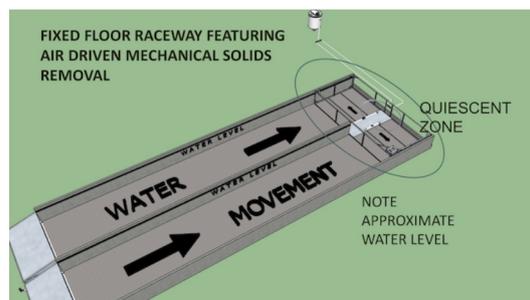
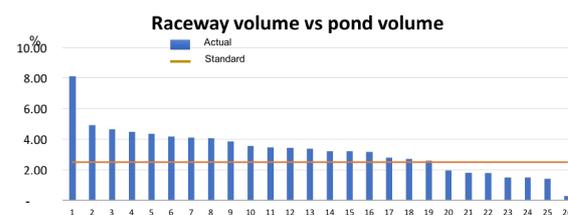
The In Pond Raceway System (IPRS) or Intensive Pond Aquaculture (IPA) technology was developed initially at Auburn University. IPRS is a technology package designed to allow dramatic increases of yield from ponds without increasing volume, based on water flowing constantly in an internally constructed raceway and the overall pond in a predictable manner. The U.S. Soybean Export Council (USSEC) then brought this technology to Vietnam to help freshwater fish farms in northern Vietnam area to overcome production constraints (for example lack of water resources, mortality issues and low yields).

Feedback from the Vietnamese aquaculture industry has been very positive. As of January 2018, there were over 100 IPRS cell unit constructed. Farmers will be able to achieve production yields from the IPRS technology that is over two to three times that from traditional pond culture approaches (37.5 MT of fed fish production per cubic hectare of water compared to 7-10 MT of fish production per hectare of pond area). This is mainly accomplished through better water quality management including constant aeration using an airlift system and physical waste removal. USSEC has a suggested standard technology package approach that should be followed and closely worked with farmers to help them for proper application.

Raceway volume vs pond volume (2.5%) is one of critical parameters to allow the pond to process waste from fish production in raceway.

While the pond acts as a biological filter, the IPRS technology allows solid waste removal prior to it entering the pond. This helps to maintain water quality for the long term and may provide additional revenue.

Raceway Design



SMART FLOATING FARMS – CHALLENGES FROM A FARMING PERSPECTIVE

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Closed containment flow through systems housed on floating structures have been in use for several years now at Singapore Aquaculture Technologies for producing sea bass. This technique has advantages in excluding the disease vectors believed responsible for the high rates of mortality seen in sea cage farming. Due to the higher running costs of diesel generated electricity the focus is on energy and labor efficient processes for filtration, disinfection, oxygenation, tank configuration and water pumping. This presentation describes some of the challenges in the implementation and running of a coastal smart floating fish farm close to an urban centre.



Smart floating farms concept ponders agriculture in a world of 9.1 billion



Javier Ponce [in collaboration with Jakub Dycha](#), conceptualized 'smart floating farms' as a vision to the future of agribusiness

IMPORTANCE OF ZOOPLANKTON INDUCTION IN WHITE LEG SHRIMP (*Litopenaeus vannamei*) INTENSIVE CULTURE PONDS

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Induction of growth of zooplankton in the shrimp ponds at the initial period of culture is very crucial to provide live feed for the animals when they are still small and not used to accept the commercial pellets. A study was conducted to investigate use of a commercial nutrient powder to induce growth of zooplankton in the white leg shrimp (*Litopenaeus vannamei*) intensive ponds during the first 20 days of culture. Nutrient powder was applied daily continuously at a dose of 1 kg per 100,000 PL during the first 10 days. Quantitative and qualitative samples of zooplankton were taken right before application of nutrient powder and every day to day 10 and at day 20 to determine species composition and abundance of zooplankton in the ponds. At the same time, shrimp specimens were also collected (10 for each pond) to dissect the digestive tract to assess the selection of zooplankton as live feed during the early stage of grow-out by the cultured shrimp. The results showed that the most abundant group was protozoa (20 species, accounting for 42%), followed by copepods (18 species, 38%) and rotifers presented only 2 species in which *Brachionus plicatilis* was the most dominant species in number. However, protozoa were hardly found in the digestive tract, instead rotifers (*Brachionus plicatilis*) and copepod nauplii were the most dominant groups encountered during the first 4-5 days and replaced by copepods afterward. Densities of zooplankton in the induced ponds were significantly higher than those in the conventional ponds. Importantly, growth of shrimp (length and weight) also had a similar pattern. Significant higher growth of shrimp was also obtained in the induced ponds. This indicated that zooplankton are very important to white leg shrimp as initial live feed during the first 10 to 20 days of culture in the intensive ponds.

RESEARCH AND PRACTICE ON CULTURING TECHNIQUES OF FRESHWATER PEARL MUSSEL *Hyriopsis cumingii* IN RECIRCULATING AQUACULTURE SYSTEM (RAS)

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Hyriopsis cumingii is the most important mussel species exploited for commercial freshwater pearl production in China. The traditional freshwater pearl culture is existing serious environmental pollution, and often affected by environmental factors. Recirculating aquaculture systems (RAS) have been widely used for fish and shrimp, but have not yet used in cultivation of filter-feeding bivalves. New techniques are developing in RAS of freshwater pearl mussel *Hyriopsis Cumingii*. A suitable environment for growth of culturing pearl mussel is provided by RAS. Water quality parameters, such as, dissolved oxygen, PH, temperature and light, in RAS are controlled by automatic control system. Through the adequate supply of microalgae from the special culture pools, the pearl mussels grow faster in RAS than in traditional pond culture, and also the quality of pearls from pearl mussels in RAS are better than in traditional culture ponds. Because zero discharge of culturing wastewater, RAS of freshwater pearl mussel will lead upgrading of freshwater pearl aquaculture in China.

THE EFFECT OF TEMPERATURE ON IFN-REGULATED ANTIVIRAL RESPONSE IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

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Due to the impact of climate change, it directly influenced the rearing temperatures and posed a threat to the global aquaculture. As fish are poikilothermic animals, the innate immune systems were deeply modulated by temperatures. To assess the effect of temperature on antiviral response of grouper, the interferons (IFN) signal transduction pathway is applied to investigate the grouper antiviral response at different temperatures. This result of study would provide information of the disease control strategy and development of fishery management in response to the impact of climate change.

In this study, we evaluated the antiviral responses in orange-spotted grouper at different temperature through investigating temperature-sensitivity of key IFN-regulated antiviral genes, such as type I IFN, Mx1, viperin and ISG15. Primary head kidney cells from orange-spotted grouper were cultured at different temperatures (20°C, 28°C and 36°C) along with immune stimulation like poly I:C or r2C I-IFN, and sampled at 0, 4, 24 and 48 h. These immune genes increased fast and significantly at 36°C, while they showed consistent high expression at 20°C at 48 h. Low temperatures could elongate the antiviral genes expression significantly while high temperature would result in the rapid and high expression. Taken together, it suggested temperature can influence on the patterns and duration period of fish antiviral responses.

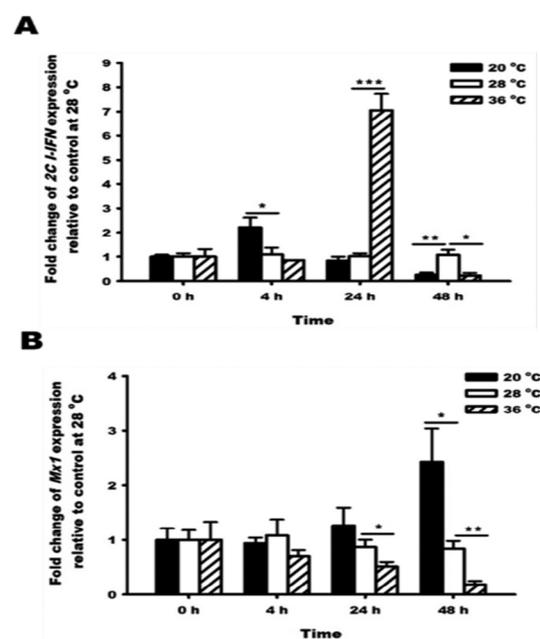


FIGURE 1. Expression analysis of grouper type I-IFNs and Mx1 in primary head kidney cells upon temperature modulation. All results represent means and standard errors (n=3), relative to the group at 28°C. Values were compared using t-test (*p < 0.05, **p < 0.01 and ***p < 0.001).

INTERACTIVE EFFECTS OF DIETARY ASTAXANTHIN AND CHOLESTEROL ON THE PERFORMANCES OF KURUMA SHRIMP *Marsupenaeus japonicus*

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A 56-day feeding trial was carried out to evaluate the interactive effects of chemically synthesized astaxanthin (Ax) and cholesterol on the performances of kuruma shrimp juveniles. 2×3 factorial designed experiment was conducted with six different experimental diets containing 2 levels of Ax (0 and 0.6 g kg⁻¹ diet) and 3 levels of cholesterol (0, 6, and 20 g kg⁻¹ diet), respectively.

The results indicated that the interaction between dietary cholesterol and Ax were not significant factor on survival, growth performances, feed utilization, and body proximate analysis. Only dietary Ax was a significant factor on growth performances (final body weight, body weight gain, and special growth rate), and the highest values were recorded by the group fed with Ax_{0.6} × CHO₆ combined diet. Only dietary cholesterol was a significant factor on survival, feed utilization (feed intake and feed conversion ratio), and crude lipid analysis of shrimp body. Further, significantly better pigmentation performances and Ax contents of different parts of juveniles (whole body, muscle, carapace, and head) were observed in higher Ax (Ax_{0.6}) supplemented groups. Meanwhile, the interactions of dietary Ax and cholesterol were also found on color reading and whole-body Ax contents parameters. In addition, juveniles fed with the diet containing higher Ax (Ax_{0.6}) supplementation three groups showed better performances on digestive enzyme (protease, lipase, and amylase) activities of hepatopancreas than the lower Ax (Ax₀) supplementation three groups. Dietary cholesterol, Ax supplementations and the interaction between these two additives were significant factors ($P < 0.05$) on lipase activity. Although the interactive effect was only found on the content of saturated fatty acid in neutral lipid, dietary Ax and cholesterol can significantly alter the composition of fatty acid in kuruma shrimp body.

It would be concluded that dietary Ax and cholesterol functioned interactively. The addition of cholesterol can significantly promote the Ax deposition in the tissues which may enhance the positive effect of dietary Ax. This enhanced positive effect was possibly due to the increasing levels of Ax found in tissues of kuruma shrimp.

ACUTE TOXICITY OF AQUASEPT™ 3.0 ON PACIFIC WHITE SHRIMP POSTLARVAE *Litopenaeus vannamei* AND NILE TILAPIA LARVAE *Oreochromis niloticus* IN LABORATORY CONDITION

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Aquasept™ 3.0 are effervescent tablets for the disinfection of hatchery water for fish and shrimp. The active ingredient in Aquasept™ 3.0 contains Troclosene Sodium, Sodiumdichloro isocyanurate (NaDCC), 3 gram releasing a free chlorine 1.97 gram per tablet. NaDCC is produced as a white crystalline powder or in granular form and a slight odor of chlorine. NaDCC is widely used at 5 mg per ml water for treating potable water and many industrial water systems. NaDCC is highly effective for cleaning ponds and convenient to transport. After dissolving NaDCC in water produces a series of complex equilibrium among a variety of chlorinated and non-chlorinated isocyanurates and free available chlorine (FAC) in the form of hypochlorous acid (HOCl) are released. In previous study, 0.77 ppm of NaDCC (0.5 ppm –FAC) could show a significant reduction of *Vibrio harveyi* in water within 30 minutes. This concentration could also provide zero residual FAC within 12 hours. According to the present Aquasept™ 3.0 label recommendation, fish and shrimp can be stocked into treated ponds within 2 or 3 days after its application to the water. However, it is interesting if this effective concentration could reduce the lead time the treated water can be used to culture fish and shrimp or it might even be possible to apply Aquasept™ 3.0 directly into fish and shrimp pond to reduce *Vibrio* load.

A study of acute toxicity of Aquasept™ 3.0 on Pacific white shrimp postlarvae (*Litopenaeus vannamei*) and Nile Tilapia Larvae (*Oreochromis niloticus*) was conducted under laboratory conditions using static bioassay. Pacific white shrimp postlarvae 15 (PL15) and Nile tilapia larvae (average 1 inch) were divided into 6 concentrations by logarithm method (5 replications/concentrations). Each replicate consisted of 10 test animals into 2-liter glass container, throughout with aerator. The result revealed that the concentration of Aquasept™ 3.0 that kill 100 percent shrimp and on Nile tilapia larvae in 48 hours were 3.47 and 1.51 ppm respectively. The 48 hr LC₅₀ of Aquasept™ 3.0 on shrimp and Nile Tilapia were 2.6276 ppm (2.4513-2.8149) and 1.2004 ppm (1.1606-1.2406), respectively. The safety period for Pacific white shrimp postlarvae after applied Aquasept™ 3.0 treatment at 3.47 ppm in water was 3 days in aeration condition. While, the safety period for Nile tilapia larvae was performed at a concentration of 1.51 ppm, there was no mortalities observed after preparation on day 2. In contrast, the concentration that provide 0 mortalities on both Pacific white shrimp and Nile Tilapia within 48 hours was 2.00 and 1.00 ppm, respectively. The above results indicate a low shrimp and fish toxicity associated with a faster water preparation or direct Aquasept™ 3.0 application during culture in the described protocols.

EFFICACY *Bacillus* spp. FOR CONTROLLING *Vibrio* spp. CAUSING WHITE FECES SYNDROME GROWTH AND SURVIVAL OF WHITE SHRIMP *Litopenaeus vannamei* IN LABORATORY CONDITION

Pannita Suvanitsuksun, Siriroj Wangsoontorn*, Arunothai Keetanon, Idsariya Wudtisin and Niti Chuchird

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Efficacy of *Bacillus* spp. on *Vibrio* spp. causing white feces syndrome, survival and growth of *Litopenaeus vannamei* was studied under laboratory condition. Twelve tanks were used and divided into 4 groups with 3 replicates per group. Control group, shrimp was fed with pelleted feed without *Bacillus* spp. Group1, shrimp were fed with pelleted feed mixed with *Bacillus* spp. powder at 2 g/kg of feed. Group2, shrimp were fed pelleted feed mixed with *Bacillus* spp. powder at 4 g/kg of feed. And in group3, shrimp were fed with pelleted feed mixed with *Bacillus* spp. powder at 6 g/kg of feed before coating with squid paste. After 30 day feeding trial, the average *Vibrio* spp. count in shrimp intestine of treatment group (group1, group2 and group3) were $12.48 \pm 3.00 \times 10^6$, $4.13 \pm 0.27 \times 10^6$ and $0.72 \pm 0.51 \times 10^6$ CFU/ml respectively, significantly lower than that of the control group ($22.57 \pm 3.01 \times 10^6$ CFU/ml) ($P < 0.05$). The treatment group mix with *Bacillus* spp. had lower number of *Vibrio* spp. than that control group. The average increase body weight of shrimp in treatment group3 was 1.42 ± 0.78 g show highest significantly different ($P < 0.05$) than (control group, group1 and group2) was 0.80 ± 0.53 g, 0.58 ± 0.57 g and 0.51 ± 0.35 g respectively. The average survival rate of shrimp in treatment group1, group2 and group3 was 35.56%, 43.33% and 48.89% respectively and control group was 24.44% with significantly different ($P < 0.05$).

MOLECULAR, TRANSCRIPTIONAL AND FUNCTIONAL CHARACTERIZATION OF TWO GSTS FROM RED-LIP MULLET (*Liza haematocheilus*)

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GSTs are well known for phase II of enzymic detoxification in host xenobiotic metabolism. These enzymes transfer SH group of glutathione to non-polar xenobiotics, thereby neutralizing and rendering product more water soluble. In this study, GST μ , and GST θ were identified from the red lip mullet (*Liza haematocheilus*) transcriptomic database and studied for tissue-specific expression, transcriptional modulation against pathogenic stimulants and functional properties. According to the *in-silico* analysis, coding sequence of MuGST μ , and MuGST θ consisted of 660 and 687 bp open reading frames encoding 219 and 228 amino acid sequences respectively.

Phylogenetic analysis and multiple sequence analysis showed that MuGSTs sequences clustered with respective orthologs distinctly. Further, MuGST μ and MuGST θ share the highest identity and similarity with *Fundulus heteroclitus* and *Lates calcarifer* respectively.

Tissue-specific expression profile demonstrated that MuGST μ and MuGST θ highly expressed in blood. According to the temporal expression in gills, MuGST μ and MuGST θ exhibited significant transcript modulation against polyinosinic:polycytidylic acid (poly I:C) and lipopolysaccharides (LPS). MuGST μ showed specific activity towards 1-Chloro-2,4-dinitrobenzene (CDNB) and p-Nitrobenzyl chloride (4-NBC). MuGST θ showed specific activity towards p-Nitrobenzyl chloride and none of the proteins did not show specific activity toward 1,2-Dichloro-4-dinitrobenzene (DCNB).

Hence, these results indicate that selected MuGSTs act against xenobiotics and as well as can be induced by PAMP stimuli with different expression profiles. Altogether, results in this study suggest that MuGST μ and MuGST θ are involving both immune responses and xenobiotic metabolism.

MOLECULAR AND TRANSCRIPTIONAL CHARACTERIZATION OF C1Q/TNF-RELATED PROTEIN 5 AND 7 FROM RED-LIP MULLET (*Liza haematocheilus*)

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The structural and evolutionary link between tumor necrosis factor (TNF) and globular C1q (gC1q) designated the C1q and TNF related superfamily (CTRP) which comprise with variety of proteins that related to the diverse function in host defense, inflammation, apoptosis, autoimmunity, cell differentiation, organogenesis, hibernation and insulin resistant obesity. In this study, CTRP5, and CTRP7 were identified from the red lip mullet (*Liza haematocheilus*) transcriptomic database and studied for tissue-specific expression and transcriptional modulation against pathogenic stimulants. According to the *in-silico* analysis, coding sequence of MuCTRP5, and MuCTRP7 consisted with 753 and 888 bp open reading frames (ORFs) encoding 250 and 295 amino acid sequences respectively. Both CTRP sequences possessed putative C1q domain and consisted with collagen region. Both MuCTRP sequences contained a signal peptide.

Phylogenetic analysis and multiple sequence analysis showed that MuCTRP sequences highly differ from each other and clustered in respective groups distinctly. Further, MuCTRP5 and MuCTRP7 share the highest identity and similarity with *Acanthochromis polyacanthus*.

Tissue-specific expression profile demonstrated that all the MuCTRPs ubiquitously expressed in all the selected tissues but *MuCTRP5* predominantly expressed in muscle and *MuCTRP7* highly expressed in the stomach. According to the temporal expression in blood, *MuCTRP7* exhibited significant mRNA expression pattern toward poly I:C and *L. garvieae* while *MuCTRP5*, showed significant expression pattern against poly I:C and *L. garvieae* and LPS. Hence, these results indicate that selected MuCTRPs can be strongly induced by pathogen associated molecular patterns (PAMP) with different expression profiles. Altogether, results in this study suggest that MuCTRP5, and MuCTRP7 are involving immune responses against invading bacterial and PAMP stimuli.

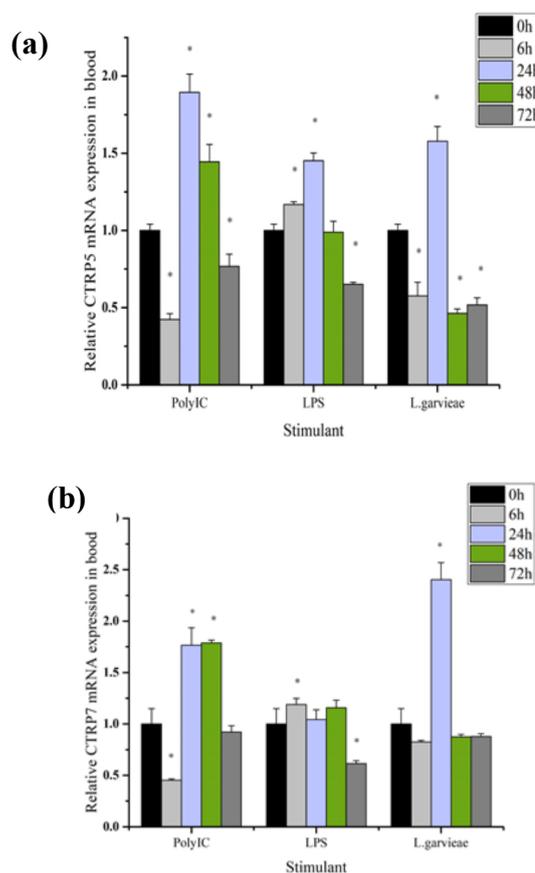


Fig 1. Temporal expression profile of (a) *MuCTRP5* and (b) *MuCTRP7*. Data are expressed as mean fold-induction (n = 3) relative to the PBS control \pm SD. (*) denotes expression significant from control. P value < 0.05.

MOLECULAR CHARACTERIZATION OF KAPPA CLASS GLUTATHIONE S-TRANSFERASE FROM DISK ABALONE (*Haliotis discus discus*) AND EXPRESSION ANALYSIS UPON IMMUNE AND STRESS RELATED RESPONSES

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Glutathione S-Transferase isoenzymes consists with a complex group of proteins, involving in phase II detoxification. In this study, Glutathione S-Transferase kappa (GST κ) in disk abalone (*Haliotis discus discus*; AbGST κ) was characterized in molecular transcriptional and functional perspectives to determine its potential capacity to perform as detoxification agent in immune stress. The deduced AbGST κ protein consists with 227 amino acids, with a predicted molecular weight of 25.6 kDa and theoretical iso-electric point (pI) of 7.78. In silico analysis revealed that AbGST κ possesses the same general fold of DsbA, consisting of a thioredoxin domain, G-sites and catalytic residue in it while having no H- site and signal peptides in it. Highest sequence identity was observed with pufferfish (*Takifugu obscurus*) (60.0%). Highest mRNA expression was observed in the digestive tract indicting its highest detoxification ability. After challenging with lipopolysaccharide (LPS), Poly (I:C) & *Vibrio parahaemolyticus*, significant upregulations were observed, showing the involvement to protect the host from various pathogens. The enzyme kinetics towards specific substrates were determined and it showed relatively low conjugation activities over CDNB. The optimum temperature and pH of AbGST κ were determined as 35°C & pH 8, respectively. Disk diffusion assay revealed AbGST κ 's significant cellular protection towards H₂O₂, CdCl₂ & ZnCl₂.

FEEDING A CALANOID COPEPOD ALGAL PASTE OR ARTIFICIAL DIATOMS – DO THEY ACCEPT THE DIETS?

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High quality live zooplankton feed is often a prerequisite for successful marine fish larval rearing. Vast amount of evidence prove copepods to pose superiority as compared to the classical rotifers and brine shrimp feed items. However, copepod rearing by the mass is considered more difficult than rearing the classical live feed items why marine hatcheries most likely cannot accomplish it. Hence, a strategy where central copepod producers continually provide hatcheries with copepod products (eggs) is proposed. The received eggs can be cold stored with a shelf-life of six months or more at the end-users where after the eggs can be hatched and nauplii used directly to feed out in fish larval tanks. The hatcheries can further grow the nauplii based on microalga food to a preferred size to match the needs for their fish larvae. If the hatcheries want to be totally independent on own algal cultures they might even wish to receive both copepod eggs and copepod feed from a producer. Among existing long lasting feed products are commercially available microalga paste based on e.g. *Isochrysis galbana*, which can be stored in a refrigerator for approximately two months. Besides algal paste, we investigate the perspective of artificially produced diatoms based on freeze dried microalgae, *Rhodomonas salina*, incorporated in a silica matrix, which can be stored indefinitely. We have tested the durability of both concepts on *Acartia tonsa*, Copepoda: Calanoida. In laboratory experiments 1) copepods were exposed for microalga paste and tested for their performance compared to when fed fresh algae of same species. Even though algal paste has similar or even better biochemical profile as compared with fresh algae no significant growth was observed in paste fed *A. tonsa*. The paste did however have a positive effect on survival when compared to starvation as well as retaining a favorable DHA/EPA ratio after hatch. Alternatively nauplii was 2) exposed for our custom made artificial diatom particles in their present form. The particles sustained the nauplii population, but resulted in poor survival and were not appropriate for rapid growth and development. We conclude that the copepod nauplii only partly accept the two alternative diets. Copepod's prey perception plays a significant role in particle intake rather than the overall quality of the food. Next step is to give up on algal paste and pursue the exploration of artificial particles. We intend to improve chemical and physical properties of these particles aiming at a suitable alternative copepod feed with indefinitely storage. This feed option will ultimately enable hatcheries to have on stock a feed concept of long lasting eggs and feed for the nauplii besides an emergency kit of ready to use copepod feed if e.g. own microalgae production fails.

AQUACULTURE RESEARCH IN TROPICAL MARINE SCIENCE INSTITUTE OF NATIONAL UNIVERSITY OF SINGAPORE

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TMSI was set up in 1996 as an initiative to gather researchers in National University of Singapore (NUS) who are keen to study marine science and engineering in the tropical region. The initial focus was on coral, marine mammals and other organisms, physical oceanography and acoustic research. In 1998, an offshore marine station was built on St. John's Island, to provide fresh sea water to marine organisms under experimentation. Currently our research scientists commute to the marine station daily through ferry services. The station is equipped with a diesel generator to power electricity. Portable water is obtained through the island service provider at a premium cost. In partnership with Ministry of Defence (MINDEF) and Agri-Food and Veterinary Authority (AVA), we are now embarking on the laying of an electric power cable and water pipelines to supply electricity and water to the marine laboratory and the other two government facilities on the island. On 1 June 2016, the marine laboratory is upgraded to become a national marine laboratory that is accessible to all marine science researchers in Singapore and from other countries. This is made possible by a generous government grant from the National Research Foundation (NRF) under the Prime Minister's Office.

Strategically TMSI continues to partner various government agencies and international research institutes to conduct targeted research contracts and innovative joint research projects. Looking into the future, TMSI will focus on developing aquaculture research to contribute to food security and sustainability and deep sea research which ventures into the unexplored bottom of the sea.

SEXUAL FATE REPROGRAMMING IN THE STEROID-INDUCED BI-DIRECTIONAL SEX CHANGE IN THE HERMAPHRODITIC FISH

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In sequential sex change fish, the timing of sex changes is regulated by age, body size, and social cues. High levels of plasma estrogen are important for ovary development. However, chemical-stimulated sex change is transient, and a reversible sex change occurs after chemical treatment is withdrawn. Thus, the sexual phase is tightly regulated by the endogenous cues. Sex is determined during the initial gonadal differentiation (primary sex determination) and the later sexual phase (secondary sex determination) in hermaphroditic fish. In present study, we try to elucidate this question by our model fish, including the protandrous (male-to-female sex change) black porgy and protogynous (female-to-male sex change) orange-spotted grouper. In black porgy, our data demonstrated that in addition to a bidirectional signal from the brain-pituitary-gonad axis which regulates temporal effects, there is a reciprocal cross-talk between both sex tissues that regulates spatial effects through the epigenetic modifications. In contrast, unlike in black porgy, chemical-induced maleness in the protogynous orange-spotted grouper is transient, and a reversible sex change occurs immediately after the chemical treatment is withdrawn. These results demonstrate that sexual fate determination is regulated by endogenous sex steroid levels. Taken together, our data indicated that the primary sex guides the secondary sex determination in the protandrous black porgy and protogynous grouper. The transition of sexual fate from one sex to the other sex is determined by the status of the primary sex.

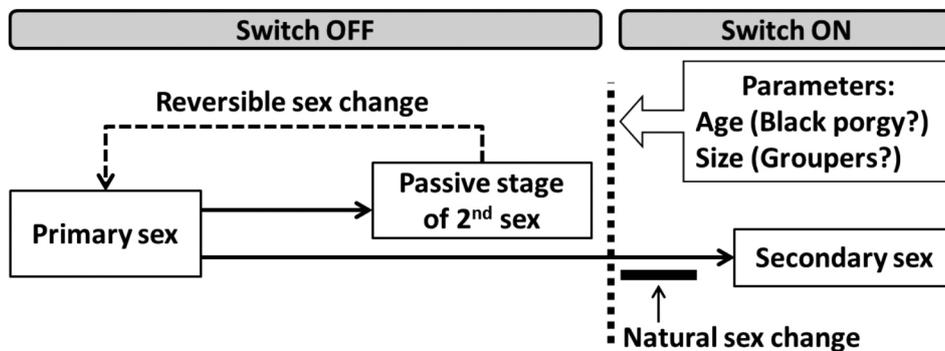


Figure 1. A reversible sex change in hermaphroditic fish

TILAPIA GRN-41 PEPTIDE DERIVED FROM SHORT-FORM PROGRANULIN PGRN1 GENE EXERTS IMMUNE MODULATION AND ANTIBACTERIAL ACTIVITIES

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A short-form progranulin *PGRN1* gene of Mozambique tilapia expresses two transcripts, PGRN1 encoding a 206 a.a. PGRN and an alternatively spliced transcript GRN-41 encoding a novel, secreted GRN peptide composed of 20-a.a. ER signal peptide and 41-a.a. GRN named GRN-41. *PGRN1* and *GRN-41* were not only abundantly expressed in immune-related organs, but also were further induced in the spleen of tilapia challenged with *Vibrio vulnificus*. Tilapia GRN-41 can significantly activate and modulate the expression of innate immune-related genes such as *IL-8*, *IL-1 β* , *IL-6*, *TNF α* , *IL-26* and hepcidin etc. in the transgenic zebrafish and also elevate the survival rate after challenged with *V. vulnificus*. The synthesized tilapia GRN-41 peptide had strong antimicrobial activity against various *Vibrio spp.*, including *V. vulnificus*, *V. alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* but weak activity on *Streptococcus iniae* and *Streptococcus agalactiae*. Tilapia GRN-41 exerted the antimicrobial MBC activity in acid (0.01 M HCl, pH 2) solution as in saline (pH7.0) and still showed MIC activity in alkaline (0.01 M NaOH, pH 12) solution or after heat treatment at 100°C for 1 hour. No hemolysis of the sheep and tilapia red blood cells was detected after treated with synthesized tilapia GRN-41 from 6.25, 12, 25, 50, 100, 200 to 400 $\mu\text{g/ml}$. In addition, transmission electron microscopy revealed morphological changes and disruption of *V. vulnificus* treated with synthesized tilapia GRN-41 peptide in 20 minutes. Our results suggest that the tilapia GRN-41 peptide is a novel, potent antimicrobial peptide and can be applied to defend against bacterial pathogens especially *Vibrio spp.* infection in aquaculture.

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DEVELOP I-TIAO GUNG (*Glycine tomentella* Hayata) EXTRACTS TO DECREASE STRESS RESPONSES AND TO INCREASE THE TRANSPORTIVE POTENCY ON GRUPER (*Epinephelus coioides*)

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I-Tiao Gung is one traditional medicine to take away the pain and to reduce inflammation in China. Our previous study had found that the extracts of I-Tiao Gung (GTE) could increase survival rate for some fish during transport. The aim of the present study just try to find what is the reasons. We found the GTE like as a natural anti-stress drops during the transport of live fish. The levels of stress indices such as antioxidative responses (SOD, and LPO) all were decreased at earlier stage upon transport with GTE addition. In addition, cortisol levels were decreased on the treatmet group. We compared the depressant effective between Eugenol (phenoxyethanol) and GTE, found the effective was similar, and the price was lower on GTE than Eugenol. All results showed GTE could increase antioxidative capacity and have a high potential to use in live fish transport.

The root of wooly glycine *Glycine tomentella*, a plant of soybean family, has been considered an effective and potent anti-inflammatory pain reliever in traditional herbal medicine. The root has proved linked to the action of isoflavonoids, and phenolic phytochemicals which are believed to have anti-inflammatory and antioxidant abilities. The present study just used the effective of wooly glycine on fish transport. The aims of the present study are to confirm if it is useful to decrease transport stress on fish. The water quality, transport density, and the affective dose of GTE will be confirmed, and cortisol, SOD and LPO of plasma, all were as indicates under transport stress.

After 2, 4, and 6 hours of transport test, we found the wooly glycine is a good nature medicine to improve the transport effective on fish, and the best dosage is 250 ppb of GTE.

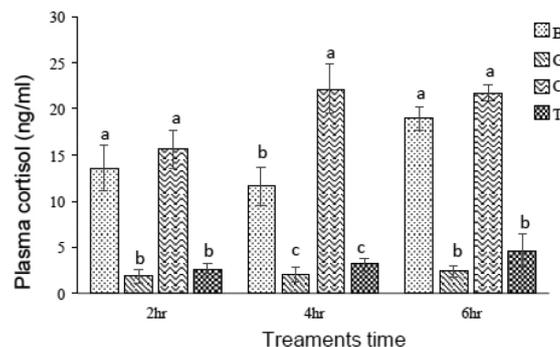


Fig. 1. Cortisol concentration in plasma of grouper after 2, 4, and 6 hours of four types of treatments, (B) without any treatment; (G) No simulating transportation but added GTE; (C) simulating transportation but didn't add GTE; (T) simulating transportation with GTE addition. The Data showed as Mean \pm S.D. (n=3), and static analysis with One-Way ANOVA. Different letters were appeared a significantly differences among them at the same treatment time ($p < 0.05$).

CHELEX AS A MEDIUM FOR SIMPLE EXTRACTION OF GENOMIC DNA FOR PCR-BASED TYPING FROM THE ORNAMENTAL SHRIMP'S SHELL

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The purpose of ornamental shrimp breeding is to breed varieties with excellent colors and textures; however, the traits of different hybrid offspring are exceedingly complex. The marker-assisted selection (MAS) can identify the genotypes of an individual, and its identification effect is not influenced by the growth period or environment of ornamental shrimps. Moreover, it has the advantage of taking shrimp shell samples for analysis, thus, it is beneficial to early strain selection, boosts selection efficiency, and significantly shortens breeding time. The objective of this study was to develop a rapid method to isolate trace DNA from the ornamental shrimp's shell for use in diagnostic DNA mutation analysis. Chelex-100 resin was evaluated with various concentrations of 1%, 5%, 10%, 15%, 20% and combined with the reaction time of 4, 6, 8, 10, 12 minutes in order to have a robust comparison (Figure 1)

This research we demonstrated that Chelex-100 efficiently makes extraction of the DNA from the ornamental shrimp's shell available for direct use in molecular analyses. Also, the quantity and quality of extracted DNA were shown to be adequate for PCR analysis. Comparatively, the quality of DNA samples isolated using 10% Chelex with 8 minutes was better than the other extracted conditions. In conclusion, the Chelex method is recommended for PCR experiments considering its simplicity and cost-effectiveness. It is expected to complete the development of chromogenic gene marker-assisted high quality freshwater ornamental shrimp identification and selection platform technology. To provide the industry can selectively accelerate the cultivation of freshwater ornamental shrimp novel strains, to achieve the MAS technology platform for the benefits of industrialization.

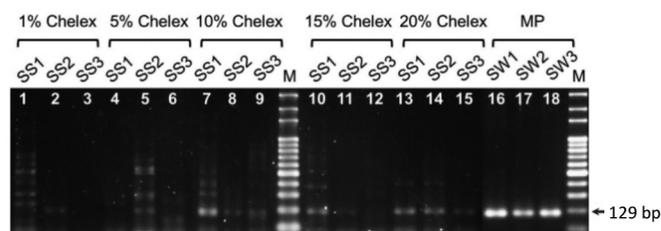


FIG 1. Electrophoresis profile of PCR performed on ornamental shrimp genomic DNA extracted from shrimp shell (SS1-SS3) by using Chelex-100 resin, which was evaluated with various concentrations of 1%, 5%, 10%, 15%, 20%. A PCR product of 129 bp was presented clearly in 10% Chelex-100

A PRELIMINARY DESIGN FOR SOFT-SHELLED TURTLE ATTRACTANT TEST

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Soft-shelled turtle is characterized by a natural lack of appetite when fed on inert feeds in captivity, a fact that has encouraged the search for attractants that increase feed intake. The inclusion of highly attractants might overcome the limitation represented by low feed intake. The objectives will use the Y-maze to measure and compare feeding stimulus of attractants fed to this species.

At attractability behavior assays were carried out with juvenile soft-shelled turtle ranging in size between 15-20 g in body weight. Animals were collected from our lab. Before any behavioral evaluations of attractability, shrimp were first subjected to an acclimation period of 2 week to adapt to laboratory rearing conditions. During the conditioning period, animals were fed libitum with a 30% crude protein diet. Before the start, will test the soft-shelled turtle eat dietary or not. And pick 20 soft-shelled turtle to conduct the study.

The Y-maze measured 12.5 cm × 9 cm × 7.5 cm × 9 cm (length × width × height × arm).For each observation, two ingredients were compared. They were offered separately in similar amounts, placed individually for the each Y-maze arms. Before the behavioral evaluations, soft-shelled turtle was stocked in the acclimation chamber and allowed to acclimate to the Y-maze for 10 min.

In the prestudy, three different raw materials will compared against each other : (1) fishmeal ; (2) squid meal ; (3) soybean meal. There will also a control without raw materials. Observations will made during red light in the presence of artificial light and lasted less than 30 min per specimen. The observer will measure soft-shelled turtle feeding stimulus, and measure whether attractiveness about each raw materials.

EFFECTS OF TRADITIONAL MEDICAL HERBS “MINOR BUPLEURUM DECOCTION” ON THE NON-SPECIFIC IMMUNE RESPONSES OF WHITE SHRIMP (*Litopenaeus vannamei*)

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This study is investigating the effect of minor bupleurum decoction (Xiao-Chai-Hu decoction) on the non-specific immune response of white shrimp (*Litopenaeus vannamei*). To determine prophenoloxidase activity (proPO), reactive oxygen species production (ROS), superoxide anion production (O_2^-), nitric oxide production (NO), phagocytic rate (PR), phagocytic index (PI), superoxide dismutase activity (SOD), total haemocyte count (THC) and differential haemocyte count (DHC). In this experiment, treating with different dosages (0, 0.25, 0.5 and, 1%) of minor bupleurum decoction to detect immune parameters on day 0, 1, 2, 4, 7, 14, 21 and 28.

Result is shown that 0.25% treatment significantly enhanced the superoxide dismutase (SOD) activity and, 0.25 and 1% treatment significantly increased the ROS production, nitric oxide (NO) production and phagocytic rate (PR) moreover, 0.5 and 1% treatment induced the proPO activity and superoxide anion (O_2^-) production.

Evidence exactly indicated that minor bupleurum decoction is able to enhance the non-specific immunity responses of white shrimp via in vivo examination.

Results

The phagocytic rate (PR: phagocytic cells/total cells) in the haemocytes of white shrimp (*Litopenaeus vannamei*), treating with different doses of minor bupleurum decoction.

The phagocytic index (PI: 0.8 mm latex beads/phagocytic cells) in the haemocytes of white shrimp (*Litopenaeus vannamei*), treating with different doses of minor bupleurum decoction.

phagocytic rate Dose(%)	Time elapsed (days)							
	0	1	2	4	7	14	21	28
0	15.05±3.87	21.71±7.39 ^b	22.09±3.39 ^c	20.75±3.54 ^b	19.26±4.12 ^b	22.53±6.20 ^b	23.63±3.90 ^c	23.91±5.93 ^b
0.25		25.99±4.14 ^b	41.55±9.86 ^b	42.11±3.33 ^a	39.53±3.06 ^a	40.33±2.69 ^a	46.86±0.89 ^a	31.35±4.50 ^{ab}
0.5		47.15±5.26 ^a	63.63±1.42 ^a	39.64±4.34 ^a	33.61±1.76 ^a	29.59±5.72 ^{ab}	39.49±5.41 ^{ab}	37.46±1.44 ^a
1		43.74±0.26 ^a	51.60±1.41 ^{ab}	37.29±2.82 ^a	33.21±2.78 ^a	32.31±8.02 ^{ab}	33.74±4.54 ^{bc}	38.87±2.77 ^a

phagocytic index Dose (%)	Time elapsed (days)							
	0	1	2	4	7	14	21	28
0	1.25±0.43	1.47±0.50 ^a	1.15±0.13 ^b	1.23±0.09 ^a	1.18±0.17 ^a	1.14±0.25 ^a	1.00±0.00 ^a	1.16±0.15 ^a
0.25		1.44±0.16 ^a	1.64±0.18 ^{ab}	1.21±0.30 ^a	1.40±0.23 ^a	1.21±0.13 ^a	1.19±0.17 ^a	1.29±0.06 ^a
0.5		1.95±0.15 ^a	1.87±0.29 ^a	1.30±0.09 ^a	1.21±0.04 ^a	1.55±0.41 ^a	1.31±0.02 ^a	1.21±0.09 ^a
1		1.71±0.01 ^a	1.57±0.20 ^{ab}	1.42±0.13 ^a	1.22±0.18 ^a	1.21±0.11 ^a	1.33±0.23 ^a	1.30±0.14 ^a

COMPARISON OF TWO IMMUNOMODULATORS “B-GLUCAN” AND “SODIUM ALGINATE” ON THE NON-SPECIFIC IMMUNE RESPONSE OF SPINY LOBSTER (*Panulirus interruptus*)

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This study mainly investigated whether β -glucan and sodium alginate enhances the nonspecific immune response of lobsters. In experiments, lobsters were fed a diet containing β -glucan or sodium alginate to determine the effect of these compounds on nonspecific immune parameters in the short term (14 days). The lobsters were then sampled to examine variance in their total haemocyte count (THC), phenoloxidase activity, superoxide anion (O_2^-) production, superoxide dismutase (SOD) activity, phagocytic ratio (PR), and phagocytic index (PI).

The effects of treatment with various doses of β -glucan and sodium alginate were analysed. The results showed that treatment with 1–2 g of β -glucan or 2–4 g of sodium alginate significantly increased the THC, O_2^- production, SOD activity, and PR.

In short, the immunomodulators β -glucan and sodium alginate can increase the nonspecific immune response of spiny lobsters.

Results

Phenoloxidase (PO) (O.D 492 nm) activity in the haemocytes of Spiny lobster (*Panulirus interruptus*) treated with different immunostimulants. a~c: mean in the same column with the different latter are significantly different ($p < 0.05$). Value represented by mean \pm S.D..

The phagocytic rate (phagocytic cells/total cells) in the haemocytes of Spiny lobster (*Panulirus interruptus*) treated with different immunostimulants. a~c: mean in the same column with the different latter are significantly different ($p < 0.05$). Value represented by mean \pm S.D.

Treatment (g/Kg)		O.D 492 nm					
		Days					
		0	1	2	4	7	14
Control	0	1.662 \pm 0.062	1.698 \pm 0.056 ^d	1.764 \pm 0.099 ^{ef}	1.467 \pm 0.028 ^c	1.668 \pm 0.035 ^a	1.611 \pm 0.108 ^{ab}
	1		1.856 \pm 0.080 ^{cd}	2.274 \pm 0.056 ^c	1.905 \pm 0.049 ^{ab}	1.682 \pm 0.103 ^a	1.518 \pm 0.074 ^a
Sodium alginate	2		2.057 \pm 0.122 ^{bcd}	2.490 \pm 0.012 ^b	1.548 \pm 0.133 ^c	1.645 \pm 0.050 ^a	1.998 \pm 0.190 ^a
	4		2.248 \pm 0.262 ^{ab}	2.085 \pm 0.017 ^d	1.613 \pm 0.047 ^c	1.686 \pm 0.036 ^a	1.960 \pm 0.217 ^a
	4		2.177 \pm 0.120 ^{abc}	2.408 \pm 0.055 ^b	1.987 \pm 0.075 ^a	1.643 \pm 0.056 ^a	1.594 \pm 0.065 ^{ab}
β -glucan	1		2.249 \pm 0.042 ^{ab}	2.678 \pm 0.059 ^a	1.677 \pm 0.066 ^{bc}	1.621 \pm 0.124 ^a	1.680 \pm 0.179 ^{ab}
	2		2.522 \pm 0.035 ^a	1.839 \pm 0.038 ^e	1.491 \pm 0.114 ^d	1.564 \pm 0.259 ^a	1.728 \pm 0.148 ^{ab}
	4						

Treatment (g/Kg)		PR					
		Days					
		0	1	2	4	7	14
Control	0	10.118 \pm 0.319	10.441 \pm 0.649 ^e	10.972 \pm 0.647 ^e	10.035 \pm 0.704 ^e	9.832 \pm 0.569 ^e	10.631 \pm 1.588 ^b
	1		10.982 \pm 0.531 ^c	17.763 \pm 0.250 ^d	21.044 \pm 0.624 ^a	10.945 \pm 0.685 ^b	10.294 \pm 0.365 ^b
Sodium alginate	2		13.289 \pm 0.431 ^b	20.864 \pm 0.828 ^{bc}	21.820 \pm 0.515 ^a	10.442 \pm 0.327 ^{bc}	10.702 \pm 0.473 ^b
	4		14.835 \pm 0.757 ^a	23.020 \pm 1.176 ^a	14.398 \pm 0.211 ^f	10.520 \pm 0.255 ^{bc}	10.520 \pm 0.255 ^b
	4		11.475 \pm 0.381 ^c	18.140 \pm 0.092 ^d	16.090 \pm 0.280 ^f	10.480 \pm 0.080 ^{bc}	10.480 \pm 0.080 ^b
β -glucan	1		10.805 \pm 0.266 ^c	22.381 \pm 0.639 ^{ab}	18.389 \pm 0.728 ^f	13.565 \pm 0.424 ^a	13.224 \pm 0.403 ^a
	2		14.835 \pm 0.575 ^a	19.078 \pm 1.024 ^{cd}	14.615 \pm 0.586 ^{cd}	10.676 \pm 0.012 ^{bc}	10.397 \pm 0.328 ^b
	4						

GENETIC SELECTIVE BREEDING AND GENOME SEQUENCING OF PENAEID SHRIMP

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Since 2008, the local cultured commercial line imported from Chia Tai, Kona Bay and OI (Ocean Institute) were collected, together with our own cultivated “Kehai 1” were selected as the breeding base group. Using the pure line breeding method combined with the cross-test breeding method, the individuals with the high performance in different families were selected. After seven consecutive generations of selection, four lines including fast growth group (A series), high survival / high fecundity line (B series), high survival / fast line (C series), high fecundity line (D series) were established, and the two-hybrid breeding system were established: the A series were mated with B series to produce the female parent (AB), which shown advantage in growth and survival. The C series and D series were mated to produce male parents (CD), which shown advantage in both fecundity and high survival. AB and CD were mated to produce commercial line (ABCD), which was named as “Guangtai NO.1”. The growth rate of Guangtai NO.1 increased by 37% and the survival rate increased by 20% compared with the local breed. In the high-level pool culture mode, the growth rate increased by 16%, breeding survival rate increased by 30% compared with the SIS.

Based on the second and third generation high-throughput sequencing technology, we obtained 828 Gb high quality Illumina data and 133 Gb PacBio sequencing data for *Litopenaeus vannamei*, covering 319 and 50 times of its genome respectively. The hybrid assembly method which was suitable for the characteristics of high repetition genome were successfully constructed, as a result we assembled the whole genome work frame. The Contig N50 is 57 KB and the Scaffold N50 is 600 KB, with the genome coverage 77%, gene region coverage reached 92%. The genome structure, the characteristics of the shrimp genome and the evolution characteristics of prawn were analyzed with comparative genomics method. Using the methods of integrating genomic method, including transcriptomic and proteomic data, the function genes related to the growth of shrimp, molting and resistance were selected and discovered, including prawn disease resistance related gene: VEGF3, SOCS, thrombospondin, Descam, genes related to the growth of the prawn molting: EH, crustacyanin, IGFB. Use similar method, we have assembled the *Penaeus chinensis* genome and obtained a better results (the Contig N50 was 53kb, the Scaffold N50 was 127kb). Further investigation and improvements of the two prawn genomes are still ongoing.

COMPARATIVE STUDY ON THE UTILIZATION OF DIETARY PROTEINS IN DIFFERENT STRAINS OF CRUCIAN CARP (*CARASSIUS AURATUS*)

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It was investigated that the differences between three different strains of crucian carp fed with diets containing different protein sources. Growth performances, plasma biochemical indexes, transcriptomic analysis and metabolomic analysis were tested in juvenile, one-year-old and two-year-old crucian carp of three strains (Strain DT, Strain A and Strain F). Three diets containing fishmeal (FM), soybean meal (SBM) and rapeseed meal (RM) as main protein sources were formulated for each growth stages, respectively.

Specific growth rate (SGR), feed efficiency (FE), protein retention efficiency (PRE) and lipid retention efficiency (LRE) of crucian carp fed SBM diet and RM diet decreased significantly compared to those fed with FM diet ($P < 0.05$). Different strains of crucian carp showed different growth performance when fed plant proteins. The juvenile fish of Strain A showed better SGR when fed FM diet ($P < 0.05$); juvenile Strain A and Strain F had higher SGR than Strain DT when fed SBM diet ($P < 0.05$); Strain F had the highest SGR when fed RM diet ($P < 0.05$). Juvenile Strain A and Strain F had higher FE than Strain DT fed FM diet or RM diet ($P < 0.05$), while Strain F fed RM diet had the higher FE ($P < 0.05$); When fed FM diet and SBM diet, juvenile Strain A had higher PRE, while Strain F fed RM diet had higher PRE. One-year-old Strain A had the higher SGR in three diet groups; FE and PRE of Strain F was higher when fed FM diet and RM diet. Two-year-old Strain A had higher SGR than Strain F; In FM diet group, Strain F had higher FE and PRE; when fed SBM diet, Strain A had better FE and PRE; Strain A had higher FE and lower PRE in RM group. Strain F had lower LRE than other two strains in three life stages ($P < 0.05$). The utilization of protein sources of crucian carp was influenced by growth stages.

It suggested that dietary plant proteins reduced the growth performances of crucian carp, and different strains had different growth performances, plasma biochemical indexes, transcriptomic and metabolomics profiles. Strain F showed better utilization on rapeseed meal. The differences between strains might be related to taurine metabolism, lipid metabolism and immune response. Fish growth stages also had effects on utilization of protein sources in crucian carp. With the increased of fish size, the larger fish showed less differences between different diets or strains.

PUFFERFISH FXYD8 MAY PLAY THE ROLE OF INHIBITORY MODULATION FOR SODIUM PUMP EXPRESSION FOLLOWING ENVIRONMENTAL SALINITY CHANGES

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The pufferfish *Dichotomyctere nigroviridis* (also known as *Tetraodon nigroviridis*) is a euryhaline ornamental fish as well as an important model teleost with genomic sequences available for molecular studies. This pufferfish, being a peripheral freshwater (FW) inhabitant, has been demonstrated to be an efficient osmoregulator upon salinity challenge. In osmoregulatory process of teleosts, sodium pump (Na⁺/K⁺-ATPase, NKA) in osmoregulatory organs (i.e. gill, kidney, and gut) maintains intracellular ion homeostasis by providing a driving force for many ion-transporting systems. FXYD proteins, the regulators of NKA, also play crucial roles in osmoregulation and ion exchange via their interaction. Hence, the purpose of this study was to elucidate the potential role of FXYD8 in modulating NKA activity in the pufferfish kidney.

The pufferfish *fxyd8* (*Tnfxyd8*) mRNA was observed in all studied organs including the gill, kidney, and gut of pufferfish acclimatized to either FW or seawater (SW). Among the three osmoregulatory organs, the highest levels of *Tnfxyd8* mRNA were found in the kidneys of both FW and SW groups (Table 1). Salinity effects on expression of renal TnFXYD8 were further investigated. Renal TnFXYD8 protein expression revealed a significant increase in the SW-acclimated pufferfish (Table 1). The association between TnFXYD8 and NKA was demonstrated via co-immunoprecipitation and immunostaining, showing that TnFXYD8 interacted with NKA in renal tubules. Furthermore, TnFXYD8 was observed to exhibit an inhibitory effect on NKA expression/activity via *Xenopus* oocyte overexpression system (Table 1). Our results suggested that the FXYD8 was able to modulate NKA activity through inhibitory effects upon salinity challenge. This study is the first to explore the physiological functions of teleostean FXYD8. The findings of the present study illustrated the potential functions of a novel NKA regulator in the kidneys of teleosts and further extended our understanding on the modulation of NKA activity by FXYD proteins in vertebrates.

Table 1. TnFXYD8/*Tnfxyd8* expression and NKA activity in the pufferfish.

Items	Expression patterns
<i>Tnfxyd8</i> mRNA expression	
Osmoregulatory organs	Kidney > Gill, Gut (both FW and SW groups)
Gill/Kidney	FW group \approx SW group
Gut	FW group > SW group
TnFXYD8 protein abundance	
Kidney	SW group > FW group
NKA activity	
Overexpression system	Control groups > <i>Tnfxyd8</i> -injection group
Kidney	FW group > SW group

ESTABLISHMENT OF THE ARTIFICIAL SEEDING TECHNIQUE OF *Sargassum ilicifolium*

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Due to global climate change as well as artificial pollution and damage to parts of the ocean, economical macroalgae which inhabited around Taiwan's coast have greatly decreased in both distributional areas and quantities. The main purpose of this research is to establish an artificial seeding technique of *S. ilicifolium*. And this research focuses on cultivation techniques of zygotes and young seedlings for mass production. The study observed the growth of *S. ilicifolium* in northeast coast of Taiwan in March to December on 2017. When the algae in the wild be mature, Gamete cells are obtained from mature algal bodies, and then the research focuses on cultivation techniques of zygotes and young seedlings. In the study of the fertilized egg and embryo, the maximum of the fertilized egg release time in group of 25°C 50 μ mole photons is earlier than other groups; and the maximum of sperm release quality in 20°C 100 μ mole is better than other groups; and the survival rate of the fertilized eggs in 25°C 50 μ mole is the highest; and in the condition of 20°C 20 μ mole, the seedling has the best condition of growing.

CITRIC ACID IMPROVE THE PERFORMANCE OF HYBRID BROWN-MARBLED GROUPER *Epinephelus Fuscoguttatus* X Giant Grouper *Epinephelus Lanceolatus* JUVENILE

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Marine fish hybrid brown-marbled grouper *Epinephelus fuscoguttatus* x giant grouper *Epinephelus lanceolatus* is a potential food fish in Asian countries. Plant meal is well accepted in replacing fishmeal for aquatic animals and studies had shown that organic acid such as citric acid can trigger beneficial effects by improving growth and feed utilization when added in the aquaculture feed. Duckweed is an aquatic plant that can be obtained easily from most fresh water bodies and it has been incorporated into the feed for poultry and pig. However, limited study is reported for fish.

Thus, the present study was conducted to investigate the utilization of duckweed (*Lemna minor*) with or without the supplementation of citric acid in feed by the hybrid grouper juvenile. Three isoproteic and isonitrogenous diet (50% protein, 16% lipid) were formulated and assigned as fish meal-based diet (Con), 5% *L. minor* supplementation (DL), and 5% *L. minor* with 3% citric acid supplementation (DLC). Triplicate groups of fish (10.30 ± 0.05 g) were randomly distributed in tanks with flow through system at stocking density of 20 tails per tank. The fish were fed twice daily until apparent satiation for 10 weeks. All experimental diets were well-accepted by the hybrid grouper. Fish fed diet DLC achieved the highest body weight gain (557.12%) and specific growth rate (3.14%), followed by fish fed DL and lastly fish fed Con. The growth performance of fish fed DLC was significantly better than fish fed Con ($P < 0.05$). Fish fed DLC showed significantly higher net protein utilization compare to Con ($P < 0.05$). Fish fed DLC also showed better feed conversion ratio and protein efficiency ratio however, without significant difference with other treatments ($P > 0.05$). The comparable apparent digestibility coefficient of crude protein and crude lipid of DLC compared to Con. The trypsin-like, amylase and lipase enzyme activities were also significantly high in fish fed DLC compared to Con ($P < 0.05$). Citric acid addition also led to higher phosphorus absorption as shown by DLC ($P < 0.05$) compared to others. This study showed that duckweed can be well accepted by the hybrid grouper juveniles. Supplementation of citric acid can help to improve the growth, feed utilization, digestibility and phosphorus absorption in fish.

SCREAMING THE PROTEIN THAT CAN INTERACT WITH GROUPEr NERVOUS VIRUS COAT PROTEIN (GNNVCP)

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Nervous necrosis virus (NNV) in grouper larvae can cause mortality rate up to 100%. Thus, it is important to understand how the virus affects the hosts. The structure of NNV coat protein (CP), the only structural protein of NNV, consists of two large sub-domains, the Shell-Domain (S-domain) and the Protrusion-Domain (P-domain). The two domains may interact with different proteins to control specific biological pathways in the hosts. Therefore, in this study, Yeast two-hybrid system was applied using the NNVCP S-domain and the P-domain respectively, as bait to hybridize with the grouper whole fry library to determine what proteins can interact with NNVCP. Some candidates were found and annotated after aligning with the known database. The results show that 40s RPS6, 60s RPL21 and CW2-1 Kunitz-type protease inhibitor can interact with NNVCP S-domain. In addition, LFABP, 40s RPS20, 60s RPL3 and CW2-1 Kunitz-type protease inhibitor were found to interact with NNVCP P-domain. Further identification is needed to confirm their interaction and biological meanings.

IDENTIFICATION OF SEX-DETERMINING LOCI IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei* USING LINKAGE AND ASSOCIATION ANALYSIS

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The Pacific white shrimp *Litopenaeus vannamei* is a predominant aquaculture shrimp species in the world. Like other animals, the *Litopenaeus vannamei* exhibited sexual dimorphism in growth trait. Mapping of the sex-determining locus will be very helpful to clarify the sex determination system and further to be beneficial for the shrimp aquaculture industry towards the production of mono-sex stocks. Based on the data used for high density linkage map construction, linkage mapping analysis was conducted. The sex determination region was mapped in Linkage Group (LG) 18. A large region from 0 cM to 21.205 cM in LG18 showed significant association with sex. However, none of the markers in this region showed complete association with sex in the other populations. So an association analysis was designed using the female parent, pool of female progenies, male parent and pool of male progenies. Markers were *de novo* developed and those showing significant differences between female and male pools were identified. Among them, three sex associated markers including one fully associated marker were identified. Integration of linkage and association analysis showed that the sex determination region was fine mapped in a small region along LG18. The identified sex associated markers can be used for the sex detection of this species at genetic level. The fine mapped sex determining region will contribute to the mapping of sex determining gene and improved understanding of sex determination for *Litopenaeus vannamei*.

DRAFT GENOME OF THE HONG KONG OYSTER *Crassostrea hongkongensis*

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Hong Kong oyster *Crassostrea hongkongensis*, a member of the phylum Mollusca, is a dominant oyster species along the coast waters of South China Sea, with a long cultivation history and has been a popular seafood with a high market demand. The identification of genes related to various physiological processes in this oyster might enhance our understanding of reproduction, possibly contributing to the production of high-quality seeds and also help understand the underlying molecular mechanisms of the traits related to immunity and stress adaptation, which may provide a basis for better strain development in aquaculture.

A total of 147.25 gigabases (Gb) of raw reads were obtained from genome mapping of the Hong Kong oysters by PE125 format of high throughput sequencing on Illumina HiSeq 2500 platform. The estimated final genome assembly (714.88 Mb), covering about 98.20% of the estimated genome size, was found to be composed of 20.34 Kb of contig N50 and 618.24 Kb of scaffold N50 respectively. A total number of 35,624 genes were predicted; of which 90.84% were annotated on the basis of available genomic databases and 1,223 gene families were found to be specific to *C. hongkongensis*. A total of 154 tRNA, 83 rRNA, 807 miRNA, 2,607 pseudogenes and 415.71 Mb repetitive sequences were predicted. Based on comparisons with other all studies of *C. hongkongensis* with other oysters and non-mollusk species it could be concluded that oyster genome might not have undergone large-scale genome duplication events. In addition, the molecular basis of hemolytic phagocytosis and shell formation were also analyzed in genomic level. In conclusion, we report the first draft genome sequence, assembly and annotation of *C. hongkongensis*. The assembled genome will provide a valuable resource for the study of essential physiological processes, phylogeny and evolution among this Hong Kong oyster.

THE CHARACTERS OF REPRODUCTIVE PHYSIOLOGY IN LARGESCALE MULLET, *Chelon macrolepis*

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Largescale mullet, *Chelona macrolepis*, is one of the economically important marine aquaculture species in south of Taiwan. The spawning season of the fish is from Decembers to February at one year old when gonadosomatic index were increased to 12% and 1.2% both in female and male fish, respectively. Previtellogenic stage of the fish is before October with diameter of oocyte between 20-100 μ m; in vitellogenic stage, oocyte diameters increased to 600 μ m in late vitellogenic stage during November to January; in maturation stage, oocyte diameters increased to 850 μ m in germinal vesicle breakdown (GVBD) stage with transparent ooplasm and a single clear oil droplet. High levels of plasma testosterone and estradiol-17 β were observed on December. Treatments with catfish pituitary homogenates (CPH), the development of the oocyte maturation could be divided into hormone-insensitive stage (insensitive to gonadotropin and maturation inducing steroid (MIS), 0-6 h after treatment), MIS-sensitive (respond to MIS, 6-12 h after treatment) and spontaneous stage (GVBD in the hormone-free condition, 18 h after treatment), respectively. In the response of various hormone *in vitro* studies, both 17, 20 β , 21-trihydroxy-4-pregnen-3-one (20 β S), and 17, 20 β -dihydroxy-4-pregnen-3-one (DHP) were the most effective steroids to induce *in vitro* maturation. 17-hydroxyprogesterone and 21-hydroxy-4-pregnen-3, 20-dione also could significantly induce oocyte maturation at higher concentrations. Fish treated with LHRH-A (D-Ala⁶, des-Gly¹⁰ LHRH ethylamide analog) \square LHRH-A with dopamine antagonist, HCG were not effect in induce oocyte maturation even in high dose. CPH treatments could significantly induce oocyte maturation but not ovulation. Only combine with catfish pituitary homogenates and MIS had a higher effectiveness both in induce oocyte maturation and ovulation. Haloperidol, The dopamine antagonist, could induce spermiation in male fish.

DETECTION OF HEPATITIS A VIRUS, FECAL COLIFORM AND *E. coli* IN CULTURED OYSTER, *Crassostrea iredalei* from SOUTHERN MALAYSIA

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This research reported the process of detection of Hepatitis A Virus (HAV), enumeration of fecal coliform, *E. coli* in oyster (*Crassostrea iredalei*) and water sample collected from Senibong Village, Southern Malaysia (N01° 28.904', E103° 48.985'). One-Step RT-PCR analysis according to the method by Wan Nurhana et al., (2011) was used for the detection of HAV in this study. HAV was absent in all of the oyster and water samples collected during the first and 2nd sampling after examination. The Most Probable Number (MPN) of fecal coliform and *E. coli* in oyster and water sample were higher during the first sampling compared to the second sampling. From the PCR assay on *HlyA* gene (361 bp), 16 strains (15.38%) out of the 104 strains of *E. coli* isolated from water and oyster samples collected during the first and second sampling showed the presence of *HlyA* gene upon conventional PCR amplification. Three strains of *E. coli* were randomly selected to represent *E. coli* isolated from oyster sample collected during the first sampling (M8, M14), second sampling (M69, M90); and water sample (W1) collected during the second sampling (M41), respectively for the sequencing analysis of 16S rRNA gene and *HlyA* gene. The BLAST analysis results for 16S rRNA and *HlyA* gene of the selected *E. coli* showed that they matched with a region of the *Escherichia coli* O157:H7 strain 8368, complete genome (GenBank accession number CP017444.1) with more than 95% query cover and sequence identity. Ammonia content in water was found to be higher during the first sampling (> 0.01 ppm) while nitrite content was found to be higher during the second sampling (> 0.01 ppm). The dissolved oxygen in water during the second sampling was higher along with lower temperature and salinity (2.4 ± 0.1 mg/L, $29.7 \pm 0.1^\circ\text{C}$, 28.0 ± 0.1 ppt) when compared to the second sampling (1.3 ± 0.1 mg/L, $30.7 \pm 0.1^\circ\text{C}$, 29.4 ± 0.1 ppt). The average length and weight of oyster collected during the first sampling (7.45 ± 0.14 cm, 6.21 ± 0.33 g) were similar to the average length and weight of oyster collected during the second sampling (7.56 ± 0.15 cm, 6.21 ± 0.29 g). Thus, this is the first detection of *HlyA* gene in cultured oyster, *Crassostrea iredalei* from Malaysia.

BACTERIAL COMMUNITY DIVERSITY ASSOCIATED WITH HEALTHY AND UNHEALTHY SHRIMP (ACUTE HEPATOPANCREATIC NECROSIS DISEASE) FROM SHRIMP FARM IN MALAYSIA

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Bacterial community diversity of healthy and unhealthy shrimp (*Penaeus vannamei*) of Acute Hepatopancreatic Necrosis Disease (AHPND) collected from shrimp farms in Kedah and Penang, Malaysia were examined using a cultivation method and 16S rRNA gene analysis. This study aimed to describe and identify the major abundance of shrimp's bacterial communities and its relationship with different shrimp health status (healthy and unhealthy with AHPND). 16S rRNA gene analysis of metagenomic and culturable bacterial numbers were found to be different between healthy and unhealthy samples in two different collection times that shows, the bacterial community diversity may potentially have contributed to the health shrimp status.

Results from this study indicated that most of bacterial genera found in healthy shrimps were typically present in the shrimps, while bacteria genera in unhealthy shrimps indicated different from the healthy one. The results revealed that there were significant different between healthy shrimp status and sampling times. Based on the results, the bacterial communities of healthy shrimp comprised of members of the genera *Pseudomonas* (30% of the total reads), *Photobacterium* (23% of the total reads), *Acinetobacter* (15% of the total reads) and *Vibrio* (10% of the total reads), while the members of the genera *Vibrio* (40% of the total reads), *Photobacterium* (25% of the total reads), *Pseudomonas* (10% of the total reads) and *Paracoccus* (7% of the total reads), were greatly predominant in unhealthy shrimp of AHPND, with highly dynamic of their bacterial communities. Other important bacterial genera also found were *Escherichia*, *Cyanobacteria*, *Pseudoalteromonas* and *Aliivibrio*. The overall data demonstrated dynamic bacterial communities in healthy and unhealthy shrimp (*Penaeus vannamei*) and its diversity from shrimp farm in Malaysia, and proves that bacterial communities were distinct between healthy and unhealthy (APHND) shrimps collected from shrimp farm.

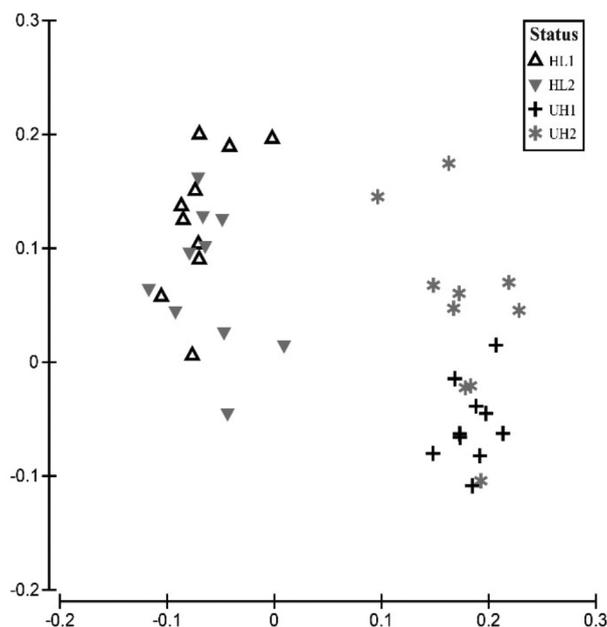


Figure 1: CAP plots comparing healthy and unhealthy shrimp affecting bacterial community diversity.

RAS HAS AN IMPORTANT ROLE IN WSSV-INDUCED WARBURG EFFECT

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The White Spot Syndrome Virus (WSSV) has caused huge economic losses in the global shrimp industry. In previous studies, metabolic changes resembling the Warburg effect occurred in WSSV-infected shrimp, with glucose consumption and plasma lactate concentrations both increased at the replication stage (12 hpi). Although the Warburg effect can be regulated by a PI3K/Akt/mTOR pathway, it is unknown if the WSSV affects that pathway. A critical oncogene in human cancers is various forms of Ras, with K-Ras being the most common. We identified two types of K-Ras (named RAS1 and RAS2) in *L. vannamei* and determined that gene expression of RAS1/2 was significantly increased after WSSV infection (12 and 24 hpi). Moreover, PI3K/Akt and RAS/ERK pathways were activated after WSSV infection. To elucidate the importance of Ras in WSSV replication, studies were conducted to suppress Ras with Salirasib or silence Ras with RAS1/2 dsRNA. Injection of inhibitor caused significant, dose-dependent decreases in copies of the WSSV genome. Moreover, in response to dsRNA silencing, gene expression of VP28 was decreased only in RAS2-silenced shrimp at a late stage (24 hpi), although WSSV genome copies were decreased in RAS1/2-silenced shrimp at a late stage (24 hpi). We concluded that RAS was of central importance in triggering this WSSV-induced Warburg effect at the viral genome replication stage (12 hpi) and that RAS1/2 has an important role in WSSV replication.

STUDIES ON THE IMPAIRMENT IN INTESTINAL HEALTH OF TURBOT CAUSED BY DIETARY SOYBEAN MEAL AND THE MITIGATING STRATEGY

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The soybean meal has been the most important alternative protein sources replacing fish meal in commercial aqua-feeds production. However, the use of soybean meal in feed for marine carnivorous fish species is still limited due to the high dietary level soybean meal induced negative impacts on fish growth performance and other physiological processes, especially intestinal health. Since intestine is the most important digestive organ of fish and plays key roles in immunity and disease resistance, health of the intestine is crucial for securing optimal fish health and growth. The first study was designed to comprehensively evaluate the impact of high dietary level soybean meal on intestinal health of turbot. Two isonitrogenous and isolipidic practical diets were formulated to contain 0% and 40% soybean protein named as FM and SBM group, respectively. Each diet was fed to sextuple groups of 30 turbot. The intestinal samples were collected after 2, 4, 8 and 12 weeks. The intestinal morphology and the gene expression of intestinal inflammatory cytokines of turbot were observed and analyzed at different time point to show the intestinal impairment of turbot fed high level SBM diet. The intestinal mucosal barriers (IMB) in terms of the tight junction proteins and mucin, the intestinal myosin light-chain kinase (MLCK) and nuclear factor- κ B (NF- κ B) responded markedly to diet with high level SBM. The microbiota results showed that dietary SBM altered turbot intestinal microbiota profiles obviously. Collectively, dietary soybean protein (40%) in the diet caused the enteropathy of turbot, and the function of IMB and the homeostasis of intestinal microbiota were closely related.

Based on these results and the function of citric acid, a 12-week feeding trial was conducted to evaluate the protective effect of citric acid against soybean meal induced enteropathy in juvenile turbot. Four isonitrogenous and isolipidic practical diets, fish meal-based diet (FM), 40% fish meal protein in FM was replaced with soybean meal protein diet (SBM), SBM with 1.5% citric acid (1.5%CA) diet and SBM with 3% citric acid (3%CA) diet, were designed to feed fish respectively. Triplicate groups of 30 fish were fed to apparent satiation twice daily for 12 weeks. Turbot fed SBM showed typical soybean meal-induced enteropathy. On the contrary, fish fed citric acid showed the integrity of intestine without obvious inflammation. In addition, the enhanced IMB function was observed in fish fed citric acid. Sequencing of bacterial 16s rRNA V4 region showed that dietary citric acid beneficially modulated the micro-ecological balance and specifically reduced the relative abundance of the *Vibrio* genus. In brief, citric acid showed a promising potential as a dietary supplement to enhance the intestinal function of turbot. The results will be helpful for exploiting nutritional methods to alleviate the intestinal impairment caused by diets with high levels of plant protein.

COMPARATIVE EFFICACY OF VARIOUS PHOSPHATE SOURCES OF THE GROWTH PERFORMANCE, PHOSPHORUS RETENTION AND DIGESTIBILITY IN WHITELEG SHRIMP *Litopenaeus vannamei*

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A study was undertaken to evaluate the effect of three supplemental phosphate sources, Windmill® Aquaphos (MAP), monopotassium phosphate and monocalcium phosphate on the growth performance, whole-body phosphorus (P) and calcium (Ca) retention, P and Ca digestibility on whiteleg shrimp *Litopenaeus vannamei*.

Material and Methods

Quintuplicate groups of 25 shrimps, were fed one of the experimental diets during 54 days. At the start of the trial, a pool of 25 whole shrimp from the initial stock and a pool of 10 whole shrimp per tank at the end of the trial, were sampled for analysis of whole-body protein, ash, phosphorus and calcium content. To determine the digestibility, yttrium oxide was added to the experimental diets as an indigestible marker. Faeces were collected by means of syphoning.

Results & Discussions

Final body weight (FBW, g) ranged between 8.00 and 13.70 g. FBW and specific growth rate (SGR, %/d) of shrimp fed the NC diet was significantly lowest among all diets. Shrimp fed the MAP showed a significantly higher FBW and lower FCR than other diets. During the entire trial, the survival rate ranged between 97.6 and 99.2%.

The whole-body composition of shrimp in terms of protein, P and Ca was significantly affected by the various dietary treatments. The retention of P (expressed as % of intake) was significantly higher for MAP group. This resulted in a higher P-retention of the phosphate source for MAP (39,8%) compared to MCP (28,5%) and MKP (23,3%).

Shrimp fed phosphate without Ca (MAP and MKP) presented calcium retention values higher than 100%, suggesting absorption of calcium from the surrounding water. While the diet containing MCP resulted in a significant lower calcium retention. In other words, there is no need to add Ca via feed ingredients. It has even a negative effect on nutrient retention.

When calculating the digestibility of phosphorus (ADC), MAP showed the highest results which was significantly higher than that of MCP and MKP ($P < 0.001$).

The overall experimental data demonstrates that Windmill® Aquaphos is an effective strategy to enhance growth performance, phosphorus digestibility and retention in whiteleg shrimp (*Litopenaeus vannamei*).

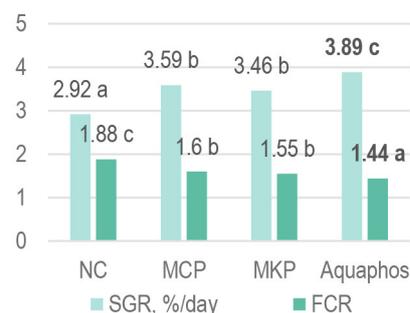


Figure 1: Growth performance after 54 days ($p < 0.001$)

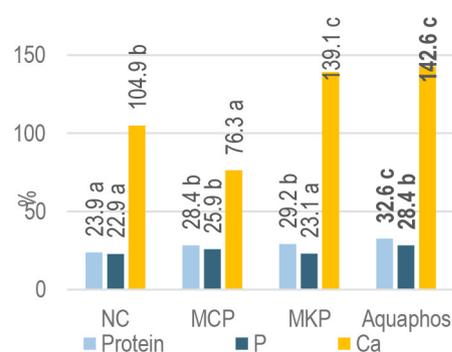


Figure 2: Nutrient retention in whole body, % of intake ($p < 0.001$)

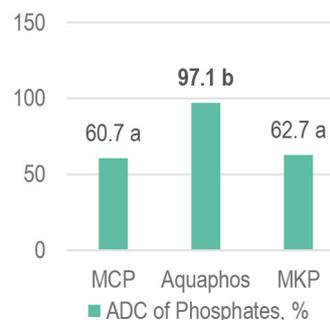


Figure 3: Apparent Digestibility Coefficient of phosphates, % ($p < 0.001$)