World Aquaculture Singapore 2022

NEXT GENERATION AQUACULTURE

INNOVATION AND SUSTAINABILITY WILL FEED THE WORLD

Nov. 29 - Dec. 2, 2022

Singapore EXPO Convention & Exhibition Centre and MAX Atria

The Annual International Conference & Exposition of World Aquaculture Society

Asian Pacific Aquaculture 2022
– Annual Meeting of Asian Pacific Chapter, WAS

Hosted by Singapore Food Agency

Conference Sponsors
Temasek Polytechnic, Nanyang Technological University
National University of Singapore, James Cook University
Republic Polytechnic

3rd International Symposium on Perch and Bass

WAS Premier Sponsors

KEMIN AQUASCIENCE · BLUE AQUA · MSD Animal Health · Zeigler nutrition through innovation · GRAND Fish Feed

USSEC U.S. SOYBEAN EXPORT COUNCIL · US SOY Delivers Solutions

WA2020 Partner

@WASAPC

APEC Policy Partnership on Food Security

@WASingapore

WASAPC

Associate Sponsors
Aquaculture Engineering Society
International Association of Aquaculture Economics & Management
WorldFish

Welcome Message for World Aquaculture 2022

Warmest greetings to everyone

On behalf of the Singapore Food Agency (SFA) I like to welcome everyone to Singapore for World Aquaculture 2022. This conference was originally intended to be held in Singapore in 2020. But the Covid-19 pandemic resulted in the conference being postponed until 2022. Therefore, we are really pleased to be able to welcome you to Singapore.

Despite your busy schedule during WA 2022, I hope that you will get the chance to do some sightseeing and try our local cuisine. We have a diverse range of food in Singapore. However, as Singapore is a small country, most of our food has to be imported. Therefore, food security is an ongoing concern for us. To ensure a stable supply of safe food, the Singapore Food Agency has embarked on a 30 by 30 goal to locally produce 30% of our nutritional needs by the year 2030. While this appears to be an ambitious goal, we believe that it can be achieved.

One of the cornerstones of our 30 by 30 goal is aquaculture. We aim to increase the volume of aquaculture products produced in Singapore by 2030. To achieve this, we want to transform our traditional net cage way of farming into high tech and highly productive fish farms where we can make use of the latest technology and farming methods to increase output.

Therefore, it is with this background that we are happy to host World Aquaculture 2022 in Singapore. We want to take this opportunity to learn from you and to also share with you the opportunities for fish farming and research & development in aquaculture in Singapore. Despite our small size, Singapore has a lot to offer.

Finally, I like to thank the conference organizers, participants from the industry, the various government agencies and all who have contributed to the planning and organization of this Conference. Thank you for your efforts and hard work.

Let us all make World Aquaculture 2022 a resounding success.

Thank you very much.

Dr Leong Hon Keong
Co-Chair of WA 2022

WELCOME MESSAGE

Dear All,

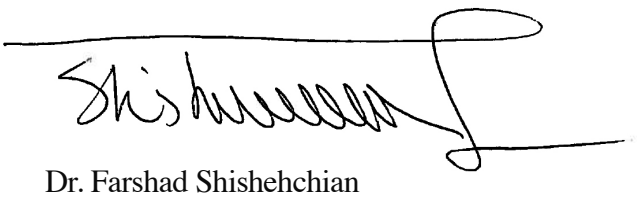
Welcome to the World Aquaculture 2022 Conference. It is an honour for us to host you in the beautiful Singapore. We are confident that during the following days, all of you will have plenty of opportunities to share knowledge and experiences, meet old friends, make new ones; and eventually start new businesses. Such moments in our academic and business careers are contributing to the exciting growth and the development of sustainable aquaculture globally. As you will see, the conference will offer some of the greatest opportunities to access key players of aquaculture in Asia and to network with experts travelling from all over the world to Singapore.

Being the world leading aquaculture association, it has been important for the World Aquaculture Society (WAS) to organize this global event in Singapore, a global hub for business and knowledge exchange. Our combined trade show and scientific conference reflect our commitment to consolidate the linkage between academia and industry as a basis for innovation.

We would like to thank the Government of Singapore and its agencies for all the supports received, especially the Singapore Tourist Board and the Singapore Food Agency. We extend our acknowledgements to the steering committee and program committee members for their hard work in preparing the event. Finally, we would like to thank all our sponsors and exhibitors for being part of the WAS family and making this event a success.

The Garden City is one of the most conducive business environments globally, providing us with incredible amenities, cultural diversity and outdoor activities. With so many varieties of food and culture, Singapore is a home to some of the best gastronomy in the world. This necessarily includes some of the locally produced aquaculture products. We are confident that this will be an unforgettable event for everyone!

Enjoy World Aquaculture 2022!



Dr. Farshad Shishehchian

Dr. Guillaume Drillet

Co-Chairs of the World Aquaculture 2022

TABLE OF CONTENTS

To find abstracts for a specific author or subject, use the pdf search features built into Adobe Acrobat.

ABSTRACTS

PILOT TESTS ON INCORPORATING THE FILAMENTOUS GREEN ALGAE, *Rhizoclonium riparium* IN THE DIET OF THE NILE TILAPIA AT PANGASINAN STATE UNIVERSITY, PHILIPPINES

Rosie S. Abalos*, Augusto E. Serrano Jr.,Barry Leonard M. Tumbokon, Raul V. Dela Pena, Ricardo A. de Guzman, and Lemark M. Bautista

College of Fisheries
Pangasinan State University- Binmaley Campus
Binmaley, Pangasinan
2417 Philippines

This study was conducted to evaluate in the field the results obtained in the controlled laboratory of feeding the Nile tilapia incorporating the filamentous green algae *Rhizoclonium riparium var implexum* in practical diet.

A super intensive culture system at PSU-Binmaley using a stocking density of 26 fish per m^2 was used to test the growth and survival of Nile Tilapia fed with a diet incorporating the filamentous green algae.

The daily feed intake of the *Rhizoclonium* group was significantly the least and thus also the feed cost while the final body weight was significantly the highest.

When the profitability is projected into one-hectare unit, the *Rhizoclonium*-based culture under a super intensive system resulted in the highest estimated gross revenue of Php 1.51 Million in just 64 days. The estimated additional profit of the *Rhizoclonium*-based culture over that of the commercial feed-based was Php 0.49 Million in just two months exclusive of other costs such as labor, tilapia fingerlings, among others.

Table 1. Growth and efficiency performance of Nile tilapia cultured in a super intensive system (26 fish per m^2) in Pangasinan State University – Binmaley Campus, fed either commercial feed, control feed or *Rhizoclonium*-based feed for 64 days

Treatment	IBW (g)	TFI (g)	DFI g day^{-1}	FBW (g)	WG (g)	FCR	SGR (% day-1)	AGR g day^{-1}	Surv. No.	Surv. rate (%)	CNP
Commercial	70.67	75.1^{b}	1.42	104.0	39.80	4.4	0.6^{b}	0.52	62	77.1	58.88^{a}
Control	57.77	113.6^{ab}	1.24	117.0	56.53	1.9	1.0^{ab}	0.93	67	83.8	26.13^{b}
Rhizoclonium	64.26	125.7^{a}	0.78	120.8	59.27	1.3	1.1^{a}	0.88	76	94.2	24.90^{b}

Superscripts indicate significant differences between diets ($p < 0.05$).

Table 2. Profitabilities of super intensive culture of tilapia pilot-tested in Pangasinan State University-Binmaley Campus.

Treatment	*DFI (g day-1)*	*AGR (g day-1)*	*FBW (g)*	*Feed cost cage^{-1} (Php)*	*Gross Rev Cage^{-1} (Php)*	*Est. Profit cage^{-1} (Php)*	*Est. Feed cost ha^{-1} (M Php)*	*Est. Gross Rev. ha^{-1} (M Php)*	*Est. Profit ha^{-1} (M Php)*
Commercial	1.42	0.52	104.0	290.97^{a}	562.67	230.00	0.97^{a}	1.38	0.41^{a}
Control	1.24	0.93	117.0	254.44^{a}	633.00	378.69	0.85^{a}	1.46	0.61^{ab}
Rhizoclonium	0.78	0.88	120.8	182.86^{b}	733.00	550.28	0.61^{b}	1.51	0.90^{b}

Superscripts indicate significant differences between diets ($p < 0.05$). DFI = daily feed intake (g day^{-1}); AGR = absolute growth rate (g day^{-1}); FBW = final body weight (g); Feed cost cage^{-1} = FBW*feed cost: Php 39.60 kg^{-1} of commercial feed; Php 33.60 kg^{-1} for the control, and Php 32.50 kg^{-1} for the *Rhizoclonium* diet; Gross Revenue cage^{-1} = FBW*survival number*Php 80 (gate farm price of 1 kg tilapia); Profit cage^{-1} = Gross rev. cage^{-1} minus feed cost cage^{-1} with the exclusion of other expenses such as labor, cost of tilapia juvenile, etc.; Feed cost ha^{-1} = feed cost cage^{-1}*3,333.33 since a cage measures 3 m^2 and there are 10,000 m^2 in an hectare; Gross Revenue ha^{-1} = FBW in kg*no. of fish stocked in 1 ha (i.e. 260,000)*0.60 (expected rate of survival)*farm gate price (Php 80 kg^{-1}); Profit ha^{-1} = Gross Revenue ha^{-1} minus Feed cost ha^{-1}.

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

Abdul Mateen*

Department of Zoology, Wildlife and Fisheries
University of Agriculture, Faisalabad, Pakistan
*mateen117@yahoo.com
mateen117@uaf.edu.pk

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

ASSESSING THE PREVALENCE AND LOAD OF PRAWN PATHOGENS IN THE NORTHERN AUSTRALIA PRAWN INDUSTRY

Thomas Ackery, Phoebe Arbon, Kelly Condon, Ellen Ariel, Roger Huerliman, Dean Jerry

JCU AquaPATH Detection Laboratory
James Cook University
Townsville, QLD, 4814, Australia
aquapath@jcu.edu.au

Our understanding of endemic and exotic prawn pathogens in *P. monodon* hatchery systems remains fairly sparse, with limited large scale studies having contributed to this topic. The hatchery phase represents a critical stage of the production cycle influencing the overall success of each farm. Furthermore, the Australian *P. monodon* industry is reliant on wild caught broodstock which poses a biosecurity risk when they enter a controlled environment from the uncontrolled natural environment they are caught from. The aim of the study presented here was to attain a "snap-shot" assessment of the pathogens that are present at the starting phase of prawn production, namely, the hatchery system.

This project analysed pleopod samples from 967 individual *P. monodon* broodstock that were sourced from four commercial hatcheries. The participating hatcheries supply over 80% of the postlarvae to the Australian prawn farming industry. The *P. monodon* broodstock in this study were wild-caught from East Coast Queensland and Northern Territory. Samples were analysed, using qPCR, for the presence of WSSV, YHV-1, IHHNV, GAV, YHV-7, HDV, When-2, Pir-A as well as the *P. monodon* Dicer-1 gene. Details of the geographic source (East Coast Queensland or Northern Territory), health status (culled, moribund, dead, dead on arrival, jumped from the tank), production status (pre- or post-spawn) and date of sample collection were provided. This allowed comparisons in viral load between stock sources used by prawn farmers in Australia to collect their broodstock. The effect spawning had on viral load of broodstock was also investigated by comparing pre and post spawned broodstock. Quantification and basic statistical paramaters for each virus were enabled through qPCR.

This study concludes Phase 1 of the NA CRC project "Improving Biosecurity in northern Australian prawn aquaculture" (Ref No. A.3.1718113). The analysis conducted in Phase 1 of the NA CRC is the most comprehensive and extensive study to date that examines endemic viruses in Australian prawn hatchery systems. This study revealed correlations between pre and post spawn viral loads as well as differences in viral load and prevalence between East coast and Northern Australian *P. monodon* stocks. Results reported here are beneficial to informing better management decisions in prawn hatcheries as well as improving economic sustainability by reducing the risk of disease outbreaks in broodstock and postlarvae.

PHENOTYPIC AND TRANSCRIPTOMIC RESPONSE OF FARMED ATLANTIC SURFCLAMS (*Spisula solidissima*) TO REPEATED HEAT STRESS AND THE FEASIBILITY OF SELECTIVE BREEDING FOR GREATER HEAT TOLERANCE

Michael P. Acquafredda[1*], Ximing Guo[1], and Daphne Munroe[1]

[1]Haskin Shellfish Research Laboratory
Department of Marine and Coastal Sciences
Rutgers, The State University of New Jersey
6959 Miller Avenue, Port Norris, New Jersey 08349
United States of America

**Presenting author*
email: Michael.Acquafredda@rutgers.edu; phone: +1 856-785-0074

Bivalve aquaculture is an important and rapidly expanding sector in global food production, yet climate change presents numerous challenges to its continued expansion. The Atlantic surfclam (*Spisula solidissima*) is emerging as an attractive alternate species grown by aquaculturists across the Northeast, USA. This species is native, grows rapidly, and complements the region's established farming framework. However, the surfclam is vulnerable to high temperature conditions, an issue that will be exacerbated by rising ocean temperatures and one that will be problematic on shallow coastal farms. This study evaluated the phenotypic and transcriptomic response of farmed surfclams to heat stress, and the ability for the heat-tolerant phenotype to be passed to subsequent generations. Initial results showed that when juvenile surfclams were exposed to prolonged lethal temperatures, the adult survivors withstood subsequent heat stress for significantly longer than non-selected (control) individuals. Transcriptomic response of heat-selected and control surfclams to a heat shock (29˚C for six hours) was also evaluated. The control or naïve surfclams had a more intense response to the heat shock, differentially expressing about 3x more genes, than the heat-selected clams that had survived a previous heat exposure. Additionally, enhanced heat tolerance was evident in first-generation progeny after selection, and the growth of heat-selected progeny was not significantly different than that of control progeny under ambient rearing conditions. This work suggests that genetic variation exists in heat tolerance, and selective breeding may be a viable strategy for enhancing summer survival of farmed surfclams.

EFFICACY OF STARCH SUSPENSIONS FROM SWEET POTATO *Ipomea batatas* FOR REMOVAL OF EGG STICKINESS DURING ARTIFICIAL PROPAGATION OF AFRICAN CATFISH *Clarias gariepinus*

Olabode T. Adebayo* and Gbemisola T. Otitolaye

Federal University of Technology
Department of Fisheries and Aquaculture Technology
PMB 704, Akure, Nigeria
otadebayo@futa.edu.ng

African catfish, *Clarias gariepinus* is one of the predominant and viable cultivable fish species in Nigeria. This fish species can easily be propagated by induced breeding in the hatchery. However, the stickiness of the eggs during artificial propagation has been a major bottleneck affecting the hatching of the eggs and invariably the survival of prospective larval. This constraint discourages many fish breeders in Nigeria from artificial propagation of *C. gariepinus*. Sweet potato (*Ipomea batatas)* is widely cultivated in Nigeria. It is also a stable and starchy food. Hitherto, there are no information on the use of starch suspension of *I. batatas* for the removal of eggs stickiness. Thus, the aim of this study was to determine the use and efficacy of *I. batatas* as egg desticking agent. The specific objectives were to determine the optimum concentration and immersion period that efficiently removed eggs stickiness, increase hatching and survival of larval.

Post fertilized eggs of *C. gariepinus* were collected in 0.25g aliquots (175eggs) into 20 experimental spawning bowls for each treatment replicate. They were rinsed in three concentrations of *I. batatas* starch suspension treatments (0.5%, 1.0% and 1.5%) and Control (0%), at immersion periods {5, 10, and 15mins}.

The 0.5% concentration level had egg detachment between 36% and 88%, 1.0% had egg detachment between 33% and 41% while 1.5% had egg detachment between 25% and 66%. The result shows no regular pattern across the concentration levels and immersion periods. The incubation periods of the post fertilized eggs was not affected by the immersion periods but by concentration levels. There was significant difference ($P<0.05$) across all concentration levels but there was no significant difference ($P>0.05$) across the immersion periods. The hatchability of post fertilized eggs in 0.5% concentration level decreased as immersion period increases while 1.0% and 1.5% had highest hatchability at 5 minutes. A deformed larva was found in 1.0% concentration level at 10 and 15minutes immersion periods. There was no regular pattern of increase in the larval survival with increase in immersion period but 0.5% and 1.5% concentration levels had highest larval at 15minutes while 1.0% had highest larval survival at 5minutes. There was no significant difference ($P>0.05$) across all concentration levels but there was significant difference ($P<0.05$) across the immersion periods.

Based on the results of this study, the optimum concentration level and immersion periods of *I. batatas* starch suspension for detachment of eggs of African catfish, *C. gariepinus* are 0.5% and 10minutes, respectively.

ASSESSMENT OF SEAFOOD MARKET STRUCTURE IN THE SOUTHWEST COASTLINE REGION OF NIGERIA

*Adeleke M. Lydia and Akinlade A. Deborah

The Federal University of Technology, Akure, Nigeria
Department of Fisheries and Aquaculture Technology
P.M.B. 704, Akure, Ondo State, Nigeria
mladeleke@futa.edu.ng

Seafood is a major commodity in the South West region of Nigeria owing to its vast economic importance. However, the demand-supply gap continues to widen hence, sustainable seafood production is therefore paramount through efficient and effective marketing to ensure the transfer of seafood products from the producers to the final consumers. This study therefore, assesses seafood marketing in Nigerian coastline: the case of Lagos and Ondo States in the South West, Nigeria. Multistage sampling technique was used to collect data from 180 respondents primarily using well-structured questionnaire. Data were analysed and seafood market structure was measured using the Gini coefficient (GC), and Lorenz curve indexes.

The results of the two States; Lagos and Ondo indicated an inequality in the sales or income of marketing actors (0.71) and (0.52). This means that there is non-uniformity in the prices of seafood in the study area that is, prices were determined based on the bargaining powers of the buyers and sellers. The result further reveals an imperfect competitive market systems in the study area. The Lorenz curve buttresses the Gini coefficient value Figure 1 and 2. The curves bowed outward towards the northeast for both States. There is a close proximity in the value of plotted data in Lagos State, Nigeria compared to that of Ondo State, Nigeria.

The study revealed that seafood market was under strong oligopoly at concentration ratio of 71% and 38% in Lagos and Ondo States respectively. The study therefore, recommends that effective programmes, policies and optimum sensitization that will enhance the sustainability of seafood marketing should be put in place. This will enhance an orderly seafood market, solve marketing challenges, break the oligopolistic nature of the seafood market and provide a market responsive to the demand and supply forces.

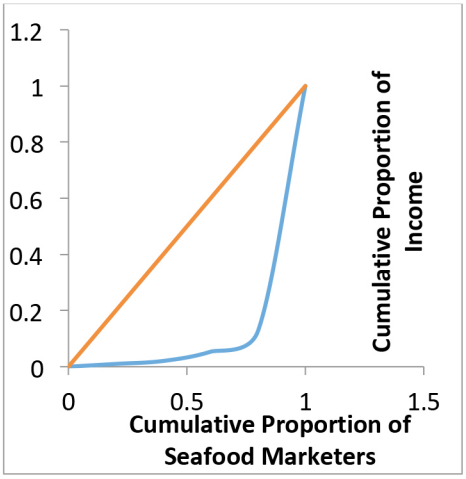


Figure 1: Lorenz Curve for Seafood Marketers in Lagos State, Nigeria

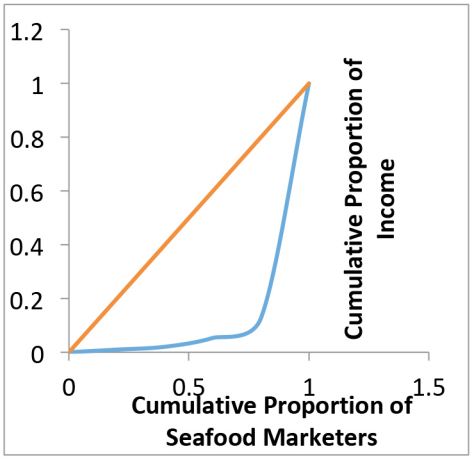


Figure 2: Lorenz Curve for Seafood Ondo State, Nigeria

Source: Computed from the field survey data, 2018

SUB LETHAL EXPOSURE AND TOXICITY EFFECT OF LINEAR ALKYL BENZENE ON HAEMATOLOGY AND SERUM BIOCHEMISTRY IN *Clarias angularis* IN A STATIC BIOASSAY

[1]Lawee, A.Y., [2]Adeyemi, S. O., and [1]Zuleideen, M.G.

[1]Aquaculture and Fisheries, Department,Nasarawa State University Keffi,, Nigeria
[2] Kogi State University Lokoja,Kogi State
Corresponding Author; laweeyusuf@gmail.com +2348032898637

Linear alkyl benzene is a toxicant that can adversely affect the health of a fish. The study was conducted to ascertain the toxicity effect of the detergent on haematological and serum biochemical indices in *clarias angularis* juveniles of fairly uniform sizes. Juveniles of *C. angularis* were exposed to sub-lethal concentrations (0.0mg/l, 0.45, 0.60, 0.75, 0.95 & 1.05mg/L) of the detergent derived from the 96hour lethal concentrations for eight weeks. The result of the study revealed that significant changes and dose dependent decreases in red blood cell (RBC), packed cell volume (PCV) and haemaglobin (Hb) values were observed in detergent exposed fish compared to the control. Progressive increases in white blood cell count (WBC) were found in detergent exposed fish compared to the control. Significant increases in biochemical parameters (glucose and protein were observed in detergent exposed fish groups compared to the control. However, cholesterol levels decreased significantly except for the highest contaminant concentration and duration of exposure. Linear alkyl benzene detergent incorporated into culture medium to juveniles of *C.angularis* was seen to have disrupted the haematological and biochemical profile of the fish.

Introduction

Fishes are grown for commercial purposes to meet the dire need of animal protein of man and to earn foreign exchange (Fagade*et al*., 1993). However, it is one of the most important non-target aquatic organism affected by industrial wastewaters discharged into the environment contain organic and inorganic pollutants in dissolved, suspended and insoluble forms. The ability of the pollutant to disrupt the biological balance or cause deleterious effect on the aquatic ecosystem in dependent on the concentration and the physicochemical characteristics of the wastewaters. Even in situations where the pollutants are discharged in low concentrations with increase in discharge rate to ecosystems, there is an accumulated effect which in turn may be hazardous (Odiete, 1999). A great deal of research has been conducted to ascertain the effect of pollutants on the physiology and survival of many aquatic organisms. Scott and sloman (2004) reported that because behaviour links physiological function with ecological processes, behavioural indicators to toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations.

A detergent is a surfactant or a mixture of surfactants with "cleaning properties in dilutesolution". These substances are usually alkyl benzene sulphonates, a family of compounds that are similar to soap but are more soluble in hard water because the solar sulphonate (of detergent) is less likely than the polar carboxyl (of soap) to bind to Calcium and other ions found in hard water. Detergents like soap, work because they are amphoteric (having the characteristics of both acid and base), partly hydrophilic (polar) and partly hydrophobic (non-polar) Werner *et al*., (2002).

Experimental Area

The experiment was carried out at the Fisheries Unit of the Experimental Farm of the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, Nasarawa State University Keffi. Lafia is located on latitude 8° 35'N, longitude 8° 32'E, altitude 181.53m above sea level with a mean temperature of 34°C, relative humidity of 40-86% and average day light of 9-12h (NIMET, 2016).

Experimental Animal and Experimental Toxicant

Apparently 200 healthy juveniles of fresh water catfish, *Clariasanguilaris*of known weight and length was purchased from the Korlem Fish Farm Ltd, which is located along Mararaba-Karu, Nasarawa State and was transported in 50 liters modified jerry-can containing a sufficient water from the farm to the department of aquaculture and fisheries management, Nasarawa state University Keffi. It was transferred carefully into the concrete outdoor tanks for acclimatization, each tank with diameter 2m x 2m breadth, and each tank was supplied with borehole water using flow through system to ensure aeration for two weeks. The fishes were acclimatized for two weeks during which they were fed twice daily (08:00am- 4:00 pm) with a commercial feed coppens 2mm and feed at 5% body weight.

(Continued on next page)

The detergent (Klin) used in this study was bought from Lafia Main Market, Nasarawa State. The chemical constituents of the detergent as stated on the detergent nylon are: Surfactants, Sodium sulfate, Sodium carbonate, Optical brighteners, phosphate, poly-carboxylates and enzymes. It was in powdery form and whitish in colour.

Determination of Sub-lethal concentrations

Sub-lethal concentrations ranges from (0.0mg/l, 0.45, 0.60, 0.75, 0.95, & 1.05mg/L) used in the current study were derived from 1/5, 1/10 and 1/20 of the 96h LC_{50} (1.2mg/l) linear alkyl benzene detergent to *C.angularis* .Measurement for the toxicity test were done following the procedure as recommended by (Oladimeji and Ologunmeta, 1987).

Experimental Design

Twelve (12) transparent plastic aquaria tanks (42cm x30cm x 24cm) were cleaned and setup for the experiment. Each tank contained 25litres of dechlorinated water. Total of one hundred and twenty (120) juveniles of C.angularis (24± 0.11 and 10± 0.12) were randomly selected and assigned to six varying sub lethal concentrations of linear alkyl benzene 0.00mg/l (as control) (0.45,0.60,0.75,0.95& 1.05mg/L) as treatments. Each treatment was duplicated in a completely randomized designed (CRD) .Each duplicate tank contained ten fish. The experiment lasted for eight weeks and was done under natural photoperiod (12:12 light-dark cycle).Water quality of the test media were monitored and sampled daily while remnants of the unconsumed feed and the excreta were siphoned .The exposed solution was renewed every 48hrs and nominal concentrations of linear alkyl benzene replaced. The fish were fed twice daily 8:00-16:00h at 5% body weight with commercial feed containing 45% crude protein.Water quality parameters such as Temperature, PH and Dissolved oxygen were monitored and estimated following the procedures of APHA (1985) and the mean of the respective parameters are reported as 28.11 ± 0.11^{0}, 6.11-6.56 and $5.86\pm0.13mg/l^{-}$ .The treatment lasted for eight weeks.

Hematological Studies

Blood Sampling

Three fish per duplicate were sampled and the end of 8 weeks for blood collection and were never returned to the respective test mediums. Collection of blood from fish specimens was done following the procedure of Blaxhall and Daisely(1973). Fish specimens were anaesthetized using tricainemethanesulphonate (MS 222) to ensure easy collection of blood. Blood was collected by severance of caudal peduncle from the caudal artery. The caudal region was cut 2cm away and blood then collected with EDTA plastic tube Iheanacho et al., (2018). Blood specimens were transferred to the laboratory unit, DalhatuAraf Specialist Hospital for hematological analysis.

Total Erythrocyte Count (Red blood cell)
Hendricks solution was used for the erythrocyte count. Neubauer's chamber haemocytometer was prepared and blood drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool to adjust the volume to exactly 0.5 mark. The pipette was filled to 101 marks with the diluting fluid and shaken for 30 minutes to ensure mixing. The diluted suspension of cells then drawn into the chamber. The haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer were counted with 4mm objectives and x 40 eyepiece microscope. The number of cells was multiplied by x 10 and this gave the total number of cells per cubic millimeter (mm3) of blood.Hesser (1960).

Total Leucocytes Count (White blood cell)
Leucocytes were counted using Shaw's solutions A and B. The blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shaken the bulb of the pipette half way and then filled to 101 mark with solution B. A few drops were dispensed into the haemocytometer. The cells in the four large squares of the chamber were counted using a 4mm objective lens at 40 × magnification. The number of cells was multiplied 10 × to obtain the total number of leucocytes per cubic millimeter (mm3) of blood Hesser (1960).

Haematocrit (packed cells volume)
Determination of packed cells volume was carried out by micro-westegren method as described by Blaxhall and Daisely(1973). The well mixed sampled blood from the severance of caudal peduncle was drawn into micro-haematocrit tube, 75mm3 long, and 1.1-1.2 mm3 internal diameter. The tubes were then centrifuged for five minutes. The reading was made with the aid of a micro- haematocrit reader and expressed as the volume of the erythrocytes per 100 cm3.

(Continued on next page)

Biochemical Parameters

Table 1: Heamatological profile of*Clariasanguilaris*juveniles exposed to sub-lethal concentrations of Linear alkyl benzene sulphonate

Parameters	0.00g Control	0.45g	0.60g	0.75g	0.95g	1.05g
PCV (%)	34.00±5.66[a]	31.00±1.41[b]	27.00±4.24[c]	26.00±1.41[d]	25.00±2.83[e]	24.00±2.83[f]
WBC ($10^{9/1}$)	92.10±11.17[f]	98.20±3.11[e]	102.10±2.97[d]	106.00±5.66[c]	110.10±14.00[b]	120.00±14.14[a]
RBC($10^{12/1}$)	3.30±0.42[a]	3.30±1.41[a]	2.60±0.85[b]	3.80±1.13[a]	2.40±0.57[c]	2.50±0.00[b]
Hb (g/dl)	11.10±1.27[a]	10.10±1.14[b]	9.00±2.83[c]	8.10±2.97[d]	7.10±1.27[e]	6.20±1.31[f]
Glucose (mmol)	3.75±0.35[b]	2.76±0.34[d]	2.91±0.13[c]	2.91±1.28[c]	4.23±0.33[a]	3.44±0.63[b]
Cholesterol (mmol)	2.75±0.36[a]	2.40±0.56[c]	2.86±1.22[a]	2.38±0.54[c]	2.63±0.90[b]	2.78±0.34[a]
Protein (mm0l)	3.0±011[a]	3.0±0.26[a]	3.46±0.42[b]	3.56±0.10[b]	3.73±0.15[c]	3.78±0.15[c]

Means with different superscript along the row are significantly different (p>0.05)
Values are Mean ± StDev

Blood and Serum Collection

Collection of blood for biochemical examination was done following the same procedure for hematology and blood was collected in 3ml non-heparinized tubes. Blood samples were immediately taken to DalhatuAraf Specialist Hospitalfor serum extraction and analysis. To obtain the serum, the blood was placed in micro centrifuge tubes, and immediately centrifuged at 1500 rpm for 10 minutes. Serum was then removed by pipetting and stored at 40C prior to immediate determination of biochemical parameters. Glucose, total protein (TP) and cholesterol levels were measured with an automatic biochemical analyzer (Olympus AU 400 biochemical analyzer, Tokyo Japan). Procedure for the analysis was done following the manufacturer's instructions.

Statistical Analysis

Data obtained from hematology and biochemical analysis were subjected to statistical analysis using statistical package for social sciences, one-way analysis of variance (ANOVA),, version 22.Results were presented as mean ± SE. Test for significant difference between treatment groups were done using Ducan multiple range test (DMRT) and significance declared at 5%.

Results and Discussion

Heamatology

Heamatological profile of*Clariasanguilaris*juveniles exposed to sub lethal concentrations of Linear alkyl benzene sulphonateis presented in Table 1below. The result revealed that the packed cell volume (PCV %), red blood cell (RBC) and haemoglobin were highest (34%, 3.30.00±0.42 and 11.10g/dl) in the control fish i.e. *Clariasanguilaris*juveniles exposed to 0.0g of linear alkyl benzene sulphonate, whereas white blood cell (WBC) was highest 120.00±14.14 x$10^{9/1}$in *Clariasanguilaris*juveniles exposed to 1.05g of linear alkyl benzene sulphonate. Glucose was highest 4.23±0.33 mmol in concentration 0.95g of the detergent while cholesterol was highest 2.86±1.22 mmol in *Clariasanguilaris*juveniles exposed to 0.60g of linear alkyl benzene sulphonate.

Sublethal Concentration

Idowu*et al*. (2017) reported a remarkable decrease in the values of haematological parameters in *C. gariepinus*fed with sunlight detergent. The result of haematology in this study is in agreement with (Joshi *et al*., 2002) that reported effect of toxicants on blood parameters in fresh water teleost fish *Clariasbatrachus*. Similar reduction of blood parameters were made by various authors.

(Continued on next page)

Jayaseelan (2001) reported that the RBC count decreased significantly in the fresh water fish, *Labeorohita*on exposure to herbicide glyphosate. Soundararajan and Veeraiyan (2014), noticed the decreased haemoglobin content in the fresh water fish, *Tilapia mossambica*on exposure to arsenic. Vasanthi*et al*., (2016) made similar observation in *Cirrhinusmrigala*exposed to detergent tide. All these observations confirm the findings of the present study.

The reduction in haematological parameters is indicative of blood loss from fish fed with diet that contains detergent compared to the fish fed with normal diet, although they were not significantly different from the initial haematological values of the fish and those fed with the control diet. The standard values shown under some tables' show that the fish were probably overfed which led to the increase of their standard values.

Increase in white blood cell of the treated fish in this study showed increase in pathogenic stress that need to shoot-up immunity to combat such pathogens. This observation was in line with that of Adejinmi (2000). Increase in total white blood cell (leucopomia) as observed in the fish fed with normal diet is attributed to increased production of leucocytes in the haematopoietic tissue of the kidney and perhaps the spleen. Lymphocytes are the most numerous cells comprising the leucocytes which function in the production of antibodies and chemical substances serving as defense against infection. The primary consequence of observed changes in leucocytes count in stressed fish is suppression of the immune system and increased susceptibility to disease (Wedemeyer and Wood, 1974). The values for fish fed poultry litter are lower than those reported for African catfish by (Agbede*et al*., 1999 and Oyelese*et al*., 1999). Lower haemoglobin level might decrease the ability of fish to enhance its activity in order to meet occasional demands (Idowu*et al*., 2017).

The marked reduction in the blood parameters (PCV, HB, RBC, Glucose and Cholesterol) of the exposed fishes confirms the report of (Wadaan and Mubarak, 2009) who reported blood parameters reduction in rabbit exposed to Sodium Dodecyl Sulfate (surfactant). The toxic effect of the anionic detergent (surfactant) has been reported by (Dehelean*et al*., 2004), to cause reduction in blood parameters and weight loss. The reduction in the blood parameters was as a result of destruction of the cells of the fish by the surfactant and traces amount of heavy metals found in detergents. Riaz*et al*. (2009), reported that blood and skin of an animal may be adversely affected by soaps and detergents used as anti-bacteria and insecticides because they do so by killing the cells of the germs and insects.

References

Abbas, H.H.H. and Mahmood, H.M. (2003). The toxicological effect of water pollution on the Nile Tilapia fish (*Oreochromisniloticus*) collected from four sites along the River Nile. *Journalof Egypt Veterinary Medical Association*. 63: 307-323.

Abbas, H.H.H. and Mahmood, H.M. (2004). Haematological and biochemical changes in *Oreochromisaurens*and *Clariasgariepinus*exposed to mixtures of copper and lead salts. *Egyptian Journal of Basic and Applied Physiology*.3: 89-1 06.

Adejinmi, O.O. (2000). The chemical composition and nutritional potential of soldier fly larvae (*Hermetiaelucens*) in poultry rations. University of Ibadan., PhD. Thesis. Pp292.

Agbede, S.A., Ogunsanmi, A.O., Taiwo, V.O., Oso, J.A. and Ogundipe, T.I. (1999). Toxic effects of poultryfaeces on *Clariasgariepinus*broodstock. *Tropical Veterinarian*. 17:181 – 191

Ainsworth, S.J.J. (1996). Linear alkyl benzene sulphonate. *Chem. Eng. News* 74: 32-54.

Alaa El-Din, S.H., Usama, M.M. and Iman, M.A. (2012).Toxic effects of 4-nonyiphenol on the embryonic development of African catfish *(Clariasgariepinus)*. *International Journal of Biology and Biological Sciences* Vol. 1(2):34-46.

Almeida, C. (1994). Synthetic Detergent and Linear Alkyl Benzene. Course Note on Chemical Technology (Organic) Module IV.

Anilkumar, V. and Janaiah, C. (2009). Imidacloprid Exposure atters serum biochemical parameters in*Channapunctatus*(Bloch). *Bulletin of Pure and Applied Sciences*, vol. 28A(2). 123-130.

Avoajah, D.A and Oti, E.E, (1997). *Nigeria Journal of Biotechnology*, 8; 40— 45.

Ayoola, S.O. (2008). Histopathological Effects of Glyphosate on juvenile African Catfish *(Clariasgariepinus) Amerisurasian*. *Journal of Agric and Environmental Science*. 4(3):362-367.

Bakirel, T., O. Keles, S. Karatas, M. Ozcan, G. Turkmen and A. Candan. (2005). Effect of linearalkyl benzene sulphonate (LAS) on non-specific defence mechanisms in rainbow trout (*Oncorhynchusmykiss*). *Aquat. Toxicol*. 71, 175 -181 (2005).

Blaxhall, P.C., Daisley, K.W., "Routine haematological methods for use with fish blood.",Fish Biology, 5: 771-781, (1973).

Chukwu, L.O. and Odunzeh, C.C. (2006). Relative toxicity of spent lubricantoil and detergent against benthic macro-invertebrates of a West African estuarine lagoon. *J. Environ. Biol*., 27, 479-484.

Cook, A.M. (1998). Sulfonated surfactants and related compounds: facets of their desulfonation by aerobic and anaerobic bacteria. *Tenside Surfactants Detergent* 35: 52-56.

(Continued on next page)

Demand, M. (2012). Historical perspective of the phosphate detergent, university of colorado 2(3):1-3.
Dehelean, C., Nastase, V., Dragomirescu, A., Heges, A. and Dinte, E. (2004). Skin toxicity of sodiumlauryl sulfate as evidenced in an animal model. *Rev. Med. Chir. Soc. Med. Nat. Iasi*., Vol(108), 169 – 172.
Department of Petroleum Resources (DPR); (2002). Environmental. Guidelines and standards for the petroleum industries in Nigeria (EGASPIN) Revised Edition.
Di Corcia, A., Casassa, F., Crescenzi, C., Marcomini, A., Samperi, R. (1999). Linear alkylbenzene sulfonate chemical structure and bioavailability. *Environ. Sci. Technol*. 33: 4112 -4118.
Dunphy, J, TW Federle, N Itrich, S Simonich, PK Kloepper-Sams, J Scheibel, T Cripe, E Matthijs (2000). Environ. profile of improved alkyl benzene surfactants, *5th Cesio World Surfactants Congress* V.2: 1489-1497, May-June, Firenze, Italy.
ECOSOL (2005), *Statistics*, Brussels.
Eniola, K.T.T. and Olayemi, A.B. (2002). Some Aspects of Bacterial-Detergents interaction in fresh water environment. *Bioscience Research Communication*. 14 (6): 645-649.
Environmental Protection Agency (EPA); (2007): Washington, DC. The national water Quality inventory, report to congress fact sheet No, EPA 841-F- 07-003.
Esenowo, I.K. and Ugwurnba, O.A. (2010). Growth response of catfish exposed to water soluble fraction of detergent and diesel oil. *Environmental Research Journal* 4(4):298-301.
Fafioye, O.O. (2001). Lethal and sublethal effects of extract of *Parkirabiglobosa* and *Raphia vinifera* on some freshwater fauna. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria. 216.
Field, J.A., Barber, B.L., Thurman, E.M., Moore, B.L., Lawrence, D.L. and Peake, D.A. (1992). Fate of Alkyl benzene sulfonates and Dialkyltetralinsulfonates in sewage-contaminated Groundwater. *Environ. Sci. Technol*. 26: 1140-1148.
Hesser, E.F. (1960). Methods for routine fish hematology. *Fish* 22(4): 164-171.
Ikele, C., Bright, M., Bernard, O.O. and Ndusuisi, (2011). Fisheries and Hydrobiology Research unit Department of Zoology, University of Nigeria Nsukka, Enugu state, Nigeria. *Animal Research International* 8(1): 1375-1379.
Idowu E.O., Adewumi A. A. and Babalola A. E. (2017). Effects of Detergent (Alkylbenzene sulfonate) on the Haematology of *ClariasGariepinus*. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*. 10 (3): Pp 69-75.
Iheanacho, S.C., Ogbu, M., Ude, E., Ayotunde, I., Ogueji, E., "Growth, Hematology and Immuno-Modulatory Potential of Ginger *(Zingiberofficinale)* Supplemented Diets in *Clariasgariepinus*Juvenile (Burchell, 1822)", Aquaculture Studies, *18*, 41-49. http://doi.org/10.4194/2618-6381-v18_1_05, (2018).
Jayaseelan, K., Muthukumaravel, K. and Rajakumar, R. (2001). Toxic effects of herbicide glyphosate on haematological parameters in the fresh water fish, *Labeorohita*. J.*Ecotoxicol. Environ. Monit*., 21(3), 277-285.
Joshi, P.K., Harish, D. and Bose, M. (2002). Effect of lindane and malathione exposure to certain blood parameters in a fresh water teleost fish *Clariasbatrachus*. *Pollution sources*. Vol 21(1): 55 – 57.
Kamrin, M.A. and Mayor, G.W. (1991). Diethyl phthalate - a perspective. *Journal of Clinical Pharmacology*, 31(5):484-489.
Kock, G., Triendi, M. and Hofer, R. (1996). seasonal pattern of metal accumulatioin Arctic char *(Salvelinusalpinus)* from an oligitrophic Alpine lake related to temperature. C*anadian Journal of Fish Aquatic Science*. 53:780-786.
Ladipo, M. K., Doherty, V.F. and Oyebadejo, S.A. (2011). Acute toxicity, behavioural changes and histopathological effect of paraquat dichloride on tissues of catfish (*Clariasgariepinus*). *Int J Bio*l; doi: 10.5539/ijb.v3n2p67
Liwarska-Bizukojc, E., K. Miksch, A. Malachowska-Jutsz and J. Kalka. (2005). Acute toxicity and genotoxicity of five selected anionic and nonionic surfactant. *Chemosphere*, 58, 1249-1253.
Mathur, A.K. (2005). Effects of dermal application of chromium and linear alkyl benzene sulphonate alone and in combination in guinea pigs. *Toxicol. Int*., 12, 9-12 .
Millar, J.S., A.C. Debra, G.M. Mary, J.R. Deniel and T.B. Jeffrey. (2005). Determining hepatic triglyceride production in mice: Comparison of poloxamer 407 with Triton WR-1339. *J. Lipid. Res*., 46, 2023-2028.
NIMET (2016).Nigerian Metrological Agency. Synoptic Office, Lafia, Nasarawa State, Nigeria.
Nomura, Y., Ikebukuro, K., Yokoyama, K., Takeuchi, T., Arikawa, Y., Ohno, S., and Karube, I. (1998). Application of a linear alkylbenzenesulfonate biosensor to river water monitoring. *Biosensors Bioelectronics* 13:1047-1053.
Ogundiran, M.A., Fawole, O.O., Adewoye, S.O. and Ayandiran, T.A. (2010). Toxicological impact of detergent effluent on juvenile of African catfish *(Clariasgariepinus)*. *Agriculture and biology Journal of NorthAmerica* 20(5): 215 1-7525.
Haematological effect of acute concentration of cypermethrin on juveniles of *Clariasgariepinus*. *International Journal of Engineering and Science Invention* 2 (31):96 100.
Okpokwasili, G.O. and Nwabuzor, C.N. (1998). Primary biodegradation of anionic surfactants in laundry. *Chemosphere* 17: 2 175-2185.
Okwuosa, V.A and Omoregie E. (1995). *Aquaculture Res*. 26: 755 — '758pp.
Omoniyin, I.A., Agbon, O. andSodunke, S.A. (2002). Effects of lethal and sublethal concentrations of Tobacco (*Nicotianatobacum*) leaf dust extraction on weight and haematological changes in *Clariasgariepinus*(Burchell). *J. Appl. Sci. Environ. Manage*. 6:37-41.

(Continued on next page)

Omoregie, E, Ufodike, E.B.C and Keke, I.R. (1990). *Aquatic Science,* 5: 33 — 36.

Oti, E.E. (2003). Acute toxicity of water extracts of bark of the Thevetiaperuviana to the African freshwater catfish "Heteroclarias" hybrid fingerling *J. Fish. Tech.,* 2, 124-130.

Oti, E.E. (2005). Acute toxicity of gammalin 20 to *Chrysichthysnigrodigitatus*(Lacepede). Proceeding of the 16th annual conference of the Fisheries Societyof Nigeria(FISON)Maiduguri. 75-l79.

Oyelese, A.O., Taiwo, V.O., Ogunsanmi, A.O. and Faturoti, E.O. (1999). Toxicological effects of cassava peels on haematology, serum biochemistry and tissue pathology of *Clariasgariepinus*fingerlings. *Tropical Veterinarian*. Vol(17):17 – 30.

Rana, S.V.S. and Y. Verma. (2005). Biochemical toxicity of benzene. *Review Article. J. Environ.Biol., 26, 157-168.*

Rani, R., S.P. Trivedi, P. Singh and R.K. Singh. (2002). Haematotoxic effect of linear alkyl benzene sulphonate (LAS) in fish, *Heteropneustesfosilis. J. Environ. Biol.,* 23, 101-103 (2002).

Riaz, S., Ahmad, A. and Hasnain, S. (2009). Antibacteria activity of soaps against daily encountered bacteria. *Afr. J. Biotechnol.* Vol (8): 1431 – 1436.

Ruiswell, R.E., Brimblecombe, P., Deut, D.L. and Liss, P.S. (1992). Environmental Chemistry, the Earth-Air-water Factory. Edward Arnold. Great Britain.

Saxena, P., S. Sharma, V. Suryavathi, R. Grover, P. Soni, S. Kumar and K.P. Sharma. (2005). Effect of an acute and chronic toxicity of four commercial detergents on the freshwater fish, *Gambusiaaffiris. J. Environ. Biol.*, 47, 119-124.

Sharma, S., S. Sharma, S. Kumar, P. Soni, K.K. Bhardwaj, R. Grover, V. Suryavathi and K.P. Sharma (2004). Comparative effects of two consumer (fish) in the oligotrophic and eutrophic microcosms. *Ind. J. Environ. Sci.*, 8, 95-102.

Shahsavani, D., M. Mohri and K. Nazeri. (2004). A study of effects of anionic detergents (shampoo) on blood parameters of gold fish (*Carassiusauratus*). Pajoushesh-va-sazandegi. In: Animal and fisheries science. pp. 99-103.

Soundararajan, M. and Veeraiyan, G. (2014). Effect of heavy metal arsenic on haematological parameters of fresh water fish, *Tilapia mossambica. Int. J. Modn. Res. Revs.*, 2(3), 132-135.

Sprague, J.B (1971). The ABC of pollutant bioassay using fish in Cairus J. and Dickson K.L (eds). Biological methods for the assessment of water quality. Philadelphia, American society for Teating and materials 528, 6-30.

Valtorta, L., Radici, P., Calcinai, D. and Cavalli, L (2000). Recent development of LAB/LAS, *Riv. It. Sostanze Grasse* LXXVII: 73-76.

Wadaan, M.A.M. and Mubarak, M. (2009). Blood chemistry an evidence of the toxic effects of anions surfactant sodium dodecyl sulfate. *Asian J. Scientific Res.* Vol(2): 113 – 118.

Wedemeyer, G.A. and Wood, J.W. (1974). Stress as a predisposing factor in fish diseases. Fish Disease Leaflet 38. 749

World Health Organization WHO (1996). International programme on chemical safety environment health criterial 169 Linear AlkylbenzeneSulfonates and related compounds. World Health Organization Geneva.

RISK-BENEFIT OVERVIEW ASSOCIATED TO THE CONSUMPTION OF SEAWEEDS TAKING INTO ACCOUNT BIOACCESSIBILITY, CASE STUDIES

Cláudia Afonso*, Carlos Cardoso, Raquel Quintã, Pedro Pousão-Ferreira, Narcisa M. Bandarra

*Division of Aquaculture and Upgrading, Portuguese Institute of the Sea and Atmosphere (IPMA)
Avenida de Brasília, 1449-006 Lisboa, Portugal
cafonso@ipma.pt

Seaweeds (SW) are a widely available resource, which can be a solution to meet an increasing global consumer's demand for sustainable and healthy alternative food sources. SW are a rich source of nutrients, as iodine (I), being its consumption advised in the prevention/delay of some diseases. Still, as for others foods, their consumption is subject to a nutritional-toxicological conflict, since some SW may contain potentially dangerous high I and contaminants levels, as arsenic (As). These elements have different health impacts, which need assessment. A realistic risk-benefit assessment (RBA) requires not only knowing a total compound's level, but also the maximum fraction released from the food into the digestive tract, bioaccessibility (Bioac). Moreover, SW may be eaten fresh, dry, rehydrated or cooked. This may affect compounds' content, Bioac, and RBA, albeit rarely studied.

This work aimed to study 12 cultivated/wild SW species, green (*Rhizoclonium riparium* and three *Ulva species*), red (*Pterocladiella capillacea*, *Osmundea pinnatifida*, *Asparagopsis armata* and *A. taxiformis*), and brown (*Petalonia binghamiae*, *Halopteris scoparia*, *Fucus spiralis* and *Saccorhiza polyschides*) consumed and/or produced/caught in Portugal/Europe. Proximate composition, fatty acids (GC), and elements (ICP-MS) were analysed by standard methods. Bioac was simulated by an *in vitro* model. The RB was assessed by calculation of the probability of exceeding the advised thresholds.

Results showed that SW have a unique nutritional profile. Fat levels were low (< 4 %) and had a specific fatty acids profile. They had high levels of essential elements, particularly I. Non-essential elements (Hg, As, Cd, and Pb) were lower and, for most of the studied species, do not represent a hazard to consumers. Bioac varied across compounds and species. Namely, I Bioac reached 50-75 % in wild *S. polyschides* and *F. spiralis*, respectively, but in green SW and *S. polyschides* cultivated in an Integrated Multi-Trophic system it did not exceed 40 %. This study enabled to set advisable consumption frequency intervals for several seaweed species.

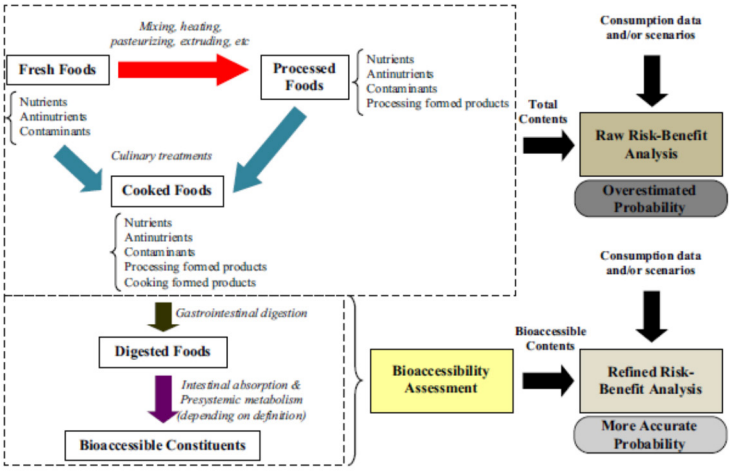


Figure 1 - Risk-benefit assessment based on compound content, cooking/hydration treatment, and bioaccessibility data.

REPRODUCTIVE CAPACITY AND POPULATION GROWTH OF CLADOCERAN ZOOPLANKTON, *Moina micrura* FED WITH DIFFERENT MICROALGAE DIETS

Umi Wahidah Ahmad Dini[1*], Fatimah Md Yusoff[1,2,3], Zetty Norhana Balia Yusof[1,4], Norulhuda Mohamed Ramli[5]

[1]Laboratory of Biotechnology Marine, Institute of Bioscience
[2]Department of Aquaculture, Faculty of Agriculture
[3]International Institute of Aquaculture & Aquatic Sciences (I-AQUAS)
[4]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences
[5]Department of Biological and Agricultural Engineering, Faculty of Engineering
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
umiwahidah2013@gmail.com

In aquatic ecosystem, zooplankton represents the channel of transmission of energy and organic matter from primary producers to top consumers. As primary producers, microalgae are the main food source for zooplankton which contributes ultimately in providing nutrients and energy for the aquatic food chain. Rapid reproduction and highly nutritious contents of zooplankton enable them to become effective live feed for the aquaculture industry. In order to become effective live feed, they should be fed using high quality nutritional food source, such as beneficial phytoplankton species. Therefore, the objective of this study is to determine the effect of different microalgae including cyanobacteria on reproductive capacity and population growth of cladoceran zooplankton, *Moina micrura*.

Moina micrura was treated with six phytoplankton treatments (Diet 1= *Chlorella* sp., Diet 2=*Chlamydomonas* sp., Diet 3=*Cyclotella* sp., Diet 4=*Pavlova* sp., Diet 5=*Microcystis* sp. and Diet 6= *Planktothrix* sp.). Experiments on reproductive capacity (parameters including longevity, age at first reproduction, total clutch, total egg and total offsprings) of each individual neonate were observed for fifteen replicates for each treatment. The population growth study was conducted in triplicates for each treatment. The culture conditions were maintained at room temperature 27.0 ± 2.0° C under a photoperiod of 12 h light: 12 h dark.

Diets 1 to 4 were beneficial for *M. micrura* because all of them promoted significantly faster maturity, higher number of eggs, offspring and clutch, longer longevity and higher population density (P<0.05) compared to Diet 5 and Diet 6 (Table 1 and Figure 1). The negative effects of cyanobacterial diets (Diet 5 and Diet 6) might be due to their toxin and poor nutritional contents. This study suggested that the highly nutritious microalgae diets can significantly increase the survival, reproductive, and growth performance of *M. micrura* which in turn can contribute to the refinement of quality live feed production for the aquaculture industry. On the other hand, cyanobacteria, which can be dominant in eutrophic waters, would have negative impacts on zooplankton growth and production

TABLE1. Reproductive capacity of *Moina micrura* fed with different diets. Means with different letters were significantly different ($P < 0.05$).

Parameters	Treatments					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Age at first reproduction (Day)	2.0±0.1^{b}	2.0±0.2^{b}	2.0±0.1^{b}	2.0±0.1^{b}	4.0±0.1^{a}	4.0±0.2^{a}
Number of eggs (Ind Female^{-1})	18.0±0.7^{a}	18.0±0.5^{a}	19.0±0.6^{a}	18.0±0.4^{a}	5.0±0.3^{c}	7.0±0.3^{b}
Number of offspring (Ind Female^{-1})	16.0±0.6^{a}	16.0±0.4^{a}	17.0±0.6^{a}	16.0±0.5^{a}	4.0±0.3^{b}	4.0±0.3^{b}
Number of clutch (Ind Female^{-1})	5.0±0.2^{a}	4.0±0.2^{ab}	4.0±0.3^{b}	5.0±0.2^{a}	2.0±0.1^{c}	2.0±0.1^{c}
Longevity (Day)	14.0±0.5^{a}	14.0±0.6^{a}	15.0±0.6^{a}	15.0±0.5^{a}	7.0±0.2^{b}	7.0±0.3^{b}

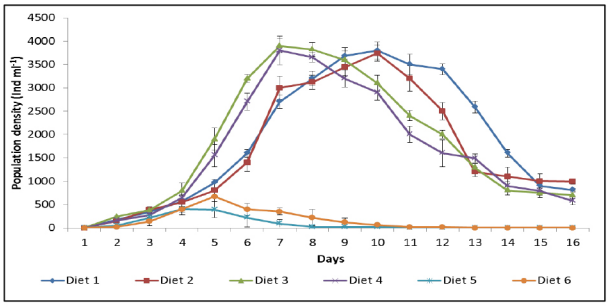


FIGURE1. Population density of *Moina micrura* fed with different diets.

REPRODUCTIVE FEATURES OF NATIVE SPECIES MUSSEL *Anodonta cygnea* IN ANZALI WETLAND IN IRAN

Mohaddeseh Ahmadnezhad*, Homayoun Hosseinzadeh Sahafi, Mohammad Sayad Bourani, Sohrab Dajandiyan, Hossein Khara

*Inland Waters Aquaculture Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran
**m_ahmadnezhad@yahoo.com*

A native freshwater mussel inhabitant of Anzali wetland in Iran is *Anodonta cygnea* whose population is in danger due to pollution and habitat destruction by human manipulation. The objective of the present study was to survey some reproductive characteristics and gametogenesis of this bivalve in order to plan for future breeding programs. Reproductive traits of this species were evaluated for one year period using biometric and histological studies by standard method. The mean length, width, height, weight and age were 10.6 ± 0.1 cm, 6.04 ± 0.04 cm, 4 ± 0.04 cm, 132 ± 3 g, 6 ± 0.1 years old, respectively. In all shells, the two sexes were separated. There was no apparent sexual appearance. The frequency of female was 1.5 times that of male. The mean diameter of mature oocyte and sperm in fresh smears were 139.95 ± 8.1 and 4.5 ± 0.26 μm and in tissue slides were 59.6 ± 0.8 and 2.2 ± 0.03 μm, and the mean area of them by the fresh observation were 15255.58 ± 1834.4 and 9.1 ± 1.16 μm^2 and by histological method were 3171.46 ± 79.4 and 3.9 ± 0.6 μm^2, respectively. The mean fecundity was 107844.6 ± 20621.9. Both of male and female gonads were in developing phase in spring (May and June) and in summer (July and August). The spawning stage occurred in the fall and the post-spawning stage in the winter. Although, gametogenesis was active in all seasons, the peak of oogenesis was observed in July and September and the peak of spermatogenesis was in September. The development of glochidia was completed in January and the release of them from the gill has been occurred between January and March.

STATUS AND PROSPECTS OF AQUAPONICS IN THE SULTANATE OF OMAN

Hussein S. Al-Masroori*, Wenresti Gallardo, Adil Al-Sulaimani, Ahmed Al-Souti, Nora Ourabah Haddad, Ghady Chedrawi, Hasna Al-Harthy, and Ziyin Zhang

Department of Marine Science & Fisheries
College of Agricultural and Marine Sciences
Sultan Qaboos University
P.O. Box 34, PC:123 Al-Khod, Oman

Oman has successfully started the development of integrated agriculture-aquaculture (IAA) production systems, particularly aquaponics, in rural and water- scarce areas. Based on existing literature review, there is limited information on integrated agriculture-aquaculture (IAA) in Oman. The present study was conducted with the aim of documenting the current status of IAA in the Sultanate of Oman and describe beliefs and awareness, knowledge, socio-economic characteristics and challenges as well as future plans of the farmers regarding the development of their activities. An online questionnaire survey was created and administered to all farmers engaged in IAA. The survey information was then checked for missing, unclear and/or doubting information, which were filled/cleared by phone or personal communication by researchers.

Results showed that around 57% of participants are practicing integrated aquaculture (non-recirculating aquaponic system) and 43% are practicing recirculating aquaponics. Integrated aquaculture farms discharge an average of 187 ± 239 (SD) m^3 of water per day from fish tanks to irrigate the plants. An average of 37% reduction in fertilizer use was reported by the four integrated farms. Except for one, all farms surveyed use ground water for farming. One farm uses water provided by the government for residential houses. Among those who use groundwater from the wells, one farm uses 'falaj' water. Tilapia is the only fish cultured in IAA systems in Oman. Cucumber is the major commercial crop from integrated aquaculture while lettuce is the major crop followed by basil, in recirculating aquaponic system.

Lack of experience and skilled labor are among the major challenges encountered in operating aquaponics system followed by lack of marketing and administrative support and then lack of fish feed and feed factory. Despite these challenges, there is a high willingness and readiness to further invest in IAA particularly aquaponics and this indicates a promising prospects for the future development of these systems in the Sultanate. Considering that Oman suffers from scarce freshwater supply and is concerned with environmental impacts, IAA system is highly recommended as a promising of on-conventional source of water to address water scarcity. Government authorities are encouraged to 1) promote IAA particularly aquaponics as a business, considering that it can be profitable and is environmentally-friendly, 2) minimize the constraints and challenges by providing the needed support services including mainly feed manufacturing that will strengthen the aquaculture sector in Oman, as currently feeds are imported, and 3) provide additional funding and promote national and international collaboration and investment partnership to further develop an aquaponic technology that is profitable and suitable for Oman.

LONG TERM MODELLING OF BENTHIC EFFECTS OF THE SEABREAM CAGE CULTURE FARM IN QURIYAT, OMAN, USING AQUAMODEL

Dawood Al-Yahyai*, Wenresti Gallardo, Gerd Bruss, Dale Kiefer and Zach Siegrist

Ministry of Agriculture Wealth, Fisheries and Water Resources
Muscat, Sultanate of Oman
dawoodalyahyai@gmail.com

Accumulation of uneaten feed and fecal matter is the main impact on marine sediments beneath the cages. Their continuous accumulation will lead to enrichment of sediment with organic carbon which can affect benthic life and may lead to an anoxic bottom environment. Different simulation models have been established and developed to examine these effects from aquaculture cages. Therefore, the main objective of this study was to use the modelling approach in quantifying the possible impacts of gilthead seabream (*Sparus aurata*) marine cage farm at Quriyat, Oman, on benthic sediment.

A modelling tool (AquaModel) was used for simulations in this study. The simulation period was for the period from 25 May 2017 to 31 December 2020. The operational farm data for this period along with the environmental water quality variables were incorporated in the model for purpose of simulation. Many sediment variables were selected for simulation with main ones including total Organic carbon (TOC), total Organic carbon rate (TOC rate), sediment sulfide and sediment oxygen.

The average monthly TOC levels of the farm were below 1.6% which was the threshold set during the simulations (Table 1). For sediment sulfide, the monthly average levels were below the threshold level of 1500 μM. For sediment oxygen, the monthly average levels were above the threshold level of 2 g_ox m^{-3}. The average TOC rate from the cage farm was 1.15 g_C m^{-2} d^{-1} (Figure 1). Modeling results showed low to moderate effects of cage farms on the sediments based on current activities of the farm. However, there is a need for continuous monitoring to determine potential changes in sediment variables with the expected increase in production capacity of the farm in the future.

Table 1: Summary of the main sediment variables from the simulation, values are mean ± SE and range.

Variables	Mean± SE	Range
TOC (frac)	0.010±0.00	0.008-0.015
TOC rate (g_C m^{-2} d^{-1})	1.15±0.15	0.01-16.64
Sulfide (µM)	93±14.4	0-3459
Sediment Oxygen (g_ox m^{-3})	2.27±0.00	1.96-2.3

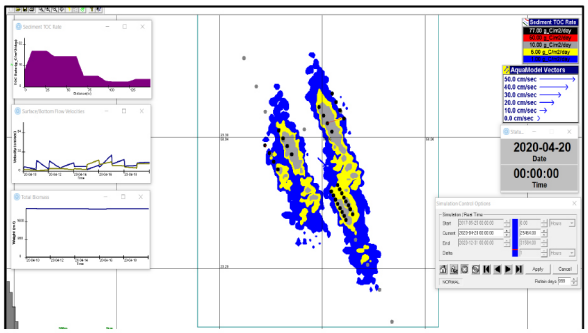


Figure 1: Contours levels of sediment TOC rate (g_C m^{-2} d^{-1}) at the period of maximum fish biomass.

WHOLE GENOME SEQUENCING AND DEVELOPMENT OF SNP MARKERS FOR ROHU CARP *Labeo rohita* (HAMILTON)

Daniel G. Peterson[1], Mark A. Arick II[1], Chuan-Yu Hsu[1], Adam Thrash[1], Emma Ruth Miller[1], Attila Karsi[1], Mark Lawrence[1], John Benzie[2], Matthew Hamilton[2], Md. Akhtaruzzaman Khan[3], Md. Samsul Alam[3]*

[1]Mississippi State University, USA; [2]WorldFish Center, Penang, Malaysia, [3]Bangladesh Agricultural University, Mymensingh
*Department of Fisheries Biology and Genetics, Bangladesh Agricultural University
Mymensingh 2202, Bangladesh
samsul.alam@bau.edu.bd

Aquaculture and fisheries have long been recognized as a means by which Bangladesh can help feed and increase the nutritional health and wellbeing of its people. *Labeo rohita* (rohu) is one of several carp species that account for much of the protein in the diets of Bangladeshis. To provide insight into genes that can be used to increase the efficiency of aquaculture of rohu, we sequenced the whole genome of *Labeo rohita*. Briefly, blood from a single male rohu was used to isolate DNA. The DNA was sequenced using Illumina and Oxford Nanopore Technologies (ONT) platforms. Contigs were assembled using the ONT reads, and the Illumina data was used to correct errors in the ONT contigs. Hi-C chromatin configuration sequencing was conducted to further assemble the contigs into scaffolds. Functional annotations were done with Uniport Swisprot and InterproScan. The rohu genome assembly indicated that the rohu genome size is about 939.5 Mb in length, a value less than half the reported size of the rohu genome as determined by Feulgen densitometry (1C = 1950 Mb). To explore this discrepancy, we performed our own flow cytometric determinations of rohu genome size and found our flow cytometry estimates to be close to our predicted genome size (1C = 968 Mb average for five male rohu). The current assembly of the rohu is composed of 6,175 scaffolds, L50 is 26, and N50 is 1.29 Mb. Functional annotations indicated there are 29,494 genes with 30,480 mRNAs predicted. The Illumina sequences from fish collected from three different river systems (Padma, Jamuna and Halda) had a mapping rate around 99% against the genome assembly. From this mapped data filtering to SNPs between known NsiI-MspI restriction sites, the number of predicted SNPs found to be 469,392. SNP analysis indicated that fish collected from the Padma and Jamuna are genetically closer compared to Halda fish. The productivity of species depends on two things, management and biological potential of the species. By completing a high quality whole genome sequencing and assembly and developing SNP markers, we have created an opportunity to improve the biological/genetic potential of rohu. For this, it is essential to utilize this information to develop improved rohu strain by marker assisted selection.

ELECTRICAL STUNNING AND MICROBIAL DIVERSITY DURING THE CONVERSION OF MUSCLE TO MEAT IN A DECAPOD CRUSTACEAN SPECIES

Amaya Albalat[1]*, Douglas Neil[2], Sasha Saugh[1], Karen D Adler[4], Ahmed Elsheshtawy[1], Sonia Rey Planellas[1], Simon MacKenzie[1], Thomas Sicheritz-Pontén[3]; Martha Clokie[4], Benjamin Gregory James Clokie[1]

[1] Institute of Aquaculture; University of Stirling; Stirling, Stirling UK
*amaya.albalat@stir.ac.uk
[2] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow; UK
[3] The GLOBE Institute, University of Copenhagen, Copenhagen,1353 Denmark
[4] Department of Genetics and Genome Biology; University of Leicester; Leicester; UK

The farming of decapod crustaceans is a key economic driver, with production reaching 11.2 million tonnes (USD 81.5 billion) in 2020. In the last decade, public interest in the welfare of decapod crustaceans has increased in many parts of the world. In the UK, for instance, decapods crustaceans are recognised as sentient. In other countries, changes in legislation on methods for stunning have occurred. Electro-stunning may have the potential for reducing noxious stimuli experienced by decapods during slaughter. However, data on activity in the central nervous system and product quality-related data are not available for most of the important decapod species. Furthermore, decapod crustaceans are highly perishable due to rapid post-harvest bacterial growth. Previous work in our group has identified multiple bacteria species that drive lobster spoilage. However, comprehensive microbiome spoilage landscape for key decapod species is missing. Therefore, the aim of this research was firstly to investigate electro-stunning in a valuable decapod species, Norway lobster (*Nephrops norvegicus*) as a humane slaughter method and secondly to characterise microbiome changes during the conversion of muscle to meat.

Recordings of nerve activity showed that electro-stunning can render *N. norvegicus* rapidly insensible qualifying it as a potential humane slaughter procedure. In contrast, placing lobsters on ice for 30 min did not suppress neural activity. Results also show that while shelf life was not affected by the electro-stunning process, this stunning method did shorten the period that the product would be considered fresh. From a microbial biodiversity perspective, results indicate a clear lag phase of up to 5 days if product is stored on ice and key representatives' families will be discussed. This research represents an important effort towards improving decapod welfare practices and increasing our understanding on the degradome of valuable seafood products.

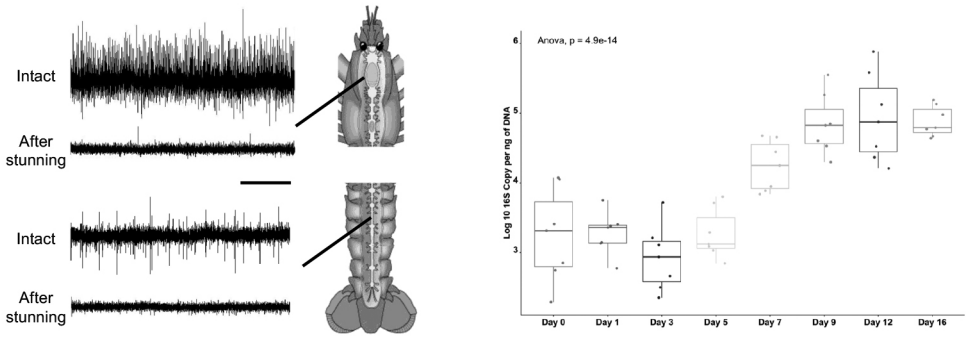


Fig 1. A) Spontaneous nerve activity recorded extracellularly in the left circumoesophageal connective and the abdominal nerve cord of a *N. norvegicus*. Scale bar 1s. and B) 16S copy numbers (Log10)/ng DNA extracted from *N. norvegicus* muscle stored on ice.

TECHNOLOGY TO SUPPORT SUSTAINABILITY IN AQUACULTURE: LEARNINGS, PROGRESS AND CHALLENGES

Juliette Alemany*, Emmanuelle Bourgois

Fairagora Asia ltd., Asoke Tower 213/10, 5 Flr
Sukhumvit 21 Road, Klong Toei Nuea Wattana
Bangkok 10110, Thailand
Juliette@fairagora.com

Sustainability in aquaculture is a recurrent topic and its geometry varies; the environmental challenges and the social impacts it creates are being questioned. Are there answers and more importantly solutions?

In this presentation, we show the forces interacting in some of the aquaculture supply chains, through a sustainability lens. From this mapping, we underline how industry solution(s) need to emerge in order to foster change for friendlier farming for the planet and for the people involved. This implies designing innovative solutions, which have at their core economic efficiency.

This paper covers a mapping and its findings from the shrimp and salmon farming industries. We showcase two very different types of production value chains, one being artisanal (shrimp) and one industrialized (salmon). From this research, we draw findings around designing innovative solutions, and the limitations imposed when the supply base is too scattered to cause a domino effect.

Sustainability itself encompasses a wide field, therefore the number of variables, indicators and metrics varies, and changes based on regulators, influencers or prescribers. This multi-faced reality of perception correlates in a complex framework and therefore pushes for better efficiency in measuring sustainability.

To measure sustainability, a first step is to collect detailed datasets that can then be merged to meet the requirements of various standards. Technology can help to easily aggregate data and identify the gap in the initial datasets. Once the metrics are finalized, they can be used to connect the variable needs of the industry and consumers through clear, transparent narratives.

Modeling of complex production chains together with multifaceted sustainability data sets, allows innovation to take place. It creates the engine to run and enable connections, clarity, understanding and transparency. This is to create a pull factor for change to responsible practices. It is also to complement the usual top-down push factor - well known and used by brands and buyers.

ELECTROCHEMICAL DETECTION OF IRIDOVIRUS IN FISH SAMPLES

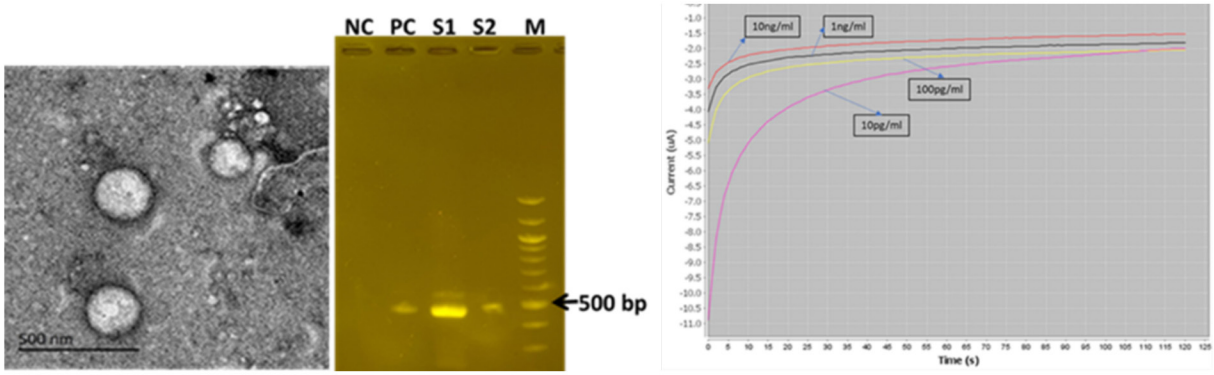
Nurshahidah Ali*, Masato Miyata, Padmanabhan Saravanan, Chan Pek Sian Diana, Lee Chee Wee

Centre for Aquaculture and Veterinary Science, School of Applied Science, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
nali@tp.edu.sg

Iridoviruses are large, icosahedral shaped, enveloped DNA viruses (~120–200 nm in diameter) that replicate in the cytoplasm of infected cells. Iridoviruses cause systemic disease and are associated with high mortality among thirty-five over marine and freshwater fish species. Current methods of detection takes long turn-around time and laboratory dependent.

The team has developed a rapid and sensitive electrochemical DNA biosensor for fish iridovirus detection that could be applied to both indoor and outdoor settings. In brief, semi-concentrated viruses from tissue homogenate were lysed using ultrasonication method and subjected to recombinase polymerase assay at 39°C for 18min. The amplified DNA was purified by affinity displacement method. The eluted sample was applied onto screen printed electrode substrate to initiate the reaction. The sample was read after 1 min using amperometric measurement and concentration of DNA was determined.

The method was found to be rapid with <20min of analysis time and sensitive. Operational advantages include user friendliness with less logistical load, fieldable, can be tailored to detect wide range of pathogens



THE STRUCTURE, CONDUCT, AND PERFORMANCE OF THE MIDSTREAM SEGMENTS OF THE AQUACULTURE VALUE CHAIN IN BANGLADESH

Hazrat Ali*, Ben Belton, Khondker Murshed-e-Jahan, Ricardo Hernandez, and Mohammad Mahfujul Haque

*Department of Aquaculture, Bangladesh Agricultural University, Mymensingh & WorldFish, Bangladesh
Email: h.ali@cgiar.org

The rapid growth of aquaculture in Bangladesh over the past 30 years has been facilitated by the proliferation of fish traders and retailers, but comparatively little is known about the organization and behavior of actors in these segments of the aquaculture value chain in Bangladesh. We conducted a comprehensive statistically representative study with 329 aquatic product traders in 31 markets from seven districts in south and southwest Bangladesh between January and May 2021 to address this knowledge gap. Five distinct categories of trader were identified: (1) wholesalers, who buy fish from farmers and traders for sale to other traders and retailers (N =50); (2) auctioneers (known as *arotder* in Bangla language) who earn commission from buyers and sellers (N =75); (3) depots, who receive shrimp and freshwater prawn from farmers and assemblers, for sale to processing factories (N=62); (4) assemblers, who mainly buy small quantities of crustaceans from farmers and transport them to market (N=42); (5) retailers, who purchase small quantities of fish from other traders for sale to consumers (N=100). The total number of traders in surveyed markets increased by more than 175% within the past 10 years. Numbers of wholesalers grew faster than any other category of traders. Wholesalers trade the largest average volumes among the five actor types (526 t/year), of which 88% is fish (Table 1). This finding is consistent with results from farm surveys conducted simultaneously in the same zone which show a big increase in fish production over this period. Trading businesses are predominantly family owned and operated, and mainly create employment opportunities for men (99%). Fish trading created an average of 14 days of employment per ton of fish across all trader types, amounting to a total 2,542 full time equivalent (FTE) jobs created by traders in our sample of which 51% family labor and 49% hired. Trader's annual working capital requirements, operating costs and gross margins are substantial, averaging $18,630, $17,000, and $22,722, respectively across all trader types. Average marketing margins earned on each transaction average 6.0% of the sales value, ranging from 2.9% for auctioneers, to 14% for retailers. These are relatively modest margins, indicating that the rate profit extracted by traders is not excessive.

Table 1: Characteristics of aquaculture product traders

Variables	Wholesaler	Auctioneer	Depot	Assembler	Retailer	Total
N	50	75	62	42	100	329
Mean volume traded (t/year)	526	313	250	23	31	235
Total volume traded (t/year)	26,312	23,473	15,524	973	3,115	69,397
Total fish traded (t/year)	23,054	20,367	1370	286	2972	48,049
Total crustacean traded (t/year)	3,258	3,106	14,154	686	143	21,349
Fish share in total volume (%)	88	87	9	29	95	69
Mean labor days per ton fish sold	7	8	13	26	20	14
Total FTE jobs created	659	751	800	96	237	2,542
Working capital (USD/year)	21,159	15,152	41,411	1,737	525	18,630
Mean operating cost (USD/year)	56,493	4,218	19,579	1,039	1,523	16,985
Mean gross margin (USD/year)	43,565	23,795	30,913	5,496	4,975	22,722
Marketing margin (%)	5.3	2.9	4.5	4.6	14	6.0

OPPORTUNITIES IN ORNAMENTAL FISH BUSINESS IN NIGERIA

Okechukwu Alum-Udensi*, Johnpaul C. Ikwuemesi and Anthony A. Nlewadim

Department of Fisheries and Aquatic Resources Management
Michael Okpara University of Agriculture, Umudike. Nigeria
*okeyalum@yahoo.com

Ornamental fish keeping is a popular hobby in developed countries and is gaining popularity in many developing countries. Ornamental fish breeding, wild-fish collection and trade and associated aquarium business provide excellent opportunities in non-food fisheries for employment and wealth creation. Pet fish keeping has made a come-back in Nigeria after about two decade's decline of the hobby in the early 1990s.

There is a great opportunity for growth in both in international market and the almost untapped domestic market with the non-existence of aquatic outlets and public aquariums in major cities of Nigeria. This paper takes a close look at the business opportunities presented by this non-food fisheries sector for employment and wealth creation.

THE ECOLOGICAL REALITY AND DOMESTICATION POTENTIALS OF SILVER CATFISH *Chrysichthys nigrodigitatus* OF THE CROSS RIVER, NIGERIA

Daniel Ama-Abasi*, Mfon T. Udo, Edak Uyoh, Sandra Uwalaka, Uduakobong Sambo

Akwa Ibom State University, Obio Akpa Campus, Nigeria

Chrysichthys nigrodigitatus is a prized food fish in Nigeria, particularly in eastern Niger Delta, with good taste and fillets with high integrity. It forms the most commercially important freshwater single species fishery in the Cross River and provides employment opportunity to the teeming population. The fish population is on the decline due to overexploitation, pollution and climate change impacts. To avert the crisis in the sector, conservation through domestication is the best bet. However, the aquaculture of the species was considered impossible because it was believed that the species is estuarine and cannot be cultured. This supposition was like a myth since the species is abundant in the freshwater during the rainy season. To unlock the apparent mystery of the ecology of the species, we embarked on the studies of its migration and aquaculture potentials. The objectives were to establish the reality or otherwise of migration and investigate the domestication potentials of the species.

Fish were tagged and monitored for two years for possible migration between the upper course of the river and its downstream/ estuary. At the end of two years 64 specimens representing 7 % of the tagged samples were recovered. However, there was no evidence of migration from the downstream to upstream and vice versa. This proved that the fish was not migrating from the estuary for spawning or returning to estuary after spawning; rather the fish is freshwater species occurring all year round with potentials of culture in freshwater milieu. With this finding we embarked on the culture of the species both in concrete and earthen ponds. Fish in the concrete ponds were reared in extensive culture system and allowed to depend on natural food especially tilapia. Fish in the concrete tanks were fed with earthworms. After six months the biomass was measured.

Result shows that *Chrysichthys nigrodigitatus* survived throughout the period of the study with appreciable growth and biomass increase especially in the earthen ponds as indicated in Table 1. Increase in biomass in earthen ponds was mainly from growth and reproduction. That the species can breed in captivity underscores the imperative of its domestication. Domestication of the species will shield it from the vagaries of overexploitation, pollution and climate change impact, thereby ensuring its conservation and food security.

Table 1. Growth performance of cultured *Chrysichthys nigrodigitatus*

Enclosure	Initial weight(g)	Final weight(g)	Biomass increase	% biomass increase
Earthen pond	8359.6	15007.7	6648.1	79.5%

EFFECT OF DIFFERENT BIOFLOC VOLUME ON GROWTH AND SURVIVAL OF *Macrobrachium rosenbergii* POST LARVAE

Md. Eilious Hosain, S. M. Nurul Amin*, Aziz Bin Arshad, Mohd Salleh Kamarudin and Murni Marlina Abd Karim

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
sm_nurul@upm.edu.my

Biofloc system is the environment friendly blue revelation technology which enhanced productivity, ensure animal healthcare and bio-security and conserved water while optimum biofloc volume is a paramount important for productivity and survival of animal culture. The study was conducted to optimize the suitable floc volume for *Macrobrachium rosenbergii* post larve (PL) culture. The experiment was conducted in five different biofloc volumes *i.e*. floc volume 2-5 ml (BF2-5), 7-10 ml (BF7-10), 12-15 ml (BF12-15) and zero-exchanged biofloc (BF-Z) with triplicates during one month period. Giant prawn post larvae stocked at the rate of 5 individuals L^{-1} (5000 PL m^{-3}) with an average initial weight ± SD of 21.83 ± 12.92 mg in 12 polyethylene tanks (100 L).

The highest survival (82.33%) was found in 2-5 ml biofloc volume and followed by 72.33%, 62.0% and 14.66% in 7-10 ml, 12-15 ml and zero-exchanged biofloc volume, respectively. The minimum FCR (2.12) was recorded in 2-5 ml floc volume while the maximum FCR (12.11) was found in zero-exchanged biofloc (Table 1). It is revealed that the density of *Vibrio* spp. was also the lowest in 2-5 ml volume and it was the highest in zero-exchanged biofloc. Thus, it might be suggested that 2-5 ml biofloc volume could be apply for commercial venture of *M. rosenbergii* post larvae nursery phase.

TABLE 1. Average final weight (AFG), weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and survival rate (SR) of giant freshwater prawn post larvae reared in different floc volume based biofloc system. Means in a column with different letters were differed ($p< 0.05$) significantly.

	Biofloc system			
	2-5	7-10	12-15	BF-Z
AFW (mg)	213.36^{a}	229.83^{a}	210.06^{a}	255.53^{a}
WG (mg)	193.26^{a}	209.73^{a}	189.96^{a}	235.43^{a}
SGR (%/day)	8.14^{a}	8.40^{a}	8.10^{a}	8.63^{a}
FCR	2.12^{a}	2.24^{a}	2.88^{a}	12.11^{b}
SR (%)	82.33^{d}	72.33^{c}	62.0^{b}	14.66^{a}

A STUDY TO STANDARDIZE PROPER GROW OUT FEED FOR *Etrplus surnensis* AND DEMONSTRATE ITS PRODUCTION POTENTIAL IN BRACKISH WATER CAGES

Javad Amiri[1] *, S. Felix[2]
[1]Dr. MGR Fisheries College & Research Institute, Ponneri, Tamil Nadu, India
[2]Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattianam, Tamil Nadu, India

* Corresponding author:
javed5160@yahoo.com
Ph. D scholar, Dr. MGR Fisheries College & Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University
Ponneri- 601204, Tamil Nadu, India

This research was undertaken to standardize a proper grow out feed for *Etroplus surentensis* and demonstrate its production potential in brackish water cages". This study involved in preparation of experimental diets with different percentage of protein and lipids followed by two rearing experimental trials. The first trial was done to assess the optimum level of protein and lipid required for *E. suratensis* and the second trial was carried out in brackish water cages. Experimental diets with three different percentage of protein (25%, 30%, 35%) and three different percentage of lipids (3%, 6%, 9%) was formulated. Around 20 numbers of pearl spot fingerling with an average initial body weight of 12 g. Among the different protein and lipid the maximum mean body weight was recorded in treatments T2 (30% Protein) and T5 (6% Lipid), being (15.09^{a}±0.02g), (14.7^{ab}±0.02g) and shows maximum survival rate (100^{a}±0.00 %). The treatments differed significantly with control as well as with other treatments respectively ($p<0.05$). Hence, it could be observed that the 30% protein and 6% lipid was be the optimal requirement level for pearl spot fingerling to achieve maximum growth and survival. Again the maximum weight and survival rate in trial II was achieved in treatment T1 (30% protein and 6% lipid) which was (16.5^{a}±0.03 g), (100^{a}±0.00%). These treatments differ significantly with the control as well as with other treatments respectively ($p<0.05$).

STUDY ON SULTAN FISH, *Leptobarbus hoevenii* (BLEEKER, 1851) PROPAGATION

Thumronk Amornsakun*, Sajeenuth Srithongthum, Sarawuth Chesoh, Sitthisak Jantarat, Lim Leong Seng, Nobuo Suzuki, Yutaka Takeuchi and Anuar Hassan

Fisheries Technology Program
Department of Technology and Industries
Faculty of Science and Technology
Prince of Songkla University Pattani Campus, Pattani, 94000 Thailand

The sexual maturity of female sultan fish, *Leptobarbus hoevenii* was studied by determining fecaundity and gonadosomatic index (GSI). It was found that the size at sexual maturity of female sultan fish was with 40.85±3.80 cm in total length and 783.00±217.46 g in body weight. Fecundity was 76,414.69±36,586.61 ova/fish and gonadosomatic index (GSI) was 8.14±3.92%.

The artificial breeding injection was carried out by using synthetic chemical (Suprefact 20 μg/kg and Motilium 5 mg/kg) for induced spawning, male and female brooders were done by once injection. The trials about hatching rate and hatching period were carried out in a 15-liter glass aquarium (water volume 12 liters), containing 1,000 eggs and there replications in each trial. It was found that the average of hatching rate was 82.6% and hatching out was 22 hours 44 minutes at 27-29 C of water temperature.

The newly hatched larvae were 4.05±0.24 mm in total length with yolk sac of 77.625 μm^3 in volume. The yolk sacs were completely absorbed within 108 hr after hatching. All the larvae opened their mouth at about 36 hr after hatching (4.42±0.20 mm TL) with mouth measuring 182.36 μm in height.

Feeding experiments were carried out in the 15-liter glass aquarium with 10 liters of water containing 600 larvae aged 2.5 days post-hatching and feed them by *Moina*. At 2-hourly intervals, 20 larvae were collected randomly from the aquarium, At 62 hr after hatching, the mouth size was measured to be 412.27 μm. The onset of feeding showed 0.05 individual *Moina*/ larvae.

A starvation of newly hatched larvae was studied in the 15-liter aquarium with 12 liters of water by three replications. In each replication contained 300 newly hatched larvae by without feeding, observed every 2 hours. This trial was found that totally larvae died within 112 hours after hatching at water temperature ranging between 27.0 and 29.5 C.

GROWTH PERFORMANCE, IMMUNE RESPONSE AND DISEASE RESISTANCE OF NILE TILAPIA *Oreochromis niloticus* FED DIET WITH DIFFERENT LEVELS OF *Amaranthus spinosus* LEAF MEAL FOLLOWING *Aeromonas hydrophila* INFECTION

Isagani P. Angeles*, Jonathan M. Bolaños, Jonie C. Yee, Levy D. Madrid and Raymond M. Pascua

Freshwater Fisheries Center
Isabela State University-Main Campus
Echague, Isabela, Philippines 3309
ipangelesjr_15@yahoo.com

Nile tilapia *Oreochromis niloticus* is one the most farmed fish species in the country and it has been globally traded fish food around the world. This is because tilapia has positive aquaculture characteristics such as fast-growing fish, high-market demand, excessive breeding and minimal environmental requirements. Commercial approaches were used to control the spread fish diseases in various aquaculture farms, such as the use of anti-microbial agents and vaccination, but they have limited success in the prevention of/or treatment of aquatic diseases. Thus, the present study was conducted to evaluate the growth performance, immune response and disease resistance of Nile tilapia *Oreochromis niloticus* fed diet with different levels of *Amaranthus spinosus* leaf meal (ASLM) following *Aeromonas hydrophila* infection.

Fish were fed with a diet containing 25% (ASLM), 50% (ASLM) and 75% (ASLM) replacement of fish meal by ASLM. Formulated diet without ASLM served as the control (C). At the end of the experiment, growth, haematological profile and disease resistance to *A. hydrophila* infection of fish were evaluated. The influence of ASLM on the immunomodulation in *O. niloticus* was observed by evaluating its survival after *A. hydrophila* infection. Based on the results, the final weight, weight gain, daily growth rate and feed conversion ratio were significantly increased in the fish fed diet with ASLM50. The results also revealed that red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) count of fish fed with ASLM50 and ASLM75 were significantly higher than that of fish fed with C and ASLM25 before and after bacterial challenge test. However, HCT count of fish was comparable among treatments. The percentage survival of fish fed diet with ASLM was higher than that of C. Overall, replacing fish meal at 50% ASLM is promising in enhancing the fish growth performance, haematological profile and resistance to *A. hydrophila* infection.

Table 1. Rearing performance of Nile tilapia fed four practical diets. Means without a common superscript are significantly different ($P \leq 0.05$).

Diet	Wi	Wf	WG	DGR	FCR	Survival
C	10.53^{a}	28.83^{c}	173.197^{c}	1.44^{c}	3.43^{a}	77.00^{b}
ASLM25	10.53^{a}	30.23^{b}	187.257^{b}	1.51^{b}	3.17^{b}	80.00^{a}
ASLM50	10.50^{a}	31.73^{a}	201.713^{a}	1.58^{a}	3.10^{b}	82.00^{a}
ASLM75	10.53^{a}	29.00^{c}	175.513^{c}	1.45^{c}	3.33^{a}	82.00^{a}

REARING PERFORMANCE AND DISEASE RESISTANCE OF FRESHWATER EEL *Anguilla* sp. FED DIET WITH DIFFERENT LEVELS OF WATER FERN *Azolla filiculoides* EXTRACT FOLLOWING *Aeromonas hydrophila* INFECTION

Isagani P. Angeles Jr.*, and Raymond M. Pascua

Freshwater Fisheries Center
Isabela State University-Main Campus
Echague, Isabela, Philippines 3309
ipangelesjr_15@yahoo.com

Fish culture is an important industry where various kinds of marine and freshwater fish are cultured with increase of annual production worldwide. The Philippines has been identified as an increasingly important source of Freshwater eels (*Anguilla* sp.). Eel is a catadromous fish, which can live in freshwater but spawn in tropical ocean waters. This has a peculiar leptocephalus larval stage that is unique to elopomorph fish. The study evaluates the effect of different levels of Water fern (*Azolla filiculoides*) extract (AFE) on rearing performance and disease resistance of eel against *Aeromonas hydrophila* infection.

The extraction of the powdered sample leaves was done through ethanol extraction method. The AFE was supplemented unto one kg formulated feeds; T1-1% AFE (10ml kg^{-1}), T2-2% AFE (20ml kg^{-1}), T3-3% AFE (30ml kg^{-1}), while 0 AFE for the control (C-basal diet). Eel was reared for ten weeks and fed thrice a day at 20% of their average body weight. The weight sampling was done every two weeks. After rearing experiment, the eel was subjected to *A. hydrophila* infection.

The W_f, WG, and SGR of T2 were increased by 37%, 55% and 61% as compared to that of C, respectively. However, no significant effect was found on FCR among treatments. In addition, percentage survival of C, T1 and T2 were 52%, 46% and 28% higher than that of T3, after ten weeks of rearing, respectively. On the other hand, percentage survival of T2, T1 and T3 were increased by 102%, 48% and 16 % as compared to that of C at three days after *A. hydrophila* infection, respectively. The improved growth performance and disease resistance could be attributed to the antioxidant capacity (TAS: 1.13 mmol/L; DPPH: 52.61%) and phytochemical constituents (tannins and saponins) of AFE. Overall, AFE at 2% improves rearing performance and disease resistance of eel against *A. hydrophila* infection.

Table 1. Rearing performance of Freshwater eel fed four practical diets. Means without a common superscript are significantly different ($P \leq 0.05$).

Diet	W_f	WG (%)	SGR (%)	FCR	SUR (%)
C	1.10^{b}	213.43^{b}	1.55^{b}	3.41^{a}	79.00^{a}
1	1.17^{b}	233.05^{b}	1.54^{b}	3.12^{a}	76.00^{ab}
2	1.51^{a}	331.81^{a}	2.49^{a}	3.08^{a}	66.33^{bc}
3	1.21^{b}	246.38^{b}	1.73^{b}	3.18^{a}	52.00^{c}

WHAT CAN HYPERSALINE LAKES AND PONDS GIVE TO AQUACULTURE DEVELOPMENT?

Elena Anufriieva*, Nickolai Shadrin

A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS
2 Nakhimov Ave., 299011, Sevastopol, Russia

In the World, a substantial part of the current human population is lacking in fresh water and food and these problems are among the vital problems currently. Agriculture itself cannot solve the problem of food lack consuming approximately 70% of the total world's freshwater consumption. Intensive aquaculture development of would overcome this. Globally, there has been no significant increase in marine aquaculture production in two past decades, but in inland freshwaters, total aquaculture production demonstrated high increase. Overwise, in inland freshwaters, total aquaculture production demonstrated high increase thus consuming freshwater and significantly contributing to the eutrophication of freshwater bodies. This reduces the freshwater reserve for drinking and other human needs. In some countries including China, it is becoming a recognized problem. The sharpest conflicts are common between different freshwater users in the arid countries. Aquaculture development needs to look for a way to overcome this. Only a way to overcome this problem is to develop aquaculture in saline and hypersaline lakes without compromising for drinking water source. There are a number of saline and hypersaline lakes and ponds in arid/semi-arid zones; they can be used for effective aquaculture development. Saline/hypersaline lake aquaculture is needed not only to overcome water user's conflicts; this may significantly intensify aquaculture development in whole. The background of this conclusion is based on high primary productivity of saline/hypersaline waters, more to up to 100 times than land agriculture. One of a key problem in aquaculture is food for cultivated fish and shrimps particularly live food for fish and shrimps larvae. Hypersaline waters can give not only *Artemia* cysts to overcome the problem. Several animal inhabiting hypersaline waters (Copepoda, Cladocera, Amphipoda, etc.) are the high perspective objects as live food. Algal, plant, and animal biomass produced in the hypersaline lakes/ponds can be used as food not only for fish, shrimps, and farm animals, but for humans also. It also can use as fertilizer or resource for different industries.

This study was supported by the Russian Science Foundation (grant 18-16-00001).

GROWTH AND METABOLIC RESPONSES OF WHITELEG SHRIMP *P.vannamei* AND NILE TILAPIA *O. niloticus* POLYCULTURE FED WITH POTENTIAL PROBIOTIC MICROORGANISMS ON DIFFERENT SCHEDULES

Juan Pablo Apún-Molina, Mauro Espinoza OrtizVladimir Medina-Alcantar & Ilie Racotta

IPN-CIIDIR, Sinaloa, Boulevard Juan de Dios Bátiz Paredes 250, Guasave, Sinaloa, México C.P. 81101
Instituto Politécnico Nacional
E-mail: japun@ipn.mx

Probiotics and co-culture of shrimp and tilapia are two strategies to improve yield and eco-efficiency of both species. However, only few studies have analyzed the combination of these two strategies. This study analyzes the effect of a mixture of potential probiotics supplied in the diet at different frequencies in a system of shrimp (10 m^{-2}) and tilapia (5 m^{-2}) in a trial lasting 84 days. The probiotics mixture was composed of four lactic acid bacteria and one yeast strain that were supplied either daily, every 5 days, or every 10 days in the diet and compared with a control without any supply of probiotics. At the end of the experiment, blood chemistry and hematology of shrimp and tilapia were analyzed as an index of physiological condition. Water quality did not differ between treatments. The final weight, feed conversion ratio, and yield of shrimp were significantly higher with daily supply of probiotics compared to shrimp that did not receive probiotics supply, with intermediate values for treatments with probiotics supply every 5 or 10 days. However, level of supplementation every 5 days could be considered as optimal because no significant differences with daily supply were observed for any variable, whereas the supply each 10 days resulted in a significantly lower yield. Significantly higher final weight, feed conversion ratio, and yield of tilapia occurred with daily supplements.

Table 3. Effect of dietary supplementation of potential probiotics at different rates on overall performance of tilapia *Oreochromis niloticus* co-cultured whit shrimp.

	Treatments Groups			
Performance	Daily	Every 5 days	Every 10 days	Control
Initial weight(g)	6.25 ± 0.43	6.22 ± 0.34	6.12 ± 0.37	6.11 ± 0.38
Final weight (g)	115.6 ± 10.71^{a}	76.93 ± 9.15^{b}	79.90 ± 1.26^{b}	72.97 ± 1.065^{b}
SGR (%/d)	3.44 ± 0.11^{a}	2.97 ± 0.12^{b}	2.99 ± 0.13^{b}	3.06 ± 0.06^{b}
AGR (g/ d)	1.30 ± 0.13^{a}	0.84 ± 0.11^{b}	0.88 ± 0.02^{b}	0.80 ± 0.02^{b}
FCR	1.22 ± 0.12^{a}	1.85 ± 0.19^{b}	1.88 ± 0.15^{b}	2.21 ± 0.15^{b}
Survival (%)	100 ± 0.0	100 ± 0.0	93.3 ± 6.66	86.7 ± 6.66
Yield (kg/ ha)	5780 ± 535^{a}	3846± 457^{b}	3731 ± 291^{b}	31603 ± 236^{b}

SALINITY PREFERENCE AND GROWTH OF GLASS EEL *Anguilla marmorata* (ANGUILLIFORMES: ANGUILLIDAE) EXPOSED TO DIFFERENT SALINITY LEVELS

Goldyn Anne G. Aquino*, Patrick C. Cabaitan, and Katsumi Tsukamoto

The Marine Science Institute
University of the Philippines
Diliman, Quezon City, 1101 Philippines
goldynanneaquino@yahoo.com

Catadromous eels undergo stepwise migration from marine to estuarine, then to freshwater habitats. A few species are able to stop their migration and settle in marine habitats, while some can migrate between seawater and freshwater habitats throughout their life. However, it is necessary to know the effects of salinity on the preference and growth performance of tropical freshwater eels found in the Philippines to optimize practices on the culture of these species. In this study, glass eels of *Anguilla marmorata*, an economically important freshwater eel, were collected from an estuary near Cagayan River, northern Philippines and observed for choice and growth experiments.

Majority of the glass eels were active towards freshwater compartment (0-5‰) and this continues to increase with time from 80% to 92.5%. After 60 days of culture, glass eels reared in freshwater (0‰) and brackish water (17.30 ± 0.01‰) have significantly higher growth performance in terms of total length and weight. On the other hand, delayed growth was observed in seawater (31.79±0.01‰) until day 45. Glass eels reared in freshwater, brackish and seawater had comparable survival, indicative of wide salinity tolerance of *A. marmorata* at glass eel stage. Examining the habitat preference and its influence on growth and survival are essential in improving the culture techniques and understanding the spatial distribution of *Anguilla marmorata* glass eels.

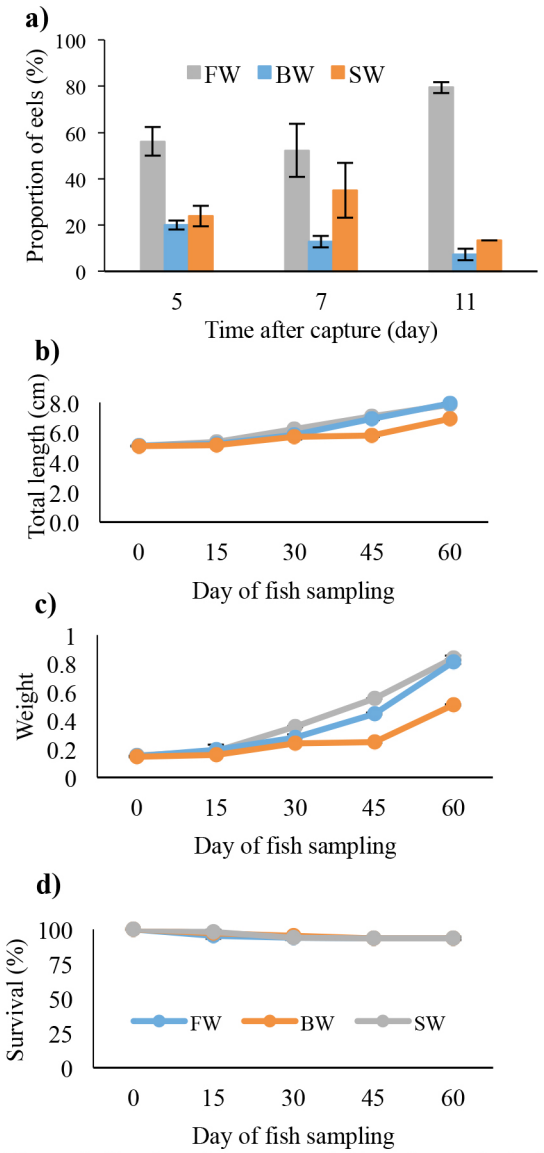


Figure 1. Results of the grouped (a) and growth (b-d) experiments conducted to *A. marmorata* glass eels. (a) Mean (±SE) proportion of glass eels counted on each compartment after the 30-minute group experiment (n=3, 60 glass eels per replicate); mean (±SE) total length (b), mean (±SE) weight (c), and mean (±SE) survival (d) of *A. marmorata* glass eels reared on freshwater, brackish water, and seawater for 60 days. Each treatment is triplicated and contains 40 glass eels

CURRENT STATUS OF INFECTION WITH INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV) IN THE PERUVIAN AND ECUADORIAN SHRIMP INDUSTRY

Luis Fernando Aranguren Caro1*, Muriel Gomez-Sanchez2, Yahira Piedrahita3, Hung N. Mai1, Roberto Cruz-Flores1,4, Rod Russel R. Alenton1, Arun K. Dhar1

1 Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences The University of Arizona, 1117 E Lowell St. Tucson, Arizona, USA 85721
2 Subdireccion de Sanidad, Dirección de Sanidad e inocuidad, National Fisheries Health Agency in Peru (SANIPES), Calle Amador Merino Reyna N° 263, San Isidro, Lima, Perú
3 Camara Nacional de Acuacultura, CNA, Avenida Francisco de Orellana y Miguel H Alcivar, Guayaquil, Ecuador
4 Centro de Investigación Científica y Educación Superior de Ensenada (CICESE), Carretera Ensenada-Tijuana No. 3918, Zona Playitas, 22860 Ensenada, Baja CA, México

* Corresponding author:
E-mail: lfarangu@email.arizona.edu (LFAC)

Infection with infectious hypodermal and hematopoietic necrosis virus (IHHNV) is a crustacean disease that caused large-scale mortality in *Penaeus stylirostris,* deformity and growth retardation in *Penaeus vannamei* and *Penaeus monodon*. We surveyed the presence of IHHNV in three major shrimp-producing regions in Ecuador, namely Guayas, El Oro, and Esmeralda. The data show that IHHNV is endemic (3.3–100% prevalence) to shrimp farms in these regions. The whole genome sequences of representative circulating IHHNV genotypes in Ecuador and Peru showed that these genotypes formed a separate cluster within the Type II genotypes and were divergent from other geographical isolates of IHHNV originating in Asia, Africa, Australia, and Brazil. In experimental bioassays using specific pathogen-free (SPF) *P. vannamei, P. monodon*, and *P. stylirostris* and representative IHHNV isolates from Ecuador and Peru, the virus did not cause any mortality or induce clinical signs in any of the three penaeid species. Although IHHNV-specific Cowdry type A inclusion bodies were histologically detected in experimentally challenged *P. vannamei* and *P. monodon* and confirmed by *in situ* hybridization, no such inclusions were observed in *P. stylirostris*. Moreover, *P. vannamei* had the highest viral load, followed by *P. monodon* and *P. stylirostris*. Based on IHHNV surveillance data, we conclude that the currently farmed *P. vannamei* lines in Ecuador are tolerant to circulating IHHNV genotypes. The genome sequence and experimental bioassay data showed that, although the currently circulating genotypes are infectious, they do not induce clinical lesions in the three commercially important penaeid species. These findings suggest a potentially evolving virus-host relationship where circulating genotypes of IHHNV co-exist in equilibrium with *P. vannamei* raised in Peru and Ecuador.

WHEN TECHNOLOGY FROM AQUACULTURE AND THE PETROLEUM INDUSTRY MEET: THE TRANSFORMATION OF THE SALMON FARMING INDUSTRY IN NORWAY

Peter Arbo*

Norwegian College of Fishery Science
University of Tromsø – The Arctic University of Norway
9037 Tromsø, Norway
peter.arbo@uit.no

The Norwegian aquaculture industry has experienced rapid growth since its infancy in the 1970s. Norway is today the leading international producer of salmonids, the bulk of which is exported. The basis for this success has been a combination of favorable natural conditions, innovative business players, extensive research efforts, and government policies that have sought to balance conflicting considerations. In the same period, another key industry has also emerged in Norway, namely the oil and gas industry. Production commenced in the North Sea in the early 1970s, and today exploration and exploitation are carried out in both the North Sea, the Norwegian Sea, and the Barents Sea. The fish farming industry and the petroleum industry have largely developed independently of each other. Both industries have derived much of their dynamism from a close interaction with specialized suppliers, but there have been few links between the respective value chains.

After 2015, there has been a change. The aquaculture industry, which has traditionally been based on open water net-pen cages, encountered increasing sustainability challenges. This limited opportunities for further growth and spurred interest in developing alternative fish farming concepts. In the petroleum industry, the drop in oil prices in 2014 led to downsizing and spare capacity. Several of the supplier companies started looking for alternative markets. This meant that the aquaculture industry began to look towards the petroleum industry, and the petroleum industry began to look towards aquaculture. At the same time, the Norwegian authorities introduced a temporary scheme of development permits in the fish farming industry with the aim of triggering large-scale and innovative projects. In recent years, we have therefore seen the development of closed and semi-closed containment systems, submersible fish cases, and an increasing focus on both offshore and land-based aquaculture.

In this paper, we analyze these new links between the petroleum sector and the fish farming sector in Norway, and we provide an overview of some of the new aquaculture concepts that have been launched. Based on Arctic Offshore Farming, which is one of the projects granted development permits, we take a closer look at the technology transfer from petroleum to aquaculture, how technology and expertise from the oil and gas industry have been used in the context of fish farming, and what challenges the new cross-industry alliances may create with regard to, among other things, project management, costs, and the fit between technology and biology. The paper ends with a discussion of future perspectives for the Norwegian aquaculture industry.

DETECTION OF ENDEMIC PATHOGENS FROM *Penaeus monodon* IN GROW-OUT SYSTEMS AND INVESTIGATION OF WILD PATHOGEN VECTORS

Phoebe Arbon*, Thomas Ackery, Kelly Condon, Ellen Ariel, Dean Jerry

AquaPath Detection Laboratory
James Cook University
Douglas QLD 4814, Australia
Aquapath@jcu.edu.au

The Australian shrimp industry is heavily reliant and thus considerably influenced by the natural environment. The presence of shrimp pathogens, including IHHNV, Pir-A, HDV, GAV, YHV-7 and When-2, in wild stocks represents a major biosecurity risk due to the highly connective disposition of shrimp farming to the natural environment. Thus, baselining and continuing assessment of the status of viral pathogens within both natural and aquacultured stocks is important if this industry is to develop adaptive and sustainable management strategies under dynamic natural conditions. This study involved the assessment of aquacultured *Penaeus monodon* stocks at the grow-out phase of production to baseline the presence and intensity of infection at this stage of production. Contiguously, sampling of wild glass shrimp was conducted to identify the extent of this common species as a potential pathogen vector when considered in the aquaculture framework.

This study concludes Phase 2 of the NA CRC project "Improving Biosecurity in northern Australian prawn aquaculture" (Ref No. A.3.1718113). The analysis conducted in Phase 2 of the NA CRC is the most comprehensive and extensive study to date that examines endemic viruses in Australian shrimp grow-out systems. Results reported from this study are beneficial to informing better management decisions and improving economic sustainability in Australian shrimp production by highlighting the epidemiological patterns of infection in shrimp grow-out systems and the potential pathogen vectors threatening production.

For the assessment of viral pathogens during the grow-out phase of production, 39 individual production ponds were sampled from commercial shrimp farms throughout the 2018-2019 production season (total n = 490). Analysis was conducted using qPCR on pleopod samples pooled from three individual shrimp for detection of pathogenic targets (Pir-A, IHHNV, HDV, YHV-7, When-2 and, GAV) at 20% prevalence in an infinite population. Glass shrimp were targeted for assessment as a natural pathogen vector threatening on-farm biosecurity and hence production. A total of 30 pooled glass shrimp samples were analysed for the same suite of pathogens as the grow-out *P. monodon* samples.

The study revealed high numbers of positive target detections in the grow-out ponds, especially for IHHNV (57% positive) and GAV (97% positive). The study also highlighted variable intensity of infection and prevalence of infection between individual production ponds. Furthermore, the glass shrimp analysis demonstrated the large potential of the species as pathogen vectors, with 97% of the samples positive for When-2 and 37% positive for HDV. These results highlight the importance of pathogen testing in commercial production systems to enable data driven biosecurity protocol development and decision-making. Baselining and continual monitoring of viral pathogens throughout the production cycle is imperative to strategic and adaptive management of stock, especially for an industry which is particularly vulnerable to environmental factors, such as widespread pathogen vector species.

IDENTIFICATION OF *Vibrio kanaloa* IN THE *Oyster crassotrea gigas* BY FLUORESCENT IN SITU HYBRIDIZATION

*Noushin Arratahery

Professor Jens Rolff
Freie Berlin University, Berlin, Germany - Berlin (Germany)
arfa.n@fu-berlin.de

Background
An ideal model for studying *Vibrio* and host and their interaction in disease dynamics is *Crassostrea Gigas* in the North Sea, exposing invasive sources to pathogens, especially (*vibrio kanaloae*).

Methods
This study used molecular fluorescent in situ hybridization (FISH) techniques to rapidly identify the diversity of bacteria in the oyster *Crassostrea Gigas* from Sylt Island and 16SrRNA gene sequence validated all strains' identification. Oysters were collected and exposed with *vibrio kanaloae* strains on different tissues were examined utilizing culture-independent methodologies.

Results
the digestive glands, gill and Muscle in *vibrio Kanaloa* were identified as metabolically active by the FISH technique, one of the advantages of the FISH technique is that it is accurate and very easy to use.

Conclusions
This technique allows rapid assessment of *Vibrio* in oysters and seafood.

IN VITRO AND IN VIVO EVALUATION OF INDUCED *Vibrio splendidus* TOWARD IMMUNE RESPONSE AND CROSS PROTECTION IN *Oyster grassostera gigas*

*Noushin Arratahery

Professor Jens Rolff
Freie Berlin University, Berlin, Germany - Berlin (Germany)
arfa.n@fu-berlin.de

Vibrio splendidus is an oyster pathogen associated with the summer mortalities affecting the production of *Crassostrea gigas oysters* worldwide. The increasing experimental evidence suggests that there are some forms of specific acquired immunity in invertebrates, but the underlying mechanism is not fully understood. In the present study, Pacific oyster (*Crassostrea gigas*) stimulated primarily by exposure to 10^5 *Vibrio splendidus* displayed stronger immune responses levels when they encountered the secondary challenge of 10^8 *V.splendidus*.we tested *Vibrio* primed by exposure to colonized in oysters to investigate their resistance against hemolymph in vivo and in vitro experiments. we investigated the immunological responses in oysters and whether this colonization results in elevated mortality.The selected strains showed higher survival in seconde challenge in oyster model and cross- protection against *Vibrio splendidus* in vitro. A population of oyster selected for resistance to the *Vibrio splendidus* showed increased in vivo survival, in addition, *V.Splendidus* reduction of pathogen shedding. In conclusion, our results suggest that the primary stimulation of *V. splendidus* led to immune priming in oysters when encountering the secondary challenge with *V. splendidus*, in addition, the increased survival of oysters almost certainly by stimulating the immune system in hosts led to prevent vibriosis in the oyster.

ANTIBACTERIAL ACTIVITY OF THE SEA CUCUMBER *Holothuria leucospilota* WHOLE BODY EXTRACT AGAINST METHICILIN RESISTANT AND ENTERTTOXIN PRODUCING *Staphylococcus aureus* STRAINS

*Noushin Arratahery

Professor Jens Rolff
Freie Berlin University, Berlin, Germany - Berlin (Germany)
arfa.n@fu-berlin.de

Introduction:
Preformed enterotoxins of *Staphylococcus aureus* are one of the most common causes of seafood-borne food poisoning worldwide. Aquatic organisms, including those used as seafood, are also a source of organic compounds of potential medical importance.

Purpose:
Within the framework of an antimicrobial activity study of marine macro-organisms from the Persian Gulf, bioactive compounds of the sea cucumber *Holothuria leucospilota* were extracted from whole sea cucumber bodies using chloroform or methanol. The extracts were evaluated for their antibacterial effects against methicillin-resistant *Staphylococcus aureus* (MRSA) and enterotoxin producing *Staphylococcus aureus strains* (SEASA, SEBSA).

Methods:
Antimicrobial activities were determined using three methods: disk diffusion tests, minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC)

Results:
The results demonstrate that methanol and chloroform extracts have an inhibitory effect on the growth of all strains tested at MIC concentrations up to 100 mg/ml. Also, the chloroform extract demonstrated bactericidal activity against SEBSA in concentrations of about 100 mg/ml. The extract below 100 mg/ml concentrations also shows bactericidal effects against MRSA and SEBSA. The highest antibacterial activity was found in the methanol extract.

Significance:
Therefore, sea cucumber extract is an interesting candidate for the identification of new antimicrobials, however, comprehensive investigations are needed to separate and identify the active compounds from *Holothuria leucospilota* from the Persian Gulf.\

CHARACTERIZATION OF TOXIN GENES ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus aureus* ISOLATES IN FISHERY PRODUCTS IN IRAN

*Noushin Arratahery

Professor Jens Rolff
Freie Berlin University, Berlin, Germany - Berlin (Germany)
arfa.n@fu-berlin.de

Introduction:
Staphylococcus aureus is one of the most common causes of seafood-borne diseases worldwide, which are attributable to the contamination of food by preformed enterotoxins.

Purpose:
The final aim of this study was to investigate the contamination of fishery products before their purchase and consumption.

Methods:
In this study, a total of 206 (34.3%) Staphylococcus aureus strains were obtained from 600 fish and shrimp samples and were tested by PCR for their antimicrobial susceptibility. We assessed the prevalence of the genes responsible for the staphylococcal enterotoxins (SEA, SEB) and toxic shock syndrome toxin 1 (TSST-1) genes.

Results:
The results indicated that 34% of aqua food samples were contaminated with S. aureus, and 23.8% of these isolates were mec-A-positive. Sixty-four percent of the strains isolated from contaminated seafood was enterotoxigenic S. aureus, and 28.2% of SEs were MRSA-positive. The most prevalent genotype was characterized by the presence of the sea gene (45.2%), followed by the seb gene (18.5%), and the tst gene encoding TSST-1 was found in eight strains (3.9%). Of the 206 S. aureus isolates, 189 strains (84.9%) were resistant to at least one antibiotic.

Significance:
Given the frequent outbreaks of enterotoxigenic MRSA, it is necessary to make revisions to mandatory programmes to facilitate improved hygiene practices during fishing, aquaculture, processing, and sales to prevent the contamination of fishery products in Iran

TRANSCRIPTOME ANALYSIS PROVIDES MOLECULAR BASES FOR THE SELECTION OF A SUPERIOR ASIAN CATFISH *Clarias macrocephalus* GÜNTHER 1864 STRAIN INTENDED FOR REINTRODUCTION

July B. Ariñez*, Augusto E. Serrano Jr., Apple Gray P. Deallo, Shellah Dee T. Canillo

College of Fisheries and Ocean Sciences
University of the Philippines Visayas
Miagao 5023, Iloilo, Philippines
july_arinez@yahoo.com

The selection of physiologically-superior strains constitutes one of the most important factors that predicts the success of stock enhancement programs. In this study, a comparative transcriptome analysis between two remaining *Clarias macrocephalus* populations from Cagayan (CmC) and Agusan del Sur (CmA), Philippines was conducted to profile and compare the expression patterns of various fitness-related genes present in the hepatic tissues and acquire molecular insights for the identification of a superior strain that is suitable for breeding and reintroduction.

By using RNA sequencing, a total of 59, 742 genes were found to be expressed in the hepatic tissues of the two catfish strains. Of these, 6,948 and 8,669 genes were identified to be uniquely expressed only in CmC and CmA tissues, respectively. Gene expression analysis of these uniquely-expressed genes using CmC assembled transcriptome also revealed that 3,407 differentially expressed genes (DEGs) were significantly upregulated while 2,400 DEGs were significantly downregulated. Functional annotation of the identified strain-specific DEGs using NCBI non-redundant (NR) and Swiss-Prot databases also confirmed the presence of various fitness-related genes associated with growth, reproduction and immunity. Significant upregulation of methylsterol monooxygenase I (MSMO1), complement component 7 (*C7*), cathepsin B (*CTSB*), glutamine synthethase (*GS03*), glutaminase 2 (*GLS2*) and carboxypeptidase B2 (*CPB2*) genes were observed in CmA tissues while mannan-binding lectin serine peptidase 2 (*MASP2*), arginase 1 (*ARG1*) angiotensin converting enzyme -2 (*ACE-2*) and mast cell tryptase-like (TPS) genes exhibited downregulation in CmC samples. Further investigation of genes involved in significantly enriched pathways (Figure 1) also indicated that growth- and immune-related genes are more abundant in CmA compared to CmC. Our study demonstrated that RNA-seq can be used to identify strain-specific DEGs which could provide valuable information to prod the selection of a superior catfish strain for breeding and reintroduction.

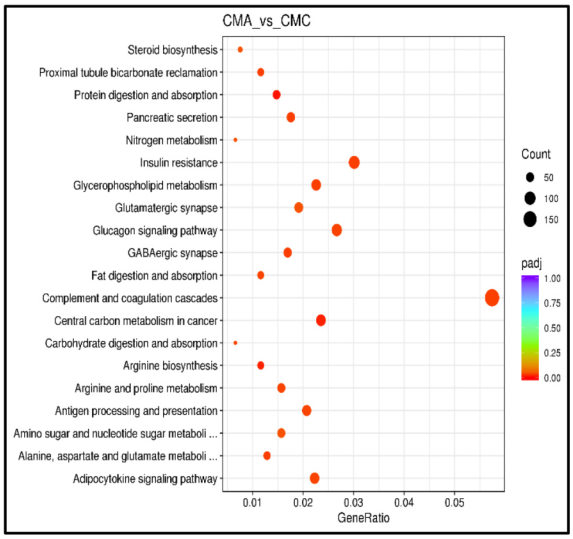


Figure 1. Pathways significantly enriched in CmA vs. CmC generated by KEGG database

DEVELOPMENT OF PCR TECHNIC WITH SPECIFIC PRIMER FOR QUICK DETECTION OF BACTERIAL PATHOGEN CAUSING *ICE ICE* DISEASE ON SEAWEED

Muhammad Aris
Tamrin Ali Ibrahim
Rusmawati Labenua

Management of ice ice free seaweed aquaculture is important in increasing seaweed production recently. For that reason, it is required a technology that is able to support the health of seaweed. This research aimed to determine the optimation, specifity, and sensitivity of specific primer and to develop molecularly quick detection method to detect the agent of *ice ice* disease on seaweed thallus. Optimation, specifity and sensitivity tests as well as detection on thallus used specific primer PCR (aSEFM-F and aSEFM-R). PCR was conducted as follows: pre-denaturation at 94°C for 5 minutes, synthesis at 72°C for 2 minutes, post PCR at 72°C for 7 minutes, and PCR reaction was stopped at 4°C. Research result showed the use of PCR with specific primer aSEFM-F and aSEFM-R was able to detect isolate *Vibrio alginolyticus* PNGK 1 from pure culture and to detect ice-ice disease directly on seaweed tissue within 6 hours with DNA concentration of 0.21 ng/μL, while at concentration bacteria cell at 2.3 x 10^3 cell/mL and high specificity indicated by the present of band electrophoresis get at 201 bp with comparative evaluation of bacteria *Vibrio alginolyticus* SKT-b, *Vibrio harveyi, Pseudomonas cepacia, Flavobacterium meningosepticum, Pseudomonas diminuta* and *Plesiomonas shigelloides*.

LATERALIZATION AND AGGRESSION IN NILE TILAPIA *Oreochromi niloticus*

TOLULOPE O. ARIYOMO*, DOLAPO S. OLATOYE, and TOPE JEGEDE

Department of Fisheries and Aquaculture Technology
Federal University Oye-Ekiti, Ekiti State, Nigeria
tolulope.ariyomo@fuoye.edu.ng

Lateralization (eye preference, preference of one side over the order or handedness) has been studied over the years in different species of vertebrates in other to study how they perform multiple tasks simultaneously with respect to their survival, tenacity, responses, predator detection, etc. Variation in personality traits (such as aggression) in different organisms has been linked to differences in behavioural lateralization. In this study, the mirror and dyadic tests were used to test, determine, and compare the lateralization and aggression levels of Nile tilapia (Oreochromis niloticus) with its mirror image, an opponent and a potential natural predator. 120 apparently healthy Nile Tilapia adults about the same weight and length were used for the experimental trials.

Results showed that individuals showed more aggression towards their mirror image than they did towards their opponents in the dyadic test (Fig 1). The individuals in the dyadic test used their left eye as much as they used their right eye when viewing their opponents (Fig 2). However, tests fish in the mirror test used their left eye more than they used their right eye when viewing their opponents (Fig 2), so they are left eye biased. Furthermore, test fish were found to also use their left eye more than they used their right when viewing a predator.

Given the difference between the levels of aggression shown towards the mirror image by the Oreochromis niloticus, compared to a conspecific, this study has provided evidence that does not agree with findings in other studies that reported that the interaction of some lower vertebrates with a mirror image and real opponent is similar and may measure the same aspect of aggression.

This experiment revealed a level of lateralization while test fish viewed a mirror image and a predator, which is in line with the results of similar studies of the right-left eye preference, which is an evidence of the right hemisphere specialization.

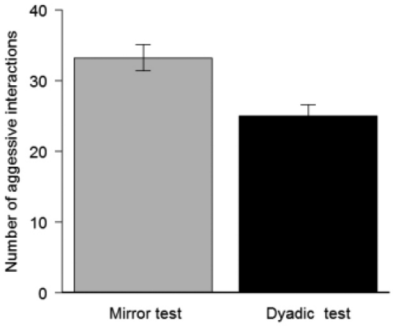


Fig 1: Mean (± SE) number of aggressive interactions in the mirror and dyadic tests.

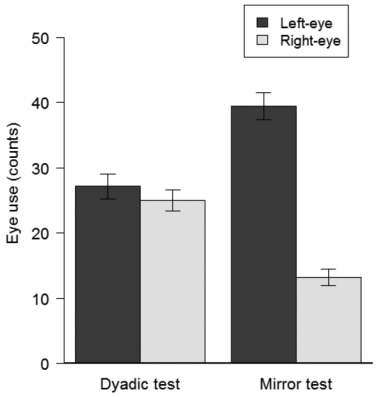


Fig 2: Mean (± SE) of left and right use in the dyadic and mirror tests.

COMMERCIAL SCALE R&D OF SUPER-INTENSIVE SHRIMP FARMING IN VIETNAM

Stuart Arnold*, Mauricio Emerenciano, Tim Perrin, Ashfaqur Rahman and Bryce Little

Commonwealth Scientific and Industrial Research Organisation (CSIRO)
144 North St. Woorim
Queensland, Australia 4507
stuart.arnold@csiro.au

Executing R&D in commercial scale shrimp grow-out systems is highly challenging and requires a delicate balance between meeting commercial imperatives whilst preserving scientific rigour. To meet this challenge, Viet-Uc in Vietnam have partnered with the Livestock and Aquaculture program at CSIRO in Australia. Since its inception in 2017, a collaborative project between Viet-Uc and CSIRO has focused on developing technologies to sustain profitable, super-intensive indoor *Penaeus vannamei* shrimp farming in Vietnam. These technologies involved taking a multi-factorial approach to how such super-intensive grow-out systems are designed and managed.

Six commercial-scale experiments (90 to 100 days of culture) were completed across different seasons using two commercial greenhouses, each containing 14 plastic lined ponds (500 m^2), which allowed for 4-6 replicate ponds per treatment. Diverse and comprehensive data was collected and analysed from 132 ponds in total. Experimental designs systematically refined and retested new protocol versions based on learnings from the preceding experiments. Complimentary R&D included economic modelling, assessing shrimp health, pathogen monitoring and biosecurity, as well as investigating precision farming strategies such as new sensor technologies and machine learning.

The culture environment was substantially improved throughout the project via optimal water quality and low pathogen loads. This led to healthier shrimp with average survival rates above 90% and consistent yields above 40t/ha equivalent. When combined with economic modelling to reduce costs, increases in net profit margins up to 25% were reported.

Our project highlights the benefits of performing production systems research directly on farm and taking a whole-of-systems approach to deliver a profitable, sustainable, and consistent indoor, high-intensity farming systems for Whiteleg shrimp, *P. vannamei*.

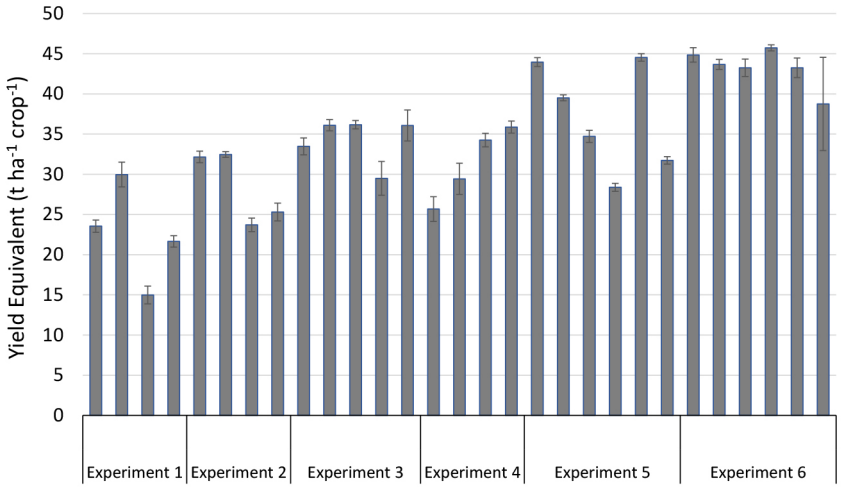


Figure 1. Means (±SEM) of yields (t ha^{-1} $crop^{-1}$ equivalent) and survival (%) per treatment for each of the six experiments executed in the project.

NOVEL PRECISION AQUACULTURE TECHNOLOGIES FOR PRAWN FARMING IN AUSTRALIA

Stuart Arnold*, John McCulloch, Mingze Xi, Joel Dabrowski, Ashfaqur Rahman

Commonwealth Scientific and Industrial Research Organisation (CSIRO)
144 North St. Woorim
Queensland, Australia 4507
Stuart.arnold@csiro.au

Aquaculture is undergoing a transformation in scale in Australia and globally, however there's a substantial yield gap between how much is harvested and how much could be harvested. The CSIRO has been collaborating with Australian prawn farmers to investigate how developments in precision farming technologies can help reduce this yield gap. In a three-year project, we worked directly with farmers to evaluate ideas and collect commercially relevant data to test and validate novel techniques and technologies in sensing, data modelling, situational awareness, and decision support.

Wearable Data Collection Suit is a smart glass application that could transform how aquaculture farmers carry out field jobs via hands-free interaction, situated pond water quality trend visualisation, automatic sensor data extraction and deep learning-enabled crop sampling. We hope that the system will play a vital role in upscaling the prawn farming industry by supporting staff to make more informed management decisions, faster than they can currently.

A decision support application was created for prawn farmers that makes use of real-time sensor data and state-of-the-art deep learning models to provide 24-hr forecasting and anomaly detection. Water quality in ponds is highly dynamic and challenging to manage for large farms with hundreds of ponds. Our application provides farmers with valuable information that allows them to be more proactive with pond management, thereby improving water quality and reducing risk. The application has been tested on a prominent Australian prawn farm.

COMPARING PERFORMNACE OF *Gracilaria tenuistipitata* AND *Enteromorpha* sp. AS SHELTER IN NURSING MUD CRAB *Scylla paramamosain* CRABLETS

Cliford P. Arsa* and Nguyen Thi Ngoc Anh

College of Aquaculture and Fisheries
Can Tho University
Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Vietnam
cliford2210@yahoo.com.ph

The present study aims to find out the suitable seaweed species as shelter to improve survival and production of mud crab *S. paramamosain* crablets. The experiment was conducted consisting of seven treatments, and randomly designed in triplicate tanks. The control treatment had no shelter (without seaweed in the culture tank) and other six treatments, red seaweed (*Gracilaria tenuistipitata*) or green seaweed (*Enteromorpha intestinalis*) were placed to the rearing tanks at three density levels of 0.5, 1 and 2 kg/m^2. Instar 2 crablets with a mean weight of 0.019 g were reared at density of 300 ind./m^2 in the 250-L tanks with salinity of 20 ppt, and provided continuous aeration. The crablets were fed frozen *Artemia* biomass to satiation for four weeks.

Results showed that survival of crabs in all treatments decreased with the rearing period. At the end of experiment, average survival of crab in the control group was lowest (17.4%) while survival of crabs were significantly improved and varied in the ranges of 53.3-70.7% and 27.8-35.9% in the *Gracilaria* and *Enteromorpha* groups, respectively. Notably, higher survival resulted in lower growth rates in the seaweed treatments but enhanced biomass and production of crab juveniles. Moreover, the concentrations of TAN and NO_2^-, in the *Gracilaria* treatments were much lower than in the control and the *Enteromorpha* treatments that improved water quality in the rearing tanks. The present finding proved that *Gracilaria* could be considered a suitable shelter for reliable production of mud crab juveniles in nursery phase.

COMPARING PERFORMNACE OF *Gracilaria tenuistipitata* AND *Enteromorpha* sp. AS SHELTER IN NURSING MUD CRAB *Scylla paramamosain* CRABLETS

Cliford P. Arsa* and Nguyen Thi Ngoc Anh

College of Aquaculture and Fisheries
Can Tho University
Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Vietnam.
cliford2210@yahoo.com.ph

Mud crabs (*Scylla* spp.) are considered luxury seafood items and high market value; therefore mud crab farming is well established throughout Southeast Asia. In Vietnam, mud crab *S. paramamosain* is one of the most promising candidates, and is often seen as an alternative to shrimp or poly-culture in the improved extensive shrimp farming in the Mekong delta. A nursery period as an intermediate step in between hatchery and grow-out, to grow postlarvae to a size appropriate for transport and release into large extensive to intensive production systems, is considered necessary. However, the prevalence of cannibalistic behavior in crabs thus using shelter in the culture system to minimize conspecific cannibalism and improve survival of crab.

The present study aims to find out the suitable seaweed species as shelter to improve survival and production of mud crab *S. paramamosain* crablets. The experiment was conducted consisting of seven treatments, and randomly designed in triplicate tanks. The control treatment had no shelter (without seaweed in the culture tank) and other six treatments, red seaweed (*Gracilaria tenuistipitata*) or green seaweed (*Enteromorpha intestinalis*) were placed to the rearing tanks at three density levels of 0.5, 1 and 2 kg/m^2. Instar 2 crablets with a mean weight of 0.019 g were reared at density of 300 ind./m^2 in the 250-L tanks with salinity of 20 ppt, and provided continuous aeration. The crablets were fed frozen *Artemia* biomass to satiation for four weeks.

Results showed that survival of crabs in all treatments decreased with the rearing period. At the end of experiment, average survival of crab in the control group was lowest (17.4%) while survival of crabs were significantly improved and varied in the ranges of 53.3-70.7% and 27.8-35.9% in the *Gracilaria* and *Enteromorpha* groups, respectively. Notably, higher survival resulted in lower growth rates in the seaweed treatments but enhanced biomass and production of crab juveniles. Moreover, the concentrations of TAN and NO_2^-, in the *Gracilaria* treatments were much lower than in the control and the *Enteromorpha* treatments that improved water quality in the rearing tanks. The present finding proved that *Gracilaria* could be considered a suitable shelter for reliable production of mud crab juveniles in nursery phase.

MICROBIOLOGICAL DIVERSITY AND COMPOSITION OF WATER AND SEDIMENTS IN THREE FARMS FROM ECUADOR

Authors: A. Artiles*[1], M. Allen[1], J. Stannard[1], R. Towner[1], K. Verbyla[1], J. Córdova[2], J. Rocha[3], J. Buchanan[1]

*Presenter author: Adriana Artiles
aartiles@aquatechcenter.com
The Center for Aquaculture Technologies. 8395 Camino Santa Fe. Suite E. San Diego, CA, 92121. USA
2. Cp-acuacultura. GYE EC
3. Texcumar S.A., San Pablo, Santa Elena, Ecuador

It is understood that there is an association between productivity and microbial diversity in aquaculture systems. For example, changes in bacterial microbiomes are implicated in animal performance, in disease development associated with both bacterial and viral origin, and in dysbiosis (disequilibrium in microbial communities) triggered by environmental stressors or diet choice. With the increasing development of novel DNA sequencing technologies, new concepts and cost-effective applications have emerged to better understand the role of microbial communities in the growth and health of farmed vertebrates and invertebrates. Evidence of any of the three main mechanisms that lead to dysbiosis, such as the decrease in diversity, the loss of beneficial bacteria and the expansion of pathogens or potentially harmful microorganisms, can be used as an indicator tool for productivity monitoring/ forecasting purposes. This work establishes a methodology and a pipeline for microbiome characterization in three commercial shrimp ponds in Ecuador. Also, we compare the merit of two types of samples (water and sediment) and detect patterns that allow inference on water quality in the ponds and evaluation of productive performance of the shrimp.

Briefly, the samples were filtered to concentrate, remove debris, and capture microbes present. DNA was extracted from the concentrated microbes sequenced to look at the type of bacteria present and the relative amount of each type. This was successful, with clear profiles produced along with some information on pathogen presence. Alpha diversity is a measurement of the microbial diversity of each sample. All sediment samples from the three ponds were very diverse with alpha diversity scores of over 600. Water samples were markedly less diverse with alpha diversity scores generally less than 250. For one of the ponds, alpha diversity was relatively high and over 250. For another pond alpha diversity was less than 100, and a single bacterial species made up over 50% of the bacteria detected in all water samples. In addition, for some water samples potentially pathogenic Vibrio species were identified.

Overall, this information reveals that monitoring the microbiome of production ponds over a grow-out cycle has the real chance to deliver data meaningful for pond management and pond performance. Possible application of this technology/analysis are 1) assessing genotype by environment effects in shrimp performance, 2) understanding of the impact of the microbes in the growth, development, and survival of shrimp, 3) evaluation of the effectiveness of probiotics or diets, and 4) predicting pond performance by evaluation of variations in microbial composition at different timepoints in the production cycle.

USING DNA TOOLS TO IMPROVE THE GENETICS OF *L. vannamei* SHRIMP

Klara Verbyla, Melissa Allen, Jason Stannard, Adriana Artiles*, Debbie Plouffe, Alan Tinch, John Buchanan

Presenting author: Adriana Artiles
aartiles@aquatechcenter.com
Center for Aquaculture Technologies, 8445 Camino Santa Fe, Suite 104, San Diego, CA, 92129, United States

Selective Breeding is the process of improving one or more desirable traits of a cultured species through the selection of superior parents for the next generation. A breeding program is the implementation of a selective breeding strategy and the set of tools needed to deliver the desired outcomes. The approach selected should be designed to maximize the economic return by balancing input costs and with the expected genetic and economic gains for a commercial aquaculture producer. The use of molecular markers is by far one of the main scientific tools used to help manage breeding programs today.

While there are several genetic marker types available that can be used for this analysis, the single nucleotide polymorphism (SNP) is the most desirable type because SNPs are much more abundant in the animals' genome than other types of markers. SNPs are also easily assayed and scored in a high-throughput and cost-effective manner. The recent development of industry-wide, single nucleotide polymorphism (SNP) genotyping panels from 200 to 50,000 SNPs provides access to such tools at a very reasonable cost. The choice of how many SNPs to use will be dependent on the objectives of the analysis and the complexity of the breeding populations being investigated; it can be customized for any program, any species, and any budget. These molecular markers can be used in the breeding practice for assessment of diversity parameters, evaluation of consanguinity, pedigree assignment and inference of the structure of the population. However, for more sophisticated applications, such as identification of trait associated markers or genomic selection, larger number of SNPs, > 10,000 must be used. We have developed a powerful 50,000 SNP tool (AquaArrayHD vannamei) designed to work in all shrimp populations to deliver sophisticated results at an industry leading price.

With the development of DNA tools and the understanding of breeding strategies, the economic costs for incorporating genomic selection to accelerate shrimp breeding programs are no longer barriers to implementation. Application of genomic selection is feasible when combining an economically efficient method for scanning the genome of broodstock for SNP (single nucleotide polymorphism) markers and often through the use of genomic imputation to reduce the overall burden of genotyping costs. Genomic selection utilizes either the effects of the genome wide markers or the realized genomic similarity to predict breeding values, rather than pedigree relationships (the expected relatedness). This allows for within family selection resulting in increased selection accuracy such that the breeding candidates with the highest genetic merit from different genetic backgrounds can be identified. Inbreeding can also be controlled in a genome wide fashion. Genomic selection is particularly powerful when selecting for difficult to measure and lowly heritable traits. Among the many benefits of the use of genomic selection for shrimp breeding, is that it would allow for the accurate incorporation of genetic data from ponds without increasing biosecurity risks.

THE EFFECT OF DIFFERENT PROBIOTICS ON GROWTH PERFORMANCE OF JUVENILE CORAL TROUT *Plectropomus leopardus*

Ni Wayan Widya Astuti*, Widanarni, Dedi Jusadi, Nyoman Adiasmara Giri

Institute for Mariculture Research and Fisheries Extension (IMRAFE)
Dusun Gondol, Desa Penyabangan, Kec. Gerokgak, Kab. Buleleng, Bali-Indonesia, 81155

Coral trout grouper *Plectropomus leopardus* aquaculture is being developed in Indonesia. Grouper aquaculture mainly use trash fish as feed. Therefore, the study to improve utilization of artificial feed is needed. Probiotic has good impact to host; one of them is to improve fish growth. Three probiotics were isolated from digestive tract of coral trout grouper in previous study. The probiotics are *B. subtilis* BS3, *B. amyloliquefaciens* BS4, and *B. cereus* BS6. The aims of this study were to evaluate the effectivity of the probiotics in improving fish growth.

Fed were formulated with protein content 44,49–45,24%, fat content 11,10–11,69% and energy 4.305,00-4.321,61 kkal GE/kg feed. This research was designed using Completely Randomized Design with 4 treatments: 1). Feed without the addition of probiotics, 2). Feed with addition 1% *B. subtilis* BS3 3). Feed with the addition 1% *B. amyloliquefaciens* BS4, 4). Feed with the addition 1% *B. cereus* BS6. Juvenile coral trout grouper with total length 4,11±0,26 cm and body weight 0,99±0,20 gram were reared in 100 liters fiberglass tanks. Fish were fed twice daily by ad satiation method.fish were reared for 12 weeks. Water quality during treatment were maintained: temperature 26.4-29.8°C, pH 8.03-8.20, DO 5,8-6,5 mgL^{-1}, ammonia <0,0523 mg L^{-1} and nitrite 0.0024-0.0056 mg L^{-1}. Parameter observed were final weight, feed consumption, specific growth rate, protein retention, lipid retention, feed conversion rate and survival rate of coral trout grouper fed four treatment diets.

Results showed that treatments feed with probiotic gave higher final weight, specific growth rate, protein and lipid retention. Feed convertion rate were higher in treatments without probiotics. The survival rate did not affected by the treatments and feed consumption increase as follow the growth.

The highest growth performance were showed by treatments 4 with the lowest feed conversion rate.

Table 1. Final weight, feed consumption, specific growth rate, protein retention, lipid retention, feed conversion rate and survival rate of coral trout grouper fed four treatment diets. Means in a column with different letters were significantly different.

Parameters	Diet			
	1	2	3	4
FW (g)	5.62 ^{a}	6.60^{b}	6.48^{b}	8.45^{c}
FC (g/fish)	11.82^{a}	11.92^{a}	11.64^{a}	15.08^{b}
SGR (%/day)	1.84^{a}	2.07^{b}	2.05^{b}	2.42^{c}
PR (%)	48.67^{a}	59.63^{b}	60.12^{b}	62.43^{b}
LR (%)	17,.24^{a}	19.29^{ab}	21.83^{b}	21.82^{b}
FCR	3.01^{b}	2.39^{a}	2.30^{a}	2.26^{a}
SR (%)	61.67^{a}	63.33^{a}	70.00^{a}	60.00^{a}

EFFECT OF ZINC-L-SELENOMETHIONINE AND CHROMIUM-L-METHIONINE ON GROWTH PERFORMANCE, BLOOD GLUCOSE AND DIGESTIVE TRACT PH IN RED TILAPIA (*Oreochromis* spp.)

Suveena Auepaiboon*, Orapin Jintasataporn and Srinoy Chumkam

Department of Aquaculture, Faculty of Fisheries, Kasetsart University
Bangkok 10900, Thailand
E-mail: suveena.au@ku.th

In aquaculture, there are many things that cause animal to stress for example water quality, environment, disease, stocking density, transportation or sampling. Animal requires a lot of energy to manage stress instead of using it for growth and muscle development. Mineral supplementation is one strategy to solve this problem. Chromium and Selenium are interesting trace minerals. Chromium plays a role in carbohydrate, protein, and lipid metabolism. Selenium plays a role in growth, antioxidant, and reproductive functions. Dietary effects of Zinc-L-selenomethionine (Zn-L-Se) and Chromium-L-methionine (Cr-L-met) on growth performance, blood glucose and pH of digestive tract were investigated in juvenile fingerling Red Tilapia (*Oreochromis* spp). The trial was a CRD with 6 treatments and 20 replications. Fish were fed isonitrogenous and isocaloric diets with 36% crude protein and 8% crude lipid. The 6 treatment groups were T1) Control diet without Zn-L-Se or Cr-L-met supplementation, T2) Control diet supplement with Cr-L-met 0.5 ppm, T3) Control diet supplement with Cr-L-met 1.0 ppm, T4) Control diet supplement with Zn-L-Se 0.3 ppm, T5) Control diet supplement with Zn-L-Se 0.3 ppm and Cr-L-met 0.5 ppm and T6) Control diet supplement with Zn-L-Se 0.3 ppm and Cr-L-met 1.0 ppm. Red Tilapia with an average weight of 35 to 40 g were stocked in 100-L tanks at a density of 1 fish/tank/replicate, with 20 fish/treatment to reduce stress. Fish were fed experimental diets 2 time/day at 3 to 4% body weight for 4 weeks. The results showed that growth performance, in terms of final weight, weight gain, average daily growth and feed conversion ratio were not significantly differences among the treatment groups ($P>0.05$). Immunity, in terms of superoxide dismutase and glutathione peroxidase differed by treatment ($P<0.05$) as lysozyme activity and blood glucose showed no significant differences ($P>0.05$). Digestive tract pH was not affected by treatment ($P>0.05$). Therefore, Zinc-L-selenomethionine (Zn-L-Se) at 0.3 ppm and Chromium-L-methionine (Cr-L-met) at 0.5 to 1.0 ppm in the diet had no significant effect on growth performance in fingerling Red Tilapia.

ADVANCES IN SEA CUCUMBER AQUACULTURE: GONAD MATURATION CONTROL OF *Holothuria (Paningothuria) forskali* DELLE CHIAJE, 1823

Francisco Azevedo e Silva*, João T. de Sousa, Pedro M. Félix, Ana C. Brito, Tomás F. Simões, Eliana Venâncio, Ana V. Pombo

MARE - Marine and Environmental Sciences Centre
Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal
Email: fhsilva@fc.ul.pt

Sea cucumbers have been in high demand in Asia for centuries. The fishing pressure placed on natural stocks drove them to levels of concern, resulting in the depletion of several natural species. Sea cucumber aquaculture, highly developed in SE Asia, has become a helpful conservation tool. Even though aquaculture already exceeds fisheries' production, it still cannot meet the increasing market demand, with the exploitation of holothuroids expanding to new areas and targeting new species. In Europe, some sea cucumbers are already targeted for international trade, one being *Holothuria forskali*, widely distributed in the Mediterranean Sea and the NE Atlantic Ocean. Aquaculture of sea cucumbers in Europe is inexistent and *H. forskali* is still understudied. Transport and maintenance of broodstock in captivity are delicate tasks that could hinder the animals' condition. The aim of this study was to widen the natural maturity peak of H. forskali in captivity, paving the way for gonad maturation control.

Forty sea cucumbers were captured in January (2018) and transported in aerated containers filled with sea water, for three hours. Sea cucumbers were monitored for any eventual evisceration and mortality. At the facilities, ten random individuals were sacrificed (t_0) to determine the baseline biometric parameters (total, gutted and gonad weights) and the Gonadosomatic Index (GI). Muscle bands were preserved for protein and lipid content analysis, to determine individual condition. The remaining animals were placed in aquaria to undergo a 1-week period of acclimation. Then, they were transferred to aquaria (3 replicates) with monitored and stable conditions and integrated in RAS. At the end of the trial (t_f), in May (2018), all parameters determined at t_0 were also collected from the individuals at t_f. At the same time, wild animals were collected to compare with the specimens from the trial. Histological analyses were done on all gonads to assess sexual maturity.

During capture 67% of the animals released Cuvierian tubules, showing signs of stress, but none during transport. This indicates stress was caused by manipulation. During captivity, mortality rate was low (6.67%), 5 animals eviscerated and there was a significant loss of fresh and eviscerated weight (ANOVA, $p < 0.01$), between t_0 and t_f. Despite this loss, protein and lipid content remained stable indicating their nutritional profile was maintained. The GI decreased but this loss was not significant (Kruskal-Wallis, $p > 0.05$). Interestingly, even though there was a slight decrease in GI, histological analysis showed *H. forskali* were mature at t_0 and maturity stages remained similar at the end of the trial. Also, at the end of the trial, wild animals had a GI close to 0 and gonads were either spent, in recovery or absent, revealing that a natural spawning event occurred between t_0 and t_f. This is an important result as it revealed that it is possible to maintain matured individuals, allowing breeding control in captivity.

STRIPED VENUS (*Chamelea gallina*) RESTOCKING ASSAY OFF PORTIMÃO (SOUTHERN PORTUGAL)

Miguel B. Gaspar*, Domitília Matias, Ana M. Matias, Cláudia Roque, Paulo Vasconcelos, Fábio Pereira, Paula Moura, André N. Carvalho, David Piló, Marta Rufino, Sandra Joaquim

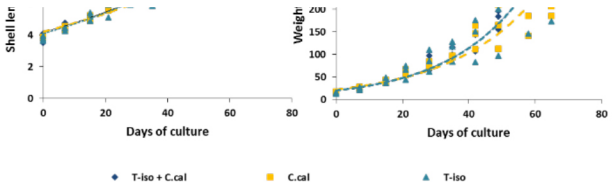
Portuguese Institute for Sea and Atmosphere (IPMA)
Research Centre of Olhão
8700-335 Olhão, Portugal
mbgaspar@ipma.pt

Bivalve dredge fisheries play an important role in the social-economical context of the Algarve coast (southern Portugal), mainly due to the size of the fishing fleet and the number of fishermen involved in this activity. Among several bivalve species caught, the striped venus (*Chamelea gallina*) constitutes a very important fishing resource because of its high economic-value. Recently, intensive fishing coupled with recruitment failure resulted in large inter-annual fluctuations in stock abundance, threatening the biological and economic sustainability of this fishery. In this context, the implementation of management strategies, such as stock-enhancement programmes based on hatchery production of juveniles, could promote the rebuilding of bivalve beds.

The present study describes the biology (growth and reproductive cycle) and distribution of *C. gallina*. This species shows a high growth rate and short lifespan, a prolonged spawning period (between April and August), matures within its second year of life and occurs preferentially on sandy bottoms between 5 and 11m depth. Overall, this information was to establish the time of the year used to collect the wild broodstock and to assess the potential of *C. gallina* for hatchery production. Several spawning techniques were tested (thermal stimulation, scarification, and flow through). Growth and survival of larvae and juveniles were compared using different nutritional regimes (larvae: *Isochrysis* aff. *galbana* (T-iso), *Chaetoceros calcitrans* (C. cal) and unfed; juveniles: (1:1) T-iso + C. cal, T-iso and C. cal) and rearing temperatures (larvae: 20 and 23°C). Among the different methods used to obtain gametes, *C. gallina* only spawned in the flow through system. The highest larval survival and growth rates were obtained from larvae fed T-iso. First *C. gallina* pediveliger larvae were found at day 8. The presence of metamorphosed larvae, found even in the unfed treatment, is an advantage for aquaculture production. After metamorphosis, there was no mortality of *C. gallina* juveniles reared without substrate regardless the diet provided. Juveniles fed the binary diet (T-iso + C. cal) and the monodiet T-iso showed a relatively higher growth in shell length and weight than when fed with C. cal. (Fig. 1).

Based on these results, a production protocol was designed for *C. gallina*, which allowed the production of millions of juveniles that were seeded in the first restocking assay with this species, in an area off Portimão.

FIGURE 1. Growth in mean shell length (mm) and mean weight (mg) and respective exponential growth curves of *Chamelea gallina* juveniles fed different diets (T-iso + C. cal, C. cal and T-iso).



COMPARATIVE STUDIES AND PERFORMANCE CHARACTERISTICS OF AFRICAN CATFISH FRYS USING MICRO WORMS *Paragrellus redivivus* AND COPPENS CRUMBLES RESPECTIVELY

*Olusola Adeniyi Babalola

Department of Fisheries Technology, Lagos State Polytechnic, Ikorodu, Lagos State, Nigeria
Correspondence should be addressed to: sola_aug@yahoo.com

The experiment was conducted on the comparative growth performance of African Catfish frys (Clarias gariepinus) using Micro worms (Panagrellus redivivus) and Coppens crumbles respectively. Three thousand pieces of catfish frys were randomly distributed into two treatments, which were replicated thrice-using complete randomized design. Clarias gariepinus frys with average initial weight of 4grams were stocked at the rate of 500 frys per replicate. Treatment one (T1) was fed with 0.2mm Coppens crumbles while treatment two (T2) was fed with Micro worms (Panagrellus redivivus). The fish frys were fed thrice a day base on 5% body biomass for the period of 4weeks. There is no significant difference (P>0.01) among all the treatments (T1 and T2) in the weight gain, feed intake, specific growth rate, protein efficiency ratio, survival rate (%) and feed conversion ratio. However, Panagrellus redivivus could be recommended for the feeding of African catfish frys due to its ease of digestion and also, fish larval respond to live food than formulated fish feed as reported by previous authors on fish nutrition. Similarly, micro worm is preferred to Coppens crumbles from economic point of view due to its importation using foreign currency.

DESIGNING NATURAL ANTIMICROBIALS TO BLOCK THE CYTOTOXIC EFFECTS OF *Vibrio parahaemolyticus* PirA^{vp}/PirB^{vp} TOXINS

Haig Yousef Babikian*, Tigran K Davtyan, Rajeev Kumar Jha, Yusef Babikyan

Research and Development, PT. Rhea Natural Sciences, Indonesia
Speaker* email: haig.babikian@cpp.co.id

Acute hepatopancreatic necrosis disease (AHPND) is caused by *Vibrio parahaemolyticus* containing toxins. The toxins are controlled by the genes pirA and pirB. The PirAvp/PirBvp toxin induces cell death in the shrimp's hepatopancreas but not in the stomach or other organs. It looks pretty likely that these putative PirAvp/PirBvp receptors will be found exclusively in the hepatopancreas membrane. Here we used protein homology modeling and protein-protein docking approaches to understand the cytotoxic mechanisms of PirAvp/PirBvp toxins, which is likely to be essential for AHPND research and agricultural applications. We selected aminopeptidase N (APN)- and alkaline phosphatase (ALP) as candidate cellular receptors for modeling cytotoxic mechanisms of *V. parahaemolyticus* PirAvp/PirBvp toxins. For this end, we chose *Penaeus vannamei* shrimps cytosol aminopeptidase cysteinyl glycine-S-conjugate dipeptidase (UniProtKB A0A423TDZ4_PENVA) as well as *Penaeus vannamei* shrimps alkaline phosphatase (UniProtKB A0A3R7PII5_PENVA) as cellular receptors for PirAvp/PirBvp, *B. thuringiensis* Cry, and Cry6Aa toxins. The PDB codes 3X0T and 3X0U for PirAvp and PirBvp and 5KUC and 1JI6 for Cry6Aa and Cry toxins were used for protein-protein docking experiments. The fasta and pdb files for *Penaeus vannamei* shrimps APN and ALP were downloaded from the UniProt database. The protein homology modeling was carried out by using SWISS-MODEL algorithms for template search (BLAST and HHblits against the template library), model building (ProMod3 coordinates which are conserved between the target and the template), and model quality estimation (QMEAN scoring function). For protein-protein docking, ezCADD and Patchdock algorithms were used, and Discovery Studio Visualizer and PyMOL software analyzed the results.

In conclusion, we presented structural views of the major pathogenic factors of AHPND, based on the structural similarity to *V. parahaemolyticus* PirBvp and *B. thuringiensis* Cry pore-forming toxins. Our protein-protein and receptor-toxin interaction models suggested that they are attractive for ascertaining unanswered questions relating to the cytotoxic mechanism of PirBvp and that this toxin may use similar mechanisms to cause cell death shrimps mortality.

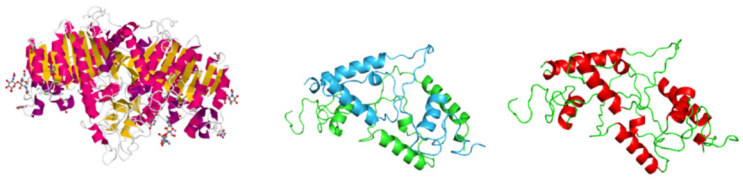


Figure: *Penaeus vannamei* shrimps ALP protein homology modeling by SWISS-MODEL algorithms.

L. stylirostris FIRST SPECIFIC PATHOGEN RESISTANT STRAIN DEVELOPMENT IN TAHITI AND NEW CALEDONIA SINCE 1975

Regis Bador* and Marc Le Groumellec

Innov'Aquaculture
BP 1001 98874 Pont des Français
New Caledonia
regis.bador@innovaquaculture.com

As no endemic penaeid shrimp were present, various species were imported into Tahiti at Centre Océanologique du Pacifique (COP) since the 70s for a shrimp aquaculture development program lead by Alain Michel and his Aquacop team: *P. occidentalis, P. japonicus*, *P. aztecus*, *P. merguiensis*, *M. ensis*, *P. monodon* and ultimately *L. vannamei* and *L. stylirostris*. Out of all candidates, *L. stylirostris* has been the only species kept especially for its ability to better resist to colder subtropical water temperatures in both Tahiti and New Caledonia.

Several batches of *L. stylirostris* post-larvae were then received in the 80s from various locations in Mexico and Panama to increase genetic diversity of a founder stock. During experiments run at COP, some batches experienced mass mortality events. Survivors were systematically kept and crossed to develop Tahiti's own domesticated stock in order to stop any more import, since first infectious diseases had already been reported to badly hit some shrimp industries. Studying the health status of COP domesticated population, typical IHHNV lesions were found present in specific tissues. However, they showed no signs of infection like what was reported in those countries. Once characterized as a parvovirus and when experimental infections were developed in University of Arizona laboratory, collaborating assays of infectious challenges were run on juveniles of this Tahitian population and from other origins. Results concluded that this population was resistant to IHHNV, while carrying the virus at low levels, while other populations were highly sensitive. That was when the hypothesis of low virus load could be protective against serious infection. This strain was then named as SPR43.

In 2005 a genetic program was initiated in New Caledonia to correct possible negative effects of inbreeding. A SPF/High Health *L. stylirostris* population was imported from Hawaii and field assays were performed with pure SPF and crossed SPFxSPR stocks, to compare their results with the original stock. Both "newly developed" stocks experienced signs of typical IHHNV infection, showing that the tolerance of SPR43 strain to IHHNV had a genetic basis, and was potentially reversible if important polygenes were lost during the "new blood" introduction. Facing a progressive increase of IHHNV infections in those "new" stocks, local partners decided in 2008 to destruct them and keep only the successive generations of "pure" SPR43, which remains nowadays the first *L. stylirostris* still successfully reared thanks to a pathogen resistance.

These results are consistent with more recent disease resistance programs in several countries, especially *L. vannamei* successful Local Pathogen Resistant (LPR) programs run in Ecuador.

A SUMMARY OF ALAIN MICHEL CONTRIBUTIONS TO MODERN EVOLVING TROPICAL AQUACULTURE

Regis Bador

Innov'Aquaculture, BP 1001, 98874 Pont des Français, New Caledonia
regis.bador@innovaquaculture.com

In 1972 the Centre Océanlogique du Pacifique (COP) was created to develop aquaculture in French Polynesia. Alain Michel was hired to lead the scientific program. He first visited main scientists doing research on shrimp aquaculture worldwide. Alain, then created, his research team joining, on purpose, varied profiles. During pioneer period, all research work has been published under the name of Aquacop team, an efficient way to keep a strong team spirit. As the local freshwater shrimp was over-fished, first research at COP focused on *M. rosenbergii*. Knowing the constraints of the green water method, the team developed a more controlled technique, later referenced as the French clear water technique.

But since the 80s the world interested more in Peneids. The COP imported 8 different species of Peneids, no marine shrimp being present in Tahiti. Main bottle neck was reproduction in captivity. Aquacop published in 1975 the eyestalk ablation allowing to control maturation and spawning of females, once inseminated. Information exchanges with Harvey Persyn allowed to progress in both artificial insemination and biofloc production. In 1979, Aquacop presented at WMS full reproduction cycle closing of the 3 *L. vannamei*, *L. stylirostris* and *P. monodon*.

In the early 80's, Aquacop team tested the biofloc technique with two purposes: grow-out and broodstock culture. Grow-out yields were astonished and transferred to a private farm. Once reproduction was under control. *L. stylirostris* was selected due to its allowance to "low" subtropical water temperatures. Locally domesticated strain showed resistance to IHHNV and was named SPR43, showing value of Specific Pathogen Resistance in adverse environment.

After main results published in 1979, the international development started: Peter Shayne, representing US Morrison brothers Group, ordered to France Aquaculture, IFREMER commercial subsidiary, the design, building follow-up and staff training of the first Ecuadorian industrial shrimp hatchery in Salinas, Ecuador. SEMACUA hatchery is still in operation in 2022, with around 200 more on same beach later called "the hatchery row". This has been the beginning of hatchery and farm design with "the French Touch" in Colombia, Panama, Cuba, Venezuela and Mexico with *L. vannamei* as well as in Indonesia, Malaysia, the Philippines, India, Sri Lanka, Thailand, Madagascar, Senegal and Mozambique with *P. monodon*.

Once retired in 2000, Alain dedicated his French experimental spirit mainly to fish culture (barramundi and tilapia), discovering the efficiency of heat shock in presence of pathogen to enhance resistant to it. Results have been astonished and contributed to significant commercial production recovery of large size fish farms who had been struggling with deadly viruses. This is just the beginning of a mindset evolution to manage pathogens in aquaculture and more…

A REVIEW ON THE EFFECTS OF DIETARY ANTIOXIDANTS TO REDUCE MERCURY TOXICITY IN OLIVE FLOUNDER *Paralichthys olivaceus* AND MICE

Sungchul C. Bai[1]*, Wonsuk Choi[1], and Ali Hamidoghli[1]

[1]Feeds and Foods Nutrition Research Center, Pukyong National University, Busan 48513, Republic of Korea
Email: scbai@pknu.ac.kr

Five experiments were conducted to evaluate the dietary effects of vitamin E, vitamin C and selenium on mitigation of mercury toxicity in olive flounder and mice. In the first experiment, six experimental diets were prepared in a 2×3 factorial design; these were three levels of vitamin E (0, 100, or 200 mg/kg diet) and two levels of mercury (0 or 20 mg/kg) for olive flounder. Results showed that dietary supplementation of vitamin E (at 100, or 200 mg/kg) increased growth in both presence and absence of Hg. Also, vitamin E at 100, or 200 mg/kg inorganic reduced mercuty bioaccumulation for Hg toxified fish (20 mg/kg) groups. In the second experiment, vitamin C (0, 100, or 200 mg/kg diet) and mercury (0 or 20 mg/kg) were added to the basal diet for olive flounder in a 2×3 factorial design (six experimental diets). Results revealed that dietary vitamin C at 100 or 200 mg/kg resulted in high growth and a low mercury accumulation for Hg toxified fish (20 mg Hg /kg). In the third experiment, synergistic effects of dietary vitamin E (0, 100, or 200 mg/kg diet) and selenomethionine (0, 2 and 4 mg/kg) in a 3×3 factorial design were evaluated in the mercury toxified (20 mg/kg) olive flounder. Results showed that dietary vitamin E more than 100 mg/kg diet with 2 or 4 mg/kg selenomethionine-supplementation could have synergistic effects on growth and liver mercury bioaccumulation in juvenile olive flounder. In the fourth experiment, synergistic effects of dietary vitamin C (0, 200, or 400 mg/kg diet) and selenomethionine (0, 2 and 4 mg/kg) in a 3×3 factorial design were evaluated in the mercury toxified (20 mg/kg) olive flounder. Results indicated the synergistic effects on growth and mercury accumulation in muscle, liver and kidney for fish fed diets containing 400 mg/kg vitamin C with 2 or 4 mg selenomethionine. In the fifth experiment, a 3×2 factorial design was used with three levels of Hg (0, 50 or 500 μg/kg and two levels of Se (0 or 2 mg/kg) with olive flounder muscle powder in constant combination with vitamin C and E (C: 0 or 400 mg/kg; vitamin E: 0 or 200 mg/kg) in mice. Results showed that dietary selenomethionine (2 mg/kg), vitamin C (400 mg/kg) and vitamin E (200 mg/kg) had no effect on reducing the mercury bioaccumulation in tissues but reduced the serum lipid peroxidation as well as prolonged the cumulative survival rate in terms of high Hg exposures in mice. Therefore, these result indicate that dietary antioxidants such as vitamin C, E and selenium could reduce bioaccumulation of mercury in fish but not mice, hence these antioxidants can reduce the toxicity effects of mercury in both fish and mice models.

INDUCED SPAWNING AND LARVAL REARING FOR THE SEA CUCUMBER *Holothuria nobilis* IN VIET NAM

Bakari A. Mariamu * and Nguyen V. Hung

College of Aquaculture and Fisheries
Can Tho University
Can Tho City, Viet Nam
mariamuahsadi@gmail.com

Holothuria nobilis is one of the economically important species for live hoods in Vietnam. However, an increase in demand of the species due to their economic value have led to depletion of the stocks in the wild. Hence, introduction of this species in aquaculture is required to reduce fishing pressure and improve live hoods of the communities. However, the knowledge of inducing spawning and larva rearing of this species is still limited, therefore, this research was undertaken to develop the breeding and larval rearing techniques for development of *H. nobilis* aquaculture.

The broodstock (150 individuals with 700-1500g) collected from the wild were induced to spawn by using thermal stimulation and combination of thermal stimulation and dry algal spirulina. The eggs and larvae were reared and their developmental stages, growth and survival rate at different experimental diets and different stocking densities were described for 18 days. Three experimental diets were used, microalgae (T1), microalgae and Protein selco (T2) and microalgae with Protein selco and (Frippak) (T3) and three stocking densities (0.3, 0.6 and 1larva/ml).

Results showed higher number of fertilized eggs (5,200,000) in thermal stimulation method compared to combination of thermal stimulation and dry algal spirulina (3,100,000 eggs). Survival and growth rates of larva were significant different in the treatment of dietary and stocking density experiments from day 3 to day 15 ($P<0.05$), however at day 18 the growth and survival rate of larva was similar ($P>0.05$) (Figure1 and Table 1). In this study, thermal stimulation was more effective method to induce spawning of *H. nobilis*, however, further research is needed to improve the survival and growth of larvae to ensure the sustainable production of this species.

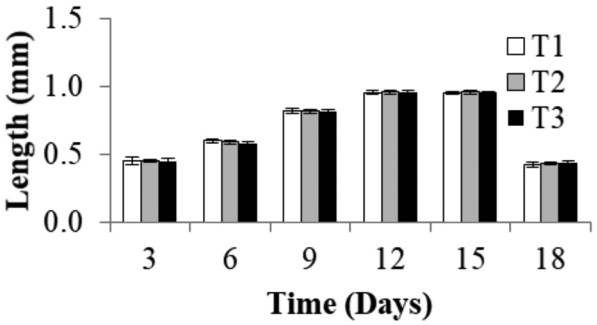


Figure 1. Growth rate (length gain (mm)) of *H. nobilis* larvae at different diets for 18 days.

Table 1. Survival rate (%) of *H. nobilis* larvae at different stocking densities larva/ml Means in the column with different letters were significant different ($P<0.05$).

	Treatments			
Days	0.3 larva/ml	0.6larva/ml	1 larva/ml	**p -value**
3	96.9 ± 1.27^{a}	97.7 ± 0.77^{a}	92.4 ± 5.14^{a}	0.150
6	95.1 ± 1.55^{b}	94.4 ± 2.93^{b}	56.9 ± 0.38^{a}	0.000
9	91.8 ± 0.9^{c}	80.2 ± 1.35^{b}	49.2 ± 0.59^{a}	0.000
12	55.2 ± 0.5^{c}	35.6 ± 1.80^{b}	21.5 ± 0.93^{a}	0.000
15	17.9 ± 1.87^{c}	11.9 ± 1.98^{b}	7.0 ± 1.18^{a}	0.000
18	1.0 ± 0.51^{a}	0.6 ± 0.05^{a}	0.3 ± 0.06^{a}	0.062

EFFECTS OF DIFFERENT LEVELS OF *Pinus tabulaeformis* POLLEN ON GROWTH AND IMMUNE RESPONSE OF MILKFISH FRY *(Chanos chanos)*

Analyn B. Baldove*, Barry Leonard M. Tumbokon, Augusto E. Serrano, Jr.

Camarines Norte State College/University of the Philippines Visayas San Roque, Mercedes
Camarines Norte 4601, Philippines
anabaldove@gmail.com

Milkfish is one of the most intensively cultured fish species in the Philippines. However, intensive fish culture is associated with chemical, biological, and physical disturbance that could lower the immune system of the fish leaving the fish vulnerable to diseases. Pollen of pine trees are sometimes used as a dietary supplement for animals due to its anabolic function which can increase growth rate. The present study aimed to determine the effects of various levels of dietary pine pollen from *Pinus tabulaeformis* Carr. on the growth and immune response of milkfish fry (*Chanos chanos*).

Milkfish fry with average body weight of 0.02 g were stocked in 15, 50L tanks at 40 fish per tank. A control diet (without pine pollen) and four experimental diets were prepared containing pine pollen at 0, 1.3, 2.6, 3.8, and 5.1 g kg^{-1}, and fed to the experimental fish for 60 days. At the termination of the trial, milkfish were subjected to sublethal dosage of ammonia to test the immune response of the fish.

Results revealed that there was no significant difference on survival rates among dietary treatments ($p>0.05$). Milkfish fed with diets containing different levels of pine pollen exhibited significantly higher growth ($p < 0.05$) in terms of weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio than did those fed the control diet. This could be attributed to the anabolic and androgenic effects of androgen from the pollen. Survival rate of milkfish fed with the diets containing 2.6 to 5.1 g kg^{-1} pine pollen were significantly higher ($p <0 .05$) than those fed with diets containing 1.3 g kg^{-1} or with the control diet. The results indicated that diets incorporated with pine pollen could enhance the immune system of milkfish. Optimum dietary PP level was estimated using the quadratic model by using SGR and FCR values and found to be 3.0 g kg^{-1}. In conclusion, pine pollen can be used as a dietary additive in milkfish diet to enhance growth and immune response against environmental stressors like dissolved ammonia.

MODELLING THE TECHNICAL AND BIO-MITIGATION POTENTIAL OF A COMMERCIAL IMTA SYSTEMS CONSISTING OF ATLANTIC SALMON (*Salmo salar)* AND SEA- CUCUMBERS (*Holothuria forskali)*

Anastasios Baltadakis *, Lynne Falconer, Trevor Telfer

Institute of Aquaculture
Faculty of Natural Sciences
University of Stirling
Stirling, FK9-4LA, Scotland
Anastasios.baltadakis1@stir.ac.uk

The concept of Integrated Multi-Trophic Aquaculture is to allow particulate and dissolved organic wastes to be used as food source for organisms at different trophic levels. So potentially offering ecosystem services, such as bio-mitigation of waste, while diversifying production, and providing an extra source of income. Previous studies have primarily focussed on capacity of organisms to directly consume wastes of others but tend to overlook technical bottlenecks and economic investment, which might arise under a commercial IMTA set-up in coastal environments. One example is the use of sea cucumbers, which are considered a strong IMTA candidate species due to their high bio-mitigation capacity and high market value.

In this study, an organic particulate depositional model was used to assess deposition of waste feed and faeces in a hypothetical system (Figure 1) based on six commercial salmon farms in Scotland. An optimal benthic zone for sea cucumber production (the Integrated Area, IA) which excluded sediments, with depleted oxygen levels and limited particulate food supply, was developed as a spatial management tool for the deployment of sea cucumbers. Furthermore, two sea cucumber production scenarios of 500 and 200 crates (each 1 m^2) were used to relate levels of stocking density to amount of waste mitigation potential based on the total area and total deposition. Finally, economic investment required for the set-up was assessed to investigate the financial cost of waste removal as an ecosystem service. Results from the models and economic analysis showed that the ecosystem services provided from this type of IMTA system for waste mitigation are very limited for full scale salmon farms in Scotland.

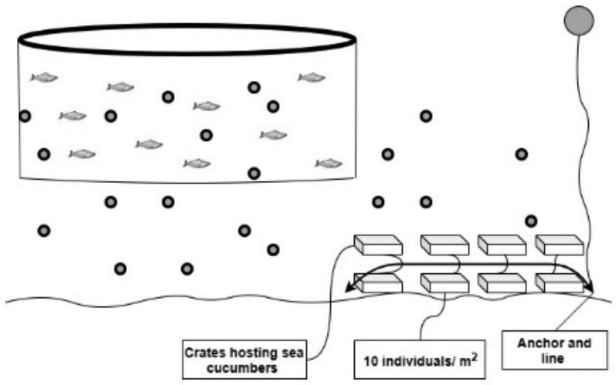


Figure 1 IMTA set-up introduced on this study. Oyster baskets (crates) deployed beneath salmon farm for the mitigation of particulate organic waste. Information about stocking densities of each crate are introduced on the figure.

AROMATASE INHIBITOR FADROZOLE AND 17B-ESTRADIOL ANTAGONISTICALLY AFFECT GONADAL DEVELOPMENT IN THE PROTANDROUS BARRAMUNDI

Quyen Q. T. Banh*, Jarrod L. Guppy, Jose A. Domingos, Julian Wilson, Dean R. Jerry

*Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering
James Cook University, Townsville, QLD 4811, Australia
Email: thiquyenquyen.banh@my.jcu.edu.au

Barramundi *Lates calcarifer* is a protandrous hermaphrodite, with all individuals initially maturing as males and then naturally sex change into females several years later. The gonadal development of barramundi provides a good model to study the genetic mechanism underlying the natural sex change. Practically, being able to control sex of barramundi to maintain the male and female status of high quality broodfish is one of the key objectives for successful hatchery production.

This research assessed the effects of a non-steroidal aromatase inhibitor, Fadrozole (FAD), administered solely or with 17β-estradiol (E_2) cholesterol pellet implants on the gonadal development of mature male barramundi. Fifteen-month-old male barramundi (2102 ± 126 g body weigh (BW)) were divided into four groups of 12 individuals each. Fish in the first group were implanted a cholesterol pellet without FAD or E_2 as control. The three treated groups of fish were implanted with E2 at 8 mg kg^{-1} (n=12), FAD 8 mg kg^{-1} (n=12), and binary of 8 mg E_2 and 8 mg FAD kg^{-1} (n=12).

Survival rates were 100% for all groups during the experiment with no damage observed in liver and kidney of all fish. At the final sampling nine weeks after treatment, 8 % (1/12) of the untreated control fish were found as early stage of sex reversion (stage T1) compared to 100% males at the commencement of the experiment, whereas 100% of FAD-treated and E_2-treated fish were either males or females, respectively. Binary treatment with FAD and E_2 resulted in 42 % (5/12) males, 42 % (5/12) females and 16% (2/12) transitional fish. In the FAD-treated fish, expression of male genes (*dmrt1* and *cyp11b*) were significantly upregulated; whilst female-biased gene (*foxl2*) downregulated. Exposure to E_2 resulted in significantly higher expression of *cyp19a1a* and *foxl2;* and lower expression of *dmrt1*, *cyp11b* and *esr1* than control fish. FAD neutralized the effects of E_2, in term of gonad morphology and gene expression.

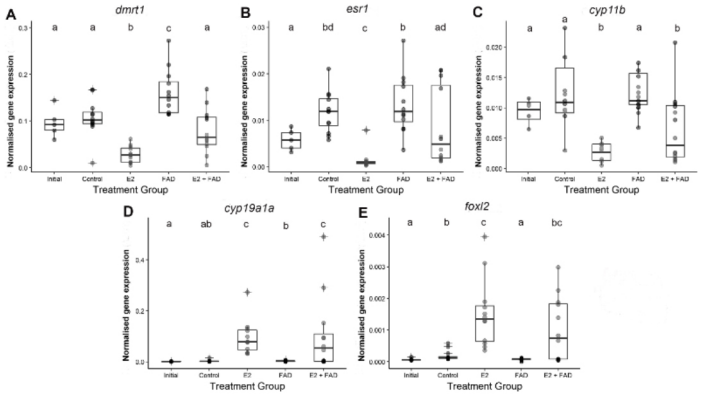


Figure 1. Relative gene expression of different sex-related genes measured by RT-qPCR in barramundi (*Lates calcarifer*) gonads implanted with E_2 sampled at 9 weeks post implant (A) *dmrt1* (B) *cyp11b*, (C) *esr1*, (D) *foxl2* and (E) *cyp19a1a*. Different letters represent statistical differences (P<0.05) between treatments (n=12 for each value).

LOBSTERS DO NOT PLAY BY THE RULES: UNDERSTANDING THE RNA INTERFERENCE RESPONSE IN THE TROPICAL ROCK LOBSTER *Panulirus ornatus* TO PROMOTE AQUACULTURE BIOTECHNLOGY

Thomas M. Banks*, Tianfang Wang, Quinn P. Fitzgibbon, Gregory G. Smith, Tomer Ventura

Centre for Bioinnovation
University of the Sunshine Coast
Sippy Downs 4556, Australia
tmb042@student.usc.edu.au

RNA interference (RNAi) is an endogenous mechanism of gene silencing and has been utilised in aquaculture as a powerful gene knockdown tool. RNAi allows for potent and specific gene silencing using custom double stranded RNA (dsRNA) molecules, and in a vast majority of crustacean models works extremely efficiently to study gene function, protect from disease, or generate desirable phenotypes. In the tropical rock lobster *Panulirus ornatus* and related spiny lobsters however, this process is difficult to induce because of the animals' intrinsic biology, which limits the capacity to manipulate the genes of these aquaculture species. This study aims to identify the mechanisms of RNAi inhibition in *P. ornatus*, which will inform methodologies to induce gene silencing in spiny lobsters to support new aquaculture biotechnology.

Preliminary studies have revealed that *P. ornatus* is deficient in the core enzymes and machinery which processes dsRNA for silencing when compared to closely related species where silencing is highly effective (Fig.1). This lack of protein expression likely translates to reduced silencing *in vivo*, even if dsRNA would be highly bioavailable. In addition, whole animal fluorescent microscopy utilising the unique larvae of these lobsters revealed potent mechanisms to sequester and secrete dsRNA molecules via the antennal gland, preventing its access to cells and tissues for silencing. These data show that dsRNA localises only in the antennal gland for subsequent secretion, and not systemically in the body of the animal (Fig.2). This implies that even if the core RNAi machinery was functional, dsRNA would still be unable to reach cells for silencing. Elucidation of these mechanisms exposes the dysfunctional RNAi system of *P. ornatus*, and in turn informs on which mechanisms must be overcome to enable effective silencing in this species.

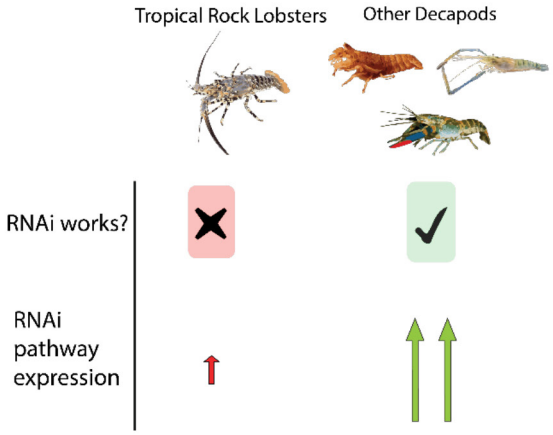


Figure 1- The RNAi system in Panulirus ornatus versus other decapod species

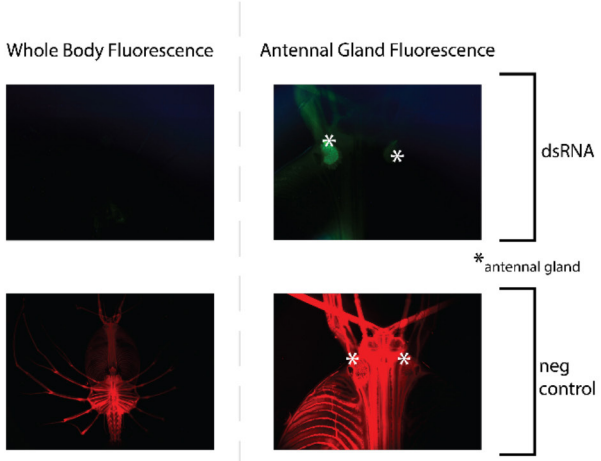


Figure 2- Double stranded RNA (green) localisation in Panulirus ornatus larvae versus non-specific fluorescent antibodies (red)

EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS ON THE GROWTH, NUTRIENTS PROFILE AND DIGESTIVE ENZYMES ACTIVITY OF *Pangasius hypothalamus* FRY

Sheeza Bano[1*], Noor Khan[1] and Sadia Nazir[1]

[1]Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore-Pakistan
Email: sheezabano4@gmail.com

An experiment was conducted to investigate the effect of different dietary protein levels (30, 35, 40 and 45% crude protein (CP) diets on growth performance, nutrients profile and digestive enzyme activity of *Pangasius hypothalamus* fry. There were four treatments each with three replicates. Experimental trial was carried out in 12 hapa's installed in earthen pond. Each hapa was stocked @20 fish and trial duration was 90 days. A formulated pelleted feed was given with feeding rate of 10%, 8%, 6%, 4% which was consecutively adjusted after each fortnightly sampling and 3% for last 4 weeks. Results showed that highest weight gain, SGR and feed efficiency were observed significantly higher ($P<0.05$) in T3 (40% CP) followed by T4, T2 and T1 while significantly higher survival rate was observed in T1 and T4 than T3 and T2. The proximate analysis of whole body revealed that crude protein content increased with increasing dietary protein level and was found significantly higher in T4 (45% CP) followed by T3, T2 and T1. Lipid contents in fish were observed significantly higher in T3 and T4 compared to T2 and T1. The protease enzyme activity in fish increased with increasing protein level while lipase and amylase activity decreased with increasing dietary protein contents in the diet.

THE CHROMOSOMAL-LEVEL GENOME ASSEMBLY OF *Tegillarca granosa* PROVIDE INSIGHT INTO THE ORIGIN OF RED BLOOD CELL HEMOGLOBIN OF BLOOD CLAM

Yongbo Bao*, Qifan Zeng, Zelei Zhang, Sufang Wang, Liyuan Lv, Qinggang Xue, Yinghui Dong, Zhenmin Bao, Shi Wang, and Zhihua Lin

Key Laboratory of Aquatic Germplasm Resource of Zhejiang
Zhejiang Wanli University
Ningbo 315100, China
bobbao2001@gmail.com

Background: *Tegillarca granosa* (*Anadara granosa*) is a marine bivalve belonging to the family Arcidae, which known as the blood cockle or blood clam due to the red hemoglobin in haemocytes. Polymorphism and multifunction of hemoglobin have been found in Arcidae. Homodimeric, heterodimeric, tetrameric and 2D polymeric hemoglobins have been found in blood clam. Thus, blood clams present a great model to elucidate the complex molecular evolution of invertebrate hemoglobins. Therefore, we reported a high-quality chromosomal-level reference genome of *T. granosa* to elucidate the complex molecular evolution of invertebrate hemoglobins.

Findings: A total of 101.96 Gb and 131.15 Gb sequencing data were obtained with the PacBio and Illumina platforms, which represented approximately 283.87× genome coverage. In addition, a total of 113.15 Gb data were also obtained to investigate the chromosome interaction. A *de novo* genome assembly of 812.61 Mb was generated, with a contig N50 of 599.92 kb. Hi-C scaffolding anchored 97.13% of the contigs into 19 chromosomes with a scaffold N50 of 42.62 Mb. The repeat sequences account for 53.56% of the assembled genome. A total of 24,398 protein-coding genes were predicted and 81.18% of them were functionally annotated. Phylogenetic analysis revealed that Arcidae diverged from the lineage of Pectinidae at approximately 349 million years ago. The expanded globin gene family and heme-synthesis pathway genes suggested clues to molluscan RBC hemoglobins origins and an evolutionary adaptation to burrowing living and sulphide rich environments.

Conclusions: The high-quality genome assembly and comprehensive transcriptomes generated in this work not only provides highly valuable genomic resources for future studies of *T. granosa*, but also lays a solid foundation for further investigation on the evolutionary origin of hemoglobin in the molluscs.

Gracilaria gracilis As A FUNCTIONAL FEED AADITIVE FOR EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

Passos, R.[1], Pires, P.[1], Simões M.[1], Ferreira, I.[1], Frias, P.[1], Gomes, E.[2], do Carmo, B.[1], Correia, A.P.[1], Afonso, C[1]., Baptista, T.[1]

[1] Marine and Environmental Sciences Center (MARE), CETEMARES, Peniche, Portugal
[2] Tourism and Maritime Technology School (ESTM), Leiria Polytechnic (IPLeiria), Peniche, Portugal
Email – teresa.baptista@ipleiria.pt

Introduction

In recent years, several studies have been developed regarding the use of immunostimulants, probiotics and prebiotics to enhance the fish immune system response towards different pathogens (Cruz *et al*., 2012; Carbone & Faggio, 2016). There are several sources of immunostimulant chemical compounds that have been proved to be effective in fish disease prevention, namely those that are extracted from marine algae (Barman *et al*., 2013). Some extracts from marine algae can possess antiviral and antibacterial activity showing encouraging results as fish feed supplementation, specially the red algae (Sahu *et al*., 2008; Rizzo *et al*., 2017; Hoseinifar *et al*., 2018). *Gracilaria gracilis*, is a multi-product source, having several interesting components such as lipids, proteins, carbohydrates, phycobiliproteins, phenols and biologically active phytochemicals (Francavilla *et al*., 2013). The present study intends to analyse *G. gracilis* potential as fish feed supplementation for growth as well as its protective effect against a *Photobacterium damselae* subsp. *piscicida* (*Phdp*) infection on seabass.

Materials and methods

The study was performed at CETEMARES (Politécnico de Leiria) facilities, with seabass (mean wet weight 17.5 ± 6.1 g) juveniles obtained from the Aquaculture Research Station-EPPO (IPMA, Olhão, Portugal). Fish were randomly distributed as a completely randomized design into 12 tanks of 60 l closed recirculation systems for the feeding trial (i.e. triplicates per experimental conditions). Using standard seabass feed, SPAROS Lda (Olhão, Portugal) manufactured five different diets: a control diet, *G. gracilis* extract (0.35%) supplemented diet, and two *G. gracilis* powder (2.5% and 5%) supplemented diets. Fish were weighed before and after the feeding trial (51 days), and then subjected to *Phdp* challenge, the same amount of fish were sham injected with phosphate saline buffer. At each sampling point, fish were anaesthetized with 2-phenoxyethanol and blood samples were collected for haematological procedures such as total and differential counting of peripheral leukocytes and total circulating erythrocytes. The remaining blood was centrifuged and plasma was collected for the assessment of innate humoral immune parameters. Liver was dissected out from the fish to evaluate some parameters of oxidative stress.

Results

No differences were registered about the effect of the different diets on the growth performance. White blood cell (WBC) counts were consistently reduced in infected fish 24h post-infection ($p<0.05$). However, fish supplemented with algae powder recovered faster, as can be seen by the differences noted between the WBC counts at 24h and 48h post-infection in the 2.5% supplemented diet ($p<0.05$). Almost all red blood cell (RBC) counts decreased when compared with the 0h counts, even the not infected fish, as well as the haematocrit ($p<0.05$). However, the fish fed with no supplementation had a significant decrease in both parameters when infected with the bacteria from 24h to 48h post-infection ($p<0.05$). Lysozyme also showed differences, as seen by the lower concentration seen in both injected groups when the fish were fed with the base feed ($p<0.05$). Catalase activity, only measured before the infection, was higher on fish fed with the 5% algae powder supplementation, compared to the 0.35% algal extract supplementation ($p<0.05$).

Discussion and conclusion

Red algae have been proven to have some characteristics that may be of use as a fish feed supplement (Sahu et al., 2008; Rizzo et al., 2017; Hoseinifar et al., 2018). WBC increased counts from 24h to 48h post-infection in fish fed the 2.5% supplemented diet may be a sign of the immunostimulant and anti-infective capacity (Abdelmalek et al., 2015) of *G. gracilis*. Leucocytes are in the first line of defence against infections, so an increased count works as a barrier against a bacterial infection (Abdelmalek et al., 2015; Talpur and Ikhwanuddin, 2013). RBC reduction might be related to stress resulting from handling, in this situation, as well as lower haematocrit (Talpur and Ikhwanuddin 2013). Yet, algae supplementation might aid with the recovery since non-supplemented diet had a significant decrease in bacterial challenged fish.

;(Continued on next page)

Stress from the handling might have affected lysozyme, causing immune suppression. Nevertheless, the non-supplemented diet showed a lower concentration, meaning that *G. gracilis* supplementation might help coping with stress, resulting from handling procedures.

Catalase, an antioxidant enzyme, activity was higher on fish supplemented with a 5% algae powder, meaning that some oxidative radicals were promptly detoxified in these fish (Monteiro et al., 2006). As such, algal inclusion might be beneficial to reduce oxidative damage in cultivated fish, and, ultimately improve growth and income due to the lower energy dispended in oxidative defence systems.

Even though growth parameters were unaffected during the time of the feeding trial, the algal inclusion in European seabass feed showed benefits in some regards. Namely in aiding against bacterial challenges, improving immunity to some extent, and oxidative damage. As such, it is necessary to assess the cost-effectiveness of algae inclusion in marine fish feed. These fish's immunity and oxidative parameters are still being studied in regards to further recognize the *G. gracilis* immunostimulative capabilities.

Acknowledgments: This study had the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2019 granted to MARE, and the project Operational Programme MAR2020 through the project 16-02-01-FMP-84 – SeaWeedFeeds.

References

Abdelmalek, B. E., Driss, D., Kallel, F., Guargouri, M., Missaoui, H., Chaabouni, S. E., Ayadi, M. A., Bougatef, A., 2015. Effect of xylan oligosaccharides generated from corncobs on food acceptability, growth performance, haematology and immunological parameters of *Dicentrarchus labrax* fingerlings. Fish Physiology and Biochemistry 41: 1587-1596. doi: 10.1007/s10695-015-0110-5

Barman, D., Nen, P., Mandal, S. C., Kumar, V., 2013. Immunostimulants for Aquaculture Health Management. Journal of Marine Science: Research and Development 3: 134. doi: 10.4172/2155-9910.1000134

Carbone, D., Faggio, C., 2016. Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish & Shellfish Immunology 54: 172–178. doi: 10.1016/j.fsi.2016.04.011.

Cruz, P. M., Ibáñez, A. L., Hermosillo, O. A. M., Saad, H. C. R., 2012. Review Article Use of Probiotics in Aquaculture. International Scholarly Research Network (ISRN) Microbiology, Volume 2012, Article ID 916845, 13 pages. doi:10.5402/2012/916845

Francavilla, M., Franchi, M., Monteleone, M., Caroppo, C., 2013. The red seaweed *Gracilaria gracilis* as a multi products source. Marine Drugs, https://doi.org/10.3390/md11103754.

Hoseinifar, S. H., Yousefi, S., Capillo, G., Paknejad, H., Khalili, M., Tabarraei, A., Van Doan, H., Spano, N., Faggio, C., 2018. Mucosal immune parameters, immune and antioxidant defence related genes expression and growth performance of zebrafish (*Danio rerio*) fed on *Gracilaria gracilis* powder. Fish & Shellfish Immunology 83: 232–237. doi: 10.1016/j.fsi.2018.09.046

Monteiro, D. A., de Almeida, J. A., Rantin, F. T., Kalinin, A. L., 2006. Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comparative Biochemistry and Physiology C 143: 141- 149.

Rizzo, C., Genovese, G., Morabito, M., Faggio, C., Pagano, M., Spanò, A., Zammuto, V., Armeli M., S., Manghisi, A., Cigala, R., Crea, F., Marino, F., Gugliandolo, C., 2017. Potential Antibacterial Activity of Marine Macroalgae against Pathogens Relevant for Aquaculture and Human Health. Journal of Pure and Applied Microbiology 11: 1695-1706. doi: 10.22207/JPAM.11.4.07.

Sahu, M., Swarnakumar, N., Sivakumar, K., Thangaradjou, T., Kannan, L., 2008. Probiotics in aquaculture: Importance and future perspectives. Indian Journal of Microbiology 48: 299-308. doi: 10.1007/s12088-008-0024-3.

Talpur A., D., Ikhwanuddin, M., 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunology 34:254–264.

TIME-COURSE IMMUNE RESPONSE OF GILTHEAD SEABREAM JUVENILES FOLLOWING INFECTION WITH *Photobacterium damselae* SUBSP. *piscicida*

Baptista, T.*, Pires, P., Passos, R., Ferreira, I., Simões, M., Santos, P., Machado, M., Costas, B.

Marine and Environmental Sciences Center (MARE), CETEMARES, Peniche, Portugal
Email – teresa.baptista@ipleiria.pt

Aquaculture plays an important role in food production worldwide. However, the presence of pathogens is currently a major constraint for its development, leading to disease outbreaks and therefore causing severe economic losses. *Photobacterium damselae* subsp. *piscicida* (*Phdp*) is the causative agent of photobacteriosis, a bacterial septicaemia. *Phdp* is able to infect a wide variety of marine fish with economic relevance such as, gilthead seabream, European seabass, sole and meagre. The aim of this work was to study haematological dynamics and oxidative stress parameters of gilthead seabream (*Sparus aurata*) juveniles infected with *Phdp*.

A time-course study was performed, with gilthead seabream (mean weight 9.8 ± 2.2 g) juveniles. Among population, 12 fish were randomly selected and sampled before infection (time 0 h). Thereafter, the remaining animals were randomly selected and intraperitoneally (i.p.) injected with 100 μl of 1×10^5 CFU ml^{-1} of *Phdp*, while control fish were sham-injected with 100 μl a phosphate buffered saline solution. Afterwards, fish were randomly distributed as a completely randomized design into 6 tanks of 60 l closed recirculation systems (i.e. triplicates per experimental condition). Two animals per tank (n=6) were randomly selected and sampled at 3, 6, 9, 24 and 48 h after i.p. injection. At each sampling point, fish were anaesthetized with 2-phenoxyethanol and blood samples were collected for haematological procedures. Liver was dissected to evaluate oxidative stress parameters. Each recirculation system was kept at 25 °C for 14 days and pathological signs and mortality were observed and recorded.

Mortalities started at 24 h in infected seabream and exhibited a maximum mortality rate of 40 % at the end of infection, whereas no mortality was observed in control groups. Regarding white blood cells, neutrophil counts increased in the infected treatment compared to the control (0 h) whereas monocyte counts decreased in the infected groups at 6h post-infection. Total circulating erythrocytes values decreased in the infected group compared to control and non-infected fish, as well as the haematocrit values that also decreased in the infected treatment. Hepatic catalase activity decreased at 6h and 9h compared to 48h post-infection in the infected group. Lipid peroxidation in the infected group increased consistently until 48 h post-infection.

Acknowledgments: The authors thank FCT, Portugal, for financial support (UID/MAR/04292/2019) and Be4AquaHealth (MAR2020-02.05.01-FEAMP-00139).

ENVIRONMENTAL DRIVERS FOR SPATIAL AND TEMPORAL DISTRIBUTION OF *V. vulnificus* AND *V. parahaemolyticus* IN TRIPLOID *Crassostrea virginica* AQUACULTURE (SOUTH ATLANTIC, GEORGIA, USA)

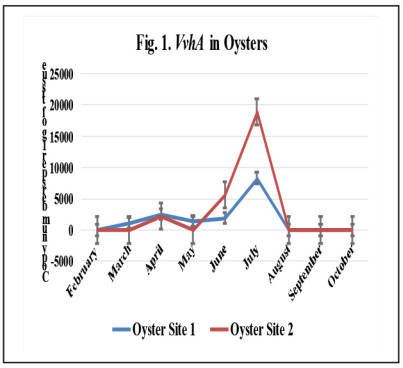
Andrei L. Barkovskii* and James Prescott

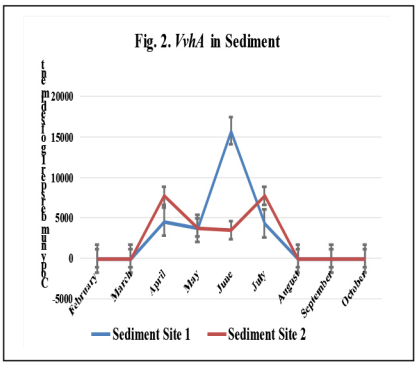
Department of Biological and Environmental Sciences
Georgia College and State University, Milledgeville, GA 31061, USA
andrei.barkovskii@gcsu.edu

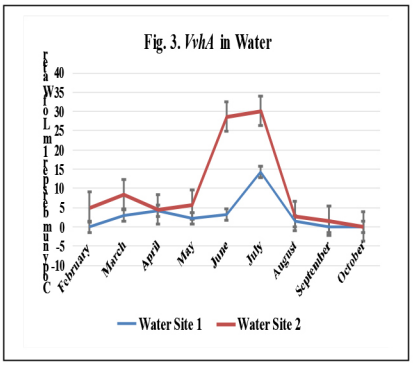
The South Atlantic accounted for 3.4% of Eastern oyster production with the majority of that coming from wild harvest leaving the oysters aquaculture almost unexploited. The occurrence of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in South Atlantic aquaculture has not been addressed. In this study, the presence, distribution and dynamics of these pathogens were evaluated at two sites cultivating triploid *Grassostrea virginica* in floating gear. *V. vulnificus* and *V. parahaemolyticus* were detected using primers with TaqMan probes for *vvh*A gene, and *tlh*, *trh*, and *tdh* genes, respectively. 16S rRNA gene was used for calculating their abundance.

The highest concentration of *tlh* gene was observed in sediments with three seasonal peaks in May, July, and September at 1.5-5.0×10^5 copies/g. Much lower concentrations of *tlh* were detected in water column with peaks at 1.0-1.5×10^3copies /mL in June and September. Concentration of *tlh* in oysters was higher than in water, lower than in sediment, and peaked in September at ~1×10^5 copies /g. These concentrations positively correlated both temporally and spatially; and the temperature and dissolved solids were the environmental drivers. No *trh* or *tdh* were detected evidencing the absence of pathogenic *V. parahaemolyticus* strains.

VvhA highest concentration (~1.8×10^4 copies/g) was observed in oysters at both sites in July (Fig. 1). Lower concentrations (0.5-1.5×10^4 copies/g) were observed in sediments with main peak occurred in June (Fig. 2). The lowest *vvhA* concentrations were detected in water column, which peaked at 15-30 copies/mL in June-July at both sites (Fig. 3). This dynamic and corresponding increase in *vvhA* abundancy in oysters' microbiota suggested repositioning of *V. vulnificus* from sediments to water column, and its bioaccumulation from there by oysters in subsequent months. Temperature, dissolved solids, and turbidity were environmental drivers for *vvhA*. Since all *V. vulnificus* are considered pathogenic, its presence in, and possible bioaccumulation by oysters need to be addressed in aquaculture.







BEHAVIOURAL RESPONSES OF *Venerupis corrugata* EARLY LIFE STAGES CULTURED UNDER ACIDIFICATION AND WARMING EXPERIMENTAL CONDITIONS

Carlos M. Barroso*, Joana G. Fonseca, Daniela B. Freitas, Isabel B. Oliveira, and Susana Galante-Oliveira

Biology Department & CESAM
University of Aveiro
Campus de Santiago
3810-193 Aveiro, Portugal
cmiguez@ua.pt

The pullet carpet shell, *Venerupis corrugata*, is an important fishery resource, traditionally harvested at the Southwestern and Mediterranean Europe. This species has a high market value (actual global average price over $21USD) and its aquaculture production is now essential to meet the market high demand, warranting natural stocks maintenance under frequent overfishing and high mortalities caused by pathogens and extreme climatic events, that will be more frequent under the climate conditions predicted for a near future. The potential impacts of two major global phenomena ongoing in the world oceans –acidification (OA) and warming (W)– on the performance of *V. corrugata* early life stages was studied by assessing the development stage and the behaviour exhibited by specimens cultured under 9 experimental scenarios for 14 and 60 days (larvae and juveniles, respectively).

Broodstock collected at the Ria de Aveiro (NW Portugal) was induced to spawn by thermal stimulation. Eggs were incubated to hatch in 1-µm filtered, UV-sterilized artificial saltwater under unmanipulated pH (~8.2) and 18±1°C (control conditions). The D-larvae collected in a 60µm-sieve 48h post-fertilization (48h pf) were exposed to the 9 OA-W scenarios presented in Fig.1, resultant from a factorial design of three pH levels (targeting 8.2, 7.8 and 7.6) at three different temperatures (18, 20 and 22°C). Larval development stage and swimming behaviour (characterized by a 6-stages scale developed for the purpose) were characterized after 14 days of exposure (at T14) and the early juveniles' burial activity and byssus production were assessed at T60.

As expected, larval development was proved to be accelerated by warming and relatively delayed under acidity. However, larval behaviour was proved to be significantly affected only by temperature, with "*searching*" and "*crawling*"

behaviours being registered at treatments at which more developed specimens were recorded. In turn, when subject to the presence of sediment, the early juveniles reared under higher acidity (pH_{target} 7.6) for 60 days revealed lower ability to bury, as well as reduced capacity to produce byssus, behavioural changes that might compromise both mobility and fixation to the substrate, essential to this clam survival.

Hence, this study suggests that despite warming may reduce the negative effects of OA on *V. corrugata* larval development, the exposure to lower pH throughout ontogeny might reduce juveniles' performance, increasing their vulnerability.

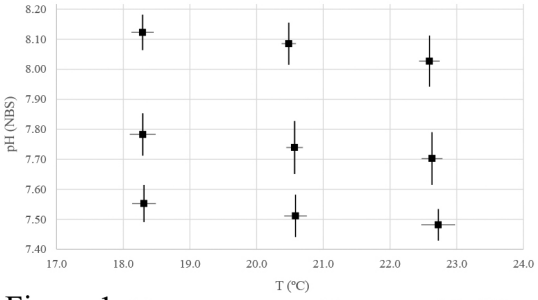


Figure 1: Mean temperature (T, expressed in ºC) and pH (NBS scale) calculated from probe measurements performed twice a day, and respective standard deviation.

ECONOMIC AND HEALTH BENEFITS OF INTRODUCING FISH IN SMALL FARMS OF NIGERIA

Amrit Bart*, Matthias Halwart, Emmanuel K. Ajani, Oluwafemi Ajayi, Bamidele O. Omitoyin, Esendugue G. Fonsah, Gary Burtle, Austin Stankus, Yuan Xinhua, Oladeji K. Kazeem, Babatunde O. Oduntan and Annika Kolbe

Amrit Bart*
Department of Animal and Dairy Sciences
College of Agricultural & Environmental Science
University of Georgia
abart@uga.edu

Background

Rural Nigerian rice farming communities suffer from access to and availability of nutritious food. Fish in Rice-fish farming system has the potential to increase essential protein and micronutrients in people's diets. Rice-fish farming is not practiced in Nigeria despite several favorable environmental advantages (temperature, soil, water) and premium value placed on fish. Rice-fish research and farm trials are limited. There are only a few publications referencing cultivation of rice with fish in Nigeria. Our project, 'Aquaculture and Rural Communities: Farm Diversification Strategy Through Integrated Agriculture-aquaculture Systems and Nutrition-Sensitive Value Chain for Better Nutrition Outcomes' performed one-year farm trials (second production cycle in on-going). The results from these trials provide insight into potential nutritional and economic benefits of adding fish into traditional rice cultivation systems.

Approach

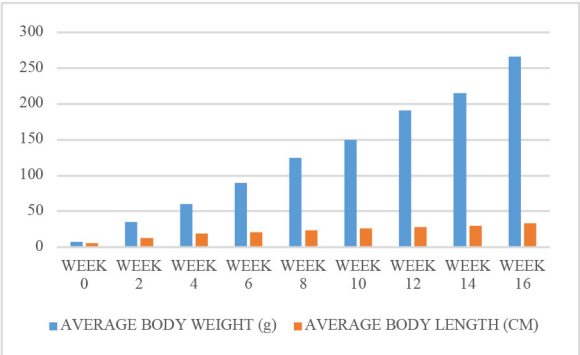
Six rice plots of various sizes were selected in Kebi and Ebonyi State for rice-fish trials. Each plot was modified by creating rectangular canal (1.5m wide and 0.7m deep) around the edges of the plots. Catfish, *Clarias gariepinus* fingerlings (6-8g; 5fish/m^2) were stocked and fed twice daily with fish feed (to satiation) formulated using locally available ingredients. No pesticide or herbicide were applied.

Results

Rice and fish were harvested after 90 days of stocking. Harvested fish ranged from 250 – 300g (4.9 tons/ha). Production varied among plots but, were higher than national average per unit area (2.5-3.0 tons/ha national average) in all six plots. No mortality was observed although, 20 % of the fish were missing at harvest.

Predatory birds were observed around the paddy. Predation and/or poaching may have contributed to the reduced number of fish at harvest.

Our preliminary results indicate high levels of receptivity to rice-fish adoption among rice farmers in both, northern and southern Nigeria. Income and nutrition benefits from inclusion of fish are significant among households under our project.

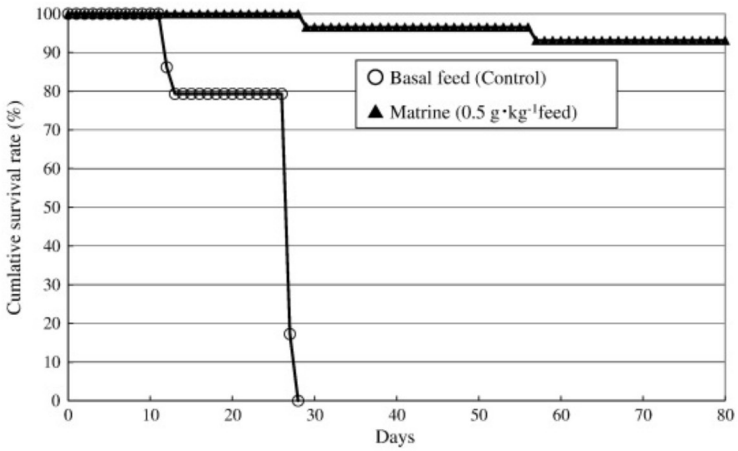


FISH NUTRITION IN RELATION TO FISH HEALTH: GOOD PRACTICES WITH APPLICATION OF PROBIOTICS, PREBIOTICS AND PHYTOBIOTICS

Gerald Bassleer

Bassleer Biofish, Achter de wereld 1, B2230 Herselt, Belgium
(www.bassleer.com)
gerald@bassleer.com

Singapore & other Asian countries (mostly Japan, Indonesia, Thailand, Malaysia, & Vietnam) are known to supply 80% of the world market for ornamental fish&shrimp with a livestock export value of US$ 500million/year. It is a common practice to use a complete food for ornamental freshwater and marine fish to provide high quality fish within a short period. Especially feed additives play an important role to control the health of fish & shrimp: to help the immune (defense) system, to assist in faster repair after damage, to prevent viral, bacterial and parasitic infections, to prevent or limit the use of antibiotics & other medications, to stimulate growth, health and color. In a short overview, with the recent studies & personal experience we will explain the health benefits of a functional fish food= a food that gives more benefits than just 'feeding fish', a food that can be seen as a "nutricament"; a nutritional medication with products from nature, avoiding (or preventing) the use of medications. The use of probiotics *Pedioccocus acidilactici*, the use of prebiotics such as yeast extracts (*Saccharomyces cerevisiae*) and also phytobiotics such as extracts Matrine from *Sophora flavescens*, extracts from brown kelp (*Laminaria japonica)* and others will be presented.



(Additive Matrine and survival rate of Flounder during infection with parasites *Cryptocaryon irritans; Goto,2015 Journal of Aquaculture*)

(White Spot disease Cryptocaryon in Clown fish) and Fancy Goldfish with bacterial infection)

INTERACTIONS BETWEEN PARASITIC PROTOZOAN AND BACTERIAL MICROBIOME IN A TROPICAL FISH FARM

Bastos Gomes G[1 *], Hutson KS[3,5], Domingos JS[2,3] , Infante Villamil S[3], Huerlimann R[3], Miller TL[3,4] and Jerry DR [2,3]

[1] Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong Kowloon, Hong Kong SAR, China
[2] Tropical Futures Institute, James Cook University Singapore, Singapore, 387380
[3] College of Science and Engineering and Centre for Sustainable Tropical Fisheries andAquaculture James Cook University, Townsville 4811, QLD, Australia.
[4] Fish Health Laboratory, Department of Fisheries Western Australia South Perth 6151, WA, Australia.
[5] Cawthron Institute. 98 Halifax Street East Nelson 7010. Nelson 7042. New Zealand
*Email: gbastosg@cityu.edu.hk

The bacterial microbiome is an important component of any aquaculture environment. The association and interaction among different microorganisms in finfish aquaculture environments during infections are rarely explored. However, the interaction between the bacterial microbiome and other microorganisms (e.g. parasites, viruses, or other bacteria) in aquaculture can prevent or contribute to disease outbreaks. This study characterised the bacterial composition associated with the abundance of a ciliated protozoan parasite, *Chilodonella hexasticha*, and their link with mortalities in a freshwater barramundi, *Lates calcarifer,* farm situated in tropical Queensland, Australia over one year. An environmental DNA (eDNA) approach was used to estimate the abundance of *C. hexasticha* (copies/μl) in water through SSU-rDNA gene qPCR and the relative abundance of bacterial species in water and fish gills through 16S rRNA V3 and V4 metabarcoding. The overall bacterial community diversity, dominated by Actinobacteria (42%), Proteobacteria (28%), Bacteroidetes (10%) and Cyanobacteria (6%), was stable among ponds over the study period ($P > 0.05$). Of those that could be identified to species level, *Flavobacterium columnare*, *Veillonella dispar* and *Bdellovibrio bacteriovorus* abundance correlated with both high *C. hexasticha* levels in pond water and high observed fish mortalities ($P < 0.05$). Results also revealed significantly higher levels of *F. columnare*, *B. bacteriovorus*, *Plesiomonas shigelloides*, *Prostecobactor debontii* and *Oxalobacter formigenes* ($P < 0.05$) in gills of fish with high infection levels of *C. hexasticha* compared to fish with no detected parasite infection. This study demonstrated, for the first time, a link between increased parasitic ciliate abundance, bacterial composition and fish mortalities in a freshwater aquaculture environment and the application of eDNA to investigate pathogen, host and environment interactions.

DECADAL IMPACTS OF HARMFUL ALGAL BLOOMS ON BIVALVE AQUACULTURE IN THE ASIA PACIFIC REGION

Leila Basti*

Marine Environmental Physiology Laboratory, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato, Tokyo 108-8477 JAPAN.
College of Agriculture and Veterinary Medicine, United Arab Emirates University, P.O. Box 15551, Al Ain, Abu Dhabi, UAE
Emails: basti@uaeu.ac.ae; bastileila@gmail.com

Several species of harmful algal blooms cause mass mortalities of shellfish in aquaculture farms, including cosmopolitan ichthyotoxic dinoflagellates and raphidophytes. Others are responsible for the contamination of shellfish, notably bivalves with potent toxins that are detrimental to human health, following ingestion. In the latter case, bivalve fishery beds and aquaculture farms remain closed to harvesting for sustained periods of time, lasting months at a time, leading to serious economic losses. Here, the decadal occurrence as well as the impacts of HAB in terms of shellfish mortalities and contamination have been analysed for the Asia-Pacific region, from the period of 1980-2020, for the most recurrent HAB species. The analysed data was driven of the IOC-UNESCO global database, HAEDAT, to identify the most recurrent HAB species and the most impacted bivalve species and geographical regions. GIS was used to represent the decadal geographical distribution of the HAB events and associated effects on bivalve aquaculture.

AQUAPONICS IN ARID ENVIRONMENTS: HISTORICAL OVERVIEW AND RECENT DEVELOPEMENTS

Leila Basti*

College of Agriculture and Veterinary Medicine, United Arab Emirates University, P.O. Box 15551, Al Ain, Abu Dhabi, UAE
Emails: basti@uaeu.ac.ae; bastileila@gmail.com

Aquaculture in arid environments is seen as an alternative to conventional agriculture and live stocks to offer sources of proteins for an increasing demand projected over the coming decade, specifically in arid environments where water resources are under increasing strains. Aquaponic systems are one of the major aquatic production systems best fit for arid environments, notably the Middle East that has been experiencing water scarcity and severe impacts from climate change. These systems offer an excellent alternative to conventional land-based aquaculture, to provide both plant and seafood products for a market that has been heavily relying on exports for food security. In addition, aquaponic offers an alternative to marine-based aquaculture in a region where the marine environment is subjected to extreme weather conditions and has been experiencing depleted stocks of fisheries. In this presentation, the historical development of aquaponic systems in arid environments is presented, and the recent and current developments of these production systems in the Middle East region are presented and discussed.

Bacillus indicus AND *Bacillus subtilis* AS AN ALTERNATIVE HEALTH SUPPLEMENT TO ASTAXANTHIN

S. Baumgärtner *, J. James, A. Ellison

Bangor University, School of Natural Sciences, Bangor LL57 2DG, UK
E-mail: smb20gzg@bangor.ac.uk

Infectious diseases are one of the biggest burdens in the growing aquaculture industry. Novel alternative strategies to improve fish health and increase disease resistance are urgently needed. The carotenoid astaxanthin is a widely used feed supplement with well-known health benefits for the host and is used as a/or colourant for fish flesh/skin. However, due to high productions cost, its use is restricted to high-value species. In the following experimental study, we tested a novel two-species probiotic - *Bacillus indicus* and *B. subtilis* - as a potential alternative health supplement to astaxanthin in mirror carp (*Cyprinus carpio*). The main goal of the study was to use experimental feed trials and 16 S rRNA microbiome profiling to compare the effects of *B. indicus* and traditional astaxanthin supplementation on fish performance and health, based on the overall microbial community of the gastrointestinal tract.

Over a 7-week carp trial, fish fed the probiotic showed a greater specific growth rate (SGR) compared to the control, however not significant. Fish fed astaxanthin demonstrated significantly increased SGR. The haematological and immunological analysis showed significant results in fish fed the probiotic (e.g., increased phagocytic activity). In carp, we found that both treatment groups significantly increased microbial diversity in the distal intestine (Figure 1). The probiotic increased the abundance of potentially beneficial bacteria including *Bacillus spp*. Overall, the probiotic blend induced a wider community shift compared to astaxanthin. In addition, a greater number of bacteria metabolic pathways were altered in carp fed the probiotic compared to astaxanthin. However, during the microbial analysis, the probiotic bacteria species could not be detected in the distal intestine.

The probiotic supplement *B.Indicus/B.subtilis* significantly increased gut microbial diversity in carp, similar to astaxanthin supplementation. However, the probiotic induced greater changes in abundance across the microbial community. Increased microbiome diversity is likely linked to healthier fish, for example, our bloodwork analyses demonstrated significantly increased phagocytic activity in fish fed the probiotic, indicating an increased immune response. Overall, we conclude that the *B.inidicus/ subtilis* probiotic has great potential to be used as a replacement for astaxanthin.

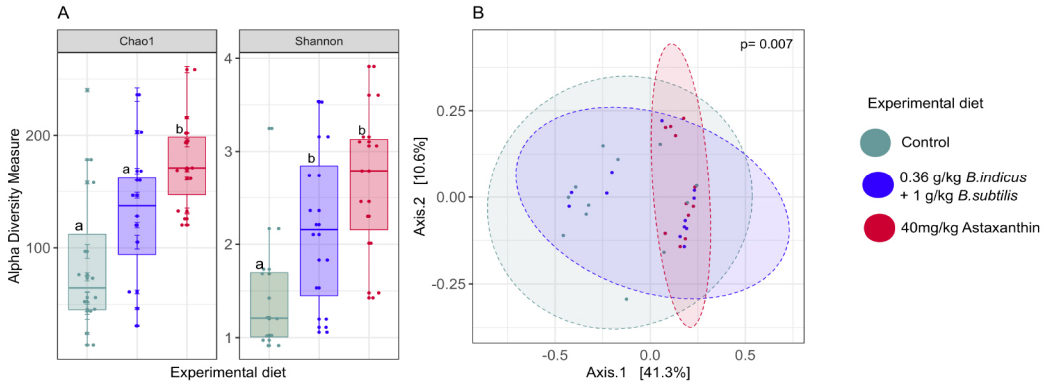


Figure 1: Diversity measures of the microbial community in carp under probiotic inclusion levels and the supplementation of astaxanthin, Alpha diversity measured by Chao1 and Shannon indices (A). NMDS ordination of Beta diversity communities (B). Ellipses indicate 95% confidence.

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

Dalhatu Y. Bawa*, Muhammad A. Sulaiman, Rasaq Ibrahim

Department of Fisheries, Faculty of Agriculture, Kebbi State University of Science and Technology, Aliero. Nigeria
*Corresponding: bawadal@hotmail.com

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

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

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

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

THE FEASIBILITY OF LANDBASED RAS PROJECTS - HOW TO MAKE THE RIGHT CHOICES

Dr. Michael Bech*

Nordic Aquaculture Consulting ApS
Denmark
E-mail: drbechaqua@gmail.com

Landbased Recirculation Aquaculture Systems (RAS) is booming worldwide and the present pandemic is pushing us even further towards local production but what is required to make a healthy business?

The most important factors will be identified including site selection, selection of species, production volume, logistics, bio-security, level of processing, selection of suppliers and financing. Financial evaluations of various scenarios will be used to illustrate the importance of each contribution of both OPEX and CAPEX.

THIN LAYER DRYING KINETICS OF TILAPIA (*Oreochromis niloticus*) IN CONVENTIONAL AND HYBRID SOLAR DRYERS DURING THE WET SEASON

Bello, Muinat Moninuola. *; Aviara, Ndubisi Aviara; Aremu, Ademola Kabir

Department of Fisheries,
University of Maiduguri,
PMB 1069, Maiduguri, Borno State, Nigeria
mmbello@unimaid.edu.ng

Oreochromis niloticus is an extremely perishable fish, spoiling soon after death due to enzymatic and microbial actions. Therefore, mathematical modelling and computer simulation gives insight, into the comparative performance of various drying systems. The research therefore aimed at providing quantitative description of drying behaviour and helps to predict quality changes during drying.

Thin layer drying of tilapia in conventional solar dryer (CSD) and hybrid solar dryers (HSD) was considered and sun drying was used as control. The experiment was conducted in completely randomized design (CRD) with three replicates during wet season. Eight popular thin layer models were selected and tested with the drying data obtained from the fish species (TABLE 1).

Results indicated that, in CSD (Figure 1), Midilli *et al*., model had the lowest standard error of estimate (0.0652) and the highest co-efficient of determination R^2 (0.9879) and for HSD (Figure 1), the Wang and Singh model had the lowest standard error of estimate (0.1569) and the highest co- efficient of determination R^2 (0.9326), while in sun drying, the Wang and Singh model had the lowest standard error of estimate (0.0810) and highest co-efficient of determination R^2 (1.0000).

The Wang and Singh model best described the drying behaviour of *Oreochromis niloticus* in conventional solar dryers and it can be used to understand the heat and mass transfer phenomena in solar drying of *Oreochromis niloticus* using the dryer.

TABLE 1: Thin layer models considered

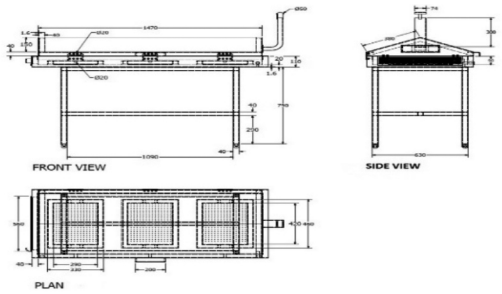


FIGURE 1: Orthographic projections of the conventional solar dryer

S/N	Model name	Model equation	References
1	Newton's	MR= exp (-kt)	(Henderson 1974)
2	Pages	MR-exp (-kt^n)	(Page, 1949)
3	Henderson and Pabis	MR= aexp (-kt)	(Zang and litchfield,
4	Logarithmic	MR= aexp (-kt) +C	(Karathanos, 1997)
5	Wang and singh	MR=1 + at + bt^2	(Wang and Singh., 19
6	Midilli *et al.,*	MR = aexp (-kt^n) +bt	(Midilli *et al*, 2002)
7	Thompson	t = ALn (MR) +B [Ln (MR)]^2-1	(Thompson *et al*., 19(
8	Diffusion Approach	MR=aexp (-kt) + (1-a) exp(-kgt)	(Karathanos, 1997)

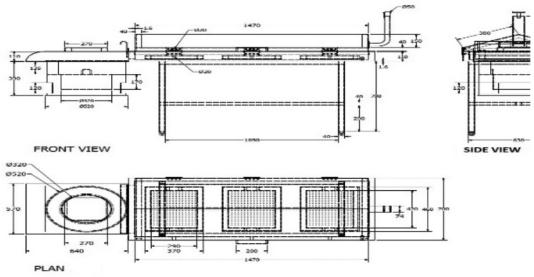


FIGURE 2**:** Orthographic projections of the hybrid solar dryer

NUTRIENT QUALITY AND SHELF LIFE OF PROCESSED *Parapenaeopsis atlantica* UNDER DIFFERENT PRESERVATIVES

Oluayo A. Bello-Olusoji; Olaronke O. Olawusi-Peters and Tinuke T. Bamgbose

Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Nigeria

Demand for seafood is increasing while production from captured fisheries is static, due to increasing population, habitat destruction, climate change, postharvest loses etc. Nigeria coastal water is rich in shrimps, if properly managed it will increase shrimp production, improve livelihood and contribute to fish-food security agenda in Africa. This work examined the potency of some natural preservatives on the nutritional values of *Parapenaeopsis atlantica* shrimp.

The nutritional values of freshly, processed *P. atlantica* collected from Igbokoda market, Igbokoda, Ondo State, were determined. Collected shrimps were subjected to five different natural preservatives: *Azadirachta indica (*treatment I*), Zanthoxylum zanthoxyloides (II), Piper guineense* (III), *Afromomum melegueta* (*VI),* and Vegetable Oil (V). Proximate, mineral, Total Volatile Nitrogen (TVN) and Free Fatty Acid (FFA) were determined for each of the treatment after 45 and 90 days.

Sodium concentration levels dropped in samples preserved with I, II,V to 1,744.30, 825.65 and 3,750.81 (ppm) respectively, while it increased in samples preserved with III and IV*;* 3,623.18 and 2,086.75 (ppm) respectively. Potassium concentration reduced in all the preservatives, while phosphorous reduced in treatment I, II, IV and V with 49.09, 258.53, 43.41 and 583.05 (ppm) respectively, but increased in treatment III as 269.41 ppm. Calcium and magnesium decreased in all except in specimens with treatment III that increased with 23.900 and 3,500 ppm respectively. TVN of 22.40, 24.50, 22.40, 15.40 and 23.10 (mgN/100g) were recorded at day 45 and 26.60, 29.40, 23.80, 19.60 and 16.10 (mgN/100g) at the end of 90 days for treatment I,II,III,IV, and V respectively with treatment IV having the lowest value. .....

Except the moisture concentration, there was no significant difference ($p>0.5$) between the proximate composition; protien, ash, fat and NFE of control and treatments samples. Reduction of postharvest loses using natural preservatives in coastal communities will enhance food security.

HARNESSING MACHINE LEARNING TO ESTIMATE AQUAUCULTURE'S CONTRIBUTIONS TO THE ECONOMY OF SOUTHWEST BANGLADESH

Ben Belton*, Mohammad Mahfujul Haque, Hazrat Ali, Amir Pouyan Nejadhashemi, Ricardo Hernandez, Khondker Murshed-e-Jahan, Hannah Ferriby

*Michigan State University, East Lansing, MI, USA, and WorldFish, Penang, Malaysia
Email: b.belton@msu.edu

Aquaculture in Bangladesh has grown quickly over the past three decades, becoming a major contributor to the country's rural economy. National systems for collecting aquaculture statistics have not kept pace with these changes, so more accurate, up-to-date information is needed to inform policymakers. We used machine learning techniques to extract information from freely available satellite images and estimate the area of waterbodies used for aquaculture in seven districts in southern Bangladesh, one of country's most important aquaculture zones producing fish for domestic markets and crustaceans for export. We combined machine learning derived estimates of aquaculture farm area per district with data from statistically representative farm surveys to estimate farm size, productivity, and total output, economic value of production, on-farm employment generation by gender, and demand for formulated and non-formulated feeds. Machine learning estimates returned a total farm area similar to that reported in Department of Fisheries (DOF) statistics, but we estimate that production of crustaceans (shrimp + freshwater prawn production) is 31% lower than officially reported by DOF in 2020, while fish production and total aquaculture production (fish + crustaceans) are 41% and 27% higher, respectively. Aquaculture makes a massive contribution to food production, farmer incomes and employment in southern Bangladesh. We estimate that there were more than 500,000 farms in 2020, producing 787,000 t of aquatic food (89% fish and 11% crustaceans), with a mean yield of 3.1 t/ha. This production was worth a total $1.45 billion (farmgate value) and generated farm profits of $0.67 billion, after subtracting production costs (Table 1). These farms support 430,000 fulltime equivalent (FTE) jobs on-farm, of which 15% worked by women, and created demand for 759,000 t of feed, of which 30% comprised of formulated pelleted feeds. Our findings reveal great potential to combine remote sensing and machine learning techniques with representative surveys to estimate a range of statistics that are difficult to obtain otherwise, with potential to expand the approach to whole of Bangladesh and other countries.

Table 1. Estimates of selected aquaculture statistics in southern Bangladesh by district.

Estimate	Bagerhat	Barisal	Bhola	Gopalganj	Jashore	Khulna	Satkhira	All districts
Farm area ('000 ha)	73.6	6.2	11.1	5.8	37.8	72.2	72.1	278.8
No. of farms ('000)	66.8	17.2	29.7	19.1	183.3	130.0	101.8	546.9
Mean farm size (ha)	1.1	0.4	0.4	0.3	0.2	0.6	0.7	0.5
Mean yield (t/ha)	2.4	5.1	5.4	2.8	4.3	2.0	2.6	3.1
Aquatic food ('000 t)	177.4	31.6	60.4	15.9	163.9	147.6	190.1	787.1
Fish ('000 t)	148.9	31.6	60.4	15.6	160.0	120.6	163.6	700.7
Crustaceans ('000 t)	28.5	0	0	0.3	3.9	27	26.5	86.4
Farmgate value (Million USD)	394	39	106	20	220	335	341	1,454
Total farm profit (Million USD)	185	14	36	9	95	172	160	670

EVALUATION OF ANTARCTIC KRILL (*Euphasia superba*) MEAL SUPPLEMENTATION IN DIETS FOR OLIVE FLOUNDER (*Paralichthys olivaceus*)

Tibiabin Benitez-Santana*, Kasun Tharaka, Buddhi E. Gunathilaka, Min-Gi Kim, Chorong Lee, Jaehyeong Shin and Kyeong-Jun Lee

Aker BioMarine Antartic AS
Oksenøyvn 10. PO Box 496NO-1327 Lysaker, Norway
tibiabin.benitez-santana@akerbiomarine.com

The supplemental effect of Antarctic krill meal (KM) into a low fish meal (FM) diet was evaluated for olive flounder (*Paralichthys olivaceus*). A 56% FM-based diet was regarded as a high FM inclusion diet (HFM), and a low-FM diet (LFM) was prepared by replacing 50% FM from the HFM. Four other diets were prepared by supplementing 3%, 6%, 9% and 12% KM into the LFM diet gradually replacing soy protein concentrate and tankage meal (designated as KM3, KM6, KM9 and KM12 respectively). Quadruplicate groups of fish were fed one of the diets for 12 weeks. The growth performance and feed utilization efficiency were improved by the dietary KM supplementation. Digestibility of dietary protein and dry matter was increased by the KM3-9 diets. Haematocrit and haemoglobin were increased by KM supplementation. The innate immunity and antioxidant capacity assessed by Ig, antiprotease, lysozyme, GPx and SOD and the condition factor of fish were significantly increased by KM3-9 diets. Moreover, goblet cell counts, villi length and fillet yield of fish were significantly improved by all the KM-containing diets (KM3-12). A 25-day-long challenge test with the *Edwardsiella tarda* pathogen showed that the cumulative mortality was higher in fish fed the LFM diet than in fish fed the HFM or KM-supplemented diets. The results indicate that dietary KM supplementation in a LFM diet can increase growth performance and feed utilization efficiency, diet digestibility, intestinal development and functions, innate immunity and disease resistance of olive flounder. The recommended level of KM inclusion in a LFM diet seems to be 6.6% according to quadratic regression analysis.

NEXT GENERATION APPROACHES IN SUSTAINABLE AQUACULTURE AND ITS ENVIRONMENT

Subha Bhassu

[1]Animal Genetics and Genome Evolutionary Biology Lab, Institute of Biological Sciences, 50603, University Malaya Kuala Lumpur, Malaysia
[2]Center for Research in Biotechnology for Agriculture, 50603, Universiti Malaya, Kuala Lumpur, Malaysia
Email: subhabhassu@um.edu.my

In line with sustainable development goals, the combination of biological, chemistry and physics are fundamental science to be revisited in aquaculture. Fundamental science coupled with new technologies such as artificial intelligence, engineering and computational biology which are essential core components to ensure aquaculture growth in the ASEAN region. The multidisciplinary approach makes an important contribution to ensure safe and healthy food for the people. Thus, the innovation and disruptive technologies demonstrated in our study which will give solutions to the aquaculture growth and its environment. Food security and safety and its nutrition benefits with the aid of next generation approaches will pave way to ensure sustainable production in the years to come. In this paper, we will discuss case examples of how next generation approaches are used in biosafety and biosecurity area, host, pathogen and environment interactions and food security and safety. Innovation approaches discussed can be short to long term approaches in sustainable aquatic production.

INTRODUCING INNOVATIVE NUTRITION SOLUTIONS- READY TO COOK FISH FOR INDUSTRIAL WORKERS IN BANGLADESH

Bhattacharjee, Debasish; Shahin, Shaima Arzuman; Arafat, Yasir; Paz Mendez, Alvaro

d.bhattacharjee@cgiar.org
WorldFish Bangladesh
House # 335/A (Old) 42/A (New), Road # 114, Gulshan-2, Dhaka-1212, Bangladesh

Bangladesh is one of the largest contributor of the RMG products, usually engaged women (~70%) in the production activity. Due to their high workload and time bound engagement in the production, they does not got chance to prepare nutritious meals in their day-to-day life, which eventually adversely affected in their family nutrition. For improving the situation Apon Wellbeing with support from WorldFish Bangladesh, introduced semi-processed fish in their pre-installed Apon Fair Stores in the factory premises. Apart from offering fish, these stores also offered health support and information associated with intake of nutritious food also being promoted among the workers of this sector. Discounts and credit facilities on every products, especially on fish, is the part of social responsibilities of this innovative social business model.

Apon Wellbeing operates 24 stores in RMG factories in 3 geographic clusters, which host the regional supply chains and from those, nine stores were selected and capacitized to handle fish. To maintain a sustainable business model, match market prices and keep the price affordable to the lower income factory workers, Apon decided to purchase fish from local suppliers as purchasing fish from WorldFish's farmers was estimated to be higher. Twenty-five farmers were contracted to supply fish to the Apon target customers. With support from WorldFish, branding, marketing, sales promotional deals and offers were strategically implemented to attract factory workers to increase fish consumption. Customers at the RMG factories were regularly communicated via PA announcement, floor visits and promoted sales by enhancing branding and marketing.

Exceeding the target of 27 tons, Apon sold a total of 31 ton of fishes over the 12 months of operation leading to a sale of BDT 166M. Almost 63% of the total fish sold were Rohu fish while Prawns and Pangasius held large shares among the rest. The introduction of the fish in such stores also triggered the demand of other perishables and identified the new business channel for Apon, which created avenue of introduction of vegetables, eggs, fruits etc. Rice contributed 35% of the overall revenue in early 2021, where fish and other perishables contributed average 38% of the overall revenue in mid-2022 and data showed a positive progressive trend in the business margin for Apon. Customer penetration followed a month-to-month trend similar to fish sales – a rise in the first six months followed a dip due to the planned closure of cash supply to introduce ERP and then gradual increase over the later months. A positive trend was observed for fish and other perishables purchase, which eventually indicated the increase of nutritious food intake among the customers, especially for the women and their family members.

The rising demand for on-premise shops with quality nutrition products at affordable prices indicated the need for expansion of the model. Besides vertical growth, focus should be given on horizontal growth in terms of greater customer penetration. Continuous improvement of the supply chain facilities is needed to increase affordability. In addition, more strategic and innovative communication approach is needed to increase awareness among the customer groups.

THE STUDY OF GUT PROBIOTICS ON ASIAN STINGING CATFISH (*Heteropneustes fossilis*) AND NILE TILAPIA *Oreochromis niloticus*) IN BIOFLOC TECHNOLOGY

Amitesh Bhattacharyya*, Swagat Ghosh, Narayan Chandra Sahu and F. H. Rahaman

IRDM Faculty Center, Narendrapur Campus, Ramkrishna Mission Vivekananda Educational and Research Institute, Kolkata, West Bengal, India
*bhattacharyyaamitesh8@gmail.com

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal balance. Due to the presence of different bacterial species in the gut of the fish body, the bacterial species are the strong candidates of probiotics in the fish health. This study aimed to characterize the gut micro- biota of Asian stinging catfish (*Heteropneustes fossilis)* and Nile tilapia (*Oreochromis niloticus)* treated through biofloc technology. The microbial community from the gut of four individuals from each fish species was identified using 16S rRNA pyrosequencing. The gut microbial community differed significantly between fish species in terms of bacterial species diverseness. However, after the analysis of the gut from the fish body, the micro biome environment were dominated by the phylum *Fusobacteria,* specifically the species *Cetobacterium somerae*. The overwhelming dominance of the genus *Cetobacterium* in all species permits further investigation into its role in the fish gut micro-biota. This study provides the first characterization of the gut micro-biota of two economically significant fishes and establishes a baseline for future probiotic trials under biofloc system in India.

THE IMPACT/ADVANTAGES OF GOOD LIVE FOOD PRACTICES: SIMPLIFICATION & STANDARDIZATION OF LIVE FOOD PRODUCTION LEADS TO MORE EFFICIENT AND MORE CONSISTENT PRODUCTION

Rudi Bijnens [*1] and Geert Rombaut[2]

Benchmark Advanced Nutrition, INVE Aquaculture
Bangkok City
Thailand
r.bijnens@inveaquaculture.com

Science and experience have proven that success in fish & shrimp farming is primarily determined by the quality of the first life stages. Live food remains a fundamental aspect to ensure optimal survival and ensure predictable production of high-quality larvae and fry. Artemia is globally recognized as the most suitable live feed for shrimp and fish hatcheries. Thanks to its unique qualities, Artemia improves hatchery performance. When used right, it will result in high-quality and robust post-larvae and fry, ready for optimal growth in nursery and farm.

Reducing live feed in the hatchery protocols is not always the way to make more profit. On the opposite well-balanced protocols relying on a sufficient amount of Artemia and high-quality larval diets will help you to find the ideal balance between cost-effectiveness and quality. The inclusion level of Artemia sp. in daily feeding protocol in the hatchery affects positively the growth, survival, and robustness of the larvae. If the use of Artemia sp. in the larval protocols is optimized, substantial effects can be observed. In the end, the outcome will be stronger, and more robust larvae will be produced for the nursery & farms.

For more than 35 years we are working with Artemia and have invested significantly in innovation resulting in improved and well-performing Artemia products highly appreciated by the marine fish and crustacean hatcheries. Clever harvesting techniques based on the SEP-Art technology together with nutritional and health solutions have been developed to make the best possible use of the available Artemia in the hatcheries (read: get a maximum of high-quality nauplii out of the cysts).

The consistent availability and production of high-quality live food in the hatchery, produced via easy and efficient protocols and tools, relying on SEP-Art technology and on complementary microbial management solutions has contributed to a more predictable, more efficient & consistent production of high-quality larvae/juveniles. This approach has shown to have a positive trend on the hatchery, nursery and farm level.

COMMERCIAL-SCALE HATCHERY PRODUCTION OF TROPICAL ROCK LOBSTER – A NEW AQUACULTURE INDUSTRY

Jennifer Blair*, Scott Parkinson, John Breen, Sandra Infante-Villamil, Steven Gill, Quinn P. Fitzgibbon, Chris G. Carter, Andrew J. Trotter, Basseer M. Codabaccus, Greg G. Smith

Ornatas Pty Ltd.
Townsville Queensland 4818, Australia
Email: jenniferb@ornatas.com.au

The hatchery production of spiny lobsters has been a technical and biological challenge addressed through aquaculture research for decades. Hatchery technology to produce the tropical rock lobster *Panulirus ornatus* has been successfully developed by the Institute for Marine and Antarctic Studies, University of Tasmania based on over 20 years of science. The life cycle of *P. ornatus* has been closed, and this has enabled the emergence of a new aquaculture industry in northern Australia. The hatchery technology has now been transferred to Ornatas, to implement at a commercial scale.

Ornatas has invested over AUD20 million from 2019-2022 in hatchery and farm infrastructure, operations and research, with a further AUD14 million committed for infrastructure development by 2025. The company plans to produce 1,000 tonnes of premium quality export product, with a value of over AUD140 million, and employ 120 people by 2032. Construction of the purpose-built hatchery facility began in 2020, and now thousands of tropical rock lobster puerulus and juveniles are routinely produced in each batch. The short-term hatchery production target is 100,000 puerulus per annum by 2025, with staged increases to 2032.

Despite hatchery production success, ongoing technical challenges include juvenile cannibalism and development of a commercially manufactured feed for grow-out. These topics form core components of R&D through the Australian Research Council, 'Industrial Transformation Research Hub – Sustainable Onshore Lobster Aquaculture'. In addition, an improved understanding of grow-out production systems, biosecurity requirements, feeding strategies and growth performance are essential to produce market-ready, premium lobster product. This is the focus of research through the 'Pioneering Tropical Rock Lobster Raft Grow-out for Northern Australia' project supported by the Cooperative research Centre for Developing Northern Australia. Ensuring the growth of this new aquaculture industry requires strong research collaboration, effective translation of science, critical infrastructure, strategic business partnerships, committed investment, sustainable practices, and capacity building of people.

Fig. 1. Hatchery-produced juvenile *Panulirus ornatus*.

MATERIAL SCIENCE INNOVATIONS FOR AQUACULTURE: ANTI THERMAL FILMS AND ANTI BACTERIA COATINGS

Freddy Boey

National University of Singapore
21 Lower Kent Ridge Road, Singapore 119077
Email: Freddy_boey@nus.edu.sg

Better anti thermal and Anti-Bacterial management will provide for more productive outcomes for Aquaculture and Agriculture. This presentation will introduce innovations based on the use of novel materials that would help to provide anti thermal and anti-bacterial coatings and films that can be used for both Aquaculture and Agriculture farming.

SPAWNING AND LARVICULTURE OF THE STREAMLINED SPINEFOOT, *Siganus argenteus* (QUOY & GAIMARD, 1825) IN THE REPUBLIC OF THE MARSHALL ISLANDS

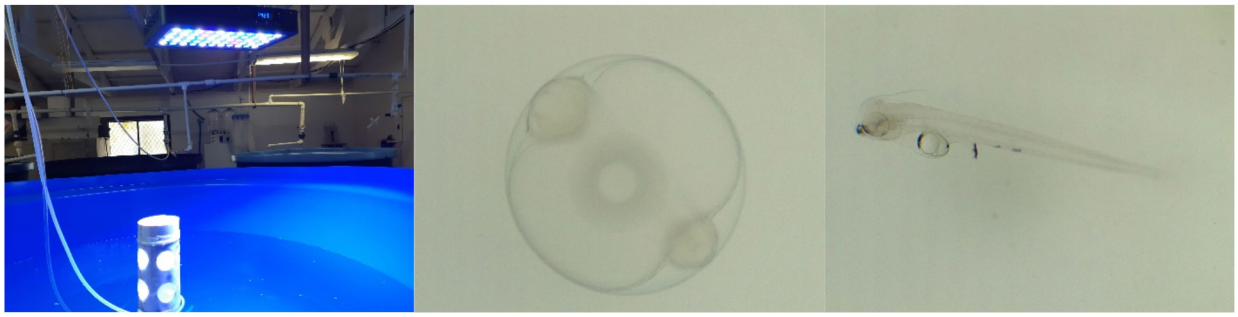
Sergio Nestor Bolasina

Arrak Campus Aquaculture Laboratory
College of the Marshall Islands
Land Grant Program (NIFA, USDA)
sbolasina@cmi.edu

The streamlined spinefoot, *Siganus argenteus* inhabits tropical coastal and inner reef slopes and lagoons, ranging from East Africa to French Polynesia. They are important components of the herbivorous reef fauna. They spawn pelagic eggs and the reproductive cycle is synchronized with lunar periods. These characteristics jointly with their economic and cultural value in the region make it a potential species for aquaculture.

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

Several attempts on the larval rearing of this species were made. Eggs obtained from natural spawning after full moon were incubated in 500 liter plastic tanks with green water. Different feeding schedules, illumination and temperatures were tested. Results showed that the seed production in captivity is feasible, the main factor to be considered is the size of the live prey at first feeding because of the small size of the mouth in the larvae of this species.



IMPLEMENTATION OF TECHNICAL SOLUTIONS THAT SUPPORT SUITABLE CONDITIONS FOR THE *Artemia* POPULATION ON GREAT SALT LAKE IN THE FACE OF DECLINING LAKE LEVELS

Thomas Bosteels and Phil D. Brown

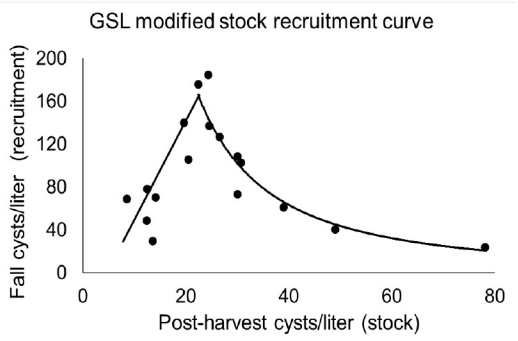
Great Salt Lake Brine Shrimp Cooperative, Inc.
1750 West 2450 South
Ogden, Utah 84401

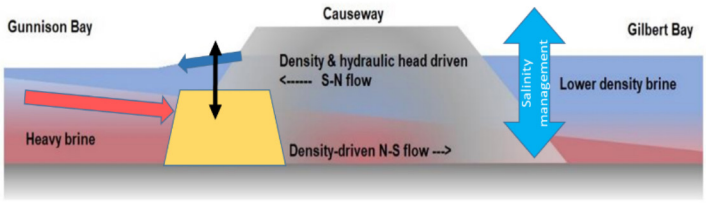
Great Salt Lake (GSL) is a hypersaline terminal lake located in northern Utah, USA. Multiple years of research on GSL strongly suggest that harvest management, nutrients and salinity are the primary drivers of the *Artemia* population on the lake. We review the implementation of technical solutions that combine to optimize *Artemia* harvest management, nutrient inflows, and salinities to support a healthy *Artemia* population in GSL.

Management of the *Artemia* cyst harvest began in 1997, and is based upon a modified Ricker recruitment curve in which leaving a post-harvest escapement stock of 21 cysts per liter results in optimal cyst densities the next autumn (Figure 1). This criteria has been re-evaluated annually, incorporating the latest year's data. Harvest results spanning the last 3 decades are presented to demonstrate the results of this adaptive harvest management model.

Gilbert bay, the south arm of GSL, relies on natural and anthropogenic sources of nutrients to replenish approximately 10% of dissolved nitrogen annually. We review how nutrients and water management in the GSL basin are intertwined and present novel solutions to nutrient management that support continued nutrient inflows into the lake such as the implementation of numeric nutrient criteria with associated ecological responses and redirected inflow of anthropogenic effluents.

Historic data and literature studies suggest the optimal salinity range for GSL *Artemia* is between 120 to 160 ppt. A causeway dividing the lake into two separate bays with different salinities and a breach with an adaptive management berm (Figure 2) offers a unique salinity management solution for Gilbert Bay. We present the principles of bidirectional flow between the bays of Great Salt Lake and how the adaptive management berm can be used to regulate salinity under a variety of environmental conditions.





ENERGY USE IN SHRIMP AQUACULTURE

Claude E. Boyd

Professor Emeritus
School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University
Auburn, AL 36849
boydce1@auburn.edu

The main direct energy use in shrimp aquaculture is for mechanical aeration. At shrimp farms where daily water exchange is practiced, considerable energy also is used to pump water. The primary embodied energy use is for feed, and feeds with a high fish meal percentage have an especially large embodied energy content. Energy use for edible shrimp meat (or shrimp protein) is greater than for equal portions of chicken, swine, or beef meat (or protein). Energy use in shrimp production could be substantially reduced by adoption of better mechanical aerators in Asia, and especially by replacement of the "long-arm" aerators in common use. Restriction in water exchange other than for prevention of excessive salinity, use of low-fish-meal content feeds, and careful feeding practices to maintain a low FCR also can lower energy use at shrimp farms.

COMPARISON OF PROTEIN PRODUCTION AND PROTEIN QUALITY AMONG TERRESTRIAL AND AQUATIC ANIMAL PROTEIN SOURCES

Claude E. Boyd* and Aaron A. McNevin

Professor Emeritus
School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University
Auburn, AL 36849
boydce1@auburn.edu

Fisheries and aquaculture combined produce about 19% of animal-derived protein for human consumption globally. Aquaculture lags slightly behind the capture fisheries in the amount of edible protein produced, and it has not yet exceeded edible protein production by beef. The order of amounts of protein produced is the same for capture fisheries and aquaculture: fish > crustaceans > molluscs. Based on essential amino acid composition and digestibility, fish and crustacean protein are similar in quality to other animal-derived protein. Molluscan protein is somewhat inferior in quality to other protein sources. Resource use for a quantity of protein needed to contain the daily, essential amino acid requirements for the average human adult is compared for the different types of animal-derived proteins.

505

COMPARISON OF RESOURCE USE FOR FARMED SHRIMP IN ECUADOR, INDIA, INDONESIA, THAILAND, AND VIETNAM

Claude E. Boyd*, Robert T. Davis, and Aaron A. McNevin

School of Fisheries, Aquaculture, and Aquatic Sciences
Auburn University
Auburn, AL 36849
boydce1@auburn.edu

Land use for whiteleg shrimp *Litopenaeus vannamei* production differed slightly between Indonesia (0.37 ha/t shrimp) and the other four, major shrimp exporting countries of Ecuador, India, Thailand, and Vietnam (0.42–0.46) ha/t shrimp). Total water use was greater in Ecuador (76,800 m^3/t) and Indonesia (55,000 m^3/t) than in the other three countries (14,000 m^3/t–45,500 m^3/t), but most water was saline. Freshwater use was mainly embodied in feed, did not differ among countries, and averaged 6.3% of total water use. Energy use ranged from 56.0 GJ/t (Ecuador) to 98.8 GJ/t (Thailand). All Asian countries had energy use above 75 GJ/t. Wild fish use for fishmeal and fish oil in feeds was greatest in Ecuador (0.891 t/t) and similar in Asian countries (0.612–0.670 t/t. Compared to *L. vannamei*, black tiger shrimp *Penaeus monodon* farming required more land, a greater amount of water, but less energy per tonne of shrimp.

Meat is considered important as a protein source in human diets. In terms of resource use per tonne of crude protein, land use for shrimp protein is similar to that of broiler chicken protein but less than for pig and beef cattle protein. Farmed shrimp protein production uses considerable brackish or sea water, but freshwater use is less than that required for the terrestrial proteins and mainly embodied in feed. Because of the high energy requirement for maintaining dissolved oxygen concentration in ponds, energy use is greater for shrimp protein than for the three terrestrial sources. Farmed shrimp protein uses much more wild fish (for fishmeal and fish oil in feeds) than do the terrestrial sources.

VARIABLES ASSOCIATED WITH A HIGH SURVIVAL RATE IN A CENTRAL PHILIPPINES SHRIMP FARM CULTURE

Neil Arvin Bretaña*, Vishwa Vijaysheel, Masum Patel, Yee Ting Chung, Mary Ann Solis, Neslly Marie Bretaña

Neil.Bretana@unisa.edu.au
University of South Australia, Adelaide, Australia

By 2030, the number of people experiencing food insecurity is expected to double. With this, the contribution of aquaculture to global aquatic food production is expected to increase 5-fold. Growth in the sector invariably affects the need to efficiently manage resources to maximize productivity. Moreover, issues such as climate change and emerging diseases contributes to the challenges in aquatic food farming. Therefore, it is essential that innovative technologies and solutions are leveraged to ensure a healthy yield outcome.

In this study, we analysed industry data consisting of 3 harvest cycles from 9 P. vannamei shrimp ponds in the Philippines. The dataset includes physio-chemical properties, feed and supplement data, and water management input data. The survival rate, indicating the health outcome of the shrimp culture at the end of each harvest cycle, was taken as the target variable for the study. The survival rate threshold was set to 80%. Statistical analysis was performed to identify important variables related to the yield outcome using statistical methods: Boruta and Random Forest. The resulting variables were validated using Pearson's correlation. All statistical tests were implemented using Python.

From the feed and supplement variables, we identified feed data labeled V3S, PVO3, V3P, V2, PVO4, PVO1, and TOP_S as significant variables for a healthy yield. From the set of physio-chemical property variables, dissolved oxygen, PH level, depth, temperature, and salinity were revealed to be important parameters. From the set of water management input variables, Ammonia and Magnesium levels were revealed to be significant parameters for farm yield. These results validate the importance of specific parameters already used in best practices in the industry. Moreover, it reveals additional variables, such as specific feed and supplement types that can inform industry practices.

Table 1. Importance values of variables associated with a high survival rate.

Variable	Boruta (Mean importance)	Random forest (Mean decrease accuracy)
V3S	33.09	50.04
PVO3	3.53	5.12
V3P	11.29	13.24
V2	6.03	5.35
PVO4	15.12	16.76
PVO1	3.37	3.92
TOP_S	16.79	23.42
DO	18.78	27.09
PH	11.44	14.10
Depth	8.62	14.33
Temperature	4.94	9.71
Salinity	15.11	19.37
Ammonia	7.78	10.57
Magnesium	5.15	4.05

MANAGING SALINITY FOR GREAT SALT LAKE *ARTEMIA* DURING DROUGHT: AN ADAPTIVE MANAGEMENT BERM PROVIDES A UNIQUE AND POWERFUL TOOL

Phil D. Brown*, Thomas Bosteels, Brad T. Marden

Great Salt Lake Brine Shrimp Cooperative, Inc.
Ogden, UT 84401
pbrown@gsla.us

Gilbert Bay, the expansive *Artemia*-producing arm of Great Salt Lake, is currently at a low stand due to drought and water use in the basin. However, a recent breach in the solid fill causeway bisecting the lake provides an unprecedented tool for managing the salinity of Gilbert Bay in the face of reduced lake volume. The causeway separates Gilbert Bay, which receives all three inflowing rivers, from Gunnison Bay, which stores a substantial portion of GSL salt away from the *Artemia*-producing waters of Gilbert. A 55-meter breach in the causeway constructed in 2017 included an adaptive management berm which can be modified to increase or reduce the flow of water and salt between the bays. Through salinity data collected from 2010-2021, we demonstrate the previously unobserved mass movement of salt through this causeway opening, observe an important changing relationship between salinity and surface elevation in Gilbert Bay, and present berm management strategies for keeping salinities within an acceptable range for *Artemia* at a variety of lake elevations.

In the 5 years preceding the breach, the relationship between Gilbert Bay salinity and elevation was linear and consistent with the annual concentration and dilution of a stable salt load. This changed in 2017, when high runoff coupled with the opening of the breach to force nearly 17% of the Gilbert Bay salt load into Gunnison Bay. The salinity-elevation relationship was lowered, resulting in more dilute salinities. The salt load of Gilbert Bay began increasing measurably again in 2020, demonstrating a return flow of Gunnison Bay heavy brine through the opening. In response, and under advisement of a scientific panel, management agencies modified the berm to restrict the return flow with the goal of preserving salinities conducive to the *Artemia* population. As of September 2022, return flow of heavy brine has reportedly been almost entirely halted.

This multiyear bidirectional transfer of salt mass across the causeway illustrates remarkable potential for managing salinity for *Artemia* during periods of limited river inflows in the near and long term. Spring runoff can export salt from Gilbert Bay to reduce salinities in individual years, with the berm utilized to prevent return flow of heavy brine. In the long term, existing hydrologic models can be refined to provide berm geometries that optimize salinity across multiple lake elevation scenarios.

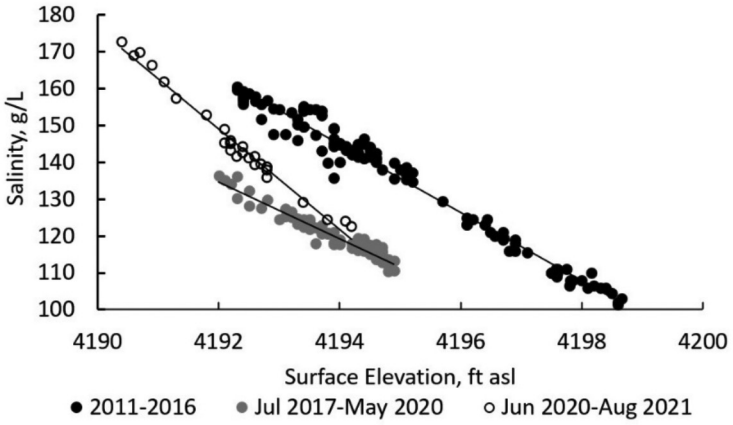


Figure 1. Changing salinity-elevation relationships in Gilbert Bay before and after the 2017 causeway opening demonstrating mass transfer of salt between the bays through the opening

GENOME EDITING AND THE FUTURE OF GENETIC IMPROVEMENT IN SHRIMP

John T. Buchanan*, Klara I. Verbyla, Debbie A. Plouffe, and Alan Tinch

The Center for Aquaculture Technologies
8445 Camino Santa Fe. Suite 104. San Diego, CA, 92121. USA
jbuchanan@aquatechcenter.com

Harnessing the power of genomics is forcing a rethinking of aquaculture breeding strategies. Successful breeding programs will always be built on the careful selection of the next generation of broodstock, detailed record keeping, and accurate collection of phenotypic data. Genomics allows this base of phenotype and selection to be enhanced, and ultimately accelerated to increase genetic gain per generation. This is currently done in finfish and shrimp through the use of Genomic Selection. However, another exciting technology is on the horizon that will fundamentally change how we deliver genetic improvement. This technology is Genome Editing.

Genome Editing is a technology that can thought of as "precision breeding". It will be an important tool in the future toolbox for genetic improvement in shrimp. The current state of the art in genetic engineering in aquaculture is impressive and improving. The basic concept is that enzymatic tools (such as CRISPR technologies) can be used to create variants in specific DNA sequences that create a desired phenotype (such as rapid growth, all-female, or disease resistance). The technique does not involve adding new DNA, so is not transgenic and does not create a GMO. It simply involves understanding the genetic variant that is needed for a trait and using natural processes to create that variant, rather than sorting through many thousands of broodstock and many generations to achieve the same effect.

The power of genomic research is that we are beginning to understand the exact genes involved in performance traits, and how variation in those genes leads to improved performance. Harnessing the power of Genome Editing allows us to transfer this knowledge to application in commercial breeding programs for heritable, quantum advances in genetic improvement.

This presentation will provide background on how genome editing works, an update on regulation, and how this tool may be used to improve shrimp aquaculture genetics in the near future.

GENOME EDITING TO PRODUCE MONOSEX AND STERILE FISH FOR AQUACULTURE

John T. Buchanan, Takeshi Umazume, Alan Tinch, and Xavier Lauth

Center for Aquaculture Technologies
8445 Camino Santa Fe Suite 104, San Diego, California, USA
jbuchanan@aquatechcenter.com

The ability to produce sterile progeny from broodstock for aquaculture has significant benefits to productivity and environmental sustainability. In addition, to responsibly introduce fish which harness the power of genome editing into commercial production systems, the farmed fish should be sterile. We describe the development of a strategy to generate, breed and mass-produce infertile fish. Our solutions rely on precise genetic modifications to create broodstock lines that can be incorporated into breeding programs. These approaches have been validated in tilapia but are transferrable to multiple species of fish. We expect that adoption of these technologies will result in broad economic and environmental benefits for aquaculture.

Our strategy for mass producing sterile fish is designed to produce monosex, sterile populations. In addition to the benefit of sterility, this allows the benefit of sexually dimorphic performance traits. We investigated gene mutations in two evolutionarily conserved pathways, one governing sex differentiation and the other sexual competency. We created edits in genes necessary for spermiogenesis and steroid hormone synthesis causing male sterility and masculinization, respectively. Double gene edit combinations for these genes produced all-male sterile populations. Likewise, we created variants in genes whose inactivation disrupted oogenesis. We further disrupted genes causing genetic males to sex reverse into females. Double gene edit combinations for these genes produced all-female, sterile populations.

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

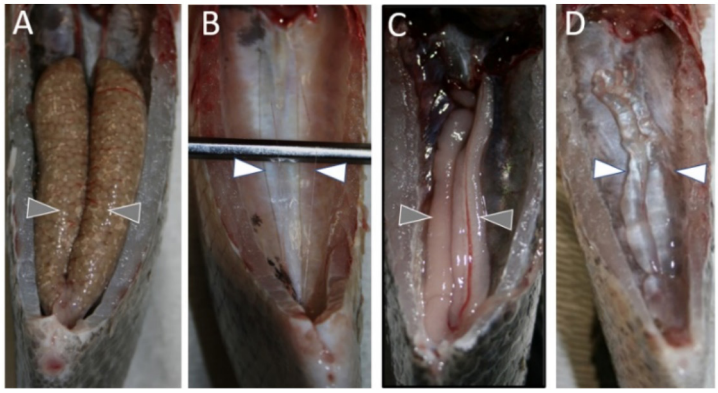


Fig 1 A-D. Dissected gonads of fertile and sterile Nile tilapia. Female (B) and male (D) with genome edited changes show string-like ovaries and translucid testes devoid of oocytes and spermatozoa, respectively. Age matched control female (A) and male (C) display mature gonads. Gray arrow heads point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

COLLABORATIVELY DESIGNED GENOMIC TOOLS MAXIMIZE BOTH GENETIC GAIN AND ECONOMIC EFFICIENCY

John. T. Buchanan*, Klara I Verbyla, Adriana Artiles, and R. G. Tait

Center for Aquaculture Technologies
8395 Camino Santa Fe, Suite E, San Diego, CA, 92129, United States
jbuchanan@aquatechcenter.com

Genomics can deliver great benefits to agricultural breeding programs, including efficient management of diversity and inbreeding, accurate parentage assignment, optimal mating designs, improved breeding value prediction, selection decisions, and breeding strategies. Using the appropriate platform for the population of interest is critical. It is often expected that optimal results require a customized tool with a higher level of initial investment and larger ongoing costs. However, it is possible to keep costs reasonable with optimal outcomes through the creation or use of a collaboratively designed universal genotyping platform.

Collaborative genotyping platform are designed using diverse populations to ensure the core market set has broad utility alongside markers that capture specific population characteristics. Many industry parties can benefit through using such platforms, creating a sample volume to keep costs reasonable and enabling results and outcomes that are easily compared and evaluated. As the platform is updated and improved, the benefit flows to all users.

A successful example of this approach in the livestock domain is widely used Illumina Bovine BeadChip arrays which were collaboratively developed with partners across the USA, Europe, and Australia. The platforms support many genomic applications, across both the dairy and beef industries, where widespread use create high demand keeping the price per sample low. More recent extensions of this concept have been deployed as GeneSeek Genomic Profiler arrays which leverage the continuously developing knowledge base about the genomic structure of a species.

This approach is also proving extremely beneficial for aquaculture species, as exemplified by the creation of a collaborative genotyping platform for *L. vannamei* shrimp designed using samples from eleven populations. This poster will present the design of the array, validating the array's results, how to achieve maximal benefit from the array, and the economic impacts of creating and using such genotyping platforms for aquaculture species in general.

MANIPULATING BACTERIAL COMMUNITIES IN CARP POLYCULTURE – THE NEXT FRONTIER FOR INTENSIFICATION?

Bui Ngoc Minh Ngan*, Jasmine Heyse, Jérôme Delamare-Deboutteville, Ruben Props, Colin Shelley

WorldFish
Jalan Batu Maung, Batu Maung
Penang, Malaysia
N.bui@worldfishcenter.org

Kytos BV
Technologiepark-Zwijnaarde 82
9052 Ghent, Belgium

Carp polyculture is the most widely practiced fish production system in the world that makes use of the diverse trophic and spatial niches of a pond to maximize fish output per unit space. In Bangladesh, with over 1.1 million tons of annual carp production (2019-20), WorldFish has supported hundreds of thousands of farmers in improving their production over several decades. While microbiome management has been hailed as the next generation of advice to farmers to improve productivity and minimize disease incidence, there exists a large knowledge gap to connect the sparse scientific data with practical farm implementation. WorldFish commissioned a review of the current status of carp polyculture microbiomes from contemporary scientific, grey literature and current best management practices.

Here we present the current state-of-the-art knowledge of carp microbiomes and highlight the knowledge gaps and opportunities for future microbiome management. Research on microbial communities in carp polyculture is still in its early stages, with most studies performing exploratory analyses of the microbial assemblages of fish organs (skin, gill, gut) and their rearing environments. As a result, disentangling interactions and identifying keystone species for specific functions in microbial communities has proven difficult because of the complex structure of these communities (viruses, bacteria, microalgae, fungi, etc.), especially when environmental impacts on population dynamics and metabolic pathways are taken into consideration. The functions of these microorganisms, as well as correlations and causation in relation to enhancing carp production, remain largely unknown. Despite industry awareness, little work has been undertaken to determine the effect of input factors such as substrate, feeds, fertilizers, sanitizers, and microbial products on carp polyculture microbiomes and how their manipulation, together with environmental parameters, can be better managed and used productively.

Technological issues, in addition to the sparse scientific data, have also hindered the research and development of microbiome management strategies. To date, most studies of carp microbiomes have relied on semi-quantitative molecular approaches, such as 16S rRNA gene amplicon sequencing. However, we argue that combining this technology with alternative and quantitative methods will be crucial to understanding and steering the compositional community dynamics in aquaculture ecosystems. Such integrated approaches, utilizing new tools such as flow cytometry (e.g. Kytos) will facilitate a far greater understanding of the microbial ecology of carp polyculture systems. Future research should be organized in a holistic way to document the composition, diversity, functional roles, and potential manipulation of carp polyculture microbiomes. This holistically curated database will lay the foundations on which novel microbiome management advice will be based and through which resilient and productive carp polyculture systems can be achieved.

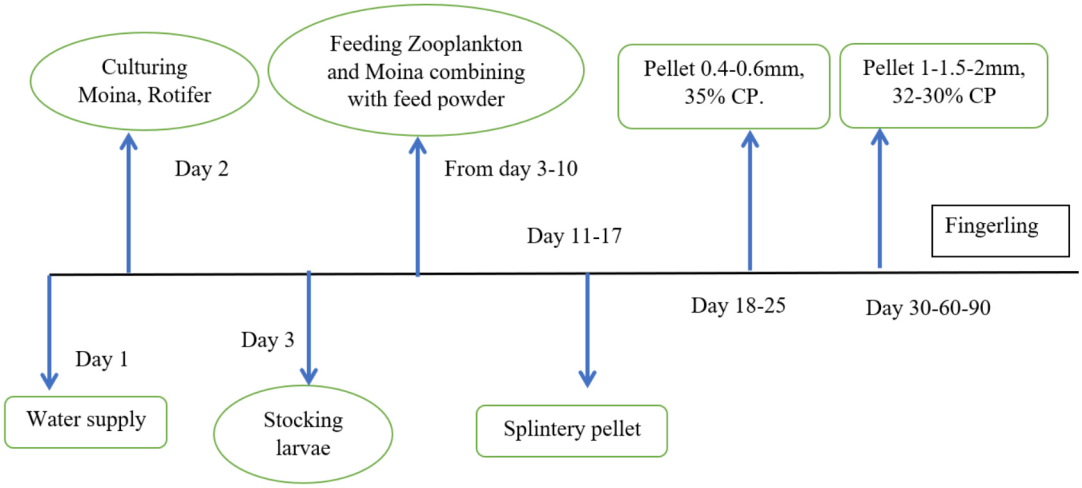
SEED PRODUCTION AND TECHNICAL TRANSFER OF SNAKEHEAD *Channa striata* TO CAMBODIA

Bui Minh Tam, Tran Thi Thanh Hien, Pham Minh Duc, Pham Thanh Liem

College of Aquaculture and Fisheries, Can Tho University, Can Tho city, Viet Nam
Email: bmtam@ctu.edu.vn

Snakehead feeding with pellet are cultured widely in Vietnam, especially in the Mekong Delta. Snakehead production increases from 5,300 ton in 2002 to 40,000 ton in 2009. In 2012 and 2013, total productions of Tra Vinh, An Giang and Dong Thap provinces are 35,818 and 36,140 tones, respectively. Snakehead are carnivorous but can be weaned onto pelleted or extruded feeds. They are air-breathers and can tolerate very low oxygen. Optimal temperatures are 20-35°C, optimal pH is 6-8 and optimal salinity is 0-6 ppt. It matures at 10-12 months old and naturally spawns during the rainy season. They produce large eggs with oil-droplets and float to the surface. The eggs hatch one day after spawning at 26-28°C. The larvae move belly-up against the surface but swim normally after 3 days when the yolk sac is absorbed. The parents take care and protect the larvae.

Under CAST-CE SAIN-CTU project, technics of seed production were tranferred to RUA (Cambodia) hatchery. Snakehead brooders were injected with HCG and they spawned naturally. Fry are fed 3 times/day (at 9:00, 14:00, and 18:00) at satiation (about 10-15% body weight) with following regime.



EFFECT OF MEDICINAL PLANT EXTRACTS ON IMMUNE RESPONSES AND BACTERIAL RESISTANCE OF SNAKEHEAD FISH (*Channa striata*)

Bui Thi Bich Hang[1*], Truong Quynh Nhu[1], Do Thi Thanh Huong[1], Nguyen Thanh Phuong[1] and Patrick Kestemont[2]

[1] College of Aquaculture and Fisheries, Can Tho University, Viet Nam
[2] Research Unit in Environmental and Evolutionary Biology, Institute of Life, Earth & Environment University of Namur, Belgium
Email: btbhang@ctu.edu.vn

The study was conducted to evaluate the effect of medicinal plant extracts (*Psidium guajava, Eclipta alba, Moringa oleifera*) on modulating growth performances, immune responses as well as disease resistance in snakehead fish (*Channa striata*) against *Aeromonas schubertii*. The experiment was randomly designed with 10 treatments including 0% (control treatment), 0.01%, 0.1% and 1% *P.guajava*; 0.01%, 0.1% and 1% *E. alba*; 0.01%, 0.1% and 1% *M. oleifera* during 4 weeks and triplicate for each treatment. Fish blood was collected after 2 and 4 weeks (W2, W4) of plant extracts supplementation for hematological analysis, lysozyme, complement activities and total Ig. At the end of experiment, fish were challenged with *A. schubertii* to evaluate the therapeutic efficacy of medicinal plant extracts. The result showed that the hematological parameters were significantly enhanced following supplemented plant extract diets in dose dependent manners. Specifically, 1% *P. guajava,* 0.1 and 1% *E. prostrata* and *M. oleifera* could statistically increased the total red blood cells (RBC) and white blood cells (WBC) compared to control. The number of lymphocytes were considerably increased in fish fed 1% *E. prostrata*, 1% *M. oleifera*. Most of plant extract treatments started to significantly enhance the neutrophil and monocytes compared to control from W2. In addition, the lysozyme activity, complement activity and total of Ig were significantly enhanced in fish fed diets supplemented with 0.1, 1% *P. guajava* and 1% *M. oleifera* in W2 and W4. Moreover, supplemented plant extracts diets could improve the mortality in snakehead fish against *A. schubertii* infection. Especially, treatment supplemented with 1% *P. guajava*, 1% *E. prostrata,* 0.1 and 1% *M. oleifera* significantly reduced the mortality in snakehead fish after challenged with *A. schubertii*. These results suggested that plant extract diets could play a positive effect in immune response as well as disease resistance in snakehead fish against bacterial infection.

EFFECT OF PREBIOTIC ON IMMUNE PARAMETERS AND DISEASE RESISTANCE OF STRIPED CATFISH (*Pangasianodon hypophthalmus*)

Bui Thi Bich Hang* and Nguyen Thanh Phuong

College of Aquaculture and Fisheries, Can Tho University, Viet Nam
Email: btbhang@ctu.edu.vn

This study aims to evaluate the effects of prebiotics (inulin and fructooligosaccharides (FOS)) on the growth performance and immune response of stripped catfish fingerling (*Pangasianodon hypophthalmus*). Experiment was randomly designed with 5 treatments including control; 0.5% inulin; 1% inulin; 0.5% FOS and 1% FOS, triplications for each treatment. The experiment was carried out for 28 days. After 21 and 28 days, several immune parameters including the total erythrocyte cells, total leucocyte cells, number of each type of leucocytes and lysozyme activity were analyzed for evaluation of fish immune response. After 28 days, fish was weighted for estimation of growth performance and challenged with *Edwardsiella ictaluri* for evaluation of bacterial resistance. Fish mortality was recorded every day for 14 days. After 3 days of infection with *E. ictaluri*, 3 fish/tank were collected for immune assay. Results showed that hematological parameters and lysozyme activity of inulin and FOS supplemented treatments were higher than those of control treatment after 28 days. Treatment of 1% inulin showed that the total number of leukocyte, monocyte, neutrophil, lymphocyte, thrombocyte and lysozyme activity were higher than those of control treatment after 28 days. The lowest mortality after challenge with *E. ictaluri* was also addressed in treatment 1% inulin and the difference was statistically significant compared to the control treatment ($p<0.05$). Base on these results, supplemetation of 1% inulin enhanced immune response of striped catfish and could protect the fish from bacterial damage.

BENEFITS OF KRILL MEAL INCLUSION IN SHRIMP DIETS

Lena Burri*, Ambasankar Kondusamy, Orapint Jintasataporn and Alberto J.P. Nunes

Aker Biomarine Antarctic AS, Oksenøyveien 10, 1366 Lysaker, Norway
lena.burri@akerbiomarine.com

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

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

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

MODULAR INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS FOR MARINE AND BRACKISH WATER SPECIES – BREAKTHROUGHTS OF THE AQUAMMIN PROJECT

Ricardo Calado*, and Ana I. Lillebø

Department of Biology & CESAM & ECOMARE
University of Aveiro
Campus Universitário de Santiago
3810-193 Aveiro, Portugal
rjcalado@ua.pt

Project AquaMMIn successfully designed and implemented modular integrated multitrophic aquaculture systems (IMTA) for the production of sea bass (*Dicentrarchus labrax*), using polychaete assisted sandfilters with ragworms (*Hediste diversicolor*) to incorporate particulate nutrients, and raft aquaponics with sea asparagus (*Salicornia ramosissima*) and biofilters with sea lettuce (*Ulva* sp.) to incorporate dissolved inorganic nutrients (namely P and N). These modular systems are equipped with *in situ* and *ex situ* real-time monitoring systems, allowing their and correction of physico-chemical parameters through automation. These systems operate on a zero water exchange regime, reusing 100% of the water regime.

This study was supported by project "AquaMMIn - Development and validation of a modular integrated multitrophic aquaculture system for marine and brackish water species" (MAR-02.01.01-FEAMP-0038) co-funded by Portugal 2020 and the European Union through Mar2020, the Operational Programme (OP) for the European Maritime and Fisheries Fund (EMFF) in Portugal.

MICROBIAL BIOMARKER DETECTION IN SHRIMP LARVAL REARING WATER AS PUTATIVE BIOSURVEILLANCE TOOL FOR SHRIMP AQUACULTURE IN NEW-CALEDONIA

Nolwenn Callac*, Carolane Giraud, Nelly Wabete, Dominique Pham, Viviane Boulo

Ifremer, IRD, Université de la Nouvelle-Calédonie
Université de La Réunion, CNRS, UMR 9220 ENTROPIE
101 Promenade Roger Laroque, 98897, Nouméa, New Caledonia
Nolwenn.callac@ifremer.fr

Aquacultured animals are reared in water hosting various microorganisms with which they are in close interactions as these microorganisms can be involved in the animal development, physiology or health during their whole lifecycle. Consequently, it is important to understand the relationships between the rearing water microbiota and the larval stage and health status; notably in New Caledonia, where the rearing of *Penaeus stylirostris* is one of the main economic activities. Unfortunately, this activity is strongly impacted by larval mortalities that impair the shrimp production and enhance major economic loss for the farmers and the Territory. This phenomenon, occurring at any larval stage since the past decade, is poorly understood. This study aimed to detect microbial biomarkers in the rearing water, specific of a given larval stage and health, to establish microbial proxies to monitor the rearing ecosystems and ultimately to help microbial management in the hatcheries.

We studied the daily microbial compositions of the water storage as well as those of the rearing water where both healthy larvae with a high survival rate, and unhealthy larvae with a high mortality rate, occurred during the same rearing cycle. We used the sequencing of the V4 region of the 16S rRNA gene coupled to zootechnical parameters and statistical analysis, to explore if any microbial lineages and biomarkers could be linked to a certain larval stage and mortality rate.

We highlighted that the active microbiota of the rearing water was highly dynamic whatever the larval survival rate, with various active lineages associated with a given larval stage and survival rate (Figure 1A). When compared to the microbiota of the storage waters, it appeared that several of these specific lineages were also detected in the natural seawater, highlighting the great role of the seawater microbiota (Figure 1B). The biomarker exploration allowed to spot out several microbial proxies specific of healthy or unhealthy larvae, that could be used as practice tools to test the natural seawater before the rearing and then during the first days of the larval rearing to monitor the larval health. *In fine*, these biomarkers might be useful to manage the rearing water microbiota and select valuable microorganisms for the larvae.

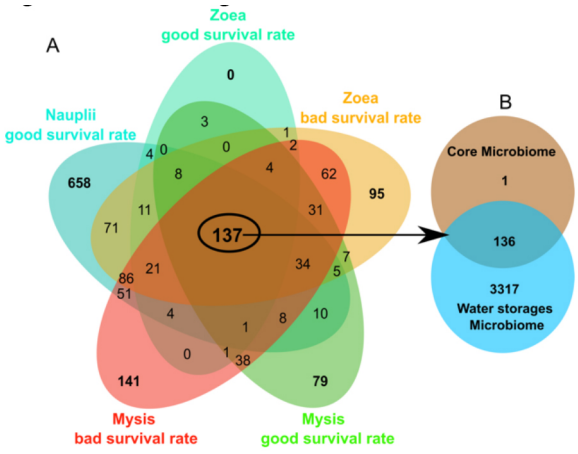


Figure 1 : **A-**Venn diagram of shared and specific taxa from rearing water hosting the nauplii, zoea with a good survival rate, zoea with a bad survival rate, mysis with a good survival rate and mysis with a bad survival rate. **B-**Venn diagram of shared and specific taxa from core rearing water and the microbiota of the storage water

RELATIONSHIP BETWEEN SWIM BLADDER INFLATION AND SPINAL DEFORMITIES IN GIANT GROUPER *Epinephelus lanceolatus*

Maximiliano M. Canepa*, Anjanette Berding, Ryan Rossi, Matthew Reason, Richard Knuckey

The Company One
Cairns, QLD 4878, Australia
mcanepa@thecompanyone.com

Giant Grouper (GG) is a valuable aquaculture species in Southeast Asia. Its farming is challenging, and the occurrence of spinal deformities is one of the bottlenecks found in aquaculture production. This study investigated the development of the swim bladder (SB) in GG and a possible causative relationship between failing to inflate the swim bladder and spinal deviations in juvenile fish. The ontogeny of the swim bladder in GG revealed that the organ is connected through the pneumatic duct to the digestive tube from day 4 to day 7 post hatching (DPH). Repeated observations of larvae over this period showed that GG SB inflation period commences on day 5 DPH and lasted until the end of day 7 DPH. However, a proportion of day 7 DPH larvae failed to inflate their SB showing histological abnormalities in the swim bladder. Later assessment of skeletal anomalies in post metamorphic fish (42 DPH) revealed a relationship between the lack of an inflated swim bladder and anomalies in the caudal region; more specifically, the urostyle ($p<0.05$). Subsequently, a morphological assessment of juvenile fish showed a direct relationship between the lack of swim bladder and lordotic/kyphotic anomalies. This assessment was confirmed by X-ray of fish with and without a SB following separation by hypersaline flotation. These findings show that failure of larval swim bladder inflation is the major contributing factor to spinal lordotic anomalies in GG. Also, this study described the timing of the swim bladder inflation period in GG and its development.

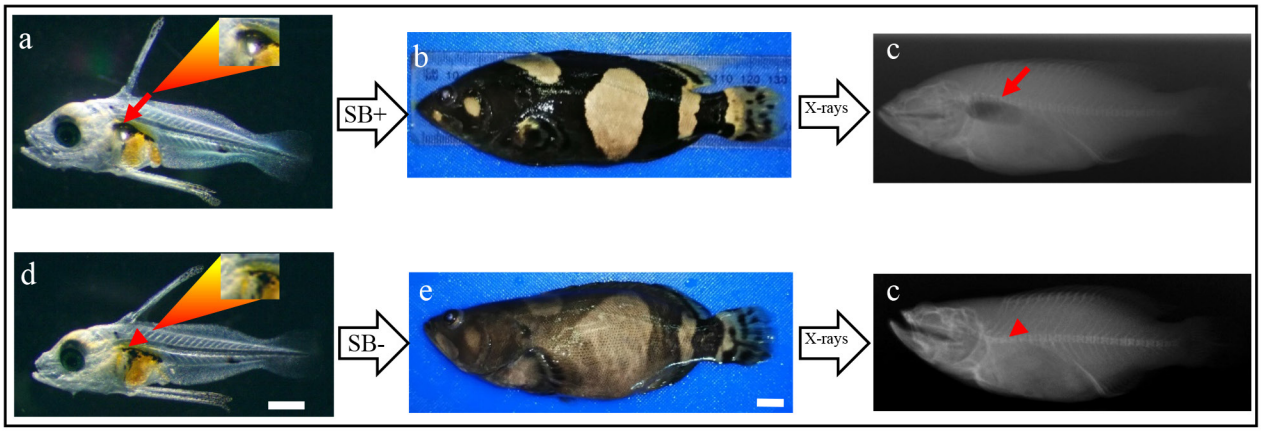


Figure. Larvae 10 DPH which successfully (a) gulped a bubble of air (arrow) and (d) a larvae that failed (arrowhead) to inflate the swim bladder. Bar 0.5mm. Floating (b) and sinking (e) juvenile GG after being placed in hypersaline seawater (bar 1cm). X-rays photographs of both floating (c) and sinking (f) fish depicting the presence (arrow) and lack (arrowhead) of swim bladder.

NEW GENERATION MULTI-STRAIN *Bacillus spp.* FEED PROBIOTIC (MICROSAF®) IMPROVES SURVIVAL, HEALTH STATUS AND ECONOMIC RESULTS OF THE WHITELEG SHRIMP (*L. vannamei*) REARED UNDER SUPERINTENSIVE CONDITIONS

Alban Caratis[1*], Nadege Richard[1], Tom Hashman[2], Alberto J P Nunes[3], Rodrigo Maggioni[3], Otavio S. Castro[1]

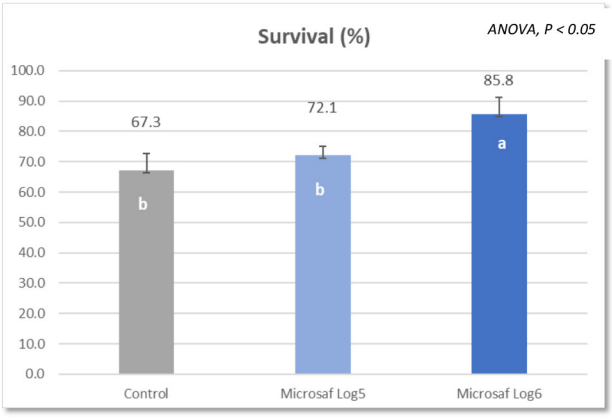
[1] Phileo by Lesaffre, Marcq-en-Baroeul, France
[2] Envera LCC, West Chester, PA, USA
[3] LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará, Fortaleza CE, Brazil
Email: a.caratis@phileo.lesaffre.com

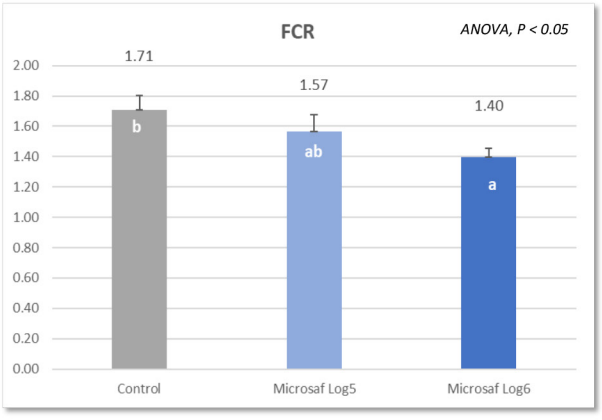
Feed probiotic adoption in aquaculture feeds is limited due to bottle necks related to feed processing such as high temperature and pressure. Top-coating applications with oil can successfully overcome this issue, enabling probiotic supplementation in commercial feed operations. However, probiotic efficiency in the gut can still be restrained by the fast feed transit rate and body temperature, especially in the case of spore-forming bacteria as *Bacillus spp*.

A trial was carried out at to evaluate the effects of a multi-strain probiotic Microsaf® (*Bacillus amyloliquefaciens*, *Bacillus licheniformis*, and *Bacillus pumilus*) primed with the GO Technology® (Germination Optimization Technology; patents US 9,447,376, US 9,932,543 and EU application 14749483.5), intended to improve and accelerate bacteria germination and activity. Shrimp (0.83 ± 0.07g) were stocked under super-intensive conditions (250 shrimp/m^2) with water exchange limited to 1% per week, only for sludge removal. Shrimp were fed twice daily for 10 weeks with commercial diets top coated with Microsaf® at Log5 and Log6 CFU per gram of feed and compared to a control group only coated with fish oil.

Supplementation of Microsaf® at Log6 CFU/g significantly increased shrimp survival and, reduced feed conversion rate (FCR). No differences were observed in biomass gain, that was numerically higher in the group supplemented with Log6 CFU/g. In the health-related parameters, a reduced *Vibrio spp*. counting in the hepatopancreas trend was observed in the probiotic supplemented groups. Phenoloxidase activity in the hemolymph was significantly higher in the group at Log 6 CFU/g. No significant differences were observed in the *Vibrio spp*. counting in the water and total hemocytes counts in the hemolymph. Feeding cost per kg of shrimp produced biomass was significantly reduced with the probiotic supplementation.

This study demonstrated that shrimp feed supplementation with probiotic at Log6 CFU/g can improve survival, productivity, and feed efficiency under super intensive system, with further health improvements and demonstrated economic feasibility.





FLOATING RAS, A CLIMATCHANGE RESILIENT WAY OF MARINE AQUACULTURE PRODUCTION

Paul-Daniel Sindilariu*, Kurt Carlsen

* Next Tuna GmbH, Londoner Strasse 3, 60327 Frankfurt am Main, Germany
paul@nexttuna.com
[1] CM Aqua, Farum Gydevej 64, 3520 Farum Denmark
kc@cmaqua.dk

Introduction

Next Tuna GmbH is planning, the first of its kind, production facility for Atlantic Bluefin Tuna (*Thunnus thynnus*) (ABT) at the shore of the Mediterranean Sea. (see as well https://www.nexttuna.com/).

Tuna in general and specially ABT is very sensitive to handling. In the early live stages, the small fish are nearly impossible to touch, and at later stages, the fish are simply too big to be handled in a meaningful way.

To address the logistic issue, Next Tuna, together with its Norwegian partner Seafarming system AS, developed the concept of a floating RAS. With the floating system, the fish can be collected at the hatchery and, when grown to the right size, transferred to a transport net-pen by joining the net-pen with the floating system. The flexibility of the floating systems solves the logistic challenges in tuna closed cycle aquaculture.

However, while developing the system adapted to the specific demands of ABT, next Tuan and its partner CM Aqua realized, that the floating facility might provide a solution to many challenges marine net-pen aquaculture is facing.

System description

The floating RAS, with a diameter of 30m and depth of 10m, is comparable in size with a net-pen however, the system is fully closed toward the surrounding environment through an insulated steel shell and covered by an industrial building (Fig. 1).

The floating RAS systems has two operational modes:
1. Production mode: the system is connected to a harbour dock, or moored in a sheltered area and receives all essential supplies from land;
2. Delivery mode: the system is disconnected from land supplies, pulled out of the sheltered/harbour area into the open sea and joint with the delivery net-pen, for safe and stress-free fish transfer and customer delivery.

Figure 2: Visualization of a floating production facility in a sheltered port environment (Curtesy of AFRY)

(Continued on next page)

During production mode, the floating RAS receives all supplies from land or, if operated in open water, from a supply vessel, including, electricity, oxygen, new (sea) water and delivers all residuals back to land for final effluent water treatment (Fig. 2). For transport, the tanks are detached from harbour or supply vessel and tugged to destination.

The fish feed is supplied by net-pen feeders, which can be centrally loaded from the feed storage in the Next Tuna harbour and connected to each floating RAS. This gives a maximum of flexibility, with no additional infrastructure for feed supply needed.

The RAS effluent streams are further treated on land. In a sludge thickening station, the particulate matter from drum-filter backwash is separated from the liquid fraction and further processed in a biogas facility, while the liquid fraction, is treated together with the system effluent in a fixed bed biofilter, integrated with an IMTA approach (multitrophic integrated aquaculture) with macro algae.

The roof top of the floating RAS is used for solar panels, to supply the energy for the temperature control inside the RAS.

Climate resilient aquaculture

The floating RAS has the same size as a net-pen but is independent from the surrounding seawater temperature and quality. With only a minimum of new, pre-treated water supply the system is suitable for any marine fish production. It is thus resilient to adverse effects of climate change like heat waves, prolonged precipitation, algal blooms or water pollution. In addition, parasites and pathogens can be excluded with efficient inflow water treatment. Another advantage over open net-cages is that no uncontrolled effluents will be washed into the surrounding waters and escapees will not be an issue. This should help to tackle typical issues in permitting processes. Overall, the system will allow to take aquaculture closer to consumption, regardless availability of land or quality of water. In short, the floating RAS offers the advantages of a land-based RAS and adds the flexibility of a floating production platform.

The operation of the floating RAS is most suitable in harbour environments (Fig. 2) or sheltered areas with a max wave hight of 0.5m. However, design parameter can be adapted to allow operation more exposed places.

Discussion

Next Tuna wants to present this novel solution to marine aquaculture to the audience and discuss the chosen solutions and their implications with the experts present at the conference.

NUTRITIONAL PHYSIOLOGY APPLIED TO ADVANCING CRUSTACEAN NUTRITION AND FEED DEVELOPMENT

Chris G. Carter*, Shuangyao Wang, Basseer M. Codabaccus, Quinn P. Fitzgibbon, Andrew J. Trotter, Greg G. Smith.

Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania, Private Bag 49
Hobart, TAS, 7001, Australia
Email: chris.carter@utas.edu.au

These are important and exciting times for research on sustainable aquaculture. Advancing our understanding about the nutritional requirements of aquaculture species and improving feeds are critical to sustainable aquaculture. Nutritional physiology is a term often used to describe nutrition research that arguably takes more holistic consideration of aquaculture organisms and their environment. The environment is particularly relevant because all aquaculture animals are aquatic ectotherms and their energy expenditure is dependent on environmental temperature. Important drivers of aquaculture research include: climate and environmental change; decreasing availability of traditional marine ingredients; increasing opportunities for new and more sustainable feed ingredients; inclusion of nutrition-based traits in selective breeding programs; and increasing awareness of meeting human nutritional needs. The aim of this presentation is to examine some current research drivers and overview research methods and approaches being used to better understand the nutritional physiology of crustacean species with a focus on rock lobsters.

Investigating energy substrate use (ESU) and protein metabolism is crucial to understanding nutritional physiology and refining feeds. This study determined ESU by stoichiometry and in relation to nutritional status of cultured *Sagmariasus verreauxi* juveniles. Additionally, the contribution of protein synthesis (PS) to energy metabolism was considered in two ways: oxygen consumption pre- and post-injection of a PS inhibitor cycloheximide; measurement using a stochastic endpoint method in lobsters fed isoenergetic diets containing 40%, 50% and 60% protein. During starvation, lipid was the primary energy substrate whereas during fasting, protein was the primary substrate (65% of oxygen consumption), with lipid accounting for the remainder (35%). Following feeding, protein contribution remained at over 50%, while lipid (0-43%) and carbohydrate (0-37%) provided considerable energy at different times. The contribution of protein synthesis to energy metabolism in starved, fasted and fed lobsters was 13%, 29% and 96%, respectively. Different dietary protein levels did not affect protein synthesis. Overall, this study illustrated the potential of a sophisticated nutritional physiological approach for developing cost-effective feeds.

The importance of nutritional physiology research remains high in ensuring sustainable aquaculture. There is tremendous opportunity provided by new technology and approaches to increase the accuracy, depth, scope and challenges of the research done.

SUSTAINABLE AQUACULTURE SYSTEMS FOR AUSTRALIA: OPPORTUNITY AND OPTIONS FROM ONSHORE TO OFFSHORE

Chris G. Carter*

Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania, Private Bag 49
Hobart, TAS, 7001, Australia
Email: chris.carter@utas.edu.au

Australia has nearly 60000 km of coastline and the World's third largest Exclusive Economic Zone. Aquaculture production is relatively small but valuable, particularly in regional areas and States. Tasmania is the country's largest seafood producing State by value which is mainly attributable to farmed Atlantic salmon. Atlantic salmon accounts for over 50% of Australian aquaculture production and 21% of the value of Australian seafood. The aquaculture industry aspires to World best practice and can be characterised as both innovative and a fast adopter of new technologies. Thus, Tasmania presents a unique location for developing sustainable aquaculture due to a range of factors including historical, organisational, commercial and environmental. For example, Tasmania is a climate change hot-spot and as higher temperatures are experienced more often the industry is having to develop responses to the impacts of climate change. Strategies are underpinned by research and include selective breeding for robustness, evolving management practices and using new technology. The location of salmon farms is also changing and moving away from some coastal zones. Land-based recirculation aquaculture systems (RAS) are being built to increase the size of salmon before they are moved into seawater. Recently, industry has started to explore moving to offshore sites in order to expand beyond the limited coastal sites for aquaculture. The seas around Tasmania are relatively high energy and pose considerable technological challenges as well as unknown questions about production biology. The integration of multiple species in aquaculture systems is increasingly of interest and supported by renewed focus on seaweed aquaculture and by new or improved technology for production of aquaculture species.

The aim of the presentation is to consider opportunity and options for sustainable aquaculture systems in Australia and with a focus on current industry initiatives and setting research priorities.

NUTRITIONAL PHYSIOLOGY APPLIED TO ADVANCING LOBSTER NUTRITION WITH A FOCUS ON PROTEIN METABOLISM

Chris G. Carter*, M. Basseer Codabaccus, Andrea Wirtz, Michael Landman, Shuangyao Wang, Quinn P. Fitzgibbon, Greg G. Smith

Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania, Private Bag 49
Hobart, TAS, 7001, Australia
chris.carter@utas.edu.au

These are important and exciting times for research on sustainable aquaculture. Advancing our understanding about the nutritional requirements of aquaculture species and improving feeds are critical to sustainable aquaculture. Important drivers of aquaculture nutrition research include: climate and environmental change; decreasing availability of traditional marine ingredients; increasing opportunities for new and more sustainable feed ingredients; inclusion of nutrition-based traits in selective breeding programs; and increasing awareness of meeting human nutritional needs. Nutritional physiology is a term often used to describe nutrition research that, arguably, makes more holistic consideration of aquaculture organisms and their environment. The environment is particularly relevant because all aquaculture animals are aquatic ectotherms and their energy expenditure is dependent on environmental temperature. The aim of this presentation is to overview research methods and approaches being used to better understand the nutritional physiology of lobster species with a focus on protein metabolism. The research approach integrates bioenergetics and protein-nitrogen flux to measure nutrient intake, biosynthesis, retention and waste to more closely examine different feed formulations. Energy substrate use (ESU) of protein, lipid and carbohydrate was calculated by combining respiration (oxygen) and excretion (carbon dioxide, nitrogen). Stable isotope incorporation revealed information about both ingredient use and biosynthesis. Combining these data types refined the approach for determining energy and macro-nutrient utilisation. Thus, adopting a nutritional physiology approach provides multiple layers of information to optimise feeds and support sustainable lobster aquaculture.

THE BLUE ECONOMY COOPERATIVE RESEARCH CENTRE PATH TO SUSTAINABLE INTEGRATED SYSTEMS FOR OFFSHORE AQUACULTURE: THE BUILDING BLOCKS

Chris G. Carter*, Lindsey White, Irene Penesis, Angela Williamson, John Whittington,

Blue Economy Cooperative Research Centre
Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania, Private Bag 49
Hobart, TAS, 7001, Australia
Email: chris.carter@utas.edu.au

The Blue Economy Cooperative Research Centre brings together over 40 partners from 10 countries to grow the blue economy around seafood and renewable energy production. There are five research programs (www.blueeconmycrc.com.au) including seafood and marine products. Australia and New Zealand have some of the world's largest Exclusive Economic Zones that offer extensive access to offshore locations with unrealised potential for aquaculture (Gentry et al., 2017) and renewable energy. The BECRC aims to tap into this potential and use new systems to move production offshore safely, economically and sustainably. The BECRC will operate until the end of this decade and this presentation aims to outline current and planned R&D building blocks to advance sustainable offshore aquaculture.

Established R&D building blocks are provided by salmon aquaculture, current production in the region is relatively small on a global scale but extremely valuable nationally. For example, Tasmania is the country's largest seafood producing State by value which is mainly attributable to farmed Atlantic salmon aquaculture: in 2019/20 Atlantic salmon accounted for well over half of Australian aquaculture (55% value, 62% production) and 29% of the value of all Australian seafood. The Australian and New Zealand salmon industries aspire to world best practice and can be characterised as both innovative and a fast adopter of new technologies. Recently, both industries have started to explore moving to offshore sites in order to expand beyond their limited coastal sites. The seas are relatively high energy and pose considerable technological challenges as well as unknown questions about salmon production biology that the BECRC partners are investigating. Seaweed aquaculture is only very recently emerging as a potential industry in Australian and New Zealand. Consequently, seaweeds require different R&D building blocks to salmon, these range from establishing farming technology to understanding the values that seaweeds may have, these include as quality human food, a source of high value extractives, nutrient sequestration and potential for hydrodynamic attenuation in exposed sites. Future R&D building blocks will be outlined and concern offshore systems in sub-tropical and tropical regions, species selection, the use of multiple species and integration among seafood production as well as with renewable energy.

The presentation aims to consider opportunity and options for sustainable integrated offshore aquaculture systems with a focus on establishing BECRC R&D building blocks and pathways.

GENTRY, R. R., FROEHLICH, H. E., GRIMM, D., KAREIVA, P., PARKE, M., RUST, M., GAINES, S. D. & HALPERN, B. S. 2017. Mapping the global potential for marine aquaculture. *Nature Ecology & Evolution,* 1**,** 1317-1324

THE DIGESTIBILITY TRAP: THE NEED FOR ADDITIVITY IN THE APPARENT DIGESTIBILITY COEFFICIENTS OF NUTRIENTS FROM INGREDIENTS

Rodrigo A.P.L.F. de Carvalho*[1], Albert G.J. Tacon[2] and Daniel Lemos[3]

[1]Aquatic Animal Nutrition Laboratory, Unidade de Ciências Agrárias, Escola Agrícola de Jundiaí (EAJ)
Universidade Federal do Rio Grande do Norte (UFRN)
*corresponding author e-mail: rodrigoplfc@gmail.com
[2]AquaHana LLC, Kailua, HI 96734 USA, [3]Aquaculture Laboratory, Oceanographic Institute
Universidade de São Paulo

Despite the several studies about ingredient digestibility being published in the last decades the applicability and usefulness of these outputs by the aquafeed industry remain uncertain. Among the different factors which may influence the quality of the apparent digestibility coefficients (ADC), the formula used for their calculation is a matter of concern as some outputs are questionable and the 70:30 inclusion rate approach is not suitable for all ingredients. The objectives of this study were: i) review the adequacy of the ingredient inclusion levels employed in the ADC studies with crustaceans, ii) study the effects of the two formulas currently employed in the calculation of the ADCs, iii) evaluate the predictability and additivity of different approaches to calculate the ADCs for ingredients. The review yielded 43 papers about *in vivo* digestibility in which 308 evaluations of 97 distinct practical ingredients were compared in respect of their inclusion levels versus the recommendation for practical inclusion levels. The effects of the two formulas were simulated with published data and the predictability and additivity of the ADCs calculated after three formulas and linear regressions were compared through diets with known formulation and digestible protein values. Results revealed that, on average 58% of the evaluations were estimated with inclusion levels more than 50% above the recommendations for practical inclusion levels. The ADCs estimated through the formulas with the inclusion levels in their terms are affected by a bias which magnifies the digestibility coefficients, especially for the less digestible ingredients at low dietary inclusion levels. This effect is softened by the conventional formula which includes the composition of the reference diet and the test ingredient. The evaluation of the different approaches to estimate the ADCs in terms of additivity revealed that the estimate with the formula that replaces the inclusion levels by the nutrient composition of the reference diet and the test ingredient, only, and the linear regression recorded the highest additivity among the test diets. This study highlights the importance of a critical analysis of the digestibility studies outcomes to improve the reliability and usefulness of the ADCs to practical diet formulation for animal production as a whole and for such past information must be reviewed and the new studies must take a proper calculation approach into consideration.

ANTIMICROBIAL POTENTIAL OF SOLID STATE FERMENTED AGROINDUSTRIAL BY-PRODUCTS' EXTRACTS IN MARINE FISH

Silva, J.F.[1*], Serra, C.R.[2], Santos, R.A.[1,2], Oliva-Teles, A.[1,2], Fernandes, H.[1,2] , Diogo, F.[1], Belo, I.[3], Salgado, J. [3], Castro, C. [2], Peres, H.[1,2]

*joaomanuelfelixsilva@gmail.com
[1]FCUP-Biology Department, Faculty of Sciences of University of Oporto
[2]CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, University of Oporto
[3]CEB-Centre of Biological Engineering of University of Minho

In order to ensure the future global fish provision, sustainable development of aquaculture is mandatory. To guarantee the total quality and consumer safety of fish provided by aquaculture, alternative nutritional strategies for bacterial disease management are required, preventing the use of synthetic drugs.

Agroindustry byproducts, like those from winery and olive oil industry constitute a low cost source of add-value bioactive compounds. Solid-state fermentation(SSF) is a biotechnological process that may be applied to increase the availability of their bioactive compounds.

The aim of this study was to develop an innovative bioactive enriched extract from the SSF of solid agroindustry by-products with *Aspergillus ibericus* and assay their antimicrobial potential against fish pathogens.

For that purpose, optimized mixture to maximize antioxidant potential of exhausted grape marc, vine trimming shoots and exhausted grape marc, were fermented by *A. ibericus* MUM 03.49. Fermented and unfermented matrixes were water extracted and their total phenolic content was determined. The antimicrobial potential of these extracts against high incidence fish pathogens in aquaculture was addressed by spot analysis and well-diffusion assays.

The unfermented optimized mixture extract has shown to be effective against bacteria from *Aeromonas, Edwardsiella, Photobacterium, Vibrio, Staphylococcus and Streptococcus genera*, while fermented optimized mixture inhibits *Streptococcus iniae and Photobacterium damselae subsp. piscicida*.

Further analysis are being conducted to address potential fermented and unfermented optimized mixture extracts against bacteria from *Tenacibaculum* genera.

Funded by:Project SPO3(ref.POCI-01-0145-FEDER-030377;FCT); project InovFeed(ref. MAR-02.01.01-FEAMP-0111;Mar2020).

POTENTIAL OF SOLID-STATE FERMENTED WINERIES AND OLIVE-OIL BY-PRODUCTS AS FUNCTIONAL INGREDIENTS FOR SEA BASS AQUAFEEDS

Castro, C.[1,2*], Fernandes, H.[1,2], Medeiros, C.[2], Silva, I.[2], Silva, J.F.[2], Diogo, F.[2], Belo, I.[3], Salgado, J. [3], Oliva-Teles, A.[1,2], Peres, H.[1,2]

✉ *carolinacastro23@gmail.com
[1]CIIMAR-Interdisciplinary Centre of Marine and Environmental Research, University of Oporto
[2]FCUP-Biology Department, Faculty of Sciences of University of Oporto
[3]CEB-Centre of Biological Engineering of University of Minho

Sustainable development of aquaculture requires the use of economic and eco-friendly ingredients in aquafeeds. The use of alternative ingredients (such as plant feedstuffs) can lead to negative consequences in fish health and welfare, including inflammatory and immune processes, and risk of disease. Functional ingredients can be used in fish nutrition as a nutritional strategy to mitigate harmful effects of diets including alternative ingredients. Winery and olive oil industries are two of the most demarked and well established Portuguese food industries that generate every year a vast quantity of by-products that are largely unused. In the context of Circular Economy Strategy, reuse and valorization of these by-products/wastes represent multiple economic and environmental advantages. Solid state fermentation(SSF) is a biotechnological low cost process that allows to convert these by-products into added-value ingredients, namely enriched in compounds with important biological activities (such as polyphenols).

The aim of this study is to evaluate the potential of unfermented or fermented mixture of three agro-industrial wastes (exhausted grape marc, vine trimming shoots and exhausted grape marc,), obtained through SSF, as functional ingredients in aquafeeds for seabass (*Dicentrarchus labrax*). The prospective beneficial effects of the extracts of the agro-industrial wastes mixture will be evaluated in seabass in terms of zootechnical performance, health and welfare. To achieve that purpose three diets rich in plant ingredients including those extracts were formulated. Each diet are being tested in triplicate in an 9 weeks growth trial with sea bass juveniles (23 ± 0.5 g; 18 fish/tank). Data will be obtained on growth performance, feed utilization efficiency, immune and oxidative status of liver and intestine.

Funded by:Project SPO3(ref.POCI-01-0145-FEDER-030377;FCT); project InovFeed(ref. MAR-02.01.01-FEAMP-0111;Mar2020).

AN OVERVIEW OF *Mastigias papua*, LIFE CYCLE, REARING PARAMETERS, NUTRIENT FEEDING, AND IDEAL MORPHOLOGICAL TRAITS IN S.E.A. AQUARIUM SINGAPORE

Vivian J.W. CAVAN*, Abel M.W. YEO, Nicholas M. DERBYSHIRE, Nicholas W.L. YAP

S.E.A Aquarium Singapore
Resorts World Sentosa
8 Sentosa Gateway
Singapore 098269

Jellyfish exhibits remain as one of the most popular exhibits for guests in aquariums worldwide, with significant resources invested to maintain them. Despite their appearance as simple, gelatinous animals, the successful culture and upkeep of these marine invertebrates for display are notoriously difficult. Two key issues pervade this: i) the availability and supply of jellyfishes, and ii) the rearing of attractive individuals. Supply of jellyfishes are often greatly limited by fluctuating catch rates and dependability on seasons, while those cultured in-house can often result in deformed individuals – unattractive to guests. Here, we focus on *Mastigias papua* (Lesson, 1855), commonly known as the 'Spotted' or 'Golden' Jellyfish, we relate our experiences into creating a sustainable supply of individuals and rearing ones attractive for display, alleviating the aforementioned problems.

Mastigias papua is a cosmopolitan species that is widely distributed across the Indo-West Pacific and are often part of aquarium displays worldwide. We define attractive individuals to have the following morphological traits for display: bell diameter of >80 mm, a distinct hemispherical-shaped bell, an exumbrella with fine granulations and white spots, eight velar lappets, and oral arms about half the length of the bell diameter. To ensure a sustainable supply of desirable individuals, we adopt strict husbandry practices. This included maintaining the cleanliness of the exhibit, providing a 100% water change, and relocation of larger individuals to more spacious enclosures. We found that adult individuals were healthier when tanks had a density of 30 individuals/m^3; crowded tanks lead to higher food competition, and insufficient space for proper growth. We also adopted in-water methods to reduce air-exposure to the jellyfish, also avoiding netting damage during transfers. These strict practices ensure the reduces deformities in *M.papua* individuals, improved their growth rates, and decreased mortality.

Further, our attention to simulate the nutrition levels and the abiotic conditions experienced by this species in the wild impacted our success in culturing healthy *M. papua*. Individuals were provided freshly hatched *Artemia* spp. nauplii larvae as feed, along with phytoplankton for enrichment. Along with nutritional supply, the jellyfish were also exposed to strong LED lighting 200PAR, with adequate photoperiods, water salinity 29 ±1 ppt, and temperatures 26 ±1 °C, corresponding to those experienced in the wild. Our experiences shared here will be relevant to partners who are interested in breeding these elegant and enigmatic marine invertebrates, be it for display, or as a source of sustainable protein for food production.

OIL LEAKAGE PHENOMENON IN TROUT FISH FEED: OPTIMISATION OF VACUUM COATING PROCESS CONDITIONS

Asma Chaabani*, Laurent Labonne, Vanessa Durrieu, Antoine Rouilly, Fabien Skiba, and Philippe Evon

Laboratoire de Chimie Agro-industrielle (LCA)
INP-ENSIACET
Toulouse, France
asma.chaabani@toulouse-inp.fr

Fish are the most efficient farmed animals for converting feed nutrients into edible meat. The quality of such fish feed is crucial to ensure this goal. Fish feed is presented in the form of extruded pellets obtained through twin-screw extrusion. It is an agro-material whose composition, nutritional value, density, and size vary in order to suit the development stages, behaviour and therefore the nutritional requirements of various fish species. We focused this study on a carnivorous species called trout. Their diets must have high lipid content to provide a source of easily available energy (up to 30% w/w). That is allowed by injecting a mixture of vegetable and animal oils (*i.e.*, rapeseed and fish oils) using a vacuum coating operation. However, pellets present a major defect, *i.e.*, the oil leakage over time, contributing to various issues like water pollution and loss in the lipid intake expected for fish consumption.

This work describes a process approach to study the oil leakage phenomenon in adult trout feeds. A Stolz (France) *MRSV 100* pilot vacuum coater was used for the study, and the latter was conducted in the form of an experimental design. Four coating parameters were studied: the stirring speed, the pressure inside the coater, its filling rate, and the time needed for going back to the atmospheric pressure. The properties measured on the obtained coated pellets were the oil leakage rate (OLR, in proportion to the pellet weight), their durability and their floatability. Coating conditions showed a real ability for influencing the OLR value, the latter varying at 40 °C from 2.7% to 1.2% depending on the coating conditions used. In fact, the vacuum level was the most effective factor for reducing this phenomenon. Indeed, a progressive reduction in OLR was observed as the pressure inside the coater was reduced at coating (Fig. 1a and Fig. 1b).

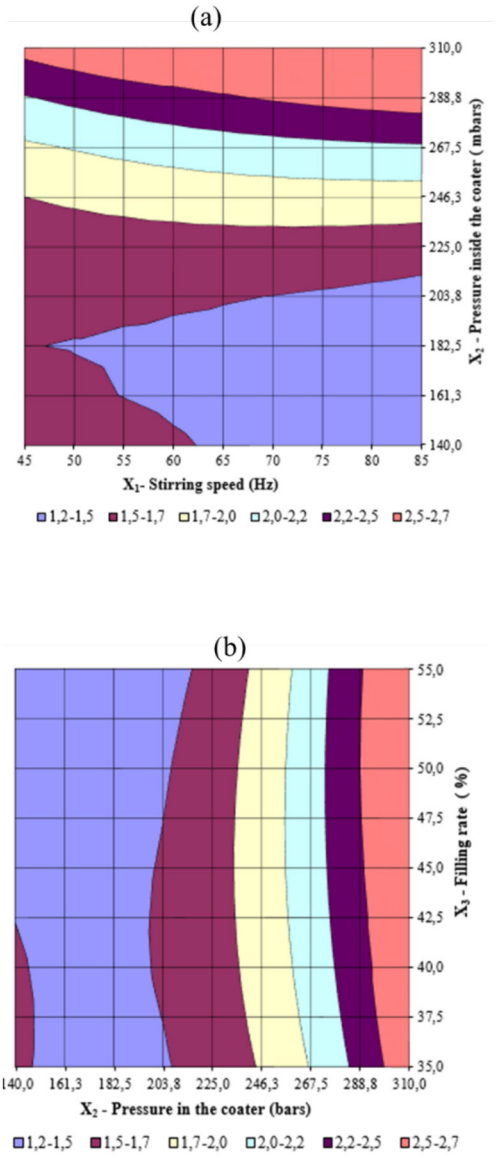


Figure 1. **Isoresponse curves for oil leakage rate at 40°C, at 45% filling rate for the coater (a), at 65 Hz stirring speed (b), and at 225 mbar for the pressure inside the coater (c).**

THE STUDY OF EFFECT OF AMINO ACIDS ARGININE, LEUCINE AND TRYPTOPHAN ENRICHED DIETS ON *Channa punctatus* EXPOSED TO COLD STRESS

*Rina Chakrabarti, Tauqueer Ahmad and JaiGopal Sharma

*Department of Zoology, University of Delhi, Delhi 110 007
e-mail: aquaresearchlab@yahoo.co.in

Temperature plays important role in the culture of aquatic organisms. The exposure of fish at low temperature especially during winter season affected the aquaculture production. The murrel *Channa punctatus* (58.93±1.72 g) were acclimated at 25°C and were fed with three experimental diets containing amino acids, arginine (2.0 g/100 g), leucine (2.0 g/100 g) and tryptophan (2.0 g/100 g) and one control diet without amino acid. After 45 days of feeding, fish were exposed at temperature 15°C. Experimental temperature was achieved at a rate of change of 1°C/12 h starting from acclimation temperature 25°C. Various tissue and blood samples were collected after the 12 h and 7 days of reaching the assigned temperature.

Significantly ($P < 0.05$) lower serum cholesterol level was found in tryptophan-enriched diet fed fish in both days of sampling. Serum triglyceride level was significantly ($P < 0.05$) higher in fish fed with arginine supplemented diet compared to others. The triglyceride level was minimum in the control diet fed fish. The glucose level was significantly ($P < 0.05$) higher in fish fed with leucine and tryptophan supplemented diets compared to others after 12 h of exposure. After 7 days, the maximum glucose level was found in leucine supplemented diet fed fish compared to others. The cortisol level was significantly ($P < 0.05$) lower in tryptophan supplemented diet fed fish compared to others diets fed fish in both days. The reduced glutathione (GSH) level was significantly ($P < 0.05$) higher in the liver of leucine and tryptophan enriched diets fed fish compared to others after 12 h of exposure. The heat shock protein 70 (Hsp 70) and heat shock protein 90 (Hsp 90) levels were ($P < 0.05$) higher in the liver of control diet fed fish compared to others. The Hsp 70 and Hsp 90 levels were minimum in tryptophan-enrihed diet fed fish.

ETHANOL AND METHANOL EXTRACTS OF *Withania somnifera* ROOT ENHANCES INNATE IMMUNITY AND PROMOTES GROWTH IN NILE TILAPIA *Oreochromis niloticus*

Suman B. Chakraborty*

Department of Zoology
University of Calcutta
Kolkata, West Bengal, India
sumanbc76@gmail.com

Efficacy of ethanol and methanol extracts of medicinal herb *Withania somnifera* roots were evaluated in tilapia, *Oreochromis niloticus* on immunostimulation and growth. *Withania* roots were extracted with water, ethanol, methanol, methylene chloride, hexane, successive methanol. Phenol, flavonoid content and antiradical activity of all the extracts were measured. Ethanol extract showed the highest phenol, flavonoid content and antiradical activity followed by methanol extract. Subsequently, Nile tilapia (mean weight 25.00±0.40 g) were fed diets containing different concentrations (0.0, 0.3, 0.5 and 0.7 g kg^{-1} feed) of ethanol and methanol extracts of *W. somnifera* roots for 30 days (n = 126, 3 replicates). Fish fed plant extract fortified diets showed significantly better (*P<0.05*) immunological, haematological, biochemical and growth parameters compared to fish fed control diet. Fish fed diet containing ethanol extract at the concentration of 0.7 g kg^{-1} feed showed the highest immunological (phagocytotic activity, respiratory burst activity, serum lysozyme, total protein, total immunoglobulin), haematological (total red blood cells, haemoglobin, hematocrit, total white blood cells, lymphocyte), biochemical (reduced glutathione, glutathione reductase activity) and growth (final weight, weight gain, daily weight gain, specific growth rate) parameters. The plant extract might act as potent free radical scavenger in fish tissues, and have tissue protecting ability, thus increasing fish health.

TABLE 1: Comparative growth and immunological parameters in Nile tilapia fed control, *W. somnifera* roots ethanol and methanol extracts at different concentrations supplemented diets after 30 days of culture. Means in columns with different superscripts are significantly different (P<0.05).

Treatment Groups (g kg^{-1})	Parameters				
	Final weight (g)	Weight Gain (g)	Phagocytic activity (OD 510 nm)	Respiratory burst activity (OD 620 nm)	Lysozyme (µg/ml)
Control	50.34±0.98^{a}	25.34±0.59^{a}	0.39±0.001^{a}	0.07±0.001^{a}	19.67±1.14^{a}
Ethanol 0.3	60.14±0.37^{bcd}	35.14±0.03^{cd}	0.69±0.002^{e}	0.14±0.003^{cd}	33.09±0.23^{c}
Ethanol 0.5	63.31±0.62^{de}	38.31±0.23^{d}	0.75±0.001^{f}	0.15±0.003^{d}	33.69±0.52^{c}
Ethanol 0.7	66.85±1.40^{e}	41.85±1.00^{e}	0.82±0.002^{g}	0.16±0.003^{e}	35.72±0.53^{c}
Methanol 0.3	56.83±0.38^{b}	31.83±0.78^{b}	0.48±0.001^{b}	0.10±0.003^{b}	20.91±0.22^{a}
Methanol 0.5	58.69±0.52^{bc}	33.69±0.12^{bc}	0.64±0.002^{c}	0.12±0.002^{c}	21.99±0.24^{a}
Methanol 0.7	62.36±0.91^{cde}	37.37±0.52^{d}	0.67±0.002^{d}	0.14±0.001^{d}	26.98±1.96^{b}

IMPACT OF COVID 19 ON AQUACULTURE AND FISHERIES IN BANGLADESH

Dr. Binay Kumar Chakraborty

Researcher & Consultant, Former Director and Supervisor
Mud Eel, Mud Crab, Aquaculture and Management Centre &
Department of Fisheries, Bangladesh & Bangladesh Agricultural University
E-mail: bborty@gmail.com

Bangladesh has an inland water area of about 45,000 km^2 and about 710 km long coastal belt. Bangladesh has vast inland freshwater resources including floodplains, ponds, rivers, oxbow lakes, large depressions (locally known as haor and beel), estuaries and a large coastal area with a sea. The fisheries sector contributes 3.52% to the national GDP, 26.37% to the agricultural GDP and more than 1.35% to the total export earnings. The total production was 4.552 m.mt fish in 2019-2020. Fisheries sector contributes significantly 62.58 g/day/capita of animal protein in daily dietary requirement. Aquaculture and fisheries sector of country is severely affected by pandemic corona virus (COVID-19). Data and information was collected by mixed-method including online questionnaire surveys, direct and telephonic interviews, key informant interviews (KII) and focus group discussions (FGD) and secondary data collected from the Department of Fisheries (DoF) and online grey literature. This study exposes a positive impact on ecosystem and increased fish stock in open water and coastal area due to stop of industrial influents and decreased fishing pressure for a certain period. The demand of spawn production decreased at the rate of 16 percent and number nursery practice was decreased at 18 percent and demand of food fish decreased at 20 percent. Finally, about (20-27)% fish farmer are not continued fish culture practice due to less demand and lower price of fish. All export and import based business is slowdown globally and nationwide lockdown situation imposed the supply chain, and processing and exporting of shrimp, crab and exportable other fish item hampered in this pandemic period. Out of 13%, about (18-21)% fisheries people somehow directly or indirectly related with fisheries sector are workless due to pandemic and a negative impact resulted a worsened livelihood. So, an action plan is needed to recover aquaculture sector from the pandemic considering enhance production, economic, social and environmental sustainability.

USE OF EMAMECTIN BENZOATE TO TREAT SEA LICE IN HYBRID GROUPER IN HONG KONG

Stephen Chan[1], Sandy Cheng[1], Karen Chan[1], Aaron Leung[1], and Sophie St-Hilaire

[1] Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong

Sea lice is an ectoparasite that affects farmed salt water fish species globally. It is particularly problematic for farmed species held in salt water net pen cages. In Hong Kong the sea lice species of the genus *Caligus* infects the oral cavity of hybrid grouper and can lead to significant losses if not treated. The most common strategy used to treat this parasite in Hong Kong is repeated formalin baths, but these are logistically difficult to perform on a large scale, and not always effective. We assessed the use of SLICE® (oral emamectin benzoate product) as an alternative treatment for sea lice in hybrid grouper in Hong Kong. Our findings suggest the dose recommended for salmonids (50 μg/kg of fish for 7 days) was adequate to effectively treat this species of fish, and the effect was long lasting. We also report tissue concentrations over time to guide practitioners on appropriate withdrawal periods for this drug in this species of fish at 17 C.

FATE AND TRANSPORT OF MODEL MICROORGANISMS IN SEDIMENTS FROM AQUACULTURE ENVIRONMENTS

Stan Chan Siew Herng*, Maria Yung Pui Yi, Elton Lim Wenxiong, Stefan Wuertz

Singapore Centre for Environmental Life Sciences Engineering
60 Nanyang Dr, SBS-01N-27
Nanyang Technological University
Singapore 637551
siewherng@ntu.edu.sg

The role of biofilms as potential environment reservoir for pathogens in marine aquaculture is unknown. Biofilms are assemblages of microbial cells that are enclosed in a self-produced matrix of extracellular polymeric substances (EPS). Here, it is proposed that biofilms in marine sediments can act as a reservoir for the retention of aquaculture-related pathogens. We used laboratory based flumes to simulate the sediment-water interface and introduced model microorganisms such as the fish pathogen, *Tenacibaculum maritimum* and a surrogate fecal indicator, *Enterococcus faecium*, to conduct a fate and transport study.

The abundance of *T. maritimum* and *E. faecium* in sediments was determined by quantitative polymerase chain reactions (qPCR) while their viability in the sediments over time was determined by propidium monoazide qPCR (PMA-qPCR) and *Enterolert,* respectively. Viable *T. maritimum* was only detected in sediments from 1 h to 10 h after spiking (Figure 1a). In contrast, viable enterococci were still detected after 24h (Figure 1a). Abundance of *T. maritimum* remained relatively constant from 1h to 24h followed by a 3-log reduction by Day 3 (Figure 1b). Levels of *T. maritimum* after Day 3 fell below the qPCR detection limit. Unlike *T. maritimum, E. faecium* only demonstrated a 2-log reduction from Day 0 to 21 in the spiked flumes. The inability of *T. maritimum* to persist for prolonged periods in sediments suggests that fish infections related to *T. maritimum* are unlikely to originate from sediments.

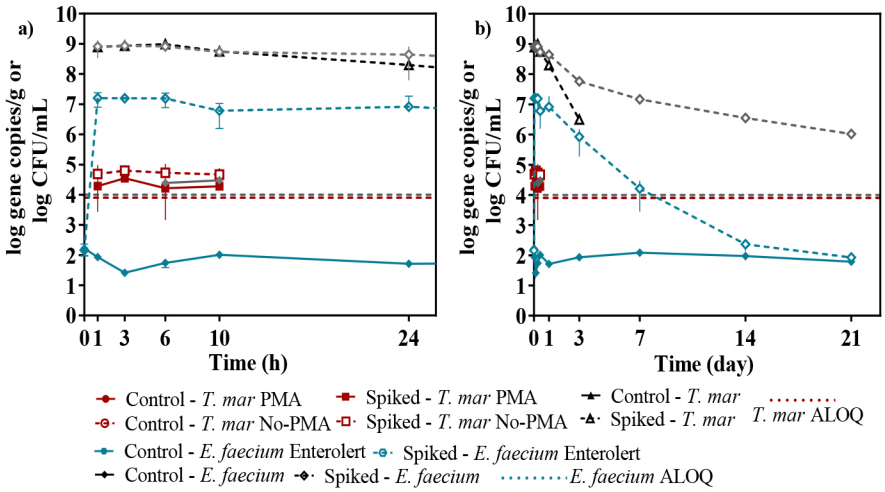


Figure 1: Viability and abundance of *T. maritimum* and *E. faecium* during the first (a) 24 h and (b) 21 days. Viability of *T. maritimum* expressed in log genes copies/g of sediment and *E. faecium* in log CFU/mL. ALOQ represents the analytical limit of quantitation for qPCR assay.

EMAMECTIN BENZOATE TREATMENT OF HYBRID GROUPER INFECTED WITH SEA LICE IN HONG KONG

Stephen Chi Ho Chan *, Sophie St-Hilaire, Tzu Hsuan Cheng, Chi Fai Leung, Ka Man Chan, Kwok Zu Lim, William Furtado, and Giana Bastos Gomes

City University Jockey Club College of Veterinary Medicine and Life Science Aquaculture Ambulatory Veterinary Team
Kowloon, Hong Kong
schchan@cityu.edu.hk

Sea lice (*Copepoda: Caligidae*) are ectoparasites which negatively impact marine aquaculture species around the world. There are a limited number of treatments licensed for use against sea lice in tropical and semi-tropical farmed fish species. Emamectin benzoate (EB) was an effective pharmaceutical drug against sea lice infestations in several salmon industries before resistance to the product developed. This drug has not been extensively tested in marine fish within Asia. The objective of this study was to determine whether this drug could be used to treat oral infections with sea lice in hybrid grouper (*Mycteroperca tigris* × *Epinephelus lanceolatus*) cultured in saltwater net-pen sites in Hong Kong. We observed an overall reduction in sea lice infections over time, starting on the last day of the treatment up to the end of our study (i.e., 14 days after the last EB treatment). We also observed a large variation in concentrations of EB in fish on the last day of the treatment, which provides an explanation for the variation in response to the treatment. It also suggests that distribution of the medication to fish in saltwater net-pens is difficult, especially when medication is hand-mixed in the feed and possibly unevenly distributed in the daily rations. Overall, this study provides preliminary evidence that EB could be used to treat sea lice found in Hong Kong and potentially in other regions of SE Asia.

FIGURE 1 | Examples of the range of sea lice infections observed on farms during the EB trial. Fish (A1–A3) were considered moderate to severe infections (category 2). Fish (B1–B3) were considered mild infections (category 1). Fish (C1–C3) were considered fish that were not infected or recovered from infection. The black arrow (on fish A1) illustrates a chronic lesion associated with sea lice. The red arrow (on fish B3) illustrates sea lice clusters (newer infections) with no tissue response. The blue arrow (on fish C1) illustrates tissue response in fish which was no longer infected with sea lice (post-treatment).

MACROSCALE BEADS WITH ANTIBACTERIAL AND ANTI-AMMONIA PROPERTIES FOR AQUACULTURE

Penghui Zhang and Mary B. Chan-Park

Nanyang Technological University
mbechan@ntu.edu.sg

As part of the 'grow basket', Singapore is planning to raise the local production of nutrients from 10% to 30% by 2030. Among these nutrients, 10% is the increase of proteins, including fishes and eggs. According to Singapore Food Agency (SFA), there are 123 fish farms that produced about 4,600 tonnes of fish in 2019, accounting for 9% of local fish consumption. To prevent the economic losses of fish farms, and increase aquaculture production in Singapore, advanced technologies to eliminate harmful pathogens and purify the water periodically are urgently required. Conventional technologies, such as coagulation, chemical oxidation, UV irradiation, *etc*., are not sustainable as they suffer from a high number of chemical usages, high energy consumption, *etc*. These technologies with a single function and high cost make them impractical in the aquaculture industry where the water environment is complicated.

Herein, multifunctional positively charged macroscale beads with high porosity were prepared by a facile method that involves a diffusion-driven layer by layer (dd-LBL) process using graphene oxide as the substrate and branched polyethyleneimine (bPEI) as the functional ingredient. The as-prepared beads then underwent a simple crosslinking reaction by which they were chemically stabilized and mechanically strengthened. Due to the properties of the dd-LBL self-assembly of graphene oxide, the size of the beads can be tuned from millimeter to centimeter level by adjusting the diffusion reaction time.

The developed crosslinked beads with negligible *in vitro* toxicity to 3T3 cells and zebrafish embryos can inactivate 99.9% of *Escherichia coli* (*E. coli*) in 30 min and, due to its chemical stability and mechanical strength, retain its antibacterial properties after 10 cycles of regeneration by a simple wash step. Meanwhile, the advanced porous beads with abundant functional groups show high adsorption capacity and good recyclability for many organic matters, including dye, fish fecal matter, organic solvent, oil, *etc*.

Further, the capability of removing total ammonia nitrogen (TAN) can be realized by adding zeolite nanoparticles, while preserving the antibacterial activity and biocompatibility. In addition, the facile preparation method makes the developed beads to be feasibly scaled up, and industrially economical. As calculated, the cost of raw material of one single bead is 0.01775 SGD. Given the recycling stability of the beads, the cost can be further reduced by recycling and reusing.

We believe that our developed multifunctional beads with economic cost are feasible to be applied and would benefit the modern aquaculture industry to deal with many issues, including purification of the recirculating aquaculture systems water, increasing the survival rate of both fresh water and marine food fishes, *etc*.

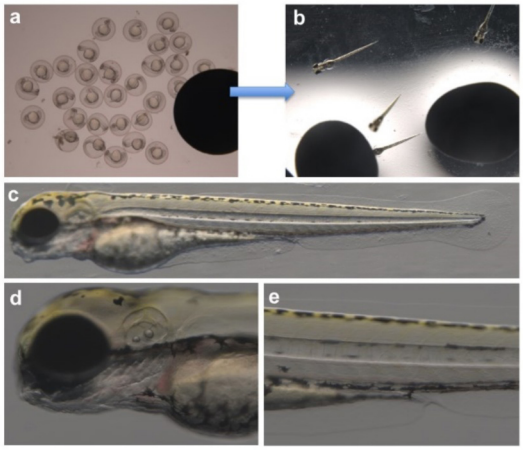


Figure. 10 beads incubated with 30 zebrafish embryos in 3 mL of E3 medium at (**a**) 1 dpe and (**b**) 3 dpe; (**d**) and (**e**) is the zoon-in images of (**c**) which is in anesthesia state of (**b**).

DETECTION OF MISLABELLED FOOD FISH IN SINGAPORE USING DNA BARCODING

Chang, J., * Pang, J., Lim, A. L. S., Thomas, N., Gwee, S., Yeo, A. W. A., and Mulyana, S.

Centre for Aquaculture and Veterinary Science
School of Applied Science, Temasek Polytechnic
21 Tampines Avenue 1, Singapore 529757
jasoncha@tp.edu.sg

The global fish industry has been the fastest-growing food production sector over the last two decades and is expected to continue growing due to the increasing demand for seafood worldwide. Trailing the rapid expansion of fish production and consumption is the unfortunate consequential rise of fish mislabelling, which is the deliberate or accidental substitution of one fish species with another. Mislabelling can negatively impact consumer finances, consumer health and endanger the survival of certain fish species. DNA barcoding is the use of DNA sequences for genetic identification and has been used to uncover widespread mislabelling in many countries. This study is the first to investigate the extent of fish mislabelling in Singapore, using novel degenerate primers for the Cytochrome c oxidase subunit I (COI) gene. A total of 106 DNA samples were extracted from raw, processed and cooked fish samples collected from eateries, supermarkets and wholesale markets across Singapore. Amplification and sequencing of the COI gene sequences were followed by interrogation of the National Centre for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLAST). Fish identity was then checked against the FishBase Information System to determine whether labelled fish names had been incorrectly assigned. Out of 106 fish samples, 105 samples were successfully amplified, sequenced and identified. Among those samples, 21 (20%) were found to be mislabelled. Of the mislabelled fish, Grouper (38.5%) and Snapper (36.4%) were the most commonly mislabelled and surprisingly, none of the 20 tuna samples were found to be mislabelled. Three of 19 salmon samples (15.8%) were found to be Rainbow trout. This study confirms that fish mislabelling is prevalent in Singapore, which is on par with the global average, and consumer finances and health are of genuine concern to people who place trust in the purchase their fish products.

EFFECT OF TANK COLOUR ON QUALITY OF JUVENILE GOLDEN TREVALLY *Gnathanodon speciosus* UNDER ELEVATED TEMPERATURE

Chau Bich Lien*, Ngo Thanh Cam, Nguyen Duc Toan, Ngo Van Manh, Le Minh Hoang

Institute of Aquaculutre, Nha Trang University, No 2 Nguyen Dinh Chieu Street, Nha Trang City, Vietnam
*Email: lien.cb.60ntts@ntu.edu.vn

The objective of this study was to evaluate the effect of tank color on quality of juvenile golden trevally such as growth, survival rate, respiratory rate and deformation rate reared at elevated temperature. The experiment was arranged with fifteen treatments corresponding to five tank colours (blue, red, yellow, grey, white) reared at three temperature (30, 32, 34°C). Each treatment was repeated three times. The quality properties were assessed after four weeks of rearing. The results showed that tank colour and elevated temperature affected the quality of juvenile golden trevally. Following the tank color as red, yellow, blue, grey and white, the growth and survival rate of fish tend to decrease gradually, but the respiratory rate and deformation rate of fish tend to increase. The growth and survival rate of fish gradually decreased and the respiratory rate and the rate of deformation gradually increased as the temperature increased from 30 to 34°C at the above tank colours. The combination of tank colour with elevated temperature did not affect the quality of the juvenile. In conclusion, the good quality of juvenile golden trevally can be reared in the condition that the tank colours were red and yellow at the temperature of 30°C.

DEVELOPMENT OF BACTERIA CONSORTIUM AS POTENTIAL PROBIOTICS AGAINST VIBRIOSIS IN PRELIMINARY IN-VIVO STUDY IN *Artemia* CULTURE

Chean Mei Yun Beryl*, Puvaneswari Puvanasundram, Murni Marlina Abd Karim and Nur Jasmin Mohd Yaminudin

School of Applied Science, Temasek Polytechnic
21 Tampines Avenue 1, Singapore 529757
beryl.chean@gmail.com

Infection of marine fish by pathogens such as *Vibrio* spp. are a common consequence in intensive aquaculture due to poor management practice. Mass mortalities translates to severe economic losses for farmers. Antibiotics used to combat disease outbreak encourages the proliferation of antibiotic-resistance among bacteria populations. Probiotics in aquaculture have been gaining traction as an alternative environmentally friendly tool for disease control. Hence, this study aims to develop and evaluate a multi-strain bacterial consortium as potential probiotics against vibriosis.

Isolated probiotic strains were screened for antagonistic activities against *Vibrio alginolyticus,* and its probiotic properties. *Lysinibacillus fusiformis* SPS11*, Bacillus megaterium* I24 *and Bacillius* sp. A2 displayed inhibition against *V. alginolyticus* and were selected and mixed as multi-strain probiotics. In biofilm formation assay, multi-strain probiotics showed significantly higher formation (1.312±0.101) at 12-hour compared with *V. alginolyticus* only (0.516±0.069). Preliminary *in-vivo* challenge assay using *Artemia* revealed that *Artemia* treated with 10^8 $CFUmL^{-1}$ multi-strain mix and challenged with 10^6 $CFUmL^{-1}$ *V. alginolyticus* (76.67±1.67%) demonstrated highest survival. It also had significantly higher survival when compared to *Artemia* treated with 10^6 $CFUmL^{-1}$ probiotic mix (65.00±0.00%) challenged with 10^6 $CFUmL^{-1}$ *V. alginolyticus*. Vibrios count showed negative reduction in *V. alginolyticus* in *Artemia* treated with 10^6 $CFUmL^{-1}$ multi-strain mix but positive and significant reduction in *Artemia* treated with 10^8 $CFUmL^{-1}$. There was a slight reduction in vibrios count in culture water across all treatments with 10^6 $CFUmL^{-1}$ and 10^8 $CFUmL^{-1}$ of multi-strain mix. Therefore, this study highlights the potential of the formulated probiotic mix as potential probiotics against vibriosis in the aquaculture industry.

CAPTIVE BREEDING OF A MARINE ORNAMENTAL FISH, BRISTLETAIL FILEFISH *Acreichthys tomentosus*

Jun Yu Chen*, Chaoshu Zeng

Centre for Sustainable Tropical Fisheries and Aquaculture
James Cook University
Townsville, QLD 4814
Bill.chen@my.jcu.edu.au

The marine ornamental fish trade is an important sector of the billion-dollar aquarium trade industry and it continues to expand. However, unlike freshwater ornamental fish trade, which mostly relies on the supply of captive-bred fish, it has been estimated that as much as 90% of marine ornamental fish are sourced from the wild. Aquaculture is considered as a sustainable alternative to reduce the fishing pressure of wild fish stocks. Unfortunately, the expansion of captive breeding of marine ornamental fish has also been limited by various hurdles, and larviculture is arguably the biggest bottleneck.

The bristletail filefish *Acreichthys tomentosus* is a popular marine ornamental fish not only because of its unique appearance, but also because of its ability to eat aiptasia, which is considered as a pest anemone by aquarium hobbyists. In this study, we naturally induced the spawning of *A. tomentosus* in laboratory and successfully reared the larvae to juveniles (Figure 1).

The eggs of *A. tomentosus* were demersal and adhesive. Under incubation at 27°C in seawater of 32 ppt, the larvae hatched out in the second evening post fertilization. The seawater for larval rearing was maintained at salinity between 32 and 34 ppt, and at temperature between 27.5 and 28.5°C. Greenwater made of commercial algae paste was used during the first week of larval rearing. The *A. tomentosus* larvae were initially fed a mixed diet of rotifers *Brachionus rotundiformis* and the copepods *Parvocalanus crassirostris* until 14 days post-hatching (DPH). Newly hatched *Artemia* nauplii were also added to feed the larvae from 10 DPH. From 15 DPH onwards, enriched *Artemia* metanauplii gradually replaced *Artemia* nauplii as the sole live feed for the larval rearing. All larvae had finished metamorphosis by 35 DPH and the survival rate of *A. tomentosus* to juvenile was around 40%. Our result suggested that *A. tomentosus* showed great potential as an aquaculture candidate species for the marine ornamental fish trade industry.

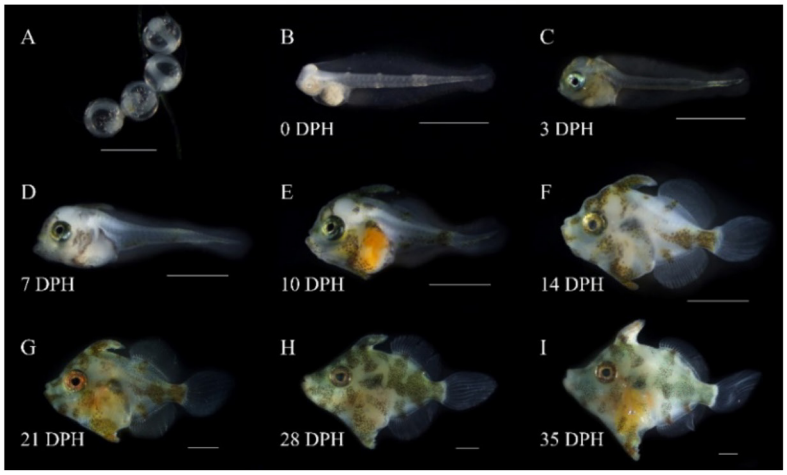


FIGURE 1. Laval development of *Acreichthys tomentosus* from eggs to juvenile. Scale bar in each picture represents 1 mm in length.

EFFECT OF LIGHT SPECTRUM AND LIGHT INTENSITY ON GROWTH AND IMMUNITY OF JUVENILE RAINBOW TROUT, *Oncorhynchus mykiss*

Xueweijie Chen*, Shuanglin Dong, Yangen Zhou, Shisheng Ma, Jianfei Yu, Yunwei Dong, and Qinfeng Gao

Fisheries College, Ocean University of China, 5 Yushan Rd., Qingdao 266003, China
cxwj@stu.ouc.edu.cn

In order to meet the demand of salmon market, Chinese scientists and entrepreneur are working on salmon mariculture far offshore in the Yellow Sea, China. Light is one of the most dominant environmental regulating the growth of salmonids. Optimal light regulation technology is vital for far offshore salmon mariculture, especially in a full-submersible cage in China. The objective of this study is to explore the effect of different light spectrum and light intensity on growth and high-temperature tolerance of juvenile rainbow trout *Oncorhynchus mykiss*. Eight treatments were designed as two light intensities of 150 lx and 1500 lx and four light spectrums of white (full-spectrum), red (615 nm), blue (455 nm) and green (518 nm). The photoperiod was 12 L: 12 D.

Juvenile rainbow trout (N=4; 73.85 g initial weight) were fed diets for nine weeks and subsequently underwent the high temperature acclimation. This study has eight treatments at a stocking density of 20 fish per tank (400 L). The fish were fed satiation twice daily (8:00 and 16:00) and fed consumption was recorded daily. At the end of the growth trial, there were no significant differences in final mean weight (231.09-256.56 g), percent weight gain (313.45-347.04%), survival (97.5%-100%) and FCR (0.93-1.07) (Table 1). On days 1, 7 and 14 after high temperature acclimation as well as at the end of the growth trial, enzyme activities such as (liver superoxide dismutase (SOD) activity, liver catalase (CAT) activity), malondialdehyde (MDA), immunoglobulin M and heat shock protein 70 (HSP70) will be determined.

Table 1 Growth performance of juvenile rainbow trout under two light intensities and four light spectrum for nine weeks.

Treatment		Final biomass (g)	Final mean weight (g)	Weight gain (%)	Survival (%)	FCR[1]
150lx	White	4509.14	231.78	318.04	97.50	0.98
	Red	5057.25	256.56	347.04	98.75	0.95
	Blue	5020.51	251.03	332.43	100.00	1.00
	Green	4988.36	249.42	337.38	100.00	0.93
1500lx	White	4505.75	231.09	313.45	97.50	1.07
	Red	4757.63	237.88	320.40	100.00	0.97
	Blue	4904.97	248.12	334.14	98.75	0.96
	Green	4852.78	245.59	339.59	98.75	0.93
	PSE[2]	82.2027	4.1358	3.2780	0.3712	0.0130
	P-value	0.5598	0.7690	0.0968	0.4662	0.1564

[1]FCR: Feed conversion ratio; [2]PSE: Pool standard error

DETECTION AND CHARACTERIZATION OF A NOVEL MARINE BIRNAVIRUS ISOLATED FROM ASIAN SEABASS IN SINGAPORE

Jing Chen*†, Xinyu Toh†, Jasmine Ong, Yahui Wang, Xuan-Hui Teo, Bernett Lee, Pui-San Wong, Denyse Khor, Shin-Min Chong, Diana Chee, Alvin Wee, Yifan Wang, Mee-Keun Ng, Boon-Huan Tan and Taoqi Huangfu

Centre for Animal & Veterinary Sciences, Professional and Scientific Services, Animal and Veterinary Service
National Parks Board (NParks), 1 Cluny Road, Singapore 259569, Singapore
Chen_jing@NParks.gov.sg

Through disease investigation of Asian seabass from a coastal fish farm in 2015 in Singapore, a novel birnavirus named Lates calcarifer Birnavirus (LCBV) was isolated in Bluegill Fry (BF-2) cell line. The LCBV-infected BF-2 cell line showed cytopathic effects such as rounding and granulation of cells, localized cell death and detachment of cells. The propagated virus, when injected intraperitoneally into naïve Asian seabass under experimental conditions, induced lesions similar to fish naturally infected with LCBV. Morphology of LCBV, visualized under TEM, revealed icosahedral particles around 50 nm in diameter. Chloroform and BUDR sensitivity assays confirmed the virus to be a non-enveloped RNA virus. Further genome analysis using NGS identified the virus to be a birnavirus with two genome segments. Phylogenetic analyses revealed that LCBV is more closely related to the Blosnavirus genus than to the Aquabirnavirus genus within the Birnaviridae family. These findings revealed the presence of a novel birnavirus that could be linked to the diseases observed in the Asian seabass from the coastal fish farms in Singapore. This calls for more studies on disease transmission, pathogenicity and epidemiology of this novel virus. The gene sequences data obtained from the study can be used for development of PCR-based diagnostic test methods.

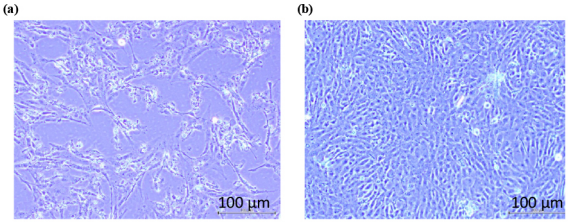


Figure 1. BF-2 cells infected with case A17/2/15 organ suspension showed CPE. (a) Day 2 post-infection of cells inoculated with case A17/2/15 organ suspension demonstrated CPE such as rounding and detachment from surface; (b) Uninfected BF-2 cell monolayer show no evident CPE.

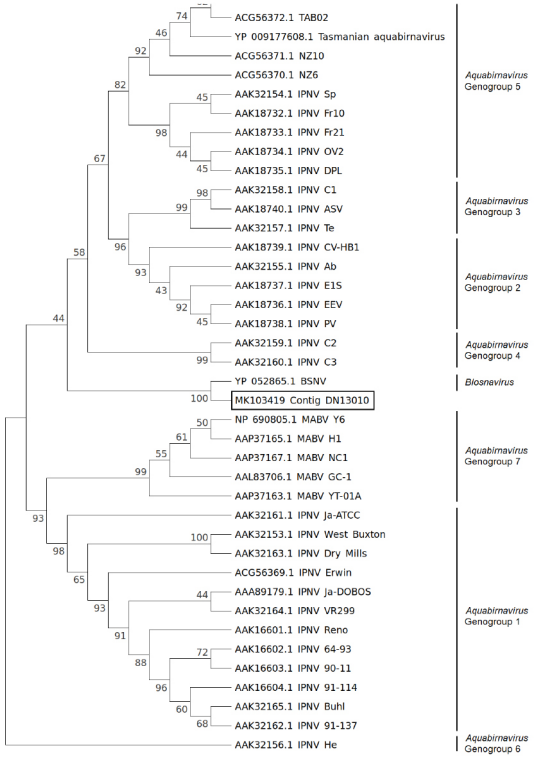


Figure 2. The evolutionary history of the VP2 protein of viruses in the *Aquabirnavirus* and *Blosnavirus* genera was inferred by using the maximum likelihood method based on the Le and Gascuel model.

POPULATION GENOMICS AND ADAPTIVE EVOLUTION OF CHINESE SEABASS *Lateolabrax maculatus*

Baohua Chen*, Wenzhu Peng, Zhixiong Zhou, Yue Shi, Peng Xu

College of Ocean and Earth Sciences
Xiamen University
Xiamen, China, 361105
chenbh@stu.xmu.edu.cn

The marine species usually show high dispersal capabilities accompanied by high levels of gene flow. The gene flow is frequently impeded by environment factors such as temperature, salinity, ocean currents and geographic barriers increasing the genetic and phenotypic differences between populations.

Chinese sea bass distributes broad latitudinal gradient spanning from the tropical to the mid-temperate zones. In the past decades, aquaculture of Chinese sea bass has developed fast in south China, but largely rely on germplasm from north China, which creates great uncertainty on wild seabass conservation and sustainable aquaculture. It's eager to perform comprehensive investigation of population studies of Chinese sea bass along the coast of China.

In this case, we performed whole genome sequencing and chromosome-level genome assembly of *L. maculatus*. We also collected numerous genome-with SNP genotypes of sea bass populations along the Chinese coast. After digging deeply into these data, we find out that the most remote two population in the Bohai Gulf and the Beibu Gulf retained significant genetic divergence which are connected by a series of intermediate populations in between. We also investigated the potential genetic basis of local adaptation correlating with population differentiation of *L. maculatus*. Genome-scale adaptive micro-evolution analysis identified many functional genes located in regions with significant selective signatures. These genes involve in many biological functions important to adaptation to different local environment, such as acid-base regulation and ion homoeostasis, neuroregulation, growth and movement ability. Overall, our genome scale analysis provided insight into population divergence and local adaptation of the highly dispersed Chinese sea bass in the continental margin seas.

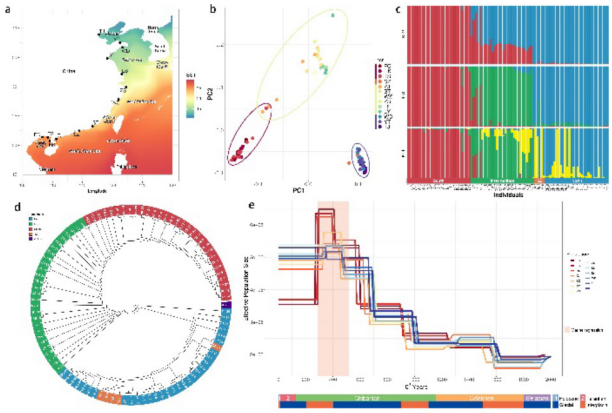


Figure 1. Population structure and effective population size of *L. maculatus*.

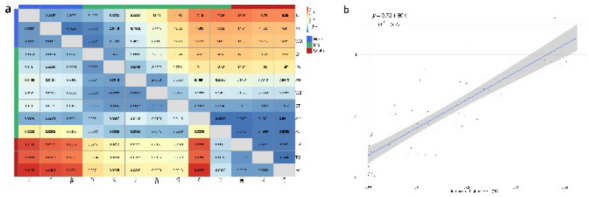


Figure 2. Correlation between genetic distance and difference of annual maximum SST.

THE USE OF MACROALGAE FOR THE REMOVAL OF NITRATE IN A MINIMAL WATER EXCHANGE RECIRCULATING AQUACULTURE SYSTEM

Paul Chen and Steven Fong

The recirculating aquaculture system (RAS) present as a solution for Singapore's strive for greater food security and resilience. Being technologically driven, RAS allows for the intensification of production without the reliance on favourable environmental conditions. However, the current impediment of the RAS is the build-up of nitrate. High nitrate level (>50 ppm) is detrimental to fish health. Traditionally, this is dealt with through partial water exchange. As macroalgae require nitrate for growth, the farming of macroalgae could potentially serve as cheaper alternative for nitrate removal. However, high stocking density in RAS could cause pH to drop. The effectiveness of nitrate removal by macroalgae under low pH condition of RAS is currently unclear. How might these change due to climate change is also unknown. Using a random block design, we investigated the nitrate uptake in two commercially important species under different pH and temperature regime. Our analyses suggest that, in general, the efficacy of macroalgae in nitrate removal decrease as pH decrease and temperature increase. While macroalgae could be used to remove nitrate in RAS, they are unlikely to be effective in high stocked RAS. The efficacy of macroalgae in removing nitrate is also probably going to be affected by climate change.

DETERMINING THE OPTIMAL STOCKING DENSITY OF BOTH JUVENILE AND EARLY GROWER HYBRID GROUPER – TIGER GROUPER *Epinephelus fuscoguttatus X* GIANT GROUPER *Epinephelus lanceolatus*

Ian C. JueQi*, Jack M. James, Chassie T. Tse Tjie and Crystal L. Liwen

Pontus Research Group
Marine Aquaculture Centre (MAC)
St John's Island, Singapore
icj@pontusresearch.com

Hybrid Grouper – Tiger Grouper *Epinephelus fuscoguttatus X* Giant Grouper *Epinephelus lanceolatus* are one of the more commonly aquaculture species. Its fast growth rates, resistance to disease, high survival and good economic value makes it an important species to be farmed commercially.

A 6.5-week performance trial was conducted with 1575 mixed sex fish (± 30 grams) for juvenile (30 – 100 grams) hybrid groupers and a 4-week performance trial were conducted with 2300 mixed sex fish (± 150 grams) for early grower (100 – 300 grams) hybrid grouper. The aim of this study was to examine the optimal stocking density and growth performance for each stage, and to mimic a commercial production. Fish were batched weighed and randomly assigned and stock in 300-liter and 1000-liter fiberglass tanks, with a recirculating aquaculture system. Ammonia, nitrite, nitrite, temperature, dissolved oxygen, and pH was measured twice a week. Fish was fed 3 times a day to satiation with a commercial grouper feed and light: dark was at 8-9 hours light: 15-16 hours dark.

After 6.5 weeks, juvenile groupers kept in density group D5 (9 kg/m^3) and D6 (10.50 kg/m^3) showed the best performance, with actual end densities of 37.48 kg/m^3 and 44.20 kg/m^3. Following the 4-week trial, the early grower groupers in density group D2 (25 kg/m^3) and D3 (30 kg/m^3) showed the best performance, with actual end densities of 45.43 kg/m^3 and 46.94 kg/m^3.

TABLE 1: Performance results for juvenile grouper.

	Internal treatment codes with target start density (kg/m³)						
	D1	D2	D3	D4	D5	D6	D7
FCR	0.84^a	0.84^a	0.85^a	0.83^a	0.82^a	0.83^a	0.84^a
SGR	3.71^a	3.85^a	3.81^a	3.91^a	4.02^a	3.97^a	3.89^a
FI	3.30^a	3.42^a	3.44^a	3.49^a	3.56^a	3.37^a	3.45^a
End weight	110.67^a	116.89^{ac}	122.89^{bc}	120.67^{ab}	125.41^{bc}	126.29^b	123.28^{bc}
Survival	100.00^a	100.00^a	100.00^a	100.00^a	99.63^a	100.00^a	100.00^a
Actual Start Density	2.91	4.80	6.25	7.37	8.86	10.61	12.14
Actual End Density	11.07	17.53	24.58	30.17	37.48	44.20	49.31

TABLE 2: Performance results for early grower groupe

	Internal treatment codes with target start density (kg/m³)			
	D1	D2	D3	D4
FCR	0.85^a	0.80^a	0.80^a	0.91^a
SGR	2.01^a	2.02^a	1.77^a	1.36^b
FI	1.99^a	1.94^{ac}	1.70^{bc}	1.44^b
End weight	272.94^a	282.23^a	262.56^a	227.89^b
Survival	97.74^a	99.60^a	99.83^a	99.75^a
Actual Start Density	19.85	25.63	30.88	40.38
Actual End Density	35.48	46.94	52.43	60.70

MULTI-TISSUE PROTEOMIC ANALYSES REVEALS THE IMMUNE REGULATORY EFFECTS OF SANACORE®GM IN MARINE FISH

I-Tung Chen* & Waldo G. Nuez-Ortín

Adisseo, 600 North Bridge Road
#15-06, Singapore 188778
i-tung.chen@adisseo.com

When a fish encounter invading pathogen, both the innate and adaptive immune systems will be actively triggered. If the pathogen overwhelms the innate immune response and persists, the adaptive immune responses is then initiated. Increasing evidence shows that production conditions such as densities, thermal fluctuations or current aquafeed formulations, negatively impact fish immunocompetence. Health promoting additives can be used as a preventive strategy to restore immunocompetence and reduce the severity of infections during production. SANACORE®GM (SNGM) is a health promoting additive widely used in marine fish feed for the prevention of bacterial and parasite infections.

This study aimed to elucidate the immunoregulatory mechanisms of SNGM to restore immunocompetence in gilthead sea bream (*Sparus aurata*). We used multi-tissue proteomics approach to differentially analyze the proteome of key immunocompetent tissues: head kidney, anterior intestine, and liver following supplementation. Specifically, we used a label-free quantitative proteomic approach that applies a large-scale high-throughput LC-MS/MS based technology with high mass accuracy and sensitivity. Combing the differentially proteomic analyses result which annotated from *Salmo salar* database (Uniport Taxon 8030), 800~1000 proteins from different key immunocompetent tissues showed differential expressions. Computational and biological networks analyses were used to establish protein interactions and identify the key regulators (Figure 1).

Results showed that SNGM elevated fish immunocompetence by regulating key modulators of programmed cell death, phagocytosis, or antigen presenting pathways. Additionally, SNGM upregulated anti-inflammatory and antioxidant pathways, and induced changes associated to cell integrity, cell proliferation, and membrane transport. It was concluded that SNGM supports fish immunocompetence via both innate and adaptive mechanisms. This regulatory action is key in helping fish in dealing with production conditions and in reducing the impact of infections.

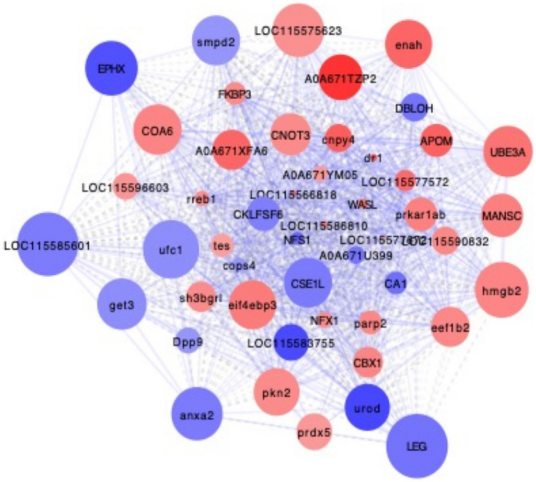


FIGURE 1. Inferred protein network for 49 proteins. Node color indicated the fold change (red: downregulated; blue: Upregulated), and size correspond to the number of connections. Galectin (LEG) was the most abundant protein in the network and exhibited the highest number of protein interactions. Galectin is a glycoprotein that promotes integrin adhesion.

SODIUM PERCARBONATE (SPC) FOR PARASITE MANAGEMENT IN TROPICAL MARINE AQUACULTURE

Kirby Chen*, Bing Liang, Naomi Koh, Mohamad Yazid Anuar

Marine Aquaculture Centre
Singapore Food Agency
11 St John Island
Singapore 098659
Kirby_chen@sfa.gov.sg

To manage periodic monogeneans parasite outbreaks, local farmers commonly use freshwater bath or formalin as a prophylactic treatment. To encourage shift towards a more sustainable aquaculture industry, hydrogen peroxide was investigated as an environmentally friendly alternative. Hydrogen peroxide has been used in parasite treatment of salmon and freshwater aquaculture species; however, no data is available for usage for tropical marine species. Additionally, hydrogen peroxide is volatile in liquid form which makes it unpopular among local farmers. The more stable sodium percarbonate (SPC) powder which decomposes into hydrogen peroxide, sodium and carbonate in water is a preferred alternative for aquaculture.

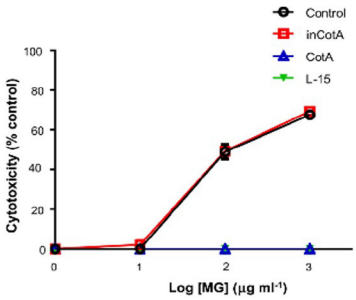
We investigated the effectiveness of SPC against commonly found ectoparasites in local coastal water such as *Neobenedenia* spp, *Zeylanicobdella arugamensis*, *Dactlogyrus* spp., and *Cryptocaryon irritans*. Different SPC concentrations and treatment timings were tested to determine the safe dosage for various tropical marine fish species at our research facility (Marine Aquaculture Centre) and local farms. A protocol for 1-hour treatment at 150ppm hydrogen peroxide was developed and found to be effective for Asian seabass and marine tilapia.

BIODEGRADATION OF MALACHITE GREEN FROM AQUACULTURE POND WATER USING BACTERIAL COTA-LACCASE

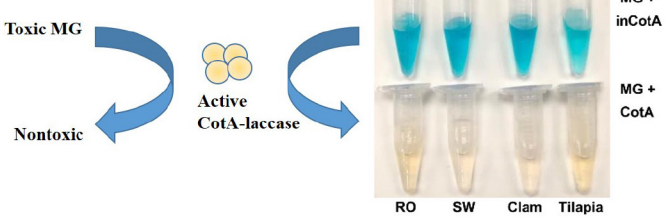
Chiu-Min Cheng*, Chia-Hua Lin

Department and Graduate Institute of Aquaculture, National Kaohsiung University of Science and Technology
No.142 Haijhuan Rd, Nanzih Dist, Kaohsiung City 81157, Taiwan (ROC)
cmcheng@nkust.edu.tw

Malachite green (MG) is often used as an effective fungicide and parasiticide in aquaculture; however, its use in food-producing animals is banned because of possible mutagenic and carcinogenic effects and persistance in aquatic organisms and the environment. Bacterial laccase can tolerate extreme environments and may be the best enzyme for removing MG. To increase bacterial laccase production, we cloned the CotA-laccase from *Bacillus subtilis* ISW1214 and efficiently expressed it in *Escherichia coli*. The optimal catalytic conditions for purified CotA-laccase were pH 5.0, 60°C, and 12 ppt for ABTS oxidation. To examine the decolorization ability, MG was incubated with CotA-laccase, heat-inactive CotA (inCotA), or phosphate-buffered saline with or without acetosyringone as a mediator, and absorbance at 620 nm was measured at different time points. Following decolorization, MG concentration in the decolorization solution was determined. To verify the cytotoxicity of MG, grouper fin (GF-1) cells were exposed to the decolorized solution and cell viability was measured. CotA-laccase decolorized MG in the presence of acetosyringone, and decolorization efficacy increased but MG concentration decreased with time. The decolorization solution was non-toxic to GF-1 cells. To verify the ability of CotA-laccase to decolorize MG in aquaculture, MG was added to the water from clam and tilapia ponds. MG was significantly decolorized by CotA-laccase but not by inCotA. Thus, recombinant CotA-laccase may be an environmentally friendly enzyme for aquaculture and may be applied on a wide scale to remove environmental pollutants, thereby reducing pollutant accumulation in aquatic organisms and ensuring safe aquaculture products.



Cytoxicity of MG following CotA-laccase decolorization. Grouper fin (GF-1) cells incubated with decolorized solution treated with active cotA, inCotA, or control for 24h, the cell viability was measured using the Cell Counting Kit-8 (CCK-8) assay.



Decolorization of MG by CotA-laccase in aquaculture pond water. we collected aquaculture pond water samples (12 containing clam and 4 containing tilapia) from southwestern Taiwan, and the mixtures were treated with either CotA-laccase or inCotA for 24 h at 37°C.

SURVIVAL, GROWTH AND ROTIFER INTAKE OF ASIAN SEABASS (*Lates calcarifer*) LARVAE REARED IN ACIDIFIED SEAWATER

Cheong J. Z. D.*, Ching F. F., Mohd Faudzi N., and Senoo S.

Borneo Marine Research Institute
Universiti Malaysia Sabah
Kota Kinabalu, Sabah
johannes_cheong_my21@iluv.ums.edu.my

Ocean acidification is a rising concern globally, and the effects on fish, especially the fragile larval stage is not well documented. This experiment was designed to investigate the effect of ocean acidification on the survival, growth and rotifer intake of Asian seabass (*Lates calcarifer*) larvae. Carbon dioxide (CO_2) gas was used in this experiment to acidify the water assisted with the use of timer system to keep the pH of water within the fixed range (5.5, 6.0, 6.5 and 7.0). A control treatment was used to compare the results with pH fluctuating from 7.8 to 8.5, mimicking the current pH value of the ocean. Asian seabass larvae (initial total length: 2.13 ± 0.23 mm) were stocked at 30 individual/L in a 6L experimental aquarium. The experiment was conducted for 30 days. At the end of experiment, the survival of Asian seabass larvae in control treatment is 30.92%. Total mortality was observed in pH 5.5 (day 3), 6.0 (day 4), 6.5 (day 4) and 7.0 (day 5) respectively. The changes in growth can only be observed up until day 2 of experiment. The total length recorded for Asian seabass larvae for day 1 and 2 in treatment pH 5.5 (2.19mm, 1.85mm), pH 6.0 (2.23mm, 2.01mm), pH 6.5 (2.16mm, 2.02mm), pH 7.0 (2.28mm, 2.16mm) and control (2.40mm, 2.50mm), respectively. The larvae in control treatment showed significantly better growth compared to other treatments with continued growth throughout the experiment period (final total length: 14.25 ± 1.02 mm). Rotifer intake also shows similar result where control treatment shows higher intake (10.7 ind) compared to other treatments, pH 6.0 (2.7 ind), pH 6.5 (1.3 ind) and pH 7.0 (2.7 ind), respectively. As conclusion, acidified seawater negatively impacts the survival, growth and rotifer intake of Asian seabass larvae. This result also shows the effect in the future if the current rate of carbon emission is not reduced. Continuation of ocean acidification will bring negative consequences towards the future of marine aquaculture industry.

NANOBODIES PROTECT *Artemia* FROM THE *Vibrio parahaemolyticus* BINARY PIRAB TOXIN THAT IS RESPONSIBLE FOR ACUTE HEPATOPANCREATIC NECROSIS DISEASE IN SHRIMP

Sylvia Cheung*, Slade Loutet, Sin Yee Lee, Filip Van Petegem and Hamlet Abnousi

NovoBind Livestock Therapeutics
Vancouver, BC, Canada
sylvia.c@novobind.com

Acute hepatopancreatic necrosis disease (AHPND) is a recently emerged disease in Penaeid shrimp aquaculture that is characterized by high mortality. A binary toxin composed of the PirA and PirB proteins expressed by *Vibrio parahaemolyticus* (PirAB^{Vp}) has been shown to be sufficient for the development of the disease. Here we show that nanobodies derived from the heavy chain only antibodies of llamas can neutralize PirAB^{Vp} activity and reduce shrimp mortality.

Vibrio parahaemolyticus PirA and PirB proteins were recombinantly expressed in *Escherichia coli* and purified. Together, the recombinantly expressed PirAB^{Vp} killed specific pathogen free Pacific whiteleg shrimp (*Litopenaeus vannamei*) and brine shrimp (*Artemia*), a surrogate model for whiteleg shrimp. To obtain nanobodies targeting PirAB^{Vp}, llamas were immunized with PirA and PirB, and, using phage-based biopanning techniques, hundreds of nanobodies specific to PirA or PirB were identified. A total of 128 unique PirA-binding nanobodies and 200 unique PirB-binding binding nanobodies were subcloned to an *E. coli* protein expression system and successfully purified.

The purified nanobodies were tested to confirm their ability to bind the expected antigen in protein-protein pulldown and/or ELISA experiments, to examine their ability to block the interaction of PirA and PirB, and to assess their relative proteolytic stability in gastrointestinal fluid extracts. From these screening experiments, a subset of 52 nanobodies were tested for their ability to protect *Artemia* from the toxic effects of PirAB^{Vp}. Shown here are the results for two PirA-binding nanobodies (NBXBG and NBXBH) and two PirB-binding nanobodies (NBXBD and NBXBJ) that can reduce PirAB^{Vp} -induced death in *Artemia* (Fig. 1).

Nanobodies represent the smallest functional antibody fragment and have the potential to be used in many applications, including animal feed. With the ability to neutralize PirAB^{Vp}, our nanobodies provide a new opportunity to reduce or prevent AHPND in shrimp aquaculture.

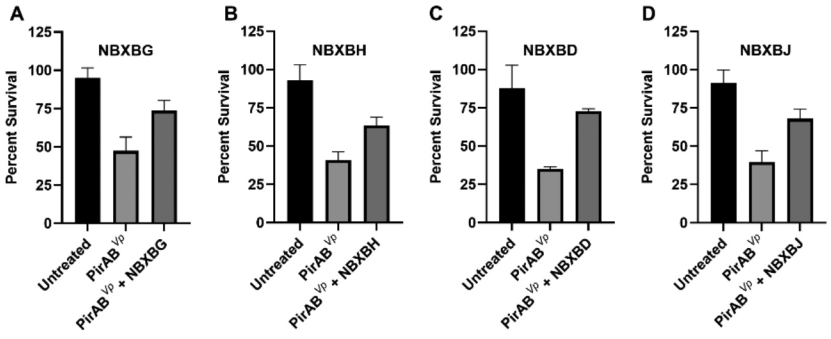


Figure 1: Survival of *Artemia* after 42 hours of treatment. Data shown are the means and 95% confidence intervals from three to four experiments. Each experiment consists of twelve wells per group with ten *Artemia* per well.

HAEMATOLOGY OF DISEASED VS CLINICALLY HEALTHY FARMED JUVENILE ASIAN SEABASS (*Lates calcarifer* Bloch)

Chew Xian Zhe[1]
Susan-Gibson Kueh[2]

[1 & 2]Tropical Futures Institute, James Cook University Singapore
[1] xianzhe.chew@my.jcu.edu.au
[2] susan.kueh@jcu.edu.au

Clinically healthy (n=435, mean wt=48.6g) and diseased (n=154, mean wt=39.3g) juvenile Asian seabass, *L. calcarifer* were sampled from a commercial fish farm in Singapore for haematology. Based on clinically healthy fish, a reference interval was developed for blood glucose, haematocrit, haemoglobin, total serum protein, total white blood cell (WBC) counts, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and relative differential WBC count, as a health assessment tool for farmed fish. Histopathology of outliers of these blood parameters showed fish had severe chronic enteritis, or were sub-clinically diseased. Significantly higher mean blood glucose, total serum protein, haemoglobin, hematocrit and lymphocytes counts were observed in clinically healthy than in diseased juvenile *L. calcarifer*. Diseased juvenile *L. calcarifer* were observed to have significantly higher mean total WBC counts and monocytes.

USE OF MIIIOME POSTBIOTICS MICROBIOME TECHNOLOGY IN MARINE AQUACULTURE

Tet-Fatt Chia*

Seatobag Pte Ltd
7 Gambas Crescent, #04-11 Ark@Gambas
Singapore 757087
chia.tetfatt@gmail.com

Current high density fish aquaculture is not sustainable and causes rapid environmental degradation. Coupled with the overfishing and pollution of the seas, coastal marine ecosystems are breaking down. At this rate if nothing is done, the world will run out of fish in 50 years.

To continue high density fish farming and make it sustainable, we must learn to control the microorganisms that are associated with the aquaculture system. Disease outbreak is a common phenomenon and poses a serious challenge to fish farming. Extensive measures taken to treat these diseases caused by the pathogens should not be based on chemical products like antibiotics and formalin. Rather, the use of beneficial microbial postbiotics to strengthen the fish digestive system, increase immunity and improving the overall health, size and weight of the fish naturally is the correct path. This microbiome improvement approach also helps strengthen the ecosystem of the fish farms naturally by reducing the pathogens load and increasing the good microbes and biodiversity. Finally, fish fed with the fermented feed pellets containing the beneficial microbial postbiotics and farmed under this good environment grew up to 50 percent faster, showed better body form, colour and meat texture.

This microbiome technology heralds a new and sustainable high density eco-friendly approach that may allow Man many more years of continued enjoyment of the benefits of eating a good and nutritious fish and at the same time improving the environment.

* Scientific Advisor for Seatobag Pte Ltd

THE EFFECT OF FLOWRATE IN RECIRCULATING AQUACULTURE SYSTEMS ON WATER CHEMISTRY AS IT CONCERNS FEEDING EVENTS OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

Jui-Che Chiang*, Giovanni Marco Cusimano, Natalie E. Panasiak, Freya Robinson, Tamás Bardócz, Shane A. Hunter, Dannie O'Brien

AquaBioTech Group, Research Development and Innovation, Naggar Street, Targa Gap, Mosta, Malta
Email: jcc@aquabt.com

Investigations were undertaken related to inlet configuration and flow rate on efficiency of solids removal, maintenance of water quality, and production and welfare indicators in farmed Rainbow trout (*Oncorhynchus mykiss*) in Recirculating Aquaculture Systems (RAS). Nozzle-bar inlet placement benefits waste removal performance and best homogenises water quality. Juvenile trout were not negatively affected by higher flowrates regarding either growth performance or welfare, but this is an interesting field of further investigation. Higher flowrates are considered to have better system efficiency in RAS in terms of waste removal and tank reoxygenation rate, further mitigating O_2 stress of fish during growth.

Two inlet configurations (nozzle bar and elbow pipe), and three flowrates (600L/h, 800L/h (normal for trout juveniles), and 1000L/h). In unstocked tanks, feed pellets were distributed as per feeding practice and movement recorded on overhead video cameras. Primary and secondary flow, pellet distribution in the tank, settling location at the tank bottom and number of pellets entering the central drain were assessed. Higher flowrate displayed stronger primary flow in both inlet configurations. Primary flow in the elbow configuration decreased with increasing depth regardless of flow rate. Higher flowrate created stronger secondary flow patterns and the nozzle bar configuration produced higher secondary flow than the elbow pipe, thus, pellets settled earlier than in elbow configured tanks with a nozzle inlet bar. Use of the nozzle bar inlet configuration with higher flowrate showed the most optimal option for waste removal of uneaten feed particles.

Efficiency of the six treatments for reoxygenation of the system was tested in unstocked tanks by using an oxygen scavenger (Na_2SO_3) to deplete the isolated tanks oxygen level to ~0% and then restoring water flow to assess time needed for each configuration to restore to 100% saturation. At lower flow rates (600L/h) there was a lower reoxygenation rate using the nozzle bar compared with the elbow pipe, but at higher flow rates the two configurations did not vary and at 1000L/h could achieve reoxygenation rates of 1.9-3%/min regardless of configuration.

In replicate tanks stocked with 9±0.35g rainbow trout at optimal production conditions (16.5°C, 1-2ppt salinity, > 6.5 mg/l DO, automated O_2 injection) nozzle bar configuration at flow rates (600L/h, 800L/h, 1000L/h), variation in DO and CO_2 level were recorded during 1 hour over feeding (2.5-3% body weight, performed by hand 3 times per day at 9.00, 12.00 and 15.00). DO consumption of each treatment during feeding increased as the fish grew and stocking density increased, but no difference was measured within the flow rate treatments in any of the sampling weeks. CO_2 generation changed as fish size, and thus stocking density, increased but no difference was measured among the flow rate treatments in any of the sampling weeks. Growth performance was assessed for fish in all treatments throughout the experimental period including final weight, FCR, SGR, visual indicators of welfare, as well as condition factor and visceral fat content. Flow rate was not found to have any effect on these factors.

YOU ARE WHAT YOUR PARENTS EAT: BROODSTOCK DIET INFLUENCE ON EGG QUALITY AND PRODUCTION IN CALIFORNIA YELLOWTAIL *Seriola dorsalis*

Li Sun Chin*, Kevin Stuart, Mark Drawbridge, David Bradshaw, Paul Wills and Sahar Mejri

Harbor Branch Oceanographic Institute
5600 US 1 North
Fort Pierce, FL 34946
Email: lchin2021@fau.edu

An optimal broodstock diet contains essential nutrients that are required for successful reproduction and healthy offspring production. Currently, a cut-bait diet is commonly used by the industry to feed broodstock. However, a formulated pellet commercial diet would be a more affordable, sustainable and nutritionally consistent alternative. The aim of this study is to use alternating diets of sardine-squid and commercially available pelleted feed to determine the amount of time needed for broodstock to incorporate nutrients, especially essential lipids, from their diet into their eggs. California yellowtail, *Seriola dorsalis,* broodstock were fed two alternating diets: Vitalis Prima pellets (World Feeds Ltd, UK) and sardine-squid. Each diet was switched after every 6 weeks within the spawning season lasting for a total of 24 weeks and 47 spawns. Egg samples along with biometric data from each spawn were collected. They will then be processed for proximate analysis, essential fatty acid content, and amino acid composition. This data will be used to compare the varying nutritional incorporation happening at each diet switch. This study aims to provide a greater understanding and insight into nutritional assimilation on a temporal scale between the maternal parent and offspring. Our findings will contribute towards the development of a more cost-effective and reliable broodstock diet for farmers and reduce the industry's reliance on cut bait diet, which will improve the economic viability of the California Yellowtail as a staple aquaculture species.

SURVEY FOR *Batrachochytrium dendrobatidis* IN SINGAPORE'S WILD AMPHIBIAN POPULATIONS

Ching Biyun, Brian Tan Zi Yan, Wendy Sng Beng Guat, Michelle Ooi Siew May, Holly Joy Siow, Lin Suxian Cyrena and Wong Wai Kwan

Centre for Animal & Veterinary Sciences, Professional and Scientific Services, Animal and Veterinary Service, National Parks Board (NParks), 1 Cluny Road, Singapore 259569, Singapore

Ching_Biyun@NParks.gov.sg

Global transmissions of *Batrachochytrium dendrobatidis*, also known as chytrid fungus, have been shown to coincide with expansions of international amphibian trade. This disease has caused widespread devastation to amphibian populations worldwide, but certain amphibians have shown resistance to it with high survival rates. The presence of *B. dendrobatidis* in local populations of wild amphibians was tested using a real-time PCR assay on swab samples obtained from individual amphibians. Following up on a previous study done in 2015, this survey was extended over various additional study sites. A total of 1105 amphibians were sampled from 2016 to 2017, out of which 38 were tested positive for *B. dendrobatidis*. The overall prevalence of *B. dendrobatidis* was estimated at 3.44%, with upper and lower 95% confidence limits of 2.52% and 4.68% respectively. No statistical difference was found between the overall *Bd* prevalence of amphibians surveyed in urban parks and the nature reserve. All amphibians tested positive did not show any overt disease symptoms, and the prevalence rates obtained may be representative of the endemic presence

HIGH MORTALITY AND SEVERE ABNORMALITY OF ASIAN SEABASS, LATES CALCARIFER LARVAE REARED IN ELEVATED TEMPERATURE AND ACIDIFIED SEAWATER

Ching, F.F*., Mohammad, M.N.S., Cheong J. Z. D.*, Mohd Faudzi N., and Senoo S.

Borneo Marine Research Institute
Universiti Malaysia Sabah
Kota Kinabalu, Sabah
cfuifui@ums.edu.my

Asian seabass (Lates calcarifer) is an euryhaline aquaculture species and one of the most highly produced fish in Asian region. It is predicted that the oceans will warm and acidify simultaneously as a result of climate change and Asian seabass is particularly vulnerable to this danger. We examined the impact of both elevated temperature and acidified seawater on well-beings of Asian seabass at early larval stage. Larvae were reared under different water temperature ranging from 28, 29, 30, 32 and 34oC and pH of 5.5, 6.0, 6.5 and 7.0 respectively. Larvae reared at 34 oC were discovered dead in 15 days after hatching (dAH) while those reared in pH of 5.5, 6.0, and 6.5 all perished as early as 3 and 4 dAH. Even before the introduction of the first feeding, larvae reared in acidified seawater were severely harmed, resulting in body abnormalities and restricted growth as early as 2 dAH. More than 80% of larvae exhibited body deformities, mostly lordosis and scoliosis when reared in acidified seawater while none were found in elevated temperature. In contrast to other temperatures, cannibalism was shown to be more prevalent and intense when reared at 32 and 34 °C in which further worsen the survivorship of Asian seabass larvae. This study concluded both elevated temperature and acidified seawater adversely affect survival and well-beings of Asian seabass larvae.

GROWTH AND SURVIVAL RATE OF PORTABLE HATCHERY AND EARTHEN POND BRED FRY AND FINGERLINGS OF *Oreochromis karongae* (TREWAVAS, 1941) IN HAPAS AND CONCRETE TANKS

Chinguo C.M.[1,2]., Valeta J.S.[1], Kassam D[1]

1 Lilongwe University of Agriculture and Natural Resources (LUANAR), Africa Centre of Excellence in Aquaculture and Fisheries (AquaFish), Department of Aquaculture and Fisheries Science, P.O. Box 201, Lilongwe, Malawi
2 Ministry of Agriculture, Irrigation and Water Development, Department of Fisheries, P.O Box 593, Lilongwe, Malawi
madachinguo@gmail.com

Aquaculture production in Malawi is low and hinges on very few tilapia species, which have poor fingerling quality, poor growth and low fingerling supply. *Oreochromis karongae* has shown potential for fast growth, but is constrained by low fecundity, hence low fingerling supply. *O. karongae* hatchability has been improved with the introduction of a portable hatchery. The demand to produce uniform size fish for commercial farming has posed a challenge for natural incubation. The research was vital to the finalization of the development of a technology that has high potential to increase fingerling availability and improve fish production in Malawi. The research is in line with Malawi Government's efforts to increase fish production using simple technologies.

A portable hatchery technology was developed to help combat low *O. karongae* fingerling availability in Malawi. *O. karongae* fry was bred in the two hatching units (portable hatchery and earthen ponds) and nursed in concrete tanks and hapas. Treatments were replicated thrice. One-way analysis of variance (ANOVA) was used to determine the significant difference in growth and survival rates of fish in all treatments at 5% significance level. The nursing technology had a significant effect on growth ($P<0.05$) but not survival of fry ($P>0.05$). Survival rate was significantly higher for fry hatched from portable hatchery than from earthen ponds ($P<0.05$).

The study has shown that earthen ponds hatching followed by tank fry nursing promoted better fry growth while portable hatchery prepares the fry for better survival to fingerling stage in both pond hapas and concrete tanks. Farmers intending to promote higher fry survival and maximize returns from selling large quantities should consider adopting portable hatchery.

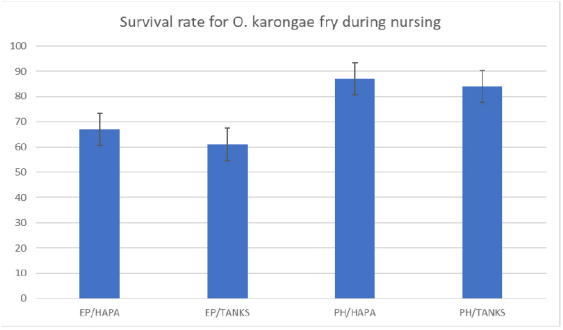


Figure 2. Survival rate of fry during nursing

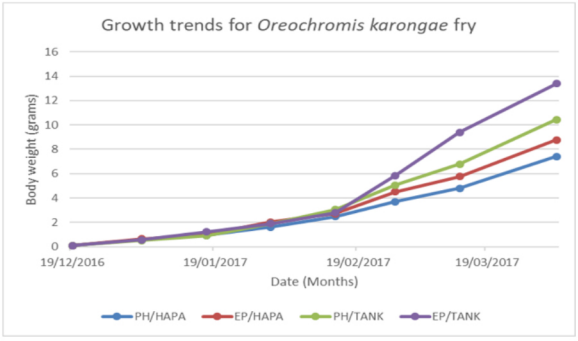


Figure 1. Growth trends for O. karongae fry during nursing.

TRANSCRIPTOMIC ANALYSIS OF RAINBOW TROUT INFECTED BY HIGH AND LOW PATHOGENIC INFECTIOUS HEAMATOPOIETIC NECROSIS VIRUS

Miyoung Cho*, KwangIl Kim, EunYoung Min, Sung-Hee Jung, Suhee Hong

Pathology Research Division
National Institute of Fisheries Science
mycho69@korea.kr

Infectious hematopoietic necrosis virus (IHNV) is one of the most important fish viral pathogens causing acute, systemic and often virulent disease in culture rainbow trout. In this study, we have investigated the infection mechanisms of high and low pathogenic IHNV in rainbow trout by RNA seq-based transcriptome analysis. Healthy rainbow trout were infected with two types of IHNV at 20°C and RNA was extracted from kidney and spleen of juvenile fish to analyze transcriptome at 1, 3, and 5days post-infection. After mapping the RNA sequences to each genome, DEG analysis and KEGG pathway analysis were performed. When IHNV were delivered by intraperitoneal injection (9.8×10^5 PFU/fish), the mortality caused by IHNV-Shizuoka ware 45~70% while IHNV-Nagano were 10~60%. In addition, FoxO signaling and NOD-like pathway had increased in IHNV-Shizuoka infected fish than IHNV-Nagano infected group in KEGG pathway. Also, RT-PCR was used to assess viral load and gene expression of selected immune genes. Analyses of *in vivo* replication showed that P and L gene of IHNV-Shizuoka are constantly more prevalent than M, U and G gene in rainbow trout injected with 1×10^4 PFU/fish for each IHNV genotypes. In analyses of the host immune response, IHNV-Nagano-infected fish showed higher expression of innate immune-related genes such as IFNg, IL 1b while IHNV-IHNV-Shizuoka-infected fish showed higher expression of TNF-α gene.

GHRELIN EXPRESSION AND GROWTH PERFORMANCE BY LONG-TERM STARVATION AND REFEEDING IN STARRY FLOUNDER *Platichthys stellatus*

Younsu Cho*, Jungyeol Park, Irfan Zidni, Hyobin Lee, Jihye Yoon, Hyunseok Jang, Taemin Kim, Hankyu Lim

Department of Fishery Biology
Pukyong National University
Busan 48512
Republic of Korea
Whdbstn12@gmail.com

In fish, appetite control is closely related to feed intake. Feed intake is an essential factor in fish production, and it is directly related to fish growth and improves aquaculture production. Ghrelin, a gene linked to appetite regulation, increases the expression of ghrelin in mammals and fish as the fasting time increases, thereby promoting the individual's appetite. Ghrelin binds to the ghrelin receptor in the pituitary gland to stimulate the secretion of growth hormone and acts on the nervous system to promote food intake. Since appetite control of starry flounder is also closely related to feed intake, basic research on appetite regulation is essential to improve the production of starry flounder.

In this study, we confirmed ghrelin expression and growth in long-term fasting of the starry flounder, and the endocrine changes in ghrelin according to the long-term fasting pattern were approved. In addition, LEAP2, which has been reported as an antagonist of ghrelin, was used as a tool to identify the endocrine pattern of ghrelin. The long-term fasting patterns of the experiments in this study were group A (regular feeding twice daily for eight weeks), group B (2-4 weeks, fed for 6-8 weeks, fasting the rest), and group C (feeding for 4-8 weeks, fasting for the rest of the period) and group D (fasting for 0-8 weeks) were all different.

Group C showed the highest daily growth rate, followed by group B and group A the lowest. According to long-term fasting and refeeding, the expression of ghrelin in the starry flounder increased slightly during the first two weeks of fasting in group B, decreased after feeding, and increased more than seven times during the 4-6 weeks fasting period. Similarly, in group C, the expression level of ghrelin continued to grow during the fasting period. Starry flounder growth hormone, according to long-term fasting and refeeding, tended to increase at four weeks after the fasting period in group B, rose at two weeks and 5-6 weeks in group C, and increased at two weeks in group D, these showed a tendency to increase at 4-5 weeks. According to the results of this study, long-term fasting in starry flounder increases the expression of ghrelin and leads to an increase in the growth hormone and acyl ghrelin. These results will be used basic data to help us understand the physiological role of ghrelin in the endocrine system of fish.

EFFECTS OF DIFFERENT SALINITY CONCENTRATIONS ON GHRELIN EXPRESSION AND GROWTH PERFORMANCE IN STARRY FLOUNDER *Platichthys stellatus*

Younsu Cho*, Jungyeol Park, Irfan Zidni, Hyobin Lee, Jihye Yoon, Hyunseok Jang, Junwook Hur, Hankyu Lim

Department of Fishery Biology
Pukyong National University
Busan 48512
Republic of Korea
Whdbstn12@gmail.com

In aquaculture, salinity is an essential factor regulating fish growth. There is a report that fish can use this energy for growth if the salt concentration is lowered because the energy cost used for osmoregulation is high when the fish usually live in the salt concentration of the sea. Also, a study showed a significant difference in food intake when changed the salt concentration. The expression of ghrelin was also thought to be changed because the word indicating the difference in food intake means a change in appetite. In this study, ghrelin gene expression and growth were analyzed to investigate changes in food intake and growth according to salinity differences. In addition, by confirming the expression of LEAP2, an antagonist of ghrelin, the effectiveness of ghrelin's appetite stimulation was indirectly confirmed, and growth hormone, acyl ghrelin, and glycogen were measured to measure growth hormone changes and growth according to additional food intake.

There was no significant difference in weight gain (%), specific growth rate (%), feed efficiency (%), and daily feed intake of the starry flounder. The ghrelin expression in the starry flounder decreased after feeding and showed a common tendency that the expression increased as the fasting time increased. In all experimental groups, the expression of LEAP2 in starry flounder exhibited an opposite trend to that of ghrelin, with expression decreasing as fasting time increased. In the 0 psu experimental group, a 7-fold increase in ghrelin expression showed an increase in the growth hormone concentration in the brain of the starry flounder. The concentration of acyl ghrelin in the starry flounder of all groups increased with a time difference with the expression of the ghrelin gene. According to this study's results, ghrelin's expression was amplified at 0 psu, leading to an elevation of growth hormone. These results provide basic physiological information for studying fish salinity and ghrelin.

DETERMINATION OF OPTIMAL DOSING REGIMEN OF FLORFENICOL IN SEABASS *(lates calcarifer)* AT DIFFERENT WATER TEMPERATURES WITH PHARMACOKINETIC-PHARMACODYNAMIC APPROACH

Chi-Chung Chou*, Tirawat Rairat, Wan-Cih Ho and Zhu-Ying Fu

Department of Veterinary Medicine, College of Veterinary Medicine
National Chung Hsing University
Taichung, 40227 Taiwan
ccchou@nchu.edu.tw

Bacterial disease is a critical issue in aquaculture industry, and antimicrobials remain the key to ensure food safety. The usage of antimicrobials for aquatic animals should consider the influence of rearing temperature because their pharmacokinetics (PK) is temperature-dependent. Through pharmacokinetic-pharmacodynamic (PK-PD) approach, we investigated the optimal dosing regimens of florfenicol (FF) at multiple minimum inhibitory concentration (MIC) levels in seabass rearing at both 25 °C and 30 °C.

The serum concentrations of FF versus time after a single oral administration (10 mg/kg, PO) at 2 rearing temperatures were shown in Figure 1. The corresponding PK parameters indicated that rearing at 30 °C resulted in longer absorption half-life, shorter elimination half-life, faster clearance, lower apparent volume of distribution and smaller area under the serum concentration-time curve than at 25 °C (Table 1). The optimal dosages were then calculated with related PK parameters using equation for multiple-dose extravascular administration. A 1.5-2.0 fold differences in dosage between 2 rearing temperatures (Table 2) at each MIC level were evident. Our results demonstrated that at the common recommended dose of 10-15 mg/kg/day, it was likely under-dosed especially in warmer rearing temperature, which might cause ineffective medication. Whether or not non-linear PK occurs at higher dosage as was discovered for Nile Tilapia warrants further study.

TABLE 2. Calculated optimal dosage (mg/kg/day) of FF in seabass (n = 4) at different temperature levels and MIC values.

MIC	25 °C	30 °C
1	7.38 ± 1.97	16.26 ± 6.02
2	14.75 ± 3.95	27.28 ± 11.07
3	22.13 ± 5.92	38.29 ± 19.21
4	29.50 ± 7.89	49.30 ± 27.86

TABLE 1. Selected PK parameters (mean ± SD) of FF (10 mg/kg, PO) in seabass at different temperature levels (n = 4).

Parameter	25 °C	30 °C
Ka (1/h)	3.32 ± 1.67	2.03 ± 0.97
$t_{1/2Ka}$ (h)	0.27 ± 0.18	0.39 ± 0.13
α (1/h)	1.07 ± 0.38	1.85 ± 0.76
$t_{1/2\alpha}$ (h)	0.72 ± 0.26	0.41 ± 0.12
β (1/h)	0.062 ± 0.019	0.097 ± 0.016
$t_{1/2\beta}$ (h)	12.09 ± 4.09	7.26 ± 1.19
C_{max}(μg/mL)	16.89 ± 6.97	25.82 ± 10.49
T_{max} (h)	0.69 ± 0.33	0.63 ± 0.20
AUC (h*μg/mL)	117.6 ± 33.2	93.50 ± 28.44
Vz/F (L/kg)	2.49 ± 1.59	1.80 ± 0.66
CL/F (L/kg/h)	0.136 ± 0.041	0.173 ± 0.058
MRT (h)	14.72 ± 5.90	7.62 ± 1.45

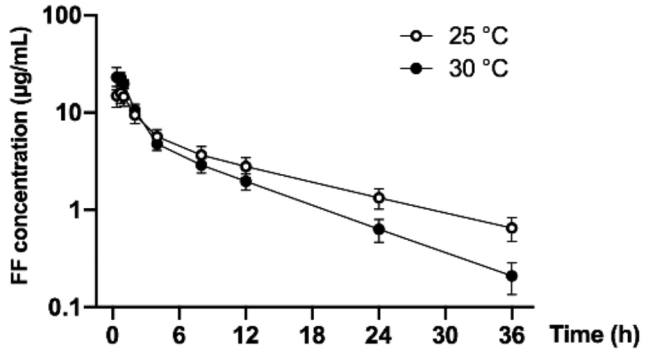


FIGURE 1. Semi-logarithmic plot of serum concentration-time profile of 10 mg/kg FF in Seabass at two temperatures (n=4).

MOVING SHRIMP FARMING INDOORS: FEEDS AND FEEDING CONSIDERATIONS

M A Kabir Chowdhury*[1] and Albert Tacon[2]

[1]Jefo Nutrition Inc., 5020 Jefo Avenue, Saint-Hyacinthe, QC J2S 7B6, Canada
kchowdhury@jefo.ca
[2]Founder & Owner of Aquahana LLC, Aquaculture Nutrition & Feeds Consulting Service
93 Pinana St., Kailua, HI 96734
agjtacon@aol.com

Since 2015, global farmed shrimp production increased from three million metric tons (MMT) to approximately five MMT. This is largely driven by Ecuador and India, where lower density production systems prevail. Ecuador has surpassed the elusive one MMT mark in 2021, the only country that achieved the feat in over a decade. Sustainability of such growth can be questionable as it happened within the existing production systems highly vulnerable to externalities and subjected to frequent disease outbreaks. The overall growth in Asia is far more modest compared to that of Ecuador with less room for improvement of the existing farming systems. In recent years, there is a global shift from extensive outdoor to intensive or super-intensive indoor farming. A trend towards more environmentally controlled systems. These systems may include bio-floc, raceways, or recirculating aquaculture (RAS) systems or a modular hybrid system. Artificial intelligence (AI) is also increasingly playing a crucial role in disease prevention, water quality, and feeding monitoring and management.

In closed systems, the feed and feeding should also consider balancing the ecosystem to maintain suitable environment for cultured animal. The feed needs to be of good physical and nutritional quality to support fast growing and healthy animals, while maintaining an optimum water quality. For example, for a stable bio-floc system, the preferred C:N ratio is 12:1 requiring focus on indigestible content when formulating a bio-floc diet. Similarly, RAS feeds should also meet several criteria. They should be balanced with essential nutrients, highly digestible, and of excellent physical quality.

This paper focuses on technological advances in shrimp farming systems, novel interventions for improved management, criteria to consider when formulating the feed, as well as feeding management.

ORGANIC FISH FARMING IN HONG KONG: PRESENT STATUS AND FUTURE PROSPECT

Ka Hou Chu[1,2], Kwok Cheong Chung[1,2], and Jonathan W.C. Wong[3]

1School of Life Sciences, The Chinese University of Hong Kong, Hong Kong SAR China
kahouchu@cuhk.edu.hk, kcchung@cuhk.edu.hk
2Department of Science and Environmental Studies, The Education University of Hong Kong, Hong Kong SAR, China
3Departmet of Biology, Hong Kong Baptist University, Hong Kong SAR, China
wcwong@hkbu.edu.hk

Presenter: Ka Hou Chu

The supply of aquatic food products in Hong Kong is largely dependent on capture fisheries. In 2019, production from the aquaculture sector was only 5% in value of the total fisheries production. Production from local inland ponds, covering an area of approximately 1,131 ha, was three times that of mariculture. With an endeavor to provide safe and reliable aquaculture products to local consumers, the Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong SAR Government launched an Accredited Farm Scheme in 1994. Moreover, AFCD started to explore organic fish farming and laid down a code of practice for organic aquaculture in collaboration with the Hong Kong Organic Resource Centre (HKORC), which commenced its certification service on organic aquaculture in 2010. Besides HKORC, the Hong Kong Organic Certification Centre, established by the Hong Kong Organic Agriculture & Ecological Research Association, also provides certification service to local and mainland organic farms.

Currently, eleven accredited organic fish farms are operating in Hong Kong with a total certified culture area of 3.5 ha, the largest being Wah Yuen Farm with 1.8 ha. In addition, two more fish farms were accredited and under conversion. Such a minute portion of pond area engaged in organic production reflects that organic aquaculture is not well received by the local fish farmers. The main challenges facing pond aquaculture practices in Hong Kong are strong competition from imported aquatic products, limited choices and supply of fry, uncompetitive production costs, and continual decrease in fishpond areas due to urban development. Switching to organic production will incur potential problems such as sourcing of organic feed and organic fry, changing operation practices, and unknown market demand due to poor public awareness of organic aquaculture. As most pond aquaculture in Hong Kong is conducted on a family basis, fish farmers are reluctant to switch to organic production methods due to unknown risks and challenges associated with the switch, which might imply increased operation costs and hence reduced profits. Meanwhile the universities in Hong Kong are undertaking research efforts to provide a reliable source of organic feed to local fish farmers at reasonable prices. It is hoped that with the efforts from universities and support from AFCD, organic fish farming in Hong Kong will grow and provide safe and quality products to consumers.

What will audience learn from your presentation?
Research and development of organic fish farming in Hong Kong, with relevance to other areas in Southeast Asia

Biography of presenting author
Prof. Ka Hou Chu received his bachelor's degree from the University of California at Berkeley in 1976 and completed his PhD study at the Massachusetts Institute of Technology/Wood Hole Oceanographic Institution Joint Program in Oceanography in 1984. After retirement at The Chinese University of Hong Kong, he is now a Visiting Professor at the Education University of Hong Kong and a Visiting Scholar at the Hong Kong University of Science and Technology. Prof. Chu has diverse research interests in marine science, published about 240 papers on marine biology and biotechnology, molecular systematics and ecology, fisheries and aquaculture, biodiversity conservation, and seafood allergy. He serves as associate editor of three international journals.

EFFECTS OF DIFFERENT FEED INCLUSION LEVELS OF ASTAXANTHIN AND CAPSANTHIN ON THE SKIN PIGMENTATION OF RED SNAPPER (*Lutjanus malabaricus)*

Gavin CHUA Shi Wei*, Xu Qunying, Wong Yao Xing, Lee Jia Ying, Ching Mei Yu Janelle, Sheryl Lim Yu Zhen, Joseph Angelo Uichanco, Dorian Baraba, Liew Woei Chang, Jose A. Domingos

Tropical Futures Institute, James Cook University Singapore, 149 Sims Drive, 387380, Singapore
Marine Aquaculture Centre, Singapore Food Agency, 11 St John Island, 098659, Singapore
gavin_CHUA@sfa.gov.sg

Fish skin pigmentation is a key indicator of carcass quality and is highly dependent on multiple factors and the ability to control skin pigmentation presents a way for farmers to reach a higher farm gate price. In this study, through two independent experiments, we investigated the effects of different dietary carotenoids and additives on skin coloration (specifically red pigmentation) of Malabar red snapper (*Lutjanus malabaricus)*. The first experiment tested the effects of natural astaxanthin, capsanthin and capsanthin supplemented with additives on juvenile red snappers (28.1 ± 5.5g) on diets of control, 100mg/kg natural astaxanthin, 100mg/kg of capsanthin and 100mg/kg capsanthin with additives (Maxilys & Aflorin BF Innovad®). The 100mg/kg natural astaxanthin diet had significantly ($P < 0.05$) increased redness in skin pigmentation (CIELAB a value = 11.67 ± 0.66) compared to the control group (a value = 3.23 ± 0.43), while the capsanthin (a value = 3.86 ± 0.50) and the capsanthin with additives diets (a value = 4.40 ± 0.42) had no significant effect ($P > 0.05$). The second experiment was carried out with adult snappers (741.7 ± 212g) on diets of control, 50mg/kg synthetic astaxanthin, 500mg/kg synthetic astaxanthin and 500mg/kg capsanthin, whereby all dietary carotenoid treatments significantly ($P < 0.05$) increased redness in skin pigmentation (CIELAB a value ~6.15 ± 2.22 to 8.04 ± 5.39) compared to the control group (a value = 4.94 ± 2.11). Both trials show the potential to increase the skin pigment redness of *Lutjanus malabaricus* through dietary addition of both natural and synthetic astaxanthin. However, an unexpected negative effect on feed consumption and growth rates were observed, suggesting that if colour is to be improved in the species via coating feeds with astaxanthin, a finishing diet should be used two to four weeks prior to harvest to avoid loss of farm productivity.

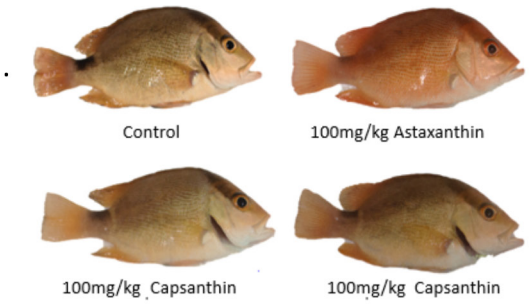


Fig 1: Trial 1 Juvenile Red Snapper Coloration Results

Fig 2: Trial 2 Adult Red Snapper Colouration Results

AQUAPONICS AS A CROSS CURRICULAR TEACHING TOOL IN THE SECONDARY SCHOOL CLASSROOM

David J Cline

203 Swingle Hall
Auburn University, AL 36849
Clinedj@auburn.edu

Aquaculture has, perhaps more than any other agriculture or academic content area, the potential for interdisciplinary and collaborative instruction. The multifaceted nature of aquaculture provides numerous opportunities and classroom activities to engage students in any number of subject matter areas. Students are confronted with complex problems that allow them to experiment and build their problem solving and cooperative learning skills. These problems help translate the academic principles; they are required to learn, into real-world applications. The hands-on nature of many of these activities helps hold student interest and provide ongoing motivation. Concepts learned can be applied in many other fields and can help better prepare students for higher education. Table 1 indicates other academic areas that relate directly or indirectly to aquaculture.

The connections to biology and chemistry are readily apparent but connections to other disciplines require more effort. Our role as Extension educators and content specialists is to help teachers make and explore these connections. This presentation describes methods to maximize the cross curricular capacity and effectiveness of an aquaculture teaching platform.

IMPACT OF NET CLEANING ON ATLANTIC SALMON, *Salmo salar*, GILL HEALTH

Benjamin Gregory James Clokie*, Ahmed Elsheshtawy, Are Nylund, Trond Einar Isaksen, Linda Andersen, Allan Beveridge, Simon MacKenzie

Institute of Aquaculture
University of Stirling
Stirling, FK9 4LA
b.g.clokie@stir.ac.uk

Sustained intensification of salmon aquaculture has resulted in the industry facing a myriad of challenges especially in the seawater grow-out stage where net biofouling provides a crucial obstacle for the global salmon industry. A diverse range of organisms can be found on on cage nets including the hydroid *Ectopleura larynx*, the amphipod *Jassa falcata*, blue mussel *Mytilus edulis*, and algae *Saccharina latissima* and *Polysiphonia stricta*. Net occlusion not only reduces water quality by limiting water exchange, reducing oxygen levels and waste removal there is also the potential to reduce the efficacy of cleaner fish by offering alternative food sources. Biofouling also impacts cage infrastructure, increasing pressure on mooring loads and reducing the net pen volume though deformation. Mitigation relies upon the use of antifouling net coatings or *in situ* cleaning with the later performed by remotely operated cleaning rigs coupled with high pressure spinning jets. Net cleaning may occur as frequently as every two weeks, dependent upon biofouling accumulation rates, cultured species requirements, and net coating. During cleaning, flushed particles are discharged directly into the surrounding environment within and around the cage leading to concerns regarding the impact on gill health.

In this project we sought to determine the impact of this procedure on gill health with particular relevance to the microbiome. Identification was performed using a novel library protocol that included a titration step prior to building 16S rRNA libraries that was optimised for low 16S copy samples.

Data obtained from these trials showed marked differences on the gill surface bacterial communities in response to treatment before and after cleaning driven by a reduction in richness. Evenness was maintained between sampling events with further analysis showing an increase in opportunistic and potentially pathogenic bacteria. Gill microbial dysbiosis may be attributed to physiochemical changes in the water quality and other biological changes related to the net cleaning event and have potential to increase the acceptability to infection. Our data relies upon a robust method for analysing microbiome diversity and provides an important tool for using the microbiome as an informative indicator of fish gill health and could be used to define when appropriate interventions are utilised.

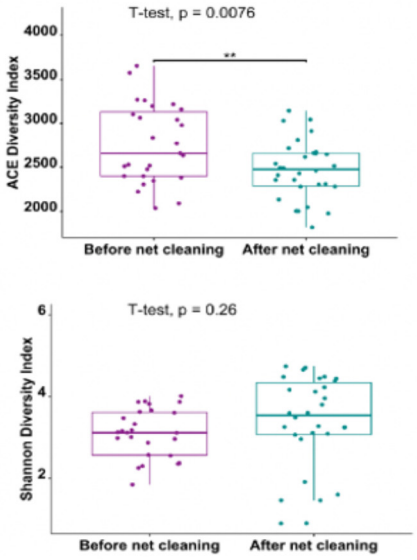


Figure 1. Comparison of richness (A) and evenness (B) following a net cleaning event. Data shows significant changes in diversity following treatment compared to evenness

OPPORTUNITIES FOR EXPANSION OF THE NORTHERN AUSTRALIAN AQUACULTURE INDUSTRY

Jennifer Cobcroft*, Kyall Zenger, Jan Strugnell, Amy Diedrich, Rob Bell, Jess Fitzgerald, Simon Irvin, Greg Coman, Kylie Penehoe, Michael Davis, Kim Hooper, Jo-Anne Ruscoe, Dean Jerry

Centre for Sustainable Tropical Fisheries and Aquaculture
James Cook University
Townsville, 4811 Queensland
Australia
jennifer.cobcroft@jcu.edu.au

The Australian Government is committed to a policy in support of doubling the Australian aquaculture industry to $2 billion over the 10 years to 2027. Around 17% of Australian aquaculture value currently comes from northern Australia, despite northern Australia representing 53% of the national land mass, at ~4.8 million km^2, with extensive options for marine and freshwater aquaculture sites based on geophysical assessment. Aquaculture has the potential to increase primary production, regional employment, and infrastructure investment in northern Australia. In response to this opportunity, the Cooperative Research Centre for Developing Northern Australia (CRCNA) commissioned a situational analysis of the northern Australian aquaculture industry. The goal of that project was to develop a realistic, inclusive, industry-supported Vision 2030, including recommendations for the industry and the CRCNA to inform and guide future investment.

Annual gross value product (GVP; FY17) from northern Australian aquaculture was around $223 million, predominantly barramundi (33%), prawns (32%), and pearls (non-edible) (31%), with several other species (tropical rock oysters, redclaw and other finfish) making up the remaining 3% of value. Aquaculture in northern Australia has demonstrated a slow increase in production compared with aquaculture in southern Australia. Through consultation with 117 stakeholders in an online survey, and over 150 stakeholders in workshops, the project collected data on the key industry challenges and explored solutions to enable the industry to achieve its Vision 2030.

Seven strategic recommendations were proposed by the CRCNA project to address key barriers to industry development, including: bolster biosecurity; infrastructure for Aquaculture Development Hubs; market development and access; increase availability of skilled staff; build Indigenous aquaculture opportunities; target RD&E to key industry needs; and stronger and adaptive governance. Investment in existing key industries (barramundi, prawns and pearls) and in emerging sectors where government and industry support and investment are driving establishment (tropical rock oysters, lobsters, sea cucumbers, redclaw, and other finfish) have high potential to underpin growth.

AQUAKULTOR: USING MOBILE GAME TECHNOLOGY TO ENHANCE TERTIARY EDUCATION IN APPLIED AQUACULTURE GENETICS

Jennifer Cobcroft*, Esther Fink, Joseph Uichanco, Emma Maynard, Mathieu Besson, Kyall Zenger

Centre for Sustainable Tropical Fisheries and Aquaculture
James Cook University
Townsville, 4811 Queensland
Australia
jennifer.cobcroft@jcu.edu.au

AquaKultor is a fish-farming simulation game for mobile devices developed by aquaculture researchers, François Allal of IFREMER (French Research Institute for Exploitation of the Sea) and Mathieu Besson of INRA (National Research Institute of Agronomy), in conjunction with TOMAGameStudio. The game was launched in August 2018 and it challenges players to run and manage every dimension of a fish farm.

A team of James Cook University (JCU) educators was awarded an internal Learning and Teaching Grant from JCU to establish the game as an alternative resource in the 'Aquaculture: Stock Improvement' subject at Australian and Singapore campuses. The team employed a Universal Design for Learning (UDL) framework with the aim to provide:

- an equitable educational experience by including subject information in different formats, incorporating game-related activities
- to help students make the connection between the game and learning outcomes in their course;
- flexible materials, including instruction scaffolds for performing game tasks, an introduction to game mechanics, opportunity for students/players to help each other through discussion, and technical support for students to use their own mobile devices for learning;
- visual and auditory cues to help learners engage with scientific principles in Aquaculture;
- an opportunity to learn from mistakes that would not be possible in the real world;
- learning experience catered for varied skills and ability.

Evaluation of use of the game in teaching demonstrated that it provided important context to students with limited previous exposure to aquaculture. For students with more prior aquaculture knowledge, it placed the application of selective breeding approaches and decision-making in the broader aquaculture business context, and was an important complement to the deep-learning material provided on the stock improvement course. There were some suggestions made to include other aspects of aquaculture, such as the ability to manipulate feed formulations, and a desire to see "more dudes on boats" to convey the practical aspects of sea-pen farming. Overall, the game was well-received by students and they encouraged its continued use in tertiary education.

EFFECTS OF DIETARY CHOLESTEROL AND PHOSPHOLIPID ON THE GROWTH PERFORMANCE OF JUVENILE SLIPPER LOBSTER *Thenus australiensis*

Mohamed B. Codabaccus*, Michael J. Landman, Chris G. Carter, Quinn P. Fitzgibbon, David S. Nichols, Greg G. Smith

Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania, Private Bag 49
Hobart, TAS, 7001, Australia
mohamed.codabaccus@utas.edu.au

Cholesterol (Chol) is often regarded an essential nutrient for decapod crustaceans owing to low to no capacity for *de novo* synthesis. Dietary requirements for cholesterol in crustaceans have been shown to depend on multiple criteria such as species, life-stage, natural diet, feeding behaviours, and nutrient interactions. This study was conducted to test the hypothesis that dietary Chol is essential for early juvenile slipper lobster, *Thenus australiensis*, and further to explore the potential for interactions between dietary Chol and phospholipid (PhosL). An 8-week experiment was conducted using six experimental feeds in a 3 x 2 factorial design using three supplemental Chol levels of 0, 0.2 and 0.4% dry matter (DM) at two different supplemental PhosL concentrations of 0% and 1.0% DM. Results showed that dietary Chol concentrations of 0.2 and 0.4% DM resulted in 2 to 3-fold greater growth responses and overall higher nutritional condition compared to 0% DM dietary Chol. With no inclusion of dietary PhosL, survival was significantly lower (46%) at 0% DM dietary Chol compared to 0.2 and 0.4% DM dietary Chol (96-100%), while survival of SL was similar for all dietary Chol concentrations at 1% DM dietary PhosL (71-92%). Survival was improved by supplementing the 0% Chol feed with 1.0% PhosL. However, all surviving lobsters at 0% DM dietary Chol, regardless of dietary PhosL level, were in poor nutritional condition upon conclusion of the experiment as judged by the lower hepatosomatic index. Apparent feed intake (AFI) was significantly higher at dietary Chol ≥ 0.2% DM but was slightly lower for each corresponding dietary Chol level at 1% DM dietary PhosL. Similar growth rates with lower AFI resulted in improved feed conversion ratios (FCR) at 1% DM dietary PhosL. This study confirms that dietary Chol is essential for early juvenile *T. australiensis*, while the provision of 1% DM dietary PhosL provided minor additional benefit compared to dietary Chol alone. The supplemental dietary Chol requirement for early juvenile *T. australiensis* appears to be relatively low at 0.2% DM in a semi-purified feed, and higher levels may not provide additional growth enhancing benefits.

SHRIMP EARTH-POND SEDIMENT MICROBIOME EVOLUTION THROUGH HALOPHYTE CULTURE FOR BIOREMEDIATION PERSPECTIVES

Marie COLETTE*, Nolwenn CALLAC, Linda GUENTAS, Luc DELLA PATRONA

marie.colette@etudiant.unc.nc
French Institute for Research in the Science of the Sea (IFREMER)
Institute of Exact and Applied Sciences (ISEA)
University of New Caledonia
98851 Noumea, New Caledonia

Drainage and drying of the shrimp earthen-pond at the end of each production cycle allows the aeration of pond bottom sediment and enhance microbial decomposition of the accumulated organic matter (Yang et al., 2017). However, pond drying is not always beneficial for the microbial communities as water stress could impaired the bacterial decomposition activities (Schimel 2018, Boyd and Pippopinyo, 1994). In New Caledonia, high parts of the internal earth dikes of shrimp farms are colonized by halophytic plants, able to thrive in extreme salinity conditions. The use of halophyte species as crops for remediation of shrimp pond sediment was explored. Plants interactions with soil environment can change surrounding soil microbial communities and thereby affect nutrient cycling and organic matter degradation. Thus, we aim to answer if **1)** *halophyte can change the composition of the microbial communities inhabiting the pond sediment*, **2)** *microbial communities are different according to the halophyte species and moisture conditions,* **3)** *distinct microbial communities differ functionally.*

We conduct experimental greenhouse study where three halophyte species (*Sarcocornia quinqueflora*, *Suaeda australis* and *Atriplex jubata)* were separately grown in pots filled with shrimp earthen-pond sediments. At the end of the experiment, upper part of the sediments was collected to explore the active microbial diversity present in each soil condition. For that, RNA extractions, reverse-transcription and sequencing of the V4 region of the 16S rRNA molecule and bioinformatics analysis were done.

Our data highlight that dry sediment shares very few common taxa with sediment colonized by halophytes species. Venn diagram evidenced great proportion of specific taxa following moisture conditions and halophytes species. Thus, at family's level, there is clear dissimilarities of sediment specific microbiome following sediment moistures condition and halophytes species.

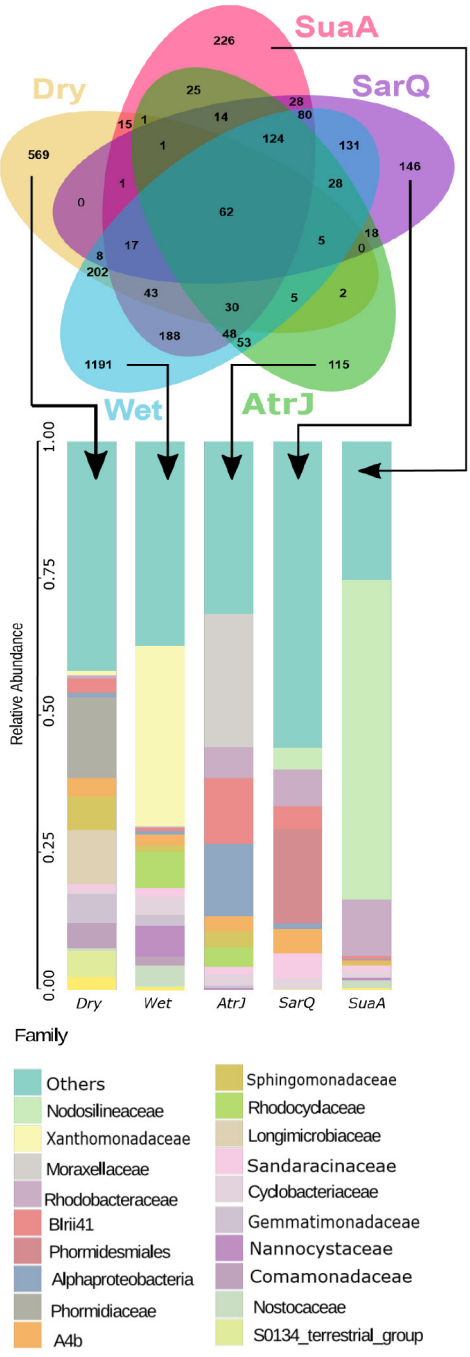


Figure 1 : Venn diagramm of shared and specific taxa from dry, wet sediment and sediment colonized by *Suaeda australis* (SuaA), *Sarcocornia quinqueflora* (SarQ) and *Atriplex jubata* (AtrJ). Stacked bar plot represent relative abundance of the different sediment specific taxa.

HYDROLYSIS-LIGNIN AS A MULTI-FUNCTIONAL ADDITIVE IN ATLANTIC SALMON FEED IMPROVES FISH GROWTH PERFORMANCE, PELLET QUALITY AND SHIFTS GUT MICROBIOME

Stefanie M. Colombo*, Zeyu Zhang, David F. Wong, Zhirun Yuan

Department of Animal Science and Aquaculture
Faculty of Agriculture, Dalhousie University
Bible Hill, Nova Scotia, Canada
scolombo@dal.ca

Functional feed ingredients are highly sought out in the aquaculture industry to improve fish health, growth, feeding efficiency and sustainability. There is also growing demand for bio-products from sustainably harvested, renewable, non-food resources. The objective of this study was to evaluate hydrolysis lignin (H-lignin), derived from wood biomass, as a multi-functional component of aquafeeds, with potential beneficial effects on pellet durability, growth, intestinal health, and gut microbiota.

Atlantic salmon parr in freshwater were fed diets that contained one of two types of H-lignin, HL1 and HL2, each at inclusion rates of 15, 30, and 50 g/kg (wt/wt) of the diet, in comparison to a control diet. Salmon (28.8 ± 1.1 g fish^{-1}) were fed to satiation twice daily for 16 weeks, with triplicate tanks per treatment. After 16 weeks, salmon were measured for weight and length, whole carcass, hindgut, and digesta contents were sampled. Pellet durability increased from the control to 30 g/kg H-lignin but decreased at 50 g/kg. Salmon fed diets with HL1 at 15 and 30 g/kg showed up to 16% higher weight gain, as well as lower feed conversion ratios compared with salmon fed the control diet. There were no significant differences in whole body composition or intestinal morphology. Microbial characterization (16S) revealed lower abundance of Proteobacteria, higher abundance of Mycoplasmataceae, and increasing Lactobacillaceae abundance with higher HL1 inclusion.

This study is the first to demonstrate that salmon fed diets with wood-derived H-lignin (HL1) at 15 g/kg and 30 g/kg inclusion exhibit superior growth performance compared with salmon fed the commercial-type control diet, and shows potential as a functional feed additive for salmon, and potentially other species.

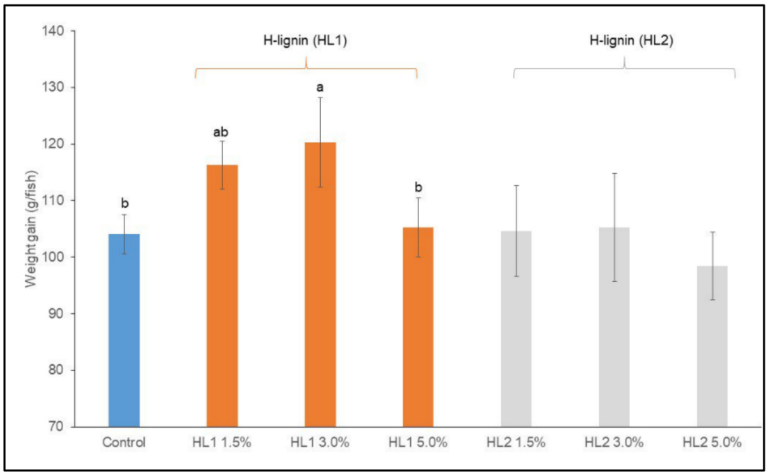


Figure 1. Weight gain of salmon fed increasing levels of feed additives H-lignin 1 and H-lignin 2 after 16 weeks of feeding

FUNCTIONAL FEEDS FOR SENEGALESE SOLE POST-LARVAE

Luís Conceição, Ana Teresa Gonçalves, Wilson Pinto, Sara Castanho, Ana Mendes, Diogo Peixoto, Mariana Hinzmann, Rita Colen, Maria Morais, Cátia Marques, Joana Silva, João Navalho, Helena Abreu, Pedro Pousão, Jorge Dias, Sofia Engrola, Benjamin Costas

SPAROS, Lda. Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
luisconceicao@sparos.pt

Senegalese sole (*Solea senegalensis*) is one of the most promising species for Southern European aquaculture. However, control of opportunistic diseases caused by pathogens such as *Tenacibaculum maritimum* is a bottleneck for sole aquaculture. Such diseases are favoured by some current production conditions in intensive aquaculture such as high density, temperature fluctuations, fish handling, and their synergistic effects, which are capable of inducing a stress response. Nutrition has been shown to have a strong impact on fish health, in particular in what concerns immune competence and intestinal health. This lead to the concept of functional feeds which in already widely adopted in ongrowing fish operations but not so much in hatchery and nursery phases. Micro and macro -algae are rich in bioactive compounds with potential to increase robustness of early life stages of fish. The aim of this study was to evaluate the immune response of S. senegalensis postlarvae when fed microdiets fortified with different algae species, and their blends, are included as functional ingredients to boost immunocompetence and overall performance.

Two trials were conducted with Senegalese sole post-larvae were fed a Control diet, and the same diet fortified with 3% to 6% inclusion of micro and/or macro -algae. The trials started at 34 days after hatching (DAH) and lasted 4 weeks. Growth performance, survival, and whole-body immune response were assessed, as well as the expression of a panel of genes related with immune response (i.e., *HAMP*, *complement C3*, *gLys, IL1b, IL10*, *TLR1* and *TRL5*). A follow-up trial was performed after the second trial to evaluate the capacity of post-larvae to cope with a *Tenacibaculum maritimum* infection inflicted by bath exposure.

The first trial indicated that biomasses of *Nannochloropsis sp*., *Skeletonema sp*. and *Tetraselmis striata*, improved the innate defence mechanisms in Senegalese sole, while *Nannochloropsis* also improved antioxidant capacity.

The second trial showed that a blend of *Nannochloropsis sp*., and *Gracilaria gracilis* enhanced growth performance of sole, as well as its innate defence mechanisms and antioxidant capacity. When challenged with *T. maritimum* sole responded with a 20% increase in survival when compared to the CTRL group, validating that algae blends inclusion in post-larvae microdiets improve immune competence of Senegalese sole post-larvae, and pave the way for the use of functional feeds in marine fish hatcheries.

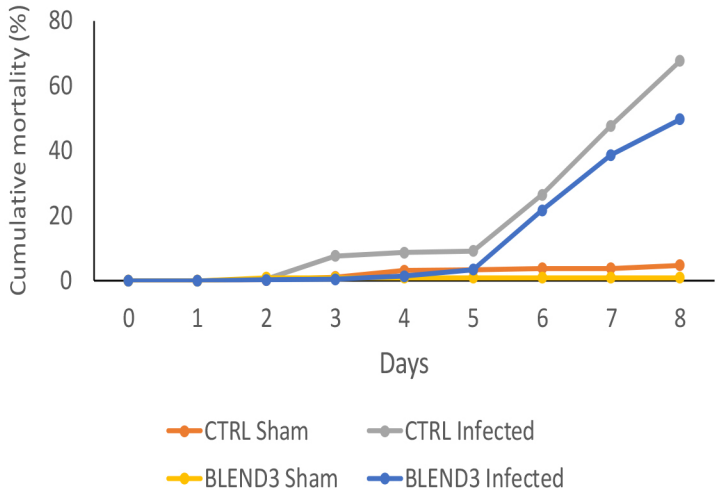


Figure 1. Cumulative mortality of Senegalese sole post-larvae challenged with Tenacibaculum maritimum after being feed with 4 dietary treatments for 28 days.

DEVELOPMENT OF A FUNCTIONAL MICRODIET FOR WHITELEG SHRIMP POST-LARVAE

Luís E.C. Conceição, André Barreto, Wilson Pinto, Benjamin Costas, Rui J.M. Rocha

SPAROS, Lda
Olhão (Portugal)
luisconceicao@sparos.pt

The whiteleg shrimp (*Penaeus vannamei*) farming industry requires that hatcheries improve rearing protocols in order to achieve better quality post-larvae (PL). The early life-stage is a critical period in shrimp development, with the weaning from live-feeds to inert microdiets being severely influenced by diet quality. Microdiet quality has a large impact on shrimp performance in the long-term, affecting the downstream production of high-quality juveniles. Therefore, there is room for optimizing a weaning diet for shrimp larvae/post-larvae, in particular to improve robustness. In fact, industrial shrimp farming is characterized by inconsistent yields as it is quite susceptible to severe pathogenic outbreaks. Once shrimp cannot be vaccinated and the use of antibiotics is legally limited, functional dietary additives can be used as a prophylactic alternative to stimulate the shrimp immune condition. This work summarizes the development of a functional premium microdiet boosting shrimp post-larval performance and quality.

DEVELOPMENT OF A FUNCTIONAL MICRODIET FOR WHITELEG SHRIMP POST-LARVAE

Luís E.C. Conceição, André Barreto, Wilson Pinto, Benjamin Costas, Rui J.M. Rocha

SPAROS, Lda
Olhão (Portugal)
luisconceicao@sparos.pt

The whiteleg shrimp (Penaeus vannamei) farming industry requires that hatcheries improve rearing protocols in order to achieve better quality post-larvae (PL). The early life-stage is a critical period in shrimp development, with the weaning from live-feeds to inert microdiets being severely influenced by diet quality. Microdiet quality has a large impact on shrimp performance in the long-term, affecting the downstream production of high-quality juveniles. Therefore, there is room for optimizing a weaning diet for shrimp larvae/post-larvae, in particular to improve robustness. In fact, industrial shrimp farming is characterized by inconsistent yields as it is quite susceptible to severe pathogenic outbreaks. Once shrimp cannot be vaccinated and the use of antibiotics is legally limited, functional dietary additives can be used as a prophylactic alternative to stimulate the shrimp immune condition. This work summarizes the development of a functional premium microdiet boosting shrimp post-larval performance and quality.

Four trials were conducted with white leg shrimp PL (initial wet weight 3-13 mg) reared under standard procedures in triplicate tanks. Shrimp were reared at RIASEARCH Lda facilities (Portugal), at 27-28ºC, and fed on microdiets ad libitum for 18-21 days.

Firstly, the protein requirement at the post-larval stage was evaluated; 6 diets ranging from 34 to 63% crude protein were used. Results show a dose-response pattern for growth of shrimp PL (3-70mg wet weight), with a break-point at 47.1% dietary crude protein.

Protein sources were subsequently tested using 4 diets containing either fish meal, squid meal, krill meal or a balanced mix of these three (52% crude protein), as main ingredients. Results show no significant differences between treatments for final body weight, and survival was lower in the fish meal-based diet.

The cost-effectiveness of 3 microdiet prototypes was then assessed. Results showed no differences for PL survival (75 - 87%) or economic feed conversion ratio, but shrimp PL fed an ultra-premium diet achieved a significantly higher final body weight and relative growth rate (19.8 %/day) than those fed the standard (15.6 %/day) or premium (17.1 %/day) diets.

Finally, 4 experimental microdiets were evaluated: a commercial like diet as positive control (PC); a negative control diet (NC) based on PC but lower in vitamin C and E; the NC diet supplemented with taurine plus methionine (T+M); and the NC diet supplemented with β-glucans (BG). Results suggest that vitamin C and E dietary levels impact susceptibility to pathogens, and that the use of β-glucans decreased lipid peroxidation and boosted the antioxidant and immune status.

In short, this study shows that: 1) A dietary protein requirement of 47.1% was established for shrimp PL (3-70 mg wet weight); 2) Squid and krill meals proved to be good sources of protein for the early development of shrimp, as well a mixture of these two with a high quality fish meal: 3) Higher quality diets can be advantageous in early phases of production, leading to a reduction of time to reach commercial size, and savings in operational costs; and 4) The use of functional dietary additives, such as β-glucans, vitamins C and E, bring benefits to the antioxidant capacity and immune condition of the shrimp PL.

Acknowledgements: This work is part of the project FA_05_2017_005 SHELLWIN, financed by the Blue Fund program of the Ministry of the Sea, Portuguese Republic.

DEFORMITIES, GROWTH AND SURVIVAL OF SABLEFISH LARVAE *Anoplopoma fimbria* FED TAURINE ENRICHED LIVE FEEDS

Matthew A. Cook*, Jonathon S.F. Lee, Ronald B. Johnson and Frederick R. Goetz

Manchester Research Station, NOAA/NMFS
7305 Beach Dr. E. Port Orchard, WA 98366
matt.cook@noaa.gov

Sablefish (*Anoplopoma fimbria*) is a high value fish found throughout the Northeastern Pacific. There is strong interest to develop sablefish as an aquaculture species. High percentages of deformities are a problem during larval rearing. To improve aquaculture protocols for sablefish larvae and reduce deformities we studied the effects of taurine, an important amino sulfonic acid, during the live feeding stage on deformities, growth, and survival from first feeding to juveniles. Reducing deformities and improving growth and survival during early larval development is necessary and important to aquaculture facilities, researchers and ultimately consumers.

In separate trials we studied the effects of feeding zero to high concentrations of taurine (~zero, 20mg/g, 40mg/g, 80mg/g) in rotifers on deformities, growth and survival of sablefish larvae at weaning and in juveniles raised from those treatments (trial one). For trial two we studied the effects of dietary taurine verses temperature during the rotifer feeding period. Larvae in cold temperature treatments (with and without taurine) spent the first 9 days at 10°C before being increased to 15°C. Larvae in warm treatments (with and without taurine) spent 4 days at 10°C degrees prior to being increased to 15°C. In the third trial we studied the effects of feeding taurine enriched *Artemia* nauplii on deformities, growth and survival of sablefish larvae.

In trial one there were no differences in the percentage of deformed fish among the ~zero to high taurine treatments, though survival and growth were improved in the 80mg taurine/g rotifers treatment. After three months of growth, survival and deformities were not significantly different among juveniles raised from the treatments. In the second trial, larvae that had remained at 10°C for 9 days and were fed taurine enriched rotifers had significantly higher ($P < 0.05$) survival compared to larvae in the other three treatments (Table 1). Weights were lower in the two taurine treatments, possibly because survival was higher compared to no taurine. There were no significant differences for deformities, growth and survival among treatments fed taurine enriched *Artemia* nauplii compared to non-taurine enriched *Artemia* nauplii. Overall, increased dietary taurine during the rotifer and *Artemia* nauplii feeding period had no effect on deformities, benefited growth in some cases and improved survival in all trials, significantly so in trial two.

Table 1. Trial 2. Percent deformities, survival and average weight at weaning.

	Cold No Tau	Cold Tau	Warm No Tau	Warm Tau
% Survival	7.3 ± 3.5^{b}	12.4 ± 3.1^{a}	3.8 ± 2.2^{b}	6.3 ± 0.3^{b}
Weight (mg)	273 ± 14^{a}	206 ± 11^{a}	408 ± 16^{b}	339 ± 12^{b}
% Deformities	54.9 ± 6.1	56.8 ± 15.2	64.2 ± 10.6	68.9 ± 14.2

INNOVATION IN PHYCOGASTRONOMY: REDISCOVERING SEAWEED IN THE NEW NORDIC CUISINE

Cheryl Marie Cordeiro*, Jaap W. van Hal

Nofima, Muninbakken 9, 9019 Tromsø, Norway
cheryl.cordeiro@nofima.no

Seaweed aquaculture is the world's largest and fastest growing aquaculture sector. In Scandinavia and Southeast-Asia, seaweed is cultivated responding to rising market demand. While seaweed is a staple in many East Asian dishes, it remains foreign in many European countries. To that end, a theory-based and industry-oriented framework for service innovation is needed in research and practice. Data collected from field studies and interviews with key stakeholders in the seafood industry is used towards an informed discussion of innovation as a process of searching and the recombination of existing elements, factors or components. These elements are usually commercially available, some with long historical use that evolve by employing different techniques and processes. In the early Viking era, seaweed washed ashore during stormy seasons was gathered and used to produce glass, fertilizer and feed. More recently, seaweed has been commercially harvested for over 70 years in Scandinavia to produce alginate. However, seaweed as a culinary resource has for the most part been overlooked in Scandinavia.

Ambidextrous Innovation Model

In this context, the paper presents an ambidextrous innovation model, from empirical observations based on key individuals such as the Belgian pioneering Seaweed Chef Donald Deschagt. His catalysing work is a good example of how seaweed is being (re)introduced into the northern European diet of this ambidextrous combination of industry leadership and gastro-entrepreneurship. He prepared for example, the 2019 Seagriculture dinner, held near his restaurant on September 25-26th 2019 in Ostend.

Noticeable in this setting were the synergies of location, event context and the food. The evening´s dining ambience with a display of familiar seafood dishes such as the Spanish paella, ushered in various novel seaweed products. New seaweed foods presented at dinner introduced diners to novel means of using seaweed in food. The dinner menu items by Deschagt, oyster sea buckthorn with sea fennel and North Sea grey shrimp with sea lettuce and cheese, combined familiar ingredients with the relative unfamiliarity of seaweed. Innovative plating techniques such as using a brush of seaweed pesto in the bowl or on plates, sparked the interest of the participants of Seagriculture 2019. The very idea is for others be inspired by the event to try their own hand at using seaweed at home.

Innovation comes often with a vision and purpose. For Deschagt that exemplifies the ambidextrous innovation model in practice. The idea is for consumers in the New Nordic cuisine to not even notice that half of the time there was seaweed in the dishes. For gastro-entrepreneurs like Deschagt, the mission is to make the wonderful world of seaweed accessible to everyone. To drive home Deschagt´s point, the wakame infused chocolate ice-cream was a great success at the conference, demonstrating that even a perfectly familiar treat can be enhanced with seaweed to give that element of "spennende" (Norwegian for "exciting").

Mussels served with seaweed pesto and a side of Arame seaweed by pioneering Seaweed chef Donald Deschagt, Ostend, Belgium for Seagriculture 2019 dinner. Photo by Jaap van Hal.

Seaweed infused chocolate ice-cream by Tivoli Boutique, Belgium. Photo by Jaap van Hal.

AN INTEGRAL THEORY PERSPECTIVE OF ECOLOGY VALUES AND ECOSYSTEM SERVICES: THE EXAMPLE OF SEAWEED AQUACULTURE

Cheryl Cordeiro*, Wang Xinxin, Philip James

Nofima, Muninbakken 9, 9019 Tromsø, Norway
cheryl.cordeiro@nofima.no

An Integral Model of Ecology values and Ecoservices

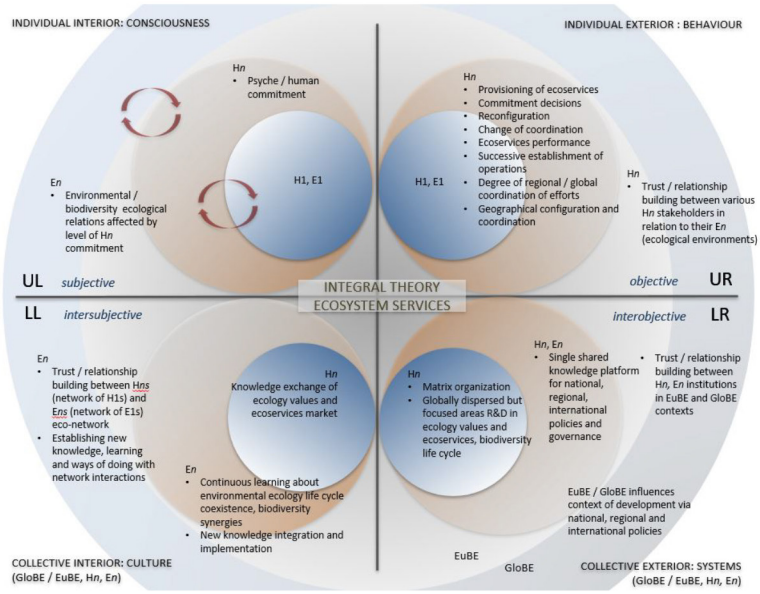
The use of marine algae/seaweed in culinary presentations and gastronomy in Far Eastern countries such as China, Japan, South Korea and parts of Southeast-Asia is well known. In Scandinavia however, seaweed is mainly associated with humble origins, local coastal activities and historical narratives. In Norway, seaweed was regularly washed ashore during storms, making harvest of the seaweed accessible for use for animal feed and as soil fertiliser. During the 18th century in the Scandinavian coastal regions, seaweed was also gathered to produce potash and soda ash that were used for cleaning and to make glass. The alginate industry blossomed in the 1960s in Norway when seaweed was harvested manually from the coast for applications in the food industry as a thickening agent.

The past decade has witnessed a (re)discovery of seaweed as a natural resource and raw produce. While there is increasing academic interest in modelling ecoservices, there seems a lack of a unified or integral view from the various disciplines in a combined knowledge of the field.

Paper objective

Taking the example of seaweed aquaculture, this paper applies an integral theoretical framework of analysis on ecology values and ecosystem services towards a unified perspective of ecosystem services. Empirical data is collected from various study visits to seaweed farms, as well as interviews with industry actors / stakeholders.

The paper presents and discusses how an integral view of the ecology values and ecosystem services in seaweed aquaculture can help towards deeper strategic management of today´s natural resources and marine environments. Seaweed is proving to be not only a valuable industry resource but one that enables an emerging services industry sector, ecoservices in seaweed aquaculture. The applied Integral Theory model also highlights challenges that seaweed aquaculture faces, and suggests means for further collaboration between research institutions, industry actors and governance.



NATURAL PIGMENT FROM PARACOCCUS CAROTINIFACIENS (PANAFERD-AX®) ENHANCED COLOR AND IMMUNE SYSTEM OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Dominique Corlay*, Nutt Nuntapong, Wutiporn Phromkunthong, Naraid Suanyuk

Aquaculture Natural Solutions, 22400 COETMIEUX, France
ansaqua.dc@gmail.com

This study investigated for the first time, the effects of the natural carotenoid, derived from the carotenoid-rich microorganism, *Paracoccus carotinifaciens,* known under the brand name Panaferd®-AX, on growth performance, survival, body pigmentation, immune responses and disease resistance of juvenile Pacific white shrimp (*Litopenaeus vannamei*). Four dietary levels of Panaferd®-AX (T1 to T4 at 0, 250, 500 and 1,250 mg kg $^{-1}$ diet) were supplemented to a basal pelletized diet. The shrimp with an initial body weight of 4.00±0.01 g were fed with these diets for 30 days. The results showed that shrimp growth, feed utilization and survival were not affected (P>0.05) by the inclusion of the Panaferd®-AX in any of the dosages tested in the basal diet, under the current experimental conditions, in accordance with other studies on fish and shrimp. On the other side, pigmentation and hemolymph immunological responses (table 1) were significantly affected by the diets containing Panaferd®-AX (P<0.05). The dietary supplementation with Panaferd®-AX enhances disease resistance against *Vibrio parahaemolyticus* (Fig.1) and mortality delayed after exposure to white spot syndrome virus (WSSV). In conclusion, the diet supplemented with Panaferd®-AX, derived from *Paracoccus carotinifaciens* can enhance pigmentation and immune competence of *L. vannamei*.

Fig.1 Survival (%) of shrimp from each dietary treatment after challenged with *Vibrio parahaemolyticus* for 17 days

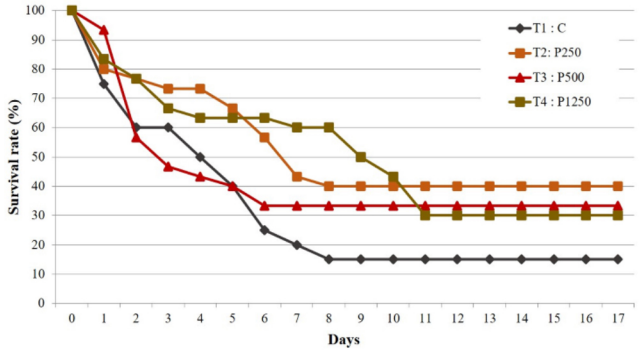


Table 1. Hemolymph parameters of Pacific white shrimp fed with the experimental diets for 30 days

Parameter	Experimental diets			
	T1 : C	T2 : P250	T3 : P500	T4 : P1250
THC (× 10^8 cells mL^{-1})	2.58±0.45^a	3.35±0.43^b	3.28±0.69^b	3.60±0.62^b
NBT reduction (OD 630)	0.485±0.111^a	0.588±0.089^b	0.597±0.108^b	0.613±0.095^b
PO activity (U mg $protein^{-1}$)	290.38±96.35^a	501.46±126.74^b	451.43±113.31^b	499.40±90.97^b
Lysozyme activity (U mg $protein^{-1}$)	13.19±3.86^a	16.53±4.74^a	17.27±4.99^a	22.80±5.53^b
Phagocytic activity (%)	24.19±1.44^a	24.60±2.24^a	31.00±3.23^b	40.90±6.49^c

Values are given as mean (n = 12 shrimps) ± standard deviation. Mean values in the same row with different letters are significantly different (P < 0.05)

LIVE FEED PRODUCTION TECHNOLOGY FOR HATCHERY PRODUCTION

Jose Lowell, Xu Qunying, Punithavathi K Haridas

Marine Aquaculture Centre
Singapore Food Agency
11 St. John's Island
Singapore 098659
Jose_LOWELL@sfa.gov.sg

Rotifers are small zooplankton that live in freshwater, brackish and marine environments. They are used extensively in aquaculture because of their high reproduction rate, optimal size for larval fish and good nutritional profile. Rotifers are essential for the first feeding of many species of marine finfish larvae. The commonly used rotifer culture methods by hatcheries in Singapore and the region are indoor batch cultures and outdoor pond cultures. However, the two methods could face culture stability issues where the rotifer population collapse overnight, and the shortage of rotifers resulting in the failure of the affected larviculture batch. In addition, an outdoor pond culture has a larger footprint compared to indoor batch culture. This topic describes the rotifer culture method based on a recirculating aquaculture system (RAS) developed at the Marine Aquaculture Centre and the management protocols adopted to maintain stability of rotifer growth. In a land-scarce Singapore, having this technology would enable production of rotifers intensively and consistently on a small footprint, which is important to support large-scale hatchery production.

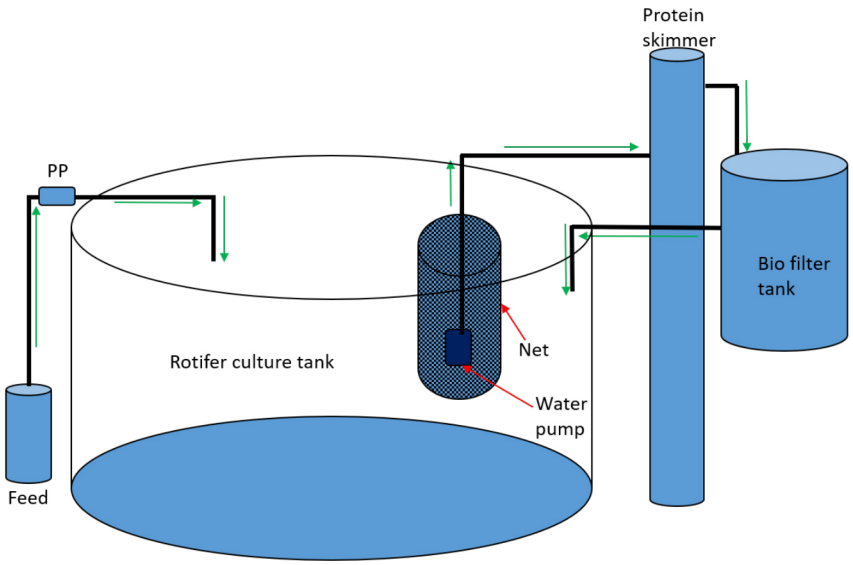


Fig 1: Example of a rotifer RAS

SOURCING PRACTICES MATTER MORE THAN INGREDIENTS FOR THE ENVIRONMENTAL FOOTPRINT OF SALMON AQUACULTURE FEEDS

Richard S. Cottrell*, Benjamin S. Halpern, Helen A. Hamilton, Beth Penrose, Chris G. Carter, Louise R. Adams, Alexandra Johne, Julia L. Blanchard

*richardstuart.cottrell@utas.edu.au
Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, 7004, Australia

Reduced dependence on wild-caught fish for aquaculture feeds has been a central tenet of sustainable aquaculture growth, particularly for carnivorous species such as salmon. Salmon aquaculture feeds are now largely dominated by crop-based ingredients in response to growing sustainability concerns surrounding the use of fishmeal and oil. Yet quantitative analyses of the environmental footprint of marine- or plant-dominant feeds and the variability of this footprint are lacking. Using global production, diet, and feed efficiency data, we present geospatial analyses of the cumulative environmental footprint (including greenhouse gas emissions, habitat disturbance, water extraction and nutrient pollution) generated from feeding the global farmed salmon industry either marine-dominant or plant-dominant feeds. We show that, relative to marine-dominant feeds, plant-dominant feeds reduce natural habitat disturbance, but their total environmental footprint tends to be similar due to trade-offs from water extraction and nutrient pollution (Figure 1). Importantly, where ingredients are sourced drives considerable variation in the cumulative environmental footprint within feeds such that the upper 97.6% of cumulative pressures scores for marine-dominant feeds overlap with the lower 97% of plant-dominant feeds. Policy and discourse surrounding the environmental sustainability of aquaculture feeds needs to better acknowledge the role that responsible sourcing can play for the sustainability of feed ingredients and the farming operations that rely on them.

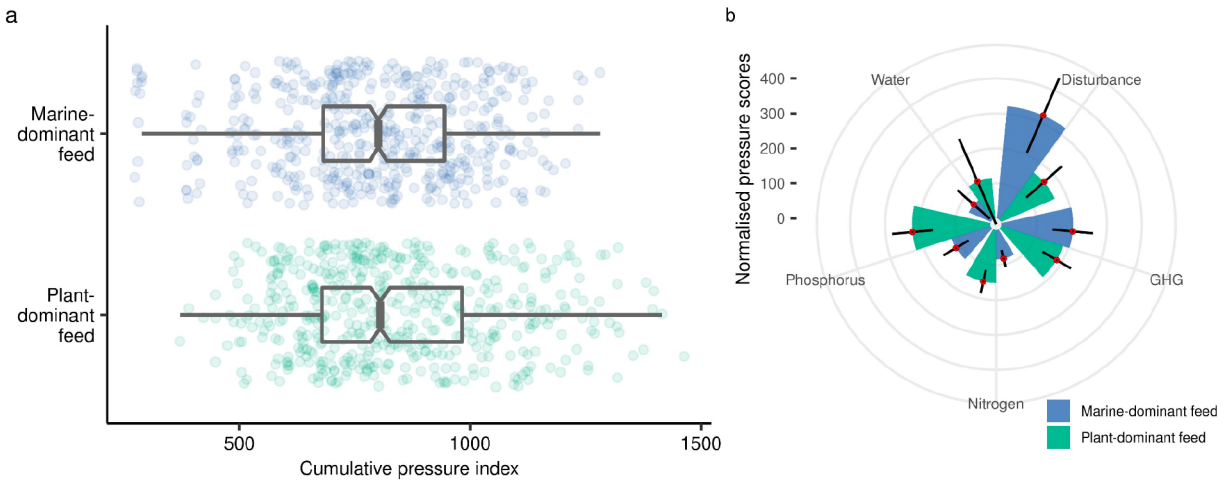


Figure 1 - a) The cumulative environmental footprint of marine- or plant-dominant feeds from 500 ingredient sourcing combinations and b) Cumulative environmental footprint disaggregated by normalised greenhouse gas emissions, water extraction, nitrogen pollution, phosphorous pollution, and habitat disturbance pressures for each feed type.

VACCINATION STRATEGIES FOR STRIPED CATFISH *Pangasianodon hypophthalmus* FARMS IN VIETNAM TO PROMOTE RESPONSIBLE USE OF ANTIBIOTICS

Margaret Crumlish*, Julie A Chambers, David A Comerford, Vo Hong Phuong, Le Hong Phuoc, and Ronan E O'Carroll

Institute of Aquaculture, University of Stirling, Stirling, United Kingdom
Email: mc3@stir.ac.uk

Vaccination programmes have demonstrably reduced antibiotic resistance (AMR) development within several intensively farmed aquaculture sectors, globally. Adoption of fish vaccination programmes has been poor in Vietnam and the continued reliance on antibiotics without a therapeutic approach has promoted multiple-antibiotic resistance, resulting in treatment failure at the farm level. Understanding the social and cultural awareness towards animal health, disease management and vaccination is critical to help promote responsible use of antibiotics in any food production system. This is the first report of using psychometrically robust tools to identify the belief's, concerns and catalysts to promote uptake of vaccination strategies within the Vietnamese catfish sector and promote responsible use of antibiotics.

A questionnaire-based survey was performed using face-to-face interviews with 400 participants in 3 provinces of Vietnam: An Giang, Dong Thap and Can Tho, between July and December 2020. The participants were representative of the striped catfish production sector and included producers with nursery, growout and combined freshwater farming systems. The questionnaire was developed using psychometrically robust measures and tools to determine: farmers perceptions and attitudes to fish disease, their beliefs about treatment of fish in their farms and their beliefs associated with vaccination and reluctance to use vaccines in their fish. A choice experiment was also conducted at the same time to identify the catalysts to promote vaccine uptake. The questionnaire was co-designed by a behavioural psychologist, aquaculture and fish disease specialists and behavioural economist. It was pilot tested prior to implementation. All data was entered into pre-formatted Microsoft Excel spreadsheet to reduce insertion errors and analysis performed using SPSS.

Table 1. Summary of Farmers Belief and Concerns regarding Fish Disease, Treatments and Vaccines

FISH DISEASE	Higher levels of worries and emotional distress was identified in those farmers with higher level of perceived problems from fish disease.
TREATMENTS	Concerns about antibiotic use outweighed their views on necessity. Higher level of perceived diseases occurring on farm was corelated with higher concerns about antibiotic use.
VACCINES	Good awareness overall of fish vaccination. Good understanding of purpose of vaccine. Higher level of disease was correlated with higher concern about vaccines. Concerns about ease of vaccine administration related to uptake.
The catalysts to secure uptake of vaccination strategies by 84% fish farmers included: • High efficacy (80%) • Simple administration route • Realist cost – 500,000VND per 1000 fish • Effective against multiple diseases	

A summary of the results is shown in Table 1. Overall 92% of Vietnamese farmers interviewed had a high level of knowledge and awareness of fish vaccination with 67% of all participants willing to use vaccines and 7% unwilling to use fish vaccines. Those reporting a better understanding of fish disease had higher belief in the helpfulness of treatments and had fewer concerns about vaccination. In contrast, farmers reporting more disease problems (undiagnosed) had higher concerns about treatments and lower belief in vaccination. This is the first description of the catalysts to help Vietnamese farmers engage with fish vaccination. These data will be used to develop future intervention strategies and training packages to promote uptake of fish vaccines as alternatives to antibiotics in this sector.

RAS SALMON PRODUCTION FOOD SECURITY PROJECTS IN TROPICAL ASIA

Yoav Dagan*, Erez Shalev, Hillel Milo

39, Robinson Road, #20-03 Robinson Point, Singapore, 068911
yoavmaof@gmail.com
AquAgro Projects Pte Ltd

Atlantic salmon provides one of the most efficient forms of protein in terms of edible yields, carbon footprint during the production process, water consumption and land usage.

Demand growth in Asia is amongst the fastest in the world, but significant supply constraints and transport costs from traditional producing regions are halting faster consumption expansion; current estimates suggest a salmon supply deficit of 585k metric tons (MT) in Asia, and growing.

The past several years have seen huge advances in salmon production technology and knowledge, including for indoor Recirculating Aquaculture Systems (RAS).

RAS technology allows for the creation and control of optimal production conditions for salmon, opening up the entire world as potential production locations, including the tropical climates of Southeast Asia.

This disruptive technology offers huge potential for the region: increased environmental sustainability of both production and the supply chain, supply of fresh salmon fillets to major consumption centres with lower transport costs and carbon footprint, and the introduction of a new and exciting technology which can spark an entirely new industry for the region.

However, RAS technology and salmon projects are not without risk: economies of scale for production mean that only larger-scale production facilities are viable, with high upfront investment costs; furthermore, whilst the technology allows for increased control over the production, managing advanced systems, and producing non-native species in the region requires a highly skilled workforce.

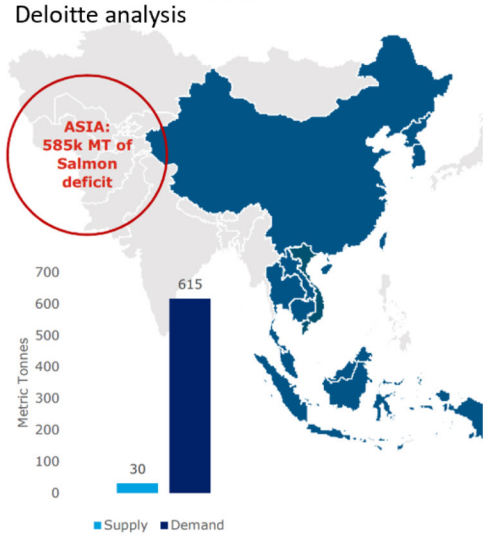
Understanding the operational risks around managing salmon RAS production are critical in ensuring the long-term viability of the projects.

Table 1: key protein benefits by meat type;
Salmon Handbook 2019, The Global Salmon Initiative

Protein benefits by meat type

Criteria	Beef	Pork	Chicken	Salmon
Protein Retention	15%	18%	34% ✓	31%
Energy Retention	27%	14%	25% ✓	23%
Edible Yield	41%	52%	46%	68% ✓
Edible Meat per 100kg fed (kg)	4-10	17	24	61 ✓
Carbon Footprint (kg CO2/kg edible meat)	30	5.9	2.7 ✓	2.9
Water Consumption (Litre/kg edible meat)	15,400	6,000	4,300	2,000 ✓
Land Use	102	11	7.1	3.7 ✓
Number of ticks	**0**	**0**	**3**	**5**

Table 2: Salmon supply deficit in Asia;
Deloitte analysis



NILE TILAPIA, *Oreochromis niloticus* EFFLUENT IMPROVES SOIL QUALITY, ENHANCES GROWTH AND SHELF LIFE OF *Amaranthus palmeri* SEEDLINGS

E. H. Alhassan, *S. O. Dandi and A Ampofo-Yeboah

Department of Fisheries and Aquatic Resources Management, University for Development Studies, Tamale-Ghana
* Corresponding author e-mail address: sdandi@uds.edu.gh

Soil nutrient management is required to maintain the plant system's constant productivity while also maintaining good soil quality. This study documented the effluent quality , soil quality, growth, shelf life, and proximate analysis of *Amaranthus palmeri* in Tamale Metropolis, Ghana as well as the effect of the resulting nutrients on soil properties, growth, and yield of *Amaranthus palmeri*. The experiment was set up in a completely randomized block design, with four factors (tilapia effluent (TE), combination of effluent and NPK 15-15-15 fertiliser (TE and FERT), only fertilizer(FERT), and only tap water(TAP)) each at six replicates per treatment (T1-T6: Tilapia effluent, T7-T12: Tilapia effluent + fertilizer, T13-T18: Only fertilizer and T19-T24: Only tap water) at the University for Development Studies. During the experimental period, the growth characteristics such as number of seed emergence, plant height, number of leaves, fresh biomass before and after shelflife,e and the proximate analysis of *Amaranthus palmeri* were evaluated. The soil properties and effluent nutrient content were measured and compared before and after the r experiment. Field and laboratory results on growth indices measured, shelf life and proximate analysis of *Amaranthus palmeri*, soil nutrient properties, effluent and tap nutrients measured were statistically analysed with ANOVA and Duncan Multiple Range Test at ($P < 0.05$). Results of the study indicate that tilapia effluent significantly influenced all the growth parameters measured compared to treatments at ($P < 0.05$). Shelf life and proximate analysis were enhanced in tilapia-treated plants. Soil quality such as soil pH, percentage nitrogen, available phosphorus, and exchangeable potassium concentration was significantly enhanced in the tilapia effluent treatment. The findings of the study suggest that tilapia effluent has the potential to improve soil quality and improve the growth and shelf life of *amaranthus palmeri* as additional benefits of culturing tilapia.

Table 5 Soil nutrient samples analysed after the termination of the experiment

Treatment	**Soil properties analysis after treatment**			
	pH	**%N**	P (mg kg-1)	K (mg kg-1)
TE	7.63 ± 1.01a	1.9 ± 0.21a	13.41 ± 1.10a	82 ± 2.39a
TE+FERT	4.83 ± 1.02b	1.5 ± 0.11b	9.62 ± 1.07b	74 ± 3.66b
FERT	4.66 ± 1.02b	0.9 ± 0.25c	7.39 ± 1.01c	66 ± 2.45c
TAP	4.47 ± 1.08b	0.3 ± 0.01d	5.11 ± 0.99d	58 ± 2.99d

EFFECT OF SALINITY ON PHYSIOLOGICAL AND IMMUNOLOGICAL RESPONSES IN STRIPED CATFISH (*Pangasianodon hypophthalmus*) IN JUVENILE STAGE

Dang Quang Hieu *, Bui Thi Bich Hang, Do Thi Thanh Huong, Nguyen Thanh Phuong, Mutien Garigliany, Frédéric Farnir, Patrick Kestemont

Research Unit in Environmental and Evolutionary Biology, Institute of Life, Earth & Environment (ILEE)
University of Namur, Namur, Belgium
Quanghieudang.87@gmail.com

Global climate change has remarkably affected on aquaculture industry, especially the consequences of salinity intrusion in freshwater regions. Striped catfish is the most important species in Vietnamese aquaculture because its turnover reaches billions US dollars every year. However, effects of salinity increase on physiology and immune capacity in the species remain largely unknown. In this study, striped catfish juvenile (20-25 g) was chronically exposed to different salinities, namely 0, 5, 10, 15 and 20 ppt with the increase 0, 0.25, 0.5, 0.75 and 1 ppt per day respectively, during 20 days (D20). Then, the fish was continuously kept in its corresponding salinity two weeks (D34) to see how it acclimate to the salinity. All treatments had very good survival rates (over 90%), except treatment 20 ppt after the first 20 days exposure. Weigh gain showed the highest level in the fish submitted to 5 ppt in D20 and the indicator exhibited elevating pattern with salinity in D34. In terms of osmoregulatory responses, osmolality, sodium and chloride concentrations significantly increased in fish exposed to hyperosmotic environment during the experiment. Red blood cell and hematocrit had the same pattern that decreased after 20 days of salinity increase. Two immune responses (plasma lysozyme and peroxidase activities) were investigated but the lysozyme activity was only significantly elevated in the hyperosmotic conditions in D20. The fish reared in higher levels of salinity had stronger activities of digestive enzymes , especially the activities of aminopeptidase, leucine alanine peptidase and pepsin in D20. Interestingly, the study found that the scoring of interlamellar cell mass in gill (ILCM) was significant lower in fish submitted to higher salinity in D20 and this effect was more remarkable in D34. These results support the hypothesis that hyperosmotic stress may affect the striped catfish juvenile through changes of physiological and immunological responses, however the fish looks like possible to acclimate salinity after two weeks, except the one in 20 ppt.

MICROBIOME HEALTH ASSESSMENTS VIA SINGLE-CELL FINGERPRINTING

Ruben Props*, Frederiek-Maarten Kerckhof, Jasmine Heyse, Peter Rubbens, Marc Indigne, Nico Boon

KYTOS BV
Technologiepark-Zwijnaarde 82
Zwijnaarde
Belgium
ruben@kytos.be

Microorganisms represent by far the most abundant life form in all aquaculture systems. Managing the aquaculture microbiome to improve farm performance requires methods that enable the measurement of all microbes in the microbiome, and that can distill its complexity into ecologically-relevant but understandable health metrics. Unfortunately, our knowledge on the aquaculture microbiome has largely been derived from cultivation-based methods which detect only a small fraction of the microbiome (e.g. < 0.1 - 1 %). Here, we showcase several applications of a novel microbial fingerprinting technique based on single-cell data that takes into account all microbial cells and is both rapid and reproducible. The technique's derived microbiome health indicators (*Figure 1*) were used to evaluate the health situation during extensive sampling campaigns conducted at commercial scale of various aquaculture systems. Based on thousands of measurements we demonstrate that these health indicators reliably capture the quality of farm management decisions such as water preparation efficacy, treatment efficiency, and farm performance in both hatchery and farm context. Moreover, we show the importance of a holistic approach to microbiome health management by highlighting the dynamic nature of the health indicators in these systems. Our novel approach to studying and managing the microbiome health offers opportunities to radically improve aquaculture practices.

Figure 1: Microbiome health management tools developed based on machine-learning based processing of single-cell data.

INFLUENCE OF WATER CHANGING FREQUENCY ON GROWTH PERFORMANCE AND NUTRIENT UTILISATION OF TROPICAL FISH SPECIES

Akeem Babatunde Dauda*, Zainab Bashir and Joseph Ikpe

Department of Fisheries and Aquaculture, Federal University, Dutsin-ma PMB 5001, Dutsin-ma Katsina State, Nigeria
Corrresponding author: Email: tdabak@gmail.com Tel :+2348062085120

Fish is a major and cheaper source of animal protein and its sustainable production has been limited to aquaculture as capture fisheries has been levelled-off for almost three decades now. However, expansion of aquaculture is being challenged by resources among which water is a major one. There are different methods of culturing fish and one of the most commonly used in tank systems is stagnant-renewal method where water is changed frequently to keep the fish at optimal performance. This is mostly done arbitrarily and sometimes rely on the visual appearance of the culture water. There is limited information on how often the water changes should be conducted. This research examined the effect of water changing frequency on two major fish species cultured in Nigeria and Africa, Nile tilapia, *Oreochromis niloticus* and African catfish, *Clarias gariepinus*. The experimented water changing frequency was 50% at two days interval, four days interval and once in a week. The results showed that water change once a week is adequate for fish stocked between 10 kg/m^3 and 20 kg/m3. The water quality parameters were all within the recommended range for tropical fish culture, and only a few parameters like total dissolved solids and electrical conductivity were significantly different among the treatments. The growth performance and nutrient utilization parameters were similar in *O. niloticus*. In *C. gariepinus*, frequency of water change influenced the growth and nutrient utilization parameter, with performance increases with increased number of days of changing the water.

While further research is suggested, changing of culture water once a week is established to support improved growth performance, nutrient utilisation and well being of the fish culture. This, if adopted will reduce water usage in fish culture and increased fish production despite reduced labour and environmental friendly as the frequency of waste discharge is reduced.

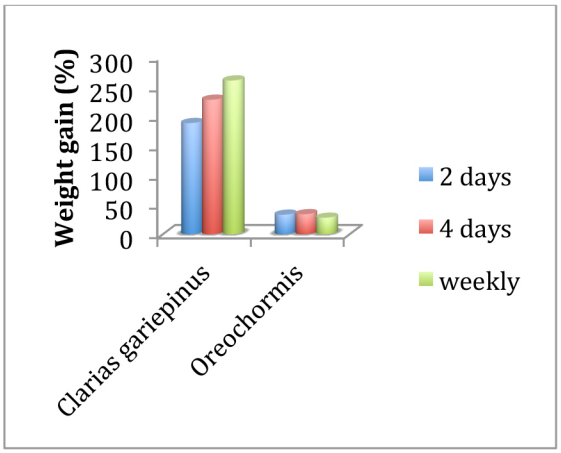


Figure 1: % weight gain of two tropical fishes reared at different water changing frequencies

DIFFERENT CARBON SOURCES AFFECT MORPHOLOGY AND PLANKTONIC COMPOSITIONS OF BIOFLOCS

Akeem Babatunde Dauda*

Department of Fisheries and Aquaculture, Federal University
Dutsin-ma PMB 5001, Dutsin-ma Katsina State, Nigeria
Email: tdabak@gmail.com

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

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

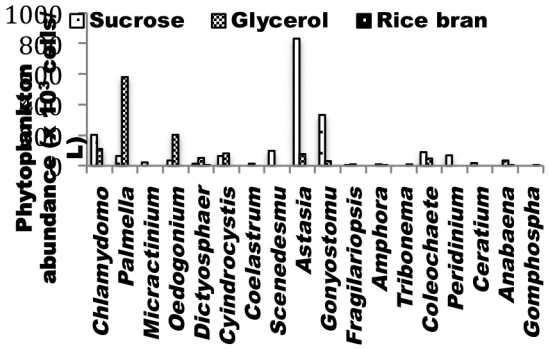


Figure 1: Phytoplankton composition of the bioflocs

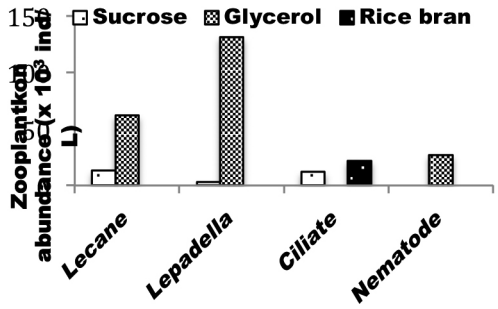


Figure 2: Zooplankton composition of the bioflocs

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

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

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

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

Gianmarco S. David·Paulo C. von Krüger, Reinaldo J. Silva, Clovis F. Carmo and Eduardo M. Brambilla

São Paulo Fisheries Institute, Avenida Francisco Matarazzo, 455 - Parque da Água Branca
São Paulo, Brasil 05033-970. PO Box 60092

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

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

Support: FAPESP 2013/50504-5

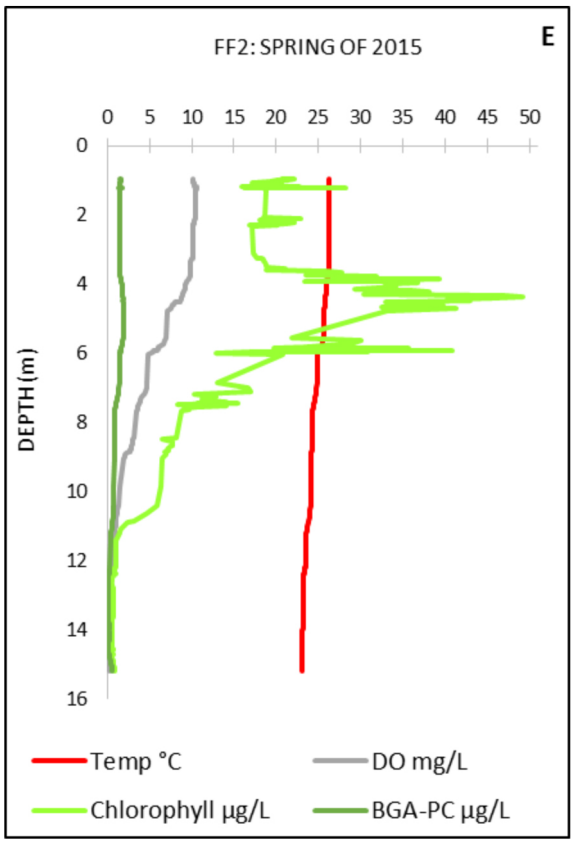


Figure 1: Vertical structure profiles of Temperature (Temp °C), Dissolved Oxygen (DO mg/l), Chlorophyll (µg / L) and Phycocyanin (BGA – PC µg / L).

ON-FARM EVALUATION ON THE USAGE OF A NUTRITIONAL EMULSIFIER ON THE GROWTH PERFORMANCE AND FEED EFFICIENCY IN NILE TILAPIA (*Oreochromis niloticus*)

M. de Jong[1]*, A. van der Aa[1], B. Bruneel[1], A. Santisukwongchote[2]

[1]Orffa feed additives; Minervum 7032, 4817ZL Breda, the Netherlands
jong@orffa.com (*presenter)
[2]Orffa Thailand
santisukwongchote@orffa.com

Nile tilapia is one of the most produced species worldwide, due to its high productivity and relatively easy husbandry. However, just like all producers of animals for human consumption, tilapia farmers are experiencing increasing feed costs. Improving the use and efficiency of raw materials is, therefore, interesting for the aquaculture sector. To reduce feed cost, the industries' focus is on improving the efficiency of digestion and retention of energy, protein and fat, which are expensive components of the diet. In previous research, it was shown that the addition of a nutritional emulsifier (NE) based on glyceryl polyethyleneglycol ricinolate (GPGR), can improve Nile tilapia protein efficiency, fat digestibility, overall growth performance, energy efficiency and health status of the fish in a research environment (Wangkahart et al., 2022). However, more interesting is to evaluate the effect on a larger scale by doing an on-farm evaluation on the addition of a NE on fish survival, growth performance and feed efficiency.

The objective of this study was to investigate the effect of adding a NE at levels of 350 g NE/MT of feed on growth performance and feed efficiency. The experiment was performed in Thailand, with a duration of 97 days. The trial consisted of seven tanks with 1500 fish per tank where two dietary treatments were tested. Fish were fed ad-libitum three times per day. They were fed either the control diet, four repetitions, which was a local commercial tilapia feed, or the treatment diet, in triplicate, where NE was added to the local commercial Tilapia feed at levels of 350 g NE/MT of feed (Table 1). Amount of feed consumed was measured and summarized in the end as feed intake (FI). Next to that average start body weight (SBW), average final body weight (FBW), growth (G) and feed conversion ratio (FCR) were measured. Additionally, survival and health were monitored during the feeding trial.

Table 1: Trial set up to test the effect of NE on Nile tilapia

Tank	Treatment	#fish	Stocking weight fish (g)
A10	NE	1500	30
A7	Control	1500	30
B25	NE	1500	30
B27	NE	1500	30
B29	Control	1500	30
B31	Control	1500	30
B33	Control	1500	30

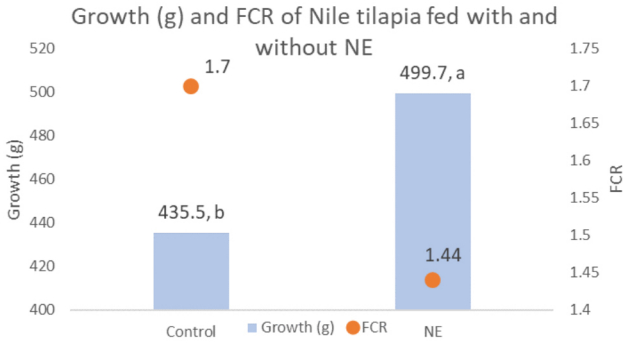


Figure 1: Growth (g) and FCR of Nile tilapia fed diets without NE and with NE supplemented at 350 g/MT of feed

Results showed that the addition of NE did not affect fish survival, health and FI. It can be observed that FBW and G was affected by dietary inclusion of NE (Figure 1; $p<0.1$). Tilapia fed NE were significantly bigger and grew significantly more compared to the fish not fed NE. Next to that, a positive trend towards FCR could be observed , where NE was shown to improve FCR slightly (Figure 1; $p=0.161$).

Previous research already showed that addition of a NE can increase feed efficiency and health status of Nile tilapia. This was confirmed by the feeding trial of this experiment. In general, it can be stated that adding a NE on farm scale can offer fish farmers a way to increase feed efficiency and, therefore, decrease feed cost.

DISCOVERY OF SINGLE NUCLEOTIDE POLYMORPHISMS AND CONSTRUCTION OF A GENETIC MAP FOR PIKEPERCH *Sander lucioperca*

Lidia de los Ríos-Pérez*, Marieke Verleih, Alexander Rebl, Ronald M. Brunner, Julien A. Nguinkal, Nadine Schäfer, Tom Goldammer, Marcus Stüeken, Dörte Wittenburg

Institute of Genetics and Biometry
Leibniz Institute for Farm Animal Biology (FBN)
Dummerstorf, Germany 18196
perez@fbn-dummerstorf.de

Pikeperch (*Sander lucioperca*) is a fresh and brackish water percid fish natively distributed in Europe and Asia. Its growing consumer demand and high potential for inland aquaculture have placed it as an attractive species for intensive rearing. Although the impact of several environmental factors has been studied on productive and reproductive pikeperch traits, information related to its genetic variation is scarce. The most common form of genetic variation is the single nucleotide polymorphism (SNP). The identification of SNPs allows its use as markers for the analysis of the genetic structure of populations, for the construction of genetic maps and to help locate genes associated to traits of interest. The main objectives of this study are to identify SNPs from whole genome sequencing of a pikeperch breed and to construct a genetic map using information of parents and offspring.

Genomic DNA from a total of 394 individuals, including broodstock, was extracted from blood or flash-frozen caudal fin for whole genome sequencing with Illumina (NovaSeq 6000). A total of 90,416,509,334 paired-end reads (151 bp) were generated with an average number of reads per sample of 229,483,526 and average coverage of 31x. Reads were aligned against the pikeperch draft assembly (GenBank assembly accession: PRJNA561467) using BWA alignment tool. SNPs were detected using the Haplotypecaller tool of the Genome Analysis Toolkit v4.0 (GATK) pipeline for DNA-Seq. A total of 4,135,096 raw SNPs were discovered, from which 1,619,874 were detected as high quality SNPs. Identified SNPs will be further processed with the genome association analysis toolset PLINK. After SNP filtering by call rate, minor allele frequency and Hardy-Weinberg equilibrium, a set of high-quality SNPs will be selected for parentage testing with ParentSearch software. This allows the construction of a linkage map using Lep-MAP3.

This study will provide the necessary input for refining the recent draft of the genome assembly and will help improving breeding programs for a more efficient aquaculture production of pikeperch. For instance, it is possible then to assess the degree of inbreeding in the population by exploring runs of homozygosity.

USING MODEL SIMULATION TO ASSESS THE FEASIBILITY OF A COMMERCIAL SCALE RECIRCULATING SYSTEM INTEGRATING SEA URCHINS (*Tripneustes gratilla*) AND SEAWEED (*Ulva rigida*)

Bas C. de Vos*, Mark D. Cyrus, John J. Bolton and Brett M. Macey

Department of Biological Sciences
University of Cape Town
Cape Town, 7701
South Africa
bascdevos@gmail.com

Global collapse of wild stocks and high demand has amplified interest in echinoculture. *Tripneustes gratilla*, found in the tropical and sub-tropical Indo-Pacific, is highly suited for aquaculture being palatable, highly valued and fast growing. Research on *T. gratilla* thus far has occurred primarily at laboratory scale but seldom tested on the intended commercial scale. This study aimed to bridges the gap between research and industry by creating a model simulation of an IMTA system with *T. gratilla* and *Ulva rigida*. This could be used to aid the design of a commercial farm and help avoid expensive trial and error of physically building multiple systems until one is successful.

Most parameters required to create this system model already exist, such as Ulva production models. Our data "fills in the gaps" of the model relating to up-scaling and optimising *T. gratilla* culture methods. Trials investigating basket design have confirmed a hypothesis by Siikavuopio et al. (2007) that urchins perform better in shallower baskets. This effect was not due to increased spine loss in deeper baskets, as previously suggested. In our experiments it is rather because of higher consumption in shallow baskets when fed either *Ulva rigida* ($t = -3.110$, $df = 4$, p-value = 0.018) or artificial pellets ($t = -3.109$, $df = 4$, p-value = 0.018) (figure 1). A 90-day analysis using 4, 6 and 8kg/m² has revealed a strong negative correlation between stocking density and growth, gonad mass and gonad quality. Further experimentation relates to consumption, feeding regimes and nutrient release.

This model will be employed to design South Africa's first pilot scale echinoculture system. This system will assist optimising the model so it can be scaled up to a commercial level.

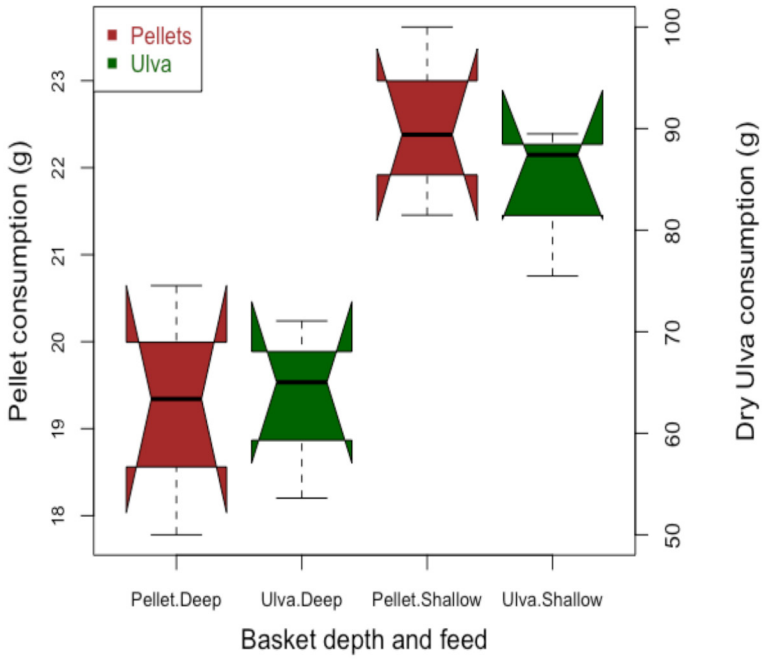


Figure 1. Boxplot demonstrating how *T. gratilla* will consume signficantly more pellets and *Ulva rigida* when cultured in shallow baskets

AN APPROACH FOR IMPROVEMENT OF SMALLHOLDER FISH FARMING SYSTEMS IN SIEM REAP PROVINCE (CAMBODIA)

Dorian Dejace*, Kabir K. Ahmed, Rotha Chork, Piraner Samoth, Nicolas Jouanard, Domenico Caruso, Jean-Michel Mortillaro, and Marc Oswald

APDRA Pisciculture Paysanne
Station Atlante - 20 rue Ampère
91300 MASSY
d.dejace@apdra.org

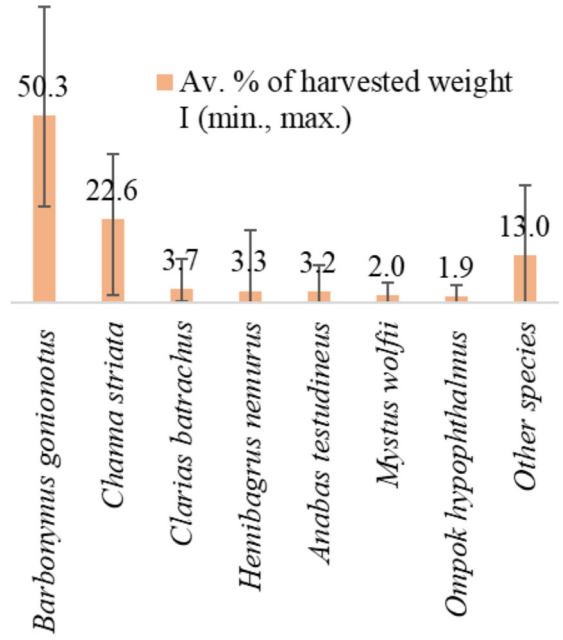
APDRA, an NGO promoting fish farming, works with farmers located in Siem Reap watershed. Aquaculture is a secondary activity taking place in small ponds (< 700 m^2) filled by rains (av. 1300 mm/year). At first sight, the context seems favourable to its development: traditional knowledge, developed feeds and fingerlings' markets. However, small-scale fish farming still appears inefficient. The projects[1] initiated a participative action-research process towards sustainable systems. Experimentation, farmers' networking, and follow up are key elements in this dynamic.

In September 2021, 17 farmers stocked silver barb *Barbonymus gonionotus* (3/m^2) in ponds connected to rice fields by canals, surrounded by dikes. To date, 9 of them fully harvested, after a 6 to 8 months cycle. The fish yield (av. 3.2 t/ha of pond) was appreciable. The survival rate (av. 60.5%) was quite low, and the relatively small size of silver barb (av. 87 gr) was deplored. Surprisingly, wild species (av. 19 s./pond) accounted for half of the total biomass (cf. chart) and 49 species were counted, including 43 fishes, 3 crustaceans, 2 molluscs and 1 frog. The income was low (av. 45 USD/ha of pond).

The high biodiversity in ponds is due to periodic floods bringing wild species, which may compete with or prey on the stocked species. Fish species known as potential predators were overrepresented (av. 70.4% of wild species weight). Moreover, floods tend to be less predictable in intensity and time, making them more difficult to manage.

As floods and biodiversity management are seen as the main challenges, the projects accompany farmers in developing solutions. Elevating bunds is an expensive investment. Putting up nets around is affordable but little effective. Selective regular fishing with traps and nets, to optimise populations' structures, appears more promising. Cage farming within the pond might be used to separate the stocked species from the wild ones. It's expected that the participatory action-research approach will lead to integrated solutions, for sustainable systems.

CHART - Harvested species



[1]DéFiP (AFD funds), AquaCAM (led by IRD, funded by France)

EFFECTS OF VINEGARS ON THE GROWTH PERFORMANCE OF BLACK TIGER SHRIMP *Penaeus monodon*

Rey J. dela Calzada*, Barry Leonard M. Tumbokon, and Augusto E. Serrano, Jr.

College of Fisheries and Ocean Sciences
University of the Philippines Visayas
Miagao 5023, Iloilo
reydelacalzada@gmail.com

A 90-day feeding trial was conducted to evaluate the effects of vinegars on growth and feed efficiency of *P. monodon* PL (0.01 g). The attractability of the diet was assessed. Results of the experiment are shown below.

Shrimp final average body weight, weight gain, total individual feed intake, specific growth rate, and feed conversion ratio were higher in vinegar group than the control group with significant difference ($P<0.05$) (Table 1). The group of shrimp fed combination of CSV and SCV vinegars exhibited the significantly best final average body weight, weight gain, specific growth rate and feed conversion ratio. Survival was statistically similar among all groups. Overall, the results demonstrated that vinegars could be used as growth enhancers in shrimp and that the combination of CSV +SCV group exhibited the significantly best growth performance.

Table 1. Growth performance and feed utilization of *P. monodon* fed with control and vinegars in 90 days.

Treatment	I_{ABW} (g)	F_{ABW}(g)	WG (g)	TIFI (g)	SGR (%/day)	FCR	SURV (%)
Control	0.01	0.53 ± 0.02^{d}	0.52 ± 0.02^{d}	1.11 ± 0.04^{c}	4.41 ± 0.05^{d}	2.08 ± 0.03^{a}	84.44 ± 1.11^{a}
CSV	0.01	0.85 ± 0.01^{b}	0.84 ± 0.01^{b}	1.50 ± 0.04^{ab}	4.92 ± 0.02^{b}	1.77 ± 0.02^{c}	84.44 ± 1.11^{a}
SCV	0.01	0.75 ± 0.00^{c}	0.74 ± 0.00^{c}	1.47 ± 0.02^{b}	4.80 ± 0.01^{c}	1.95 ± 0.04^{b}	84.44 ± 1.11^{a}
CSV+SCV	0.01	0.99 ± 0.02^{a}	0.98 ± 0.01^{a}	1.64 ± 0.02^{a}	5.10 ± 0.01^{a}	1.65 ± 0.01^{d}	84.44 ± 1.11^{a}

Means ± SE indicated. Means within columns with the same superscripts are not significantly different ($P>0.05$). IABW=initial average body weight; FABW=final average body weight; WG=weight gain; TIFI=total individual feed intake; FCR=food conversion ratio; SURV=survival rate; CSV=coconut sap vinegar; SCV=sugar cane vinegar.

Table 2. Attractability of *P. monodon* to the experimental diets.

	Number of shrimp (%)			
Diet	*1 min*	*5 min*	*10 min*	Total
Control	1.7 ± 1.7^{a}	6.7 ± 2.1^{a}	0.0^{a}	8.3 ± 1.7^{a}
CSV	11.7 ± 4.0^{ab}	18.3 ± 4.0^{b}	6.7 ± 3.33^{ab}	33.4 ± 6.7^{b}
SCV	1.67 ± 1.7^{a}	8.3 ± 3.7^{ab}	0.0^{a}	11.7 ± 1.7^{a}
CSV+SCV	16.7 ± 5.6^{b}	18.3 ± 4.0^{b}	11.7 ± 4.0^{b}	46.7 ± 6.7^{b}

Means in the same column with different superscripts indicate differences between diets ($p<0.05$). CSV= Coconut Sap Vinegar diet; SCV=Sugar cane vinegar; CSV+SCV= Combination of CSV and SCV.

MAKING FLOATING AND SINKING FISH FEED WITH TWIN-SCREW EXTRUSION TECHNOLOGY

Hadrien Delemazure*

Clextral
1 rue du Colonel Riez
42700 Firminy France
hadrien.delemazure@clextral.com

Twin-screw extrusion has been extensively used to manufacture quality aqua pellets for the last 40 years.

Recipes are getting more complex and diversified, fishmeal and fish oil are being replaced by alternative raw materials, which sources may vary according to international prices fluctuation and availability.

Furthermore, in order to produce both optimal floating and sinking feed, a precise control of the pellet expansion and density is necessary.

Aquafeed production lines are today expected to offer more process flexibility, reliability and durability that can be achieved using twin-screw extrusion technology.

BRAND-LEVEL STRATEGIC PRICE RESPONSE IN THE U.S. CANNED TUNA MARKET

Madan M Dey* and Prasanna Surathkal

Department of Agricultural Sciences, Texas State University, San Marcos, TX 78666

Canned tuna is among the most popular seafood products in the U.S. in terms of per capita consumption. There are currently three major brands of canned tuna in the U.S. retailing industry. These are: Bumble Bee Foods (BB), Chicken of the Sea International (CS), and StarKist Seafood Co (SK). The canned tuna category is noted for a high degree of competition among the brands. However, retailers have alleged that the three brands colluded to fix prices. The U.S. Department of Justice (DOJ) initiated investigation into price-fixing allegations, and there are reports the companies involved have reached agreements. In this paper, we analyze the strategic price responses among the top brands in the canned tuna market using national-level barcode scanner data.

We use nationally representative brand-level data on monthly sales quantity, prices and promotional efforts for canned tuna in U.S. superstores (stores with annual turnover of at least $2 million) for the July 16th 2005 to June 12th 2010 period. The data are barcode scanner data on canned/ shelf-stable tuna products, procured from The Nielsen Company. Data consist of information on the three major brands (BB, CS and SK) and a composite brand representing all store brands (SB) or private label brands. Names other identifiable features of the brands are not shown in the results to protect confidentiality.

Figure 1 shows the average unit prices for the four brands. There are three brands (brands 1, 2 and 3), whose prices move more closely together and show larger variations. Whereas, the price of brand 4 is the lowest among all brands and shows smallest variations.

Our econometric model is a system of demand functions and price reaction functions that incorporates both short-term and long-term dynamic responses. We use impulse response functions (IRF) to characterize the response to a one standard deviation shock in the price of the prices. We use Forecast Error Variance Decomposition (FEVD) to decompose the change in a price index into contributions of innovations in the different endogenous variables of the system.

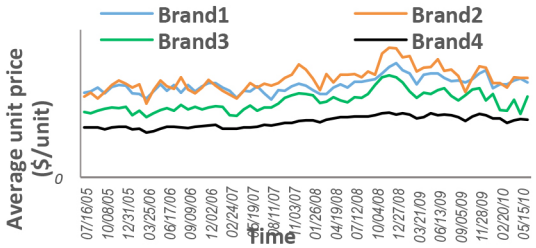


Figure 1. Brand-wise average unit prices.

EFFECT OF A NOVEL FUNCTIONAL FEED INGREDIENT ON THE GROWTH PERFOMRANCE AND DISEASE RESISTAMCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Richardson Andrew*[1] Lima Joao[2] Lefranc Maxime[1] Walraven Maye[1]

[1]Innovafeed SAS, Paris, France
[2]IMAQUA, Ghent, Merelbeke, Belgium
*Corresponding author at:
Tel.: +447867384167.
E-mail address: (andrew.richardson@innovafeed.com)

This study was performed as part of developing a novel functional feed ingredient for Pacific white shrimp (*Litopenaeus vannamei*). Here we assess the effects of dietary inclusion of a novel functional ingredient (NFI) derived from defatted Black Soldier Fly (*Hermetia illucens*) larvae meal on growth performance, tolerance to salinity stress, and disease resistance when challenged with *Vibrio parahaemolyticus* (the causative agent of acute hepatopancreatic necrosis disease) or with a strain of white spot syndrome virus (WSSV). A control diet (CTRL) was used for comparison to three test diets formulated to include 4.5%, 7.5%, and 10.5% of the novel ingredient (NFI4.5, NFI7.5 and NFI10.5) . After 28 days, all three diets including the NFI led to significantly improved weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) compared to CTRL. The results showed that even the lowest level inclusion of the NFI (4.5%) created a marked effect, with SGR significantly increasing by 13.6% and FCR showing a decreasing trend – reducing 16.5% During the growth trial the survivability was not affected by diet. NFI in the diets showed no significant effect on resistance to salinity stress or to *V. parahaemolyticus*, but a positive pattern towards WSSV resistance was recorded. Overall, we show that there is strong evidence that the use NFI derived from *H. illucens* has a strong potential to improve the performance and health of *L. vannamei* and could generate value creation for shrimp farmers at low inclusion rates.

EFFECTS OF SALINITY ON SELECTED REPRODUCTIVE PHYSIOLOGICAL PARAMETERS OF STRIPED SNAKEHEAD (*Channa striata*)

Do Thi Thanh Huong*, Le Thi Ngoc Thuy, Ngo Minh Nhut, Nguyen Tinh Em, Nguyen Thi Kim Ha and Nguyen Thanh Phuong

College of Aquaculture and Fisheries, Can Tho University
Campus 2, 3-2 street, Ninh Kieu district, Can Tho city, Vietnam
dtthuong@ctu.edu.vn

This study aimed to investigate the effects of salinity on reproductive physiological parameters of striped snakehead (*Channa striata*). Striped snakehead (body weight of 400 g) were cultured in three salinities including 0‰ (control), 3‰ and 6‰ with three replicates for each treatment. Thirty fish were stocked in each tank.

After 4 months of culture, the weight gain of fish was highest in 0‰ (279±165 g) and the lowest was in 6‰ (196±142 g). Physiological parameters such as haemoglobin, haematocrit were significantly higher in 6‰ if compared to those of 3‰ and 0‰. Red blood cells ranged between $3.07\pm0.3x10^6$ and $3.44\pm0.3x10^6$ cells/mm^3, while the highest of white blood cells was at 6‰ ($116.7\pm39.3x10^6$) which was significantly higher than that in 0‰ ($62.9\pm16.9x10^6$). Osmolality and Cl^- ion concentrations in 6‰ (301±5.5 mOsm/kg and 104±3.86 mM, respectively) were higher than that of 3‰ (294±6.20 mOsm/kg and 99.9±3.23 mM) and 0‰ (287±6.9 mOsm/kg and 95±5.31 mM). The results also showed that the highest of gonadosomatic index (5.8±2.62%) was in 3‰, while the lowest (3.85±1.95%) was in 0‰. The vitelline concentration was highest in 6‰, and lowest in 0‰. The absolute fecundity and relative fecundity were highest at the 3‰ and the lowest were in 6‰ for the absolute fecundity and in 0‰ for relative fecundity.

INDUCED SPAWNING OF AFRICAN CATFISH *(Clarias gariepinus)* USING PITUITARY GLANDS FROM PIG *(Sus scrofa domesticus)* AND GOAT *(Capra aegagrushircus)*

Jemuel S. Doctolero*, Danica S. Sotero** Lorenz J. Fajardo
*Presenting Author **Corresponding Author

Department of Aquaculture, Central Luzon State University
Science City of Muñoz, Nueva Ecija, Philippines
jemsado09@yahoo.com.ph
jemueldoctolero@clsu.edu.ph

The study was conducted to examine the potential of pituitary glands of pig and goat for the induced spawning of the African catfish by evaluating the latency period, fecundity, fertilization rate, and hatching rate. Further, the study also aimed to determine the acceptable dosage to be utilized on the induced spawning of African catfish. The treatments evaluated were: Treatment 1 (control: 4mg / kg, catfish pituitary gland), Treatment 2 (4mg / kg, pig pituitary gland), Treatment 3 (200mg / kg, pig pituitary gland), Treatment 4 (4mg / kg, goat pituitary gland) and Treatment 5 (200mg / kg, pig pituitary gland).

The pig and goat pituitary gland extracts have successfully induced the spawning activity of African catfish. All female breeders of experimental treatments attained ovulation within 12 hours which is under the prescribed latency period. Treatments with 4mg/kg of pig and goat pituitary glands gained the highest number of egg spawned followed by the treatment with 200mg/kg of pig pituitary gland, then the treatment control with 4mg/kg of catfish pituitary gland and lastly, the treatment with 200mg/kg of goat pituitary gland. For the fertilization rate, control treatment attained the highest value however, the pituitary extract from pig and goat with 4mg/kg dosage obtained a value that was comparable to the control treatment. In terms of hatching rate, treatments that utilized pituitary extract from goat both 4mg/kg and 200mg/kg dosages gave the highest result.

The study concluded that pituitary glands of pig and goat can provide positive results in terms of latency period, fecundity, fertilization and hatching rate and can be used as other source of pituitary gland on the induced spawning of African catfish. The study also concluded that the dosage of 4mg/kg of both pig and goat pituitary glands attained better result and the recommended dosage to be used on the induced spawning African catfish.

Table 1. Mean number of eggs, fertilization rate and hatching rate (±SD) of African catfish eggs induced from different pituitary extracts and dosages.

Treatment	Description	Mean Number Of Eggs	Mean Fertilization Rate (%)	Mean Hatching Rate (%)
1	4mg/kg (Control)	39,500±22,331.92 [a]	69.03±0.78 [a]	34.35±0.00 [b]
2	4mg/kg (Pig)	67,600±11,239.96 [a]	58.90±0.44 [b]	33.53±0.44 [c]
3	200mg/kg (Pig)	43,000±6,594.72 [a]	58.63±0.81 [b]	33.40±0.81 [c]
4	4mg/kg (Goat)	49,600±41,155.70 [a]	66.37±0.91 [a]	34.70±0.91 [a]
5	200mg/kg (Goat)	36,200±26,082.67 [a]	54.12±2.08 [c]	34.79±2.08 [a]

GENOTYPE BY ENVIRONMENT INTERACTIONS OF HARVEST GROWTH TRAITS FOR BARRAMUNDI *Lates calcarifer* FARMED IN MARINE AND FRESH WATER CONDITIONS

Jose A. Domingos*, Julie A. Goldsbury, Giana Bastos Gomes, Christopher Tomlinson, Brett Smith, Tim Bade, Justin Forrester and Dean R. Jerry

Tropical Futures Institute
James Cook University Singapore
149 Sims Drive, Singapore 387380

Barramundi (*Lates calcarifer*), also known as Asian seabass, is a commercially important tropical aquaculture species farmed in diverse culture systems (tanks, raceways, ponds and cages) and salinity conditions (from freshwater to seawater). Despite its adaptability to different culture conditions, selective breeding programs to improve growth rates in barramundi should consider the impact of genotype by environment (GxE) interactions on realization of genetic gains. Overlooking the effects of GxE interactions could result in the development of strains that would perform well in the same particular environment where breeding candidates were selected (for example sea water conditions), but not necessarily perform as well in a diverse environment (for example fresh water conditions).

To evaluate the effects of GxE interactions for harvest growth traits between extreme salinity conditions, fish pedigrees were reconstituted through DNA parentage analysis using two multiplex PCRs of 16 microsatellite markers. Barramundi juveniles from 146 families, originating from 26 dams and 54 sires allocated in four spawning tanks for simultaneous spawning, were farmed in a seawater raceway in Bowen (QLD, Australia) and a freshwater pond environment in Townsville (QLD, Australia) - both operated under commercial culture conditions. Animals were sampled at 15 months post hatch (mph) in the seawater raceway with 1,718 ± 309 g weight (W), 454 ± 28 mm total length (TL) and 141 ± 11 mm body depth (BD) (n = 752) and sampled at 21 mph the freshwater pond with 1,905 ± 426 g W and 451 ± 39 mm TL and 144 ± 15 mm BD (n = 752). Moderate heritability estimates were found for Asian seabass body size (W h^2 = 0.46 ± 0.10; TL h^2 = 0.41 ± 0.12; BD h^2 = 0.49 ± 0.13) and body shape (H h^2 = 0.41 ± 0.12) and lower heritability for Fulton's K condition factor (K h^2 = 0.15 ± 0.07). Significant GxE interactions were found for harvest growth traits in this study (W GxE r_g = 0.81 ± 0.11; TL GxE r_g = 0.64 ± 0.18; BD GxE r_g = 0.78 ± 0.13; H GxE r_g = 0.71 ± 0.17), and markedly interactions were found for Fulton's K condition factor (K GxE r_g = 0.36 ±0.31; $P > 0.05$). Genetic correlations between linear morphological traits and ratio traits indicative of body condition K and shape H were low and inaccurate. For instance, r_g between W and K was 0.30 ± 0.24, and r_g between W and H was 0.08 ± 0.04.

This study reveals the presence of weak to moderate re-ranking of genotypes for harvest growth traits in *Lates calcarifer* farmed in marine and freshwater conditions. Incorporation of sib-information from extreme salinity environments into the selection criteria of a breeding program may optimize the realization of genetic gains across distinct commercial conditions.

HERITABILITY OF SCALE DROP DISEASE VIRUS RESISTANCE IN BARRAMUNDI *Lates calcarifer*

Jose A. Domingos*, Loic Monteil, Celestine Terence, Yann Monteil, Emmanuel de Braux, Markus Schrittwieser, Joep Kleine Staarman, Saengchan Senapin, Ha Thanh Dong, Shannon Clarke, Rudiger Brauning, Andrew Hess, Joseph Angelo Uichanco, Xueyan Shen, Susan Kueh, Dean R. Jerry

Tropical Futures Institute
James Cook University Singapore
149 Sims Drive, Singapore 387380

Scale drop emerged as a new and devastating disease of barramundi *Lates calcarifer* in 1992. The syndrome is characterized by fish losing their scales which in addition to the effect of the disease itself opens them up to other pathogens. Subsequent work has identified the causative agent as a Megalocytivirus (Iridoviridae). This disease is one of the biggest impactors on production in Asia and cause 40-75% mortalities in juveniles. Currently there are no effective commercial vaccines to protect fish from this virus making a genetic breeding approach targeting resistance the most practical and long-term solution to lower risk. For selective breeding to be effective, however, the trait under selection needs to have a heritable component (so called additive genetic variability).

To understand the heritability of Scale Drop Disease in barramundi, we genotyped 2,305 scale drop diseased juveniles (116.5 ± 65.3g) and 2,282 survivors (417.7 ± 249.8g) from four different commercial batches reared in seacages in Southern waters of Singapore. Scale drop virus was confirmed as the main causative agent of the disease by qPCR on spleen tissues of a subsample comprising 85 sick (95.3% infected with 38,196 ± 74,469 viral copies/200ng DNA) and 95 survivors (15.6% infected with 124 ± 331 viral copies/200ng DNA), although a low co-infection with Lates Calcarifer Herpes Virus (LCHV) was also observed in 12.9% of sick (15.3% infected with 26 ± 30 viral copies/200ng DNA) and 4.1% of survivors (10.4% infected with 21 ± 20 viral copies/200ng DNA). A double digest (*PstI* & *MspI*) restriction-site associated DNA sequencing (ddRAD-seq) at a mean depth of 4.07 was used as genotyping-by-sequencing (GBS) approach. Reads mapped against the V3 barramundi genome yielded 231,329 SNPs post-filtering. A genomic relationship matrix (GRM) adjusted for the depth of sequence reads at each individual SNP, combined with survival data adjusted to a liability scale allowed for estimation of the additive genetic variance through linear mixed models using Residual Maximum Likelihood approaches.

A resulting moderate heritability of 0.33 ± 0.01 for SDDV resistance (or survival at sea) indicates that selective breeding approaches have the potential to deliver similar rates of genetic improvement as those seen for growth traits in barramundi and other commercially important aquaculture fish.

THE EFFECT OF REARING BEFORE STORAGE ON QUALITY AND FRESHNESS OF PACIFC OYSTER

Shiliang Dong*, Yabin Niu, Ryosuke Bizen, Xin Lu, Chunhong Yuan

United Graduate School of Agricultural Sciences, Iwate University
Ueda 3-18-8, Morioka, Iwate, Japan
u3220004@iwate-u.ac.jp

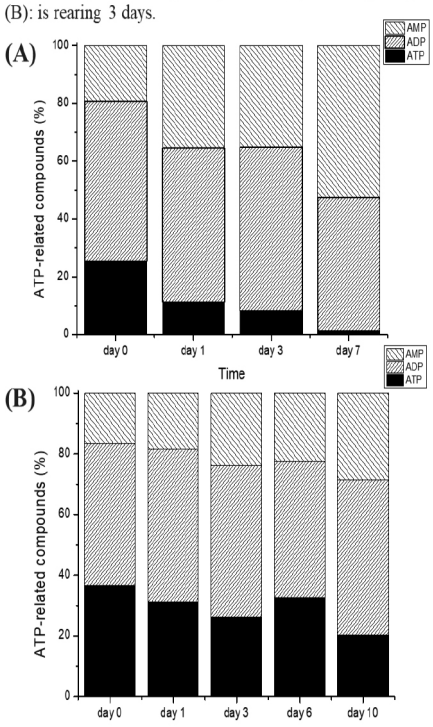
Oyster is one of the most popular shellfish in the world and the Pacific oyster (*Crassostra gigas*) is the main species of oyster consumed. With the popularity of oyster bars in recent years, demand for live oysters is on the rise. However, during the harvesting and transportation, the energy of oyster will be affected due to the forcing stress and oysters with low initial energy could also affect the shelf life. Therefore, there is an essential need to recover the energy of oyster before preservation. Rearing is one of the common methods to recover the vitality of fish or shellfish. According to the results of our previous experiments, shelled Pacific oysters were still alive and could be accepted for raw consumption at 3 weeks during the superchilling storage at -1°C and shelled oysters could have a longer shelf life when they are alive. Therefore, oysters with higher initial vitality could have a longer shelf life or not is unclear. Our study was to investigate the effects of rearing on quality and freshness of pacific oyster during storage.

Live cultured Pacific oysters (n = 40) was divided into two groups: rearing 1 day (A) and rearing 3 days (B). Oysters were rearing by deep ocean water before storage. All the groups were storage at -1°C for 10 days. The determination of pH value, ATP-related compounds was taken to indicate the changes in freshness. The AEC value has been used as a parameter that can reflect the quality of aquatic products.

AEC value (%) = 1/2 (2 ATP + ADP) / (ATP+ADP+AMP) ×100

After rearing, the group of rearing 3 days had a higher level of ATP than group of rearing 1 day. Only ATP, ADP and AMP could be detected, because all the oysters were alive. The AEC values of group A decreased from 53% (day0) to 24.35% (day 7). However, in group B, the initial AEC value is 59.78%. In contrast to group A, the AEC value only reduced to 45.52% after 10 days. The pH value of group A dropped from 6.78 to 6.49. In group B, the pH value was kept around 6.7 during the storage. The results indicated that rearing has positive effect on the quality and freshness of pacific oyster.

FIGURE 1. Change of ATP-related compounds in adductor muscle of oysters during storage time. (A) is rearing 1 day. (B): is rearing 3 days.



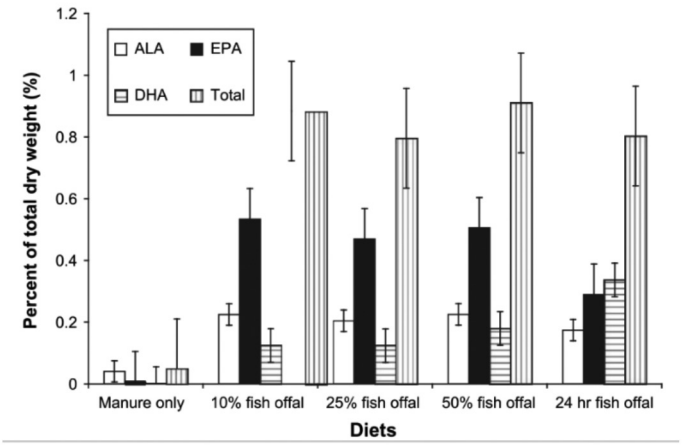
FOOD FOR THOUGHT: A CIRCULAR PRODUCTION MODEL FOR BLACK SOLDIER FLY LARVAE *Hermetia illucens* IN AQUACULTURE

Katarina Helena Doughty*, Michael Salini, Luke Wheat, Craig Lawrence, Julian C. Partridge, and Jan M. Hemmi

School of Biological Sciences & UWA Oceans Institute
University of Western Australia
Katarina.doughty@research.uwa.edu.au

The Black Soldier Fly (*Hermetia illucens*; BSF) has gained attention as a potential alternative feed source for aquaculture of finfish, as the fly's larvae have the ability to convert organic waste products into valuable fats and proteins that can be incorporated into aquaculture diets. This has the potential to allow the aquaculture industry to sustainably grow, producing more fish-based protein into the future. With the global increased in aquaculture production and fish consumption has come an associated increase in fisheries by-products, such as inedible products. These fisheries by-products are considered high value due to the protein and fat content. Studies have found that the substrate on which BSF are grown directly influence the fat and protein content of the BSF larvae. If BSF nutrient content can be manipulated by changing the diet fed to larvae, then perhaps incorporation of fisheries by-products would yield larvae with nutritional profiles targeted towards their use in aquafeeds. This would provide a mechanism to address growing fisheries waste management concerns and tailoring BSF insect meal product specific for feedstock purpose.

The study focused on the incorporation of fisheries by-products in BSF feeding substrate to assess any value-added potential to the final BSF product as it pertains to aquaculture feeds. We assessed inclusion fisheries by-products in the base substrate and as a finishing diet, described as feeding general organic waste (e.g., from horticulture) for most of the life cycle, with high-quality feed (e.g., fisheries products) in the critical time before harvest. St-Hilaire *et al*. (2007) showed that omega fatty acid profiles can be enhanced by supplementing a cow manure-based diet of larval BSF with processed fish waste (e.g., fish carcasses from canneries) in the last 24hrs of larval feeding (Figure 1.). Barroso *et al*. (2017), further supported this finding with the addition of omega-3 lipids to BSF diets resulting in BSF meal with three times more omega-3's than the control group. Finishing diets may allow the low-cost addition of high value food product (e.g., fish meal, or fish offal) resulting in high value insect meal. This would offer the potential to enhance the nutritional profile of BSF meal tailored for aquafeeds, with the potential of higher inclusion levels. This study determined how the incorporation of fisheries by-product influences BSF larval nutrient composition, as well as the potential to utilise BSF technology to address the growing concerns around increased quantities of fisheries wastes and by-products. The ability to utilise waste products from aquaculture in BSF farming promotes circularity, thereby reducing inefficiencies through reduction of resources from a range of industries required to produce aquafeeds.



TOWARDS MORE SUSTAINABLE FEED: SCALING OF CUTTING-EDGE CO_2-TO- MICROBIAL PROTEIN PRODUCTION

Draganovic, V., Johannessen, A., Dragvik, J. and Divjak, S.

Gas2Feed, Richard Johnsens gate 4, Stavanger, Norway

Introduction

As of today, producing 1 kg salmon leads to direct and indirect emissions of 5 kg CO2eq, with 80% of these emissions linked to fish feed (Winther et al., 2019). A major source for this greenhouse gas (GHG) is the usage of soy protein concentrate (SPC) imported from Brazil, while soy cultivation also causes major deforestation and biodiversity loss. To secure sustainability in aquaculture new sources of protein feed ingredients are needed, consuming less of the planet's limited area and water resources. Sustainable feed with low GHG footprint requires using non-fossil sources as the carbon storage time in such materials is short (months). Efficient production should therefore mimic photosynthesis in an efficient industrial setting not influencing arable land or habitats with high biodiversity.

Overall Idea

Among the various microbes suitable for production of protein as a feedstock for growing aquaculture needs, autotrophic HOB constitutes a special and unexplored potential for recovery and upgrade of inorganic matter. The metabolic features of autotrophic hydrogen-oxidizing bacteria (HOB) allow them to grow on hydrogen (electron donor) and oxygen (electron acceptor) while fixing carbon dioxide into cell material and assimilating nitrogen (inorganic) into high quality protein (Parkin and Sargent, 2012). Gas2Feed (G2F) aims to establish the world's first commercial production of sustainable microbial protein (MP) from air, water, and renewable energy for fish feed. We will use HOB microorganism able to fix CO2 as carbon source. By using a proprietary reactor design, we will reach low-cost fermentation. The product is a high-quality MP (80% crude protein), approved in EU (EU 68/2013), well suited for e.g. aquaculture applications.

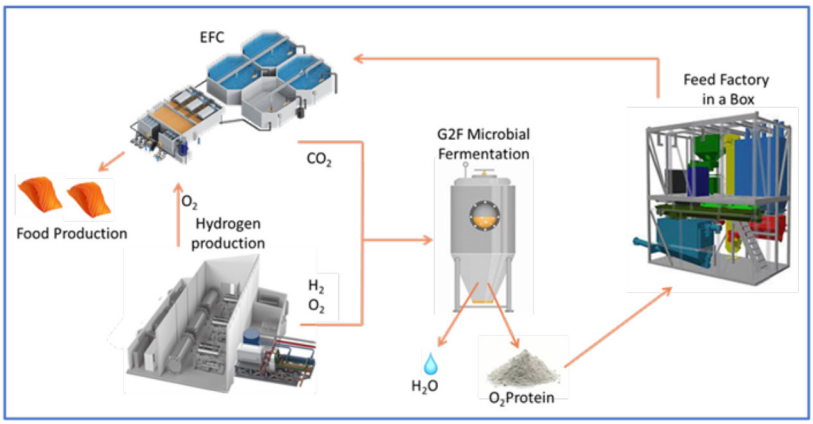


Fig. 1 Schematic overview of the concept of producing microbial protein from electricity, water and air.

Results and Discussion- The Circular concept

Schematic overview of the overall concept to address environmental challenges is given in figure below. The CO_2 for microbial production is derived from the fish tanks while hydrogen and oxygen are produced by water electrolysis (using renewable power). As such, this technology platform produces valuable products through Carbon Capture and Utilization (CCU). Further, oxygen from hydrolysis of water will be used to oxygenate the water in the fish tanks, while the hydrogen produced is consumed by the process presented in Fig. 1. The overall concept is a true circular economy concept.

References

Parkin, A., Sargent, F., 2012. The hows and whys of aerobic H2 metabolism. *Curr. Opin. Chem. Biol.* 16, 26-34.

Winther, U., Hognes, E.S., Jafarzadeh, S., and Ziegler, F. 2019 :01505, URL: file:///C:/Users/Administrator/OneDrive%20-%20EcoFishCircle%20AS/Skrivebord/SINTEF.pdf

IMAPCTS OF THE MANDATORY COMMISSIONING TESTING OF BALLAST WATER TREATMENT SYSTEMS ON THE RISK MANAGEMENT OF PATHOGEN TRASNFER ACROSS AQUATIC ECOSYSTEMS

Guillaume Drillet, Claudio Gianoli, Li Gang, Aristea Zacharopoulou, Gerd Schneider, Peter Stehouwer; Robert Goldring, Vladimiro Bonamin, Lisa A. Drake

Guillaume Drillet (PhD)
SGS Testing & Control Services Singapore Pte Ltd
30, Boon Lay Way, #03-01
Singapore 609957
Guillaume.Drillet@sgs.com

The International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) entered into force in 2017. The number of ships installing ballast water management systems (BWMS) to meet their compliance obligations has risen steeply in the last years. When BMWS are installed, "commissioning testing" occurs to determine the suitability of the installation and since June 2022 there is a requirement following the BWM Convention to include biological efficacy testing during all commissioning tests to verify that the installation allows for the ship to discharge water with organisms concentration below the "D-2 performance standard".

We present findings from >600 tests carried out in 28 countries on more than 25 technologies. Notably, nearly all failures to meet the D-2 performance standard occurred in the largest size class (≥50 μm) of organisms. Between November 2019 and March 2022, the failure to meet the D-2 standard during commissioning as decreased from >20% failure to <7% failure. The data set suggests that experience (spurred by mandatory, third-party testing) has allowed all stakeholders involved in the selling, design, planning, installation and operations of BWMS to correct many issues over time. This information suggest that regular testing of discharges from ship could improve the protection of aquatic environment from the transfer of harmful aquatic organisms and Pathogens

VARIATIONS IN FLAVOR ACCORDING TO FISH SIZE IN RAINBOW TROUT *Oncorhynchus mykiss*

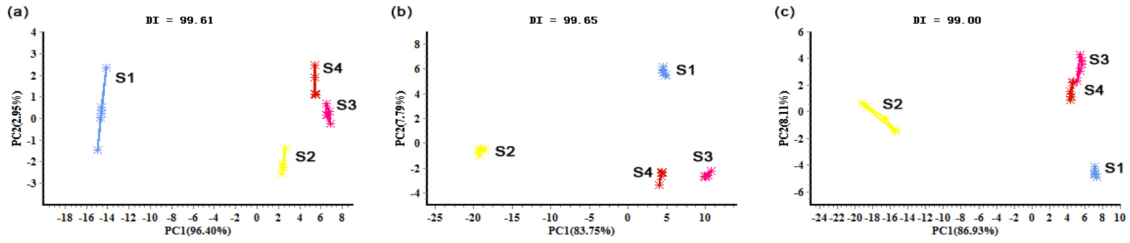
Zelin Duan*, Yangen Zhou, Wenjuan Liu, Chaojun Cathy Shi, Li Li, Yunwei Dong, Qinfeng Gao, Shuanglin Dong

Fisheries College, Ocean University of China, 5 Yushan Rd., Qingdao 266003, China
zelinduan@163.com

Rainbow trout (*Oncorhynchus mykiss*) is popular with consumers because of its delicate meat, smooth taste, bright color and high nutritional value, and is widely cultured in many countries. Fish quality is influenced by fish size as well as several other factors, including intrinsic (species, sex, and sexual maturity) and extrinsic factors (season, water temperature, salinity, and source of nutrients). The objective of this study was to evaluate the flavor qualities of the dorsal, tail, and abdominal muscles of four sizes of the rainbow trout.

Fresh triploid rainbow trout were collected at the same time in July 2018 from net cages in Liujiaxia Reservoir, Gansu Province, China. China. Four groups of fish were caught randomly from four different net cages; the average weights were 0.25 ± 0.06, 1.14 ± 0.09, 2.26 ± 0.24, and 2.89 ± 0.22 kg (Abbreviated as S1, S2, S3, and S4 groups, respectively). Results of this study showed the changes of flavor compounds in three tissues from rainbow trout of four sizes (Fig. 1). Notably, dorsal and tail tissues of rainbow trout seem to have superior quality than abdominal tissues. The S2 group had the highest levels of total free amino acid (FAA), the S1 and S2 groups had higher levels of sweet amino acids, and the S4 group had the highest levels of lactic acid and succinic acid. In fresh rainbow trout, the predominant nucleotide was Inosine-5'-Monophosphate (IMP). Electronic tongue analyses demonstrated taste differences for three tissues from rainbow trout of varying sizes. Differences were observed in fatty acids profiles for dorsal tissues, with large fish having significantly higher monounsaturated fatty acid (MUFA) contents and significantly lower polyunsaturated acid (PUFA) contents. The smallest fish (S1 group) had the highest docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contents. Based on these data, it indicates that fish size and tissues had variable effects on the flavor profiles of rainbow trout. In addition, using fish size to describe fish flavor may have applications in markets and could provide important information for consumers. However, the molecular mechanism of flavor changes in rainbow trout during growth is not clear, its molecular mechanism needs to be further studied.

Fig. 1. Results of PCA of the electronic tongue. (a) dorsal tissues; (b) abdominal tissues; (c) tail tissues.



HATCHERY-BASED MASS PRODUCTION OF MOLA (*Amblypharyngodon mola*) SEED TO SCALE NUTRITION-SENSITIVE AQUACULTURE

Sourabh K. Dubey, Francois Rajts, Kalpajit Gogoi, Rashmi R. Das, Saurava K. Biswal, Bibhuti B. Das, Arun Padiyar, Suresh Rajendran, Shakuntala H. Thilsted, Chadag V. Mohan, Ben Belton

WorldFish, Cuttack, Odisha 753001, India

*s.dubey@cgiar.org

Nutrition-sensitive approaches to aquaculture promote the production of micronutrient-rich small indigenous species (SIS) such as mola *Amblypharyngodon mola*, which is particularly rich in essential micronutrients, including vitamins A and B12, calcium and zinc, to address malnutrition in the developing world. However, to date, a lack of standardized hatchery-based mass production techniques for SIS seed has proven a key technical barrier to realizing the full potential of nutrition-sensitive aquaculture. To address this crucial bottleneck, WorldFish is implementing a project titled "Taking nutrition-sensitive carp-SIS polyculture technology to scale" in the Indian states of Assam and Odisha with funding from GIZ. A key goal of the project is to develop scalable mass production techniques for *A. mola* seed, based on a standardized protocol for hatchery-based breeding.

During breeding trials in Odisha, India, mola broodstock were collected from multiple sources to ensure genetic diversity and conditioned in broodstock ponds for two months at a partner hatchery (Lat 20°12'45.84"N/Long 86°20'3.32"E). Brooders were fed to satiation with 42% CP floating extruded feed twice daily. Six breeding experiments were conducted during peak breeding season (June–August 2022), with a natural photoperiod (12:12). Selected breeders were identified based on secondary sexual characteristics and transferred to a concrete conditioning tank (capacity: 10 m^3) with constant water flow for stimulating spawning readiness. A combination of hormone administration and environmental manipulation was used to increase seed production. Synthetic GnRH-based inducing hormone was administered through the peritoneal cavity of mola brood (mean body weight 8.0 ± 0.55 for females and 3.75 ± 0.81 g for males, with a 1: 2 sex ratio) between 16–20.5 h, at the rate of 0.5 ml kg^{-1} body weight for females and 0.25 ml kg^{-1} for males. The fish were returned to double hapas inside breeding tanks and exposed to a constant shower of oxygen-rich water from an overhead tank equipped with an aeration tower. At water temperatures of 28-29.5°C, the latency periods were observed as between 6–8 h, when male brooders began chasing the females within 6-8 hours of hormone administration. Each male was found to be actively and aggressively paired with a single female, while eggs were released and fertilized by the male. The released eggs were collected in 250 micron mesh outer hapa and became slightly adhesive following fertilization. The breeding activity was repeated several times. Hatching of larvae was observed after 12 h of fertilization at 28-29.5°C water temperature. Average performance values from the six breeding cycles are depicted in Table 1. The fertilization, hatching, and survival rates were 89%, 91% and 92% respectively (Table 1). Hatchling production was significantly correlated with female body weight ($r= 0.82$; $P \leq 0.05$). Average production over 6 breeding cycles was 0.28 million hatchlings kg^{-1} of female mola brood. Average physico-chemical parameters during the trials were: air temperature 32 °C, water temperature 28.75°C, pH 7.6, dissolved oxygen 7.2 ppm, and alkalinity 116.25 ppm. The six breeding trials produced a total 5.48 million mola hatchlings. Mola hatchlings were ready for sale after 3-4 days and 2.13 million hatchlings and 37000 fry (21 days old) were purchased by 15 local farmers. This ground-breaking commercial mass seed production trial with mola will facilitate large-scale adoption of carp-SIS polyculture to increase farm incomes and consumption of micronutrient-dense fish in regions of India where undernutrition is prevalent.

TABLE 1. Breeding performance of *Amblypharyngodon mola* during six induced breeding trials.

Particulars	Average value*
Body weight per female (g)	8.0 ± 0.55 (7.0-8.5)
Body weight per male (g)	3.75 ± 0.81 (2.7-4.5)
Injected females (kg)	3.28 ± 0.51 (2.5-4.0)
Fertilization rate (%)	89.28 ± 5.45 (81.8-96.86)
Hatching rate (%)	90.86 ± 7.53 (75.99-95.76)
Survival rate (%)	92.12 ± 4.47 (88.07-98.01)
Hatchlings harvested (million)	0.91 ± 0.30 (0.4-1.20)
Total hatchlings produced (million)	5.48

*Data were presented as Mean ± SD of six production cycles. Figures within parentheses indicted the ranges.

GENETIC DIVERSITY OF OF MALAYAN LEAFFISH (*Pristolepis fasciata* Bleeker, 1851) IN THE MEKONG DELTA

Duong Thuy Yen*, Nguyen Thi Ngoc Tran and Tran Dac Dinh

College of Aquaculture and Fisheries
Can Tho University, Viet Nam
Email: thuyyen@ctu.edu.vn

Malayan leaffish has a high potental for aquaculture and thus is a target species for domestication. This study was aimed to evaluate levels of genetic diversity of Malayan leaffish wild populations in the Mekong Delta to provide information for domestication programs of the species. Fish samples were collected from three locations representing for two types of habitats, including wetland conservation areas (Long An and Ca Mau) and inland water bodies in Hậu Giang (Figure 1). Six ISSR (Inter-simple sequence repeats) markers were used to amplify 95 samples. Results (Table 1) showed that 56 bands (5 to 12 bands per marker) were yielded with the polymorphic rate (P %) of 86.9% and expected heterozygosity (He) of 0.250. Long An population (n=33) had the highest genetic diversity parameters (P = 98.2%; He=0.289), which were not significantly different (P>0.05) from those of the other two populations in Ca Mau (n=30; P=80.4%; He=0.239) and Hau Giang (n=32; P=80.4%; He=0.245). The three populations had high levels of genetic identity and a large number of migrant per generation (Ne=9.3). Analyses of Nei's genetic distance and phylogenetic tree indicated Ca Mau and Hau Giang had a genetically closed relationship, smaller than those between these two populations and Long An.

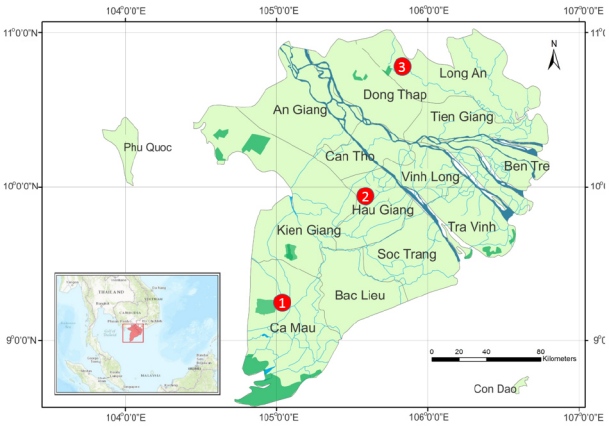


Figure 1: Map of the Mekong Delta with three sampling sites

Table 1: Genetic diversity parameters (±SD) of Malayan leaffish populations in the Mekong Delta

Population	N	(% P)	Ne	uHe	I
Ca Mau	30	80.4%	1.375 (0.042)	0.239 (0.022)	0.366 (0.032)
Hau Giang	32	80.4%	1.388 (0.044)	0.245 (0.023)	0.373 (0.032)
Long An	33	98.2%	1.474 (0.045)	0.289 (0.023)	0.435 (0.029)
Total	95	86.9%	1.412 (0.025)	0.258 (0.013)	0.391 (0.018)

N:sample sizes, %P: polymorphic ratio, Ne: effective number of allleles; uHe: unbiased heterozygosity; I: Shannon idex

EFFECTS OF INTRA-SPECIFIC CROSSBREEDING ON GROWTH AND SURVIVAL RATES OF BIGHEAD CATFISH (*Clarias macrocephalus*)

Duong Thuy Yen*, Nguyen Thanh Tuan, Huynh Thi Truc Ly and Pham Thanh Liem

College of Aquaculture and Fisheries
Can Tho University, Viet Nam
Email: thuyyen@ctu.edu.vn

Bighead catfish (*Clarias macrocephalus*) is an important aquaculture species in Southeast Asia countries but farming of this species has faced difficulties due to its slow growth and high mortality. This study was aimed to evaluate effects of intraspecific crossbreeding on growth and survival rates from fingerling to grow-out stages in order to provide information for selective breeding programs that can improve two important traits of the species. Three broodstock sources used for crossbreeding included wild bighead catfish in Ca Mau (CM) and Hau Giang (HG) and cultured fish in Can Tho (CT) populations (Figure 1). Nine diallel crosses were artificially produced from 15 to 18 pairs of each broodstock sources. Fish were cultured in 500 liters tanks (with 3 to 4 replicates) in a recirculating system. The experiment was divided into two stages, including 2 months from 60 days-old fingerling to juveniles (stage 1) and 3 months for grow-out (stage 2). Results from two stages showed a similar trend that growth rates of crossbreeds were influenced stronger by maternal parent than by paternal source. Crosses from cultured CT females grew fastest and those from wild HG females were lowest. Although differences among treatments were not significant (P>0.05), effect sizes were large and increased with experimental periods. Hybrids did not show heterosis on growth in compared with pure crosses. Survival rates were not significant among treatments (P>0.05). Results from this study indicate that genetic improvement programs of bighead catfish in the Mekong Delta can apply strain selection rather than crossbreeding.

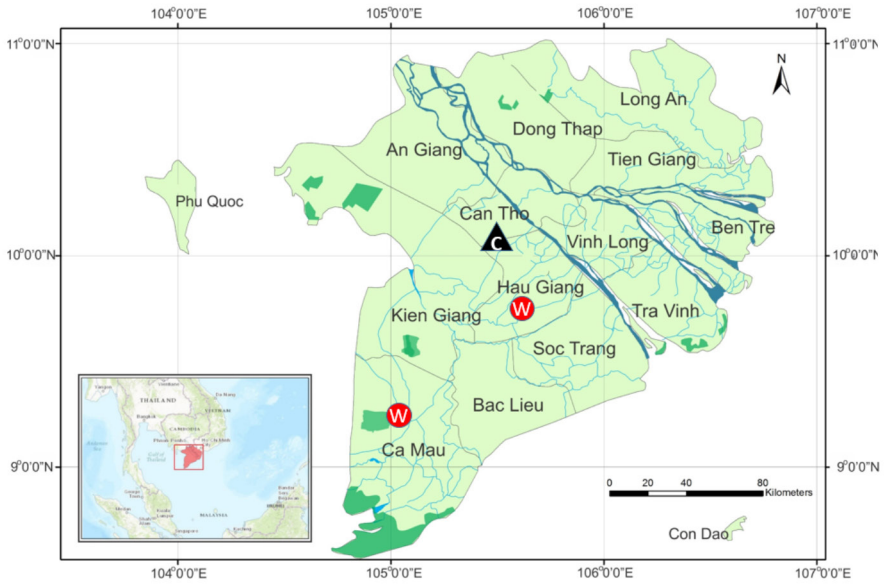


Figure 1: Sampling sites of two wild (W) and one cultured (C) populations of bighead catfish in the Mekong Delta, Viet Nam

DEVELOPING A MICRO AND MACRO ALGAE SECTOR IN NEW ZEALAND: AQUACULTURE, NUTRACEUTICAL AND PHARMACEUTICAL OPPORTUNITIES

Charles T. Eason*, Andy I Selwood, Craig Waugh, Tim Harwood, Lesley L. Rhodes, Lincoln Mackenzie, Serean L. Adams, Donato Romanazzi, Jonathan Puddick, Nick King, Mike A. Packer, Kirsty F. Smith, Marie Magnusson, Rocky De Nys and Johan Svenson

Cawthron Institute, 98 Halifax St East, Nelson, 7010 New Zealand

Email: Charles.Eason@cawthron.org.nz

Capability and capacity for algae R&D gained over 30 years, in New Zealand, includes commercial applications linked to aquaculture, food safety and biotechnology. Current research on high value products from micro and macro algae will be presented in an aquaculture context. Algae research with a focus on bioactives began in New Zealand in the 1980s and stepped up in 1993 when a major *Karenia* spp. bloom temporarily halted shellfish harvesting. For > 30 years the aquaculture industry has taken a lead with monitoring programmes alongside regulators aligned with proactive research to ensure seafood safety. New knowledge of *Alexandrium* species has been generated using such molecular tools as quantitative PCR (useful for cyst bed risk assessments) and next generation sequencing. To facilitate food safety internationally we are extracting and purifying various marine biotoxins (e.g. gymnodimine, pectenotoxin, paralytic shellfish toxins) for use as reference analytical standards for LCMS analysis and developing novel rapid detection and monitoring technologies for laboratory and field use.

Algae are a core component of the major collaborative R&D programmes, which include cryopreservation of microalgae, an internationally significant microalgae collection, microalgae production and extraction of bioactives, microalgae for aquaculture feed and experience with macroalgae cultivation. Novel compounds originating from algal biomass have been chemically determined and explored in collaborative R&D programmes to produce novel pharmaceutical applications, as well as analytical standards. Research on how to culture large volumes of microalgae and efficiently purify high-value bioactive compounds from them has positioned our researchers as leaders in the extraction of certain classes of these compounds with line-of-sight through to food, nutraceutical or pharmaceutical applications.

Recent research has confirmed that the marine macroalgae *Asparagopsis taxiformis*, when included in the feed of cattle at 0.05% to 0.2%, significantly decreased methane production which is increasing the demand for this seaweed. Progress on research underway in New Zealand to better understand the life-cycle of *Asparagopsis* and to optimise cultivation and aquaculture production systems will be outlined.

MARINE PECTIN FROM *Spirulina maxima*: MULTIFUNCTIONAL MODULATOR OF IMMUNE RESPONSE, WOUND HEALING AND REGENERATION IN ZEBRAFISH

S. L. Edirisinghe[1*], Chulhong Oh[2], Do-Hyung Kang[2], and Mahanama De Zoysa[1]

College of Veterinary Medicine, Chungnam National University, Daejeon 34134
Republic of Korea
Email: shan.lakmal09011@gmail.com

Pectin is a complex, branched and acidic polysaccharide polymer consisting a core of alternating α-(1-4)-linked D-galacturonic acid and α-(1,2)-L-rhamnose units. Recently, pectin has been investigated for its use in various biomedical applications such as food use, drug delivery, cosmetics, and scaffolding for cells. In this study, pectin was isolated from cyanobacterium *Spirulina maxima* (SmP), and its immunomodulatory, wound healing and regeneration were assessed using zebrafish (*Danio rario*) model. Upon the zebrafish larvae exposure with SmP (25 and 50 μg/mL) and SmP (4%) supplemented zebrafish were resistant to both *Edwardsiella piscicida* and *Aeromonas hydrophila* infections. Transcriptional profile described that the SmP exposed larvae, up-regulated the antimicrobial enzyme (*lysozyme*), mucin (*muc5.1, muc5.2, a*nd *muc5.3*), and anti-oxidants (*catalase* and *superoxide dismutase*). SmP fed zebrafish gut showed >2-fold induced pro-inflammatory cytokine (*il1β*) and chemokines (*cxcl18b*, *ccl34a.4* and *ccl34b.4*).

The wound healing function of SmP was investigated by *in-vitro* using the human dermal fibroblast cells (HDFs). SmP exposed (12.5 and 25 μg/mL) HDFs were significantly ($p<.001$) reduced the open wound area% as 32 and 12 %, respectively compared to control group (44%) at 18 hpe. Furthermore, zebrafish adult was also displayed significantly higher ($p<.05$) WHP upon the tropical application of SmP (46%) than the control (38%). Expression of wound healing related genes such as *tgf*β1, *timp2b*, *mmp9*, *tnf*α, *il-1β*, and chemokines (*cxcl18b*, *ccl34a.4* and *ccl34b.4*) were up-regulated in both muscle and kidney tissues suggesting SmP is a modulator of wound healing activity in zebrafish.

The capacity of SmP on fin regeneration in larval zebrafish was assessed by exposing to SmP (25 and 50 μg/mL). Regenerated fin area was significantly ($p<.001$) increased upon SmP exposure (50 μg/mL) at 2 and 3 dpa). Collectively, these results demonstrate that marine pectin derived from *S. maxima* displays multifunctional modulatory role in immune response, wound healing and regeneration hence, SmP could be applied for broad range of biomedical applications.

INTRODUCTION OF COMMERCIAL PRODUCTION OF *Oreochromis niloticus* IN IN-POND RACEWAY SYSTEM AQUACULTURE IN BANGLADESH

Md. Amimul Ehshan*, Md. Akbor Hossain, Md. Enaet Hossain and Shams M. Galib

Department of Fisheries (DoF)
Chapainawabganj 6300
Bangladesh
amisan72@yahoo.com

A study was carried out on first commercial introduction of in-pond raceway system (IPRS) aquaculture technology in Bangladesh in 2020-2021. *Oreochromis niloticus* a commercial aquaculture species tilapia was stocked at the density of 1.97 kg/m^3 in a 22m × 5m × 2m culture area raceway and at the end of 183days of production cycle harvest density was recorded 48.63kg/m^3. Specific growth rate was 1.96%d and overall FCR was found 1.55. Fishes were fed with 25% protein content commercial feed. As co-cultured fish species additional 692.31kg/ha, 503.85kg/ha, 1743.59kg/ha 294.87kg/ha 115.38kg/ha and 50kg/ha of *Labeo rohita*, *Catla catla*, *Hypopthalmicthys molitrix*, *Aristicthys nobilis*, *Ctenopharyngodon idella* and *Mylopharyngodon piceus* were harvested from outside raceway pond. Water quality parameters were more or less in suitable range. Results reveals that *Oreochromis niloticus* can be considered as a suitable species for IPRS aquaculture in Bangladesh.

EFFECT OF FEEDING FREQUENCY AND STOCKING DENSITY ON TILAPIA AND LETTUCE PRODUCTION IN AQUAPONICS SYSTEM UNDER THE UNITED ARAB EMIRATES CONDITION

Adil I. El Awad*, Ahmed M. Abdelrahman, Alyazyah S. Alkindi, Sabhah A. Alhmoudi, Lateifah A. Alsedrani, Fatima M. Al Sheaili and Eihab M. Fathelrahman

Department of Integrative Agriculture
College of Food and Agriculture
United Arab Emirates University, UAE
a.alawad@uaeu.ac.ae

United Arab Emirates (UAE), as an arid land country witnessing shortage of water and lacking in arable fertile land. However, UAE has made important progress to enhance its food security, but still food security and agriculture stability strongly addressed as real future challenges. In latest years, aquaponics system has gained high attention especially in connection of the efficient use of water and nutrients for an optimized aquaponics system. As a result, aquaponics would contributes to the sustainable agriculture in the dry areas by producing organic vegetables, fruits, and fish. The current experiments presented in this research were aimed to investigate the impact of varying Tilapia (*Oreochromis niloticus*) feeding frequency and stocking density on production of fish and quality of organic lettuce produced in an aquaponics system.

Experiment 1. Feeding Frequency: Three fish tanks, each stocked with 100 fish/m^3, were used to investigate the effect of feeding frequency of one time, two times and three times per day.

Experiment 2. Fish Stocking Density**:** Three fish tanks, each stocked with 100 fish/m^3, 120 fish/m^3 and 140 fish/m^3 of *Oreochromis niloticus* fingerlings with approximately an average weight of 15-20g. The Nile tilapia were fed while floating with a commercial feed of 36% protein.

Both experiments were lasted for three months with a replicated study. Lettuce was seeded in a culture raceway in each experiment. At the end of each experiment, the tilapia growth and growth parameters namely, fish weight gain (WG), feed intake (FI), and feed conversion ratio (FCR), protein and fat deposition values were measured or calculated. In parallel, the aquaponic system water quality (pH, temperature, total dissolved solids, dissolved Oxygen, total ammonia, nitrite, and nitrate) and water consumption were analyzed at specified intervals. Total production for fish and lettuce are shown in Table 1. & Table 2.

In conclusion, feeding frequency of three times per day and stocking density of 140 fish/m^3 are shown better lettuce production. Fish production was not affected by both feeding frequency and stocking density. Water quality also affected by increasing feeding frequency and stocking density.

	Feeding Frequency		
Parameter	1	2	3
Fish production (kg/m^3)	7.6 a	9.3 a	11.7 a
No. of lettuce heads (head/m^2)	28	28	28
Lettuce production (kg/m^2)	5.7 a	6.3 b	8.1 c

Table 1: Fish production and L. sativa production at different feeding frequency.

Table 2: Fish production and lettuce production at different stocking density.

	Stocking Density		
Parameter	100	120	140
Fish production (kg/m^3)	8.20 a	9.84 a	12.14 a
No. of lettuce heads (head/m^2)	28	28	28
Lettuce production (kg/m^2)	6,21 a	7.22 b	8.65 c

THE PROTECTIVE ROLE OF ALGAL EXTRACT AGAINST THE OXIDATIVE STRESS INDUCED BY DIMETHOATE IN ADULT ZEBRAFISH *Danio rerio*

Tamer El-Sayed Ali

Oceanography Department
Faculty of Science
Alexandria University
Egypt

Dimethoate is a widely used organophosphate (OP) insecticide. It was patented and introduced in the 1950s by American Cyanamid. Like other organophosphates, dimethoate is an acetylcholinesterase (AChE) inhibitor which disables cholinesterase, an enzyme essential for central nervous system function. It acts both by contact and through ingestion. It is readily absorbed and distributed throughout plant tissues, and is degraded relatively rapidly. Moreover, organophosphate pesticides cause irreversible inhibition of AChE which leads to neuronal overstimulation and death. Thus, the target of OP pesticides is AChE, but many authors postulate that these compounds also disturb cellular redox processes, and change the activities of antioxidant enzymes. Interestingly, it has also been reported that oxidative stress plays also a role in the regulation and activity of AChE. The aim of this study was to investigate the effect of dimethoate on oxidative stress biomarkers and acetylcholinesterase activity in zebrafish and the protective effect of marine algae *Sargassum* sp. extract against toxicological effect of such pesticide.

Several chemicals and biochemical assays were measured such as total antioxidants capacity, oxidative stress, minerals, fatty acids and phytochemical proprieties and the harm effect of dimethoate was detected. The *Sargassum* extract showed a potent antioxidant capacity, high mineral contents, fatty acids and different type of phytochemical compounds .The current study revealed that the marine bioactive extract has a protective effect against pesticides due to their potent antioxidant capacities resulting from their highly contents of phenolic and flavonoids.

THE RELATIONSHIP OF FEED INTAKE, GROWTH, NUTRIENT RETENTION AND OXYGEN CONSUMPTION TO FEED CONVERSION RATIO IN CHINOOK SALMON *Oncorhynchus tshawytscha*

Jordan E Elvy*, Jane E Symonds, Zoë Hilton, Seumas P Walker, Louis A Tremblay, Paula Casanovas and Neill A Herbert

Institute of Marine Science
University of Auckland
Auckland, New Zealand
jordan.elvy@cawthron.org.nz

Improving feed conversion ratio (FCR) can reduce both production costs and environmental impacts of farmed fish. To improve FCR, a better understanding of how it differs within a species and what factors might also be associated with driving FCR variation. Chinook salmon (*Oncorhynchus tshawytscha)* is a premium farmed salmonid in which very little is known about. This study looked to identify FCR variation within this species. Feed efficient and inefficient Chinook salmon, in both freshwater and saltwater, were identified using ballotini beads and X-radiography that tracked individual feed intake across three assessment periods under satiated feeding. This provided growth, feed intake and FCR values for individuals across two time points. Comparison of physiological traits and metabolism between the two FCR phenotypes and across timepoints was then carried out.

Daily feed intake (DFI), daily weight gain (DWG), and protein, lipid and energy retention were selected to compare to FCR. In saltwater salmon DWG negatively correlated with FCR efficiency while DFI was positively correlated. However, in freshwater salmon the relationship between FCR and DFI and FCR and DWG was variable between time points. FCR and DWG was not correlated at the first time point and was negatively correlated at the second time point. In contrast, FCR and DFI was positively correlated at the first time point but not the second. DWG and DFI was correlated in both freshwater and saltwater fish. Overall, FCR efficient fish had higher growth rates, ate smaller meals and had higher retention of protein, lipid and energy. Whole-animal metabolic oxygen consumption rates were measured to determine their minimal resting metabolic rate, maximum metabolic rate, aerobic scope as well as the amount of oxygen consumed for digestion, absorption and assimilation (a metabolic process known as specific dynamic action, SDA). There was no detectable difference in metabolism between the two FCR phenotypes with respect to maximum metabolic rate or aerobic scope for both freshwater and saltwater fish. However, the minimal resting metabolic rate was significantly lower in saltwater Chinook salmon but not in freshwater Chinook salmon. SDA parameters were measured only in the freshwater Chinook salmon and did not differ between phenotypes when fed a set ration.

In conclusion, metabolism did not differ between FCR phenotypes. However, feed efficient Chinook salmon are faster-growing fish that regulate their feed intake and retain a higher proportion of ingested nutrients, all of which are expected to reduce feed costs and environmental loading without impacting production.

POTENTIAL AND APPLICATION OF PHYCOREMEDIATION TECHNOLOGY USING *Chlorella vulgaris* FOR PALM OIL MILL EFFLUENT (POME) TREATMENT: AN EXPERIMENTAL STUDY

Quin Emparan*, Razif Harun, Rozita Omar, Dayang Radiah Awang Biak, and Aida Isma Mohd Idris

Department of Chemical and Environmental Engineering, Faculty of Engineering
Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia
gs46981@student.upm.edu.my

Palm oil mill effluent (POME) highly consists of organic and inorganic pollutants that can arise environmental issues if not treated properly. In Malaysia, a series of ponding system is the common conventional method to treat POME. Removal of inorganic nutrients and organic pollutants was limited by an ineffective ponding system that requires long hydraulic retention time and emission of greenhouse gases. The utilization of microalgae for simultaneous wastewater treatment and biomass production is currently of global interest due to the high capacity for nutrient uptake for their metabolic and photosynthetic activities. The aim of this study is to determine the efficiency of *Chlorella vulgaris* in treating POME by investigating the effects of POME concentration on the removal of chemical oxygen demand (COD), ammoniacal nitrogen (NH_3), and total phosphorus (TP) as well as biomass production. The microalgae cultivated with 10% POME yielded the highest biomass concentration of 0.603 g/L with a removal percentages of 80%, 99%, and 71% for COD, NH_3, and TP at the end of treatment ($p < 0.05$). Furthermore, the pseudo-first-order kinetic model for the nutrients removal showed a decreasing pattern proportionate to the increase in phycoremediation time. This treatment system could be a viable technology for sustainable POME treatment considering the shorter treatment time required and its effectiveness in reducing the nutrients from POME.

EX VIVO MODELISATION TO DETREMINE THE EFFECT OF A STANDARDIZED DRY GRAPE EXTRACT TO PROTECT SIBERIAN STURGEON *Acipenser baerii* RED BLOOD CELLS FROM OSMOTIC STRESS

P. Engler*, Y. Sakr, G. Le Reste, P. Garsi, S. Calvez and P. Chicoteau

Nor-Feed
3 rue Amedeo Avogadro
49070 Beaucouze, France
paul.engler@norfeed.net

In aquaculture, oxidative stress can be induced by water parameters such as salinity changes. Previous work showed that a standardized dry grape extract (SDGE) could be used in an *ex vivo* challenge to improve the stability of rainbow trout (*Oncorhynchus mykiss*) red blood cells (RBC) when exposed to an osmotic stress. The aim of this work was to extend the research on another anadromous species of fish, Siberian sturgeon (*Acipenser baerii*).

RBC solutions (RBCS, 25% v/v) were prepared from blood collected on healthy sturgeons, then mixed with phosphate buffer saline (PBS, pH = 7.4) and solutions of SDGE (Nor-Grape® WS, Nor-Feed) at various concentrations (1, 2, 3 and 6g/L in PBS). They were incubated at 56°C for 30 minutes, to induce a hemolysis reaction and were then centrifuged at 5000 rpm for 10 minutes at room temperature. The released hemoglobin was measured by spectrophotometry at 560 nm.

Results evidenced that SDGE at the two highest concentrations (3 and 6 g/L) reduced hemolysis of RBC to 32.4% and 35.6% respectively (Figure 1), compared to the lowest concentration (1g/L, 95.5% hemolysis).

This work showed the positive impact of a SDGE on the stability of RBC from Siberian sturgeon when exposed to an osmotic stress, in a similar way than with rainbow trout RBC.

Further research is required to establish if a dietary supplementation with a SDGE in the fish diet could evidence the same effect to manage oxidative stress, induced by the fluctuation of salinity, in order to reduce oxidative damage fish cells.

LESS IS MORE – A NEW DIETARY STRATEGY TO OPTIMIZE WHITELEG SHRIMP *Penaeus vannamei* PIGMENTATION

Paul Engler, Vianney Rolland, Aung Win, Thanh Chau Nhut, Yin Min Thant, Loc Tran

Nor-Feed SAS
49070 Beaucouzé, France
paul.engler@norfeed.net

Whiteleg shrimp (*Penaeus vannamei*) production is on the rise worldwide and whilst one of the qualitative aspects of the final product for the consumer is its pigmentation, the cost of dietary carotenoids in the shrimps' diets is far from negligeable. The aim of the present trial was to assess the potential of a standardized natural plant extract (PX100, Nor-Feed, France) to optimized shrimp pigmentation with and without reduction of the astaxanthin (AST) content in the shrimps' diet. 360 SPF *P. vannamei* weighing 1.00±0.11g were randomly divided into 3 groups of 120 shrimps each, subdivided into 3 tanks. stocked with 40 shrimps each (100pcs/m^3). The 3 groups received the same basal diet with either 40ppm AST added on top for the control diet (CTL), 40ppm AST and 600ppm PX100 (PX100) or 20ppm AST and 600ppm PX100 (50% AST) for 42 days. At the end of the supplementation period, 24 shrimps per tank were slaughtered in an ice bath and then simultaneously cooked. Their color was then assessed blindly using a DSM SalmoFan. Statistical analyses were carried out using R software. The supplementation with PX100 led to a significant improvement of shrimp pigmentation in all groups receiving it, irrelevant of their astaxanthin content ($p<0.05$). Interestingly, removing 50% of astaxanthin in presence of PX100 did not lead to an alteration of skin pigmentation, but to the contrary, to a significant increase of it. Overall, zootechnical performances were not significantly different between group. Interesting numerical improvement were however observed on FCR, SGR (Fig. 1) and on final biomass with an increase of +1.1% and +2.9% final biomass on average for groups 50% AST and PX100 vs. CTL group respectively (average final biomass per tank: CTL: 381g, 50% AST: 385g, PX100: 392g)

PX100 has shown a very interesting beneficial role in shrimp coloration and could thus be used in the pigmentation strategy to attenuate the overall cost of the latter at the same time as improving performances.

Figure 1. Shrimps' SGR and FCR during the trial

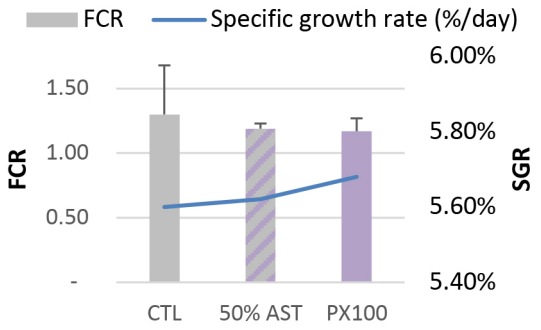
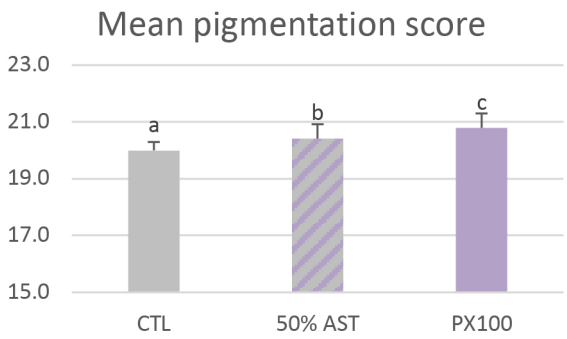


Figure 2. Mean fan pigmentation score of cooked shrimps



IS NATURAL COMPOUNDS SUPPLEMENTATION IN EGGS A STRATEGY TO IMPROVE DIGESTIVE EFFICIENCY IN MARINE FISH LARVAE?

S. Engrola*, D. Dias, G.M. Dardengo, A. Lopes, R. Colen, C. Navarro-Guillén

Centre of Marine Sciences (CCMAR), *Campus* de Gambelas, Faro, Portugal
*E-mail: sengrola@ualg.pt

Enhanced sustainable production of high quality and healthy fry is a key target for a successful and competitive expansion of the aquaculture industry and for attaining the seafood demand for direct human consumption in the near future. Early life environmental interventions are a promising way to modulate regulatory mechanisms, with the potential of improving growth rate, biological efficacy, and feed utilization efficiency. Nutrition is a strategy of early intervention that affect animal early development and later phenotype. The potential of early nutritional programming to improve gut maturation and digestive efficiency at larval early stages is enormous. This, in the long-term, may minimise the impact on the environment by lowering the biogenic emission from aquaculture facilities, while increasing productivity through a higher biological efficiency.

The present work assessed the effect of the polyamine spermine supplementation at egg stage, as promoter of early intestinal maturation in Senegalese sole larvae (*Solea senegalensis*). An innovative *in ovo* nutritional modulation technique based on low-frequency ultrasounds was used to enhance the transport of spermine across the embryo membranes. An early stimulus with three different spermine concentrations (Low, Medium and High) was applied in Senegalese sole embryos at 21 h post-fertilization. Spermine incorporation efficiency, hatching rate, growth performance, digestive enzyme activities and gut functionality biomarkers were analysed at different developmental stages to evaluate the larval nutrition-induced metabolic plasticity and the effects of early programming on fish digestive efficiency.

The preliminary results showed that hatching rate was not affected either by the sonophoresis technique or spermine supplementation ($p>0.05$). The results of the present work present a new bioactive compound that may act as promoter of early intestinal maturation, improved digestive capacity and growth performance in fish larvae, supporting sustainable self-sufficiency in fish aquaculture by integrating dietary strategies and relevant data obtained from new tools.

Acknowledgments: The present study was supported by projects ALG-01-0145-FEDER-029151 “PROLAR – Early metabolic programming in fish through nutritional modulation”, and UID/Multi/04326/2019 financed by the FCT (Portugal). Sofia Engrola acknowledge a FCT investigator grant (IF/00482/2014/CP1217/CT0005) funded by the European Social Fund, the Operational Programme Human Potential and FCT.

DIETARY ANTIOXIDANT SUPPLEMENTATION IN FLATFISHES: A COMPARATIVE STUDY

Sofia Engrola*, Maria João Xavier, Carolina Castro, Wilson Pinto, Renata Serradeiro, and Luis E.C. Conceição

Centre of Marine Sciences (CCMAR)
Campus of Gambelas, University of Algarve
8005-139 Faro, Portugal
sengrola@ualg.pt

Marine fish larvae present very high growth rates, hence, are quite susceptible to stress. Thereby, a tailored nutrition is essential to fulfil larvae growth potential and promote resilience. Several plant-derived extracts are considered promising feed additives, since is possible to maximise protein accretion, improve metabolic plasticity, and reduce stress while increasing fish resilience. The aim of this study is to provide a nutritional approach, to respond to some of the current challenges of marine hatcheries. Therefore, the potential of these natural additives as promoters of oxidative status, and muscle protein accretion are explored, at early stages of development of Senegalese sole (Solea senegalensis) and turbot (Scophthalmus maximus).

A 4-week growth trial with Senegalese sole postlarvae (45 days after hatching, DAH) fed with one of four experimental diets - control (CTRL), and supplemented with curcumin (CC), green tea (GT), and grape seed (GS) extracts. A second 5-week growth trial with turbot postlarvae (23DAH) fed with one of three experimental diets - control (CTRL) and supplemented with curcumin low dose (CCL) and curcumin high dose (CCH). After 3 weeks of feeding with the experimental diets, turbot (65DAH) were fed a commercial diet until the end of the experimental period. A thermal stress test was conducted at the end of both trials to assess how the dietary treatments modify the fish physiological responses.

Sole growth was improved by CC and GS diets when compared to those fed the CTRL. CC and CTRL postlarvae presented the lowest oxidative damage. Stress-related biomarkers decreased in CC fish compared to those fed the CTRL diet. Turbot growth was negatively affected by the higher inclusion dose (CCH) at 50DAH. After the feeding period with the commercial diet, all turbot presented similar weight independently of the dietary treatment. However, turbot fed CCL that presented similar weight to CTRL fish, presented a better total antioxidant capacity when compared to no-supplemented fish.

In conclusion, while dietary curcumin supplementation was able to promote growth and robustness through a modulation of the oxidative status in Senegalese sole, only the latter was observed in turbot (Fig. 1).

Acknowledgements: This work is part of MAXIMUS.PT project (ref. 69769) supported by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020; and co-financed by the Portuguese Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 to CCMAR.

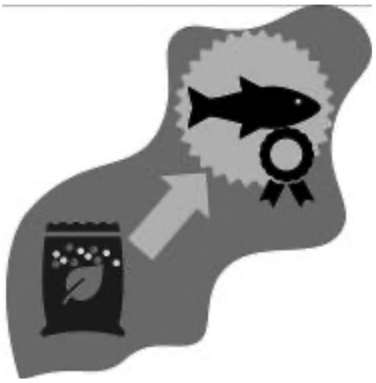


Figure 1 – Dietary antioxidants supplementation at early developmental stages may improve fish robustness in the long-term.

DIETARY VITAMIN B5 REQUIREMENT OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Gunho Eom*, and Kyeong-Jun Lee

Department of Marine Life Science
Jeju National University
Jeju 63243, South Korea
gheom@jejunu.ac.kr

No study has been conducted on dietary pantothenic acid (PA) requirement for Pacific white shrimp, while several studies have been done with fish species. Thus, this study was conducted to determine dietary PA requirement for the shrimp.

A control (Con+) diet was formulated to meet the nutrient requirement of shrimp without PA supplementation. A negative control (Con-) diet was formulated with 0.4% tetracycline hydrochloride for the evaluation of the effects of PA synthesis by intestinal microbiota in shrimp. Six other diets were prepared with graded levels of calcium pantothenate at 30, 60, 90, 120, 150 and 180 mg/kg (designated as P30, P60, P90, P120, P150 and P180, respectively). The PA content of both Con+ and Con- diets were not detected and determined to 28.3, 51.5, 92.8, 127.5, 169.1 and 229.9 mg kg^{-1}, respectively, for P30, P60, P90, P120, P150 and P180. Total 800 shrimp (1.20±0.01 g) were stocked into 32 acrylic tanks (180 L) in quadruplicates per dietary treatment. Shrimp were fed the diets six times a day for 42 days with a feeding rate of 4–10% of the biomass.

Dietary PA supplementation have a significant effect on growth performance and feed utilization of shrimp. Final body weight (FBW), weight gain (WG) and specific growth rate (SGR) of PA supplemented groups, except for P30 and P60, were significantly higher than those of Con groups. A ratio of total *Lactobacillus*/heterotrophic bacteria counts was significantly higher in P90 - P180 groups than Con groups. Phenoloxidase and lysozyme activities were significantly increased in P120 group than in Con groups. These results indicate that the optimum dietary PA requirement of Pacific white shrimp is about 90-120 mg PA/kg diet and that PA seems to be synthesized in the shrimp intestine but the amount is not enough to meet PA requirement of Pacific white shrimp.

TABLE 1. Growth performance, feed utilization and survival of Pacific white shrimp (*Litopenaeus vannamei*) fed the experimental diets for 42 days

Dietary treatments	FBW(g)	WG(%)	SGR(%)	FCR	FI (g/shrimp)	Survival (%)
Con+	9.02±0.31^{ab}	653±24.5^{ab}	4.81±0.08^{ab}	1.26±0.07	9.81±0.54^{ab}	86.0±5.16
Con-	8.41±0.40^{b}	605±37.0^{b}	4.65±0.12^{b}	1.34±0.14	9.63±0.90^{b}	83.0±8.25
P30	9.36±0.13^{ab}	685±7.62^{ab}	4.91±0.02^{a}	1.28±0.05	10.5±0.43^{ab}	83.0±2.00
P60	9.40±0.41^{a}	681±40.9^{ab}	4.89±0.12^{ab}	1.25±0.11	10.2±0.53^{ab}	85.0±6.00
P90	9.45±0.56^{a}	691±49.1^{a}	4.92±0.15^{a}	1.35±0.15	11.1±0.44^{a}	79.0±3.83
P120	9.58±0.53^{a}	698±43.7^{a}	4.94±0.13^{a}	1.24±0.10	10.3±0.46^{ab}	84.0±5.66
P150	9.54±0.13^{a}	691±9.23^{a}	4.92±0.03^{a}	1.26±0.11	10.5±0.90^{ab}	85.0±6.00
P180	9.79±0.55^{a}	723±50.0^{a}	5.01±0.14^{a}	1.27±0.09	10.9±0.43^{ab}	78.0±4.00

HIGH-FAT DIET AND COLD SHOCK IN ZEBRAFISH INDUCE BAT-RELATED MOLECULAR CHANGES

H.-J. Eom, S.-E. Nam, **J.-S. Rhee**

Incheon, Republic of Korea
119, Academy-ro, Yeonsu-gu
Ksks80@naver.com

Brown adipose tissue (BAT) is known to consume ATP via mitochondria and thermogenesis. There have been many studies on differentiating BAT through Cold exposure and chemical treatment, However, studies on fish are unclear. Here, we present how to induce BAT in zebrafish without genetic modification. First, in brown preadipocyte t37i, a drug that induces BAT differentiation was treated, changes in gene and protein levels were confirmed. Second, at the animal level after exposing zebrafish to a high-fat diet and cold, check the differentiation of white adipose tissue (WAT) and BAT generation. This was reconfirmed by changes in gene and protein expression. This study can be used as a basis for the study of brown fat in fish and provides inspiration for the use of zebrafish as a brown fat model species.

Aeromonas dhakensis IS THE LEADING CAUSE OF MOTILE AEROMONAS SEPTICEMIA IN VIETNAMESE STRIPED CATFISH FARMING

Vera Irene Erickson*, Yaovi Mahuton Gildas Hounmanou, Tu Thanh Dung, Le Minh Khoi, Tran Minh Phu, Anders Dalsgaard

Department of Veterinary and Animal Sciences
University of Copenhagen
Stigbøjlen 4, 1870 Frederiksberg, Denmark
vera.erickson@sund.ku.dk

Aeromonas species are responsible for the disease motile Aeromonas septicemia (MAS) in striped catfish (*Pangasius hypopthalmus*). *A. hydrophila* is so far considered the most common cause of MAS in Vietnam, but other species have also been isolated from diseased fish. We analyzed 86 bacterial isolates collected from the kidneys of striped catfish with MAS symptoms. The isolates were collected over a 5-year period (2017-2021) from nine provinces in the Mekong Delta. Sixty-eight isolates were identified as *A. hydrophila* by MALDI-TOF while remaining isolates belonged to other *Aeromonas* spp.. The minimum inhibitory concentration (MIC) of all isolates was determined for 14 antimicrobials. Thirty representative isolates were selected for whole genome sequencing (WGS) based on their sampling year and province of origin. Surprisingly, from the WGS analysis, only 5/30 isolates were confirmed as *A. hydrophila* while 25/30 isolates were identified as *A. dhakensis*, making *A. dhakensis* the predominant species in the analyzed collection. All of the *A. hydrophila* isolates belonged to sequence type (ST) 251 and all *A. dhakensis* belonged to ST 656. This finding was also supported by the phylogenetic analysis where isolates of one species grouped in a separate cluster (Fig. 1). All of the *A. hydrophila* isolates had an MIC ≤ 0.12 for enrofloxacin, ≤ 2 for tetracycline and oxytetracycline and ≤ 1 for florfenicol. For *A. dhakensis* the MIC ranged from ≤ 0.12 – 2 for enrofloxacin, ≤ 0.12 – > 8 for tetracycline and oxytetracycline, and ≤ 1 – > 8 for florfenicol. The strains harbored resistance genes like *sul1*, *sul2* and *floR* (Fig. 1). All genomes carried genes coding for translocation of proteins (type two and type six secretions systems), motility (polar flagella), motility and adherence (Tap type IV pili) and genes coding for aerolysin and cytolytic enterotoxin (*aer* and *act*). Additionally, all *A. hydrophila* isolates carried genes coding for biofilm and autoagglutination (Flp type IV pili) and cytotonic enterotoxin (*ast*). Each of the mentioned virulence factors contributes to the virulence of *Aeromonas* spp.. A nucleotide BLAST of the contigs where resistance genes were located revealed that the *sul2*, *qnrS2* and *floR* genes were plasmid mediated. Because *A. hydrophila* has been considered the leading cause of MAS, current vaccines include *A. hydrophila* strains. Our study indicate that *A. dhakensis* is the dominating species associated with MAS in the Mekong Delta, but further studies of this apparent change in species are warranted, e.g. *A. dhakensis* needs further characterization and it should be determined if current vaccines also protect against *A. dhakensis*. Improved methods for correct identification of the different *Aeromonas* species are needed.

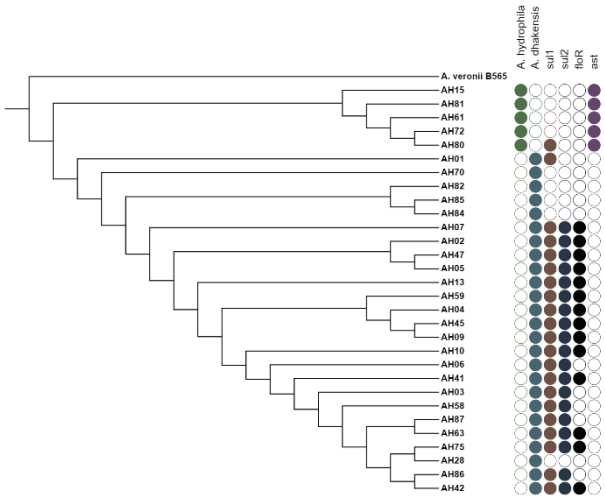


Figure 1. Phylogenetic tree showing the species and presence or absence of the sul1, sul2, floR and ast genes. Aeromonas veronii B565 was used as reference genome.

EFECTO DE LOS EFLUENTES DE CULTIVO DE CAMARÓN *Litopenaeus vannamei* MANEJADO CON BIOFLOC Y ALTA DENSIDAD SOBRE EL DESARROLLO Y PRODUCCIÓN DE TOMATE *Solanum lycopersicum* L

Héctor M. Esparza-Leal*, Carlos H. Iribe Bórquez, Adolfo D. Armenta Bojórquez, Ely S. López-Álvarez, Nadia Vazquez-Montoya, Mariel López-Espinoza, Wenceslao Valenzuela-Quiñónez, Magnolia Montoya Mejía

*Instituto Politécnico Nacional-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes # 250, Guasave, Sinaloa 81101, Mexico
hesparza@ipn.mx

A limitations for aquaponic farming with tomato-shrimp is that while the optimum temperature for tomatoes fluctuates between 15 and 25 °C, that of shrimp fluctuates between 25 and 30 °C. Therefore, the integration of aquaponics with tomato-shrimp represents a challenge that can be addressed. For this reason, the present work aimed to determine the effect of the effluents of a shrimp culture *Litopenaeus vannamei* managed with biofloc with high stocking density and enriched with 100 mg/L of nitrate, on the development and production of tomato *Solanum lycopersicum* L in autumn-winter. We worked with four treatments, which were based on watering with two types of water sources and two densities of tomato planting: T1 = tomato crop with high planting density (4.1 plants/m^2) irrigated with the effluents from the shrimp strengthened with 100 mg/L nitrate, T2 = tomato crop with low planting density (2.7 plants/m^2) irrigated with effluents from the shrimp crop fortified with 100 mg/L nitrate, T3 = tomato crop with high density sowing (4.1 plants/m^2) irrigated with Steiner solution (740 mg/L nitrate) and T4 = tomato crop with low planting density (2.7 plants/m^2) irrigated with Steiner solution (740 mg/L of nitrate). During the study, salinity fluctuated between 1 and 6 ‰. In the shrimp culture there was a 36% survival and a growth of 0.58 g/week. With respect to tomato cultivation, it was determined that using the shrimp effluents and strengthening them with no more than 100 mg/L of nitrates, a good development of tomato plants can be maintained. During flowering and fruit formation, the thickness and height of the stem of the plants showed significant statistical differences between the treatments. The thickest stems (T3 = 0.92 and T4 = 0.97 cm) and higher height (T3 = 207.47 and T4 = 201.27 cm) were observed where it was watered with the Steiner solution more that where it was watered only with the shrimp effluents enriched with nitrate (T1 = 0.73 and T2 = 0.79 cm; T1 = 162.28 and T2 = 158.67 cm, respectively), regardless of the tomato planting density. Also, there were significant statistical differences in production between treatments, obtaining a lower production where it was watered only with the effluents from the shrimp culture enriched with nitrate (T1 = 32 and T2 = 38 ton, extrapolated values) than where it was watered with the Steiner solution (T3 = 56 and T2 = 68 ton, extrapolated values).

EFFECTS OF DIETARY pH ON GROWTH AND FEED EFFICIENCY OF THE NILE TILAPIA *Oreochromis niloticus*

Ryan V. Fabay*, Augusto E. Serrano, Barry Leonard M. Tumbokon

National Institute of Molecular Biology and Biotechnology
University of the Philippines Visayas
Miagao 5023, Iloilo
ryanfabay1990@yahoo.com

Two feeding trials were conducted to investigate the effects of pH level (pH 2.5, pH 3.7, pH 4.6, pH 5.7-control, pH 7.0, pH 8.0) and different acids (acetic acid, citric acid, hydrochloric acid and control-no acid) on growth, feed utilization and survival of tilapia. In experiments 1 and 2, the attractability of the diet were assessed. Results of the experiments are shown below.

The experimental diet at pH 4.6 resulted in significantly the best growth and feed utilization. Results showed that addition of any acid in the diet (hydrochloric, citric and acetic acid) resulted in better growth than that of the control group. This study demonstrated that pH and not actually the kind of acid diet promoted growth and efficiency in the Nile Tilapia fry.

Figure 1. Attractability test of the basal diet and of test diet in experiment 1 and 2 after 3 minutes of feed placement

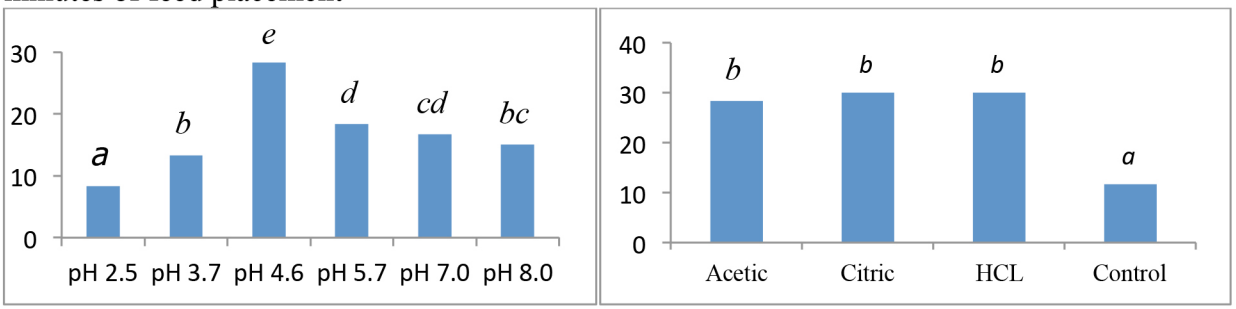


Table 1. Growth efficiency and survival of tilapia fry after 56 days of feeding at various dietary pH

pH level	IABW	ABW	FI	WG	SGR	FCR	SR
2.5	0.2	1.0±0.1^{a}	2.7±0.1	0.8±0.1^{a}	1.5±0.1^{a}	3.6±0.3^{c}	86.7±2.7^{a}
3.7	0.2	1.9±0.0^{b}	3.2±0.1	1.7±0.0^{bc}	3.3±0.1^{bc}	1.8±0.0^{b}	100.0±0.0^{c}
4.6	0.2	2.7±0.2^{c}	3.4±0.2	2.6±0.2^{d}	4.9±0.3^{d}	1.3±0.0^{a}	100.0±0.0^{c}
5.7	0.2	2.1±0.0^{b}	3.3±0.0	1.9±0.0^{c}	3.7±0.1^{c}	1.8±0.0^{b}	100.0±0.0^{c}
7.0	0.2	2.0±0.1^{b}	3.2±0.1	1.8±0.1^{bc}	3.5±0.1^{bc}	1.8±0.1^{b}	100.0±0.0^{c}
8.0	0.2	1.9±0.1^{b}	3.1±0.1	1.6±0.0^{b}	3.3±0.2^{b}	1.9±0.0^{b}	92.0±2.3^{b}

Table 2. Growth efficiency and survival of tilapia fry after 56 days of feeding at various dietary acid

Acids	IABW	ABW	FI	WG	SGR	FCR	SR
Acetic	0.1	2.7±0.1^{b}	3.8±0.1	2.6±0.0^{b}	4.7±0.1^{b}	1.5±0.3^{a}	100.0±0.0
Citric	0.1	2.7±0.1^{b}	3.8±0.1	2.6±0.0^{b}	4.7±0.1^{b}	1.5±0.0^{a}	100.0±0.0
Hydrochloric	0.1	2.7±0.1^{b}	3.8±0.1	2.6±0.0^{b}	4.8±0.2^{b}	1.4±0.0^{a}	100.0±0.0
Control	0.1	2.1±0.1^{a}	3.5±0.1	2.0±0.1^{a}	3.6±0.2^{a}	1.8±0.1^{b}	100.0±0.0

Initial average body weight (IABW), average body weight (ABW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI), and survival rate (SR). Means in the same column sharing the same superscript are not significantly different (p>0.05).

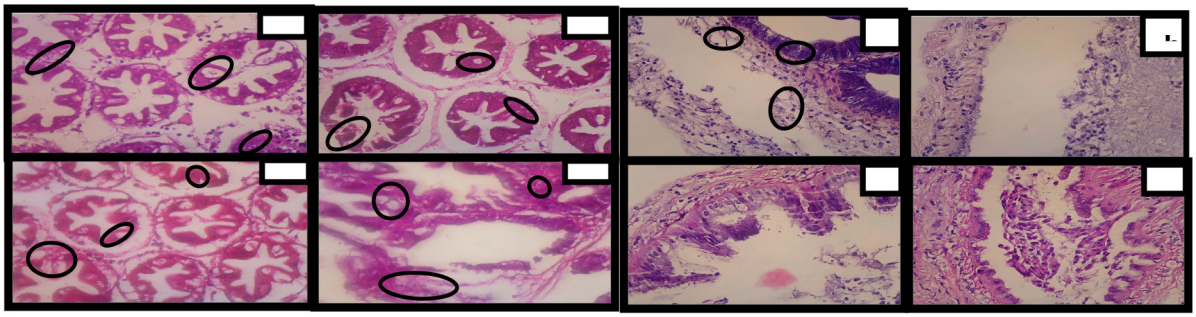
DIGESTIVE ENZYME ACTIVITY AND HISTOPATHOLOGY OF WHITE SHRIMP (*Litopenaeus vannamei*) ORGAN INFECTED WITH WHITE FECES SYNDROME (WFS) TREATED WITH SQUID (*Loligo* sp.) INK EXTRACT IN DIFFERENT MEDIA SALINITY

Mohamad Fadjar*, Nafa Aulia Ramadani, Ivana Agustin, Jefri Anjaini, and Laksono Radityo Suwandi

Aquaculture Departement, Faculty of Fisheries and Marine Science, University of Brawijaya
Malang, Indonesia
f4dj4r_02@ub.ac.id

White shrimp (*Litopenaeus vannamei*) is one of the shrimp species that is widely cultivated because of economic value and one of the national superior commodities. Salinity is one that influences the shrimp, if it does not fit the shrimp, it will stress and cause an outbreak of disease, it is easier to infect shrimp, one of them is white feces syndrome (WFS). The use of squid ink is one of the solutions used to inhibit WFS because squid ink contains oleic acid which can kill bacteria directly by damaging the cell wall. The purpose of this study was to determine the effect of squid ink extract (*Loligo* sp.) on histopathology of hepatopancreas and gut, also digestive enzymes in white shrimp (L. *vannamei*) infected with WFS in different media salinity.

The effect of salinity test was carried out by infecting 30 DOC (Day of Culture) white shrimp with 10^6 CFU/mL V. *harveyii* using immersion method, addition of blue green algae (BGA), and continued with tissue histology. Using 3 treatments, 25 ppt (A), 27 ppt (B), 30 ppt (C), and negative control (33 ppt, healthy shrimp). 8 ppm squid ink extract was add to the feed for 2 weeks treatment. The best media salinity was 27 ppt which affected the histopathology of the white shrimp hepatopancreas, while it didn't affect the histopathology of gut tissue. The average increase in digestible enzime was higher in 27 ppt than other treatments, protease enzyme activity was 0,378 U/mL, amylase enzyme was 4,029 U/mL, and lipase enzyme was 3,613 U/mL. The highest survival rate was 85% in 27 ppt salinity. Conclusion from this research was using 27 ppt salinity and squid ink extract for white shrimp (*L. vannamei*) with WFS case.



1. Hepatopancreas 2. Gut

PRELIMINARY STUDY: PRODUCTIVITY OF *Isochrysis galbana* IN AN OUTDOOR SCALE-UP MULTIPLE BAGS REACTOR SYSTEM

Farahin Abd Wahab*, Razif Harun, Divyashini Mohan, Abdulrahman Ahmed Qanaan, Quin Emparan, Yoshiki Takayama, Masahiro Otake, Natrah Ikhsan, Fatimah Md Yusoff, and Tatsuki Toda

Department of Chemical and Environmental Engineering,
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor Malaysia
nfarahin_wahab@yahoo.com

Various outdoor microalgae cultivation systems have been designed and optimized for outdoor microalgae culture such as open raceway pond, horizontal, vertical tubular, flat panel photobioreactors, etc. However, the production of high microalgae biomass under outdoor cultivation is influenced by several abiotic and biotic factors, which impact its profitability and sustainability. This study aims to evaluate the factors affecting the production of microalgae, Isochrysis galbana, using an outdoor scale-up multiple bags reactor system installed under Malaysia's tropical conditions. The outdoor scaled-up multiple bag reactor systems is a novel, low-cost, low-energy closed photobioreactor deployed on the water's surface, which can cool the reactor evenly. Designed with a closed system, it reduces contamination risks, which is a clear advantage for producing high-value microalgal biomass. Two parameters were investigated, including the effects of i) inoculation ratio to the culture volume (15 to 50% v/v) and ii) cultivation mode (batch and continuous). Based on the preliminary investigation, the 15% of inoculation ratio showed the maximum specific growth rate (0.99 day^{-1}) compared with the 33% (0.68 day^{-1}) and 50% (0.09 day^{-1}) inoculation ratio. However, the 15% inoculation ratio required a longer time (eleven days) to achieve the maximum growth compared to 33% (8 days). Considering the cultivation cost, energy, and contamination risk, the 33% sample was chosen as the best inoculation ratio for the cultivation mode experiments. The results showed that the semi-continuous mode achieved a higher specific growth rate (0.943-1.02 day^{-1}) than the batch mode (0.68 day^{-1}). Also, the semi-continuous culture provides higher quality biomass composition than the batch culture. Overall, the biomass productivity level achieved in this study which is in keeping with that of existing outdoor cultivation technologies endorses the system and offers a promising technology for outdoor microalgae mass production.

AQUAPONICS AND RENEWABLE ENERGY SYSTEMS INTEGRATION IN THE UNITED ARAB EMIRATES

Eihab Fathelrahman * Reem Bawazir, Mariam Alazeezi, Shaikha Alblooshi, Asma Albedwawi, Hussain Shareef, Zahi Osman Mohamed, and Adel Ismail AlAwad

Department on Integrative Agriculture
College of Food and Agriculture
United Arab Emirates University, Al Ain, UAE
eihab.fathelrahman@uaeu.ac.ae

United Arab Emirates (UAE) government offers and support business opportunities for small and medium size entrepreneurships to explore and expand aquaponics projects that are relevant to addressing the country's food security challenges. The UAE government goal is to support the development of projects that can succeed and become sustainable under conditions of scarce natural resources such as lack of usable water and fertile land and reduce energy consumption.

The first objectives of this research is to describe the advantages of the aquaculture and aquaponics and fish-vegetables integrated systems based on several technical and economic indicator. The second objective estimates the Total Revenue (TR), Variable Costs (VC), Fixed Cost (FC), and Net Return per the integrated renewable (solar) energy system when assuming growing Tomatoes and raising Tilapia fish (*Oreochromis niloticus*).

The system is suitable for UAE due to the scarce natural resources conditions. The case study describes the advantages of the fish-vegetables integrated system based on on-site experiments.

The exploratory study findings showed that:
1- The project addresses UAE Food Security elements of availability and accessibility.
2- Business proposition of the project showed economic viability of the integrated system at the scale of a greenhouses when the system is operating of –the-grid relying on solar system – Figure 1.
3-Prodcut/Technology description indicated the need for appropriate battery to enable continuity of power source day and night for both the cooling system and fish tanks water pumps – Figure 2.
4- Sustainability analysis upon scaling summarizes the advantages and disadvantages of the economic size of the system.

Figure 1. Integrated Aqquapic System

Figure 2. Added Solar System Componet.

PILOT SCALE COMMERCIAL PRODUCTION OF *Labeo rohita* IN IN-POND RACEWAYS SYSTEM IN PAKISTAN

Shafaq Fatima*[1], Wajeeha Komal[1], Saeed Anwar[1], Farkhanda Manzoor[1], R.S.N. Janjua[2]

[1]Department of Zoology, Lahore College For Women University, Lahore, Pakistan
[2]SoyPak, Pvt. Ltd.
Email: shafaq.fatima@y7mail.com

A pilot scale commercial-scale, in-pond raceways system was constructed in 2019 on a commercial fish farm in Punjab, Pakistan. The in-pond raceway system was installed in a 8 acres earthen pond with an average depth of 2.25 m. It consists of three raceways, each with dimensions of 22 m (L) × 5 m (W)× 2.25 m (D). With an operating depth of 2 m, the volume of water enclosed is 220 m^3. The total culture volume of the raceways for holding and growing fish is 660 m^3, equivalent to 2.33 percent of the total surface area of the production pond. Raceways are equipped with five regenerative blowers of 1.6 kW horsepower each. Additionally, the pond has three whitewater units, a jet aerator and a paddlewheel aerator installed in the open water area, which helps in mixing, accelerating and circulating water around the pond. One raceway was originally stocked with 7,700 labeo (*Labeo rohita*) fingerlings weighing between 300 – 310 g. During the 2019 production season, mean survival was 99.90 %. A total of 8,121 kg (1,160/acre) of labeo were harvested from raceway. Growth rate and specific growth rate were 4.93 g/fish/day and 0.25 % body weight/day, respectively. The average feed conversion ratio (FCR) for labeo was 1.8. An additional 3368 kg of catla and 4401 kg of silver carp were harvested from the pond as co-cultured species. The results indicated a high potential for efficient production of labeo with other co-cultured species in in-pond raceways system in Pakistan.

THE SHRIMP IMPROVEMENT PROGRAM (SIP) IN INDONESIA: A JURISDICTIONAL APPROACH TO RESPONSIBLE AQUACULTURE

Satya Reza Faturakhmat*, Achmad Fuad Fathurrahman, Ateng Supriatna, Dane Klinger, Garrett Goto, and John N. Kittinger

Conservation International Indonesia
Jl. Pejaten Barat No. 16 A
Kemang, Jakarta 12550, Indonesia
sfaturakhmat@conservation.org

The Indonesian government has set ambitious medium-term production targets for its aquaculture sector to address economic growth and food security concerns. Rapid expansion of the Indonesian aquaculture sector has outpaced the development and implementation of strong governance and management, exposing market, environmental, and disease risks that occur beyond individual farms. A jurisdictional approach to sectoral improvement that addresses such risks at politically, economically, and ecologically relevant scales can provide a foundation for projected growth without jeopardizing environmental performance or disease risks. Conservation International, in partnership with Sustainable Fisheries Partnership, IDH – the Sustainable Trade Initiative, and Longline Environment is implementing a jurisdictional approach improvement program with the shrimp aquaculture sector in the Banyuwangi Regency of Indonesia to increase responsible production and attract innovative finance vehicles that will help the sector achieve their targets. The program will archetype the ability of jurisdictional initiatives to address multiple commercial, political, and environmental barriers that catalyze responsible growth in the Indonesian aquaculture sector as a model for improvement that can be replicated in other geographies and aquaculture sectors. This talk will highlight key lessons learned during implementation and novel approaches to responsible production for Indonesia and beyond.

ENZYMES OBTAINED THROUGH SOLID-STATE FERMENTATION OF BREWER'S SPENT GRAIN INCREASE DIGESTIBILITY IN EUROPEAN SEABASS (*Dicentrarchus labrax*) – AN *IN VITRO* AND *IN VIVO* APPROACHES

Helena Fernandes[1,2*], Carolina Castro[1,2], Nelson Fernandes[1], Patrícia Ferreira[2], Margarida Gonçalves[1], Francisco J. Moyano[3], Tiago Aires[5], Isabel Belo[4], José Salgado[4], Aires Oliva-Teles[1,2], Helena Peres[1,2]

[1]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal
[2] CIMAR/CIIMAR-Centro Interdisciplinar Marinha e Ambiental, Universidade do Porto, Portugal
[*]*e*-mail address: helenafernandes012@gmail.com

Low-value agro-industrial by-products are excellent substrates to produce bioactive compounds through solid-state fermentation (SSF). In the present work, brewer's spent grain (BSG), the main by-product generated by the brewery industry, was fermented by *Aspergillus ibericus* (MUM 03.49), producing an extract (BSG/SSF extract) with high cellulase (1343 U g^{-1} lyophilized extract) and xylanase (15885 U g^{-1}) activities. A practical diet (60% plant feedftuffs+15% FM; 48% CP; 16% CL) was supplemented with this extract at increasing levels (0, 0.1, 0.2 and 0.4%). The digestibility of these diets was determined *in vitro* using a gastro-intestinal model, with enzyme extracts obtained from the seabass stomach and intestine, to evaluate the release of pentoses and amino acids (AA). The *in vitro* trial was followed by an *in vivo* digestibility trial with European seabass, using the same diets. For that purpose, 12 groups of seabass juveniles (IBW 35g) were established in tanks with feces settling columns, and the diets were randomly assigned in triplicate to these groups.

The *in vitro* trial showed that pentoses release was increased by the dietary supplementation with 0.4% of the BSG/SSF extract, indicating a positive effect of this inclusion level in the digestion of non-starch polysaccharides. Regarding AA release, no differences were detected between dietary treatments. Both pentoses and AA releases were higher during the intestinal than the stomachal stage of digestion. It was also observed that the activity of the BSG/SSF extract was reduced, to some extent, by the endogenous fish enzymes.

The *in vivo* trial confirmed that dietary supplementation with 0.4% of the BSG/SSF extract increased the apparent digestibility of dry matter, energy and starch, while the apparent digestibility of protein was not affected by the dietary treatment.

Overall, results indicate that SSF adds value to a waste, the BSG, producing an enzyme-rich extract with a high potential of application to increase the digestibility of non-starch polysaccharides. Also, that *in vitro* assays can be used as good predictors of the nutritional response observed *in vivo*. Further studies are needed to fine-tune the optimum level of supplementation, taking into account the reduction of enzymes activity due to the endogenous fish enzymes.

Acknowledgments: Present study was funded by FEDER-Operational Programme Competitiveness and Internationalization and FCT under the project SPO3 (ref. POCI-01-0145-FEDER-030377) and by Programa Operacional Mar2020, Portugal 2020 under the project InovFeed (ref. MAR-02.01.01-FEAMP-0111)

TECHNICAL EFFICIENCY OF MILKFISH PRODUCTION IN PANGASINAN

Jennie B. Fernandez

Pangasinan State University, Binmaley, Pangasinan, 2417, Philippines
jfernandez@psu.edu.ph

Humans and fish have been inextricably linked for millennia, not only because fish is an important source of animal protein, the production and trade of which provide millions with livelihood and food security. Aquaculture is regarded as a lucrative and important endeavor in terms of income generation and supply of animal protein to the majority of population. Milkfish (*Chanos chanos*) is an important cultured fish in the Indo-Pacific region, particularly in the Philippines, Indonesia and Taiwan. It is the major species farmed in the Philippines, contributing more than 80% of the total national brackish water pond production annually. Further, Pangasinan is the leading producer of milkfish in the country.

In aquaculture, stochastic frontier production functions have been used to determine economic efficiency. The frontier production function (FPF) has been most commonly applied to carp, tilapia, and shrimp. Fishpond operators were selected as respondents in the study to assess the level of technical efficiency and its determinants such as technology use, use of inputs, and efficiency of milkfish production in fishponds in Pangasinan.

The salient features of inputs and output variables involved in the stochastic production frontier and of farm-specific variables included in the technical efficiency function. Feeds, stocking density and area were the important material inputs used by most of the farmers.

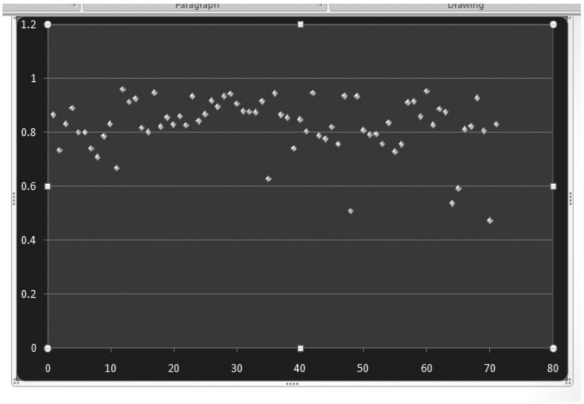


Figure 1. Average technical efficiency of Milkfish Production in Pangasinan is 0.82 or 82 percent (Minimun = 0.4733286; Maximum = 0.9555722)

MONITORING PHYTOPLANKTON PHENOLOGY USING OCEAN COLOUR REMOTE SENSING: POTENTIAL AS A GAMECHANGER FOR MANAGING MARINE OFFSHORE AQUACULTURE

Afonso Ferreira*, Carla Palma, Vanda Brotas, Carlos Borges, Carolina Sá, Ana C. Brito.

MARE – Marine and Environmental Sciences Centre
Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa
1749-016 Lisboa
ambferreira@fc.ul.pt

Over the past decades, the use of remote sensing as tool for marine aquaculture has become increasingly popular. Remote sensing has a remarkable advantage that gives it a unique potential: low-cost acquisition of continuous data across medium-large spatiotemporal scales. Moreover, with the expected rapid advances in technology and increase in Earth observation satellites, the role of remote sensing in marine aquaculture is only expected to grow. One of the main applications of remote sensing for ocean observation is the monitoring of marine phytoplankton - ocean colour remote sensing (OCRS). Phytoplankton, photosynthetic microorganisms, is considered as one of the most important biological components of the marine ecosystem and is widely regarded as a good indicator of changes. Several parameters can be used to evaluate phytoplankton using OCRS. Chlorophyll a (chl *a*) is the most common as it allows for the monitoring of changes in the biomass in regions of interest. Among the main applications of using chl *a* in is the identification of productive areas for the establishment or redefinition of aquaculture sites or the monitoring of harmful algal blooms. Chl *a* can also be used to study phytoplankton bloom phenology, i.e., the timings of the blooms and its main properties. Phenology enables the identification of the blooms, as well as analysing irregularities in the annual and seasonal phytoplankton cycles for a given region. The main goal of this work is to test and evaluate the potential of OCRS for marine offshore aquaculture, using the Western Iberian Coast (WIC; SW Europe) as a case study. This will be done by performing four tasks: 1) assess the mean climatological and seasonal cycles of chl *a*; 2) evaluate the phenology of phytoplankton blooms; 3) definition of preferential regions for aquaculture using a mixture of phenological, biological and physical indices; and 4) in-depth study of phenology for a given potential aquaculture site. Results will be presented (see figure 1 for examples of preliminary results) and are expected to contribute to establishing OCRS as a gamechanger for managing marine offshore aquaculture.

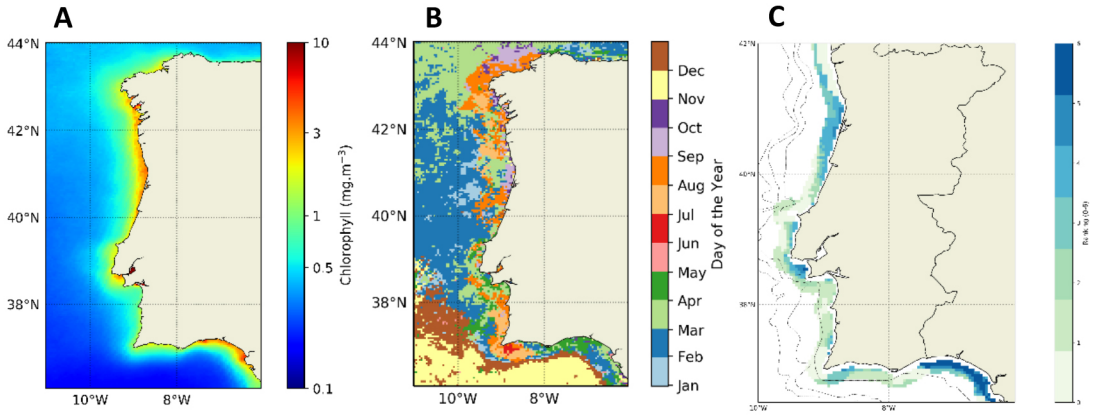


Figure 1 : A) Climatological mean of chl *a* during 1998-2018 for WIC; B) Mean date of phytoplankton bloom initiation off WIC (1998-2018); C) Preliminary results of a suitability analysis of areas for marine aquaculture from 0 (white; non-suitable) to 6 (dark blue; most suitable) off WIC.

VEGETATIVE PROPAGATION OF *Chondrus crispus*: IDENTIFYING OPTIMAL N AND P CONCENTRATIONS FOR INDOOR CULTURE

Martiña Ferreira*, Paula Frade, Mohamed Soula

ANFACO-CECOPESCA, Division of Aquaculture. Estrada Colexio Universitario 36310 – Vigo, Pontevedra (Spain)
Corresponding author: martina@anfaco.es

Macroalgae are interesting organisms for the eco-intensification of European aquaculture, particularly in land-based IMTA. Rhodophyta have a low degree of cell specialisation, which would potentially allow vegetative propagation. In this work we assess the vegetative growth of *Chondrus crispus* thallus fragments, in order to define the most suitable nutrient conditions for the scale-up of *C. crispus* cultivation in tanks.

Experiment 1. Wild harvested *C. crispus* thalli were cut to obtain apical, medium and basal fragments and placed in Petri dishes with 9 different conditions of NaNO3 (N) and NaH2PO4 (P) concentrations. Commercial micronutrients were also added at 0.5 ml l-1. Light was supplied by white LED tubes following a 14h:10h light:dark cycle. Fragments were weighed at the onset of the experiment and weekly. Culture medium was replaced by fresh medium every week. *C. crispus* apical fragments showed the highest growth rate, up to 250%, whereas medium and basal fragments only attained maximum 90% of weight increase. The effect of each combination of N and P concentrations and N:P ratios was different for each type of fragments (Figure 1).

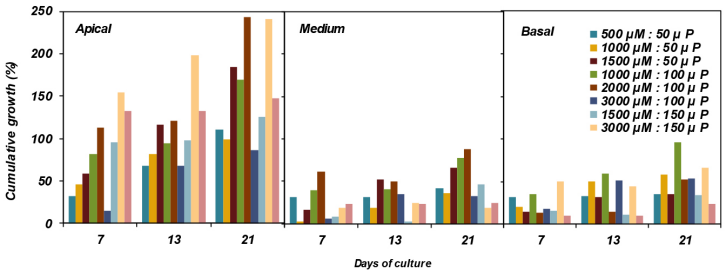


Figure 1. Cumulative growth of apical, medium and basal *C. crispus* fragments in different N and P concentrations.

Experiment 2. Whole individuals, plus apical and basal fragments were cultured under the most appropriate N and P concentrations according to Experiment 1, in 1-l flasks, under aeration and the same light conditions as Experiment 1. Weekly, algae were weighed and culture medium was replaced by fresh medium. Whole individuals showed higher biomass increase, up to 100 % and performed best in the lowest N and P concentrations, whereas fragments reached highest growth in high nutrient concentrations (Figure 2).

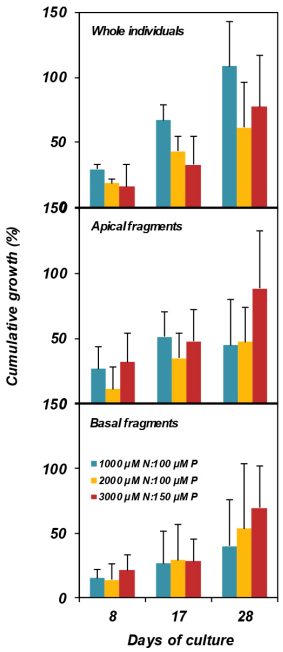


Figure 2. Cumulative growth of whole individuals and apical, and basal *C. crispus* fragments under different N and P concentrations in 1-l flasks.

Our results suggest the potential of *C. crispus* to be incorporated to IMTA schemes, thanks to its high biomass growth and the potential of being vegetatively propagated.

This work is part of project Green Aquaculture Intensification in Europe (GAIN), funded by the European Union's Horizon 2020 research and innovation programme; grant agreement No 773330.

EFFECT OF A PURIFICATION PROCESS ON BACTERIAL LOAD IN MUSSELS BROODSTOCK REARED IN HATCHERY

Samah FETTACH*, Jawad KASSILA, Yassine OUAGAJOU,
Rachid CHERKAOUI, El Mostafa TALBAOUI.

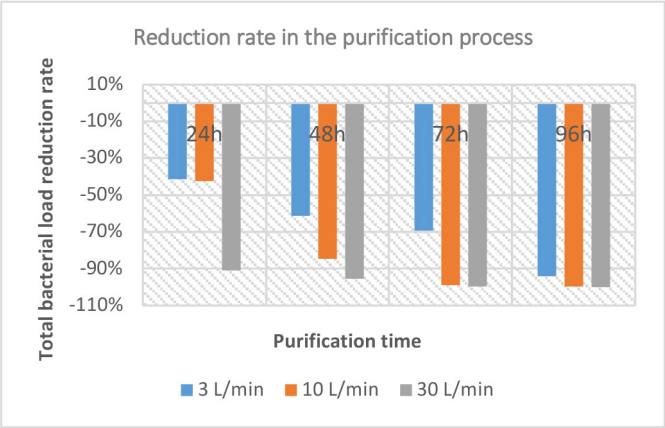
*Shellfish Farming Station, National Institute of Fisheries Research (INRH)
Amsa, B.P 93022, Morocco
* Fettach@inrh.ma

In bivalve hatchery, broodstock founds one of the most important bacterial sources for larval cultures due to the vertical transmission of bacteria. In Amsa shellfish station (INRH_Tétouan, Morocco), the discharge of bacterial load from reared broodstock originated from natural beds is one of the main challenged goals to resolve pathogenic bacteria causing massive mortality during early stage larvae. Hence, this study has been carried out to reduce the bacteriological load (particularly *Vibrio sp*) of mussel broodstock (*Mytilus galloprovincialis*), without any further use of antibiotics in order to ban or minimize their application in aquaculture.

The trials were applied on adult specimens of the Mediterranean mussels collected between May and July 2021 from a natural bed located in M'diq, Morocco (35°40'55.9"N 5°18'36.0"W). Three different water flows (3, 10 and 30 L. min^{-1}) were investigated herein to perform the effect of a purification system after 24h, 48h, 72h and 96 h. This study was held in 400 liters rectangular tanks with an open circuit of microfiltred (0.2 μm) and sterilized (Ultraviolet) seawater at optimal physicochemical conditions (temperature, pH, salinity and dissolved oxygen) with a stocking density of 1 kg. L^{-1}.

The results showed that the highest reduction was obtained after 96 hours of purification by reaching 99.83%, 99.64% and 94% of bacterial discharge at 30, 10 and 3 L. min^{-1} respectively. However, the lowest bacterial reduction was obtained after 24 hours and reaches 90.9%, 42.2% and 30.10% respectively at water flows of 30, 10 and 3 L. min^{-1}. The statistical analysis (ANOVA) revealed that flow rates have significant effect on the bacterial discharge in mussels at 24h ($F=134.6$, $p < 0.001$), 48h ($F=55.02$, $p < 0.05$), and 72h ($F=47.13$, $p < 0.05$), except 96h where no significant influence was observed between flow rates ($F=1.18$, $p = 0.3936$). As regards the effect of purification period, the bacterial load was reduced significantly using 3 and 10 L/min ($p < 0.001$) among all tested periods. As results, the bacterial depuration of mussels during conditioning could be held using high flow-through (30 L. min^{-1}) during a short period (< 24h) or low to moderated flow (3 to 10 L. min^{-1}) during long period (> 24h).

The originality of this study is based on proposing a reliable and sustainable practices during controlled shellfish spat production that can be easily transferred to commercial and industrial purposes.



Lactobacillus spp. AS AN EFFECTIVE PROBIOTICS FOR MARRON (*Cherax cainii*) AQUACULTURE

Md Javed Foysal*, Ravi Fotedar, Chin-Yen Tay

School of Molecular and Life Sciences, Curtin University, WA6845, Australia

Marron (*Cherax cainii*), a native iconic species in Western Australia is the largest farmed fresh water crayfish. Commercial culture of marron has a lot of positive aquaculture attributes including high consumer preference, high market value, and disease resistance. However, the production of marron has remained stagnant over the last few decades. Probiotics including *Lactobacillus* species has long been used in commercial aquaculture as a growth promoter and immune enhancer. In marron aquaculture, however, effects of *Lactobacillus* spp. as feed supplements has yet to be fully investigated.

A 60-days feeding trial was conducted with four different test diets, fishmeal based control (CT), *L. acidophilus* (LA), *L. plantarum* (LP) and *L. casei* (LC) supplemented with CT. The ingredients of CT were purchased from Glenn Forest, Perth, Australia, and probiotic feed was formulated by supplementing 10^9 CFU/mL of probiotic species per kg of feed according to standard methods. A total of 80 were transported to Curtin Aquatic Research Laboratories under live conditions and distributed randomly into 16 different tanks. After acclimation for a week, marron were fed with the respective test diets at 1.5% of the total body weight per day.

The results showed no significant differences in growth with any of the test diets, however, P-value of 0.059 for *L. plantarum* fed marron suggest that LP could be a diet of interest to marron. All probiotic supplemented diets positively influenced the total haemocyte counts in the haemolymph while tail muscle protein and gross energy were improved only by *L. plantarum* supplementation. Further microbiome analysis revealed significant ($P<0.05$) enrichment of gut microbial communities in terms of alpha-beta diversity in the probiotic fed diets wherein *L. plantarum* augmented higher ($P<0.05$) lactic acid bacteria in the hindgut of marron (Table 1). Finally, increased ($P<0.05$) marron survival was observed in the probiotic fed marron after 14 days of challenge with *Vibrio mimicus* (Figure 1A). The probiotic diets also significantly upregulate the expression of prophenoloxidase (proPO) and cathepsin L (PcCTSL) at 48 h post- injection with *V. mimicus*, the genes linked to innate immune response of crayfish (Figure 1B). of marron after trial.

Table 1. Comparison of differential abundance of bacteria in the gut of marron at genus level fed different diets

CT	LA	LP	LC	P-value	Genus
5.1	12.8	3695.4	36.8	0.001	*Streptococcus*
12383.0	36.0	780.0	76.0	0.002	*Vibrio*
10006.6	330.6	59.8	430.6	0.003	*Shewanella*
0.1	0.1	25.0	4.1	0.007	*Lactobacillus*
8649.4	4733.6	183.4	2738.6	0.012	*Aeromonas*

Mean of reads (non-parametric Kruskal-Wallis test in QIIME).

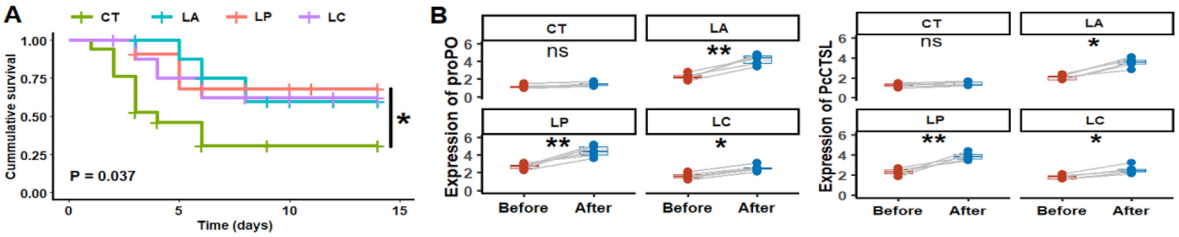


Figure 1. (A) Survival of marron after 14-days of challenge with *V. mimicus*. (B) Relative expression level of immune genes in terms of fold changes in the gut of marron after trial.

PRELIMINARY RESPONSE OF A NEW FEED SOLUTION TO IMPROVE RESISTANCE OF WHITE SHRIMP *Penaeus vannamei* POST LARVAE AGAINST *Enterocytozoon hepatopenaei* (EHP) DISEASE

Stephane Frouel*, Julie Castier, Thomas Pierrot and Maxime Hugonin

Mixscience
2, Avenue de Ker Lann
CS 17228
35172 BRUZ CEDEX
stephane.frouel@mixscience.eu

Aquaculture industry is highly challenged by diseases outbreaks, introduction and spread of specific new pathogens like parasites. This pathogenic pressure significantly impacts the economics of the farmers. In recent years, chemical solutions have been applied. However the massive use of chemicals in aquaculture is nowadays a high public health concern and dramatic effects on environment appeared. The current research works aimed to develop a specific solution against parasites in aquaculture, in particular against endoparasites.

Enterocytozoon hepatopenaei (EHP) is a microsporidian parasite that was first characterized and named from the giant or black tiger shrimp *Penaeus monodon* from Thailand in 2009. It was discovered in slow growing shrimp but was not statistically associated with slow growth at that time. EHP is restricted to the shrimp hepatopancreas (HP) and its occurrence is usually not heeded because of the overwhelming focus on Acute HepatoPancreatic Necrosis Disease (AHPND) commonly referred as Early Mortality Syndrome (EMS). However, although EHP does not appear to cause mortality, feedback from shrimp farmers indicates that it is associated with severe growth retardation in *Penaeus vannamei* leading (sometimes) to early harvest meaning economical losses for the producers.

An experimental 64-days trial has been conducted at lab facilities in Vietnam to evaluate the potential of a new feed solution (supplied by MiXscience, France) to improve the response of white shrimp *Penaeus vannamei* post larvae (average initial weight 0.5g) challenged by EHP. Shrimp were challenged under a standardized cohabitation method.

Three groups were compared:

1. Non Challenged Control (NC)
2. Positive Challenged Control (PC)
3. Experimental group (EXP)

Each group had 5 replications.

Growth performance parameters (Bodyweight gain, FCR) as well as final survival and final EHP load were monitored during the trial.

The trial results indicated that the experimental product (applied at 3.5 Kg/tonne of feed) expressed some positive effects on growth performance and a significant reduction of EHP load in HP of the infected shrimp was observed (Table 1). Survival was also enhanced even if there was no statistical difference with PC ($p>0.05$).

Table 1: White shrimp performance after EHP exposure (a, b: ANOVA $p<0.05$)

	NC	PC	EXP
iIBW (g)	0.54a	0.54 a	0.51a
fIBWG (g)	8.83a	7.52b	8.85 a
FCR	1.16a	1.60b	1.38ab
Survival rate (%)	90a	80b	82ab
Final EHP load	0a	5964b	254a

iIBW : initial Individual BodyWeight (g)
fIBWG: final Individual BodyWeight Gain (g)
FCR: Feed Conversion Ratio
EHP Load expressed at 10e5 copies/g of shrimp hepatopancreas

These preliminary results demonstrated that this new feed solution seems to counteract the EHP's effects on the host's performance and can be considered as a promising preventive product for shrimp farmers who are impacted by microsporidians and the associated economical losses.

YEAST EXTRACT IMPROVES THE EFFICIENCY OF A FISHMEAL-FREE DIET IN RAINBOW TROUT BY MODULATING THE GUT RESPONSE

Laura Frohn*, Sandrine Skiba-Cassy, Frédéric Terrier, Pierre Aguirre, Cervin Guyomar, Sarah Maman Haddad, Julien Bobe, Christophe Klopp, Benjamin Costas, Carla Teixeira, Diogo Peixoto, Nadège Richard, Karine Pinel

Phileo by Lesaffre
137 rue Gabriel Péri
59700 Marcq-en-Baroeul
l.frohn.ext@phileo.lesaffre.com

The use of fishmeal (FM) in aquafeeds is considered ecologically unsustainable for the long-term development of aquaculture. Consequently, fishmeal has been largely and successfully taken out from diets of several omnivorous farmed species using substitutes from plant proteins and processed animal proteins (PAP). However, this approach has proven to be more difficult to implement in carnivorous species without affecting their growth performances and welfare. According to their levels of inclusion in aquafeeds, poultry co-products and pork blood meal have proven to be suitable substitutes to marine ingredients but further efforts are required to enhance the efficiency of those aquafeeds. In that concern, the use of feed additives, such as yeast and its derivatives, appeared to be noteworthy to counteract the adverse effects of fishmeal-free diet.

In this context, a 17% PAP-based diet (FM-free) supplemented or not with 3% of yeast extract (Prosaf®, YE) was compared to a 19% FM-based control diet in rainbow trout through a 12 weeks feeding assay. Zootechnical performances were evaluated during the feeding period and intestinal histology was assessed at the end of the trial. Moreover, transcriptomic response of distal intestine was determined through mRNA and miRNA high throughput sequencing.

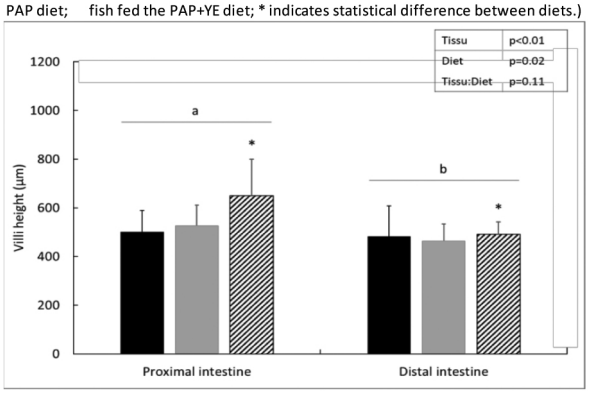
The growth performances of fish fed the PAP diets were lower to those obtained with a FM-based diet. However, the addition of 3% of YE improved the efficiency of the PAP diet by significantly increasing fish growth (Tab. 1). Moreover, fish fed the YE-supplemented diet showed higher intestinal villi size (Fig. 1). Additionally, RNA-seq analysis revealed that the diets affect the expression of a small number of genes and miRNAs in the distal intestine, mainly associated with inflammation, immunity, and structure.

In conclusion, our study showed that yeast extract supplementation improved the performance of a fishmeal-free PAP diet in rainbow trout, by potentially improving nutrient absorption capacity and modulating mechanisms related to gut health, leading to better growth.

TABLE 1. Zootechnical parameters during the feeding trial

	FM	PAP	PAP+YE
Final body weight (g)	231±8 **a**	182±14 **c**	207±14 **b**
Feed efficiency	1.16±0.01	0.99±0.04	1.15± 0.03
Daily feed intake (%/day)	1.49±0.01 **b**	1.61±0.04 **a**	1.44±0.04 **b**

FIGURE 1. Intestinal villi height at the end of feeding trial (■ CTL group; ■ fish fed the PAP diet; ▨ fish fed the PAP+YE diet; * indicates statistical difference between diets.)



MICROBIOLOGICAL SURVEY OF SEAFOOD IN SINGAPORE: A CROSS-SECTIONAL STUDY

FUA'DI Muhd Tarmidzi*, Jia Quan Oh, Manogaran Vijitha, Selvaraj Renuka Zhi Ning Wong, Angela Li, Kyaw Thu Aung

National Centre for Food Science
Singapore Food Agency
52 Jurong Gateway Road, JEM Office Tower, Singapore 608550
Muhd_Tarmidzi_Fuadi@sfa.gov.sg

Increasing consumer demand for seafood is expected to continue worldwide as well as in Singapore. Based on literature, consuming seafood particularly that are raw or undercooked can pose food safety risk to consumers. To assess the microbiological quality and safety of seafood sold at retail in Singapore, a cross-sectional study was conducted over a 3-year period of 2016-2018.

A total of 1,147 seafood (raw, ready-to-eat) samples were purchased from various retail outlets such as markets, supermarkets and food establishments for the testing of standard plate count, coliforms count, *E. coli* count and common foodborne pathogens. *Salmonella*, *Campylobacter* and *Clostridium perfringens* were not detected in any of the ready-to-eat seafood samples collected from food establishments in this study. However, these pathogens were detected, though at relatively low occurrence (<5%), in raw seafood from markets and supermarkets. *Vibrio parahaemolyticus* was detected in both raw (15.6%) and ready-to-eat (0.5%) seafood respectively, while *Listeria monocytogenes* was detected in less than 1% of the raw and ready-to-eat seafood samples tested. Compared to the ready-to-eat samples (mean 4.1 log CFU/g), raw seafood (mean 4.8 log CFU/g) samples were found with a relatively higher standard plate count which serves as a proxy for hygiene assessment.

Our study provides baseline microbiological data of seafood available in Singapore. The findings reinforce the significance of proper food hygiene and safety measures from farm to fork to prevent contamination in seafood.

NUTRITION-SENSITIVE FISH FARMING IN GRAM PANCHAYAT TANKS BY WOMEN SELF-HELP GROUPS IN ODISHA, INDIA

Amar Gaikwad[1,2*], Neetha Shenoy[1], Arun Padiyar[1],Chadag Vishnumurthy Mohan[3], Debananda Bhanj[4], Sasikanta Acharya[4], Nivedita Palita[4], Sushreeta Sahoo[4], Binod Kumar Jena[5]

[1]WorldFish, Cuttack, Odisha 753001, India
[2]Centurion University of Technology and Management, Odisha 752050, India
[3]WorldFish, Penang, Malaysia
[4]Directorate of Fisheries, Odisha, Cuttack-753001, India
[5]Mission Shakti Department, Government of Odisha, Bhubaneswar
*Corresponding and presenting author: amargcife24@gmail.com (Amar Gaikwad)

India ranks number 2nd in the list of countries by population and 973.3 million people who were unable to afford a healthy diet. Odisha state located in northeast India is one of the least developed, particularly in rural areas, which has contributed to high levels of food insecurity and malnutrition. In response, scientific and nutrition-sensitive aquaculture (NSA) programs have emerged, which promote activities such as species diversification, nutrition education, and women's empowerment by evolving in aquaculture activities. In India, Women are predominantly involved in subsistence aquaculture, however, these women are not targeted for further empowerment because of the lack of women-centric aquaculture policies coupled with cultural and social constraints that prevent women's participation in commercial aquaculture. This study investigates how women self-help groups (WSHGs) in Odisha has incorporated scientific fish farming in underutilsed Gram Panchayat (GP) ponds practices into a socially equitable, gender-sensitive, economically profitable, nutritionally-sensitive and environmentally sustainable fish farming. The survey was conducted on WSHGs that participated in the program over first years of implementation in 2018–2019 and 2019–2020. Data were collected through personal interview of 944 WSGHs, chosen randomly, (512 Carp–mola polyculture WSGHs and 432 carp-only polyculture WSGHs) using a structured questionnaire. Mola broodstock were stocked in Gram Panchayat (GP) ponds, at an average density of 24.46 $kgha^{-1}$. The results indicated that BCR and profitability of carp–mola polyculture system are significantly higher ($P<0.05$) than carp-only polyculture systems. Smaller ponds proved to have higher productivity of mola than larger ponds.Pond area, water depth, size at harvest and crop duration was found to be identical for both systems. Feed conversion ratio (FCR) and size at stocking were significantly higher in carp–mola polyculture compared to carp–mola polyculture system though stocking density of carp fingerlings lower. More than 93% WSHGs expressed a willingness to renew their lease agreement to further continue fish farming at their GP tank beyond period of the program. The findings of this study highlighted the superiority of the involvement of WSHGs in aquaculture and carp–mola polyculture system. The adoption of carp–mola polyculture in GP tanks by WSHGs improved food and nutritional security, gender equity and increased fish production and productivity of the GP tanks in villages across Odisha.

AQUAPONIC PRODUCTION OF HIGH-VALUE SPECIES IN A GREENHOUSE: TECHNICAL AND FINANCIAL FEASIBILITY

Wenresti G. Gallardo*, Mohammed Al-Mahfudhi, Michel Claereboudt, and Shekar Bose

Department of Marine Science and Fisheries
College of Agricultural and Marine Sciences
Sultan Qaboos University, Oman
gallardo@squ.edu.om

Aquaponics is becoming popular in many countries including the Sultanate of Oman where there is lack of freshwater supply. However, there is a need to determine the plant and fish species that can be grown and the financial feasibility of aquaponics considering the environmental conditions in Oman. The main objective of the research was to assess the technical and financial feasibility of growing high-value species in an aquaponic system in a greenhouse. The two specific objectives are: 1) To determine the most suitable plant species in combination with koi carp or tilapia within an aquaponic system in a greenhouse and 2) To analyze the costs and benefits of such aquaponic system in a greenhouse setting.

Two experiments were designed. The first experiment tested growing tilapia with high-value plants (spinach, cabbage and lettuce) in floating versus media-based growing system, for 3 months. The second experiment compared the growth of tilapia versus *koi* carp in combination with strawberry and mint in floating versus media-based aquaponic system., 4 four months. The fish were fed with 2mm sinking pellets at around 5% of their average body weight per day and given in 2-3 rations per day. The weight and length of the fish were measured every month. Growth of plants in terms of height and number of fruits in the case of strawberry and number of stems in the case of mint was determined every month and the total numbers of surviving plants were also counted. Statistical tools such as ANOVA, t-test and Tukey's test were used to analyze variables, such as weight, length and mortalities of species.

In the first experiment final weights of spinach and tilapia were slightly higher in the media-based than in the floating system but their differences were not significant. In the second experiment, both tilapia and *koi* in the media-based system had higher average growth and survival; however, there was no significant difference between the two systems. The media-based system also showed higher average growth in terms of the number and weight of fruits (strawberry) and also in the average number of stems (mint), however, there was no statistical difference between the two systems. The financial feasibility study was conducted using cost-benefit analysis. Based on the data from the experiments, the total revenue calculation considering the market value of each of the plant and fish species, investment and operating costs, it can be concluded that it is feasible and profitable to use either floating or media-based system for growing the tested plants especially lettuce and mint along with *koi* carp in a greenhouse setting. Investment costs can be recovered in two years. If implemented commercially, aquaponics can help address issues such as water management, food security and sustainable production of fish and vegetables to meet the increasing demand.

COMPARATIVE ANESTHETIC EFFICACY OF CLOVE OIL, ALFAXALONE, KETAMINE, LIDOCAINE, MIDAZOLAM, AND PROPOFOL FOR IMMERSION ANESTHESIA IN ZEBRAFISH *(Danio rerio)*

Randy Manuel GALUTAN, DVM, MSc, MBA, FPSVSCA

Pangasinan State University, Philippines
dr.galutan@gmail.com

The study was conducted to determine the comparative anesthetic efficacy of Clove oil, Alfaxalone, Ketamine, Lidocaine, Midazolam, and Propofol for immersion anesthesia in zebrafish (*Danio rerio*). To identify the different effects and margin of safety of the 6 anesthetic drugs by carefully monitoring and gathering data on the time when the experimental fish loss its equilibrium, the recorded time on loss of reflex from soft stimuli, the recorded time on loss of reflex from painful reflex, the time of recovery, the time when the experimental fish starts to eat, and the recorded opercular movement per minute before and during the deep stage of anesthesia prior to recovery. The data gathered was treated by statistical tool Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Each anesthestic tank treatment was represented by 10 zebrafish with 3 replications of treatment. The research study was conducted on August 12, 2022, at Animal Recovery Veterinary Centre, located at 482 Serangoon Road 01-01, Singapore 218149.

There were significant differences observed in the study with mean time loss of equilibrium, exposure to anesthetic drug Alfaxalone has shortest time of 0.95 minutes, followed by Propofol with 5.66 minutes, followed by Clove oil with 8.06 minutes, followed by Lidocaine with 9.2 minutes, Midazolam with 110.33 minutes then Ketamine at 233.16 minutes. There were significant differences observed on mean time recorded for the loss of reflex from soft stimulus, the use of Alfaxalone has recorded mean time of 1.73 minutes, followed Propofol with 7.77 minutes, followed by Clove oil with 12.23 minutes, then Lidocaine with 22.67 minutes, followed by Midazolam with 131.83minutes and Ketamine with 270.5 minutes. There were significant differences observed on mean time recorded for the loss of reflex from painful stimuli, Exposure to Alfaxalone gathered 2.3 minutes, Propofol 10.17 minutes, Clove oil 19.87 minutes, Lidocaine with 42.47 minutes, Midazolam with 150.5 minutes and Ketamine with 347.67 minutes. There were significant differences observed on mean time recorded on anesthetic recovery time, for Propofol 10.7 minutes, followed by Alfaxalone with 11.97 minutes, Lidocaine with 17.975 minutes, Clove oil with 39.67 minutes, Ketamine with 72.83 minutes and Midazolam with 188 minutes.

There were significant differences observed on mean time recorded on the experimental fish starting to eat after recovery, Clove Oil has 42.93 minutes, Alfaxalone with 22.53 minutes, Ketamine with 78.17 minutes, Lidocaine with 21.39 minutes, Midazolam with 214.33 minutes, Propofol with 21.47 minutes. There were differences observed on mean time recorded on the opercular beat per minutes before anesthesia, Clove Oil has 177.8 beats per minutes, Alfaxalone with 185.1 beats per minutes, Ketamine with minute of 188.33 beats per minutes, Lidocaine with 190.33 beats per minutes, Midazolam with 188 beats per minutes and Propofol with 186.37 beats per minutes. There were differences observed on mean Opercular beats per minute during anesthesia on the experimental fish, for Clove Oil with 4.3 beats per minutes, Alfaxalone with 2.13 beats per minutes, Ketamine with 4.67 beats per minutes, Lidocaine with 1.92 beats per minutes, Midazolam with 5.07 beats per minutes, Propofol with 3.47 beats per minutes.

CARBON ISOTOPIC COMPOSITION OF GEODUCK SHELLS FROM PUGET SOUND INDICATES OCEAN ACIDIFICATION

Yongwen Gao* and Russell Svec

Makah Fisheries Management
P.O. Box 115
Neah Bay, WA 98357, USA
E-mail: gaoy@olypen.com

Carbon isotopic composition of clam shells has been used as an indicator in detecting climate change and ocean acidification, because these shells are composed of calcium carbonate ($CaCO_3$) and ocean acidification will affect the ability of clams to build protective shells and skeletons. When the anthropogenic CO_2 sinks into ocean, it will produce HCO_3^- and CO_3^{2-} and this affects the carbonate saturation state Ω. A number of investigations suggested that a steady decrease of $\delta^{13}C$ values throughout a calcifying organism's life span can be used as signatures of ocean acidification, because ocean acidification will lead to a shift in DIC (dissolved inorganic carbon) equilibria and result in higher CO_2 and lower carbonate ion (CO_3^{2-}) concentrations.

The Pacific geoduck, *Panopea abrupta*, is an important species and mainly cultured in the southern Puget Sound of Washington State, USA. Geoduck shells are composed of calcium carbonate, and exist in polymorphism as aragonite and calcite. There are also clear rings or layers that precipitated on geoduck shells as annual growth records. The chemical composition and a time series are especially suitable for stable carbon and oxygen isotope ratio analyses ($^{13}C/^{12}C$ or $\delta^{13}C$, and $^{18}O/^{16}O$ or $\delta^{18}O$). In this study, we review the theory and practice of using stable carbon isotope ratios in detecting ocean acidification, and report new research examples from geoduck shells from Puget Sound. Two geoduck shell samples were selected, with a length of about 116 and 90 mm from the umbo to the ventral margin, respectively. The 1st geoduck sample was cultured from 2002 for 10 years (2002-2011), and the 2nd sample was cultured from 2011 for 7 years (2011-2017). The $\delta^{13}C$ values of the 1st shell ranged from -0.62 to +1.28‰, whereas the $\delta^{13}C$ values of the 2nd shell ranged from +0.12 to +1.22‰. The $\delta^{13}C$ profiles showed some seasonal variation but more strong decreasing trends over the geoduck's life span, reflecting a growth event of about 15 years in southern Puget Sound. The $\delta^{18}O$ values of the shell samples, in contrast, showed a clear life history that was consistent with the annual growth lines on the outside of the shell. In summary, the carbon isotopic composition of geoduck shells revealed isotopic signatures of ocean acidification in Puget Sound, and the shell carbonate are a unique proxy for reconstructing the life history and marine environmental changes that a clam was encountered.

FUNCTIONAL ADDITIVES TO REDUCE THE IMPACT OF CO-INFECTIONS IN INTENSIVE SHRIMP FARMING

Ruth Garcia Gomez*, Martha Mamora and Martin Guerin.

BDM Aquatic Health and Farm Care APAC/ISC
Adisseo Thailand
127/14 Panchathani Tower Nonsi Rd. Khwang Chong Nonsi, Khet Yan Nawa
Bangkok 10120
ruth.garcia@adisseo.com

Co-infections and multiple infections are extremely common in aquatic environments and therefore, in aquaculture production. Co-infections and multiple infections are defined as infectious diseases caused by two or more different pathogens either simultaneously or as secondary infections, thus two or more infectious agents are active together in the same aquatic host. Co-infections bacteria + virus (e.g. *Vibrio* spp. + WSDV), and bacteria + parasite (e.g. *Vibrio* spp. + EHP) are extremely common in shrimp farming, having devastating effects worldwide in a broad range of farming scales and systems. Co-infections can significantly modify the course and the severity of different shrimp diseases, being either synergic or antagonist. Moreover, the susceptibility of shrimp to different infectious pathogens could be increased during concomitant infections, causing the appearance of acute shrimp outbreaks with higher incidence.

In this presentation, we report on the development of preventive and corrective feed additive strategies to minimize the impact of common co-infections and multiple infections in white-leg shrimp intensive farming. The results of trials conducted both in the laboratory and in the field in situations of co-infection (WSDV + *Vibrio* spp. and EHP + AHPND/EMS) and multiple infection (WSDV + IMNV + EHP + *Vibrio* spp.) will be presented. These results are encouraging in terms of preventive and corrective medicine through the use of functional additives. As a result of these farm and laboratory trials, preventive and corrective feed additive strategies have been validated as effective tools to minimize the impact of co-infections and multiple infections in shrimp farming and therefore, improve profitability of shrimp farming activities.

SHRIMP PRODUCTION IN EUROPE AND DIVERSITY OF POTENTIAL PATHOGENIC BACTERIA IN DIGESTIVE TRACTS OF SHRIMP

ALTERNATIVE 2
DIVERSITY OF PATHOGENIC VIBRIO IN THE DIGESTIVE TRACT OF (HEALTHY) SHRIMPS

Yustian Rovi Alfiansah, Henrike Hohnholz, and Astrid Gärdes
Working group (WG) Applied and Molecular Microbiology, Alfred Wegener Institute (AWI)
Helmholtz Centre for Polar and Marine Research
Am Handelshafen 12, 27570 Bremerhaven, Germany

astrid.gaerdes@awi.de

Pacific white-leg shrimp (*Penaues vannamei*), blue shrimp (*Litopenaeus stylirostris*) and kuruma shrimp (*Marsupenaeus japonicus*) are the most cultured shrimp species in Europe. In 2020, European shrimp production reached up to 450 tonnes, produced by 25 aquaculture companies using bio-flocs and clear water systems. In addition, 9 companies in Europe serve as hatcheries and produce post-larvae for European shrimp farms. Productions of market-size shrimps as well as post-larvae in Europe are predicted to be increased due to consumer's preference. No mass mortality of cultured shrimp due to bacterial diseases has been reported from the European shrimp farms. However, effort to minimize bacterial disease outbreaks which may devastate the European shrimp farming is still challenging. Therefore, it is important to understand bacterial community composition in the digestive tract of shrimps including potential pathogens, and compare them to the disease status. Moreover, a robust detection method is needed to predict the pathogenic bacteria rapidly and precisely. We examined bacterial community composition in healthy and diseased shrimp samples covering fresh-water and saline shrimps such as *Macrobrachium nipponense, P. vannamei*, *L. stylirostris* and *M. japonicus*. We found that pathogenic bacteria of the genus Aeromonas, Alteromonas, Flavobacterium, Photobacterium, Pseudoalteromas, and Vibrio are the most common in the intestines of shrimps. We developed specific primer pairs to detect the thermolabile hemolysin (*tlh*) gene, a toxin inherent but not exclusively to the Vibrio genera to predict the risk of *Vibrio*-related disease outbreaks. Here, we developed a SYBR®Green qPCR assay to simultaneously target the *tlh* gene. Primers were experimentally validated against *V. alginolyticus*, *V. campbellii*, *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus*, as well as *V. anguillarum* and *Bacillus subtilis* to constrain their taxonomic coverage and determine their specificity for *Vibrio*, thereby enabling the quantification of pathogenic *Vibrio* without the need of multiple species-specific markers. We obtained a couple of primers (tlh-G-vibrio-0515-a-S-22: GCTGGTTCTTRGGDCAYTTCTC, tlh-G-vibrio-0771-a-A-22: TGGAACGCYACGGTTRTAGTTC) as the best primer pair candidate that is able to amplify *tlh* with a melting temperature range of 83.5 - 85°C, a limit of quantification of log 3 gene copies/ng genomic DNA, and a limit of detection at qPCR cycle 34-36. Then, we tested the system over 89 shrimp samples reared in recirculating aquaculture system (RAS) and obtained 21 positive samples with a range of 3.5 ± 1.5 to 4.3 ± 1.6 log copies tlh gene/ng genomic DNA, which equal to 3,200-20,000 Vibrio cells. Our approach offers versatile applications for monitoring and disease prevention management in commercial aquaculture. In addition, this primer pair is also suitable for detecting *tlh* via conventional PCR.

VALIDATION OF TWO PCR TESTS FOR SURVEILLANCE OF WHITE SPOT SYNDROME VIRUS (WSSV) IN SHRIMP USING A BAYESIAN LATENT CLASS MODEL

Ian Gardner*, Nicholas Moody, Peter Mohr, Lynette Williams, John Hoad, David Cummins, Joanne Slater, Axel Colling, Nagendrakumar Singanallur, and Mark Crane.

* Atlantic Veterinary College, University of Prince Edward Island
Charlottetown, Prince Edward Island C1A 4P3, Canada
E-mail: iagardner@upei.ca

Most validation studies for WSSV PCR have been limited to analytical experiments and those that used diagnostic validation procedures, following the pathway prescribed by the World Organisation for Animal Health (OIE) in chapter 1.1.2. of the Manual of Diagnostic Tests for Aquatic Animals, assumed that the reference test was perfect. One example of the latter is the two tests for WSSV that are certified as "fit for purpose" in the OIE test registry. Bayesian latent class models (LCM), which don't require an assumption of a perfect reference test, provide a practical solution for statistical analysis of the accuracy of 2 tests (++, +-, -+, and --) in at least 2 distinct populations and allow for inclusion of relevant prior knowledge about diagnostic sensitivity (DSe) and specificity (DSp) of the tests under evaluation. The analysis can be readily implemented in open-source software such as OpenBUGS (http://www.openbugs.net/w/Downloads) but users should ensure that model assumptions of conditional independence or dependence, constant diagnostic accuracy across populations, and distinct prevalences are critically assessed in each analysis and appropriate code is used to run the model.

The purpose of the present study was to diagnostically validate two PCR (OIE test and CSIRO Taqman qPCR) for purpose of surveillance in apparently-healthy shrimp and shrimp products assuming neither test was perfect. Previously, both tests had been analytically validated to the end of stage 1 of the OIE test validation pathway. The DSe and DSp of the two qPCR assays were estimated for 2 conditionally-dependent tests in 2 populations assuming that neither test was perfect (Branscum et al. Prev. Vet Med 2005: 68:145-163). A conditional dependence model was preferred to an independence model because both PCRs target similar sequences albeit in different locations in the WSSV genome. The conditional dependence model requires informative prior information for at least 2 parameters to help ensure model identifiability (i.e. a unique set of parameter values generated the joint test results, namely the 4 cell counts in the cross-classified data tables). Informative beta (a,b) distributions were specified for the DSe (beta 130.7, 15.4) and DSp (beta 15.7, 1.3) of the OIE qPCR based on the expert opinion of a co-author (NM). The priors for the OIE qPCR were readily justifiable as that assay had been used in the CSIRO Fish Diseases Laboratory in Geelong since 2010 and relevant test accuracy data had been obtained in a preliminary study. Priors for the other model parameters were flat (beta 1,1), indicating that any value between 0% and 100% was equally likely.

The median DSe and DSp values and 95% probability intervals were estimated for 3 testing scenarios that would be expected if the assays were used in other laboratories including duplicate wells that were both positive for a positive interpretation. The Bayesian model estimates of DSe for the 2 assays were almost identical: 88.9% (CSIRO) and 89.9% (OIE) and both tests had DSp of 97.6%. In conclusion, both assays had comparable DSe and DSp and were deemed fit for the purpose of surveillance for WSSV in apparently-healthy infected shrimp and shrimp products.

STRIPED VENUS (*Chamelea gallina*) RESTOCKING ASSAY OFF PORTIMÃO (SOUTHERN PORTUGAL)

Miguel B. Gaspar*, Domitília Matias, Ana M. Matias, Paulo Vasconcelos, Fábio Pereira, Paula Moura, André N. Carvalho, David Piló, Sandra Joaquim

Portuguese Institute for Sea and Atmosphere (IPMA)
Research Centre of Olhão
8700-335 Olhão, Portugal
mbgaspar@ipma.pt

Bivalve dredge fisheries play an important role in the social-economical context of the Algarve coast (southern Portugal), mainly due to the size of the fishing fleet and the number of fishermen involved in this activity. Among several bivalve species caught, the striped venus (*Chamelea gallina*) constitutes a very important fishing resource because of its high economic-value. Recently, intensive fishing coupled with recruitment failure resulted in large inter-annual fluctuations in stock abundance, threatening the biological and economic sustainability of this fishery. In this context, the implementation of management strategies, such as stock-enhancement programmes based on hatchery production of juveniles, could promote the rebuilding of bivalve beds.

The present study describes the biology (growth and reproductive cycle) and distribution of *C. gallina*. This species shows a high growth rate and short lifespan, a prolonged spawning period (between April and August), matures within its second year of life and occurs preferentially on sandy bottoms between 5 and 11m depth. Overall, this information was to establish the time of the year used to collect the wild broodstock and to assess the potential of *C. gallina* for hatchery production. Several spawning techniques were tested (thermal stimulation, scarification, and flow through). Growth and survival of larvae and juveniles were compared using different nutritional regimes (larvae: *Isochrysis* aff. *galbana* (T-iso), *Chaetoceros calcitrans* (C. cal) and unfed; juveniles: (1:1) T-iso + C. cal, T-iso and C. cal) and rearing temperatures (larvae: 20 and 23°C). Among the different methods used to obtain gametes, *C. gallina* only spawned in the flow through system. The highest larval survival and growth rates were obtained from larvae fed T-iso. First *C. gallina* pediveliger larvae were found at day 8. The presence of metamorphosed larvae, found even in the unfed treatment, is an advantage for aquaculture production. After metamorphosis, there was no mortality of *C. gallina* juveniles reared without substrate regardless the diet provided. Juveniles fed the binary diet (T-iso + C. cal) and the monodiet T-iso showed a relatively higher growth in shell length and weight than when fed with C. cal. (Fig. 1).

Based on these results, a production protocol was designed for *C. gallina*, which allowed the production of millions of juveniles that were seeded in the first restocking assay with this species, in an area off Portimão.

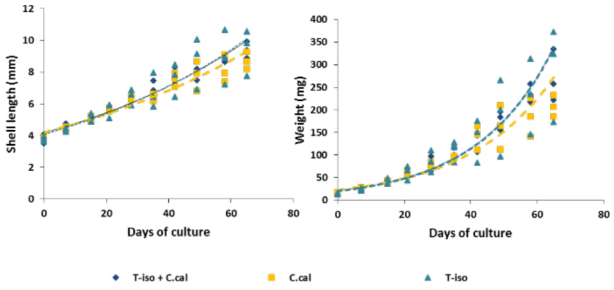


FIGURE 1. Growth in mean shell length (mm) and mean weight (mg) and respective exponential growth curves of *Chamelea gallina* juveniles fed different diets (T-iso + C. cal, C. cal and T-iso).

REPRODUCTIVE CYCLE AND SIZE AT SEX CHANGE OF *Patella vulgata* AND *Patella ulyssiponensis*: FIRST STEPS TOWARDS THE PRODUCTION OF THESE SPECIES

Miguel B. Gaspar*, Uthesh Umapathy, Paula Moura, Fábio Pereira, André N. Carvalho, and Paulo Vasconcelos

Portuguese Institute for Sea and Atmosphere (IPMA)
Research Centre of Olhão
8700-335 Olhão, Portugal
mbgaspar@ipma.pt

Sustainable commercial or recreational harvesting activities, as well as aquaculture production of any species, require comprehensive information on the reproductive biology of the species. The common limpet (*Patella vulgata*) and the rough limpet (*Patella ulyssiponensis*) are protandrous hermaphrodite species, which are common on intertidal rocky shores of Portugal. This study describes the reproductive cycle of these two species from the Algarve coast (southern Portugal) and estimates their size at sex change. Between January 2017 and December 2018, individuals of *P. vulgata* and *P. ulyssiponensis* were collected monthly on a rocky shore at Praia da Luz in Lagos (37º05′06.5”N, 08º43′45.1”W) southern Portugal. In the laboratory, individuals were measured for shell total length (SL) and weighed for total wet weight (TW). To describe the main features of the reproductive cycle of both limpet species, classical histological technics were applied.

Both species had balanced sex ratios (*P. vulgata* = 1M: 0.98F; *P. ulyssiponensis* = 1M: 1.03F), similar size-frequency distribution between sexes, and equivalent mean shell lengths (SL) and total weights (TW) between males and females. In general, the reproductive cycles of *P. vulgata* and *P. ulyssiponensis* were characterised by a short resting period mainly during summer. In *P. vulgata*, ripe and spawning gonads were mostly recorded from early autumn until early spring, with a main spawning season in early spring to early summer. In *P. ulyssiponensis*, ripe and spawning gonads were scattered almost year-round, with a main spawning season also in early spring to early summer. The occurrence of ripe and spawning individuals throughout the year probably denotes sequential gonadal re-ripening and partial spawning events in both species. It was found correlative evidence of direct or indirect effects of surface seawater temperature (SST) on the reproductive cycle of both species, with the resting period coincident with warmer SST in summer, gametogenic development and gonadal maturation synchronised with decreasing SST during autumn, and main spawning season related to the increasing of SST throughout early spring to early summer. The extended spawning period of both species will allow obtaining larvae during most of the year, by artificial spawning induction of wild broodstock. In these protandrous hermaphrodites, the size at sex change (SL_{50}) was estimated at approximately 29 mm for *P. vulgata* and 36 mm for *P. ulyssiponensis* (Fig. 1).

The present study was performed within the framework of the research project PESCAPANHA (funded by the Fisheries Operational Programme -MAR 2020- and co-financed by the European Maritime and Fisheries Fund - EMFF 2014–2020).

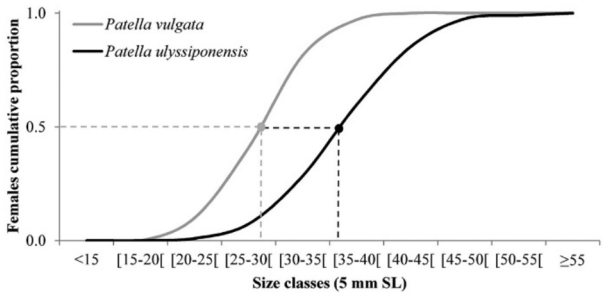


FIGURE 1. Size at sex change (SL_{50}) in *Patella vulgata* and *Patella ulyssiponensis* (grouped into 5 mm SL classes). SL50 of each species highlighted with dashed lines.

CONSTANT OR DIEL-FLUCTUATING TEMPERATURE INDUCES SALINITY ACCLIMATION IN JUVENILE RAINBOW TROUT *Oncorhynchus mykiss*

Jian Ge*, Yangen Zhou, Ming Huang, Qinfeng Gao, and Shuanglin Dong

Key Laboratory of Mariculture
Ministry of Education, Ocean University of China
Qingdao, Shandong Province 266000
gejian@stu.ouc.edu.cn

An initiative of offshore salmon mariculture in the Yellow Sea Cold Water Mass (YSCWM) has been launched since 2012. One key step of the project is the 'mountain-sea relay' mode, meaning that salmons need to be transferred to seawater appropriately. Temperature is one of the most dominant environmental stimuli initiating the smoltification in salmonids. Besides, aquatic animals reared at constant temperature and diel-fluctuating temperature perform differently in growth and physiology. Hence, our purpose of this study focuses on salinity acclimation in juvenile rainbow trout *Oncorhynchus mykiss* inducing by different constant temperatures or diel-fluctuating temperature. Two trials were carried out at the Key Laboratory of Mariculture in Ocean University of China (Qingdao, China).

In trial I, rainbow trout were distributed to three treatments (9 °C, 12.5 °C and 16 °C) with three replicates, maintained in fresh water for four weeks. In trial II, fish were distributed to three treatments (13 ± 0.2 °C, ± 1 °C and ± 2 °C) with four replicates, reared for six weeks. The water temperature was adjusted according to sine function by AI controller. After that, the salinity in both trials were elevated from 0 to 15 immediately, then elevated to 30 gradually. After that, fish were sampled at the 1st, 4th, 7th, 14th and 21th day.

In trial I, final weight (FW), percent weight gain (WG) and specific growth rate (SGR) in rainbow trout at 12.5 °C were significantly higher than those at 9°C. Thermal growth coefficient (TGC) at 16 °C was significantly lower than other treatments (Table 1). In trial II, there is no significant difference in growth among treatments (Table 2). Since growth before salinity acclimation is usually poor indicator, hematology, enzymes, and metabolomics will be determined for select tissues in trial II.

Table 1. Growth performance of rainbow trout (initial weight: 94.73 g) under constant temperature. Means within columns with different letter are not significantly different ($p > 0.05$).

Temp. °C	FW (g)	WG (%)	SGR	TGC
9	174.13^{b}	83.95^{b}	2.03^{b}	3.70^{a}
12.5	202.86^{a}	114.22^{a}	2.54^{a}	3.50^{a}
16	190.73^{ab}	101.14^{ab}	2.33^{ab}	2.49^{b}

Table 2. Growth performance of rainbow trout (initial weight: 62.28 g) under diel-fluctuating temperature.

Temp. °C	FW (g)	WG (%)	SGR	Survival (%)
13 ± 0.2	152.46	144.71	2.13	100.0
13 ± 1.0	156.25	150.91	2.19	97.5
13 ± 2.0	151.96	144.51	2.13	87.5

GENETIC GUIDELINES FOR CAPTIVE BREEDING OF ENDANGERED FRESHWATER FISHES AND MUSSELS IN AQUACULTURE FACILITIES

Juergen Geist*

Aquatic Systems Biology Unit
Technical University of Munich
Muehlenweg 22
D-85354 Freising, Germany
geist@tum.de

Many species of freshwater fishes and mussels have experienced serious declines, both in terms of their spatial distributions as well as their population sizes. Captive breeding in aquaculture facilities can be an important rescue tool to bridge critical life stages such as insufficient reproduction in the wild, and it can help to increase populations above minimum viable population sizes by stocking of captive-bred animals. Currently, several aquaculture facilities throughout Europe actively contribute to the captive breeding of endangered freshwater fishes and mussels which then become released into the wild.

This contribution addresses the question of how such captive breeding programmes related to conservation of endangered freshwater fishes and mussels can be most effective. Using the examples of the endangered Danube salmon (*Hucho hucho*), the freshwater pearl mussel (*Margaritifera margaritifera*) and the European nase (*Chondrostoma nasus*), the usefulness of genetic monitoring in such programmes will be highlighted. This includes the deduction of guidelines for the informed choice of source populations and parents based on genetic pre-screening, and the consideration of selection, genetic drift and adaptation to certain environmental conditions during the breeding and rearing process. The examples will also be used to illustrate possible pitfalls such as inbreeding and outbreeding effects as well as measures to avoid such effects.

The three presented case studies show that conservation-oriented captive breeding in aquaculture facilities need to be managed different to classical food-production oriented aquaculture systems, e.g. in terms of origin and numbers of spawners, their sex ratios and exchange rates. Rearing conditions need to mimic natural habitat and avoid selection of features that are disadvantageous in the wild. The findings also suggest that, depending on the differences in life histories of the species, multiple production cycles and long-terms programmes can reduce the risk of deleterious genetic effects. In many cases, populations of freshwater fishes and mussels would go extinct in the nearby future without captive breeding and stocking actions taken. In addition, juveniles available from such programmes provide a valuable source for ecotoxicological testings and bioindication experiments.

THIRD GENERATION PORTABLE SEQUENCING: A NOVEL APPROACH FOR RAPID DETECTION AND PRECISE CHARACTERIZATION OF EUKARYOTIC ALGAL BLOOMS IN SINGAPORE

Christaline George*, Avneet Kaur, Adriana Lopes Dos Santos, Federico M Lauro

The Asian School of the Environment
Nanyang Technological University
Student's office, N2-01-36c
50 Nanyang Avenue
Singapore 639798
christal002@e.ntu.edu.sg

Harmful algal blooms (HABs) are an alarming concern for the tropical waters of Singapore causing significant economic losses to the fishing and aquaculture industries as seen during the latest HAB event in 2015 leading to a loss of S$1.3 million per farmer. The combination of factors contributing to HAB occurrences remain poorly understood, making their forecast and mitigation difficult. Early and precise characterization of bloom causing microalgal species is key in any mitigation and/or contingency measures. This is challenging because conventional characterization methods (e.g. microscopy, HPLC, culturing, etc.,) are tedious, require expertise, lack adequate taxonomic resolution. Newer molecular methods for the characterization of microorganisms are gaining popularity due to their relatively lower costs, precise and rapid results particularly when coupled with the long-reads sequencing capacity of third-generation sequencers. Here we present a novel approach for the rapid and precise taxonomic characterization of eukaryotic microalgal species through amplicon sequencing of the 18S-ITS-28S rRNA gene using portable MinION™ sequencers from Oxford Nanopore Technologies (ONT). Amplification and sequencing of the 18S-ITS-28S rRNA gene was tested using a mock community comprising of four microalgal species, environmental samples obtained from the coastal waters of Singapore and a series of dilution experiments that mimic HAB-like conditions to determine the sensitivity and specificity of this approach for the rapid detection of HABs. The DNA sequencing reads obtained from the MinION™ device were analyzed using a bioinformatics pipeline which incorporates quality filtering and error correction of the reads upon clustering them, based on the rapidly evolving ITS regions. Limitations arising from the ONT sequencing platform leads to 12-22% error rates in the raw reads which were corrected to contain equal to or lower than 0.7% error in the corrected consensus. The consensus sequences generated from the error corrected reads were used to build a reference database of long rRNA genes exclusively of the eukaryotic microalgal species, which will be hosted on the LASeR (Long Amplicon Sequence Repository) website funded by the Marine Sciences Research and Development Programme (MSRDP). This reference database coupled with regular monitoring would allow for early detection of HABs even in the most remote locations where expensive laboratory facilities and professional expertise are not readily available. It will also provide insights into investigating the nature of biotic interactions and dynamics which may cause, perpetuate or lead to the decay of HABs. To fortify food security measures Singapore's aims to increase local protein production to 10% by 2030 (Singapore Food Agency, SFA). As a result of which the aquaculture industry would be on the rise and being equipped with the tools to better predict and mitigate HABs would be imperative to ensure sustainable aquaculture practices.

PREDICTION OF FISH AVAILABILITY USING MULTIVARIATE REGRESSION ANALYSIS

Sobha Merina George* and Joe Prasad Mathew

Department of Zoology, St. Berchman's College, Kottayam, Kerala, India
georgesobhamerina@gmail.com

Study was conducted in lower Kuttanad (9°252 303 N 76°272 503 E) fresh water aquatic system in Kerala, India. Kuttanad lies below the sea level in the western region of Kerala, stretches across three districts. Sixty five fish species were identified during the three year study period. Multivariate regression analysis was performed to estimate the relationship between fish yield (FY) (dependent variable) and independent variables (Mn, Cu, Fe, Na, K, Mg, Ca, DO (dissolved oxygen), BOD (Biological Oxygen Demand), pH, salinity, temp (temperature)) through SPSS 20.0. Most dominant fishes and significantly related variables were selected for the regression analysis. Dominant fish species were *Pseudoetroplus maculatus*, *Puntius amphibius, Aplocheilus lineatus, Hyporhamphus limbatus, Puntius mahecola, Dawkinsia filamentosus*.Results showed that predictive factors for the fish yield in this region is K, Fe, pH and dissolved oxygen.

SOCIAL, ECONOMIC, AND PRODUCTION CHARACTERISTICS OF AQUACULTURE FOR THE FRESHWATER WETLANDERS OF WEST BENGAL, INDIA

Swagat Ghosh*

Sasya Shyamala Krishi Vigyan Kendra
Ramakrishna Mission Vivekananda Educational and Research Institute
Ramakrishna Mission Ashrama, Narendrapur, Kolkata, West Bengal, India
*swagotor@gmail.com

Farming of Indian Major Carp, the freshwater fishes in wetland region, popularly known as 'IMC,' has been expanding in India. The present study was conducted using Participatory Rural Appraisal (PRA), Semi-structured interviews, focus group discussion and questionnaire survey to assess the present state of production of IMC, the freshwater fishes in wetland region in South 24 Parganas District of West Bengal, India. The socio-economic and technical survey on 80 wetlanders was conducted during May, 2021- April, 2022. Most of the farmers (78 nos.) had completed elementary level of schooling and had approximately five years of experience as owner and/or manager. Most of the respondents (12 nos.) obtained information on aquaculture from their neighbours and received no formal training. Polyculture with catfishes, prawns and Indian Major Carps was the dominant system (65 nos.), followed by monoculture. The most common management strategy included nursing of fingerlings for 45 to 60 days and harvesting with the combined method, culling only the largest market-sized individuals beginning at 9 months. Culture practices at the time of this survey were traditional or extensive. Very few farmers followed semi intensive culture system. Most farmers stocked at densities below 10 pieces m^{-2} and average production was 3,800 kg ha^{-1} yr^{-1}. Commercially produced, nutritionally complete feed was most common, water exchange and aeration methods were used to maintain suitable water quality. Water quality management throughout the cycle was practised where respondents had the resources. Variables that significantly affected yearly gross IMC production (kg ha^{-1} yr^{-1}) included feed inputs and stocking of IMC directly. Major problems identified were diseased or poor quality seed supply, disease outbreak within the crop, and external pollution. Most of the farms were operated by private owners. There is no much scope for additional income from other sources. There is need to provide an additional income generation source to the fishers of the target area based on the fisheries related activities which they will be able to take up with minimal technical advisory to the beneficiaries. Most of the farmers moving as migrant labour. Despite few possibilities of alternate livelihood such as high valued indigenous fish farming and freshwater ornamental fish culture which requires more skill and area of operation and possibility of sustainable income for the fishers and family. Hence, an alternate solution is needed with minimal effort and maximum benefit.

A COMPARATIVE STUDY OF THE PATHOLOGY ASSOCIATED WITH SCALE DROP VIRUS AND LATES CALCARIFER HERPESVIRUS IN ASIAN SEABASS *Lates Calcarifer*

Susan Gibson-Kueh[1]*, Sunita Awate[2], Markus Schrittwieser[2]

1. Tropical Futures Institute
James Cook University
149 Sims Drive, Singapore 387380
susan.kueh@jcu.edu.au

2. UVAXX PL
203 Henderson Road, #12-01 Henderson Industrial Park, Singapore 159546

The last decade has seen the emergence of three novel viruses in Asian seabass, *Lates calcarifer*. They are scale drop virus (SDV) first described in 2012, Lates calcarifer herpesvirus (LCHV) reported in 2018 and recently a novel birnavirus in 2019. While molecular detection tools for these virus infections are not lacking, there is a paucity of information on the origin of these viruses and their contribution to disease observed in the field. In this study, we present the pathology observed in association with detection of these viruses, and correlate pathology to viral loads in specific organs. Fish from both sea cages and land based nursery tanks are included in this study. These diseases are difficult to distinguish based on clinical signs or gross pathology.

Preliminary studies suggest varying severity of splenic changes in fish tested positive for SDV by PCR. In presumably early cases of SDV infections, there is mild to moderate lymphocytic-monocytic infiltration of splenic ellipsoids to more progressive stages with multifocal to extensive coalescing splenic necrosis, often involving entire segments of spleens and resembling infarcts. Some SDV PCR positive fish with early splenic changes were clinically healthy, that is, did not show external signs of disease. SDV positive clinically diseased fish usually have severe renal glomerular necrosis.

LCHV PCR positive fish had remarkable liver pathology, ranging from atrophied lobulated livers to marked loss of hepatic acini leaving behind remnants of exocrine pancreas in what is normally hepatopancreas (liver). Extensive renal tubular necrosis and attenuated tubular epithelium are observed in LCHV PCR positive fish.

The pathology associated with the novel Lates birnaviral infection is currently unclear. It is also unclear the exact contributions of these novel viral infections to pathology observed in co-infections. The authors hoped that we will have more information by WAS 2022 in Singapore, as this is a study in progress.

COPEPODS AS FIRST FEED FOR MARINE FISH LARVAE PROVIDE IMPROVED GROWTH, HIGHER SURVIVAL AND INCREASED EARLY JUVENILE QUALITY

Jan Giebichenstein[*1]
Maren R. Gagnat[1]
Tore M. Remman[1]

1: CFeed AS (Norway)

Copepods are the natural prey and thus primary first feed for many fish larvae. Their populations are abundant and provide a supreme nutritional profile to the early life stages of fish. CFeed AS produces eggs of the copepod *Acartia tonsa*. With a nauplii size of 100μm, it is the ideal organism for the first feeding of many marine species and can thus replace the rotifer phase in hatcheries. CFeed copepods are easy to store and use. They can be delivered all over the world in large quantities to supply commercial-scale hatcheries, aquarists, and researchers. Although small in size, a bottle of copepod eggs can contain more than 100 million copepods. The eggs are easily hatched into copepod nauplii and can be further grown to larger copepod stages or directly fed to the fish larvae. No enrichment is necessary. The copepods retain their nutritional quality and can be kept alive in storage and fish tanks for long periods. The drastically improved water quality and production environment give users the option of reducing the water exchange rates to make sure all copepods are consumed. In our unique intensive land-based copepod farm in Vanvikan, Norway, we can ensure stable quality and high biosecurity standards of our copepods. CFeed copepods have been tested on many different species in countless projects ranging from small research up to full-scale commercial production. All experiments have shown a substantial increase in the most critical areas of larval production. Showing a drastic increase in survival, better growth, and improved larval quality.

BENEFICAL EFFECTS OF A MARINE *Bacillus* MULTI-STRAINS CONSORTIUM ENCAPSULATED IN ALGAE ON GROWTH PERFORMANCE, DIGESTIVE AND IMMUNITY GENE EXPRESSIONS, VIBRIO RESISTANCE, MICROBIOTA MODULATION AND TRANSCRIPTOME PROFILING OF WHITE SHRIMPS *Penaeus vannamei*

Carine Le Ker, PhD & Fanny Giudicelli, PhD

MARINE AKWA
Saint Jacut de la Mer, 22750 France
fanny@marineakwa.com

20-day and 60-day feeding trials were conducted respectively on healthy *Penaeus vannamei* PL10 (n=3500, triplicate) and PL25 (n=350, triplicate) to evaluate the effects on growth and health parameters of a marine probiotic consortium (MPC) made of four marine *Bacillus* strains encapsulated in algae, used as dietary supplement.

At the end of the 60-day trial, the treatment with MPC at 1% significantly increased final body weight, weight gain, specific growth rate, feed conversion ratio of White Shrimp *P. vannamei* ($P < 0.05$). MPC also significantly enhanced the mRNA expressions of digestive genes in hepatopancreas, such as Trypsin, α-Amylase, Triacylglycerol lipase and Chymotrypsin BII; of immune-related genes, such as proPO, crustin gene in hepatopancreas and Dual Oxidase, mucin-like peritrophin, Penaeidin-3α in intestine genes ($P < 0.05$). Diets supplemented with MPC also decreased the occurrence of total *Vibrio spp*. count in shrimp's hepatopancreas under normal conditions. Under challenge conditions, MPC at 5% significantly increased disease resistance of shrimp larvae against *V. parahaemolyticus* (VpAHPND) ($P < 0.05$).

The results of high-throughput sequencing showed a significant improvement in White Shrimps digestive tract bacterial communities with probiotic supplementation. Moreover, the MPC enhances the hepatopancreas gene pathway related to metabolisms of endocrine system, immune system and digestive system. In addition, the beneficial effects observed in intestine gene pathway are mainly related to translation, transport catabolism, and signal transduction.

In conclusion, this study demonstrated the ability of the marine *Bacillus* spp. consortium encapsulated in algae to improve White Shrimp growth performance, digestive tract and immune status and disease resistance during both larval and grow-out phases.

TABLE.1 Growth performance of *P. vannamei* (control and MPC diets, after 60 days). Means in a column with different letters were significantly different in T test ($P < 0.05$)

Parameters	Control	MPC
Final body weight (g)	8.03 ± 0.49^{a}	8.88 ± 0.46^{b}
Weight gain rate (%)	3877.75 ± 490.97^{a}	4903.46 ± 290.04^{b}
Specific growth rate (%/d)	6.69 ± 0.22^{a}	7.11 ± 0.10^{b}
Survival Rate (%)	83.34 ± 6.92^{a}	87.81 ± 2.78^{a}
Total body length (cm)	8.65 ± 0.18^{a}	8.86 ± 0.92^{a}
Hepatopancreas index (%)	4.72 ± 0.24^{a}	4.88 ± 0.056^{a}
Condition Factor (%)	1.22 ± 0.040^{a}	1.27 ± 0.046^{a}
Feed coefficient	0.9358 ± 0.13^{a}	0.7961 ± 0.016^{b}

PADDY CUM FISH INTEGRATED FARMING TO SUSTAINABLE AQUATIC FOOD PRODUCTION: A CLIMATE-SMART APPROACH FOR LOW LYING AREAS IN ASSAM, INDIA

Kalpajit Gogoi, Neeta Beypi, Nabamika Sonowal, Dharitri Baruah, Sourabh K. Dubey, Suresh Rajendran, Arun Padiyar, Chadag V. Mohan

WorldFish, Guwahati, Assam-781022, India

k.gogoi@cgiar.org

Assam is a state in northeastern India where paddy is a major crop and the staple food for almost all households. About 75% of the state population is directly or indirectly dependent on agriculture, while about 69% of the workforce in the state is engaged in agricultural activities. The traditional practice of raising fish in the paddy fields probably began with the beginning of paddy cultivation itself in the region because the waterlogged paddy fields create a natural habitat for fish. However, over the years, the practice has evolved with recognition of its multi-ecological benefits. In Assam, paddy-fish integration is mostly practiced in flooded river basins, unmanageable vast waterlogged areas, and perennial waterlogged wet paddy lands. Fishes enter the paddy fields during monsoon and grow along with paddy. Fishing activities start after the recession of water during November-December and the farmers use various fishing gears and indigenous traps either operated in the paddy-free spots of the field or are fixed at appropriate water entry and exit points in the fields. These lands often remain dry from December to April. Physically, the aquatic phase starts from May to November and possesses varying water depths depending on land topography, local rainfall patterns, water tables, soil quality etc. The paddy-fish integration in Assam can broadly be classified into three categories viz., perennial system, synchronous refuge pond system and enclosure system. In the perennial paddy fish farming system, a single crop of fish is raised along with two crops of paddy viz. *Ahu* (autumn paddy) and *Sali* (winter paddy) cover nearly both seasons. In a synchronous refuge pond system, the fish crop is raised synchronously with *Sali* paddy during the monsoon period. In the enclosure system, the fish crop is raised with deep water paddy (*Bao*) in deep water areas by enclosing the plot with pegged screens. Traditionally, the waterlogged paddy fields were one of the most common fishing grounds for small indigenous fish species (SIS) for the rural people of the region during the wet season (June to November). Hence, the paddy fields were the major source of SIS production and were contributing to household nutrition in rural areas. It is an extensive level of farming practice using low to moderate input technology. In the World Bank-funded APART project, WorldFish and Govt. of Assam are working together to improvise the traditional paddy-fish culture system through multi-locational demonstrations at farmers' fields with the objectives of introducing climate resilient paddy-fish integrated farming for improving the livelihood, income, and nutrition of smallholder farmers along with gender-equitable employment. The cost and return evaluation showed that the paddy-fish culture is much more profitable than the mono-crop paddy. The integrated paddy-fish systems have high reliability and stability and therefore better adapted to future changes. Paddy-fish systems are promising climate resilient models for climate changes and challenges that will reduce risks for smallholders and maintain productivity and sustainability.

UNRAVELING THE POTENTIAL OF MANGROVE-DERIVED *Streptomyces* AS PROBIOTICS IN AQUACULTURE

Joanna X. H. Goh*, Loh T. H. Tan, Jodi W. Law, Kok G. Chan, Kooi Y. Khaw, Vengadesh Letchumanan, Learn H. Lee, Bey H. Goh

Novel Bacteria and Drug Discovery Research Group (NBDD)
Microbiome and Bioresource Research Strength (MBRS)
Jeffrey Cheah School of Medicine and Health Sciences
Monash University Malaysia
Selangor Darul Ehsan 47500, Malaysia
joanna.goh@monash.edu

Background:

The intensiveness of shrimp farming is constrained because increasing stocking density often compromises animal growth and increases the risk of infectious disease transmission. For instance, a single episode of acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio parahaemolyticus* can potentially wipe out the entire shrimp batch within a short time frame and incur severe financial losses. There is indeed an urgency to seek a sustainable solution to break through this bottleneck in shrimp farming.

In contrast to conventional antibiotics applications, probiotics might represent a safer and more sustainable alternative in disease control among cultured shrimp species. It is hypothesised that the beneficial bacteria in the natural habitat of shrimps confers a protective effect on the animals against opportunistic pathogens. Our research aims to study the suitability and benefits of introducing mangrove-derived bacteria as probiotics in shrimp farming.

Method:

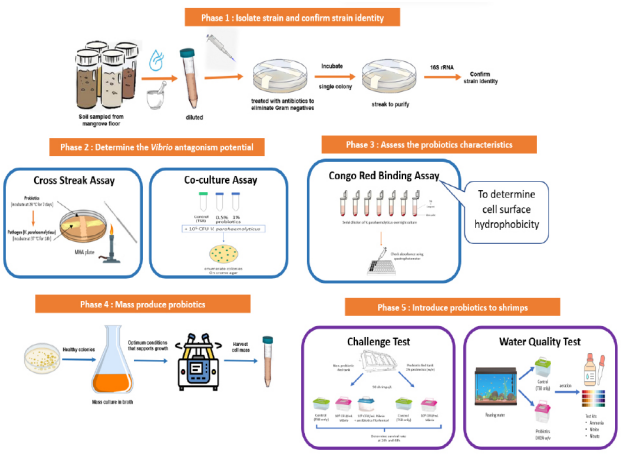


Figure 1: Methods applied in the five phases of the experiment.

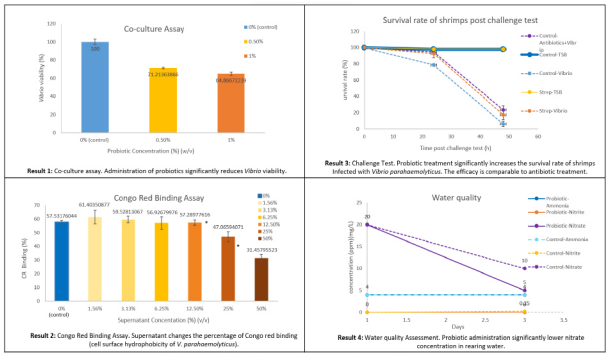


Figure 2: Experiment results.

APPLICATION OF PARENTAGE AND RELATEDNESS ANALYSIS TO IMPROVE SELECTIVE BREEDING IN BARRAMUNDI AQUACULTURE

Julie A. Goldsbury*, Jose A. Domingos and Dean R. Jerry

Centre for Sustainable Tropical Fisheries and Aquaculture
College of Science and Engineering
James Cook University
Townsville, 4811
Australia
julie.goldsbury@jcu.edu.au

Selecting premium broodstock is fundamental to improving aquaculture productivity, however, the nature of simultaneous broadcast spawning in many species creates difficulties in assigning parentage and relatedness to progeny. One such species is Barramundi (*Lates calcarifer*) which is farmed extensively in the Australasian tropics and increasingly elsewhere, often with reliance on unimproved broodstock resulting in significant variation in the quality of progeny. Accurate assignment is vital to monitor broodstock performance and reduce the incidence of inbreeding. One effective molecular technique to identify genetic lineage is microsatellite genotyping which targets nucleotide tandem repeats in DNA sequences.

A suite of 16 markers has been developed to amplify microsatellite regions of Barramundi (*L. calcarifer*) using multiplex PCR reactions. Amplicons are size selected through capillary separation (3100 Genetic Analyzer) and Genemarker® is used to verify allele size. Data is processed using Cervus 3.0.7 where allele frequencies within the population are analysed. Parentage output files assign parents to a particular offspring using a strict confidence level (95%) and no detectable mismatches as a measure of certainty. Cohorts of progeny are then conveniently sorted into family groups with the most dominate females and males being assigned a high value breeding classification. Relatedness analysis is generated using CoAncestory 1.0.1.7 where each individual is compared with all other individuals in a population. Relatedness (Rxy) values range between -1.0 (distantly related) to 1.0 (highly related) and are presented as a coloured heat map for ease when selecting breeding candidates.

These results provide useful information for selection of appropriate broodstock groups prior to spawning events by not only ensuring that mating groups for mass spawning events can be created with minimal inbreeding, but also accounts for spawning success and contribution to progeny cohorts. The analysis can be modified to suit most aquaculture species and readily integrated into genetic improvement programmes. This technology could become a useful tool in genetic studies to identify important commercial traits and has the potential to compare Genotype by Environment effects which would aid industry in producing consistently high quality seedstock.

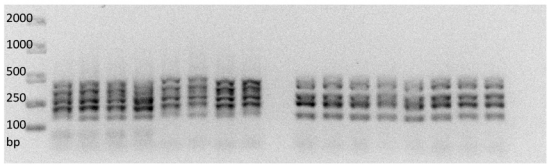


Figure 1. Multiplex amplicons visualized using electrophoresis and then size selected using Capillary Separation (3100) Genetic analyser.

	DAM 1	DAM 2	DAM 3	SIRE 1	SIRE 2	SIRE 3	SIRE 4	SIRE 5	SIRE 6	SIRE 7	SIRE 8	SIRE 9
DAM 1		-0.0272	-0.1149	-0.0001	0.2925	0.1416	0.1231	0.7055	0.7352	0.6514	0.0710	0.6205
DAM 2	-0.0272		0.1673	0.2486	0.5384	0.3148	0.4910	0.2698	-0.0976	0.1057	0.4133	0.2731
DAM 3	0.1149	0.1673		-0.0149	0.0281	0.0299	0.0866	0.2703	-0.0510	0.0572	0.1361	0.0462
SIRE 1	-0.0001	0.2486	-0.0149		0.3036	0.2395	0.3985	0.3988	0.1188	0.0391	0.3800	0.1599
SIRE 2	0.2925	0.5384	-0.0281	0.3036		0.2724	0.3330	0.5945	0.4940	0.6011	0.2027	0.7599
SIRE 3	0.1416	0.3148	-0.0299	0.2395	0.2724		0.4261	0.5881	0.2270	0.1425	0.3511	0.0347
SIRE 4	0.1231	0.4910	0.0866	0.3985	0.3330	0.4261		0.4395	0.0529	0.0132	0.3648	0.2108
SIRE 5	0.7055	0.2698	0.2703	0.3988	0.5945	0.5881	0.4395		0.5478	0.4101	0.3180	0.5298
SIRE 6	0.7352	-0.0976	-0.0510	0.1188	0.4940	0.2270	0.0529	0.5478		0.8141	0.0008	0.5960
SIRE 7	0.6514	0.1057	0.0572	0.0391	0.6011	0.1425	0.0132	0.4101	0.8141		0.0159	0.6899
SIRE 8	0.0710	0.4133	0.1361	0.3800	0.2027	0.3511	0.3648	0.3180	0.0008	0.0159		0.1530
SIRE 9	0.6205	0.2731	0.0462	0.1599	0.7599	0.0347	0.2108	0.5298	0.5960	0.6899	0.1530	

Table 2. Coancestry 1.0.1.7 relationship matrix of potential brood stock. Relatedness values range from -1 (distantly related) to 1 (highly related). Heat map represents green (distantly related) to red (highly related).

MYCOTOXINS CARRY-OVER IN AQUACULTURE: A NEGLECTED THREAT?

Rui A. Gonçalves*

* Lucta S.A. Innovation Division – Feed Additives
UAB Research Park, Edifici Eureka – 08193 Bellaterra, Barcelona, Spain

When considering plant-based meals for aquafeeds, it is commonly agreed that one of the main negative aspects is the presence of anti-nutrients (*e.g.* cyanogens, saponins, tannins, etc.) which are detrimental to fish and shrimp (Krogdahl *et al*. 2010). Conversely, the negative impact of mycotoxins is often overlooked. The disbelief in the negative effects of mycotoxins on aquatic species might be related to the lack of observable clinical signs in aquatic species directly related to mycotoxin ingestion compared to terrestrial livestock species where the effects are more pronounced. However, the awareness of mycotoxin-related issues in the aquaculture industry has grown in recent years as feed manufacturers and producers have recognised the importance of mycotoxins and their potential to impact production, final product quality (García-Morales *et al*. 2013) and safety for consumers (Michelin *et al*. 2017).

Carry-over denotes the conveyance of undesired compounds from contaminated feed into food of animal origin. The potential of carry-over of several mycotoxins in terrestrial animals such as poultry, swine and cows issue was highlighted by the European Food Safety Authorities (EFSA; EFSA, 2004b) and FAO (FAO, 2001). However, no guidelines are available regarding carry-over in farmed fish and shrimp species. The present communication will summarize the state-of-the-art knowledge on mycotoxin occurrence and co-occurrence in aquaculture finished feeds, focusing on carry-over of these contaminants in farmed fish and shrimp species as reported by Gonçalves *et al*., 2018.

From the available carry-over studies, was observed that deposition of mycotoxins into edible tissues may be higher than in terrestrial species and it is, therefore, imprudent to assume the same transfer factors for aquaculture species as for livestock species. In general, aflatoxins seem to be particularly prone to deposition in several fish and shrimp tissues (Table 1) representing a risk for human consumption, especially in species that are eaten as a whole.

Mycotoxin limits need to take into consideration animal health and welfare but also human health. Particular attention needs to be focused on aquaculture edible tissues and regional guidance limits should be advised depending on local mycotoxin occurrence and the edible tissues consumed. Risk assessment of imported aquaculture foods needs to take into account the mycotoxin occurrence, especially in those products imported from highly mycotoxin contaminated regions, or regions known to use potentially contaminated animal by-products.

Table 1: Example of some of the documented aflatoxin carry-over on aquaculture species (adapted from Gonçalves *et al.*, 2018)

Reference	Species	Tested dosage	Mycotoxin level (µg kg^{-1})	Transfer factor
Suzy *et al.* 2017	**African sharptooth catfish** *(Clarias Gariepinus)*	10[1], 17[2] and 20[3] µg AFB_1 kg^{-1}	M[1] = 0.05±0.12 µg AFB_1 kg^{-1} M[2] = 0.08±0.10 µg AFB_1 kg^{-1} M[3] = 0.08±0.12 µg AFB_1 kg^{-1}	M[1] = 0.005 M[2] = 0.005 M[3] = 0.004
Huang *et al.* 2011	**Gibel carp** *(Carassius gibelio)*	3.2, 11.3, 20.2[1], 55.2[2],95.8[3], 176.0[4] and 991.5[5] µg AFB_1 kg^{-1}	L[1-5] > 5 µg AFB_1 kg^{-1} M[5] = 2.35 µg AFB_1 kg^{-1}	HP[1-5*]> 0.090 M[5] = 0.0024

MYCOTOXINS MANAGEMENT IN AQUACULTURE - PAST, PRESENT AND FUTURE

Rui A. Gonçalves*

* Lucta S.A. Innovation Division – Feed Additives
UAB Research Park, Edifici Eureka – 08193 Bellaterra, Barcelona, Spain

Research characterizing the adverse effects of mycotoxins on the performance and health of animals has in large part focused on terrestrial livestock species (D'Mello and Macdonald 1997; Pestka 2007; Rotter *et al*. 1996). Only recently research has been carried out on the effects of mycotoxins in aquaculture species. This became even more important with the high cost of fish meal and the need to identify more economical protein sources, namely plant meals. The high cost and limited availability of fishmeal have led the aquaculture industry to gradually increase the levels of alternative protein sources as a substitute for fishmeal in their feeds (Davis and Sookying, 2009). However, contrary to the livestock industry, aquaculture is a diverse and complex industry composed of 362 finfishes and 62 crustaceans species (FAO 2018). Within the large variety of important species in aquaculture, we find herbivorous, carnivorous and invertebrates species (focusing in shrimps; scavenger/ detritivore-collector feeding behaviour). The pertinent question raised is: „How mycotoxins impact aquaculture and which species are or will be more impacted in future?"

Focusing on raw materials used in aquaculture, the sector faces a second paradigm shift. While firstly the main goal of the industry was to identify alternative protein sources to reduce the use of fishmeal, presently, the industry starts looking to alternative protein sources to partially replace plant meals as well. Overall, a wide range of products, *e.g*. animal by-products, fishery by-products, insect meals, macro-algae meals or single-cell protein, have been explored as alternatives to fishmeal and/or less sustainable plant meals sources. However, in the context of mycotoxin management, what does the use of this alternative protein sources means?

In order to correctly identify the possible impact of mycotoxins in aquaculture and therefore design a correct mycotoxin management programme, is essential to understand the current aquafeed formulation status in terms of raw materials used for the different group of species identified. Moreover, it is important to understand how novel ingredients will be adopted by the different species reared in aquaculture and what challenges in terms of mycotoxins management will these novel ingredients add to the present scenario.

The present communication will analyse the past and present mycotoxin management programmes, focusing on how novel trends in the aquaculture sector, namely the use of novel protein sources may impact the way how mycotoxin management in aquaculture is presently performed.

MARINE-ORIGIN INGREDIENTS REPLACEMENT STRATEGIES IN AQUAFEEDS: LESSONS FROM THREE ASIAN AQUACULTURE SPECIES

Rui A. Gonçalves*, Sofia Morais, Yirong Le, Maolong He

* Lucta S.A. Innovation Division – Feed Additives
UAB Research Park, Edifici Eureka – 08193 Bellaterra, Barcelona, Spain

Feed is the most costly item in aquatic production. Because of this, the aquaculture industry strives to reduce feed costs by buying cheaper feeds. Adding to the pressure for being cost-effective, customer awareness and therefore enforcement of sustainable ingredients use is now a reality in the aquafeed industry. Reformulating diets to include non-traditional feed sources (marine-origin) will be necessary to fulfil this objective, and should be viewed as a worthwhile investment that yields returns for the producer and improves the aquaculture sector sustainability.

The increasing reliance on less costly protein sources and low nutrient-dense diets will most likely see greater utilization of raw materials with lower protein digestibility, higher amino acid imbalance, and higher carbohydrate and fiber content. This can lead to an opposite effect from what initially desired, *i.e.,* inefficient utilization of the nutrients in the feed, resulting in increased feed usage and higher production costs, in addition to a sub-optimal animal performance increasing its susceptibility to diseases and ultimately higher environmental impact.

The present communication will report the results of four trials performed with Pacific white leg shrimp (*Litoenaeus vannamei;* 2 trials), snakehead fish (*Channa maculate*♀×*C.argus*♂*)* and black seabream (*Spondyliosoma cantharus*) where the level of fishmeal was reduced to minimal levels. The replacement strategy was supported by the utilization of a functional sensing enhancer product containing selected sources of umami amino acids and nucleotides, none of them of animal origin. Studies for the fishmeal reduction to a level of six per cent, in shrimp, were further supported by attractability assays in order to guarantee the minimum leaching time possible. Results obtained show that besides the reduction of fishmeal from eighteen per cent to six per cent, a significant improvement of growth performance was observed. Furthermore, the utilization of the functional sensing enhancer product at diets containing only six per cent of fishmeal increased the attractability of the diets. Figure 1 shows that shrimp fed 6% fishmeal diet plus functional sensing enhancer, approached feed 88% faster than same feed whithout the testing product and once in presence of the pellets, shrimps initiated the pellet consumption 65% earlier than control feed, decreasing the potential decrease of nutrients by leaching.

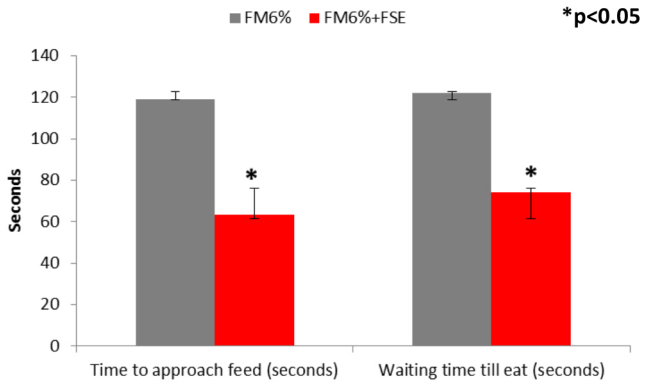


Figure 1: Attractability assay: Time that shrimp (first 5) spent to approach and eat a 6% Fishmeal diets with (FM6%+FE) or whithout (FM6%) a Functional Sensing Enhancer.

5

EFFECT OF MEDICINAL PLANTS, YEAST AND *Bacillus licheniformis* ON THE GROWTH, SURVIVAL, IMMUNE RESPONSE AND DIGESTION OF SHRIMP (PENAEUS VANNAMEI) CHALLENGED WITH *Vibrio parahaemolyticus*

Héctor Abelardo González-Ocampo*, Karime Anahi Valdez-Chavez and Arturo Fierro-Coronado

Comisión de Operación y Fomento de Actividades Académicas del Instituto Politécnico Nacional. CIIDIR-Sinaloa
Juan de Dios Batis Paredes #250, Guasave, Sinaloa. C.P. 81000
Scopus ID: 6504578835
ORCID: 0000-0003-2179-1166
hgocampo@yahoo.com

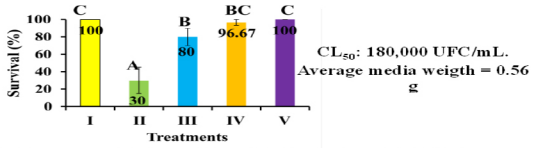
In shrimp farming viral diseases produce important economic losses. The use of prophylactic methods as natural additives wthout inmmune resistance and environmental problems were proben. Medicinal plants powder, yeast and *B. licheniformis* were used in the survival in the digestive and immune systems of *P. vannamei* challenged against *V. parahaemolyticus*.

Four bioassays were carried.Postlarval white shrimp stage were used to analyze the digestive and immune-related genes expression by RT-qPCR.

The combination of MP in the food and *B. licheniformis* in the water (3 x 106 CFU / L) significantly improved the survival of *P. vannamei* challenged with *V. parahaemolyticus*. *B. licheniformis* inoculated in the water, did not alter the expression of the trypsin digestive gene.

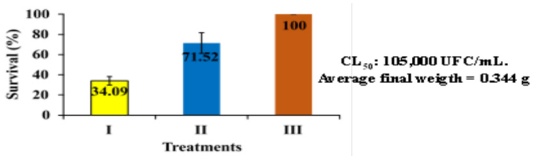
B. licheniformis in the water decreased the expression of the SOD gene (related to the immune system), which plays an important role as an antioxidant, decreasing the concentration of superoxide anion, the product of the phagocytosis process. *B. licheniformis* in the water did not alter the expression of the genes of the immune system penaeidine4 and lysozyme. The mixture of MP added in the feed and *B. licheniformis* in the water prevent the AHPND in *P. vannamei* cultivated in the laboratory.

Bioassay 1. Medicianl Plant (MP) powder added to feed every 2 d and two concentrations of *B. licheniformis* in the water after 7 d were evaluated.



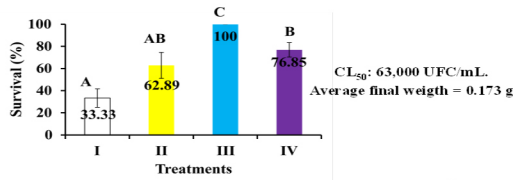
1) Control (-); II) Control (+); III) MP 4 g/kg c/2 d + Vibrio CL_{50}; IV) BL 1 x 10^6 UFC/L c/7 d + Vibrio CL_{50}; V) MP 4 g/kg c/2 d + BL 1 x 10^6 UFC/L c/7 d + Vibrio CL_{50}.

Bioassay 2. Best results with MP and *C. parapsilopsis* were added to feed after 2 d and *B. licheniformis* in water after 3 d.



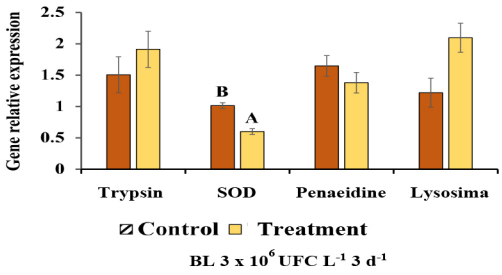
I) Control (+); II) MP 4 g kg^{-1} c/2 d + BL 2 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}; III) MP/2 d^{-1} + BL 3 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}.

*Bioassay 3.*The yeast added to feed each 2 d and *B. licheniformis* in water after 3 d were analizaed.



Control (+) ; II) Yeast 2 g kg^{-1} 2 d^{-1} + *Vibrio* CL_{50}; III) BL 3 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}; IV) DKT 3 g kg^{-1} 2 d^{-1} + *Vibrio* CL_{50}.

Bioassay 4. The yeast in two different concentrations and *B. licheniformis* in the water. All four bioassays were challenged against *V. parahaemolyticus*.



EFFECT OF MEDICINAL PLANTS, YEAST AND *Bacillus licheniformis* ON THE GROWTH, SURVIVAL, IMMUNE RESPONSE AND DIGESTION OF SHRIMP (PENAEUS VANNAMEI) CHALLENGED WITH *Vibrio parahaemolyticus*

Héctor Abelardo González-Ocampo*, Karime Anahi Valdez-Chavez and Arturo Fierro-Coronado

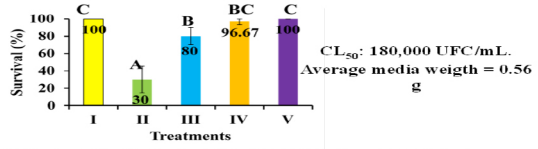
Instituto Politécnico Nacional, CIIDIR-Sinaloa.
Juan de Dios Batis Paredes #250, Guasave, Sinaloa. C.P. 81000
Scopus ID: 6504578835; ORCID: 0000-0003-2179-1166
hgocampo@yahoo.com

In shrimp farming viral diseases produce important economic losses. The use of prophylactic methods as natural additives wthout inmmune resistance and environmental problems were proben. Medicinal plants powder, yeast and *B. licheniformis* were used in the survival in the digestive and immune systems of *P. vannamei* challenged against *V. parahaemolyticus*.
Four bioassays were carried.Postlarval white shrimp stage were used to analyze the digestive and immune-related genes expression by RT-qPCR.

The combination of MP in the food and *B. licheniformis* in the water (3 x 106 CFU / L) significantly improved the survival of *P. vannamei* challenged with *V. parahaemolyticus*. *B. licheniformis* inoculated in the water, did not alter the expression of the trypsin digestive gene.

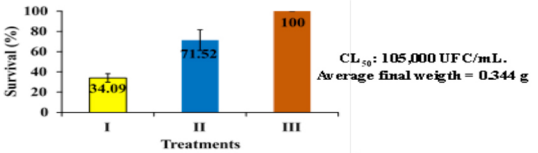
B. licheniformis in the water decreased the expression of the SOD gene (related to the immune system), which plays an important role as an antioxidant, decreasing the concentration of superoxide anion, the product of the phagocytosis process. *B. licheniformis* in the water did not alter the expression of the genes of the immune system penaeidine4 and lysozyme. The mixture of MP added in the feed and *B. licheniformis* in the water prevent the AHPND in *P. vannamei* cultivated in the laboratory.

Bioassay 1. Medicianl Plant (MP) powder added to feed every 2 d and two concentrations of *B. licheniformis* in the water after 7 d were evaluated.



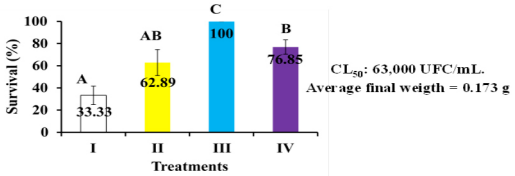
1) Control (-); II) Control (+); III) MP 4 g/kg c/2 d + Vibrio CL_{50}; IV) BL 1 x 10^9 UFC/L c/7 d + Vibrio CL_{50}; V) MP 4 g/kg c/2 d + BL 1 x 10^9 UFC/L c/7 d + Vibrio CL_{50}.

Bioassay 2. Best results with MP and *C. parapsilopsis* were added to feed after 2 d and *B. licheniformis* in water after 3 d.



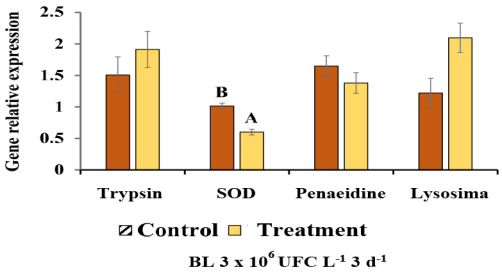
I) Control (+); II) MP 4 g kg^{-1} c/2 d + BL 2 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}; III) MP/2 d^{-1} + BL 3 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}.

*Bioassay 3.*The yeast added to feed each 2 d and *B. licheniformis* in water after 3 d were analizaed.



Control (+) ; II) Yeast 2 g kg^{-1} 2 d^{-1} + *Vibrio* CL_{50}; III) BL 3 x 10^6 UFC L^{-1} 3 d^{-1} + *Vibrio* CL_{50}; IV) DKT 3 g kg^{-1} 2 d^{-1} + *Vibrio* CL_{50}.

Bioassay 4. The yeast in two different concentrations and *B. licheniformis* in the water. All four bioassays were challenged against *V. parahaemolyticus*.



TRACING THE SOURCE OF BLACK TIGER PRAWN *Penaeus monodon* USING ISOTOPIC AND ELEMENTAL FINGERPRINTING TECHNIQUES

Karthik Gopi*, Debashish Mazumder, Jesmond Sammut, Neil Saintilan, Jagoda Crawford and Patricia Gadd

Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, The University of New South Wales, UNSW Sydney 2052, Australia.
k.gopi@unsw.edu.au

There has been a significant rise in the global demand for seafood and with the declining productivity of world fisheries stock, aquaculture is going to play a vital role in supplying seafood to the masses. However, farmed seafood produce is typically sold alongside wild-caught seafood at a lower price, these differences in price can lead to opportunities for fraudulent businesses to make a quick profit. Therefore, determining the provenance of seafood is necessary to reduce the occurrence of food fraud and lower the risk of food safety and biosecurity threats. There are several methods available for determining the provenance of seafood, however, they all have advantages and disadvantages. To our knowledge there is no single method which can be used to determine both the production method and geographic origin of seafood with a high accuracy. Therefore, the aim of this study was to use stable isotope analysis (SIA) and X-ray fluorescence (XRF), using Itrax, to test if the geographic origin and production methods of black tiger prawns (*Penaeus monodon*) from a range of Asia-Pacific locations could be determined. The stable carbon and nitrogen isotope along with the elemental composition of 31 different elements were analysed using multivariate analyses. Both the linear discriminant analysis (LDA), and randomForest produced consistent results. In both cases, SIA had a lower accuracy than XRF through Itrax when distinguishing the provenance of *P. monodon* (95% vs 100% respectively). While the elemental data had a 100% accuracy, there were still a number of incorrect predictions, which was likely to be caused by the large amounts of data used to build the models. However, when the same model was built using a combined SIA and XRF dataset, there were no incorrect predictions, even though the accuracy was lower at 97%. Therefore, a combination of SIA and XRF through Itrax can provide regulatory bodies with the tools necessary to ensure that seafood is unadulterated and from the correct origin.

MABÉ PEARL CULTURE IN THE KINGDOM OF TONGA: OPPORTUNITIES, INNOVATION AND OUTLOOK

Sophie E. Gordon*, Max Wingfield, Tuikolongahau Halafihi, Siola'a Malimali, David Schoeman and Paul C. Southgate

Australian Centre for Pacific Islands Research
School of Science and Engineering
University of the Sunshine Coast
Sippy Downs, Queensland 4556, Australia
Sophie.gordon@research.usc.edu.au

Pearl farming is an environmentally benign income source to many remote Pacific island communities and is the region's most valuable aquaculture commodity. The culture of 'mabé pearls,' or half-pearls, requires lower financial and technological investment, has a shorter culture duration than round pearl culture and is compatible with the lifestyles of many coastal communities. The mabé pearl sector offers a variety of livelihood opportunities to communities, including oyster collection and sales, the production of mabé pearls, and mother-of-pearl or mabé pearl jewellery and handicraft items.

The winged pearl oyster, *Pteria penguin*, is the traditional species used for mabé pearl production and has been used to sustain the mabé pearl industry in Tonga since the mid-1970s. Mabé pearl production in Tonga remained an artisanal industry until recently, with the country now recognised as the primary producer of mab**é** pearls in the south Pacific. Rapid recent development of the sector has resulted in the establishment of 18 mabé pearls farms, which produce > 4,000 pearls with an estimated annual revenue of > USD 275,000, annually. Aquaculture commodity analysis identified mabé pearl culture as having high feasibility for development and the greatest potential to support livelihood benefits in Tonga.

Here we discuss the key factors and underlying research that has sustained the recent growth of the Tongan mabé pearl sector, specifically through improvement and optimisation of: (1) hatchery procedures; (2) culture site selection; (3) husbandry techniques; (4) mabé pearl culture techniques, and; (5) quality control and grading. We discuss how this research will increase productivity and profitability for pearl farmers and aid the sustainable expansion of the Tongan mabé pearl sector. Lastly, we demonstrate the suitability of mabé pearl farming for alternative livelihood development in Tonga and discuss its potential applicability for other international coastal communities.

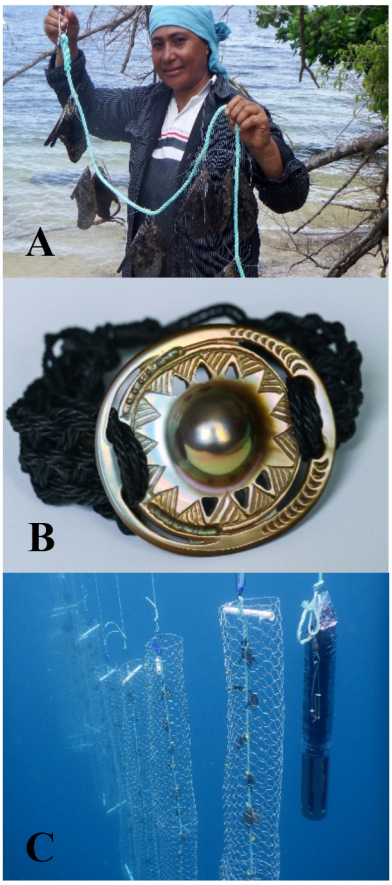


FIGURE 1. a) Pearl farmer Mele prepares her oysters for harvest; b) a piece of high quality Tongan mabé pearl jewellery presented to HRH Duke of Sussex, and; c) *Pteria penguin* in culture cylinders alongside a multiparameter water quality sonde.

GROWTH AND SURVIVAL OF SANDFISH *Holothuria scabra* JUVENILES REARED IN MARICULTURE IMPACTED AREA

Jay R C. Gorospe*, Marie Antonette Juinio-Meñez, Paul C. Southgate

School of Science and Engineering,
University of the Sunshine Coast,
Sippy Downs, Queensland, 4556 Australia
jay.r.gorospe@research.usc.edu.au

The impact of anthropogenic enrichment due to mariculture activity and substrate mesh size on periphyton characteristics and its consequent effects on the growth performance of post-settled (~5 mm) sandfish juveniles was evaluated in three sites (i.e. ENRiched, INTermediate, CONtrol) along Guiguiwanen Channel in Bolinao Northwestern Philippines using two different substrate mesh size (i.e. 400 µm and 1 mm) in a 30-day field experiment. Growth and survival of sandfish juveniles were measured twice and periphyton chlorophyll *a* and ash-free dry weight (AFDW) were monitored every 5-days for 30 days. Sea slugs that recruited inside the mini-*hapa* nets were identified and counted during monitoring.

Mean length and absolute growth rates (AGR) was not significantly different between mesh size and across sites by D15; however, differences were significantly higher in enriched site by D30. Juveniles reared in the enriched site were significantly larger and grew faster compared to the juveniles reared in sites farther from the fish farm. Nevertheless, growth rate of juveniles across sites was relatively lower by D30 compared to D15. The decrease in average AGR by D30 could be attributed to reduced food availability as indicated by AFDW due to inter- and intraspecific competition. Periphyton chlorophyll *a* content was relatively higher during the second half of the experiment, however, total biomass as indicated by AFDW of periphyton generally decreased overtime. The decreased in food availability coincided with the increased in the number of sea slugs that recruited inside the mini-*hapas* by D30. Survival significantly differ across sites but not mesh size by D15. Survival was significantly higher in intermediate site by D15. Differences in survival was significantly higher in juveniles reared in 400 µm size across all the sites by D30. Overall periphyton characteristics and biomass was better and higher near the enriched site. Additionally, sandfish juveniles were larger and exhibited higher growth rates, indicating the viability of culturing post-settled sandfish juveniles near mariculture areas.

Table 1. Mean (SE) length, AGR and survival of sandfish juveniles reared in the three sites using two substrate mesh size. Different letters within each substrate on D15 and across sites on D30 for length and AGR; and across sites on D15 and between substrates on D30 for survival were significantly different.

Site	D15		D30	
	1 mm	400 µm	1 mm	400 µm
Length				
CON	1.38 (0.04)^{a}	1.09 (0.19)^{a}	1.98 (0.12)^{ab}	1.93 (0.05)^{ab}
INT	1.45 (0.04)^{a}	1.27 (0.05)^{b}	1.75 (0.20)^{b}	1.79 (0.13)^{b}
ENR	1.46 (0.07)^{a}	1.38 (0.12)^{a}	2.31 (0.21)^{a}	2.26 (0.12)^{a}
AGR				
CON	0.06 (0.003)^{a}	0.04 (0.01)^{a}	0.05 (0.004)^{ab}	0.05 (0.002)^{ab}
INT	0.07 (0.003)^{a}	0.05 (0.003)^{b}	0.04 (0.01)^{b}	0.04 (0.004)^{b}
ENR	0.07 (0.004)^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.004^{a}
Survival				
CON	37.8 (3.9)^{b}	46.4 (20.3)^{b}	58.6 (5.1)^{b}	76.9 (7.3)^{a}
INT	80.6 (3.5)^{a}	79.2 (3)^{a}	45.8 (9.6)^{b}	65.6 (7.7)^{a}
ENR	55.6 (3.2)^{ab}	65.3 (9.4)^{ab}	63.1 (12.2)^{b}	81.9 (6.7)^{a}

INFLUENCE OF INTRA- AND INTERSPECIFIC COMPETITION ON PERIPHYTON BIOMASS AND GROWTH PERFORMANCE OF SANDFISH *Holothuria scabra* JUVENILES

Jay R C. Gorospe*, Marie Antonette Juinio-Meñez, Paul C. Southgate

School of Science and Engineering
University of the Sunshine Coast
Sippy Downs, Queensland, 4556 Australia
jay.r.gorospe@research.usc.edu.au

Grazing has a key influence on periphyton biomass. Here we investigated the effects of intra- and interspecific competition on periphyton biomass using varying density combinations of sandfish (SC) *Holothuria scabra* juveniles (~5 mm) and the sea slug (SS) *Stylocheilus striatus* in a 28-day experiment in 0.28 m^{-2} circular *hapa* nets containing; SC15, SS15, SC15/SS15, SC50, SC100 and SC0/SS0 (control). Periphyton chlorophyll *a* and ash free dry weight (AFDW) contents were measured weekly. Food consumption was estimated from the faecal production of SC and SS after 24 h and weekly thereafter. Growth performance and survival of sandfish juveniles were measured on days 14 and 28.

Chlorophyll *a* and phaeopigment were significantly higher by day 28 and lowest by day 7. Periphyton AFDW was significantly higher in the SC15 treatment and was lowest in the SC15/SS15 treatment. Notably, AFDW in the control was lower than in the SC15 treatment, although differences were not significant. Sandfish juveniles reared in the SC15 treatment were significantly larger and absolute growth rate was higher (Table 1) compared to that in all other treatments on days 14 and 28. Lowest growth rate occurred in the SC100 treatment. Coefficient of variations (length) was significantly higher in the SC100 treatment on days 14 and 28; but was not significantly different to those of SC15/SS15 and SC50 juveniles by day 28. Survival was significantly higher in the SC15 treatment on days 14 and 28; however, differences were not significant compared to juveniles reared in the SC15/SS15 and SC50 treatments on day 14. Differences in food consumption among treatments was significant only at 24 h and on day 21 with greatest consumption in the SS15 treatment.

Sea slugs in the SS15 treatment consumed a higher amount of periphyton (262.3 ± 45 mg) than those in all other treatments after 24 h and on day 21. Lowest food consumption was observed in the SC100 treatment (0.05 ± 0.01 mg) on day 21. Those in the SS15 consumed 17.5 mg periphyton ind^{-1} day^{-1} (dry weight) compared to 4.5 mg ind^{-1} day^{-1} by sandfish juveniles reared in the SC15 treatment. The higher grazing rate of sea slugs may limit the growth performance of sandfish juveniles.

TABLE 1. Mean length, AGR, CV and survival (SE) of sandfish juveniles reared in different conspecifics treatment. Different letters within monitoring period were significantly different.

Parameters	D14	D28
Length (cm)		
SC15	1.16 (0.04)^{a}	1.43 (0.12)^{a}
SC15/SS15	0.73 (0.05)^{b}	0.82 (0.11)^{b}
SC50	0.7 (0.04)^{b}	0.76 (0.08)^{b}
SC100	0.61 (0.04)^{c}	0.58 (0.03)^{c}
AGR (cm day-1)		
SC15	0.05 (0.003)^{a}	0.028 (0.008)^{a}
SC15/SS15	0.02 (0.003)^{b}	0.007 (0.006)^{b}
SC50	0.02 (0.003)^{b}	0.004 (0.003)^{b}
SC100	0.01 (0.002)^{c}	-0.003 (0.003)^{c}
CV (%)		
SC15	29.8 (2.12)^{b}	34.4 (3.56)^{b}
SC15/SS15	36.5 (5.82)^{b}	49.2 (7.58)^{ab}
SC50	46.4 (2.01)^{b}	62.8 (4.08)^{ab}
SC100	48.1 (0.83)^{a}	67.3 (2.52)^{a}
Survival (%)		
SC15	90 (5.64)^{a}	84.4 (4.77)^{a}
SC15/SS15	76.7 (4.8)^{ab}	61.1 (4.7)^{b}
SC50	79.7 (4.3)^{ab}	63 (3.34)^{b}
SC100	66.5 (6.24)^{b}	49 (4.95)^{b}

CAPTIVE BOLT INDUCED CRANIAL CONCUSSIVE STUNNING TO IMPROVE FISH WELFARE FOR EUTHANASIA AND SLAUGHTER IN STURGEON AQUACULTURE

Jackson Gross, Zarah Deutsch

jagross@ucdavis.edu

The American Veterinary Medical Association stipulates that appropriate slaughter processes must first render the animal insensible quickly and painlessly to be considered a humane practice for good animal welfare. Many techniques used to create insensibility or brain death in sturgeon aquaculture do not accomplish this goal due to their size and anatomy. The sturgeon's cartilaginous skeleton requires greater force to cause brain damage when compared to bony fish. Current techniques for cranial concussion utilize a bat, fish priest or another blunt instrument, or a non-penetrating captive bolt (NPCB). While NPCB provides superior reliability in both placement location and force, there are no studies to date describing the appropriate force required to ensure brain death in sturgeon. This study aims to provide the appropriate NPCB operating pressure (PSI) for juvenile meat fish and adult caviar-ready white sturgeon. Adult caviar-ready, 6-9 yr old female white sturgeon, (mean mass = 27.1kg) and 2-3 yr old juvenile male white sturgeon (mean mass = 8.4kg) were stunned with a Jarvis HPS-1 pneumatic NPCB with a 2.5 cm striking surface. The caviar-ready sturgeon were slaughtered on a commercial farm using 175, 200, and 225 PSI delivered by compressed nitrogen gas monitored by a regulator. Control adult sturgeon experienced multiple strikes between 120 - 145 PSI by a commercial electric air compressor which is the current farm practice. All fish were exsanguinated as a second step termination method. Juvenile sturgeon were stunned at 120, 145, and 170 PSI. Skull and brain were removed by a reciprocating saw, fixed in 37% formaldehyde, and histology of the brain was performed to quantify tissue damage. This study validates the use of NPCB as an effective tool as part of a humane slaughter protocol when operated at specified settings for sturgeon, and improves upon currently available techniques and fish welfare for all cartilaginous fish.

EFFECTS OF MICROALGAE FEED-BASED IMMUNOMODULATORS ON GROWTH, HEALTH AND IMMUNE RESPONSE IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Thomas Grimm*[1,2], Roshan P. Shrestha[1,2], Annabell Palacios Escobar[2], Maria de Lourdes Neder Munoz[2], and Jorge Tamayo[2]

[1] PhycoVax LLC, 4600 Carlsbad Blvd, Carlsbad, CA 92008, USA
[2] PhycoVax Ecuador S.A., Edificio World Trade Center Ofic 305A, Av. Francisco de Orellana, Guayaquil 090512, Ecuador

Shrimp is the second most valuable global aquaculture product after carp. *Penaeus vannamei* (Pacific whiteleg shrimp) and *Penaeus monodon* (giant tiger prawn) are the most common farmed species, which account for roughly 80% of all farmed shrimp worldwide. Infectious diseases, however, often cause havoc in shrimp farms. Microbes, including Vibrio bacteria, are omnipresent in shrimp farms and can lead to disease outbreaks in favorable conditions, including Acute Hepatopancreatic Necrosis Disease (AHPND) caused by *Vibrio parahaemolyticus*. Disease control in shrimp farming currently relies upon preventative measures. The lack of commercially available therapeutics for shrimp urged urgent development of orally delivered vaccines/therapeutics, which can be adapted widely for farm application.

Immunomodulating proteins were produced in microalgae and incorporated into shrimp diets. Experiments were conducted in aquariums and cages-in-pond to evaluate the effects on shrimp morphometric changes, survival percentage, and level of Vibrios loads, followed by the histological and pathological analysis of hepatopancreas, immune parameters quantification and feed conversion ratio. In a laboratory experiment, 15 juvenile shrimps (5 gm each) were assigned to three 40-liter aquariums per test diet. Animals were fed 8 times a day for 14 days. In the cages-in-pond experiment, shrimp post larvae (PL 18-24) were stocked in the density of 1000 animals/m^2 in 3 cages per test diet. Animals were fed 6 times a day for 7 days. The experimental results indicate an increase in the survival rate compared to controls fed with a commercial diet and high immunity as indicated by elevated superoxide dismutase (SOD) levels in the hemolymph. Moreover, growth performance parameters such as the average weight of animals and feed conversion ratio (FCR) were positively impacted. Test diets also decreased the level of *V. parahaemolyticus* loads in the hepatopancreas resulting in healthier hepatopancreas tissues

SUPPLEMENTATION OF LYSO-PHOSPHOLIPID-BASED FEED ADDITIVE MAINTAINS GROWTH PERFORMANCE OF PANGASIUS FISH *Pangasianodon hypophthalmus* UNDER DIETARY OIL REDUCTION STRATEGY

Martin Guerin*, Waldo Nuez-Ortin, Martha Mamora, Dedi Jusadi

ADISSEO Asia Pacific Pte Ltd,
600 North Bridge Rd., Unit 15-06 to 08 Parkview Square, 188778 Singapore
martin.guerin@adisseo.com

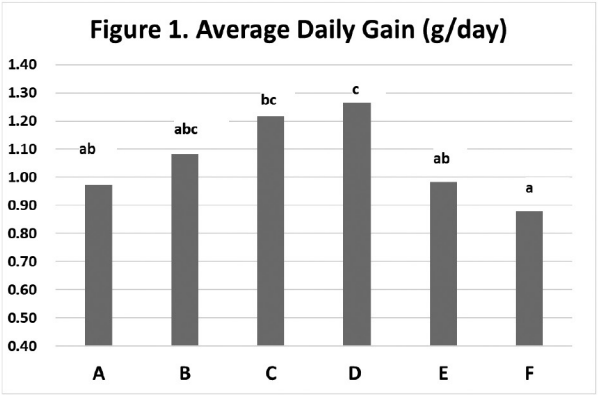
Lyso-phospholipids (LPL) are obtained by enzymatic hydrolysis of phospholipids. This hydrolysis removes one fatty acid and renders the molecule more hydrophilic, giving LPL the ability to better emulsify and digest lipids. Aqualyso STD (AQLS) is a low-inclusion feed additive based on highly hydrolysed LPL and that aims to maximize lipid digestion and utilization of aquafeeds. The objective of this study was to evaluate the efficacy of AQLS to support the performance of Pangasius feeds with progressively reduced fat levels (i.e., oil inclusion).

A feeding trial was conducted at the Department of Aquaculture, IPB University, Indonesia. Juvenile Pangasius fish (12g/pc.) were stocked in 30 net cages (2 x 1 x 1.5m). Fish were fed six isoprotein and fishmeal-free feeds (n=5), for 62 days until they reached around 100g. The six experimental feeds are summarized in Table 1 and consisted of one control base feed with 31% CP and 7% fat (A), and then five feeds with 31% CP and fat levels between 6.5 and 5% and supplemented with AQLS (B, C, D, E and F). Fat levels were progressively reduced by reducing the inclusion of fish oil and palm oil. At the end of the trial fish were harvested and feed performance was evaluated.

Results (Fig. 1) showed that feed D, with 1.5% reduction in oil content and supplemented with 0.1% AQLS, generated significantly ($p<0.05$) better growth than the control feed A. Feeds B, C, and E, respectively with 0.5%, 1% and 2% lower oil inclusion and supplemented with 0.1% AQLS, resulted in numerically higher growth as compared with the control feed A. In this line, the supplementation of 0.1% AQLS under lower oil inclusion numerically improved feed conversion efficiency (FCR) consistently across feeds. These results open interesting cost-saving strategies for fish feed formulators.

Table 1: Experimental diets

Diet	A	B	C	D	E	F
%CP	31%	31%	31%	31%	31%	31%
% fat	7%	6.5%	6%	5.5%	5%	5%
Palm oil	2.5%	2.25%	2.0%	1.75%	1.5%	1.5%
Fish oil	1.2%	0.95%	0.7%	0.45%	0.2%	0.2%
Filler	0%	0.4%	0.9%	1.4%	1.9%	1.85%
AQLS	0%	0.1%	0.1%	0.1%	0.1%	0.1%



MATURATION AND SPAWNING OF ESTROGEN-INDUCED PRECOCIOUS FEMALE BARRAMUNDI *Lates calcarifer* AND THE IMPLICATIONS OF THEIR USE IN ADVANCED SELECTIVE BREEDING

Jarrod L. Guppy*, Adrien F. Marc, Dean R. Jerry

Centre for Sustainable Tropical Fisheries and Aquaculture
College of Science and Engineering, James Cook University
Townsville, Queensland, Australia
jarrod.guppy@my.jcu.edu.au

Barramundi (*Lates calcarifer*) are a premium white fleshed fish, with rapidly increasing demand and with a number of breeding programs now operating. However the progress of selective breeding has been impeded by the complex sexual development of barramundi. Barramundi are protandrous hermaphrodites, initially maturing as males at 2-3 years (2-4 kg BW), before male to female sex change occurs at 4-6 years (>6-8 kg BW). Recently, we have successfully produced precocious female barramundi using a single intramuscular E2 implant. While this is a significant step forward for selective breeding the functional maturation of precocious females, and their ability to spawn remained untested. The present study investigates the potential of utilising precocious female barramundi in selective breeding programs.

Male barramundi (n = 22, ~2.2 kg BW, 57.8 cm TL) were hormonally induced to sex change and fed a commercial grow-out diet before transitioning to a commercial maturation diet after 6 months. The female barramundi were cannulated at 6 (T1), 8 (T2), 10 (T3), 11.5 (T4), 13 (T5) months after sex change, with oocytes measured and staged to determine reproductive condition. Furthermore, four females with >350 um oocytes (4.4 kg BW, 67.8 cm TL) at T3 were relocated to a 15,000 L spawning tank along with four males (4.8 kg BW). Mass spawning was induced with a single LHrHa injection, and eggs released on the first and second night were collected to assess fertilisation rate, hatching rate and larval survival at 24 and 48 hr post-hatch. Spawns were conducted at T4 and T5 with the same eight individuals to assess repeated spawning performance.

Females with mature oocytes ready for spawning were observed across all cannulations (T1 – T5). However, oocytes that were not already mature at T1 often did not mature further beyond pre-vitellogenic stage. A large number of eggs were collected across all spawning nights, with 7.7 million (T3), 10.9 million (T4) and 9.1 million (T5) eggs obtained across the two nights of spawning. Fertilisation was not observed for the T3 spawn (<0.0%), however a small number of larvae hatched (0.04%), but did not survive past 24 hr on the second night. Spawning performance improved considerably for T4 and T5 spawns, with the highest fertilisation rate (64%) and hatching rate (72%) observed in the T5 night one spawn. Furthermore, larval survival, 24 and 48 hr after hatching was 99% and 94% respectively. Total larval production across both nights of the T5 spawn exceeded 3 million individuals. These results are comparable with spawns of routine commercial stock that utilise three females (10 kg) and six males (4 kg), and confirm that precocious female barramundi could be effectively integrated into a selective breeding program.

CONSTRUCTION OF A GENETIC LINKAGE MAP FOR GENOME WIDE INVESTIGATION OF ECONOMICALLY IMPORTANT TRAITS IN BLACK TIGER SHRIMP *Penaeus monodon*

Jarrod L. Guppy*, David B. Jones, Mehar S. Khatkar, Herman W. Raadsma, Maria G. Nayfa, Nicholas M. Wade, Dean R. Jerry, Kyall R. Zenger

Australian Research Council Industrial Transformation Research Hub for Advanced Prawn Breeding
College of Science and Engineering, James Cook University
Townsville, Queensland, Australia
jarrod.guppy@my.jcu.edu.au

Black tiger shrimp (*Penaeus monodon*) are the second most cultured shrimp species globally, with over 700,000 tons produced per annum. However, like many aquaculture species the underlying drivers of economically important traits in black tiger shrimp are poorly understood, and the observed performance across individuals is highly variable. In particular, it is critical to uncover the genomic architecture of economically important traits to facilitate advanced selective breeding approaches (i.e. marker assisted selection or genomic selection). Such approaches are reliant upon a robust understanding of the placement and contribution of individual genetic markers in the prediction of an individual's performance or merit (genomic estimate breeding value).

In this study, we utilised 2,745 individuals obtained from 19 families in an Australian commercial breeding population to construct a high-density genomic map containing over 9,000 SNP markers obtained using genotype by sequencing. Overall, the genome-wide distribution of the SNP markers was confirmed with 44 linkage groups identified and subsequently anchored to genomic scaffolds obtained from an ongoing genome assembly project. By combining genotype data and phenotypic records obtained at harvest, we undertook quantitative trait mapping (QTL) and genome-wide association studies (GWAS) for the primary production traits, growth and sex. In both QTL and GWAS analysis, a single sex-associated region was identified, confirming earlier studies undertaken with divergent Indian and Mozambique black tiger shrimp breeding populations and the white leg shrimp, *Litopenaeus vannamei*. Current studies of genetic drivers of growth are underway; with preliminary results indicate a highly polygenic trait architecture with moderate heritability ($h^2 \approx 0.24$). Understanding the genomic architecture of commercial traits is critical to inform genetic improvement strategies for black tiger shrimp and lays the foundation for advanced methods of selective breeding.

PATHOGENESIS OF *Photobacterium damselae* subsp. *damselae* INFECTIONS IN AUSTRALIAN YELLOWTAIL KINGFISH (*Seriola lalandi*)

Nipa Gupta*, Gavin J. Partridge, Nicky Buller, Andrew Currie, and Alan J. Lymbery

School of Veterinary and Life Sciences, Murdoch University, 90 South St, Murdoch, WA, 6150, Australia
Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh
nipagupta@hstu.ac.bd

Photobacterium damselae subsp. *damselae* is a marine halophilic bacterium, which may cause fatal infections in a wide variety of hosts, including fish, molluscs, crustaceans, humans and other mammals. It is an emerging pathogen of economically important aquaculture species with increasing geographical distribution worldwide. The major virulence factors of Pdd are plasmid (pPHDD1)-encoded phospholipase-D damselysin and pore-forming toxin phobalysin P. However, strains of Pdd without plasmid have also demonstrated pathogenicity due to the presence of chromosome I-encoded virulence factors. The present study was conducted to characterize the intraspecific variability in 16 isolates of *P. damselae* subsp. *damselae* collected from cultured yellowtail kingfish (YTK) in Australia by means of biochemical and molecular typing and to get the insights of pathogenicity of Pdd infections in YTK.

The intraspecific variability was conducted by means of biochemical and phenotyping characteristics while sequence analysis was done based on *toxR* gene to investigate genetic heterogenicity. An infection trial was conducted with healthy, unvaccinated cultured YTK, weighing an average 152.0±17.7 g. Three isolates of Pdd (one plasmid-positive, AS-16-0963#3; and two plasmid-negative, AS-15-3942#7 and AS-16-0963#1) were selected for experimental infection. There were six experimental treatments, with intraperitoneal injection, one of the three test isolates of Pdd at two different concentrations, 10^4 CFU/fish and 10^7 CFU/fish, and three replicate tanks per treatment. Control fish in two replicate tanks were sham-injected. Fish mortality was recorded daily for 10 days post-challenge. Blood samples from infected fish after 4 days of infection were collected to measure hematology indices.

There are considerable differences in phenotypic traits among isolates and five biotypes were established accordingly. *toxR* sequence analysis demonstrated substantial genetic diversity among isolates, suggesting the presence of several different clonal lineages of Australian Pdd isolates. Mortality rates for the plasmid-positive isolate, AS-16-0963#3, were 47% and 100% at 10^4 CFU/fish and 10^7 CFU/fish respectively, compared to13-20% and 3-13% respectively for the two plasmid-negative isolates. No mortality was observed in the control fish. There was no significant difference in hematology parameters among isolates. These results suggest that, while presence of the pPHDD1 plasmid is not essential for pathogenicity, the plasmid does increase the virulence of *Photobacterium damselae* subsp. *damselae* to *Seriola lalandi* in Australia.

A NEW PARADIGM OF NURSERY FEEDING : CASE OF TILAPIA

Gaetan Gutter*, Thomas Raynaud**, Navid Pormehr, Frederic Baron, Delphine Weissman

Bern Aqua NV
Hagelberg 3
B-2250 Olen
Belgium

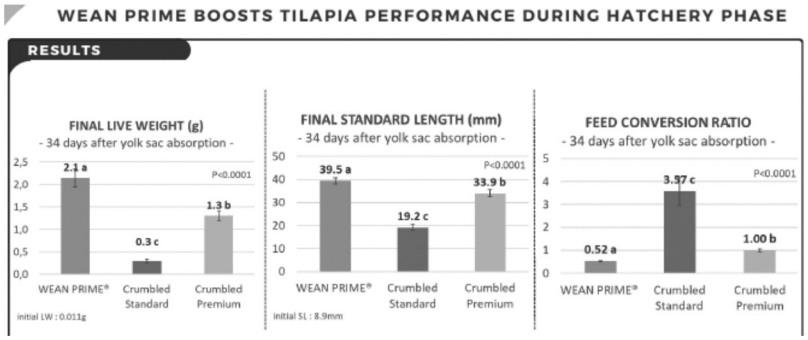
gaetan.gutter@adm.com
thomas.raynaud@adm.com

The introduction of a nursery phase is becoming a regular practice in aquaculture farming. A complete diet formulation is required to reach fish nutrients requirement at this critical and stressful phase for the fish before the transfer practice at grow-out ponds.

A new generation of nursery feed is better adapted to the natural metabolism of the animal with a balance between animal and plant components in the function of each specie. About tilapia, a new approach with some marine sources ingredients (such as algae, krill, squid, etc.) with high levels of digestibility, organic acids and immunostimulant added to the diet can provide a better performance in nutrition and health. With this new formulated nursery feed, containing less protein than a classic competitor feed, we can demonstrate that the quality of protein content is more important than the quantity of protein. This allows us to have a more sustainable offer to reduce the waste of protein.

Moreover, keeping this sustainable approach, to counter the impacting effects of stress, BernAqua (a company of ADM group) has developed a feed concept that improves the digestibility of feed and improve the health of animal: Life Defense.

Life Defense is a blend composed of feed additives intended to help fish to better cope with stress. It is a triple action formula acting at the intestinal level which finality is to improve absorption of nutrients during stress events and optimize gut function. Life Defense is included in WeaN Prime, a nursery feed for tilapia produced by BernAqua and the addition of this feed additives blend to support technical and economic performance at the farm level shows excellent results.



	Protein	Fat	Ileal Digestibility (Boisen)
WEAN PRIME	45 %	8 %	85 %
Crumble Standard	55 %	9 %	79 %
Crumble Premium	55 %	8 %	74 %

Ref : Experiment made at ADM Research center in Brazil.

WATER QUALITY DYNAMICS IN EARTHEN PONDS WITH AND WITHOUT FISH

S. Gyamfi*, R. E. Edziyie, K. A. Obirikorang, D. Adjei- Boateng and P.V. Skov

Kwame Nkrumah University of Science and Technology
Denmark Technical University (DTU Aqua)
Email:stephengyamfi40@gmail.com

Introduction

Tilapia is currently cultured in over 100 countries and increasingly becoming one of the most cherished protein sources globally. The global development of tilapia culture has passed through several phases of improvement over the last two decades. That notwithstanding, water quality management in the main culture system (ponds) for this species is yet to be fully optimised. This study was conducted to assess the water quality dynamics in tropical earthen ponds with and without fish.

Materials and Methods

The study was conducted for three (3) months in four ponds (10×15×1m each) at an experimental fish farm in Kumasi, Ghana. Two treatments i.e. fed pond which comprised two ponds stocked with all male tilapia (38g) and fed twice daily with a commercial feed (CP-30%) and a control- two other ponds without fish/feed. Growth and feed utilization of the fish were assessed at the end of the study. Physicochemical and biological water quality parameters were monitored every four days and every three weeks, respectively. Twice a week, the dissolved oxygen (DO), pH, temperature and conductivity levels were measured *in-situ* with a multiparameter probe (Hach, Hd40Q) between 7 and 8 am while water samples were taken to the laboratory for turbidity, total suspended solids (TSS), total dissolved solids (TDS), alkalinity, NH_3, NH_4,ortho-phosphate, NO_2, NO_3 biological and chemical oxygen demand (BOD & COD), organic matter (OM) and organic carbon (OC) analysis. Furthermore, every three (3) weeks, a 24h O_2 monitoring was done in the experimental ponds and sludge accumulation and sludge characteristics were also determined. Data on water quality, growth and feed utilization were expressed as means and standard deviation using Microsoft Excel and graphs generated with GraphPad Prism 5 software. Data on nutrients were subjected to the Mann Whitney test while all physico-chemical and biological parameters were subjected to Students T-test (α =0.05).

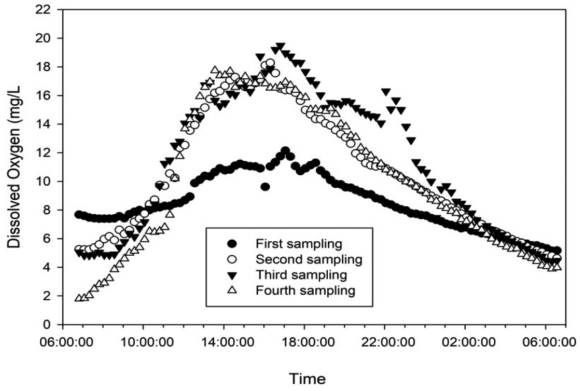


Fig 1. Diel oxygen cycles in the control pond at depths of 20 cm beneath the pond water surface. Sampling was done every three weeks with 15 min interval oxygen readings during the study

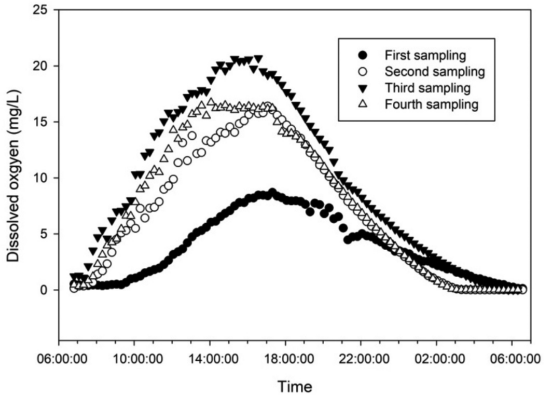


Fig 2. Diel oxygen cycles in the fed pond at depths of 20 cm beneath the pond water surface. Sampling was done every three weeks with 15 min interval oxygen readings during the study

Results

Physico-chemical parameters were relatively better in the control treatment ponds than the fed treatment ponds with highly significant differences observed in DO, temperature, TDS, TSS, turbidity, alkalinity and nutrients (NH_3, NH_4, NO_2, NO_3). Organic and biological parameters were significantly higher in the fed treatment ponds with BOD, COD, OM, OC and chlorophyll-*a* recording 22%,20.1%,14%,10.2%,35% respectively than the control treatment pond. Sludge measured was relatively higher in the fed ponds with better sludge characteristics in the control treatment. Water quality parameters recorded in the control ponds were within the recommended ranges for tilapia growth. Diel O_2 variation in both treatments decreased along sampling times.

EFFECT OF EXOGENOUS MELATONIN ON SEXUAL MATURATION OF JAPANESE EEL *Anguilla japonica*

Ji Eun Ha*, Jeong Hee Yoon, Dong Woo Kim, Joon Yeong Kwon

Dept. of Aquatic Life Medical Sciences, Sunmoon University, Asan 31460, Korea
jykwon@sunmoon.ac.kr

Melatonin is secreted during the dark and has been suggested to be a controlling factor of reproduction in various animals including fish. Eels show diel vertical movements, rising at night and going down during the day as they migrate toward the spawning ground (Seinen Chow et al., 2015). Thus, it is quite probable to think that the sexual maturation of eels is associated with photic conditions and melatonin. No relevant studies, however, have been conducted to explore any relationship between female eel reproduction and melatonin. This study investigated the effects of SPE (salmon pituitary extract) and melatonin treatment on the maturation induction of female eel.

Female eels were divided into four groups, each treated with saline (control), melatonin (M), SPE (S), or SPE+melatonin (SM) by using osmotic pumps. All osmotic pumps were implanted at the beginning of the experiment except the SM group in which osmotic pumps with melatonin were implanted on day 35. Fish were killed, and the blood, gonad, brain + pituitary gland were sampled on day 65. Gonadal development was estimated by means of histology and GSI. The level of plasma melatonin was measured by ELISA. Expression levels of LH, FSH, and melatonin receptor genes were analyzed from tissues of the brain+pituitary gland.

The delivery of SPE and melatonin by the osmotic pump contributed to the induction of sexual maturation and elevation of melatonin levels in female eels, respectively. Eels treated with SPE showed significantly higher GSI and LH gene expression than control eels. The level of melatonin ranged from 228 to 1123 pg/ml in melatonin treated group which was higher than that of the untreated group. However, the administration of exogenous melatonin did not promote or inhibit maturation at all. On the contrary, the levels of plasma melatonin in eels with mature gonads were significantly lower than the levels of eels with immature gonads. At the moment, it cannot be concluded that the sexual maturation of eel is not associated with melatonin. Further studies are required to prove whether the exogenous melatonin works properly or not in the body of eels.

ALTERNATIVES TO ANTIBIOTICS: CURRENT STATUS AND FUTURE TRENDS

Ha Thanh Dong*

Aquaculture and Aquatic Resources Management Program
Department of Food, Agriculture and Bioresources, School of Environment, Resources & Development (SERD)
Asian Institute of Technology (AIT)
PO Box 4, Klong Luang, Pathumthani 12120
Thailand
htdong@ait.ac.th

Aquaculture, particularly in Asia, is critical for global food security and nutrition. It provides protein to approximately 4.5 billion people on a global scale and employs 19.3 million people. Like other food sectors, Asian aquaculture has been confronted with increasing challenges with infectious diseases. Control of these diseases using antimicrobials has led to a rise of antimicrobial resistance (AMR) and serious concerns for public health. Alternatives to antimicrobial products are therefore urgently needed in all sectors of food production. This presentation will provide an overview of the current status of non-antibiotic approaches to controlling bacterial diseases, which include probiotics, phytochemicals, bioactive compounds, feed additives, bacteriophages, vaccines, and emerging nanobubble technology. Each mentioned approach has a number of advantages, but none is a miracle cure. Combating AMR in aquaculture is a lengthy battle that will require a strategic combination of multiple non-antibiotic approaches, including the development of novel antibiotic alternatives as feed additives, coherent with the One Health philosophy.

TWO-PHASED SUPER-INTENSIVE FARMING OF WHITE SHRIMP *Litopenaeus vannamei*: IMPORTANT INNOVATION IN VIETNAM

Tran Ngoc Hai*, Nguyen Thanh Dat, Chau Tai Tao

College of Aquaculture and Fisheries
Can Tho University, Vietnam
tnai@ctu.edu.vn

Shrimp culture is a very important industry in Vietnam with total culture area and production of 721,000 ha and 683,000 tons recently, from various farming systems, such as traditionally improved extensive shrimp farming systems, integrated mangrove-shrimp farming systems, alternative rice-shrimp farming systems, intensive and supper-intensive shrimp farming systems. Introduced to Vietnam since early 2001, white shrimp *Litopenaeus vannamei* culture have developed rapidly, which reach to 98,000 ha and 427,000 tons by 2017, mainly with intensive and super-intensive systems. The targets for the year 2025 are 600,00ha and 400,000 tons for tiger shrimps and 150,000ha and 700,000 tons for white shrimps. Under climate change condition, there has been a great technical improvement, especially super-intensive farming systems in HDPE small round tanks under green house for better control and higher production. A detail study on the practice and technical efficiency of super-intensive culture of white leg shrimp (*Litopenaeus vannamei*) was therefore conducted at a commercial company in Bac Lieu province as an example model. Shrimp were cultured with two-phases in HDPE round tanks under net houses. Shrimp postlarvae were nursed in four 100-m^3 circular tanks at stocking density of 2500 PL/m^3 for 16 days, juvenile was then transferred to the second phase with four 500-m^3 tanks at 500 shrimps/m^3 for 75 days. Molasses were added daily to the culture tanks at the C/N ratio of 15:1. Culture water was casually exchanged during culture and salinity was maintained at around 20ppt. Shrimp initial body weight and length were at 0.036 g and 1 cm, respectively. The results showed that after 90 days of culture, water quality parameters were in suitable ranges for shrimp growth. The daily weight gain and daily length gain of shrimp during 2-phased culture were at 7.27 %/day and 2.88 %/day, respectively. Rather high survival rates in nursing and grow-out phases (87% and 83.25%, respectively) and very good shrimp production (57.68kg/100m^3 at nursing phase and 3009.5 kg/500m^3 at grow-out phase) were achieved at harvest.

Table: Shrimp production and survival rate at nursing and grow-out phase

	Tank 1	Tank 2	Tank 3	Tank 4	Average
Nursing					
Production (kg/100m^3)	65	55	50.9	59.8	57.68±6.09
Survival rate(%)	85	82	90	91	87.00±4.24
Grow-out					
Production (kg/500m^3)	3041	3112	2530	3355	3009.50±346.79
Survival rate(%)	83	85	78	87	83.25±3.86
FCR	1.57	1.53	1.67	1.58	1.59±0.06

DEVELOPMENT AND APPLICATION OF BIOFLOC TECHNOLOGY FOR NURSERY OF TIGER SHRIMP *Penaeus monodon* POST-LARVAE FOR EXTENSIVE AND ALTERNATIVE RICE-SHRIMP FARMING IN THE MEKONG DELTA, VIETNAM

Tran Ngoc Hai*, Chau Tai Tao, Ly Van Khanh, Tran Nguyen Duy Khoa, Nguyen Thi Ngoc Anh

College of Aquaculture and Fisheries, Can Tho University, Vietnam
tnhai@ctu.edu.vn

Extensive shrimp farming and alternative rice-shrimp farming are of very important systems in the Mekong Delta of Vietnam which cover over 330,000ha and 170,000ha, respectively. The systems have many advantages but are also challeging to serval issues under climate change condition and croping seasons. Improving quality and size of shrimp seeds for stocking is one of important solutions to shorten grow-out phase, improve shrimp survival rates and production.

A series of triplicate experiments on the development of bioflocs technology for nursing of postlarvae of tiger shrimps were conducted in 0.5-m^3 tanks at Can Tho University. After 30 days of nursery, the best results from different experiments were found at thet treatments (i) with sugar mollas (survival and productivity at 92.9% and 558 PL/m^3 respectively); (ii) with C/N ratio at 15/1 (94.7%, 556 PL/m^3), (iii) with salinity at 15ppt (95.5% and 573 PL/m^3); and (iv) with stocking density of 600 PL/m^3 (95.5%, 573PL/m^3).

With the above findings, during 2018-2019, a total of 24 farms were selected for nursing shrimp postlarvae at farm sites, applying biofloc technology, with HDPE lined earthen tanks of 100m^2 as the average for each farm. After 20-30 days of nursery, the results showed that shrimp reach to 3-5cm in body length, and survival rates were in ranges of 81.93 ± 13.29%.

The results of the project contributed significanly to development of two-phased extensive and alternative rice-shrimp farming systems in the region.

KERNEL PCA AND ENSEMBLE LEARNING FOR PREDICTING WATER ORGANIC MATTERS AND HARDNESS OF PONDS IN PENAEUS VANNAMEI CULTIVATION

Water quality monitoring is one of several methods to control the risk in shrimp farming. Despite its importance, monitoring water quality during shrimp farming can be costly. This research was conducted to develop prediction models that would give farmers insight about water quality characteristic of their ponds. The data were collected from 31 ponds that used the JALA platform. This research tried to predict biological and chemical properties based on physical properties of water such as temperature, dissolved oxygen, salinity, and pH. This research combined Kernel Principal Component Analysis and machine learning algorithms (Random Forest and Gradient Boosting). The results of this research showed biological and chemical conditions of water (Total Organic Matter, Hardness, Calcium, Magnesium) with R2 Score higher than 0.8 on most parameters. This study also found that the Gradient Boosting model performed better in predicting water chemical properties than Random Forest model.

LESSONS FROM INTRODUCTION OF INTEGRATED RICE-FISH SYSTEM IN NORTHERN AND SOUTHERN REGIONS OF NIGERIA

Matthias Halwart*, Amrit Bart, Emmanuel K. Ajani, Oluwafemi Ajayi, Bamidele O. Omitoyin, Esendugue G. Fonsah, Gary Burtle, Austin Stankus, Yuan Xinhua, Oladeji K. Kazeem, Babatunde O. Oduntan and Annika Kolbe

Matthias Halwart
Fisheries and Aquaculture Division
Food and Agriculture Organization of the United Nations
Matthias.Halwart@fao.org

Introduction

Rice-fish farming as a farm diversification strategy was introduced to smallholder rice farmers as an approach to transform their traditional ricefield environments into a more productive, resilient, and biologically diverse agro-ecological landscape, with efficient use of land and water resources to produce fish in addition to rice within their existing ricefields. The intervention focused on generating entry-level advice for rice farmers with little or no knowledge of aquaculture.

The integration process

The process kick-started through series of community engagements with rice farmers, farmer groups and community leaders, to assess existing rice farming practices, identify wetland areas suitable for rice-fish farming and to understand farmer needs for ricefield adaptation to include fish. The diversification process involved ricefield modification- digging fish trench of 1.5m width and 0.7m depth round the edges of an 18m X 30m rice production area. Rice variety (FARO-40-National code: NGOS-91-40)) was planted at 20 X 20 cm spacing, and 1, 800 juveniles of Catfish (*Clarias gariepinus*) of 8-10 g average size were stocked and fed two times a day with fish feed developed by the project. After 3 months of co-culture of rice, 250 – 300g fish were harvested, and processed for smoked fish market. The process was carried out in 6 different pilot plots in the Northern and Southern regions of Nigeria.

Lessons learned and recommendations

Water retention in rice field trenches: Leaching of water and rapid evaporation were observed during the dry season. In the absence of a working irrigation structure, additional cost of pumping water for replenishment may not be sustainable for farmers with limited financial resources. The initial adaptation measure employed was to lay plastic bags inside the trenches to hold water for longer period. However, for environmental sustainability consideration, the plastic lining was discouraged, and thus rice-fish farming is encouraged during the wet/rainy season.

Fish seed availability: Limited access to fish seed was reported by the farmers. The solution underway is to train farmers on low-cost fish breeding techniques, thus improving availability within their locality.

Predation from birds: Loss of fish due to predation from birds was recorded. The use of net covering and stocking of fish of bigger size were recommended to prevent actions of the predatory birds.

Fish size at harvest: The fish were harvested at 250-350 g after 3 months of co-culture with rice. Although the size was not competitive for the desired size in Nigeria fresh fish market, value-addition through smoking was introduced to boost the economic value of the fish, noting that smoked fish commands high market value irrespective size.

Expensive commercial feed: The cost of commercial fish feed was challenging to the farmers. The project therefore introduced alternative feeds, using locally sourced feed ingredients (rice bran, fish offal) for feed formulation. Also, there is an ongoing process to develop a training protocol for farmers on using black soldier fly larva as alternative fish feed.

Knowledge sharing among project beneficiaries: Farm diversification processes slightly differ from plot to plot. Therefore, farmer-farmer knowledge exchange and experience sharing are considered an important aspect of knowledge transfer and information dissemination among practitioners.

Conclusion

The trials demonstrated how promoting small-scale, inexpensive integrated agriculture-aquaculture farming not only provides an immediate boost to the local supply of nutritious food (fish) in addition to rice for home consumption but can also generate significant income through sales. These are the reports from the farmers that has adopted the technology.

PREVALENCE AND SEROTYPES DISTRIBUTION OF STREPTOCOCCUS PARAUBERIS ISOLATED FROM OLIVE FLOUNDER, PARALICHTHYS OLIVACEUS IN KOREA

Hyun-Ja Han*, Tae-Ho Kim, Hye-Sung Choi and Sung-Hee Jung

Pathology Division
National Institute of Fisheries and Science
Busan 619-902, Korea
hjhan77@korea.kr

Streptococccus parauberis is etiologic agent of Streptococcosis in olive flounder, *Paralichthys olivaceus*. Formalin-inactivated *S. paraubeirs* vaccine currently marketed in the Korea are authorized for use in olive flounder from 2009.

The present study serotype distribution and multilocus sequence typing (MLST) of *S. parauberis* isolated from olive flounder cultured in Korea. Eighty *S. parauberis* isolates were analyzed by biochemical, serological and molecular analyses.

Of these 80 *S. parauberis* isolates, serotype Ia, Ib, Ic, II, non-typeable were 38, 29, 8, 4 and 2 strains respectively. The sequences of the six chosen loci were determined for MLST. The *S. parauberis* strains were divided to 2 MLST genogroups, which were highly related with those of serotypes. Serotype replacement by non-vaccine serotype (Ia and Ic) has been detected in Korea since *S. parauberis* vaccination began. The serotype Ib of *S. parauberis* strains were dominantly isolated from flounder before 2009, but the prevalence of *S. parauberis* serotype Ia and Ic had been increased from 2010 in Korea.

EFFECTS OF COMPOUNDS AND METABOLITES INVOLVED IN PHOSPHOLIPID AND STEROL METABOLISMEN ON DIGESTION AND TRANSPORT OF LIPID ACROSS THE INTESTINAL MUCOSA OF ATLANTIC SALMON *Salmo salar*

Anne Kristine Hansen*, Trond M. Kortner, Hans J. Grav, Ingemar Björkhem, Vegard Denstadli, Åshild Krogdahl

*Biomar AS, Trondheim, Norway
E-mail: annha@biomar.com

The aquaculture feed industry continuously works to decrease the marine share in the diets, by searching for alternative feed ingredients. The increased use of such ingredients, on one hand exposes the fish for new components, and on the other hand reduces the levels of components typical for diets rich in marine ingredients. These changes may present challenges for the aquaculture industry. The lipid content in salmonid diets have increased steadily over the last decades. For many years, symptoms of impaired lipid digestion, lipid malabsorption syndrome (LMS), have been commonly observed in cultivated Atlantic salmon in Norway. Severe outbreaks are characterized by steatorrhea visible in the environment as floating faeces accumulating on the water surface. In most cases, the symptoms are less pronounced and only visible upon opening of the abdominal cavity, macroscopically as a white and "cauliflower" appearance of the pyloric intestine, and histologically as hypervacuolation of the enterocytes. The symptoms are accompanied by high content of lipid in the intestinal mucosa, indicating disturbance of lipid digestion, absorption and transport across the mucosa and/or delivery to the peripheral circulation. The objectives of the present work were to investigate the effects of compounds involved in phospholipid and sterol metabolism on the development of LMS.

Three independent feeding experiments, duration 11-13 weeks, with Atlantic salmon (*Salmo salar*) (W_0 = 383 ± 68g) were carried out at *ad libitum* feeding. Commercially relevant low fishmeal diets were used in all three studies. The low fishmeal basal diets were supplemented with either fish meal, phosphatidylcholine, choline chloride, cholesterol, taurocholate, taurine, methionine and cysteine.

The effects of these compounds in preventing the development of LMS in fish fed low fishmeal diets will be presented at the conference.

MICROALGAE PRODUCTION FOR BIOMASS AND HIGH-VALUE PRODUCTS

Razif Harun, Farahin Abd Wahab, Natrah Ikhsan, Fatimah Md Yusoff, and Tatsuki Toda

Department of Chemical and Environmental Engineering, Universiti Putra Malaysia
43400 UPM Serdang, Selangor Malaysia
*Presenting author: Razif Harun (E-mail: mh_razif@upm.edu.my)

Algae are a diverse group of autotrophic organisms that have gained a vast amount of interest due to their growth requirements, such as the use of light energy, their rapid growth ability as well as in fixing atmospheric CO2, and the fact that they can produce more biomass per acre than land plants. They represent an intriguing solution to the nexus of energy, food, and waste. They are cultivated for their various benefits, e.g., high protein, lipid, vitamins, and bioactive compounds, making them excellent candidates for various bio-product developments. These photosynthetic microorganisms are also superior in terms of biomass productivity compared to their land-based counterparts, i.e., plants, as they grow much faster, enabling biomass production to be optimized within a smaller land footprint. Such advantages justified the increasing interest in these organisms as potential resources for renewable and sustainable products, which are available worldwide in the form of pharmaceutical and nutraceutical products, cosmetics, animal feed, fertilizer, and biofuel. These versatile organisms are also applied in wastewater treatment and carbon dioxide sequestration. This further highlights the use of microalgae in solving issues related to environmental sustainability. And with that potential, the commercial production of microalgae must also consider the other two pillars of sustainability, which are economical and social considerations. Researchers are attempting many efforts to make microalgal cultivation sustainable, environmentally friendly, and cost-effective.

FORTITUDE OF GLUTATHIONE REDUCTASE IN REGULATING THE REDOX HOMEOSTASIS IN FISH; AN *IN-VITRO* STUDY

Jayamini Harasgama*, Thilina Kasthuriarachchi, Qiang Wan, and Jehee Lee

Department of Aquatic Life Medicine and Fish Vaccine Research Centre,
Jeju National University, Jeju Self-Governing Province, 63243, Republic of Korea
Email: jayaminiharasgama@yahoo.com

The glutathione enzyme system is considered as one of the most exciting enzyme system present in almost all the organism because of its ability to engage in regulating the cellular redox homeostasis of the cells. Being an antioxidant, glutathione (GSH) is capable of prevent the oxidative damage in cellular components which is caused mainly by the reactive oxygen species, peroxides and heavy metals. Conversion of oxidized glutathione (GSSG) into its reduced form GSH is the most vital reaction to keep the cellular GSH levels in the appropriate levels. This reaction is catalyzed by the glutathione reductase enzyme (GSHR); therefore GSHR can be considered as one of the most significant enzymes present in the cells. As aforementioned, this enzyme can be found in almost all the living organisms and considering fish, it has a vital role in fish cells by providing the appropriate concentrations of GSH because fishes have to combat the oxidative damages caused by a number of factors including microorganisms and heavy metals, which are prominently present in aquatic environments.

Considering the important motives of GSHR, the current study was planned to investigate the enzymatic properties of GSHR using the recombinant enzyme, cloned from cDNA extracted from red-lip mullet, *Liza hematocheila*. The coding sequence of the gene was identified by a previously constructed cDNA database and it was cloned into the pMAL-C5X vector. Then the successfully recombined plasmids were purified, sequence confirmed and transformed into *E. coli* BL21 cells. The protein was then overexpressed in Luria Bertani broth (LB) and induced with IPTG. Afterward protein was purified and quantified by the Bradford method and the purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Purified protein was used in further assays. GSHR activity was measured by the Glutathione colorimetric detection kit and optimal enzymatic conditions such as temperature and pH were determined using the same enzyme assay. The disk diffusion assay was conducted to determine the participation of the enzyme in protecting the cells from oxidative damage create by H_2O_2 and heavy metal ions such as Cu^{2+}, Cd^{2+} and Zn^{2+}. Indirect DPPH radical scavenging assay was conducted to analyze how GSHR assist the antioxidant activity of GSH.

According to the results of the activity assay, activity of the enzyme has increased with the increasing enzyme concentration endorsing the proper activity of *Lh*GSHR. Results of the disk diffusion assay confirmed the protective effect of *Lh*GSHR against the oxidative damage occurred by H_2O_2 and all the tested metal ions. Contribution of *Lh*GSHR for the antioxidant activity of GSH could be clearly depicted through the results of the DPPH assay, as the increasing concentration of GSHR has resulted in increasing the antioxidant capacity up to an optimal level. All together these *in-vitro* evidences highly support to conclude that, *Lh*GSHR is an important contributor in regulating the redox homeostasis in the tested fish species and further *in-vivo* studies have to be come upon to clearly understand the mechanisms underline its regulating activity.

IDENTIFICATION AMD MOLECULAR CHARACTERISATION OF INTERLEUKIN-1β-LIKE HOMOLOG FROM BLACK ROCK FISH *Sebastes schlegelii*

Thilina Kasthuriarachchi, Jayamini Harasgama*, Qiang Wan and Jehee Lee

Department of Aquatic Life Medicine and Fish Vaccine Research Centre, Jeju National University
Jeju Self-Governing Province, 63243, Republic of Korea

The cytokine network is an important homeostatic system in an organism's body. It is actively contributing in immune surveillance, development, growth, and repair processes. The interleukin-1b (IL-1b) is considered a key pro-inflammatory cytokine, especially in host –defense in response to inflammation, injury and pathogenic invasion. IL-1β is produced and secreted by various types of cells including monocytes and macrophages. IL-1β is produced as a precursor called as pro-IL-1β in response to receptor binding of pathogen associated molecular patterns (PAMP's). The pro-IL-1β is cleaved by the action of caspase-1. After the maturation mature IL-1β is secreted from cells.

In the current experiment, homologues sequence of IL-1β-like was identified from constructed cDNA library of rock fish. *in-silico* analysis of the respective nucleotide and amino acid sequences was carried out using appropriate bioinformatics tools and software. Tissue specific expression of the IL-1β-like gene was an

Tissue specific expression of IL-1β-like gene was analyzed by quantitative real time PCR (qPCR) using cDNA synthesized from twelve different tissues of fish. Healthy Rock fish discerned into four groups were individually immune challenged with *Streptococcus iniae*, Poly I: C, lipopolysaccharide (LPS) and phosphate buffered saline (PBS) as control. In different time points, selected tissues of the immunized fish were dissected, mRNA was extracted and cDNA was synthesized and used in qPCR to determine the expression of IL-1β-like gene under different immune challenges.

In- silico analysis revealed that the complete ORF of the IL-1β-like of rock fish consisted of 792 base pairs encoding 263 amino acids with a predicted molecular weight of 29.19 kDa. The multiple sequence alignment show that the IL-1 family signature is highly conserved through the evolution from fish to human. Similarly, amino acids which comprised in receptor binding sites are much conserved. 3D modeling of IL-1β-like indicate that IL-1 superfamily domain composed with β sheets and other parts are composed with α helixes.

Tissue specific mRNA expression examination indicate that spleen tissues give the highest expression of IL-1β-like gene. Second highest expression was observed in head kidney tissues. Both of the spleen and head kidney are very important immune tissues in fish immune system. In the immune challenge experiment, IL-1β-like gene has highly up regulated by LPS and poly I:C at the early time points of challenge and gradually reduced with time. The live *Streptococcus iniae* challenge cause in up regulation of the gene at the later hours of the challenge. This might due to increase the bacterial number with time which provide more stimulants to up regulate inflammatory cytokines. Collectively, another homolog of IL-1β gene from black rock fish was identified and characterized. The results indicate that the IL-1β-like homolog also plays an active role in defense mechanism in black rock fish.

POTENTIAL OF BOESENBERGIA PANDURATA AND SOLANUM FEROX AS IMMUNOSTIMULAN WHITE SHRIMP (*Litopenaeus vannamei*)

Esti Handayani Hardi*, Gina Saptiani

Environmental Microbiology, Aquaculture Department Faculty of Fisheries and Marine Science
Mulawarman University, East Borneo Indonesia

Email : estie_hardie@yahoo.com

The objectives of this study were to evaluate the effect of different dietary levels of Boesenbergia pandurate and Solanum ferox on growth performance and feed utilization efficiency of juvenile white shrimp, Litopenaeus vannamei (mean initial weight 2.65±0.1 g). The extracts were collected from Borneo Island, Indonesia. The study used a completely randomized design with 5 treatments of dietary different extract levels (0, 10, 20, 30, and 40 g algae meal/kg feed) in triplicates. The results showed that the different levels of dietary mix extract did not significantly affect (P>0.05) average daily growth but significantly on survival (80-95%) of the shrimp after a 60-day feeding period. However, the test diets significantly affected (P<0.05) feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER). This finding indicates that the supplement of dietary 4% mix extract (40 g/ kg feed) can increase the feed utilization efficiency or reduce the FCR.

Inhibition zone from number of fraction to *A. hidrophila* EA-01 strain is very varied, but generally each number of fractions has a grown inhibition. Any 48 fractions of 103 fractions or 46.6% had more inhibition zone of 10 mm and only 27 fractions or 26.2% inhibition zone has more than 10.75 mm, or inhibition zone by whole cells of *Pseudomonas* sp. against *A. hydrophila*. This indicates that *Pseudomonas* sp. in the fish body is a limiting factor for the *A. hydrophila* infection in nila tilapia. Whole cells of *Pseudomonas* sp. able to inhibit the *A. hydrophila* growth by forming inhibitory zone of 10.75 mm. At the time, culture together both bacteria in BHIB media shown that growth of *A. hydrophila* decreased . highest inhibition zone by number of ECP fractions are number 40 and 73 (17 mm). Heat whole cells and killed whole cell product some strains of *Pseudomonas* sp. can suppress the growth of several strains of bacteria pathogen *A. hydrophila* (Das *et al*., 2006).

Potency to suppress the growth of bacteria *A. hydrophila* is due in ECP protein fractions of *Pseudomonas* sp. containing antibiotics, bacteriocyn, siderophor (Gram and Melchiorsen, 1996), lysozym and other proteases (Sugita *et al.,* 1998). Whole cells of *Pseudomonas* sp. (W3) can produce alkaline protease in extracellular products that can suppress the growth of bacteria that cause disease luminous vibriosis in shrimp. The components used are the supernatant of the bacterial suspension with a dose 0 : 45 and 0 : 22 µL. That capability was due to *Pseudomonas* sp. W3 produce proteolytic enzyme and lysozyme (N-acetylmuramidase) and lytic enzyme. (Rattanachuay *et al*., 2010). The supernatant of *Pseudomonas* sp. (I-2) contains antibacterial ingredients such as proteolytic, lipolytik and amylolitic enzyme that suppresses the *Vibrio harveyi* growth (Vijayan *et al*., 2006).

(Continued on next page)

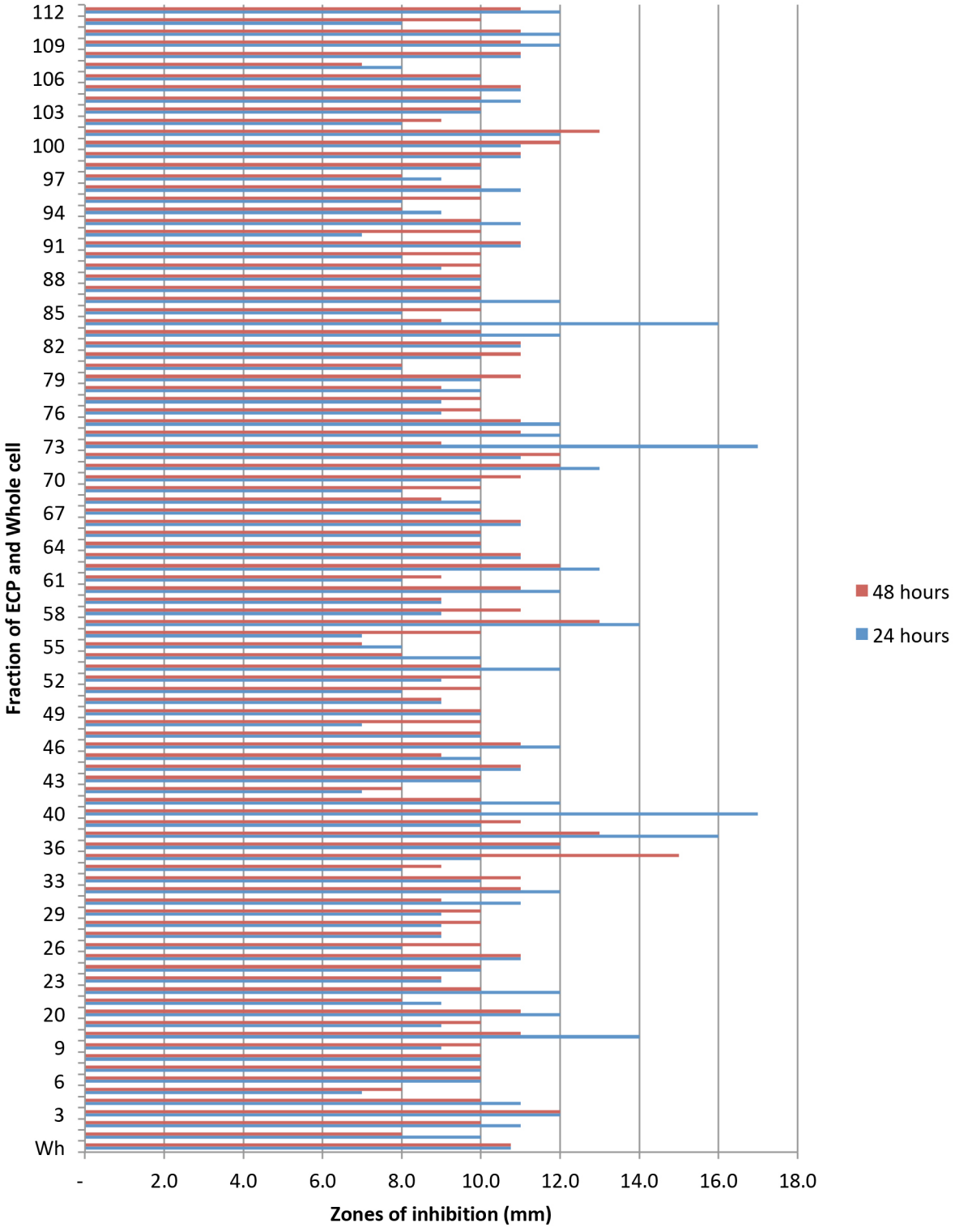


Figure 3. Zones of inhibition of ECP fraction and Whole cell of *Pseudomonas* sp. against *A. hydrophila*.

THE USE OF VISUALISATION MODELS TO ANALYSE AND NAVIGATE THROUGH SEAFOOD DISTRIBUTION NETWORKS IN SINGAPORE

Sri Harminda Hartantyo*, Ahmad Zhafir Ariff, Chau Man Ling

National Centre for Food Science
Singapore Food Agency
52 Jurong Gateway Road, JEM Office Tower, Singapore 608550
Sri_Harminda_Hartantyo@sfa.gov.sg

The unusual Singapore 2015 Group B Streptococcus (GBS) foodborne outbreak has prompted the exploration of newer analytical strategies to deal with emerging contaminants in complex food trade networks. A proof-of-concept study was conducted to map and characterise the local seafood distribution network, with the application of simulation tools for public health risk assessment and outbreak preparedness. A total of 286 companies were approached to gather information on their sources of seafood ingredients, trade volume and methods of maintaining traceability records. The seafood distribution network was mapped using the Gephi software. Point-source contamination scenarios were simulated using the Food Chain Lab application to visualise the scale of impact in the network. The participation rate of this study was about 40% (122/286). The seafood distribution network was found to be largely stable; retailers generally have fairly long-term business relationships with their suppliers. Occasionally, retailers engaged multiple suppliers for a single seafood ingredient as certain suppliers may only provide seafood meant for raw consumption while the others supply seafood meant for cooking. Retailers were found to kept traceability records mainly for accounting and taxation, rather than for food safety reasons. While the data gathered were biased towards companies that sell seafood for raw consumption, our preliminary findings have provided useful insights that can be used to guide the development of more sophisticated models and risk ranking tools to identify and characterise risk points in the network that may impact food safety.

NUTRITIONAL QUALITY OF DIFFERENTLY PROCESSED *Moringa oleifera* SEED BEFORE AND AFTER OIL EXTRACTION FOR INCLUSION IN *Clarias gariepinus* BASED DIETS

Muhammad A. Haruna* Ipinjolu J. Kayode, Sani A. Aminu and Obaroh O. Israel

Dept. of Fisheries and Aquaculture, Federal University Dutse, Jigawa, Nigeria
a4globalfisheries@gmail.com

Introduction

Previous investigations have reported on a range of different fish species that were fed relatively high inclusions of plant proteins, including African catfish (Goda *et al*., 2007). The inclusion of plant protein sources in the ration of fish requires investigation on proper processing for effective utilization (Francis *et al*., 2001). Presence of certain limiting factors in plant ingredients such as high crude fibre content and anti-nutritional factors have been demonstrated (Alegbeleye *et al*., 2005). The inclusion of plant protein sources in the ration of fish requires investigation on proper processing for effective utilization (Francis *et al*., 2001). The objective of this study was to evaluate the effect of different processing methods on the nutrient and anti-nutrient profiles of *Moringa oleifera* seed.

Materials and Methods

The experiment was conducted in Sokoto, Nigeria on latitude 13^0 07' 47.6''N and longitude 05^0 12'11.3''E. Processing and analyses were conducted using standard procedures. For each of the processing methods (200g) of the seeds were used for proximate analysis, amino acid assay and anti-nutrient determinations.

Results

The anti-nutrients detected in the raw Moringa seeds were (oxalate, saponins, alkaloids, phytic acid, tannin, cyanide and phytate) with the following contents; 0.86±0.13, 1.50±0.06, 2.32±0.06, 269.84±1.62, 1.21±0.14, 0.56±0.04 and 69.82±0.86, respectively (Table 1). It was only B90mins/S72hrs that significantly ($p<0.05$) reduced phytic acid to FAO (2009) permissible limit of (≤5mg/kg) amongst all the processing methods and this processing method was finally selected for preparation of the experimental diet.

Discussion and Conclusion

Reduction trend was observed in all the treatments for all the anti-nutrients with increasing processing periods in this study. This is in agreement with the finding of Siddhuraju and Becker (2003), who reported reduction in anti-nutritional factors in mucuna seeds with different processing techniques leading to enhanced growth performance in *Oreochromis niloticus*. The results suggest that boiling for 90mins and soaking for 72hrs was the most effective processing method that significantly ($p<0.05$) reduced the phytic acid content from 269±2.79 in raw to 0.00±1.61 in processed *Moringa oleifera* seeds.

This study has shown that boiling for 90min and soaking for 72hrs effectively reduced the anti-nutrients to safe levels and revealed the high nutrient profile of *Moringa* seed meal.

Table 1: Anti-nutritional factors of processed (B90mins/S72hrs) *Moringa oleifera* seed after oil extraction

	Anti-nutrients						
Treatment	Oxalate %	Saponins %	Alkaloids %	Phytic acid (mg/100g)	Tannins %	Cyanide (mg/100g)	Phytate (mg/100g)
BS90/72h	0.18±0.01	0.20±0.02	0.52±0.05	0.01±0.01	0.06±0.01	0.17±0.01	0.00±0.00
SEM	0.004	0.008	0.020	0.003	0.004	0.005	0.000

Values are presented as means SD of triplicate samples. B90/S72h = Boiling for 90 minutes and soaking for 72 hours; SEM = Standard error of means

Table 2: Proximate composition of raw and treated (BS90/S72hrs) *Moringa oleifera* seed after oil extraction

	Treatments	
Composition	Raw	Processed
Crude protein	37.63±0.17^b	55.05±0.16^a
Fat	18.78±0.11^a	9.39±0.11^b
Ash	4.22±0.10^b	6.01±0.10^a
Crude fiber	4.05±0.05^a	3.93±0.07^b
Moisture	2.65±0.67^a	1.50±0.06^b
NFE	32.68±0.25^a	24.13±0.25^b

Mean with same letter in row are not significantly different ($p>0.05$), NFE = Nitrogen free extract

COMMERCIALISING YOUR POND: DON'T GET CAUGHT BY LEGAL DISRUPTORS!

Katherine Hawes

Principal Solicitor, Aquarius Lawyers
L3/213 Clarence Street
SYDNEY NSW 2000
AUSTRALIA
marketing@aquariuslawyers.com.au

Introduction

Most aquaculture farmers usually work with professional researchers, equipment suppliers, specialists, technology providers, water specialists, feeding companies and other farmers to name a few when they set up their farm.

When is it time throughout that process for farmers to engage with business experts to manage your risks, protect your assets and meet your regulatory and compliance requirements. This presentation will take aquaculture professionals and farmers through the commercialisation process, from startup to a growth trajectory, and outline touchpoints with business and legal professionals.

The start-up **process** for ensuring that future generations are looked after by you minding your business begins with upstream and downstream agreements for suppliers, employees, and sub-contractors. Disruption occurs when terms and conditions of employment, such as, terminations, social media management and pandemics are unarticulated.

As you hit critical mass and your business expands, success means your customer expectations change and there are a range of tools to ensure your customer experience is of a high standard. Fish brokers are always looking for product. We will explain the critical role agreements play in export markets and the delineation of responsibilities to avoid disruption.

As you grow, partnerships or joint ventures may be on the table and these scenarios require agreements that are instrumental in monetising your business. You may want to amalgamate or sell!

Aquaculture is one of the fastest growing food production industries and technology can be both friend and foe. In 2020:

78 tonne Aquatic animal production - 30% Mariculture 37% Aquaculture
$265b Global value of aquatic production
250% Production increase
200% Supply chain growth
>77% Farms without resilient supply chain agreements
100% Risk of legal disruptions to species
47 Countries engage in modern slavery

This presentation will provide an understanding of the legal framework that your aquaculture business requires to operate without costly disruption.

POLICY AND REGULATORY RESPONSES TO DECLINING WATER LEVELS AT GREAT SALT LAKE WITH IMPLICATIONS FOR THE BRINE SHRIMP *Artemia franciscana* RESOURCE

Timothy D. Hawkes

Utah State House of Representatives
Great Salt Lake Brine Shrimp Cooperative
1750 West 2450 South
Ogden, Utah 84401
thawkes@gsla.us

Declining water levels at Great Salt Lake have generated significant media attention as well as speculation as to what continued declines mean for the Lake. The Lake holds particular significance for the aquaculture industry because *Artemia fransciscana* cysts harvested from the Lake make up more than 40% of the *Artemia* cysts used in global aquaculture each year.

In the past couple of years, news about lake declines have driven an unprecedented response from the Utah State Legislature, regulatory agencies, and, increasingly, members of the U.S. Congress. In 2022 alone, the Utah State Legislature invested more than $450 million USD in water conservation, including $50 million directly to protect the Lake and its water supply. In addition, the legislature passed ten bills related to water conservation, with six of those bills aimed directly at Great Salt Lake. The State of Utah is also finding creative ways to manage salinity to keep it within optimal ranges for *Artemia* and other species. These policy responses pave the way for innovative solutions that balance competing needs for water while restoring the natural resilience of the Lake ecosystem.

We present a historic overview of these and other policy responses leading up to and including the 2022 Utah legislative session. These policy changes and accompanying investments will help ensure that the *Artemia* and other resources remain healthy and sustainable for years to come.

EFFECT OF UTILIZATION OF FRESH, FROZEN, AND PRESERVED *Daphnia magna* MASS CULTURED USING FERMENTED ORGANIC WASTES ON GROWTH AND NUTRITIONAL QUALITY OF TILAPIA (*Oreochromis niloticus*) LARVAE

Vivi Endar Herawati, Johannes Hutabarat, Pinandoyo, YS Darmanto, Nurmanita Rismaningsih, Ocky Karna Radjasa

Department of Aquaculture
Faculty of Fisheries and Marine Science
Diponegoro University
Semarang 50275, Indonesia
Viviendar23@gmail.com
+62 821 53505993

Daphnia magna is the best natural feed for tilapia larvae because of the nutritional quality that is suitable with the needs of the fish. The fermentation process was carried out to increase the number of microorganisms, increase metabolism in feed, increase growth, and enrich the nutrition of tilapia larvae feed. The purpose of this study was to find an increase in production and nutritional quality of tilapia fed by fresh, frozen, and preserved *Daphnia magna*. This study was conducted by using completely randomized experimental design with 3 treatments and 3 replicates. Feeding using fresh, frozen, and preserved *D. magna* mass cultured using fermented culture media of chicken manure, quail manure, goat manure, expired bread and tofu waste fermented by probiotic bacteria then cultured for 28 days. Tilapia larvae used in this study had stocking density of 48 individuals/L. Feeding was done in the first week of 1,022 *D. magna* per liter of water and 1,467 of *D. magna* per liter of water in the second week. *D. magna* was given as much as 5 times a day, *ad libitum* and feeding were carried out for 14 days. Observed data included relative growth rates (RGRW and RGRL), feed utilization efficiency (FUE), protein efficiency ratio (PER), and survival rate (SR). The results showed that different types of feed gave an effect ($P<0.05$) on RGRL. The values for RGRW, RGRL, FUE, PER, and SR are 10.05, 11.62%/day, 14.24, 0.23, and 60.18%, respectively. Based on the results of this study, it was concluded that the best feeding treatment for tilapia larvae was fresh *D. magna*.

BLACK SOLDIER FLY PRODUCTION FROM LIGNOCELLULOSIC SIDESTREAMS VIA MICROBIAL BIOCONVERSION

Christian Hermansen*[a], Aaron Thong[a], Ashriel Yong[a], Coleen Toledo[a], Megan Teo[a], Gi Gi Chua[a], Rowanne Siao[a], Jerome Yeo Sam Roy[b], Nathaniel Phua[b], Eric Charles Peterson[a,c]

[a]Agency for Science Technology and Research (A*STAR)
Singapore Institute of Food and Biotechnology Innovation (SIFBI)
31 Biopolis Way, Singapore 138669
[b]Ento Industries, 31 Mandai Estate, #04-03 Innovation Place Tower 4, S729933
[c]Institut National de la Recherche Scientifique – Eau Terre Environnement (INRS-ETE)
490, rue de la Couronne, Québec (Québec) G1K 9A9, Canada
*Christian_Hermansen@sifbi.a-star.edu.sg

Black Soldier Fly (BSF) presents an attractive ingredient for aquafeeds, but production costs are still too high. Abundant and low cost lignocellulosic sidestreams can be fed to BSF, but as cellulose is poorly digestible, feed conversion ratio (FCR) is poor. In this work Single Cell Protein (SCP) has been tested as a BSF feed ingredient with success. The SCP was derived from short-chain fatty acid (i.e. acetate), which has been demonstrated to be produced from cellulose. Specifically, a microbial bioconversion technology capable of converting cellulosic sidestreams into SCP intended for use in animal and aquafeed applications was presented at WAS 2022.

Bioreactor production of a high-density SCP produced from purified acetate was done, and prepared as a slurry for BSF feed additive, representing a novel feedstock for production of this promising aquafeed ingredient. It can be seen in that Figure 1 that while addition of untreated cellulose unfavourably raised the FCR, requiring 63% more feedstock at 30% inclusion, while SCP inclusion noticeably lowered the FCR, needing 27% less feedstock. Thus, this confirms that SCP is an improvement not only over cellulose, but also okara, a typical feedstock used for BSF. Currently efforts are underway to intensify the cellulosic bioconversion technology (Figure 2), and directly produce this SCP from cellulose and generate data for life-cycle analysis and costing. This approach can reduce costs for BSF and increase the application of in aquafeed with much improved sustainability and food security in Singapore and beyond.

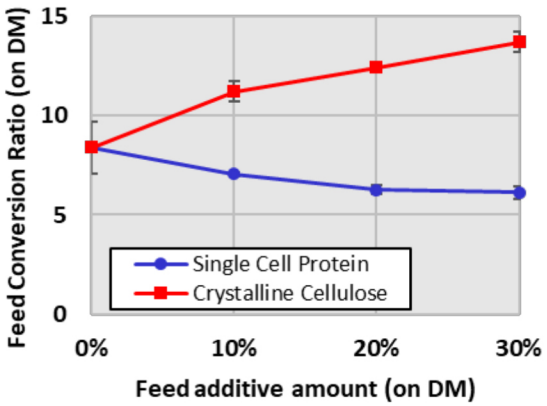


Figure 1: Black Soldier Fly feed trial on okara with feed additive substitution.

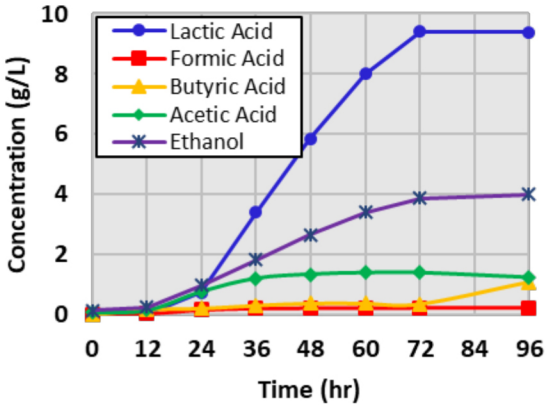


Figure 2: Intensified short-chain fatty acid and ethanol production from cellulose.

ENVIRONMENTAL MONITORING OF OYSTER FARMING IN A TEMPERATE ESTUARY IN THE NORTH-EAST ATLANTIC (SADO, PORTUGAL)

Joshua Heümuller*, Joana P. C. Cruz, Luciane R. Favareto, Pedro M. Oliveira, Ana C. Brito

MARE – Marine and Environmental Sciences Centre
Faculdade de Ciências da Universidade de Lisboa
Campo Grande, 1749-016 Lisboa, Portugal
jaheumuller@fc.ul.pt

Aquaculture production has been gaining an increased worldwide importance over the last decades, equalling worldwide fisheries production and is expected to surpass it rapidly. While intensive aquaculture productions attain high productivity, they require the feeding of the species being produced and often involve the addition of antibiotics and other medicaments. On the other hand, extensive aquacultures rarely involve the addition of pharmaceutics and feeding of the farmed species, as they rely on the food provided by the natural environment. Although most worldwide aquaculture production is focused on fish farming there is an increasing demand for multicultures where bivalves, such as mussels and oysters, are farmed along with fish and other organisms (e.g. sea cucumbers). Bivalves can reduce waste dispersal of fishes as they are filter feeders. Moreover, they also have a high gastronomic value and can reach high prices for reduced quantities, as is the case with oyster, especially if they are farmed in a sustainable fashion. This study was focused on the assessment of the environmental conditions in an extensive oyster aquaculture in the Sado estuary in Portugal, with a production tank of approximately 1.5 ha. The main purpose of this assessment was to determine how the local aquacultures affect the water quality of the Sado estuary and vice-versa. More concretely, this study will determine how the oyster aquaculture affects nutrient availability, dissolved oxygen and the composition of suspended particles, in particular, the dynamics of phytoplankton communities and other suspended particles in the water column.

Weekly water samples were collected, from June 2019 onwards, with an expected duration of one year. Samples were taken from the aquaculture tank as well as from the estuarine adjacent channel, which can exchange water with the production tank through a manual gate. During sample collection several water parameters (e.g. temperature, salinity, dissolved oxygen, pH) were recorded using a multiparameter sonde. In the laboratory, water samples were filtered for pigment analysis through HPLC (High Performance Liquid Chromatography), SPM (Suspended Particulate Matter) allowing to obtain SOM (Suspended Organic Matter) and SIM (Suspended Inorganic Matter). Dissolved nutrient concentrations (Nitrogen, Phosphorus and Silicate) in the water were also measured.

Preliminary results show no significant differences between the water quality observed in the estuary and in the aquaculture, for most parameters. In the production tank, temperature and salinity observations show seasonal variation, ranging from 24ºC and 44ºC, in summer, to 9.6ºC and 22.5ºC, in winter, respectively. Chlorophyll *a* peaked in July and October. Ammonium concentrations reached almost 12.5 µM in August and dissolved Oxygen sat% reached a minimum (69%) both in September and October. The main difference observed between the aquaculture and the estuary is that the water characteristics of the tank are much more constant and have lower variability than those from the estuary. This suggests that regular opening, every two weeks during the spring tide, seems adequate, promoting water renewal that has no apparent negative effects on the estuary as well as on the aquaculture. However, it may be worth monitoring oxygen and ammonium levels to avoid toxicity to oysters.

HIGH-RESOLUTION MONITORING OF *Litopenaeus Vannamei* LARVICULTURE SYSTEMS REVEALS DYNAMIC, TANK-DEPENDENT MICROBIOMES

Jasmine Heyse*, Ruben Props, Celine De Maesschalck, Geert Rombaut, Tom Defoirdt & Nico Boon

Center for Microbial Ecology and Technology (CMET)
Department of Biochemical and Microbial Technology
Ghent University
Belgium
Jasmine.Heyse@UGent.be

The frequent outbreaks of bacterial diseases and the economic losses associated with these outbreaks are one of the main impediments for the sustainable expansion of the aquaculture sector. The establishment of effective microbial management strategies is currently hampered by a substantial lack of knowledge on the microbial community dynamics in these systems. One could expect the rearing water microbiomes to be very dynamic, as there are constant disturbances thought the addition of live and dry feeds, water exchanges, addition of probiotics, etc. Many of these disturbances are associated with an active addition of microbes to the rearing water.

In our study, we have assessed the dynamics of five replicate tanks of *Litopenaeus vannamei* larviculture. The tanks and all sources that contributed to the rearing water microbiome, including dry feed, live feed, algae and exchange-water, were monitored over 18 days (life stages N5 to PL10). The rearing water was sampled at a resolution of 3h for flow cytometry and 1 day for Illumina sequencing. We observed highly-dynamic communities in the rearing water, that differed between the replicate tanks, both in terms of bacterial density and composition. The bacterial densities increased rapidly from approx. $1x10^6$ cells/mL at the start-up to maximum $1x10^8$ cells/mL during the cultivation process. Additionally, the sources that contribute to the rearing water were found to be highly dynamic as well. Our data illustrate the need for conscientious monitoring of both the rearing water microbiome and the sources towards the rearing water.

IMPROVED AQUACULTURE MANAGEMENT USING IOT ENVIRONMENTAL MONITORING SYSTEMS

Alex Hing*, Brock Houston

In-Situ Inc.
1 Gateway Drive
#07-01 Westgate Tower
Singapore 608531
ahing@IN-SITU.COM

Water quality monitoring in an aquaculture farm is imperative for maintaining healthy stock and therefore profitability. Traditional monitoring has involved employees taking handheld instruments into the field to manually collect measurements, a method that is time consuming and labor intensive. Technology advances beyond traditional monitoring systems have increased the availability of IoT devices and instruments that allow for remote monitoring of reliable and accurate water quality, giving farmers better visibility into changing conditions without having to physically be onsite. Integrated solutions can autonomously collect a full profile of water quality data throughout the water column and telemeter that data in near real-time to the cloud, where it can be stored, downloaded, and analyzed. Having this data available at multiple stations within an aquaculture farm can allow the facility to make informed decisions based on real-time environmental conditions, making it easier to respond to emergency situations and thereby reducing mortality. Overall, this can accelerate the effort toward food security and sustainability in global fisheries markets.

EFFECTS OF FEEDING RATE AND TEMPERATURE ON LARVAL QUALITY OF GOLDEN TREVALLY *Gnathanodon speciosus*

Ho Thanh Tuyen, Nguyen Tan Khang, Ngo Van Manh, Le Minh Hoang

Institute of Aquaculutre, Nha Trang University, No 2 Nguyen Dinh Chieu Street, Nha Trang City, Vietnam
*Email: tuyen.ht.60ntts@ntu.edu.vn

The objective of the present study was to evaluate the effects of feeding rate and temperature on the quality of larvae of golden trevally *Gnathanodon speciosus*. The experiment was carried out in three feeding rate (50, 75 and 100%) at three temperatures (30, 32, 34°C). Each treatment was repeated five times. The quality of larvae included growth, survival rate, feeding ability and respiration was determined after two weeks of experiment. The results showed that the growth and survival rate increased gradually but the respiratory rate, oxygen consumption and the ability to catch prey tended to decrease gradually with the feeding rate. In the case of temperature was, on the other hand, increased up to 34°C caused gradually decrease growth, the survival rate and feeding ability but these parameters increased the respiratory rate and oxygen consumption. Besides, the quality of larvae of golden trevally in the combined treatment of 34°C with the 50% feeding rate was lower than that of the other treatments. At the temperature of 30°C combined with 100% feeding rate gave the highest survival rate, growth rate, feeding ability and respiration. Thus, if the water temperature is high and the feed in nature condition will be less, it will affect the quality of the larvae of the golden trevally.

EFFECTS OF DIFFERENT CARBON SOURCES AND CARBON NITROGEN RATIOS ON GROWTH, SURVIVAL AND PROXIMATE COMPOSITON OF *Macrobrachium rosenbergii* POST LARVAE IN BIOFLOC SYSTEM

Md. Eilious Hosain*, S. M. Nurul Amin, Aziz Bin Arshad, Mohd Salleh Kamarudin and Murni Marlina Abd Karim

Department of Aquaculture, Faculty of Agriculture, Universiti Putra, Malaysia, 43400 Serdang, Selangor, Malaysia
mehosain83@gmail.com

Giant prawn *Macrobrachium rosenbergii* is a high demand aquaculture commodity globally and culture in many Asian countries. Biofloc aquaculture system provides food, nutrition and robustness to culture animal where appropriate carbon source and carbon nitrogen (CN) ratio are prerequisite for removal of hazardous nitrogenous compound from culture environments. Five different carbon sources *viz*. wheat bran (WB), rice flour (RF), molasses (MO), maize starch (MS) and palm kernel expeller (PKE) and four different carbon nitrogen ratios of 10 (CN10), 15 (CN15), 20 (CN20), 25 (CN25) and control (without carbon source) were examined under five treatments with triplicates of two separate experiments to get the suitable carbon source and CN ratio for *M. rosenbergii* post larvae. A total of 15 tanks (125 L each) were used for evaluating the water quality conditions, microorganisms and giant prawn PL growth performance and proximate composition of prawn and biofloc.

The highest survival (88.67%) and lowest FCR (2.21) was found using maize starch carbon source (Table1). MS biofloc contained (1.97%) higher ($p< 0.05$) crude lipid which also led higher lipid (4.02%) level in prawn carcass. Higher final weight (116.34 mg) and lower FCR (2.44) was observed in CN ratio 20 based biofloc system. CN ratio 20 biofloc was showed significantly ($p< 0.05$) higher crude protein (29.57%) which also led to higher ($p< 0.05$) protein (65.85%) in prawn carcass. Higher rotifers were observed in CN ratio 20 based biofloc system (Table 2). Thus, we recommend maize starch carbon source and CN ratio 20 for prawn nursery operation in biofloc system which would be applied in commercial venture to enhance productivity.

TABLE 1. Giant prawn post larvae growth performance in different carbon sources based biofloc system

	Carbon sources				
	WB	RF	MO	MS	PKE
AFW (mg)	266.06^{a}	352.40^{a}	324.80^{a}	401.60^{a}	316.01^{a}
WG (mg)	244.13^{a}	330.47^{a}	302.87^{a}	379.67^{a}	294.08^{a}
SGR (%/day)	6.02^{a}	6.71^{a}	6.50^{a}	6.98^{a}	6.45^{a}
FCR	4.77^{b}	3.12^{ab}	3.59^{ab}	2.21^{a}	4.39^{ab}
SR (%)	61.0^{a}	73.0^{ab}	68.34^{a}	88.67^{b}	56.34^{a}

TABLE 2. Giant prawn post larvae growth performance in different CN ratios based biofloc system

	Control	CN ratios			
		CN10	CN15	CN20	CN25
AFW (mg)	112.63^{bc}	83.96^{a}	94.26^{ab}	116.34^{c}	114.23^{bc}
WG (mg)	96.73^{bc}	68.06^{a}	78.36^{ab}	103.13^{c}	98.34^{bc}
SGR (%/day)	9.35^{bc}	8.03^{a}	8.43^{ab}	9.47^{c}	9.34^{bc}
FCR	2.86^{ab}	4.21^{c}	3.80^{bc}	2.44^{a}	2.84^{ab}
SR (%)	80.33^{a}	77.0^{a}	76.66^{a}	87.34^{a}	80.34^{a}

[NB: AFG: Average final weight; WG: weight gain; SGR: specific growth rate; FCR: food conversion ratio; and SR: survival rate. Means in a column with different letters were differed ($p< 0.05$) significantly]

A REVIEW OF THE STATUS AND DEVELOPMENT REQUIREMENTS FOR CAGE AQUACULTURE IN THE SOUTH CASPIAN BASIN (IRANIAN OFFSHORE)

Hosseinjani,A[1*].SayyadBourani,M[2].Ahmadnezhad,M[3].Sadeghinejad,E[4]

1, 2, 3, 4- Inland Water Aquaculture Center *, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Anzali, Iran
Corresponding Author:Adel_nd@yahoo.com

In recent years, cage aquaculture has been introduced as modern technology in the world. Although the use of cages for fish's holding and transporting for short periods can return two centuries ago in Asian, commercial cage aquaculture was pioneered in Norway in the 1970s and 1980s. Today, due to population growth, climate change and more attention to the productivity of water use in the aquaculture, The focus has been on dense systems. The presence of various water sources such as lakes, reservoirs, rivers and coastal, The use of cage technologies can contribute to aquaculture development. According to FAO's 2016 statistic, cage aquaculture accounts for 35% of the world's total aquaculture production. Also according to Iranian fishery organization statistics in 2016, cage aquaculture production was 10162 tons Equal to 2.2 percent of the total aquaculture production in Iran. The cage culture development has challenges in Iran such as Petrochemical pollution, Lack of liquidity, Not introducing the suitable species, Lack of standards, Incorrect site selection, Lack of suitable cage with Caspian Sea structure, The success and development of cage aquaculture depends on several factors. To develop sustainable, industries need to recognize the challenge, the advantages and disadvantages.The marketing, increasing private sector investment and government supervision are challenges that need to be considered in the cage aquaculture development.

EFFECTS OF DIFFERENT SALINITY REGIMES ON GROWTH OF ASIAN SEABASS *Lates calcarifer* LARVAE

Virakbot Hou*, Mat Nget, Sengheang Poeung, Seiha Hok and Michael A. Rimmer

Department of Aquaculture Development, Fisheries Administration
186 Preah Norodom Blvd., Sangkat Tonle Bassac, Khan Chamcar Mon, Phnom Penh, Cambodia.
houvirakbot@gmail.com

Asian seabass *Lates calcarifer* is widely farmed throughout the Asia-Pacific region, including in Cambodia. A significant advantage of this species for aquaculture is its euryhaline nature, making it tolerant to a wide range of salinities from freshwater to full seawater. However, the salinity requirements for larval rearing of *L. calcarifer* are poorly known. This experiment evaluated the effects of different salinity regimes during the hatchery phase for Asian seabass.

In this experiment three different salinity regimes were evaluated using randomized block design. Newly hatched larvae were stocked in 600 L circular plastic tanks and reared using rotifers (3–30 DAH), brine shrimp (8–35 DAH) and compounded feed (7–38 DAH). For the first 10 DAH, the water salinities in all treatments were maintained at 30 ppt. The reference treatment (T1) was maintained at 30 ppt throughout the 38-day experiment (Table 1). The other three treatments (T2 – T4) had regular reductions in salinity as listed in Table 1.

Water in all tanks was changed daily and the tanks were cleaned to maintain water quality, remove debris and uneaten feed. Salinity, temperature, DO and pH were observed twice each day throughout the experimental period and total ammonia and nitrite were checked every three days before changing water. Total lengths of each treatment were analyzed using one-way ANOVA. Duncan's multiple range tests were used to estimate the difference between treatment means.

At the end of the experiment (38 DAH) fish in T2 and T3 were significantly larger than fish in T1 and T4 (Table 2).

The results indicate that reducing salinity in hatchery tanks can improve growth of *L. calcarifer* larvae.

TABLE 1. Salinity regimes used to rear Asian seabass larvae.

Larval age (DAH)	Salinity (ppt) T1	T2	T3	T4
0–10	30	30	30	30
11–17	30	25	20	15
18–24	30	20	15	10
25–31	30	15	10	5
32–38	30	10	5	0

TABLE 2. Total lengths (mean±SD) of Asian seabass at each sampling. Different superscript letters denote significantly different (ANOVA, $P<0.05$) means.

Larval age	T1	T2	T3	T4
10	3.29 ± 0.20^{a}	3.40 ± 0.28^{a}	3.36 ± 0.33^{a}	3.42 ± 0.22^{a}
17	7.07 ± 1.10^{a}	7.75 ± 1.00^{b}	7.10 ± 1.50^{a}	7.15 ± 0.71^{a}
24	15.80 ± 1.74^{b}	15.88 ± 1.73^{b}	15.86 ± 2.16^{b}	13.46 ± 3.20^{a}
31	18.53 ± 3.15^{a}	20.45 ± 2.77^{bc}	21.28 ± 1.91^{c}	19.23 ± 2.53^{ab}
38	26.76 ± 2.98^{a}	28.01 ± 3.60^{b}	29.59 ± 2.90^{b}	25.92 ± 3.46^{a}

NITRIFICATION IN RAS: MORE COMPLEX, MORE PLAYERS AND MULTIPLE PATHWAYS – WHICH ONE(S) DOMINATE?

Timothy A. Hovanec*

DrTim's Aquatics
780 Chambers Lane, Ste 200
Simi Valley, CA, USA 93065
drtim@drtimsaquatics.com

Molecular research, using culture-independent methods, on samples taken from many RAS has demonstrated that there are multiple microorganisms and pathways involved in the oxidation of ammonia to nitrite and nitrite to nitrate, commonly called nitrification. There may be no 'one-size' fits all solution to rapidly starting-up a bio-filter. The microorganism community responsible for nitrification in RAS varies depending upon ambient water quality factors as least in the first several months after start-up. However, obtaining a stable biofilter, defined as one with minimum fluctuations in ammonia and nitrite concentrations may depend more on providing a stable biofilm environment rather than a hard set of water chemistry values. Research has also shown that as a bio-filter ages there may be a succession of microorganisms responsible to the nitrification process. Knowledge of these factors will assist RAS operator start-up, maintain and troubleshoot biofilters in their RAS.

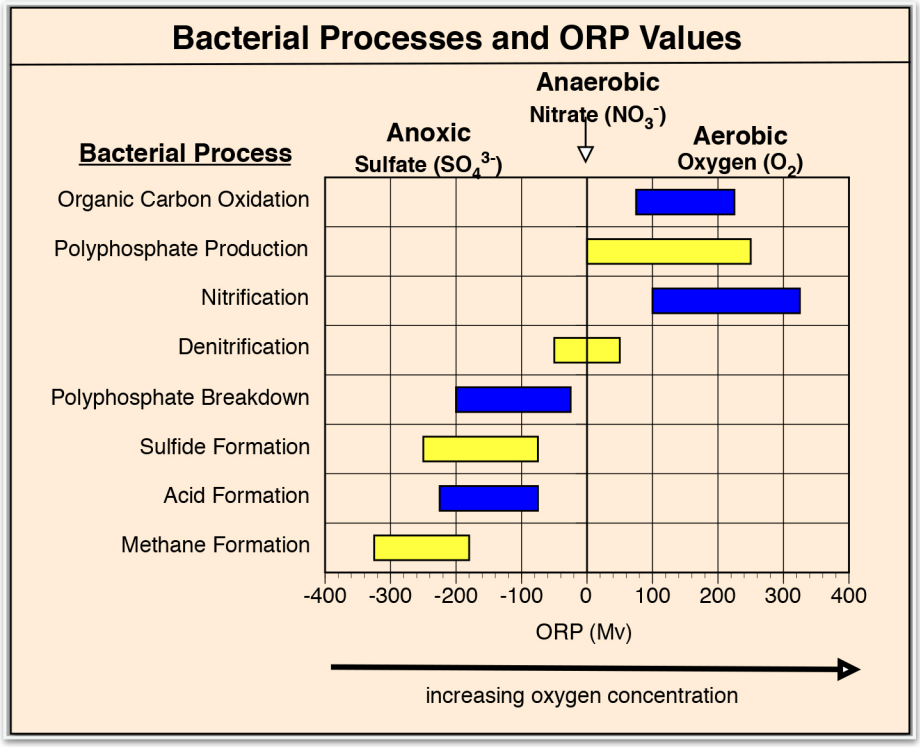
AEROBIC AND ANAEROBIC HETEROTROPHIC MINERALIZATION OF ORGANIC MATERIAL PRODUCED IN HYDROPONIC SYSTEMS – WHAT ARE THE DIFFERENCES?

Timothy A. Hovanec*

DrTim's Aquatics
780 Chambers Lane, Ste 200
Simi Valley, CA, USA 93065
drtim@drtimsaquatics.com

A key marketing feature of hydroponic systems is to be certified 100% organic. To obtain this certification one of the many restrictions is not using chemical fertilizers. Since, by definition, hydroponic systems do not contain any fish or other animals who's waste products could serve as a source of nutrients for the plants grown in the system operators of hydroponic facilities must look at other external organic sources for nutrients. These external sources must then be mineralized using micro-organisms producing nutrients such as nitrogen (in the form on ammonia which is then oxidized to nitrate via nitrification) and phosphate in the form of soluble reactive phosphate (SRP) along with many micro-nutrients.

Successful mineralization depends on heterotrophic bacteria present in sufficient quantities operating either aerobically or anaerobically. Aerobic digestion depends on large amounts of aeration to maintain high oxygen levels and prevent the creation of foul odors and bulking of solids. Anaerobic mineralization is enticing as it can result in more stable water conditions (higher pH, increased alkalinity) but there is a greater chance of system failure due to the production of nitrite or hydrogen sulfide. The bacterial processes occurring in hydroponic systems are subject to many myths and misconceptions including the bacteria species responsible, how fast they may react to changing conditions and what conditions will promote which bacteria. A framework of basic knowledge regarding aquatic microorganisms in hydroponic systems will be presented along with methods for introducing and maintaining beneficial bacteria to the system. A better understanding of the microorganisms will allow operators to more efficiently manage their systems saving time, money and resources.



EFFECTS OF LOW LEVELS OF FISH MEAL ON GROWTH PERFORMANCE AND GUT HEALTH OF LARGEMOUTH BASS *Micropterus salmoides*

Katheline Hua*, Anne Kristine Hansen, Svend Jørgen Steenfeldt, and Dean R. Jerry

*Tropical Futures Institute, James Cook University, Singapore
E-mail: katheline.hua@jcu.edu.au

Largemouth bass (*Micropterus salmoides*) is a high value aquaculture species that has been increasingly cultured in Asia. Commercial feeds of largemouth bass commonly contain high levels of fish meal, which not only contribute to high costs of feeds, but also are environmentally and ecologically unsustainable. Therefore, sustainable largemouth bass culture development warrants a reduction of the inclusion levels of fish meal in feeds while maintaining optimal fish growth performance and nutrient utilization. Our current knowledge on the effects of reduced fish meal levels in largemouth bass feeds is very limited. Therefore, the objectives of the present study were to investigate the effects of low levels of fish meal on the growth performance and gut health of largemouth bass.

An 8-week growth trial was carried out with largemouth bass in a recirculation system. Four experimental diets were formulated to be isonitrogenous and isoenergetic with fish meal and plant ingredients. The experimental diets contained fish meal at 5, 15, 25 and 35% of diets. Triplicate groups of fish with an average body weight of 23 g were randomly allocated to each of the four experimental diets. Fish were hand-fed ad libitum twice per day.

Preliminary results on body weight gain, specific growth rate, thermal-unit growth coefficient, and feed efficiency showed no significant differences among treatment groups. These results suggest that low fish meal feeds can support optimal growth performance of largemouth bass, a carnivorous fish species. Further analyses on whole body composition, nutrient retention efficiency, and histology of liver, proximal intestine and distal intestine are ongoing. The results will be presented and discussed in respect of optimal and sustainable feed formulations for largemouth bass.

RESPONSE OF GROWTH AND SALINITY ADAPTABILITY OF JUVENILE RAINBOW TROUT *Oncorhynchus mykiss* TO DIETARY SUPPLEMENTATION WITH TAURINE

Ming Huang*, Yangen Zhou, Xiaogang Yang, Jian Ge, Qinfeng Gao, and Shuanglin Dong

Key Laboratory of Mariculture (Ocean University of China), Ministry of Education
Fisheries College, Ocean University of China, 5 Yushan Rd., Qingdao 266003, China
huangming@stu.ouc.edu.cn

In order to meet the demand of salmon market, Chinese scientists and entrepreneur are working on salmon mariculture far offshore in the Yellow Sea, China. Rainbow trout was selected as one of the main culture species for its vital economic value. The objective of this study is to evaluate the growth and salinity adaptability of rainbow trout in response to different dietary taurine levels. Four diets (45% protein, 16% lipid) were formulated with the supplementation of taurine at 0%, 0.5%, 1%, and 2% (abbreviated as T_0, $T_{0.5}$, T_1, and T_2) respectively.

Juvenile rainbow trout (N=3; 80.09 g initial weight) were fed the experimental diets for six weeks and subsequently underwent salinity acclimation. The fish were fed to apparent satiation twice (0830h and 1630h) daily and fed consumption was recorded. The fish were cultured in a non-circulating system with supplemental aeration. Water was changed by hand twice daily to allow an approximately 100% water exchange. At the end of the six-week period, all fish were weighted. After that, the freshwater was abrupt changed to brackish water with a salinity of 15 ppt by mixing with natural seawater, and salinity was increased to 30 ppt by a rate of 3 ppt per day. On days 1, 4, 7 and 14 after salinity acclimation as well as at the end of the growth trial, the serum osmolality, serum ion concentration, serum cortisol content, serum free amino composition, gill Na^+, K^+-ATPase (NKA) activity, liver superoxide dismutase (SOD) activity, liver catalase (CAT) activity, and muscle malondialdehyde (MDA) content were determined. At the end of the six-week growth trial, there were no significant differences in survival rate (91.67-100%), final weight (182.35-198.49 g), weight gain (127.68-147.92%) and FCR (1.20-1.68) (Table 1). All physiological data will be further analyzed and reported.

Table 1 Growth performance of rainbow trout fed four experimental diets (initial weight of 80.09 g, 6 weeks, N=3)

Diet	Final mean weight	Final biomass	Weight gain	Survival	FCR	TGC
T_0	188.27	2259.25	134.92	100.00	1.68	0.20
$T_{0.5}$	195.45	2345.38	144.14	100.00	1.20	0.21
T_1	198.49	2381.93	147.92	100.00	1.29	0.21
T_2	182.35	2013.45	127.68	91.67	1.67	0.19
PSE	9.1481	153.8494	11.4407	4.1667	0.1942	0.0136
P-value	0.6159	0.3823	0.6117	0.4411	0.2499	0.6698

Note: FCR: Feed conversion ratio TGC: thermal growth coefficient PSE: Pool standard error

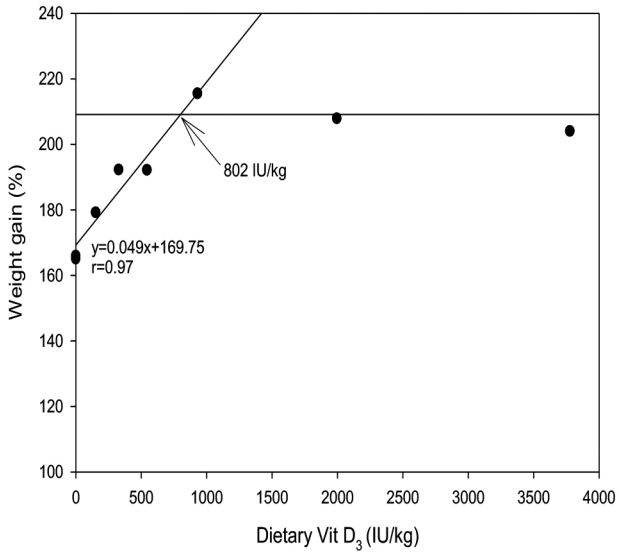
DIETARY VITAMIN D REQUIREMENT OF SOFT-SHELLED TURTLE *Pelodiscus sinensis*

Chen-Huei Huang * and Yun-Jung Wu

National Chiayi University
Department of Aquatic Biosciences
300 University Road, Chiayi 600, Taiwan
Email: chuhuang@mail.ncyu.edu.tw

A feeding trial was conducted to evaluate the effects of dietary cholecalciferol level on the growth and bone calcium of juvenile soft-shelled turtles, *Pelodiscus sinensis*. Soft-shelled turtles, with an average weight of 3.5 g, were fed diets containing 1~3778 IU/kg of vitamin D for nine week.

The growth of turtles increased when dietary vitamin D level increased from 1 up to 900 IU/kg. Beyond this level, the growth leveled off. Hepatic vitamin D and hard tissue Ca showed similar pattern as the growth. In contrast, the strength of carapace decreased when dietary vitamin D increased. Using a broken-line model with weight gain and bone Ca as the indicators, the estimated dietary vitamin D requirement was approximately 800~1000 IU/kg (Fig).



ANTIBACTERIAL EFFECT OF MANGROVE EXTRACT IN WHITE SHRIMP

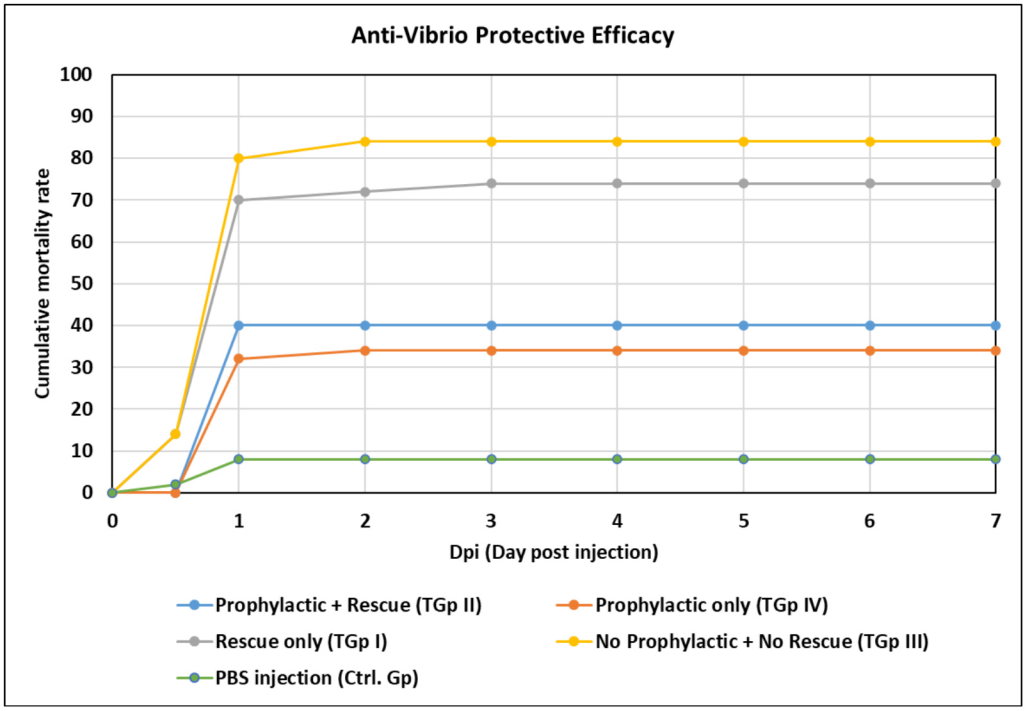
Huang Yun-Tzu*, Lau Gek Khin Germaine, Padmanabhan Saravanan, Chan Pek Sian Diana, and Lee Chee Wee

Aquaculture Innovation Centre, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
HUANG_Yun_Tzu@tp.edu.sg

Disease control is one of the most critical issues in sustainable aquaculture. In the past, antibiotics were used for bacterial disease as an effective treatment. However, as the Blue transformation is the future trend, antimicrobial resistance as an issue has been raised. Therefore, scientists seek a sustainable and eco-friendly method to control aquaculture diseases.

In this study, mangrove plant-based extract was tested for its anti-vibrio efficacy. The product has been tested for the presence of antibiotic residues and heavy metals. Subsequently, the product anti-vibrio activity was assessed in *in vitro* for minimum inhibition concentration (MIC). *In vivo* protective efficacy was studied using LD50 dose of vibrio challenge strain on shrimp. Immersion treatment of the experimental shrimps was performed, and a PBS injection group was included as a negative control.

All the experimental shrimp groups were monitored for up to 7 days post *vibrio* challenge. Prophylactic treatment of the shrimps demonstrated a significant protection against vibrio infection found with up to 50% reduction in mortality rate compared to the no treatment groups.



MICROBIOMES OF TILAPIA AQUACULTURE: MICROBES IN THE SPOTLIGHT

Jonabel Huavas*, Jasmine Heyse, Jérôme Delamare-Deboutteville, Ruben Props, Colin Shelley

WorldFish
Jalan Batu Maung, Batu Maung,
Penang, Malaysia
J.huavas@worldfishcenter.org

With the rapid growth of global tilapia aquaculture, improving tilapia health and production through better farm management is critical. Studies have recognized the significant role that microorganisms can potentially play in improving both. Microbiome exploration has been able to identify and characterize the core and associated species of the microbial communities present in the different organs of tilapia. Aside from the host microbiota, microbial communities in the rearing environment have also been found to influence fish health. This review summarizes the studies that determined the core constituents of the microbiomes in the different organs (skin, gills, mouth and gut) in tilapia. In addition, research that has characterized the microbiomes of tilapia's rearing environment; water, sediment and tank surfaces, are also reviewed. However, despite tilapia microbiome research to date, interactions between tilapia and its associated microbial communities remain poorly understood. A better understanding of how these microorganisms impact tilapia can potentially lead to better managing microbiomes to improve tilapia health and productivity. This review also sheds light on the different factors that influence tilapia microbiomes including their developmental stage, organ tissues and type of culture systems. The effects of on-farm practices such as diet, feeding regimes, use of probiotics, prebiotics, synbiotics, antibiotics, disinfection, and pond fertilization on tilapia microbiome were also investigated and summarized. By putting microbes in the spotlight, this review determined both the current status and knowledge gaps in tilapia microbiome research. This work identified critical, future research needs that can support the development of a deeper understanding of the link between microbiomes, tilapia health and productivity. In the future, harnessing microbiome-based management protocols will lead to enhanced, next generation tilapia best management practices, with major economic benefits.

FROM POND TO GENOME: GENOME ASSEMBLY AND ANALYSIS OF THE BLACK TIGER SHRIMP (*Penaeus monodon*)

Roger Huerlimann*, Yinan Wang, Kenneth Chan, Jafar Jabbari, Kirby Siemering, Gregory E Maes, Mehar S Khatkar, Herman W Raadsma, Nicholas M Wade, Jeff A Cowley, Greg J Coman, Dallas Donovan, Gopala Krishna, Kyall R Zenger, Dean R Jerry

ARC Research Hub for Advanced Prawn Breeding
Centre for Sustainable Tropical Fisheries and Aquaculture
College of Science and Engineering
James Cook University
Townsville, QLD 4814, Australia
roger.huerlimann@jcu.edu.au

Crustaceans, and especially shrimp, are an important product of fisheries and aquaculture; however, genomic resources are still few and far between due to difficulties in sequencing and genome assembly. In contrast to most vertebrate genomes, crustacean genomes are generally large, heterozygous and highly repetitive. Furthermore, while DNA extracted from shrimp can be readily sequenced using short read technology, undetermined contaminants make long-read sequencing difficult. Improvements in genome sequencing and genome assembly were made possible through optimising the DNA extraction procedures, using long-read PacBio sequences for the main assembly in redbean, Illumina short reads for polishing, and scaffolding using 10X Chromium and Hi-C data.

Here we report the assembled genome of the Black tiger shrimp (Penaeus monodon) covering approximately 86.3 % of the genome (1.9 Gbp), with a scaffold N50 of 447 Kbp, longest scaffold of 20 Mbp and a BUSCO completeness of 84.3 %. The genome is dominated by 32.8 % simple repeats, contains 9.5 % other repeat classes, with only 57.7 % of the genome not containing repeats. The genomic resource is rounded out with the integration of a linkage map, extensive transcriptomic data of multiple tissues, and gene annotation. This will provide an invaluable resource for future genome assisted breeding efforts and expanding our knowledge of shrimp biology in general.

RICE BRAN PROTEIN CONCENTRATE AS A SUSTAINABLE FEED INGREDIENT IN PRACTICAL DIETS OF JUVENILE BLACK TIGER SHRIMP *Penaeus monodon*

Fredson H. Huervana* and Rex Ferdinand M. Traifalgar

Institute of Aquaculture
College of Fisheries and Ocean Sciences
University of the Philippines Visayas
Miagao 5023, Iloilo, Philippines
fhhuervana@up.edu.ph

The intensive farming of *Penaeus monodon* in Asia has been sluggishly growing at an annual growth rate of only 1.2% in the past 10 years. Similar to other Asian countries, the production of this shrimp in the Philippines is slow with annual growth rate of 0.2%. Aside from diseases, the major constrain of shrimp aquaculture is feed cost. This is may be due to the need to import feed ingredients for black tiger shrimp aquaculture in developing countries. One locally available feed ingredient in the Philippines is rice bran. It is cheap and available in large quantities. However, rice products are not normally used in shrimp feeds because of its high fiber content, low protein and the presence of antinutritional factors. Several methods have been developed to convert rice bran into an alternative and economic source of plant-based, high quality protein. Among these products is rice bran protein concentrate (RBPC). To date however, there are no published reports on the use of RBPC in *P. monodon* feeds. Thus, the present study was conducted to evaluate the feed value of RBPC as a replacement for soybean meal (SBM) in the diet of juvenile *P. monodon*.

Analyses indicate that the RBPC contains 89.7% dry matter, 34.2% protein, 20.2% lipid, 2.2% ash and 2.1% fiber. This feed ingredient was tested in a feeding trial designed to replace 0%, 12.5%, 25%, 37.5% and 50% of SBM with RBPC in the diets of *P. monodon*. Five isonitrogenous diets were formulated to contain 44% protein. Fifteen juveniles (0.47 ± 0.002 g) were randomly assigned in twenty 60-l rectangular tanks equipped with a recirculating seawater system. Each dietary treatment was run in 4 replicates and the animals were fed ad-libitum for 50 days with the experimental diets.

Results show no significant differences ($P>0.05$) among the treatments in terms of weight gain (%), daily feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and survival. This feed material was found to be highly digestible with apparent dry matter (ADMD) and protein (APD) digestibility coefficients at 83.05 ± 0.02% and 87.20 ± 0.30%, respectively. Also, the essential amino acids were found to be complete with essential amino acid index (EAAI) of 84 and amino acid chemical score index (ACSI) of 25 with tryptophan as the limiting amino acid. There were no significant differences in whole body composition (dry matter, protein, lipid, ash) of shrimp fed with different levels of rice bran protein replacement. Correspondingly, no significant effects on lipid retention were observed but protein retention exhibited increasing trend with significant increase observed up to 25% replacement level. In addition, amino acid of shrimp fed with 0% and 50% RBPC diets were not significantly different. Collectively the data suggest that RBPC could replace up to 50% of dietary soybean meal without affecting the growth performance and biochemical composition of *P. monodon*.

RICE BRAN PROTEIN CONCENTRATE AS A SUSTAINABLE FEED INGREDIENT IN PRACTICAL DIETS OF JUVENILE BLACK TIGER SHRIMP *Penaeus monodon*

Fredson H. Huervana* and Rex Ferdinand M. Traifalgar

Institute of Aquaculture
College of Fisheries and Ocean Sciences
University of the Philippines Visayas
Miagao 5023, Iloilo, Philippines
fhhuervana@up.edu.ph

The intensive farming of *Penaeus monodon* in Asia has been sluggishly growing at an annual growth rate of only 1.2% in the past 10 years. Similar to other Asian countries, the production of this shrimp in the Philippines is slow with annual growth rate of 0.2%. Aside from diseases, the major constrain of shrimp aquaculture is feed cost. This is may be due to the need to import feed ingredients for black tiger shrimp aquaculture in developing countries. One locally available feed ingredient in the Philippines is rice bran. It is cheap and available in large quantities. However, rice products are not normally used in shrimp feeds because of its high fiber content, low protein and the presence of antinutritional factors. Several methods have been developed to convert rice bran into an alternative and economic source of plant-based, high quality protein. Among these products is rice bran protein concentrate (RBPC). To date however, there are no published reports on the use of RBPC in *P. monodon* feeds. Thus, the present study was conducted to evaluate the feed value of RBPC as a replacement for soybean meal (SBM) in the diet of juvenile *P. monodon*.

Analyses indicate that the RBPC contains 89.7% dry matter, 34.2% protein, 20.2% lipid, 2.2% ash and 2.1% fiber. This feed ingredient was tested in a feeding trial designed to replace 0%, 12.5%, 25%, 37.5% and 50% of SBM with RBPC in the diets of *P. monodon*. Five isonitrogenous diets were formulated to contain 44% protein. Fifteen juveniles (0.47 ± 0.002 g) were randomly assigned in twenty 60-l rectangular tanks equipped with a recirculating seawater system. Each dietary treatment was run in 4 replicates and the animals were fed ad-libitum for 50 days with the experimental diets.

Results show no significant differences ($P>0.05$) among the treatments in terms of weight gain (%), daily feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and survival. This feed material was found to be highly digestible with apparent dry matter (ADMD) and protein (APD) digestibility coefficients at 83.05 ± 0.02% and 87.20 ± 0.30%, respectively. Also, the essential amino acids were found to be complete with essential amino acid index (EAAI) of 84 and amino acid chemical score index (ACSI) of 25 with tryptophan as the limiting amino acid. There were no significant differences in whole body composition (dry matter, protein, lipid, ash) of shrimp fed with different levels of rice bran protein replacement. Correspondingly, no significant effects on lipid retention were observed but protein retention exhibited increasing trend with significant increase observed up to 25% replacement level. In addition, amino acid of shrimp fed with 0% and 50% RBPC diets were not significantly different. Collectively the data suggest that RBPC could replace up to 50% of dietary soybean meal without affecting the growth performance and biochemical composition of *P. monodon*.

THE EFFECTS OF WATER TEMPERATURE CHANGES ON OXYGEN CONSUMPTION AND HEMATOLOGICAL FACTORS OF OLIVE FLOUNDER, *Paralichthys olivaceus*

Jun Wook Hur*, Hyun Woo Gil, Han Kyu Lim, and Hyon Sob Han

Faculty of Marine Applied Biosciences, Aquaculture and Aquatic Science major, Kunsan National University, Korea
junwhur@kunsan.ac.kr

Water temperature (WT) is a major environmental factor of metabolic rate in fishes, and directly affects food intake, ammonia excretion, oxygen consumption, growth, and survival. Thus, the purpose of this study was to investigate the change of oxygen consumption (OC) and hematological response of olive flounder, *Paralichthys olivaceus* on WT changes. In Exp. I, WT increased from 20°C to 29°C within 18 hours, and then maintained at 29°C for 96 hours. WT decreased from 29°C to 20°C within 18 hours, and maintained at 20°C for 24 hours. In Exp. II, WT decreased from 20°C to 11°C within 18 hours, and maintained at 11°C for 96 hours. WT increased from 11°C to 20°C within 18 hours, and maintained at 20°C for 24 hours. The Exp. III maintained that the Exp. I and II was consecutively.

In Exp. I, the oxygen consumption increased from 116.7 mg O_2 $kg^{-1}hr^{-1}$ to 317.5 mg O_2 $kg^{-1}hr^{-1}$ with increasing WT. After, OC decreased to 130.3 mg O_2 $kg^{-1}hr^{-1}$ with decreasing WT. In Exp. II, the OC decreased from 96.5 mg O_2 $kg^{-1}hr^{-1}$ to 71.3 mg O_2 $kg^{-1}hr^{-1}$ with decreasing WT, and increased to 96.7 mg O_2 $kg^{-1}hr^{-1}$ when WT was increased to 20°C and maintained during 24 hours. In Exp. III, the OC, when the WT was maintained at 20°C for 48 hours, was found to be 117.1 mg O_2 $kg^{-1}hr^{-1}$. OC was 226.1 mg O_2 $kg^{-1}hr^{-1}$ during 3 days at 29°C, and was decreased to 108.4 mg O_2 $kg^{-1}hr^{-1}$ during 3 days maintenance of WT to 20°C. When WT was decreased to 11°C, and maintained for 3 days, OC was decreased to 54.4 mg O_2 $kg^{-1}hr^{-1}$. After, OC, when WT was kept for 48 hours after increased to 20°C, was increased 110.2 mg O_2 $kg^{-1}hr^{-1}$. In Exp. I, cortisol, glucose and AST (aspartate aminotransferase) values of olive flounder were increased with increasing WT. In Exp. II, Cl^-, osmolality, AST and ALT (alanine aminotransferase) values were significantly changed during experimental period. Glucose value was increased, and cortisol values was decreased with decreasing WT. But cortisol and glucose values were no significant differences between the initial and the final of experiment. In Exp. III, hematocrit, cortisol, glucose and AST were significantly increased, and Cl^-, osmolality and ALT were significantly decreased during experimental period. The other parameters were not significantly changed. Exp. III is likely more stressful environment to olive flounder than Exp. I and Exp. II. The results of our study would be useful for evaluating current culture procedures of olive flounder, and also for developing techniques to minimize stress during aquaculture.

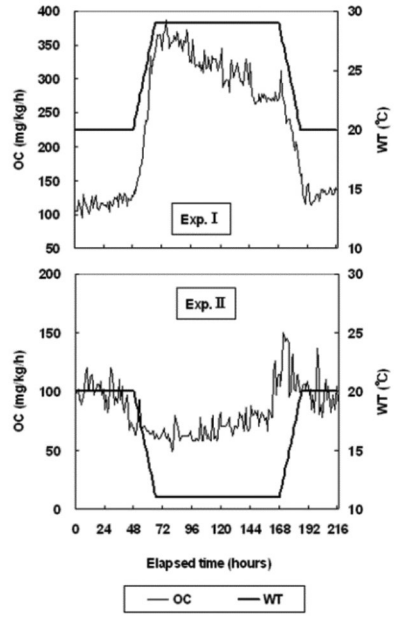


FIGURE 1. Changes of oxygen consumption (OC) in olive flounder, *Paralichthys olivaceus* by sudden changes of water temperature (WT) in Exp.I and II.

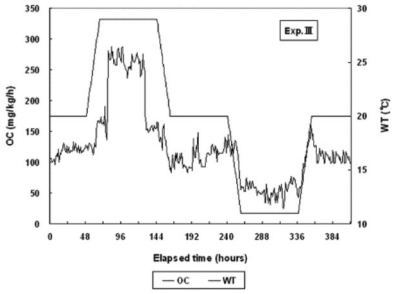


FIGURE 2. Changes of oxygen consumption (OC) in olive flounder, *Paralichthys olivaceus* by sudden changes of water temperature (WT) in Exp.III.

DEVELOPING A SYNBIOTIC CONTAINING *Lactobacillus* sp. AND NATURAL PREBIOTIC EXTRACT FOR IMPROVING THE GROWTH PERFORMANCE, INTESTINAL MICROBIOTA, AND DIGESTIVE ENZYME ACTIVITY OF WHITE SHRIMP, *Litopenaeus vannamei*

Huynh Truong Giang*, Vu Ngoc Ut, Truong Quoc Phu, Tran Trung Giang, Vu Hung Hai, and Pham Thi Tuyet Ngan

College of Aquaculture and Fisheries
Can Tho University
Campus II, 3/2 street, Ninh Kieu district, Can Tho City, Vietnam
Corresponding: htgiang@ctu.edu.vn

The aim of this study was to develop a synbiotic, in which selected natural prebiotic extract could improve the growth and enzyme activities of probiotic, *Lactobacillus* sp. and then incorporated it into feed to promote the growth performance, digestive enzyme activity, and intestinal microbiota of white shrimp, *Litopenaeus vannamei*. Firstly, white shrimp collecting from 12 farms in Soc Trang, Tra Vinh, Bac Lieu, and Ca Mau provinces, Vietnam were used for isolating and screening the *Lactobacillus* spp. strains in the intestines with high probiotic activities including inhibitory activity against *Vibriosis* and production of extracellular enzymes. Secondly, four common natural prebiotic extracts from fruits and roof vegetables, such as *Arcera* banana, *Siamese* banana varieties (*Musa acuminata*), orange-fleshed sweet potato, and white sweet potato varieties (*Ipomoea batatas*) were screened to support the growth of selected *Lactobacillus* sp. The extracts able to support the growth of probiotic were further examined for the inducing bacterial enzyme activities comprising of protease, *leu*-aminopeptidase, β-galactosidase, and α-amylase. The results showed that 23 *Lactobacillus* isolates were collected, of which 10 isolates showed the strong inhibitory activity against pathogenic bacteria *V. parahaemolyticus*. *Lactobacillus* TV32 could be used as a potential strain isolated from white shrimp intestine for further synbiotic development. Of interest, the results from *in vitro* prebiotic utilization indicated that *Lactobacillus* sp. was able to utilize all extracts from banana and sweet potato as the soles carbon sources, in which white sweet potato extract induced the highest activities of *leu*-aminopeptidase and β-galactosidase, and α-amylase by *Lactobacillus* sp. The results revealed that white sweet potato extract was more suitable for combining with *Lactobacillus* sp. as a synbiotic for shrimp. Hence, for the *in vivo* test, four experimental diets including a basal diet supplying with no extract or *Lactobacillus* (control), 0.4% white sweet potato extract (PRE), 10^8 CFU of *Lactobacillus* sp. (kg of feed)^1 (PRO), and 0.4% white sweet potato extract in combination with 10^8 CFU of *Lactobacillus* sp. (kg of feed)^1 (SYN) given to shrimp. At the end of 60-day feeding trial, shrimp fed the SYN significantly induced growth performance, lactic acid bacteria (LAB) and digestive enzyme activities in the intestine of shrimp. It is therefore concluded that dietary synbiotic comprising of *Lactobacillus* sp. and white sweet potato extract had a positive effect on improving growth performance, intestinal microbiota and digestive enzyme activity of *L. vannamei*.

VALIDATION OF A SNP PANEL FOR SPECIES-PURITY CERTIFICATION OF TAMBAQUI (*Colossoma macropomum*)

Patrícia Ianella*, Noéliton Teixeira de Araújo Junior, Eduardo Sousa Varela, Michel E. B. Yamagishi, Alexandre Rodrigues Caetano

Embrapa Recursos Genéticos e Biotecnologia
Brasília-DF, Brazil
patricia.ianella@embrapa.br

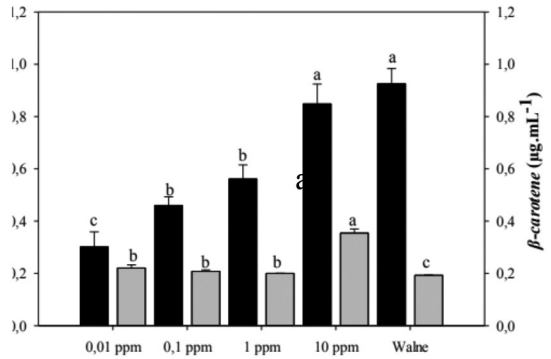
The South American freshwater fish Tambaqui (*Colossoma macropomum*) is the most popular native species raised in Brazil. In the last two decades, producers have been using tambaqui (*Colossoma macropomum*) and two other Serrasalmidae species - Pacu (*Piaractus mesopotamicus*) and Pirapitinga (*Piaractus brachypomus*) - in terminal crosses to obtain hybrids resistant to lower temperatures and diseases, and with higher growth rates, replacing the parental species production in some regions in Brazil. Production of Tambaqui and its hybrids accounts for almost 30% of all finfish cultured domestically. Resulting interspecific hybrids are phenotypically indistinguishable from pure Tambaqui at juvenile stages and are fertile when sexually mature. Inadvertent crosses between pure species and fertile hybrids can negatively impact wild populations and captive purebred broodstocks. A database containing SNPs identified in a previous high-coverage genome-wide sequencing study with complexity reduction for these three species was analyzed to identify 55,295 candidate SNPs with species-specific alleles (Tambaqui≠Pacu=Pirapitinga). SNPType® assays were designed for 144 SNPs and used to test 140 samples used for SNP discovery (Tambaqui=63, Pacu=23, Caranha=48 and six known hybrids). Technical replicates were carried out to evaluate genotyping consistency. The best 96 markers were selected considering call rate and species-specific allelic concordance. Additionally, samples from six different commercial broodstocks (n= 288) of pure Tambaqui, Pirapitinga and known hybrids were tested with the 96-SNP panel, as a blind study. Genotyping results from a subset of 70 markers (after quality control pruning) were consistent with information received subsequently from producers, with the exception of three samples (one Tambaqui and two Pirapitinga), which showed a genotyping pattern consistent with undesired introgressions of up to 6%, corroborating the tool´s effectiveness to identify individuals with small introgressions. Power analysis estimates suggest that 54 independent markers are enough to detect a fourth backcross (3% contamination) with an error rate < 5%. This initiative represents initial step for broodstock certification in Tambaqui production system.

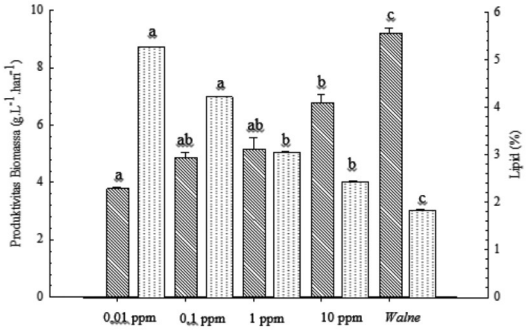
GROWTH, LIPID AND B-CAROTENE CONTENT OF *Dunaliella salina* (Dunal) Teodoresco CULTURED IN ACADIAN MARINE PLANT EXTRACT POWEDER (AMPEP) MEDIA

Wa Iba[1,2*], Andi Septiana[2], Sendry Yosalina[2], Iis Daniar[2]

[1] Aquaculture Department, Faculty of Fisheries and Marine Sciences Halu Oleo University Kendari Indonesia
[2] Biotechnology Department, Faculty of Mathematic and Life Sciences Halu Oleo University Kendari Indonesia
[*]Corresponding email: wa.iba@uho.ac.id

Acadian Marine Plant Extract Powder (AMPEP) is an organic fertilizer derived from extracts of brown algae (*Ascophyllum nodosum*) that is commonly used to increase the productivity of agricultural crops. However, it has the potential to be used as a cheap and sustainable microalgae culture medium for the production of lipid and β-carotene. This study aims to determine the effect of variations in the concentration of AMPEP on the growth, lipid and β-carotene content of *Dunaliella salina,* a well-known microalgae species using in food industries. This study used 4 AMPEP concentration (0.01, 0.1, 1, 10) ppm and Walne in triplicates. The initial density of the culture was 10^5 cells.mL^{-1} cultured for 7 days in plastic bottles (v=600 mL) with a culture volume of 300 mL. Microalgae density was calculated every other days using an improved Neubaur haemocytometer and a hand counter under a microscope with 40x magnification. Microalgae were cultured at light intensity 16.2 moles.m^2.s^{-1} , temperature 28°C, pH 7-8 and salinity 29-30 ppt. Microalgae were harvested using pre-dried, pre-weighed Whatman microfiber filter paper (Θ=25 mm), dried (1 hour, 100°C) and ashed (5 hours, 450°C) then weighed to calculate dry weight and ash-free dry weight. Density data during the culture period were analyzed using Repeated *Measure ANOVA* while data on specific growth rate, *dry weight* (DW), *ash free dry weight* (AFDW), biomass productivity and lipid content used *One-Way ANOVA*. Lipid analysis was performed using modified *Bligh and Dyer* method. The content of β-carotene was analyzed using spectrophotometer UV-VIS at absorbance of 450 and 665 nm. The interaction between day and concentration of AMPEP media affected the growth of *D. salina* (p=0,000). The final density, relative growth rate and the highest biomass productivity were found at the AMPEP media concentration of 10 ppm (p=0,000) with values 418,8×10^4 cells.mL^{-1} , 1.003 cells.day^{-1} and 1.35 g.L.day^{-1}, respectively. The highest lipid content was found at a concentration of 0.01 ppm that was 5.26%. The highest β-carotene content was obtained at a concentration of 10 ppm AMPEP medium of 0.3545 ug.mL^{-1} (p=0,000), whereas in concentrations of 0.01, 0.1, 1 ppm and Walne, the β-carotene content did not differ, ranging from 0.1-0.2 g.mL^{-1} . This study showed that the concentration of AMPEP media of 10 ppm may be used for *Dunaliella salina* culture for the production of β-carotene as a natural antioxidant although the lipid content was considerably low.





EFFECTS OF FEEDING TIME ON SURVIVAL RATES, GROWTH PERFORMANCE AND FEEDING BAHAVIOUR OF JUVENILE CATFISH

Abdullahi Ibrahim

Umaru Ali Shinkafi Polytechnic, Sokoto
Email:aidangevet16@gmail.com

The culture of Clarias gariepinus for fish production is becoming increasingly essential as the fish is contributing to the food abundance and nutritional benefit to family health, income generation and employment opportunities. Effect of feeding frequency was investigated over a period of ten (10) weeks; the experiment was conducted to monitor survival rates, growth performance and feeding behavior of juvenile catfish. The experimental fish were randomly assigned to five treatment groups; (i.e with different feeding frequency intervals) of 100 fish each. Each treatment was replicated twice with 50 fish per replicate. All the groups were fed with floating fish feed (blue crown®). The five treatments (feeding frequency) were T1- once a day feeding of night hours only, T2- twice a day feeding time of morning and night hours, T3- trice a day feeding time of morning, evening and night hours, T-4 four times a day feeding of morning, afternoon, evening, and night hours, T-5 five times a day feeding at four hours interval. There were significant differences ($p > 0.05$) among treatments. Feed intake and weight gain improved significantly ($p<0.05$) in T-4 and T-3. The best of the feeding time on weight gain, survival rate and feed conversion ratio were obtained at three times a day feeding (T-3) compared to other treatments especially those fed once and five times feeding regiment. This might be attributed to the high level of dissolve oxygen and less stress. Feeding fish three times a day is therefore recommended for efficient catfish production to maximize profits as the feed represents more than 50% of aquaculture inputs, particularly in intensive farming systems.

PRODUCTIVITY OF MICRONUTRIENTS FROM INTEGRATED AQUACULTURE-AGRICULTURE SYSTEMS: EVIDENCE FROM BANGLADESH

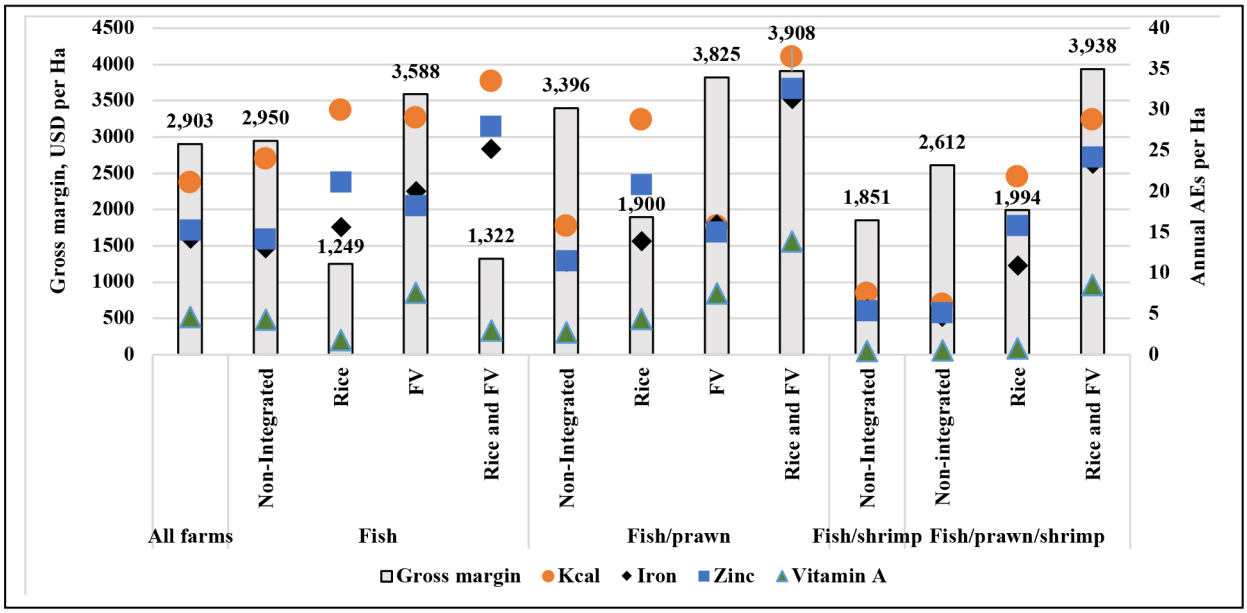
Liz Ignowski* and Ben Belton

WorldFish Center, Phnom Penh, Cambodia
e.ignowski@cgiar.org

Integrated aquaculture-agriculture (IAA) has been widely promoted as a form of farm diversification that can promote more efficient resource use, improve productivity, and lead to higher household incomes and more diverse diets. Farm productivity is usually measured in terms of biomass or income produced per area of land. Here, we extend the concept of productivity to measure production of energy (kcals) and micronutrients, and explore the relationship between the economic and nutritional productivity for 12 distinct types of IAA system, identified from a survey of 721 farms in Southwest Bangladesh.

Nutrient productivity is expressed as the number of adults able to meet their total recommended annual intakes of selected nutrients from the food produced on one hectare of land (AEs/ha). We present productivity of energy (kcal), iron, zinc, and Vitamin A under different IAA systems. Farms integrated with fruits and vegetables, and farms producing fish with freshwater prawn tend to have higher economic productivity than non-integrated farms, and those producing fish only. (Fig.1). Farms integrated with rice have higher energy productivity. Farms integrated with fruit and vegetables produce slightly more vitamin A. OLS regressions confirm that, in general, integrated farms produce more nutrients per hectare than non-integrated farms. Vegetable production is a key driver of both economic and nutrient productivity. These findings have important implications for the design of Nutrition Sensitive Agriculture programs that can enhance the contributions aquaculture makes to nutrition security in Bangladesh and other countries.

Figure 1: Economic and nutrient productivity by IAA system (USD/ha & AE/ha)



ANTIMICROBIAL RESISTANCE IN AQUACULTURE AND ITS MITIGATION STRATEGIES

Ikhsan Natrah*, Sarmila Muthukrishnan, Norfarrah Mohamed Alipiah, Md Yasin Ina-Salwany, Chong Chou Min, Low Chen Fei and Norhariani Mohd Nor

Aquatic Animal Health and Therapeutics Laboratory, Institute Bioscience, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia

*Corresponding author: natrah@upm.edu.my

Intensive farming increases stress on the host and introduces new diseases or pathogens to emerge. This scenario has increased the usage of antibiotics in aquaculture worldwide. Antimicrobial drugs can be very useful in combating pathogenic bacterial infections in humans and animals. However, it has negative implications as it increases the number of anti-microbial resistance (AMR) micro-organisms that can spread from animal to human by direct exposure or consumption of food containing AMR. In aquaculture systems, antibiotics are generally added in the feed or directly into the water system. Once, the antibiotics get into the water; it starts to disperse evenly by the aid of the paddlewheel aerators and impose a selective pressure, which eventually changes the ecosystem of the environment. Consequently, some bacteria increase their fitness in the new environment via intrinsic resistance or acquired resistance. In this paper, the threat and potential risk of AMR particularly in shrimp aquaculture will be discussed. This includes potential mitigation strategies to reduce AMR involving quorum quenching mechanisms and others.

DEVELOPMENT OF THE EARLY MICROBIOME IN HATCHERY PRODUCED SHRIMP AND ITS IMPLICATIONS FOR MANAGEMENT. THE CASE OF TWO AUSTRALIAN FARMED SPECIES *Penaeus monodon* AND *Penaeus merguiensis*

Sandra Infante-Villamil*, Roger Huerlimann, and Dean R. Jerry

Centre for Sustainable Tropical Fisheries and Aquaculture
College of Science and Engineering
James Cook University
Townsville, QLD 4814
sandra.infantevillamil@my.jcu.edu.au

Understanding the role microorganisms play in the health, development and function of aquaculture species is important to allow better management of resources. High throughput sequencing technology (HTS) can be used to expand the knowledge in microbial diversity allowing the aquaculture sector to fully benefit from its characterization with the final aim of improving productivity outcomes.

In shrimp culture the origin of the host associated microbiome is poorly understood and in fact many hatchery practices may be detrimental to the formation of a healthy natural microbiome. This is because such practices aimed at preventing pathogens taking over the system (e.g., use of antibiotics) may also be harming the natural beneficial microbiome.

In this study we have collected and sequenced the microbiomes of hatchery samples from egg, nauplii, and post-larvae in the two most important commercial shrimp species in Australia, *Penaeus monodon* and *Penaeus merguiensis*. We used HTS to characterize the microbiome associated with early developmental stages of these two species and will present results on how the microbiome develops and is associated with various hatchery production stages. This information is valuable to determine whether current antimicrobial management practices require modification to improve productivity.

WHAT THE HEMATOLOGICAL, METABOLIC AND MOLECULAR STRESS RESPONSE COULD TELL US ABOUT CLIMATE CHANGE-INDUCED HYPOSALINE EXTREME WARM WATER: THE CASE OF EUROPEAN SEABASS, *Dicentrarchus labrax*

M. Jakiul Islam[1,2*], Andreas Kunzmann[2], Rajko Thiele[1], Matthew James Slater[1]

[1]Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven, 27570, Germany
[2] Leibniz Center for Tropical Marine Research, Bremen, 28359, Germany
Email: jakiul.islam@awi.de

There is evidence that extreme weather events are becoming more frequent and intense than ever as a result of climate change. To date, studies revealing the modulation of hemato-physiological potential as a compensatory response to extreme warm events combined with different salinities are rather lacking. To elucidate the extreme warm event impacts on European seabass, *Dicentrarchus labrax* acclimated at different salinities, the study aimed to assess the growth performances, hemato-physiological response and molecular response of European seabass.

Fish reared at 32 ppt and 2 ppt showed significantly ($P<0.05$) lower final body weight (FBW), weight gain (WG), specific growth rate (SGR), and protein efficiency ratio (PER) compared to fish maintained at 12 ppt and 6 ppt. Significantly ($p<0.05$) higher mortality rate was observed for fish at 30 ppt and 2 ppt during the 10 days of extreme warm acclimation periods. Similarly, the condition factor (CF) value was also influenced by salinity and temperature stress. Overall, all the growth performance and feed utilization parameters were significantly lowest in fish reared at 30 ppt and 2 ppt, followed by exposure of 10 days warm extreme (33°C). A significant ($P<0.05$) reduction of VSI, ISI, HIS, SSI was observed in fish reared at 2 ppt (Table 1). RBCs, hematocrit, and hemoglobin content were significantly decreased, while WBCs, erythrocytic cellular abnormalities (ECA) and erythrocytic nuclear abnormalities (ENA) were found to increase in 32 ppt and 2 ppt fish.

With the progression of extreme warm stress, plasma glucose tended to decrease in all salinity groups. Plasma lactate was found to increase significantly ($p<0.05$) in 32 ppt fish on day 10. Glutathione peroxidase (GPx), glutathione reductase (GRx), superoxide dismutase (SOD), catalase (CAT) and TNF-α expression increased significantly ($p<0.05$) in fish reared at 32 ppt and 2 ppt fish. Overall results indicate that growth, erythrocytic abnormalities, antioxidant activities and gene expression mediated by the experimental conditions were exacerbated in European seabass during extreme warm exposure.

This study suggests that low salinity acclimation particularly at 12 ppt and 6 ppt salinities, fish can cope better during extreme warm exposure. However, none of the repeated measured parameters in this study indicated the adaptation and compensation capacity through physiological adaptation over the 10 days of extreme warm exposure.

Table 1.Growth response and somatic parameters of European seabass juveniles reared at different salinities for four weeks and followed by 10 days extreme warm temperature (33°C) stress. *

Parameter	Salinities (ppt)				P-value
	32 ppt	12 ppt	6 ppt	2 ppt	
IBW	12.19^{a}	12.42^{a}	12.18^{a}	12.05^{a}	0.569
FBW	20.64±1.12^{b}	29.38±1.32^{a}	26.81±1.43^{a}	21.47±0.47^{b}	<0.05
WG	91.75±3.17^{b}	168.67±6.56^{a}	134.23±5.68^{ab}	79.41±5.96^{c}	<0.05
SGR	2.03±0.08^{b}	3.12±0.15^{a}	2.78±0.17^{a}	1.93±0.08^{b}	<0.05
PER	2.46±0.05^{b}	3.04±0.10^{a}	2.09±0.13^{c}	1.57±0.06^{d}	<0.05
CF	0.47±0.01^{c}	0.68±0.02^{a}	0.62±0.02^{b}	0.48±.007^{c}	<0.05
SUR	86.67±0.96^{b}	94.45±0.56^{a}	92.78±0.56^{a}	85±0.96^{b}	<0.05

*Values are means of triplicate groups ±SEM. Values followed by different letters within the same row are significantly different by Tukey test ($P<0.05$). P values from one-way ANOVA analysis are also provided.

PRODUCTION AND CONSUMERS ACCEPTABILITY OF FISH CRACKERS - AN INNOVATIVE PRODUCT FOR THE SNACKS INDUSTRY OF BANGLADESH

Md Tariqul Islam*, Zannatun Naeem, Sonia Khatun, Shaima Aktar, Md Amanullah, Md Yeamin Hossain

Department of Fisheries, University of Rajshahi, Rajshahi 6205, Bangladesh
*Email: tariqul.fish@ru.ac.bd

Fish crackers are a popular snack item in many Southeast Asian countries. Good quality crackers must have sufficient expansion on puffing for crispness, low moisture content, and low oil absorption. Many previous studies have attempted to improve the quality and acceptability of fish crackers. We have prepared tilapia and tuna fish crackers as ready-to-eat (RTE) products by optimizing processing parameters using a required amount of fish flesh (Tilapia/Tuna) mixed properly with different ingredients, including tapioca starch, sugar, salt, ice, sodium bicarbonate etc. The colour, texture and nutritional quality were also determined by the standard method. Then the shelf-life of ready-to-eat (RTE) crackers was evaluated by biochemical and microbiological analysis (pH, TVBN, FFA, TBARS and APC) under air pack (control), modified atmosphere packaging with 100% N_2 (MAP-1) and 50% CO_2 & 50% N_2 (MAP-2) in a multilayered pouch in a month interval during storage at room temperature. Consumer's acceptability of packaged RTE fish crackers was investigated by survey method utilizing a structured questionnaire in three sales outlets such as Amana big Bazar, Rajshahi; RU souvenir shop, Rajshahi University campus; BARC canteen, Farmgate, Dhaka in June 2022. A 9-point hedonic scale method (9 like extremely and 1 dislike extremely) was used to evaluate the sensory quality during the survey.

In both crackers, around 11% protein was estimated. The lightness (L*) was high (60.67) in tilapia and low (32.85) in tuna crackers, while redness (a*) was high (13.44) in tuna and low (6.50) in tilapia. The hardness (N) was high (5.43N) in tuna crackers and low (4.90 N) in tilapia crackers. Considering the biochemical and microbial parameters, the shelf life was determined at approximately 4^{th} month in both crackers for the air pack sample and 5^{th} month in tilapia crackers and 6^{th} month in tuna crackers for both MAP-1 & MAP-2 samples. In the survey, 24 consumers responded, and all opined on introducing this kind of MAP packaged fish crackers in the market. The sensory score of tilapia and tuna crackers was 7.6 (like very much) and 7 (like moderately), respectively, in terms of colour, flavour, taste, texture and overall appearance.

Considering the above findings, there is a scope to produce MAP packaged fish crackers which can be easily sold for a longer period at room temperature, ultimately making the fish crackers a convenient and nutritious snack item in Bangladesh.

Table 1: Proximate composition (%) of fish crackers

Parameters	Tilapia crackers	Tuna crackers
Moisture (%)	4.39±0.09	3.52±0.95
Ash (%)	11.93±1.89	11.77±0.61
Protein (%)	11.03±0.25	10.93±0.10
Fat (%)	22.00±2.26	22.35±3.89
Carbohydrates (%)	50.66±4.00	51.43±4.14

THE IMPORTANCE OF CHOOSING THE RIGHT ORGANIC ACIDS BLEND FOR AQUACULTURE FEED

Dr Dafna Israel

Research manager and animal nutrition expert at Phibro Aqua

The quest for the best performing feed additive is a major mission in the aqua feed world. There is a variety of useful additives that raise the crucial debate regarding the selection of the best option. Acidifiers are functional feed additives with acidic properties. There are many organic acids available to the animal feed industry, including formic acid, acetic acid, propionic acid, lactic acid, fumaric acids, citric acid, sodium formate, butyric acids, sorbic acids and malic acids and the question is: how to choose the appropriate and most beneficial acid blend. Many factors need to be taken under consideration such as technological and nutritional aspects to achieve a unique blend that will able to be incorporated in aqua feed and offer the best solution.

GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF *Clarias gariepinus* FED SKINNED AND DEGLANDED TOAD MEAL AS REPLACEMENT FOR FISH MEAL

Ufon-Ima Jimmy Jackson*, Lateef Oloyede Tiamiyu and Sola Gabriel Solomon

Department of Fisheries and Aquaculture
University of Agriculture Makurdi, PMB 2373, Makurdi, Nigeria
E-mail: jelvy87@gmail.com

The high cost of Aquaculture production in many parts of the world can be attributed among other factors to the high cost and scarcity of conventional feed ingredients which is competitively used by livestock industries and for human consumption. Fishmeal is one of such nutritionally high but expensive conventional feedstuffs, hence, emphasis of many nutritional studies has been to identify, alternative protein sources that are cheaper, less competitive, yet nutritionally comparable to this conventional feed ingredient. Toad meal is an example of such alternative, unconventional feed ingredients which lack competitive use probably due to the presence of toxin in its parotid gland and skin. However, its protein content and amino acid profile are comparable to many conventional protein sources (including fishmeal). In our earlier study, we successfully removed the sources of the toxin (parotid gland and skin) and established the nutritional characteristics of the processed toad, hence producing a safe Toad meal that could be incorporated in feed without fear of toxin poisoning. This study therefore, investigates the nutrient utilization of the skinned and deglanded toadmeal (SDTM) as a possible replacement for fishmeal in the diet of African catfish *Clarias gariepinus*.

Clarias gariepinus juveniles (9.24 ± 0.70g) were stocked in designated triplicate aquaria stationed at the indoor hatchery facility of the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria. Toads were harvested and processed to remove the skin and the parotid glands before oven drying at 60^{0}C to a constant weight. The SDTM was used to replace fishmeal (at 0%, 25%, 50%, 75% and 100%) which was originally incorporated in the control diet at 30%. The fish were fed these experimental diets at 5% of the fish body weight for eight (8) weeks.

The results obtained suggest that SDTM can completely replace fishmeal without any adverse effect on growth or survival. Similarly, the carcass analysis also showed that 100% replacement of fishmeal with SDTM led to highest crude protein in the fed fish. Also, cost of production of feed and rearing the fish to market size (1kg) was substantially reduced as SDTM was substituted for fishmeal. It was therefore concluded, that SDTM can be incorporated in fish feed to completely replace conventional but expensive fishmeal.

Growth Performance of *Clarias gariepinus* fingerlings fed experimental diets of skinned and deglanded toad meal as replacement for fishmeal.

Parameters	Diet 1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet 4 (75%)	Diet 5 (100%)	P-Value
MIW (g)	9.24 ± 0.04^{a}	9.21 ± 0.01^{a}	9.23 ± 0.03^{a}	9.22 ± 0.02^{a}	9.28 ± 0.02^{a}	0.123
MFW (g)	29.16 ± 0.81^{a}	32.90 ± 0.22^{b}	37.11 ± 0.36^{c}	42.86 ± 0.95^{d}	50.59 ± 0.61^{e}	0.010
MWG (g)	19.92 ± 0.84^{a}	23.69 ± 0.22^{b}	27.88 ± 0.39^{c}	33.64 ± 0.98^{d}	41.31 ± 0.45^{e}	0.009
%MWG	215.56 ± 9.93^{a}	257.30 ± 2.15^{b}	302.00 ± 5.30^{c}	364.80 ± 11.46^{d}	445.10 ± 8.32^{e}	0.008
SGR (%day^{-1})	2.05 ± 0.06^{a}	2.27 ± 0.01^{b}	2.48 ± 0.02^{c}	2.74 ± 0.04^{d}	3.03 ± 0.03^{e}	0.005
FCR	2.03 ± 0.06^{d}	1.90 ± 0.03^{c}	1.84 ± 0.02^{c}	1.65 ± 0.02^{b}	1.49 ± 0.06^{a}	0.015
PER	1.41 ± 0.04^{a}	1.52 ± 0.02^{b}	1.55 ± 0.01^{b}	1.74 ± 0.02^{c}	1.92 ± 0.03^{d}	0.013
ANPU	26.65 ± 0.75^{a}	27.11 ± 0.04^{a}	28.09 ± 0.51^{ab}	28.75 ± 0.06^{b}	29.30 ± 0.07^{b}	0.035
%Survival	95.50 ± 2.50^{a}	92.50 ± 2.50^{a}	95.00 ± 0.00^{a}	95.00 ± 2.50^{a}	95.50 ± 2.50^{a}	0.170

Means on the same row with different superscripts are significantly different ($p<0.05$)

16-YEAR PATTERN OF SHRIMP LANDINGS AND ITS RELATIONSHIP TO METEOROLOGICAL PARAMETERS IN KOTA KINABALU, SABAH

Madihah Jafar-Sidik*, Nura Nabila Johari, Muhammad Ikhlas Zabidi, Siti Nor Fatihah Zakaria

Borneo Marine Research Institute
Universiti Malaysia Sabah
88400 Kota Kinabalu Sabah
Email: madihah@ums.edu.my

The temporal variation of shrimp landings over 16-year (2000-2015) and the long-term trend of meteorological parameters in Kota Kinabalu were examined. The correlation between the abundance and diversity of shrimp landing with meteorological parameters (temperature, rainfall, relative humidity, wind speed), as well as the Southern Oscillation Index (SOI), were also interpreted in the study. Results indicate that the highest shrimp landings in Kota Kinabalu were in 2000 with 735.9 tonnes, while the lowest was in 2012 with 195.4 tonnes. The dominant shrimp species during the study period was sand shrimp (*Metapenaeopsis barbata*). There were minor changes in the trend of meteorological parameters with a gradient rate of +0.03 for temperature, -0.02 for rainfall, and -0.01 for relative humidity and wind speed per year in Kota Kinabalu. Results indicated that shrimp landings were positively significant with precipitation and relative humidity with correlation coefficients of 0.308 and 0.219. Results also showed shrimp landings were negatively important with temperature, $r = -0.161$, $P < 0.026$. However, there was no correlation between shrimp landings with wind speed and SOI with a correlation coefficient of 0.057, $P > 0.583$ and -0.123 $P > 0.090$. The consequence of fluctuations of meteorological parameters may affect the marine species, affecting the fisheries economics in Kota Kinabalu.

EFFECTS OF MUTAGENESIS CHEMICALS ON THE PHYSIOLOGY OF ZEBRAFISH *Danio rerio*

*Juhyeon JANG[c], Ki-hyuk KIM[a], Hye-na MOON[a], Jin NAMGUNG[b],
and In-kyu YEO*

a. Department of Major of Aquatic Life Medicine, Jeju National University, Jeju 63243, Republic of Korea
b. Education & Research Group for Future Strategy of Aquatic Life Industry, Jeju National University
Jeju 63243, Republic of Korea
c. Department of Major of Aquatic Life Science, Jeju National University, Jeju 63243, Republic of Korea
Email : jjhbill@naver.com

The current aquarium fish industry is a complex industry that links seedling farming to breeding-related supplies and breeding management and exhibition services, and the development of valuable and rare aquarium fish can generate huge profits. The mutagenesis mechanism and toxicity evaluation of the mutagenesis chemicals (ENU (N-ethyl-N-nitrosourea), Colchicine and Hydroxylamine) used in this study have been studied a lot, but studies on how mutagenesis chemicals affect zebrafish (*Danio rerio)* in physiological terms are insufficient. In this study, we analyzed the physiological changes of zebrafish exposed to mutagenesis chemicals.

The subject of the experiment was the second progeny (F2) of zebrafish treated with a mutagenesis chemical, with pH 6 to 7, photoperiod 10/14 h, about evaporation of the water in the system is exchanged with fresh water, and tetrabits were fed to the zebrafish twice a day. ENU, Colchicine, and Hydroxylamine were used as the experimental materials. In the experimental method, wild type of zebrafish was immersed in the experimental material at a certain concentration and time, and F1 was secured by crosslinking between objects immersed in the same material for 10 weeks, and F2 was secured.

After 10 weeks, the difference in expression genes was compared through the ΔΔCt method via Relative Quantification PCR for growth (GHra), immunity (Lysozyme) and nerves (NR4A2b) suitable for each tissue through sampling. Table 1.

In this study, the physiological change of Zebrafish and its progeny F1 treated with mutagenesis chemicals was compared and analyzed through the difference in genes expressing physiological changes in F2, the descendant of F1. In addition to the above genes, research and additional experiments on other genes involved in growth, immunity, and body color are needed.

Table 1. Gene expression of F2 group in mutagenesis-treated group

	ENU		Colchicine		Hydroxylamine	
Gene	male	female	male	female	male	female
GHra	88.2	6.72	3.51	0.8	0.8	0.14
NR4A2b	1.7	2.38*	1.26	0.34	0.57	0.83
Lysozyme	0.79	0.69	0.85	8.76	1.22	19.7

-This table is $2^{-\Delta\Delta Ct}$ value of a calculated as a relative quantity using House keeping gene(GAPDH) as a reference gene
-* means that the p-value is less than 0.05 in tukey HSD post hoc tests after one-way ANOVA within 95% of the confidence interval

PILOT SCALE COMMERCIAL PRODUCTION OF *Oreochromis niloticus* IN IN-POND RACEWAYS SYSTEM IN PAKISTAN

R.S.N. Janjua[1], Shafaq Fatima*[2]

[1]SoyPak, Pvt. Ltd.
[2]Department of Zoology, Lahore College For Women University, Lahore, Pakistan
Email: janjua@soypak.org

A pilot scale commercial-scale, in-pond raceway system was constructed in 2019 on a commercial fish farm in Punjab, Pakistan. The in-pond raceway system was installed in a 8 acres earthen pond with an average depth of 2.25 m. It consists of three raceways, each with dimensions of 22 m (L) × 5 m (W)× 2.25 m (D). With an operating depth of 2 m, the volume of water enclosed is 220 m^3. The total culture volume of the raceways for holding and growing fish is 660 m^3, equivalent to 2.33 percent of the total surface area of the production pond. Raceways are equipped with five regenerative blowers of 1.6 kW horsepower each. Additionally, the pond has three whitewater units, a jet aerator and a paddlewheel aerator installed in the open water area, which helps in mixing, accelerating and circulating water around the pond. One raceway was originally stocked with 13,000 tilapia (*Oreochromis niloticus*) fingerlings weighing between 30 – 32 g. During the 2019 production season, mean survival was 99.70 %. A total of 9,841 kg (1,405/ acre) of tilapia were harvested from raceway. Growth rate and specific growth rate were 3.50 g/fish/day and 0.65 % body weight/ day, respectively. The average feed conversion ratio (FCR) for labeo was 1.03. An additional 3368 kg of catla and 4401 kg of silver carp were harvested from the pond as co-cultured species. The results indicated a high potential for efficient production of labeo with other co-cultured species in in-pond raceways system in Pakistan.

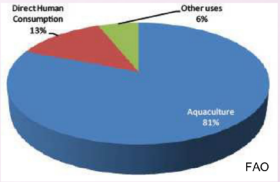
EXTRUSION PROCESSING OF FISH FEED PELLETS: INFLUENCE OF SILICA AND OIL CONTENT ON THE PRODUCT PROPERTIES

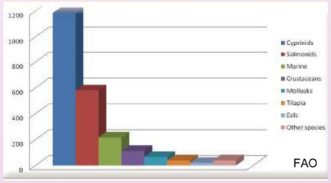
M. Gräfenhahn*, J. Wiertz, C. Schillinger, C. Bader, V. Stohl, I. Huismann

Hochschule Fulda University of Applied Sciences

EVONIK Leading Beyond Chemistry

Motivation:





- Growing aquaculture market and demand for high fat fish feed especially for species like salmon and trout
- Extension of extrusion know-how and new business
- New application for Evonik silica in the feed industry

Challenges:

- High fat content → Decrease in pellet stability
- Mild process conditions needed to avoid oxidation of essential fatty acids → Influences overall pellet properties and quality[1] + additional coating step, i.e., vacuum coating, is needed
- Post-processing steps: Increase in overall production costs

Hypothesis:

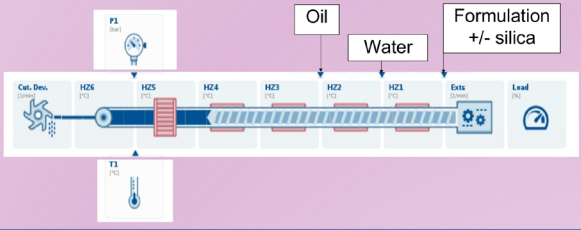
- Silica absorbs fat + stabilizes the starch structure due to network formation → Addition of silica influences extrusion processing conditions and therefore the resulting properties of pellets

Aim:

- Determination of influence of silica grade/concentration on the processing conditions, pellet abrasion, and fat leakage for pellets with high fat contents

Approach:

- All in one process → Extrusion process with addition of high amount of oil + addition of silica to counteract the lubricating effect of oil. **No coating-step after extrusion**

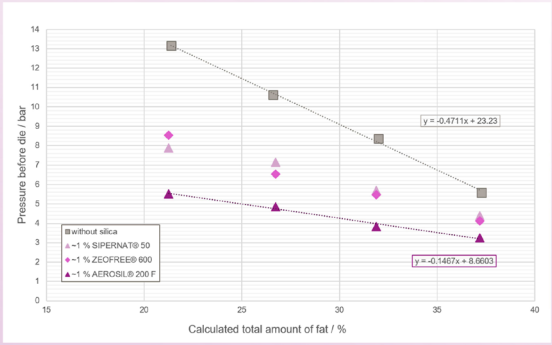


Results – Nutritional values (~32 % fat pellets):

Sample	Calculated nutritional values [g/100g] (dry matter)				
	Protein / %	Fat / %	Carbohydrate / %	Fiber / %	Ash / %
commercially-available reference ~ 30% fat	42.5	30.5	14.3	1.45	5.5
w/o silica	39.1	32.0	12.7	1.7	6.2
SIPERNAT® 22	38.8	31.9	12.6	1.7	6.9
SIPERNAT® 50					
ZEOFREE®600					
AEROSIL®200F					

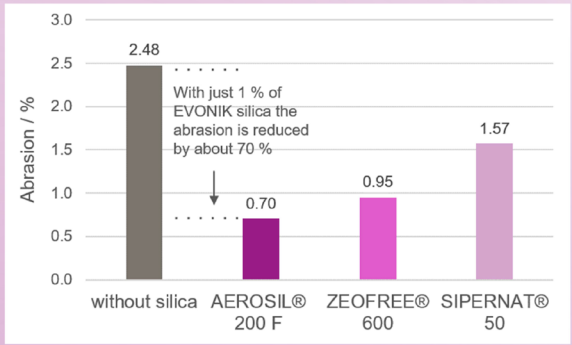
- *Development of formulation with 1 % silica to obtain comparable nutritional values with commercially-available vacuum-coated reference pellets successful!*

Results - Extrusion processing:



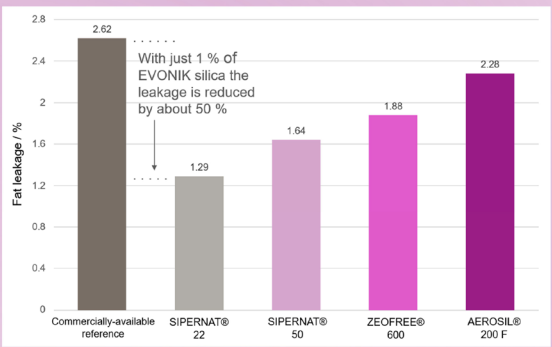
- *ddition of Evonik silica reduces the lubricating effect induced by addition of oil → Changed viscosity and resulting thermo-mechanical energy input → Enhanced product quality!*

Product quality – Abrasion (~32 % fat pellets)



- *Depending on the silica type an addition of only 1 % leads to approx. 40 – 70 % less abrasion*

Product quality – Fat leakage (~32 % fat pellets)



- *Depending on the silica type an addition of only 1 % leads to approx. 10 – 50 % less fat leakage compared to coated pellet*

Conclusion:

One step approach → More stable pellets → Less abrasion → Less downtime → More efficient feeding

- Extrusion process + addition of silica was used to produce quality pellets with high fat contents without coating step!

* Contact: maria.graefenhahn@brabender.com

[1]Huber, G.R. (2000). Twin-Screw Extruders. In Extruders in food applications, M.N. Riaz, ed. (Lancaster, Pa.: Technomic Pub. Co.), pp. 108-112

Spirulina maxima DERIVED MODIFIED PECTIN NANOPARTICLES PROMOTES THE EXPRESSION OF INTESTINE ALKALINE PHOSPHATASE FOR MAINTAINING GUT HOMEOSTATIS IN MOUSE MODEL

E. H. T. Thulshan Jayathilaka*, H.P.S.U. Chandrarathna, T. D. Liyanage, S. L. Edirisinghe, Chulhong Oh, Do-Hyung Kang and Mahanama De Zoysa

College of Veterinary Medicine, Chungnam National University, Daejeon, 34134
Republic of Korea
Email: thimira.thulshan.jayathilaka@gmail.com

Intestine alkaline phosphatase (iALP), which is secreted by enterocytes plays an important role in maintaining gut homeostasis and protection. Furthermore, iALP was reported to act as an anti-inflammatory factor by inhibiting both Toll like receptor 4 (Tlr4) and Myeloid differentiation primary response gene 88 (Myd88) dependent inflammatory cascades. Spirulina pectin is a heteropolysaccharide derived from marine *Spirulina maxima*. Since recent studies have proved nanoparticles are more efficient in delivery of therapeutic agents, we produced nano-particle form of modified Spirulina pectin and investigated its effect in iALP modulation in mouse model.

Modified spirulina pectin nanoparticles (MSmPNPs) were prepared by modification of Spirulina pectin (SmP) by high temperature and pressure exposures followed by a sonication. In this study, to evaluate MSmPNPs activity on intestine alkaline phosphatase, C57BL/6 mice were used. Mice were administrated MSmPNPs (a dose of 1.62 ± 0.01g/kg per day) orally with drinking water for four weeks. Control group was maintained with parallel to test group and after four weeks, mice duodenums were isolated. Part of the duodenum was used for western-blot analysis to determine the relative protein expression level of iALP and it was quantified. Furthermore, qRT-PCR was performed for Alkaline phosphatase 3 (Akp3) and alkaline phosphatase intestinal (Alpi) for iALP related gene expression. Tlr4 and Myd88 gene expressions were tested to confirm the anti-inflammatory action by induced iALP.

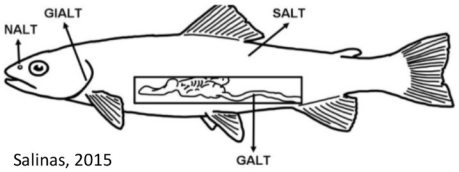
Western blot data showed significant relative iALP protein expression (1.92-fold) in MSmPNPs treated mice compare to control group. Gene expression of Akp3 had significant ($p<0.05$) 5.21-fold upregulation and Alpi expression had 1.25-fold upregulation. Meanwhile Tlr4 and Myd88 gene expressions had significant ($p<0.05$) 0.11-fold and 0.43-fold downregulation, respectively. Overall results suggest that MSmPNPs could induce the intestine alkaline phosphatase expression both at transcriptional and translational levels, while repressing the expression of inflammatory Tlr4 and Myd88 cascade and promote anti-inflammatory activity in the mouse gut.

EARLY CARING OF FISH DRIVES GROWTH PERFORMANCE

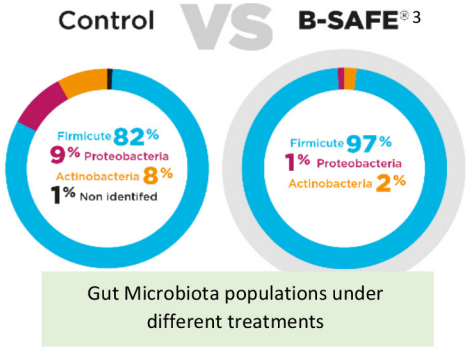
François Jégou*[1]

francois.jegou@adm.com

Juvenile fish are subject to numerous events in aquaculture operations: transportations, transfers, gradings, diverse handlings, various infectious pressures, water quality variations, first complete feed ingestion, mixing of populations, new environments and rearing structures, vaccinations. All these events represent important stressful moments that may severely impact their health, immediately and on the long term, subsequently hampering their physiology and future growth performance.



At the same time, situated between hatchery and grow-out stages, nursery phase represents a crucial intermediary phase with fundamental physiological evolutions including morphological, immunological, and digestive features and capacities. Such changes have been described on Tilapia by Fujimara *et al*. in 2007.



Gut Microbiota populations under different treatments

Joining both external challenges and ontogenetic evolution is paramount in building a program to optimize the organism readiness to present and future challenges, thus performance. ADM[1] has focused on this subject and developed *Life Defense*, a state of the art approach in order to better accompany fishes in these phases. This comprises both specific nutritional requirements at the considered physiological stage, as well as the help brought by selected feed ingredients that facilitate and promote particular physiological functions.

As first line defense, mucus is of capital importance in aquatic species. Salinas in 2015 reviewed its immunological roles at the skin, gut, and gills. More than just a physical barrier, mucus displays dynamic defensive interaction with its environment, including innate immunity such as antimicrobial peptides secretion and entrapment of microbes, as well as acquired immunity on specific targets.

Selection of effective immune modulators that allow for fast and reactive response and reducing long-term inflammation will decrease its vicious circle and favor fish recovery and resume to performance. Moreover, Peisker *et al*. in 2017 demonstrated the structural characteristics advantages of *Pichia guilliermondii*[2] yeast on bacterial aggregation properties compared to other yeasts, while further studies showed three times faster Vibrio clearance from infected innate immune shrimp hemolymph.

Beyond its primary function in digestion, the intestine plays a fundamental role in immunity, both activities being closely related via its microbiota. Strategies involving weak and strong organic acids, or fostering firmicutes populations while reducing proteobacteria, allow to stabilize the gut microbiota and optimize its activities to promote performance and health.

Numerous plant extracts have proven biological activities, among which certain may be of particular interest when it comes to digestive epithelium protection, anti-oxidative activity, or decreased parasitic prevalence associated with their inclusion in feed (Fridman *et al*., 2014).

In conclusion, special attention and caring during this critical life stage that is nursery, on nutritional needs but also on challenges met during this period, provide return on the investment. Conversely, skipping or neglecting it often leads to laborious and continuous farm management hazards including disease outbreaks, compromising farm economics and sustainability. As mentioned by Valente *et al*. in 2013, successful nursery is a key target for a competitive expansion of the aquaculture industry.

[1] Archer Daniels Midland (ADM) ; [2]whole-cell inactivated yeast *Pichia guilliermondii*, AquaTrax, PANCOSMA ;

[3] B-Safe is a patented synergistic feed solution aiming at securing digestive process

The uses and claims should be adapted to comply to the current local/ regional regulatory environment. This information does not imply any express recommendations for the cure, mitigation, treatment, or prevention of diseases.

All bibliographical references available on demand.

TARGETED HYBRID CAPTURE AND RESEQUENCING USING MOLECULAR INVERSION PROBES (MIPs) FROM *Haliotis discus*

Tae Hyug Jeong[1,*], Sukkyoung Lee[1], Hyerim Yang[2], Chaehyeon Lim[2], D.S. Liyanage[2], W.K.M. Omeka[2], and Jehee Lee[1,2]

1 Marine Science Institute, Jeju National University, Republic of Korea, Jeju 63333
2 Department of Marin Life Sciences, Jeju National University, Republic of Korea, Jeju 63243
*hmntsj@hanmail.net

Abalones belong to the genus of *Haliotis* and are economically important marine invertebrates for fisheries and aquaculture industries worldwide. The disk and pacific abalone, *Haliotis discus*, is an important species in Korean abalone culture. However, the global climate changes steadily warming the water temperature in coastal areas of Korea in summer, causing higher mortality in abalone farms. In addition, to prevent the potential inferiority in hatchery stock due to prolonged farming activities in limited areas, efforts are also being made to improve the genetic diversity of *H. discus*. Accordingly, single nucleotide polymorphisms (SNPs) analysis using molecular inversion probes were performed to analyze genetic characteristics related to thermotolerance and growth.

Initially, Genotyping By Sequencing (GBS) was done to find genome modification of 55 abalones which are F_0 parents to produce F_1 offsprings. After the sequence mapping and variant calling, 202,434 SNPs were confirmed. As a result of specific filtering steps, 27,771 SNPs were filtered as high-quality SNPs. Among them, 500 SNPs were selected to analyze the genotypes as molecular inversion probes (MIPs). Subsequently, MIPs and adapters containing a barcode sequence were designed.

For the MIPs analysis, 973 and 673 abalones were selected for the growth and thermotolerance tests respectively. MIPs experiments were performed with several steps such as probe pooling, target capture & library construction, gap filling & ligation reaction, degradation, multiplex PCR & purification. As a result, insert sequences of 180 bp were obtained from the genomic DNA of 1,646 abalones and processed to collect the data by Illumina Next-Seq. Based on the sequencing data, individual genotyping was profiled for the selected specific variations of growth and thermotolerance.

In conclusion, the specific MIPs were suitably developed from *H. discus*. Also, the genotype data were constructed and characterized for important traits such as growth and thermotolerance. In future, these results will be applied to improve the abalone with desirable qualities for the abalone aquaculture industry.

THIOREDOXIN REDUCTASE 1 (TXNRD1) FROM BIG-BELLY SEAHORSE (*Hippocampus abdominalis*)

W.K.M. Omeka, Tae Hyug Jeong*, D.S. Liyanage and Jehee Lee

Department of Marine Life Sciences, Fish Vaccine Research Center, Jeju National University, Jeju Self Governing Province 690-756, Republic of Korea
E-mail: malithiomeka@gmail.com

The thioredoxin - thioredoxin reductase system is one of key redox homeostatic system which response to environmental stress factors such as ROS, UV light, gamma rays and heat shock. Thioredoxin reductase (TXNRD) restore the oxidized Thioredoxin (TXN) to reduce state, thus keep the sustainability of the redox system. TXNRDs are homodimeric proteins which belong to the flavoprotein family of pyridine nucleotide disulfide oxidoreductases. It contains FAD prosthetic groups, NADPH binding sites and a redox-active disulfide catalytic site. Recently, three isoforms of TXNRDs; cytosolic (TXNRD1), mitochondrial (TXNRD2), Testis specific (TXNRD3) has been identified in mammals. TXNRD1 is the most abundant isozyme found in the cytosol and active site composed of a conserved Cys-$(X)_2$-Cys motif near the N-terminus of each subunit and a conserved Gly-Cys-Sec-Gly-OH motif at C-terminal of each monomer. With the presence of NADPH, tightly folded TXNRD1 accept electrons to reduce covalent disulfide and selenenyl sulfide bonds into dithiol and seleno thiol groups. Then seleno thiol groups are exposed and act as nucleophile to reduce TXN and other substrates.

Hence, the aim of present study is to characterize molecular structure of TXNRD1 from *Hippocampus abdominalis* to get better understanding of redox homeostasis. Therefore, we conducted *insilico* and transcriptional analysis of TXNRD1 ortholog that identified from Big-belly seahorse transcriptome. In this study, various bioinformatics tools were used to identify the sequence characteristics and qPCR was used to investigate the expression profiles of TXNRD in normal and immune stimulated conditions.

The open reading frame (ORF) of TXNRD1 is 1800bp and encoding 600 amino acids. According to In-silico analysis performed, TXNRD1 enzyme has 65.86 kDa molecular weight and theoretical isoelectric point is 5.54. The amino acid sequence contained 2 N-linked glycosylation sites at ^{6}NETG^{9} and ^{84}NKTH^{87}. TXNRD1 contain Glutaredoxin (GRX) domain at 25 to 106 amino acid residues which are similar to human GRXs. GRX is a glutathione (GSH) dependent reductase and it catalyze the reduction of disulfide in proteins such as ribonucleotide reductase. GRX is involved in many cellular functions including DNA synthesis, signal transduction and the defense against oxidative stress. The qPCR results indicated that highest mRNA expression was observed in blood followed by ovary and gill among fourteen different tissues from healthy seahorses. The different levels of TXNRD1 expression detected in the tissue may reflect distinct levels of metabolic activity. Mainly phagocytic blood cells can produce excessive amounts of ROS in response to the invading pathogens as a first-line host defense mechanism to activate immune signaling pathways. Therefore, TXNRD1 from seahorses can be identified as an immunologically important gene.

IDENTIFICATION OF *V. parahaemolyticus* PirA^{vp}/PirB^{vp} TOXINS POTENTIAL INHIBITORS IN ESSENTIAL OIL BLEND FORMULATION BY STRUCTURE-BASED VIRTUAL SCREENING APPROACH AND MOLECULAR DOCKING

Rajeev Kumar Jha*, Haig Yousef Babikian, Tigran K Davtyan, Le Van Khoa, Yusef Babikyan

Technical Research and Development, PT. Central Proteina Prima & PT. Rhea Natural Sciences, Indonesia
Speaker* email: rajeev.kumar@cpp.co.id

Acute hepatopancreatic necrosis disease (AHPND), caused by *V. parahaemolyticus* PirAvp/PirBvp toxins, is a significant threat to the shrimp farming industry. We successfully formulated an essential oil blend against it. The ProteinPlus and ezPocet binding site modeling revealed that the ligand-binding pockets of the toxins are possibly located on the central part of the protein and play an essential role in the small molecule ligand interaction cellular target receptors binding. To gain further insights into the interactions present at the ligand-toxins interface, each of the selected molecules in essential oil blend formulation (EOBF) was docked into *V. parahaemolyticus* PirAvp/PirBvp toxins protein using AutoDock Vina. Selected molecules in EOBF composition are first voxelized into 8 different pharmacophoric-like features. After that, they have been used as input for a DCNN model. AutoDock Vina molecular docking results for the selected molecules from EOBF were visualized and analyzed by PyMol and Discovery Studio software. We set the EOBF components to identify *V. parahaemolyti*cus PirAvp/PirBvp toxins potential inhibitors in EOBF using a structure-based virtual screening approach and molecular docking, based on the quantitative composition and biological activity data. A total of 15 molecules were selected for molecular docking into *V. parahaemolyticus* PirAvp/PirBvp toxins. These 15 EOBF molecules interacted with amino acid residues located on C-terminal alpha-helixes and the central part of the beta-strands of Cry domains II and III of the *B. thuringiensis* Cry toxin Po-1 Cry ligand-binding pocket with high druggability (Figure). All of these EOBF molecules interacted with key amino acid residues responsible for PirBvp/PirBvp homodimers formation. Moreover, all the EOBF compounds (except anethole) can disturb and prevent the PirBvp/PirAvp heterotetramer structure. Thus, 6 EOBF compounds could be potent suppressors of 3 primary potential pathogenic mechanisms of *V. parahaemolyti*cus–induced AHPND in *P. vannamei*. All the rest 9 EOBF compounds could be potent inhibitors of 2 primary possible pathogenic mechanisms of *V. parahaemolyti*cus–induced AHPND in shrimps.

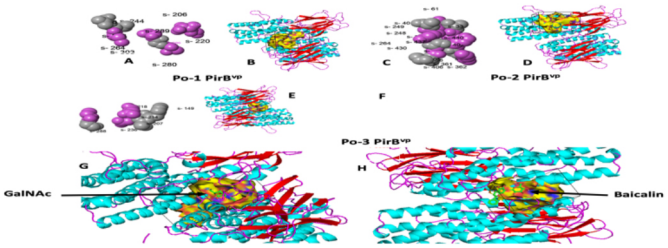


Figure : *V. parahaemolyticus* PirAvp/PirBvp toxin ligand-binding pockets EOBF as a natural antimicrobials could be effective in reducing bacterial pathogenicity via direct or indirect anti-virulence activity. The formulation is widely applied in the ponds in Indonesia and outside with successful outcome.

A PRACTICAL AND EFFECTIVE ARTEMIA HATCHING METHOD TO ELIMINATE COVERT MORTALITY NODAVIRUS (CMNV)

Yan Wang*, Zhangwang Lu, Liyong Miao, Kun Ying, Wucheng Yue, Xiangzhao Guo, Hui G. Jiang

College of Marine Science, Hainan University
Haikou, 570228, China
ywang@hainanu.edu.cn

The covert mortality nodavirus (CMNV) is an emerging pathogen that infects several species significant in aquaculture including marine shrimp, freshwater prawns, and crabs. CMNV has been detected in both wild Artemia and commercial Artemia cysts, and studies have demonstrated that Artemia could be a vector and reservoir host of CMNV. Utilization of Artemia from unknown sources can impose considerable biosecurity risks in hatchery operations, effective hatching methods to eliminate the potential infection from CMNV-positive (CMNV+) Artemia cysts are urgently needed.

Three treatments were applied to assess their efficacy of disinfecting CMNV$^+$ Artemia cysts during hatching: (1) Decapsulation of Artemia cysts with Na_2CO_3 (sodium carbonate) and NaClO (sodium hypochlorite) at various durations before hatching, (2) Application of different concentrations of formalin in hatching water, (3) Combinations of decapsulating Artemia cysts and formalin-treated hatching water. Hatching CMNV$^+$ Artemia with disinfected seawater only serves as the control.

Upon treatments, the total viral loads of cyst shells and Artemia (both larvae and adult) collected from post-hatching were quantified using a commercial diagnostic kit based on the specific TaqMan fluorescence probes. The results showed that: (1) The virus located on the cyst shells attached/infected Artemia larvae during hatching and remained active for a prolonged time (7 days), (2) The viral load of empty shells and the activity of infected larvae decreased with the decapsulation time. After a 45-minute treatment of decapsulation, CMNV on shells, as well as both larvae and adults, were eliminated successfully. Furthermore, decapsulation shortened the hatching time of the cysts to nearly half and improved the hatching synchrony, (3) 10ppm formalin in the hatching water could block the transmission of CMNV from the shells to the newly hatched larvae, although such a level was insufficient to eliminate the virus from the shells. Formalin of 30ppm or higher could eliminate CMNV, however, it also reduced the hatching rates of the Artemia cysts. (4) Combination of decapsulation and 10ppm formalin in hatching water effectively eliminated the CMNV and significantly enhanced the hatching synchrony. This study developed a practical, effective, and reliable treatment method for hatching Artemia to ensure biosecurity in aquaculture hatcheries.

VALUE CHAIN ASSESSMENT OF LOKAN (*Geloina expansa*) AND CHALLENGES POSED BY COVID-19 IN SALUT, TUARAN

Michelin Collyen Jimmy*, Madihah Jafar Sidik and Saleem Mustafa

Borneo Marine Research Institute
Universiti Malaysia Sabah,
88400 Kota Kinabalu, Sabah, Malaysia
Email: cjmichelin22@gmail.com

The COVID-19 pandemic was a serious challenge to seafood security in Sabah, which is very blessed to have a large coastal area and the rest of the country. In Salut, Tuaran, a commercially valuable marine organism, was the mangrove clam, or *Geloina expansa*, also known as lokan. The grilled mangrove clams and coconut desserts are among the famous street foods around Kota Kinabalu. However, the information on the status, distribution, and supply chain of the mangrove clam is poorly documented in Sabah. This study aimed to establish the structure of the value chain of lokan (*Geloina expansa*) in Salut, Tuaran, identify the disruptions caused by the lockdown, and examine the impact of the supply chain disruptions on each level of the chain. A set of questionnaires concerning the Delphi method for interviewing the fishermen, middlemen, and stall owners in Salut. The questionnaires are divided into three parts: the first was socio-demographic data, part 2 was the challenges posed by the COVID-19 pandemic, and the last part was the comparison of the business operations before and during the pandemic. The interviews were conducted from November 2021 to January 2022. There are 16 out of 46 stall owners who were interviewed. According to a series of interviews, most stall owners were between 32 and 41 years old and were Bajau ethnic. In addition, most stall owners only get to the primary school level. Most mangrove clam businesses are family businesses established around 1997 to 2022. The range of income for most of the stall owners was less than RM1830 per month during the COVID-19 pandemic. There are four layers of the value chain of mangrove clams in Salut, Tuaran, which consist of fishermen, middlemen, stall owners, and customers. According to interviewed stall owners, the challenges they face during the pandemic are lower demand. It is hard to meet the middlemen, such as the closure of the country and state border from March 2020 to May 2020 and the strict documentation movement regulations. In addition, some of the stall owners do not use any online platform for marketing their products. Some stall owners' lack of information technology (IT) knowledge might be due to age and health factors in selling their products. The state government could devise appropriate measures or offer incentives to combat digital poverty in this state, particularly among small business owners. Digital development in this state should be prioritised to ensure the community is familiar with an online business.

EFFECTS OF PROPHYLACTICS ON GROWTH AND HEMATOLOGY OF AFRICAN CATFISH *Clarias gariepinus* (BURCHELL, 1822) FINGERLINGS

Jeremiah O. Jimoh*, Ademola M. Akinsorotan, and Bunmi J. Olasunkanmi

Department of Fisheries and Aquaculture
Federal University
Oye-Ekiti, Nigeria

African catfish dominate aquaculture in Nigeria. However, despite the wide acceptability of *Clarias gariepinus*, its full aquaculture potential has not yet been realized. High mortality in the fingerling stage and the resulting seed scarcity is a major problem. One probable cause of fingerlings mortality is disease infection. Successful treatment of diseases depends on selecting the most effective therapeutic drug or chemical and applying it in the most appropriate legal manner. This research was conducted to determine the effects of formalin, H_2O_2 and $KMnO_4$ used as prophylactics on the growth performance and hematology of *Clarias gariepinus*.

Catfish fingerlings were treated with 2, 150, and 100 ppm of $KMnO_4$, H_2O_2, and formalin respectively. After a 70-day culture period, growth, and haematological indices were measured. Data were subjected to one-way ANOVA and the mean were separated using the New Duncan Multiple range test at $p \leq 0.05$.

Results indicated that fish treated with $KMnO_4$ had the highest weight gain (5.31g) while the least (3.70g) was recorded in fish treated with H_2O_2. The control fish had the highest (1.91g) FCR while the least (1.47) was recorded in catfish fingerlings treated with $KMnO_4$. White blood cells were more (6.23) in fish treated with formalin and lowest in control fish. Hb values were similar in control fish (12.45) and $KMnO_4$ (13.09) treated fish. The least Hb was recorded in fish treated with formalin (10.52). Apart from PCV that decreased in fish treated with H_2O_2 and formalin, other red blood indices increased significantly.

Although all the used prophylactics had effects on growth and physiology of *C. gariepinus* fingerlings, $KMnO_4$ appeared to have the least negative effect on the fish. The study therefore, recommends the use of potassium at 2 ppm for treatments in catfish fingerlings aquaculture.

Table 1: Growth of the *C. gariepinus* fingerlings under different prophylactic treatments

Parameters	T1	T2	T3	T4
Initial weight	2.43^a	2.45^a	2.43^a	2.46^a
Final weight	6.39^a	7.76^a	6.13^a	7.73^a
Weight gain	3.96^a	5.31^b	3.70^a	5.27^b
FCR	1.91^a	1.47^a	1.82^a	1.59^a
SGR (%/day)	0.57^a	0.68^a	0.62^a	0.67^a

Table 2: Haematological indices of the *Clarias gariepinus* fingerlings under different treatments

Treatments	T1	T2	T3	T4
WBC (10^9/L)	4.13^c	4.57^c	5.50^b	6.23^a
RBC (10^{12}/L)	26.70^a	27.60^a	14.00^b	9.20^c
Hb (g/L)	12.45^a	13.09^a	11.34^{ab}	10.52^b
MCV	138.58^c	146.12^{bc}	202.36^b	356.85^a
MCH	46.63^a	47.43^a	81.00^b	114.35^c
MCHC (g/L)	33.65^b	32.46^b	40.03^c	37.80^a
PCV (%)	37.00^b	40.33^b	28.33^a	27.83^a

PARASITIC DISEASE TRENDS IN FARMED OLIVE FLOUNDER *Paralichthys olivaceus* IN JEJU ISLAND, KOREA

Chang Nam Jin, Han Chang Sohn, Sang Phil Shin and Jehee Lee

Marine Science Institude of Jeju National University
Jeju Self-Governing Province 63243
Republic of Korea
jincn2000@hanmail.net

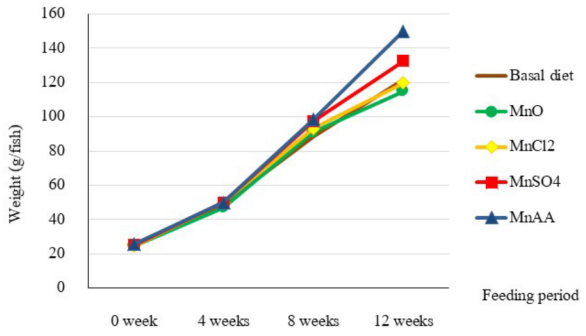
Jeju Island in Korea has a suitable environment for land-based flounder culture with its coastal seawater temperature of 13–28°C. Jeju Island's cultured flounder has a production volume of 20,243 M/T and a production value of $157 million (2019), accounting for 54% of Korea's cultured flounder production. However, with the expansion of the flounder culture industry and the long history of aquaculture, various diseases have been found in the species. Herein, we selected 20 flounder farms in Jeju Island, with independent environmental conditions, and examined parasites in cultured flounder once a month from January to December, 2019, for a total of 240 times. From the results, a total of 342 parasitic infection cases were identified; comprising 215 (62.9%) ectoparasitic and 127 (37.1%) endoparasitic infections. Seven ectoparasitic infections were identified, namely, *Trichodina* sp., 86; Scuticosiliate (*Miamiensis avidus*), 79; *Ichthyobodo* sp., 16; Gill protozoa, 16; *Amoeba* sp., 7; *Cryptocaryon irritans*,4; *Gyrodactylus* sp., 4; and *Kudoa* sp., 3 cases. *Trichodina* sp. comprised the highest number of infection cases but the damage on cultured flounder was relatively insignificant, and it did not cause much problem if the water circulation was smooth. Scuticosiliate comprised the second highest number of infection cases; it penetrates the skin, muscle, gill, and brain tissues in young flounders (< 200 g), causing necrosis, and eventually death. Other ectoparasites did not cause many infections or significant damage. Six species of endoparasites, which infect flounders' internal organs, muscles, and bladders were identified, namely, *Parvicapsular* sp., 59; *Enteromyxum leei*, 34, *Myxodavicia* sp., 16; *Sinuolinea* sp., 10; *Owrtholinea* sp., 5; and *Kudoa* sp., 3 cases. Many cases of endoparasitic infections, mostly Myxosporea, were found recently. Besides the *E. leei* infection, the damage caused by these parasites is either mild or uncertain. However, when *E. leei* infects the intestines, it causes emaciation disease, which can result in secondary bacterial infection and death.

COMPARATIVE EFFICACY OF DIFFERENT MANGANESE SOURCE FOR GROWTH PERFORMANCE OF ASIAN SEABASS (*Lates calcarifer*)

Orapin Jintasataporn, Srinoy Chumkam and Lalipat Prompol

Department of Aquaculture, Faculty of Fisheries, Kasetsart University
Bangkok 10900, Thailand
Email:ffisora@ku.ac.th

The study on efficacy of different manganese source for on growth performance, immunity and deposition in bone of Asian seabass (*Lates calcarifer*) was assigned in CRD with 5 treatments and 3 replicates. The treatments were control without manganese supplementation. The other three treatments of manganese were 10 ppm at different form of inorganic (oxide, chloride, sulfate) and manganese amino acid(Availa-Mn) 10 ppm. Asian seabass with average weight of 24.67±1.09 g were stocked in 500 liter tanks at the density of 10 fishes per tank and fed treatment diet 3% of body weight twice daily for 12 weeks. The results showed that there were statistical differences (P<0.05) on growth performance in term of weight gain, average daily gain, specific growth rate and feed conversion ratio. The growth performance of Asian seabass fed diet supplemental organic Availa-Mn was higher than the other groups (P<0.05). The immunity in term of erythrocyte count, leucocyte count, hemoglobin, serum protein and immunoglobulin (IgM) showed no significantly differences (P>0.05). The deposition of manganese in bone of Asian seabass fed diet supplemental organic Availa-Mn was numerical higher than the other groups but was not significantly differences (P<0.05). Therefore, supplementation organic manganese amino acid(Availa-Mn) in Asian seabass diet can promote growth performance and manganese accumulation in bone better than inorganic form.



Form	Manganese	
	mg/kg^{-1}	Relative accumulation (%)
Basal diet	7.55±2.08^{c}	0
MnO	7.95±0.54^{bc}	5.29
MnCl	8.04±0.4^{bc}	6.49
MnSO4	9.62±0.52^{ab}	27.41
Availa-Mn	10.61±0.96^{a}	40.52
P-Value	0.005	

SALMON MOVEMENT WITHIN A CAGE IS RESTRICTED BY A COMBINATION OF WAVES AND CURRENT

Ása Johannesen*, Jóhannus Kristmundsson, Øystein Patursson, Signar P. Dam, and Pascal Klebert

Ecology department
Fiskaaling
FO-430 Hvalvík, Faroe Islands
asajoh@fiskaaling.fo

Due to increasing consumer demand for salmon, salmon farmers are constantly seeking to expand their production. However, environmental barriers such as parasite load and site carrying capacity are preventing expansion in established sites. Therefore, salmon farmers seek to move or expand their production to more exposed sites. In exposed sites, salmon are likely to experience stronger currents and larger waves than what they would normally do. Some previous work has established some baseline information on swimming capacity and behaviour of salmon in currents, but it is not well established how currents and waves interact, and how well salmon are able to cope in conditions where they are exposed to both.

In this study we investigate the effects of strong currents and waves on the behaviour of salmon and how they choose to use the space available to them. Using video cameras and echo sounders, we show that fish prefer to use the entire water column, narrowing their range only as a response to cage deformation (Figure 1), waves, or daylight.

Conversely, fish show strong horizontal preference, mostly occupying the portions of the cage exposed to currents. Additionally, waves cause salmon to disperse from the exposed side of the cage to the more sheltered side. Even when strong currents decrease the amount of available space, salmon choose to occupy the more exposed part of the cage.

This indicates that at least with good water exchange, the high density caused by limited vertical space is not so aversive that salmon choose to move to less desirable areas of the cage. However, the dispersal throughout the entire available water column indicates that making cages deep enough to compensate for deformation in strong currents would be beneficial to salmon welfare.

APPLICATION OF MULTIPLEX PCR AND MINION SEQUENCING FOR DIRECT SEQUENCING OF WHOLE GENOMES OF *Piscine Orthoreovirus-1* (PRV-1) FROM TISSUE AND ENVIRONMENTAL SAMPLES

Stewart C. Johnson*, Lenora D.M. Turcotte and Mark P. Polinski

Pacific Biological Station
Fisheries and Oceans, Canada
Nanaimo, BC, V9T-6N7
Stewart.Johnson@dfo-mpo.gc.ca

There are no methods for culturing PRV-1; the majority of genome sequences have been assembled from direct sequencing of infected tissues. This approach is limited to samples which have high PRV-1 loads. Like many fish viruses, PRV-1 loads in wild salmonids and environmental substrates (e.g. seawater) are typically too low for successful direct sequencing.

In 2017, Quick *et al*. developed a multiplex PCR method for targeted enrichment of Zika virus genomes and optimized library preparations for MinION and Illumina sequencing. They obtained full genome sequences from clinical samples with as few as 50 genome copies. We have adapted these techniques and applied them to obtain PRV-1 genomes directly from tissue and seawater samples.

PRV-1 genome sequences from British Columbia were used as reference to design 36 sets of overlapping primer pairs using Primal Scheme software (http://primal.zibraproject.org). RNA extraction and cDNA synthesis followed standard methods (Polinski et al. 2019) and provided template for multiplex tiling PCR. Amplified products were sequenced using MinKnow high-accuracy base calling and aligned to the reference genome using Nanopipe (http://bioinformatics.uni-muenster.de/tools/nanopipe2/index.hbi?lang=en).

We routinely sequence 24-48 barcode-separated samples per flow cell and obtain 2-3 million reads for each 12-sample run. At this depth of sequencing we can generate accurate near full-length genome sequences from samples with as low as 10 PRV-1 copies per μl of sample template. Samples which had received improper handling or had been stored under suboptimal conditions for long periods of time (months to years) often had significant loss of amplification for some primer sets resulting in low read depth across portions of the genome. Nevertheless, even these mistreated samples typically yielded acceptable sequence coverage for the majority of the genome. The PRV-1 consensus sequences generated using the multiplex-MinION protocol are near-perfect matches (>99.9%) to lllumina consensus sequences from the same samples providing read coverage is greater than 20 reads per position.

We have obtained large numbers of PRV-1 genome sequences from fish and environmental samples, as well as recovered partial genome sequences from 43 year-old histology samples. Our plan is to integrate PRV-1 sequence data, epidemiological data and data from hydrodynamic modeling to determine transmission pathways within and between farmed and wild salmon populations over a range of temporal and spatial scales. Over the longer term our goal is to develop robust methods and analytical procedures that are suitable for application to a wide range of infectious agents in aquatic environments.

APPLICATION OF MULTIPLEX PCR AND MINION SEQUENCING FOR DIRECT SEQUENCING OF WHOLE GENOMES OF Piscine Orthoreovirus-1 (PRV-1) FROM TISSUE AND ENVIRONMENTAL SAMPLES

Stewart C. Johnson*, Lenora D.M. Turcotte and Mark P. Polinski

Pacific Biological Station
Fisheries and Oceans, Canada
Nanaimo, BC, V9T-6N7
Stewart.Johnson@dfo-mpo.gc.ca

There are no methods for culturing PRV-1; the majority of genome sequences have been assembled from direct sequencing of infected tissues. This approach is limited to samples which have high PRV-1 loads. Like many fish viruses, PRV-1 loads in wild salmonids and environmental substrates (e.g. seawater) are typically too low for successful direct sequencing.

In 2017, Quick *et al*. developed a multiplex PCR method for targeted enrichment of Zika virus genomes and optimized library preparations for MinION and Illumina sequencing. They obtained full genome sequences from clinical samples with as few as 50 genome copies. We have adapted these techniques and applied them to obtain PRV-1 genomes directly from tissue and seawater samples.

PRV-1 genome sequences from British Columbia were used as a reference to design 36 sets of overlapping primer pairs using Primal Scheme software (http://primal.zibraproject.org). RNA extraction and cDNA synthesis followed standard methods (Polinski et al. 2019) and provided template for multiplex tiling PCR. Amplified products were sequenced using MinKnow high-accuracy base calling and aligned to the reference genome using Nanopipe (http://bioinformatics.uni-muenster.de/tools/nanopipe2/index.hbi?lang=en).

We routinely sequence 24-48 barcode-separated samples per flow cell and obtain 2-3 million reads for each 12-sample run. At this depth of sequencing we can generate accurate near full-length genome sequences from samples with as low as 10 PRV-1 copies per μl of sample template. Samples which had received improper handling or had been stored under suboptimal conditions for long periods of time (months to years) often had significant loss of amplification for some primer sets resulting in low read depth across portions of the genome. Nevertheless, even these mistreated samples typically yielded acceptable sequence coverage for the majority of the genome. The PRV-1 consensus sequences generated using the multiplex-MinION protocol are near-perfect matches (>99.9%) to lllumina consensus sequences from the same samples providing read coverage is greater than 20 reads per position.

We have obtained large numbers of PRV-1 genome sequences from fish and environmental samples, as well as recovered partial genome sequences from 43 year-old histology samples. Our plan is to integrate PRV-1 sequence data, epidemiological data and data from hydrodynamic modeling to determine transmission pathways within and between farmed and wild salmon populations over a range of temporal and spatial scales. Over the longer term our goal is to develop robust methods and analytical procedures that are suitable for application to a wide range of infectious agents in aquatic environments.

CONTINUOUS ROTIFER PRODUCTION WITH AI-ASSISTED COUNTING TO MONITOR ROTIFER POPULATION

Jose LOWELL*, Qunying Xu, Punithavathi K Haridas

Marine Aquaculture Centre
Singapore Food Agency
11 St John Island
Singapore 098659
Jose_LOWELL@sfa.gov.sg

Rotifers are microscopic zooplankton used extensively in aquaculture because of their high reproduction rate, optimal size for larval fish and good nutritional profile. Rotifers are essential for the first feeding of many species of marine finfish larvae. Common rotifer culture methods include indoor batch cultures and outdoor pond cultures. However, both methods face a common issue of culture instability where the rotifer population could collapse overnight. In addition, an outdoor pond culture has a larger footprint compared to an indoor batch culture, an important factor in land-scarce Singapore. A continuous rotifer culture method based on a recirculating aquaculture system developed at SFA's Marine Aquaculture Centre and the management protocols adopted to maintain stable rotifer growth is described here.

Maintaining desirable levels of rotifer population density at different stages of the continuous rotifer culture is also critical for a successful production. Harvesting, feeding, or initiation of new culture for upscaling depends on the density of the culture, which requires counting of rotifers under the microscope. To reduce manhours and simplify the counting process, an artificial intelligence (AI) assisted rotifer counting mobile web application was developed. Pictures of the rotifer samples taken with a phone camera just needs to be uploaded onto the server hosting the AI-assisted counting software for analysis, drastically reducing the time required to deduce the rotifer density in any culture .

With the ability for stable indoor continuous rotifer production coupled with technology to reduce reliance on manpower, rotifers can be produced intensively and consistently on a small footprint to support large-scale hatchery production.

THE IMPORTANCE OF MULTI-DISCIPLINARY EVALUATION STRATEGIES TO ASSESS THE DIET PERFORMANCE OF REDCLAW (*Cherax quadricarinatus*): HISTOLOGICAL SURVEYS, GROWTH, FEED EFFICIENCY AND DIGESTIBILITY

Melissa Joyce*, Leigh Owens, and Igor Pirozzi

Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville AUS
melissa.joyce@my.jcu.edu.au

Redclaw (*Cherax quadricarinatus)* are a species of freshwater crayfish native to Australia and have been farmed since the 1980s. However, the industry is still small and a major limitation to growth is the lack of a commercially available feed formulated for redclaw. The aquaculture industry is increasingly using less fishmeal in feed, often substituting with plant based protein sources. The suitability of an ingredient requires determining its digestibility, this is important for feed formulation as it quantifies the potential availability of nutrients that can be absorbed and utilised to support metabolic process such as maintenance, reproduction and growth. Determination of diet digestibility and performance of fish in feeding trials are standard. However, there is the assumption is that the animals being used in experiments are healthy individuals. In crustaceans, such as redclaw, signs that an individual is compromised is sometimes only apparent upon refusal to ingest feed or sudden death. External signs such as marks or discoloration on the exoskeleton are not useful identifiers. Nutrient absorption and secretion of digestive enzymes for crustaceans occurs in the hepatopancreas with changes structure reported to occur from changes environmental conditions, different diets and feeding regimes. A digestibility study on redclaw fed a variety of plant based and animal-rendered products showed diet related effects upon examination of the hepatopancreas. A number of structural abnormalities were observed including: degradation of the myoepithelial cells, sloughing of cell contents, hypertrophy of b-cells, and thinning of epithelial lumen. Additionally, granulomas, bacterial, and viral infections (CqBV and reovirus) were also present. The study raised questions as to the potential effects of ingredients for long durations and the importance of using multiple disciplines to assess diet performance in redclaw. It is clear that there needs to be a greater awareness of the known viruses and diseases that are present in redclaw populations when conducting nutrition studies. Recommendations on the use of ingredients are often supported with performance parameters, such as growth and survival; however, this ignores vital information. Histological examination of hepatopancreas is an important technique to consider in nutrition studies. It is a well-established but often overlooked method that is simple to implement and can provide a greater understanding of the health of the animal in relation to diet.

DOES AGE AT RELEASE OF *Holothuria scabra* IN THE OCEAN NURSERY MATTER?

Marie Antonette Juinio-Meñez*, Olivier Josh C. Caasi, Minin J. Sinsona

The Marine Science Institute
University of the Philippines Diliman
Diliman, Quezon City, Philippines 1101
ajmenez@msi.upd.edu.ph

Floating fine mesh net (1 mm) enclosures (2 m × 1 m × 1.2 m) have been established as a viable ocean nursery system for the mass production of release size (> 3 g) juvenile *H. scabra* in the eutrophic coastal waters of Bolinao, Pangasinan, Philippines. The sizes of post settled juveniles of from the same spawning batch vary widely. Thus juveniles from the same batch are released in the ocean nursery at different ages. This study examined the growth rates and survival of post settled juveniles from a single batch released 30 days, 60 days and 120 days post settlement. Each net was stocked with 1,000 juveniles. Juveniles were reared for up to 3 cycles, at ~30 days per cycle. Absolute growth rates (AGR g/day) and survival rates (%) after cycle 1 (i.e. first 30 days) and cycle 3 (i.e. last 30 days) were compared. Results are important in optimizing ocean nursery yield and quality of juveniles to be released for grow-out.

The average initial sizes at release were not significantly different among all releases at 3.95 ± 0.48 mm, 3.75 ± 0.34 mm, and 4.77 ± 0.37 mm for 30D, 60D, and 120D, respectively. Survival rates were high and did not differ significantly among releases after cycle 1 (Fig. 1).

AGR for 60D was significantly higher than 120D. However, mean weight did not differ significantly across releases (0.6-1.3 g). At the end of cycle 3, while AGRs were similar across releases, mean weight of 30D (1.5 ± 0.7 g) was significantly lower than 60D (3.6 ± 2.3 g). Only juveniles in the 60D release reached the recommended release size. AGR of all age groups are comparable with the poorest performing batches reared in the same site (0.01-0.04 g day $^{-1}$).

After cycle 3, initially smaller/stunted juveniles reared for a longer period (60D and 120D; ave. AGR ~0.03 and 0.02 g day^{-1}, respectively) in the hatchery prior to release in the ocean nursery produced relatively faster growing juveniles than "shooters" released after 30D (0.01 g day^{-1}). This may be attributed to the colder temperature during the period of rearing. Additionally, thinning out was done only once for 30D, and twice for 60D and 120D releases. Whether differences with respect to age at release will affect growth performance during the grow-out phase needs to be determined.

DIETARY NON-STARCH POLYSACCHARIDES INFLUENCED NATURAL FOOD WEB AND FISH PRODUCTION IN SEMI-INTENSIVE POND CULTURE OF NILE TILAPIA

K. A. Kabir, M.C.J. Verdegem, J.A.J. Verreth, M. J. Phillips, J.W. Schrama

Sustainable Aquaculture Program, WorldFish Bangladesh, House # 22B, Road #7, Block # F, Banani, Dhaka-1213
and
Aquaculture and Fisheries Group, Wageningen Institute of Animal Sciences, Wageningen University
PO Box 338, 6700 AH
k.kabir@cgiar.org
kazi.kabir@wur.nl

Dietary non-starch polysaccharides (NSP) changes the nutrient digestibility and faecal characteristics in fish. This study assessed the effect of the type of dietary NSPs on fish production and the contribution of natural food to the total fish production in semi-intensively managed tilapia ponds. Twelve ponds, each divided into three equally-sized compartments, were assigned to test the effect of the type of dietary NSPs (i.e. "PecHem-Diet", a diet with easily fermentable NSP, vs "LigCel-Diet", a diet with slowly fermentable NSP). Fish were restrictively fed, based on the crude protein content of the feed. Three feeding levels ("no=0", "low=9 g.kg-0.8.d-1" and "high= 18 g.kg-0.8.d-1") nested in pond were analysed in a split plot design. Initial fish biomass was 3084 g.compartment-1 and the experiment lasted 56 days. With the "LigCel-Diet" biomass gain was higher (2599 vs 2192 g.compartment-1) and feed conversion ratio (FCR) was lower (1.4 vs 1.9; $P<0.001$) than with the "PecHem-Diet". Diet had no effect on fish survival and specific growth rate (SGR). For both diets, increasing feeding level increased ($P<0.001$) biomass gain, fish survival, FCR and SGR. There was a significant interaction effect ($P<0.05$) between diet and feeding level on FCR. Fish body composition was the same in both diets. With the "LigCel-Diet", the apparent digestibility coefficient (ADC) was higher ($P<0.001$) for crude protein, fat, phosphorus and calcium and lower ($P<0.05$) for ash compared to the other diet. Neither feeding level nor the interaction between diet and feeding level influenced the apparent digestibility coefficient (ADC) of any nutrient. Diet composition did not alter the organic matter (OM) composition of the faeces. δ13C and δ15N from the stable isotope analysis revealed that N gain in fish originated from both feed and natural food of the pond. Natural food abundance in the pond increased over time for both diets. Chlorophyll-a was higher in the pond fed with "LigCel-Diet". Fish gut content and calculated N gain indicated an enhanced contribution of natural food to fish growth in ponds fed with "LigCel-Diet". In conclusion, the type of dietary NSP determines tilapia productivity in semi-intensive managed ponds by altering food web productivity.

EFFECTS OF DIFFERENT SOYBEAN MEAL INCLUSION LEVELS ON GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP IN TRANG PROVINCE, THAILAND

Chuchai Kanjanamayoon*, Tanakorn Lothaka, Lukas Manomaitis, Levy Manalac and Hsiang Pin Lan

U.S. Soybean Export Council (Southeast Asia) Ltd
541 Orchard Road, #11-03 Liat Towers, Singapore 238881
Chuchai@ct.ussec.org

Shrimp intensive culture are now common practices in Thailand. Farmers are suspect whether using high inclusion of soybean meal will have side effects in term of growth, diseases, high mortality, etc. This experiment is shown that intensive shrimp culture by using different inclusion rate of soybean meal of 25%, 50% and 100% fishmeal replacement in feed formulation compare with normal commercial feed. USSEC and cooperator in this project want to do the comparison to see the result of the different feeds.

A comparative demonstration was conducted from May 2022 to August 2022 at a shrimp farm in Trang province, Thailand. Total of four ponds with volume of 3,000 m^3 in each pond will be used for the feeding demonstration. Three ponds will be used to demonstrate the effectiveness of different soybean inclusion levels, and another one pond will use a standard commercial feed as a control pond. Shrimp obtained from commercial hatchery and the post larvae will be acclimatized in cooperator's nursery pond as needed before stocking into the grow-out ponds. The stocking density is 200,000 pcs/pond. The post larvae will be approximately 5.0 gram/piece at the time of stocking and will be grown to 25 gram/piece (40 pieces/kg) for 52-53 days of culture.

All four ponds were stocked on the same day after all ponds were properly prepared. All water parameters had no significant differences when they were stocked. The feeding was operated by auto feeding machines during 0600-1800 hrs.

All ponds were managed to be in normal condition and no major issue during the culture. However, total ammonia level was accidentally raised during the culture (*table2*) which resulted in lowering the growth at the late culture period.

The result of harvesting of 4 demonstration ponds (*table 1&2*) showed no significant difference between those 4 experimental groups in terms of growth, FCR, biomass and survival rate. This clearly indicated that feed formulation can use soybean meal to replace fishmeal without significant effect.

Table 1: Growth performance of 4 different experimental groups

Growth Parameters	Control	25% US Soy	50% US Soy	100% US Soy
Biomass (kg/pond)	2,910[a]	2,904[a]	2,852[a]	2,834[a]
Average BW (g/piece)	23.8[a]	23.3[a]	24.2[a]	23.3[a]
Survival rate (%)	61.1[a]	62.3[a]	58.9[a]	60.8[a]
ADG (g/piece/day)	0.34[a]	0.35[a]	0.38[a]	0.36[a]
FCR	1.74[a]	1.78[a]	1.76[a]	1.74[a]

The different alphabet in the same roll indicates significant difference ($p < 0.05$)

Table 2: Water quality profile of 4 experimental ponds

Water Parameters	Control	25% US Soy	50% US Soy	100% US Soy
DO (mg/l)	6.69±0.17[a]	6.64±0.44[a]	6.72±0.19[a]	6.71±0.20[a]
pH	8.00[a]	8.00[a]	8.00[a]	8.00[a]
Alkalinity (mg/l)	151.53±12.90[a]	153.67±14.00[a]	150.61±14.43[a]	150.45±14.74[a]
Ammonia (mg/l)	1.55±1.00[a]	1.85±0.89[a]	1.94±0.90[a]	1.70±1.02[a]

The different alphabet in the same roll indicates significant difference ($p < 0.05$)

POTENTIAL PROBIOTIC *Bacillus amyloliquefaciens* AS A BENEFICIAL MEDIATOR FOR CONTINOUS SUSTAINABILITY IN AQUACULTURE

Murni Karim[a,b*] Fatimah Md Yusoff, Fadhil Syukri, Puvaneswari Puvanasundram, Sow Cyn Shieng, Danial Iman

[a]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia
43400 UPM Serdang, Selangor, Malaysia
[b]Laboratory of Sustainable Aquaculture, International Institute of Aquaculture and Aquatic Sciences
Universiti Putra Malaysia, 70150 Port Dickson, Negeri Sembilan, Malaysia
*Corresponding author: murnimarlina@upm.edu.my

The use of probiotics in aquaculture as a viable alternative has paved the way to ensure continuous sustainability in aquaculture. This study concentrated on evaluating the properties of *Bacillus amyloliquefaciens* isolated from the blue swimming crab (*Portunus pelagicus*) as a potential probiotic. This strain was used to develop multi-strain probiotics (MSP) incorporated feed for red hybrid tilapia as disease control and growth-promoting tool. *Bacillus amyloliquefaciens* is combined with two other probiotic strains from the genera of *Lysinibacillus* and *Enterococcus* to create MSP. The inhibitory zone displayed by MSP against *Aeromonas hydrophila* (13.0± 0.6 mm) and *Streptococcus agalactiae* (15.3± 0.8mm) was significantly higher than the single probiotic. When *A. hydrophila* was co-cultured with MSP at a concentration of 10^8 CFU mL^{-1}, no growth of *A. hydrophila* was observed, indicating that the pathogen was completely inhibited. Furthermore, *B. amyloliquefaciens* also demonstrated antimicrobial activity against *Vibrio parahaemolyticus* in spot and well diffusion assays with significant inhibition zones of 5.3 ± 0.01mm and 4.4 ± 0.06 mm respectively. In a co-culture assay, this strain at a concentration of 10^6 CFU mL-1 showed a significant reduction of *V. parahaemolyticus* after 12 hours of incubation compared to the control group. In *in vivo* study, this strain significantly improved the survival rate (88%) of white shrimp, *Litopeneaus vannamei* compared to the control group with no probiotic added (43%). Biofilm can be considered as a good source of inorganic nutrients for promoting the growth and immunity of the cultured organism. The ability of *B. amyloliquefaciens* to form biofilm was tested and the highest biofilm formation was observed at 24hr, with an optical density of 3.67 mm. Thus, *B. amyloliquefaciens* can be considered to have a high potential for use in aquaculture industries. It has been demonstrated that this strain has excellent properties in terms of pathogen antagonism, biofilm formation, and host survival rate.

SKIN MICROBIOTA OF PRUSSIAN CARP INFECTED BY ECTOPARASITIC CRUSTATIONS

Elena N. Kashinskaya*, Evgeniy P. Simonov, Karl B. Andree, and Mikhail M. Solovyev

Research group of physiology and genetics of hydrobionts
Institute of Systematics and Ecology of Animals SB RAS
Novosibirsk, Russia, 630091
elena.kashinskaya@inbox.ru

The aim of the present study was to investigate the microbial community of skin mucus of infected and uninfected Prussian carp caused by parasitic crustaceans from the genus *Argulus* and *Lernaea* in an eutrophic lake with parallel studying of associated microbiota of their parasites and environmental compartments. In this study the libraries containing 16S ribosomal RNA genes from these distinct biological sources were studied using next-generation high-throughput sequencing and bioinfomratic software for identification and classification of operational taxonomic units (OTUs). In the microbial community associated with the parasitic crustaceans *Argulus* sp. and *Lernaea* sp., along with representatives of the normal microbiota, there were identified microorganisms that could be potential agents of infectious diseases in fish (*Flavobacterium* sp., *Aeromonadaceae* sp., *Corynebacterium* sp. and *Streptococcus* sp.). Each parasite is characterized by a specific structure of its associated microbiota, which, apparently, may indicate their role as vectors of different infectious disease.

Significant perturbation of dominant microbiota of skin mucus of unhealthy fish in comparison with healthy fish was registered (ADONIS, $p \leq 0.05$). Microbiota of intact skin mucosa of unhealthy fish were dominated by *Corynebacterium* (10.03±1.82%), *Arcobacter* (5.47±0.97%), and unclassified genus from Comamonadaceae family (4.44±0.48%). The microbiota of intact skin mucosa of healthy fish was opposite dominated by *Arcobacter* (12.57±3.49%), unclassified genus from Comamonadaceae (6.40±1.13%) and Helicobacteraceae families (4.37±1.47%). According to the Spearman rank correlation test the increase of ulceration load in skin of Prussian carp were significantly correlated with reduce the abundance of *Acrobacter*, bacteria C39 from Rhodocyclaceae family, *Rheinheimera,* unclassified bacteria from Helicobacteraceae, Aeromonadaceae family, and *Vogesella* ($p \leq 0.05$). Relative abundance of known opportunistic pathogens (*Flavobacterium* and *Acrobacter*) found in intact skin and ulcers of infected Prussian carp were significantly correlated with the ability of *Argulus* sp. and *Lernaea* sp. The microbiota associated with *Lernaea* sp. was significantly different from microbial communities of intact skin mucosa of both infected and uninfected fish and skin ulcers (ADONIS, $p \leq 0.05$) and represented by unclassified bacteria from Comamonadaceae and Aeromonadaceae families (33.93± and 8.44±5.97%, respectively). Associated microbiota of *Argulus* sp. was dominated by *Flavobacterium* (17.95±16.19%) and *Corynebacterium* (10.61±5.0%). Results of conducted studies indicate that ectoparasites have the potential to alter skin microbiota and play possible role in transmission of secondary bacterial infection in fish.

GENETIC PARAMETERS FOR GROWTH RATE IN THE FORTH GENERATION OF SELECTIVE STRIPED CATFISH *Pangasianodon hypophthalmus* IN VIETNAM

Kasigwa Howard Nelson*, Nguyen Van Sang, Tran Huu Phuc

College of Aquaculture and Fisheries, Campus 2
Can Tho University
Vietnam
hkasigwa@gmail.com

Of all the species in the Mekong Delta, striped catfish (*Pangasianodon hypophthalmus*) is the most economically important fresh water fish farmed in Vietnam. However, the challenges to its culture have been associated with many reasons but above all, lack of high quality seed, water pollution, diseases epidemic and lack of elite brooders with high genetic variability which subsequently have led to prolonged production cycles and hence high costs of production. The direct selection responses from the previous three generations for BW traits was 9.3% genetic gain per generation and this justifies the success of the selection. This study aims at estimating heritability and the selection responses for growth traits in the 4th selected generation of striped catfish of a breeding program using a nested mating design (30 sires and 60 dams) where 7200 individual fingerlings will be independently raised in hapas from 120 families and afterwards tagged using Passive Integrated Transponder (PIT) tags after 120 days. They will be communally tested in an outdoor rectangular pond (2,000m^2) till harvest at an average body weight of 1.0 kg (6 months of culture). Univariate and bivariate linear animal mixed models will be used to estimate variance and covariance components of four groups of traits (body, carcass, fish body condition and survival) during grow-out period. Findings of this study will generate information that will be used in broodstock selection with desired additive genetic traits for sustainable increased commercial production of striped catfish in the Mekong Delta.

PREVALENCE OF MEGALOCYTIVIRUS INFECTION AMONG INLAND CULTURED BARRAMUNDI (*Lates calcarifer*) DURING 2018 OUTBREAKS IN THAILAND: A PROACTIVE STUDY

Pattanapon Kayansamruaj*, and Pattarawit Kerddee

Department of Aquaculture, Faculty of Fisheries, Kasetsart University
Bangkok, Thailand 10900
Pattanapon.k@ku.th

The virus in the genus *Megalocytivirus*, family *Iridoviridae*, has been considered one of the major pathogens causing mass mortalities in both wild and captive finfish in the Indo-Pacific region. In this study, disease investigation carried out between February and November 2018 focused on naturally diseased barramundi in inland growout farms located in Central/Eastern region of Thailand. According to PCR screening from liver and spleen, 60% of samples (20 out of 33) from 8 distinct outbreaks (30-90% cumulative mortalities) were infected by megalocytivirus. Viral taxonomy was subsequently identified by nucleotide sequencing of MCP and ATPase genes which indicated that infectious spleen and kidney necrosis virus (ISKNV) was the major megalocytivirus associated with die-off. In addition, scale drop disease virus (SDDV) was identified from one outbreak manifesting extensive scale loss lesion. Concurrent infection of megalocytivirus and pathogenic bacteria, e.g. *Flavobacterium columnare*, were often found (Table 1). Phylogenetic analysis revealed that all ISKNV isolates shared 100% sequence identity and was categorized as genotype I, based on MCP gene sequence. Cumulative mortality of 90% (n = 50) was observed in juvenile barramundi at 21 days after experimental challenge via i.p. injection with GF cell propagated ISKNV. Affected fish exhibited ascites, darkened body and hemorrhage, while necrosis, inflammation and nuclear pyknosis/karyorhexis was observed histopathologically. Lastly, high-throughput sequencing of naturally infected specimen was able to recover the complete genome of ISKNV (111.4 – 111.6 kb in length, Fig. 1) which was almost identical to ISKNV reference strain (0.02% genetic difference).

Table 1 Epidemiological metadata

Farm	Mortality (%)	Bacterial identification	Virus identification	
			ISKNV	SDDV
A	50	ND	+ (6/7)	-
B	30	ND	-	-
C	70	*F. columnare* and *Aeromona* sp.	+ (3/3)	-
D	70	*F. columnare*	+ (3/4)	-
E	50	ND	+ (3/3)	-
F	50	ND	+ (1/1)	-
G	80	*F. columnare* and *Streptococcus* sp.	-	+ (7/7)
H	30	*F. columnare*	+ (4/4)	-

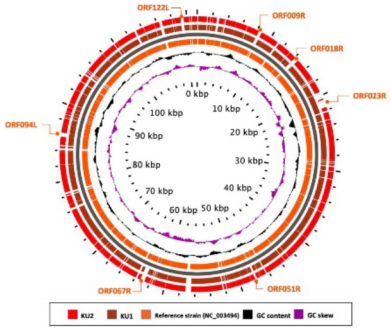


Figure 1 Circular map of ISKNV KU1, KU2 and reference genomes

RECENT STATUS AND ADVANCES ON AQUACULTURE AND GENETIC BREEDING ON IVORY SNAIL *Babylonia areolata* IN SOUTHERN CHINA

Caihuan Ke *, Jiangqiang Fu, Weiwei You, Xuan Luo

College of Ocean and Earth Sciences
Xiamen University
Xiamen 361102, PR China
chke@xmu.edu.cn

The ivory snail, *Babylonia areolata,* is dioecious mollusk that is mainly distributed across tropical and subtropical coastal areas in Southeast and East Asia, which is considered as a recently emerging maricultured species. In recent years, the aquaculture area is expanded gradually, with annual economic benefit of nearly half billion US Dollar. However, because of intensive breeding and the breeding environment was worsened, leading to the high mortality, thus causing great economic losses. In 2011, our group launched the project on genetic breeding of *B. areolata*. This research analyzed and compared the protogenesis traits of several geographic populations of *B. areolata* on the basis of growth and survival, and carried out mass selection, pedigree selection and crossbreeding. The selective response and realized heritability of four successive generations were conducted through mass selection. Microsatellite was used to evaluate the genetic variation level of these populations after successive selection. The hybridization effect of *B. areolata* by taking two populations were also analyzed with diallel crossing method, while the interaction effect between four genotypic groups and the environment were conducted at the same time. The new strain "Haitai I" was conferred for "New Variety Certificate" by Ministry of Agriculture in 2019, which displayed better growth trait and stronger stress resistance. This project would be better to solve the problems occurred during breeding process at present, and make *B. areolata* industry achieve better development.

MIXED-HABITAT ASSIMILATION OF ORGANIC WASTE IN COASTAL ENVIRONMENTS – IT'S ALL ABOUT SYNERGY!

Nigel Keeley*, Thomas Valdemarsen, Tore Strohmeier, Xavier Pochon, Thomas Dahlgren, Raymond Bannister

Institute of Marine Research, Tromsø, Norway
nkeeley@hi.no

Fish farms are increasingly situated in strong current sites above or near to mixed-bottom habitats that include organisms not normally considered in the context of organic enrichment. This study takes a holistic view of the benthic enrichment process by combining different survey techniques on complimentary spatial scales: conventional macrofaunal cores, larger-scale visual quantification of epibiota and eDNA metabarcoding of microbial communities. A large tube forming polychaete (*Arenicola marina*), normally found intertidally and living too deep for conventional sampling, was observed occupying an opportunistic niche in areas of high deposition and in very close association with Capitellid worm complexes. The surface-dwelling brittlestar, *Ophiocomina nigra*, was abundant at distances of 250 - 1000 m from Farm-B, suggesting a positive response to enrichment, but was displaced where sedimentation exceed 5 g m2 d-1. A corresponding gradient was evident within the sediment microbial communities, supporting established theories about ecosystem engineering and multi-species synergies for organic waste assimilation. Many of the bacteria present in the near-farm sediments were linked to the farmed fish and fish health issues suggesting one or two-way inoculation pressures. These functionally different benthic organisms are intrinsically linked and the resulting synergy has the potential to assimilate significant quantities of anthropogenically produced organic waste contributing to environmental sustainability.

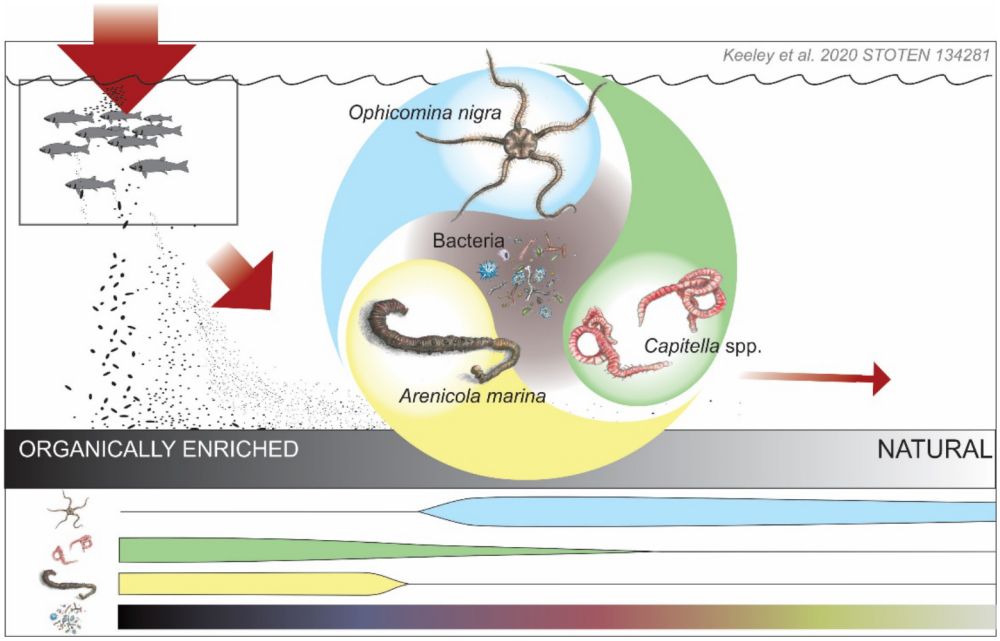


Figure 1 Stylistic representation of synergistic processes that combine and play and important role in the assimilation of fish farm wastes.

EVALUATION OF DIETARY SPRAY-DRIED PORCINE PLASMA ON IMMUNE PARAMETERS IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) AND PREVENTION OF *Vibrio parahaemolyticus* INFECTION

Arunothai Keetanon*, Niti Chuchird, Tirawat Rairat, Putsucha Phansawat, Chi-Chung Chou, and Joy Campbell

Department of Fishery Biology
Faculty of Fisheries
Kasetsart University
Bangkok, Thailand 10900
arunothai.ke@ku.th

Spray-dried porcine plasma (SDPP) is a protein-rich blood by-product obtained from abattoirs. It contains diverse biological components including immunoglobulins (Ig), albumin, peptides, enzymes, and growth factors. As a protein source, SDPP has been reported to promote the health of animals including pigs, chickens, and fish. Nevertheless, the effect of SDPP on shrimp health is less known. The aim of the current study was to investigate the health-promoting effects of SDPP in the diets of Pacific white shrimp.

Postlarvae-12 shrimp were randomly distributed into 20 fiberglass tanks with 200 L of water (27-29°C and 25 ppt salinity) with a stocking density of 80 shrimp/tank. Five experimental diets were formulated with different concentrations of SDP (AP 820, APC Europe, Granollers, Spain): control, SDPP at 1.5, 3, 4.5, and 6% diet. These experimental diets were fed to the shrimp 4 times/day. On day 45, five shrimp from each group were randomly selected for the immunological study which consists of total hemocyte count and phenoloxidase activity, whereas all survived shrimp were challenged with *Vibrio parahaemolyticus* by immersion method at the concentration of 10^5 CFU/mL for another 4 days. The shrimp body weight and mortality rate of each group were recorded at the end of the feeding trial.

Our result demonstrated that the immune parameters of the shrimp fed on 3-6% SDPP were significantly (p<0.05) improved compared to the control and 1.5% SDPP groups (Table 1). At 4 days after being challenged with *V. parahaemolyticus*, the mortality rate of the 3-6% SDPP groups was 52-55%, significantly (p<0.05) lower than that of the control shrimp (71%) (Table 2). The highest final body weight was observed in the 4.5 and 6% SDPP-fed shrimp which were 3.12 and 3.21 g, respectively, and significantly (p<0.05) different from the control (2.62 g) (Table 2).

TABLE 1. Immune parameters of the shrimp after feeding with different concentrations of SDPP for 45 days

Group	Total hemocyte count (10^6 cell/mL)	Phenoloxidase activity (units/min/mg protein)
Control	2.24±0.22^b	261.34±6.07^b
1.5% SDPP	2.40±0.10^b	265.12±9.37^b
3% SDPP	2.78±0.04^a	275.51±1.95^a
4.5% SDPP	2.85±0.05^a	276.13±2.78^a
6% SDPP	2.93±0.08^a	275.54±2.66^a

TABLE 2. The mortality rate (%) and body weight (g) after exposure to 10^5 CFU/mL *V. parahaemolyticus* for 4 days

Group	Mortality (%)	Body weight (g)
Control	70.83±1.67^b	2.62±0.06^d
1.5% SDPP	68.33±1.92^b	2.78±0.12^c
3% SDPP	55.00±1.92^a	2.98±0.08^b
4.5% SDPP	55.00±4.30^a	3.14±0.12^a
6% SDPP	51.67±1.92^a	3.21±0.04^a

FREE AMINO ACIDS MIX SUPPLEMENTATION ON DIET IMPROVES GROWTH PERFORMANCES, FEED UTILIZATION, ANTIOXIDANT AND IMMUNE RESPONSES, DIGESTIVE ENZYMES AND FATTY ACID COMPOSITION OF NILE TILAPIA FINGERLING *(Oreochromis niloticus)*

Kersanté Pierrick*, Eakapol Wangkahart, Le Reste Guillaume

BCF Life Sciences, Boisel, 56140 PLEUCADEUC – France
pkersante@bcf-lifesciences.com

Introduction

Mixes of free amino acids (MFAA) obtained from extensive hydrolysis of full protein chains are interesting candidates for aquaculture feeds. In addition to their specific amino acids profile, their low molecular weight ensure a fast and high level of assimilation with synergetic effects during the first development stages. Previous investigations on shrimp *(L.vannamei)* underline MFAA positive effects on zootechnical performances (Le Reste et al., 2019) and survival in case of bacteriological and viral challenges (Kersanté et al., 2021). The results presented here have been obtained from an eight-week study conducted to investigate the effects of a mix of 17 amino acids obtained from extensive hydrolysis of poultry keratin, on the growth performances, biochemical parameters, innate immune response, muscle composition, digestive enzyme activity and muscle fatty acid composition of Nile tilapia (*Oreochromis niloticus*) (Wangkahart et al., 2022).

Protocol

450 healthy juvenile Tilapia (*O. niloticus*), 4.76 ± 0.05g initial weight (IW) were purchased from Maha Sarakham province, Thailand. The fish were acclimatized to laboratory conditions in circular fiberglass tanks for 2 weeks, in the aquarium of the Division of Fisheries, Mahasarakham University. During this period, fish were fed with a commercial diet containing 32% of protein and 4% of fat (Charoen Pokphand Foods, CP, Thailand). After acclimatization, fish were allocated in 15 floating cages (2*1.5*1.5m^3) in five triplicates groups at a stocking density of 30 fish per cage, in an open circulatory freshwater system.

In order to meet or exceed the nutritional requirements of tilapia established by the National Research Council (2011), we formulated five isonitrogenous (32% crude protein) and isolipidic (4.2% crude lipid) diets, supplemented with MFAA.

Four different concentrations of MFAA were tested: 2.5 g/kg of feed, 0.25%, 5 g/kg of feed 0.50%, 7.5 g/kg of feed, 0.75% and 10 g/kg of feed, 1.00%, respectively MFAA0.25, MFAA0.50, MFAA0.75 and MFAA1.00. The MFAA was included into the pellet mix, before pelletizing.

Fish were fed twice a day (8am, 4pm). A daily feeding rate of 5% of the biomass was applied. It was adjusted every 2 weeks, according to fish growth. The experimentation was conducted during 8 weeks.

Results

The data underline interesting improvements of growth parameters with MFAA supplementation. A dose effect could be seen with best performances achieved for animals fed with MFAA1.00 treatment, generating significant gains on biomass evolution with final weight (FW) improved by 28.9%, weight gain (WG) by 31.5%, specific growth rate (SGR) by 8.9% and a feed conversion ratio (FCR) reduction of 24% after 8 weeks ($P<0.05$).

Interestingly, growth performances are also correlated with modifications in body composition indices and feed utilisation. These parameters clearly underlined better performances for all MFAA treatments ($P<0.05$), with significant gains, related to a dosage effect of MFAA, on protein efficiency ratio (PER) improved by 29.2%, carcass yield by 9.3% and a reduction of the viscerosomatic index (VSI) of 19.5% for MFAA1.00 ($P<0.05$).

(Continued on next page)

Fillet composition analysis underlined significant influence of MFAA on the fillet lipid content (+88% with MFAA0.75). Thirty-seven different fatty acids were exanimated in the muscle tissues of the fish following the different diet treatments. Most of the analysed individual monounsaturated fatty acids (MUFA) such as palmitoleic acid (C16:1), oleic acid (C18:1 n9c), and erucic acid (C22:1 n9), as well as the total amount of MUFA, were found to be higher in the muscle from fish fed any MFAA diet compared to the control group ($P<0.05$). Interestingly, linoleic acid (C18:2 n6c) and α–linolenic acid (C18:3 n3) levels were higher in the fish fed MFAA0.75 and MFAA1.00 diets ($P<0.05$).

In relation with these observations, the fillet color measured on ventral and dorsal regions was also significantly influenced with higher value of *L*, expressing the lightness and white color of the fillet, respectively +13.7% and +7.2% for the *L* value of the fillet ventral and dorsal regions with MFAA0.75 ($P<0.05$).

Digestive enzyme activies (amylase, lipase and proteinase) were significantly increased with MFAA0.75 and MFAA1.00 treatments, respectively from 34.9%, 10.4% and +39.9%, in comparison with the negative control ($P<0.05$).

Discussion

This study underlined some particularly interesting effects of MFAA when applied on Nile tilapia fingerling feed. Firstly, regarding growth parameters with positive effects on biomass and feed utilization. Interestingly, we underline strong improvements of VSI, fillet composition and fillet color. We can hypothesize a relation between these observations and a better assimilation of the feed due to an optimisation of the energy by the digestive system. In addition, we can hypothesize that the more pronounced white color of the fillet is probably in relation with a reduction of fat oxidation.

Interestingly, we noted a reduction of the VSI, potentially linked with a higher amylase activity generating a better utilization of the polysaccharides. This parameter probably induce a lower fat deposition around the viscera and a higher lipid transfer to the fillet. The results also revealed higher contents of important fatty acids such as oleic acid (C18:1 n9c), linoleic acid (C18:2 n6c) and α–linolenic acid (C18:3 n3) in fish fed with MFAA.
The strong effects of MFAA on digestive enzymatic parameters with positive impact on body condition indices and fillet quality open new development possibilities to improve fish feed utilization and farming performances.

This new field of application confirms their interest as a sustainable protein source converted into an efficient functional ingredient for fish nutrition.

Bibliography

Le Reste Guillaume, Kersanté Pierrick, Duperray Joël. 2019. Free Amino-Acids Mix made of Poultry Keratin as a new Functional Ingredient for White Shrimp (Litopeaneus vannamei) feed. Universal Journal of Agricultural Research 7(6): 203-209. https://www.hrpub.org

Kersanté, P., Le reste, G., Diringer, B., Quimi, J., Sergheraert, R., Duperray, J., 2021. Free amino acids mix made of poultry keratin improves survival of whiteleg shrimp post larvae (*Litopenaeus vannamei*) challenged with acute hepatopancreatic necrosis disease and white spot syndrome virus. Aquaculture International. 29, 879-890. https://doi.org/10.1007/s10499_021-00665-x.

Eakapol Wangkahart, Pierrick Kersanté, Po-Tsang Lee, Onanong Sanbut, Supap Nontasan, Anut Chantiratikul. Effect of Kera-Stim®50, a feed additive containing free amino-acid mix on growth, antioxidant and immune responses, digestive enzymes, and fatty acid composition in Nile tilapia (*Oreochromis niloticus*). Aquaculture 551 (2022) 737874. https://doi.org/10.1016/j.aquaculture.2021.737874

EFFECT OF INCREASING CONCENTRATIONS OF OMEGA 3- FATTY ACIDS BY SUPPLEMENTATION OF VEGETABLE OIL AND FISH OIL ON THE PERFORMANCE AND FATTY ACID PROFILE OF GENETICALLY MALE TILAPIA (GMT)

Noor Khan[1], Tahreem Iqbal[2] and Murat Arslan[2]

[1]Institute of Zoology, University of the Punjab, Lahore-Pakistan
[2]Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences
[3]Department of Fisheries, Ataturk University, Erzurum, Turkey
Corresponding address: noor.khan@uvas.edu.pk

The effect of vegetable and fish oil at different concentrations in genetically male tilapia was evaluated during 90-days trial. Fish were assessed on the basis of five diets with inclusion of 2 % fish oil (FO) for T1, 5 % FO for T2, 2 % linseed oil (LO) for T3, 5% LO for T4 and control. The observed results were significantly different ($P < 0.05$) among all treated groups in growth performance with best values of net weight gain, specific growth rate, percent weight gain, feed conversion ratio and feed efficiency for fish fed on 5 % LO rather than others. Proximate analysis for both periods showed significant difference ($P < 0.05$) in between all treated groups that showed similar trends, where crude protein contents were maximum in 2% FO fed feed and crude fat contents were highest in5% LO fed feed. However, fatty acid composition of liver was influenced by dietary treatment and sampling period. The 14:0, 16:0, 18:2 n-6, 18:3 n-3, 20:2 n-6, ∑SFA, ∑PUFA, n-6 and n-3/ n-6 fatty acids showed significantly ($p<0.05$) different results among all treatments, depending on the source of supplemented oil. EPA, DHA, ∑PUFA and n-6 showed highest values in muscle of fish fed upon 5% LO. Moreover, significant ($p<0.05$) results were observed in both periods with prominent values of 16:1, 17:1, EPA, ∑PUFA and n-6. These results concluded that linseed oil improved the fatty acid profile without compromising growth performance, feed efficiency and fish health of Tilapia.

PHYSIOLOGICAL EFFECTS OF DIFFERENT ANAESTHETICS IN COMMON CARP, *Cyprinus carpio*; OPTIMIZATION OF EFFECTIVE DOSAGE

Hossein Khara[1*], Mohaddeseh Ahmanezhad[2], Mina Rahbar[3], Ali Khodadoust[3]

Department of Fisheries, Lahijan Branch, Islamic Azad University, Lahijan, Iran, P. O. Box: 1616
Inland Waters Aquaculture Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran. P. O. Box: 66
Young Researchers and Elites Club, Lahijan Branch ,Islamic Azad University, Lahijan, Iran, P. O. Box: 1616
h.khara1974@yahoo.com

Despite frequent application of different anaesthetics, many questions regarding their effects on fish remain unresolved. The response of the fish species to anaesthetics are differ, so that the screening of dosages is often necessary. The objectives of this study were to identify the possible application of alternative anaesthetic agent instead of tricaine methane sulphonate (MS 222) and its optimal concentration for anaesthetizing fish. In this regard, particular attention was drawn to reduce detrimental effect of lethal dose of anaesthetic agents on stress-related changes in blood. For each anaesthetic test, fish were divided into two groups, blood samples immediately were collected after appearance of anaesthesia and the second blood sampling was done after recovery time. The results revealed significant changes in haematological parameters including white blood cells (WBCs), red blood cells (RBCs), haematocrit (Ht), and haemoglobin (Hb) between induction and recovery times of anaesthetics agents. Similar results were observed in some plasma parameters including cortical, glucose and lactate among all experimental treatments. Induction and recovery times for juvenile common carp and grass carp anesthetized by anaesthetic agents were dose-dependent. Based on blood biochemical parameters, tobacco can be recommended as novel and promising anaesthetics alternative to MS-222 with caution in view of its possible side effects.

APPLICATION OF GENOMIC PREDICTION AND MACHINE LEARNING FOR EFFICIENT BREEDING PROGRAMS IN BLACK TIGER SHRIMP (*Penaeus monodon*)

Mehar S Khatkar *, Nick N Wade, Md Mehedi Hasan, Casey Bajema, Jarrod Guppy, Peter C Thomson, Greg J Coman, Kyall R Zenger, Dean R Jerry and Herman W Raadsma

ARC Research Hub for Advanced Prawn Breeding
Sydney School of Veterinary Science
Faculty of Science
The University of Sydney
Camden, NSW 2570 Australia
e-mail: mehar.khatkar@sydney.edu.au

Large scale recording of phenotypes and DNA information under commercial pond environment is critical for implementing efficient breeding programs in aquaculture species. DNA information can help to overcome the limitations and challenges of pedigree recording and individual animal tagging. In addition, recent development in sensor technologies and imaging combined with pattern recognition are providing the avenues for developing the high-throughput recording systems in aquaculture. We generated a large resource population of black tiger shrimp (*Penaeus monodon*) using high-throughput phenotyping and genotyping for genetic studies and gene discovery, and implementing genomic selection, as part of the ARC Industrial Transformation Research Hub for Advanced Prawn Breeding, an Australian organisation consisting of a partnership among universities, CSIRO, and the private sector. This resource population consisted of 400 families of varying sizes across three generations.

We developed a custom-made phenomics system for recording images of shrimps in batches. This system was used to record digital images on a large number of animals (>30,000) at harvest under commercial pond environments. We also recorded manual weights and components traits of a proportion of these samples for constructing a training dataset for deep learning models. We will describe examples of high-throughput recording and accuracy of predicting of phenotypes from RBG images using deep learning models. We will discuss the results of analysis of genetic architecture of body size traits, accuracy of genomic prediction within and across generations, and different factors affecting the accuracy of genomic selection in black tiger shrimp.

INFLUENCE OF DIFFERENT LEVELS OF DIETARY ARACHIDONIC ACID SUPPLEMENTATION ON GROWTH PARAMETERS AND SURVIVAL OF ZEBRAFISH (*Danio rerio*)

Alireza Khiabani*[1&2], Abdol Samad Keramat[1], Hossein Oraji[1], Abolghasem Esmaili Fereidouni[1], Homayoun Hosseinzadeh Sahafi[3]

1. Department of Fisheries, Faculty of Animal Science and Fisheries, University of Agriculture and Natural Resources, Sari, Iran
2. Department of Agriculture & Natural Resources, University of Applied Science and Technology, Tehran, Iran
3. Iranian Fisheries Sciences Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO) Tehran, Iran
*Corresponding author: khiabani@uast.ac.ir

Long-chain polyunsaturated fatty acids (such as Arachidonic acid, ARA, C20:4n-6) have unique roles in controlling and regulating growth performance, lipid metabolism, cell membrane fluidity, and immune function in fish. This research was conducted to produce higher quality and resistant Zebrafish (*Danio rerio*) using arachidonic acid diet to achieve the best growth and survival rate. Five experimental groups were established and fed diets with different levels of ARA corresponding to 0 (Control), 0.5, 1, 2 and 4% of total fatty acid. Zebrafish larvae (20-days post-fertilization) were fed ad libitum 5 times daily for 10 weeks. Both weight and length were recorded to determine growth indices. Although there were significant differences in general growth demographics (length/weight) after the 10-week feeding trial, no significant differences in the overall survival rate of Zebrafish. Results of multivariate regression analysis showed that increasing arachidonic acid levels in the diet had a direct relationship with carcass fatty acid levels (R=0/97, P>0.05). Increase in dietary Arachidonic acid (up to 2%), maximum final weight (0.38 ± 0/01 g), body weight gain (0.32 ± 0/16), body weight gain percent (613.42 ± 28/44), daily growth rate (0.46 ± 0/02) and the lowest feed conversion ratio (2.24 ± 0/05) were higher than other treatments. The results show that ARA has a significant influence on the growth factors of Zebrafish.

EFFICACY OF NATURAL ANTIMICROBIALS FROM BLENDED ESSENTIAL OILS FORMULATION AGAINST AHPND IN *Penaeus vannamei* IN VIETNAM

Le Van Khoa*, Haig Yousef Babikian, Tigran K Davtyan, Rajeev Kumar Jha, Yusef Babikyan

Aquaculture Expert, Vietnam National University of Agriculture, Vietnam
Speaker* email: lvkhoa@vnua.edu.vn
lvkhoavn@gmail.com

The shrimp diseases like the White spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) have appeared in almost all major shrimp-producing countries of South East Asia. Most of the available products in the market could not achieve the successful outcome as per expectation. A formulation was developed using blended essential oils as an anti-pathogen like, V. *parahemolyticus* and White Spot Syndrome Virus of shrimp. Vietnam is one of the significant suffering countries due to AHPND and was selected for the trial site. The formulation was first tested and optimized at Can Tho University and the Vietnam National University of Agriculture, Vietnam. The essential oil blend formulation (EOBF) was applied in the tanks before the challenge to the shrimp. Shrimp were challenged by V. *parahemolyticus* -AHPND strain, obtained from the University of Arizona, USA, after seven days of EOBF application. There was 100 % survival recorded in the EOBF applied groups, whereas 57 % cumulative mortality was recorded in the control groups. Similar results were obtained against the White sport syndrome virus. Later, field trials and observations were performed in several farms in the Quang Ninh, and Nam Dinh provinces of Vietnam using dose 0.4 ppm applied two times a week. The antimicrobial essential oil blend could significantly reduce the virulence of *V. parahaemolyticus* and WSSV in the shrimp ponds. The use of essential oil blend formulation resulted in better survival, growth, and productivity in the shrimp.

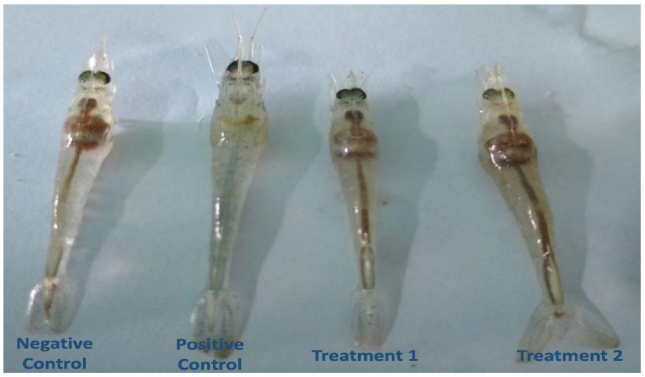


Figure: Healthy and moribund Shrimp after the AHPND challenge in a bioassay lab trial. Treatment and negative control shrimp are healthy, whereas positive control shrimp have typical clinical signs of AHPND.

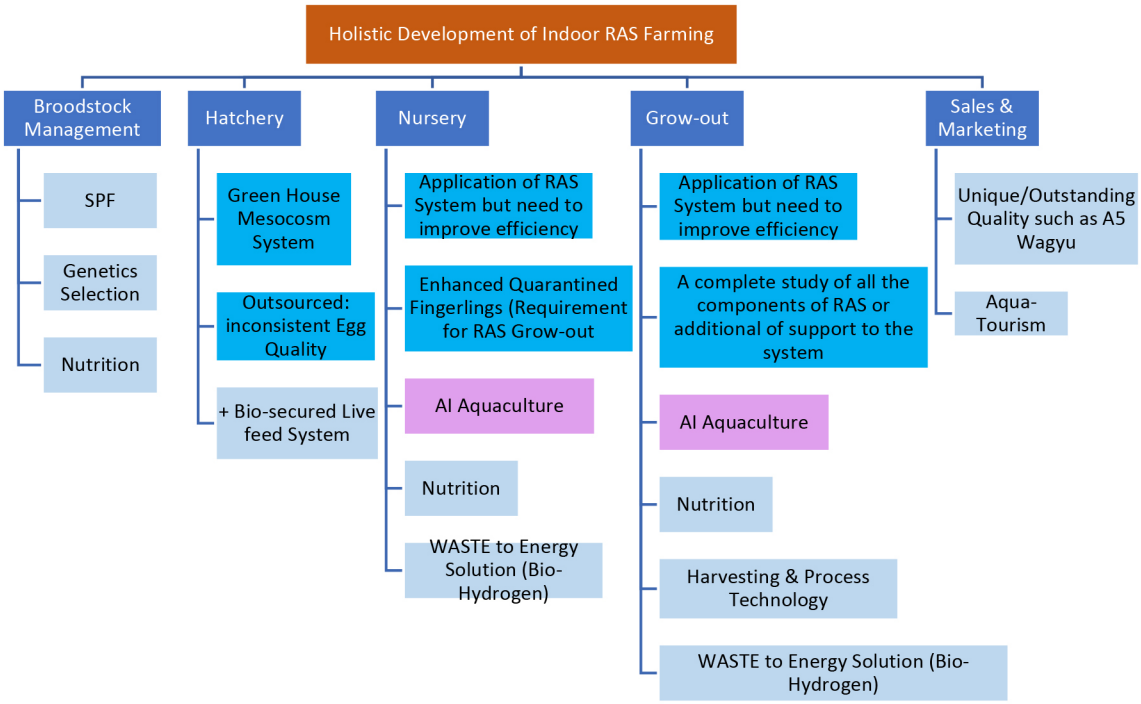
HOLISTIC DEVELOPMENT OF INDOOR RAS FARMING FROM FARMER TO FARMER

Jia-Zin Khoo* and Eng-Wah Khoo

Sepang Today Aquaculture Centre Sdn. Bhd.
1, Jalan Tasik Ara 2
Pantai Sepang Putra
43950 Sg. Pelek Selangor, Malaysia

RAS which also known as Recirculating Aquaculture System is very common in European countries. Most of the farmed species is Salmon or trout. In Asia wise, especially in China there are RAS farms for Seabass, Hybrid Grouper, Turbot & etc. Malaysia is in a very early stage of development for RAS farming even though started very early in 2008, while Singapore is picking up quick due to the food security policy since pandemic covid-19. However, the development of RAS shall not be focused on the system equipment itself only. It should be in several areas holistically such as broodstock management, hatchery technology (Green House Mesocosm), optimized RAS design factor a.k.a Nutricix™ (which include culture tank design, optimized flow rate, solids removing rate, CO_2 degassing rate, bio-treatment optimized sizing) for nursery (Enhanced Quarantine Fingerlings a.k.a EQF) & grow-out, solids waste recycles as well as Product Marketing (taste) & Quality Assurance. These areas should also be integrated with Artificial Intelligence for an optimization along the development. And Hybrid Grouper *Epinephelus lanceolatus x Epinephelus fuscoguttatus* would be the species that should be focused on Southeast Asia RAS players.

FIGURE 1: Development areas to be focused for indoor ras farming



SURVEY OF POTENTIAL FEED INGREDIENTS FOR ASIAN SEABASS (*Lates calcarifer*) FARMING IN CAMBODIA

Sros Khum*, Ramana Oem, Phearum Hoeun, Chankakada Mak, Asda Laining, Usman, Michael A. Rimmer, Viseth Hav and Somony Thay

Department of Aquaculture Development, Fisheries Administration
186 Preah Norodom Blvd., Sangkat Tonle Bassac, Khan Chamcar Mon, Phnom Penh, Cambodia
sroskhum@gmail.com

The expansion of cage aquaculture, particularly for Asian seabass (*Lates calcarifer*), in Cambodia is increasing the demand for low-value 'trash' fish and fishmeal as feed sources. The negative aspects of using 'trash' fish as an aquafeed are well documented, and in Cambodia the cost of 'trash' fish is increasing year by year because of increasing demand. To reduce feed costs and increase sustainability of cage aquaculture, we have been evaluating the availability of local feed ingredients that can be used for compounded aquafeeds.

To determine the availability of feed ingredients, we surveyed several sources in Preah Sihanouk province. Maximum availability of fishmeal is 216 tonnes per month from April to May which is the period of peak catches, and minimum availability is about 180 tonnes from October to November when catches decline because of the rainy season. The availability of squid followed a similar pattern with peak catches, and availability, in April, and minimum availability in October. In addition, the Angkor beer factories in Preah Sihanouk province produce fermented brewery yeast as a waste product, with 9–13 tonnes available monthly. Other potential ingredients that are readily available include rice bran, soybean, *Leucaena* leaf, and *Moringa* leaf.

Proximate analysis of a sub-set of ingredients was carried out according to AOAC (1999) methods to provide data for feed formulation (Table 1). Future research will focus on developing feeds for Asian seabass that incorporate local ingredients. In particular, we are evaluating the use of brewery yeast as a protein source to reduce the proportion of fishmeal in diets for Asian seabass.

TABLE 1. Proximate composition of potential feed ingredients sampled in Preah Sihanouk province, Cambodia.

Ingredients	Proximate composition (%)				
	Protein	**Lipid**	**NFE**	**Ash**	**Moisture**
Fish meal	61.1	5.2	1.32	21.0	11.5
Soybean meal	33.9	13.7	33.4	5.0	4.3
Brewery yeast	36.4	0.2	52.0	5.6	3.8
Leucaena leaf	24.9	6.4	42.1	10.8	5.9
Moringa leaf	24.3	8.1	37.3	9.5	6.4
Coconut meal	9.1	64.7	1.3	2.6	3.3
Rice bran	13.5	13.7	46.7	7.8	9.0

GENOME SEQUENCING OF BARRAMUNDI (ASIAN SEABASS; *Lates calcarifer*) FOR DEVELOPMENT AND VALIDATION OF A SNP GENOTYPING TOOL

James Kijas[1*], Sean McWilliam[1], Dan Richards[2], Bob Richards[2], Wagdy Mekkawy[3], Curtis Lind[3] and Moira Menzies[1]

[1] CSIRO Agriculture and Food, Queensland Bioscience Precinct, Brisbane, Queensland, 4067, Australia
[2] Humpty Doo Barramundi, Hobart, Tasmania, 7004, Australia
[3] CSIRO Agriculture and Food, Hobart, Tasmania, 7004, Australia

The development, implementation and improvement of applied breeding programs in aquaculture is an area of intense activity. The Australian farmed barramundi sector now has sufficient scale to justify the development of family-based breeding. We describe the development of a SNP tool formatted for routine commercial DNA profiling and test it in terms of i) informativeness and allele frequency distribution, ii) ability to detect genetic relatedness and iii) assigning parentage in the progeny of group spawning events. To begin development of the SNP tool we performed whole genome resequencing using a discovery panel of 10 Australian barramundi. The resulting Illumina short read data set was trimmed and mapped against the available 670 Mb reference assembly (Vij et al 2016). First pass variant calling identified a total of 3,731,369 raw variants. These were subsequently subjected to a series of quality filters to identify a set of 39,873 high confidence loci. Given the discovery panel consisted of individuals from a single population, published sequence data from an additional 17 wild caught barramundi from Australia and South East Asia was downloaded (Vij et al 2016) and SNP calling performed using the same parameters. Intersect analysis revealed 83% of SNP detected in the Australian population were independently called in the set of 17 wild caught fish (33,135 / 39,873 SNP). A key objective was to develop a low-density SNP genotyping tool that could be cost effectively deployed in applied breeding. Additional prioritisation steps then identified SNP that were located within the coding genes (1,637 SNP) before a final set of 337 SNP were formatted for analysis using kompetitive allele-specific PCR (KASP) at the Center for Aquaculture Technologies (CAT, San Diego California).

A set of 771 individuals were genotyped, resulting in high rates of both SNP (93%) and samples (97%) passing quality filtering based on minimum call rates and genotype frequency testing. Genotypes from samples processed in multiple batches were compared to estimate genotyping accuracy. A total of 1107 comparisons were evaluated, resulting in an estimated genotyping error rate of 0.18% (2/1107). To investigate levels of informativeness, estimation of minor allele frequency (MAF) revealed the majority of loci (215/282 or 76%) had high MAF (> 0.30), confirming the SNP tool can be expected to have high utility for applications in applied breeding.

We tested the SNP tool's ability to distinguish between different types of pairwise relationships using R_{XY}_*LR* (Pew et al 2015). Full-sibs and parent-offspring pairs were unambiguously distinguished from unrelated animals, while the majority of half-sib pairs were convincingly distinguished from unrelated animals. We next tested the tools ability to detect population substructure. Clear blocks of elevated allele sharing (A_S) were evident, and comparison with the results of formal parentage revealed A_S blocks identified separate full-sib families. Taken together, the results confirmed a SNP tool containing only a few hundred loci is sufficient to support applied breeding applications in group spawning species such as barramundi.

MOLECULAR CHARACTERIZATION AND EXPRESSION ANALYSIS OF THE C-TYPE LYSOZYMES IN YELLOWTAIL CLOWNFISH (*Amphiprion clarkii)*

Gaeun Kim, K.P. Madushani*, Seongdo Lee, Hanchang Sohn, W. M. Gayashani Sandamalika, Jehee Lee

Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province 63243, Republic of Korea
E-mail: sa02048@naver.com

Lysozymes are the important enzyme factor of the innate immune system to protect from bacterial infections. In this study, an ortholog of chicken-type lysozyme (*AcLysC*) from the yellowtail clownfish (*Amphiprion clarkii)* was identified and characterized structurally and functionally. The full-length cDNA sequence (595 bp) of AcLysC is comprised of an open reading frame made up of 429 bp, encoding a polypeptide of 127 amino acid (aa) with a predicted molecular mass of 14.2kDa. *In silico* analysis of AcLysC revealed the presence of signal peptide at 1-17 aa and there are two catalytic residues (Glu^{50}, Asp^{67}) and three Ca^{2+} binding sites (Thr^{98}, Leu^{103}, Ala^{104}). Homology studies indicated that AcLysC showed the highest sequence identity (98%) with of *Amphiprion ocellaris*. In healthy clownfish, *AcLysC* mRNA showed a constitutive expression in all the tissues examined, with the highest expression in liver and the least expression in blood. In response to the immune challenges lipopolysaccharide (LPS), *Vibrio harveyi*, and poly I:C injection, the expression of AcLysC was significantly upregulated. The recombinant protein of AcLysC was produced by cloning in to pMAL-c5X vector and purified using maltose affinity chromatography method. In protein assay, lytic activities of AcLysC were highly active at pH 3.0 to 4.0. These results provide evidences for the vital immunological role and bacteriolytic potential of AcLysC for the protection of yellowtail clownfish.

OBSERVATION OF GROWTH AND NEURONAL-RELATED GENETIC CHANGES IN ZEBRAFISH *Danio rerio* USING THREE CHEMICALS (ENU, COLCHICINE AND HYDROXYLAMINE)

*Ki-hyuk KIM [a], Ju-hyeon JANG [c], Hye-na MOON [a], Jin NAMGUNG [b] and In-kyu YEO [c]

a Department of Major of Aquatic Life Medicine, Jeju National University, Jeju 63243, Republic of Korea
b Education & Research Group for Future Strategy of Aquatic Life Industry, Jeju National University, Jeju 63243, Republic of Korea
c Department of Major of Aquatic Life Science, Jeju National University, Jeju 63243, Republic of Korea
Email : rlgur7896@jejunu.ac.kr

Chemical mutagenesis substances have been studied in various ways, such as body color change by inducing mutations for the purpose of developing application technologies for the aquarium fish industry. In this study, three chemical mutants of three chemical mutagenic substances were immersed in concentrations of 1, 2, 3 and 5 mM, and then treated to find an appropriate concentration. As a result, the optimal concentration of zebrafish was confirmed at 2 mM. As a result, they acquired the F1 generation and three chemical mutagenic substances, 1-ethyl-1-nitrosourea (ENU), colchicine and hydroxylamine, were used to investigate the effect on zebrafish (*Danio rerio*) immunity (lysozyme), growth-related (ghra) and nerve-related (nr4a2b) genes.

The zebrafish breeding water was immersed for 1 hour 3 times for 6 days at a concentration of 2 mM of each chemical mutagenic substances. And then after7 days, the brain, liver and kidney were extracted and total RNA was extracted. After total RNA was synthesized into cDNA, changes in genes were analyzed using quantitative PCR.

After immersion in 2 mM concentration, ghra did not show a sex difference in ENU and hydroxylamine in gene expression analysis, but showed more than double the expression in males of colchicine compared to females of zebrafish. In contrast to growth, lysozyme, an immune-related gene, did not show a male and female difference in colchicine, and showed higher expression in females than in males in ENU and hydroxylamine. In addition, it showed a significant increase compared to the control. The nr4a2b gene expression showed similar to that of the control in F1 crossed with normal males and hydroxylamine females. Our results, ENU and hydroxylamine at 2 mM affect the cleavage, and colchicine can be male of zebrafish. And results related to immunity and growth related to mutant substances in previous studies have not been reported much, and it is judged that the results of this study can be used as basic data. And also, these results will compare and analyze the mutation changes in F2, the offspring of F1, following the previously conducted in zebrafish treated with mutation inducing chemicals and their progeny F1.

APPLICATION OF RAS TECHNOLOGY IN HATCHERY PRODUCTION FOR ASIAN SEABASS *Lates calcarifer*

Amos Chee Boon KOH*, Rudy Hidajat, Wei Li Quek, Yong He, Yek Seng Foo

Marine Aquaculture Centre
Singapore Food Agency
11 St John Island
Singapore 098659
KOH_Chee_Boon@sfa.gov.sg

The use of outdoor ponds has been the prevalent method for hatchery production in South East Asia. In Singapore, due to constraints in land availability and seawater accessibility, farms are transforming towards the use of Recirculating Aquaculture System (RAS) for hatchery production. RAS overcomes the constraints of outdoor pond culture and indoor flow-through system as it requires a small foot-print, reduces seawater usage and allows better control of water parameters. The closed recirculating aquaculture system also helps to minimise the risk of disease with proper treatment of incoming water. With the application of RAS Technology in hatchery production, we have developed the hatchery protocols for a low-salinity RAS to manage Big-belly disease syndrome (BB), a highly pathogenic bacterial disease that affects Asian Seabass causing mortality to reach up to 70% within a week. By lowering the salinity in a RAS, we could prevent BB disease outbreak as the culture condition becomes unfavourable for the halophilic bacteria. This topic describes the hatchery protocol developed for Asian Seabass in low-salinity RAS and include some examples of RAS application in commercial hatcheries.

A LABORATORY BIOASSAY FOR EVALUATING THE EFFICACY OF ANTI-FOULING PAINTS ON *Ectocarpus siliculosus* USING ITS CIELAB COORDINATES, UNDER A FLOW-THROUGH CONDITION

Ryuji Kojima*, Nobuyoshi Nanba, and Cyril Glenn Perez Satuito

National Maritime Research Institute
6-38-1, Shinkawa, Mitaka, 181004 Tokyo, Japan
kojima@m.mpat.go.jp

Biofouling of ships leads to increasing fuel consumption and may possibly cause translocation of non-indigenous species to another marine environment where they may cause significant impact on the local ecosystem. Anti-fouling paints play an important role in the prevention of biofouling in ships. In this regard, the authors have already conducted efficacy tests of anti-fouling paints using mussels and barnacles. The authors focused on using algae as the test organism in a bioassay. The laboratory bioassay was designed and its applicability for testing the efficacy of antifouling paints was investigated. Five types of antifouling paints with hydration type coatings containing 0, 5, 10, 20 and 40 wt.% of Cu_2O were prepared as test paints. PVC plates used in the laboratory bioassay were 50 mm x 50 mm x 2 mm in size. The test plates were coated on one side with the test paint. The panels were aged using a dynamic rotating device under controlled condition with light shielding (water temperature: 20 ºC, rotation speed: 10 knots, period: 45 days) prior to the bioassay. The filamentous brown alga, *E. siliculosus*, which is one of the most common fouling macroalgae in ships, was provided by Kobe University Macroalgal Culture Collection. An algal suspension of 10 ml (ca. 1mg as algal dry weight) was filtered through a nitro cellulose membrane filter (*f*=47 mm, pore size 8.0 mm) under vacuum filtration. The membrane filter with algae was cultured in PESI medium (temperature: 20 ºC, light cycle: 12 h. light with irradiance of 20 mmol photons mmol/m^2/s / 12 h. dark, period: 5 days) in a still water condition. After culturing the algae, the surface of the membrane with algae was affixed to the test and control panels. The laboratory bioassay with a flow-through system was conducted under a controlled condition (flow rate: 0.66 turnover/h, water temperature: 20 ºC, light condition was same as with the culturing condition, period: 1 day), using boiled seawater as the test medium. The efficacy of antifouling paints was evaluated by the parameters (*e.g., L**) of *E. siliculosus* after the bioassay. The value of *L** increased with increasing Cu_2O content (Fig.1). A linear regression analysis was performed on the relationships between values of *L** and survival rate of the algae. Results showed that the value of *L** decreased with an increase of survival rate (Fig.2). The newly designed bioassay using brown algae under a flow-through system was successfully tested for screening anti-fouling paints. The assessment of the estimated paint performance from the bioassay was confirmed using correlation patterns between the value of CIELAB (*L**, *a**, *b**) and the survival rate of the algae. This study also proved to be a significantly consistent method for assessing the effectiveness of existing or future antifouling paints.

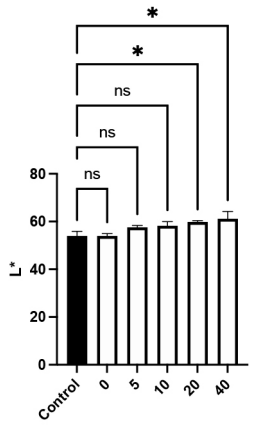


Fig.1.Difference in *L between different Cu_2O contents.**

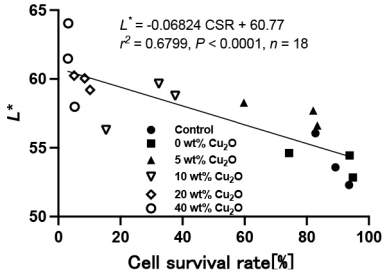


Fig.2. Correlation between *L and cell survival rate.**

COMPLEMENTARITY BETWEEN HEAT-SHOCK TREATMENTS AND VACCINATION IN BARRAMUNDI AND TILAPIA: EMPIRICAL AND LABORATORY EXPERIENCES BASED ON ALAIN MICHEL METHODOLOGY AND FINDINGS BETWEEN 2005 AND 2022

Cedric Komar*, ALAIN MICHEL†, Hubert Crieloue, Eric Vigne, Lauke Labrie, Adrian Astier, Roberto Cascione, Luc Grisez, Neil Wendover, Mark Amechi & Jérôme Bosmans

*Ictyodev, 12 Route de Bizeneuille 03170 Deneuille-Les-Mines, France
Cedric.komar@ictyodev.com

Diseases affecting larval stages and juvenile fish have long been a major bottleneck in the development of barramundi farming. From an adverse Barramundi husbandry incident, Alain Michel empirically discovered that larval fish affected by VNN could be treated by daily heat-shock treatments during few days.

From this observation, Alain Michel proposed a new paradigm: “in aquaculture, it is an utopian dream to fully exclude pathogens from the environment; they are part of the aquaculture equation and so we should find a way to live with them”.

Methodically, protocols were then developed using heat-shock treatment and controlled presence of the disease to treat the other diseases of the larval and juvenile stages of barramundi. Once they were 5-10 g, they were healthy and resistant to several viral and bacterial diseases and could be vaccinated against *S. iniae* before transfer to sea cages. Several years later, heat-shock treatments were also developed directly in sea cages to address other common diseases. A complementary approach of heat-shock treatment and vaccination has since been the key to address major diseases observed during the barramundi farming life cycle.

In 2019, a massive iridovirus outbreak wiped out almost the entire commercial Tilapia production of Lake Volta (Ghana) (15,000 tons annual production). In collaboration with a large commercial farm, Tropofarms, Alain Michel developed a similar heating protocol to treat tilapia juveniles. This heat-shock treatment allowed the survival of Tilapia that could subsequently be vaccinated at 10 g against *S. agalactiae,* a deadly bacteria found not sensitive to heat shock treatments. Tropofarms continues to use the heat-shock therapy today and lives with the virus. Alain saved the tilapia industry in Ghana.

It is unlikely that all diseases can be controlled by heat-shock treatments. Heat-shock treatments were observed most effective in cases of viral infections and bacterial infections for which the optimal growth temperatures of the pathogens were below the heat treatment temperatures. It is probable that the heat-shock treatment is effective in these cases because it reduces pathogen replication, allowing the fish to mount an immune response. In tilapia farming, like in barramundi farming, the major diseases can be controlled by a complementary approach of heat-shock treatment and vaccination.

Today the methodology developed by Alain Michel is successfully used in many South-East Asia farms with several tropical species. Over the past years, Alain has also been testing his methodology on other aquatic species, both temperate and tropical.

TRANSCRIPTOME ANALYSES ON AMBERJACK SKIN FLUKES, *Benedenia seriolae* AND *Neobenedenia girellae*

Hidehiro Kondo, Yuki Tanabe, Keigo Kobayashi, Germaine Lau Gek Khin, Reiko Nozaki, Sho Shirakashi, Hirofumi Yamashita, Hidemasa Kawakami, Ikuo Hirono

Laboratory of Genome Science
Tokyo University of Marine Science and Technology
Minato, Tokyo, Japan 1088477
h-kondo@kaiyodai.ac.jp

Amberjacks *Seriola* sp. are the most abundantly farmed fish in the Japanese marine aquaculture industry. The industry has been suffered from various infectious diseases. Various antibacterial agents have been used to control bacterial diseases and more recently vaccines have been developed for various bacterial and viral diseases. On the other hand, the industry is chronically suffering from infectious diseases caused by monogenean parasites, such as skin flukes. However, unlike bacterial and viral diseases, no effective prophylactic and long-lasting treatments against the parasites have yet been developed. Thus, vaccination for monogeneans is in great needs and is expected to overcome the economic damages caused by the monogenean diseases.

To develop an effective vaccine against infectious pathogen, cultivation of the target pathogen is normally required to prepare the antigen. However, there are no successful methods to propagate the monogenean parasites *in vitro*. Thus, subunit vaccine might be a solution for the vaccine development. In this study, we performed transcriptome analyses on two species of problematic skin flukes in amberjack farming, *Benedenia seriolae* and *Neobenedenia girellae* aiming to identify the antigenic protein genes.

Firstly, we performed transcriptome analysis on the adults *B. seriolae* and identified more than 20,000 of potential transcripts. Among the identified transcripts, we focused on the protease genes, which play important roles during initial infection in mammalian parasites, and identified serine proteases, cysteine proteases, aspartic proteases and metalloproteases. Next, we performed the analyses on *N. girellae* of different developmental stages, namely eggs, hatched larva and adults. When the abundance of mRNA levels were compared, certain transcripts were differently regulated between the developmental stages. These results provided not only candidate genes for the vaccine antigen but also important insights into the infection mechanisms of the skin flukes.

WHOLE GENOME SEQUENCE COMPARISON OF RED SEABREAM IRIDOVIRUS ISOLATED FROM CULTURED MARINE FISH IN JAPAN

Hidehiro Kondo*, Channapha Sakseepipad, Reiko Nozaki, Yutaka Fukuda, Hidemasa Kawakami and Ikuo Hirono

Laboratory of Genome Science
Tokyo University of Marine Science and Technology
Tokyo 108-8477, Japan
h-kondo@kaiyodai.ac.jp

Background: Red Seabream Iridovirus (RSIV) is a causative agent of Red Seabream Iridoviral Disease (RSIVD). RSIV causes mass mortality in more than 30 species of cultured marine fish in Japan. This virus is a member of the genus Megalocytivirus belongs to the family Iridoviridae. The comparative genomic analysis to classify Megalocytiviruses based on the major capsid protein (MCP), ATPase and DNA polymerase gene sequences were showed that RSIV-type Megalocytivirus can be divided into two subclusters: genotype I and genotype II. RSIV Ehime-1, the type strain of RSIV isolated in Japan, belongs to genotype I. RSIV genotype II is recently predominates, and includes other major RSIV strains isolated in Japan, East and Southeast Asia. In addition, other isolates such as rock bream iridovirus (RBIV), orange-spotted grouper iridovirus (OSGIV) giant seaperch iridovirus (GSIV) are also classified into the genotype II of RSIV clade. The objective of the present study is to obtain deeply insights on the comparative variations of Megalocytivirus by comparing whole genome sequences of RSIV isolates in Japan.

Methods: In this study, we sequenced the whole genome of 20 RSIV-type Megalocytivirus isolated from infected fishes in Oita and Ehime prefecture in 2008-2019 by next generation sequencer MiSeq (Illumina) and analyzed these genomic relationships with representative Megalocytiviruses from Genbank.

Results and Discussion: The complete genome sequence of 20 isolates had lengths of 112-113 Kbp. The comparative genome analysis of RSIV isolates with representative Megalocytiviruses members showed that the Japanese isolates categorized as the RSIV genotype II cluster. The phylogenetic network showed relationship of 18 haplotypes of RSIV whole genomes of 7 isolates from Ehime and 13 isolates from Oita. The number of polymorphic sites were detected as 3,577 sites. Whole genome sequence information is a helpful tool to identify genetic variations and provide more precise knowledges in RSIV-type Megalocytivirus in Japan. In future, the relationship between the individual RSIV isolates with their host species, culturing situation, and so on, might help in a better understanding of transmission and prevention of RSIV disease.

NAUPLII QUALITY CRITERIA FOR *Penaeus vannamei* SHRIMP

Kongnuan Pantipa[1], Phuthongphan Rattayaporn[1], De Maesschalck Celine[2] and Wouters Roeland[2]

[1]INVE Tropical Aquaculture Research Center (ITARC), Chonburi, Thailand
[2]INVE Aquaculture, Hoogveld 93-9200 Dendermonde, Belgium
Contact person: p.kongnuan@inveaquaculture.com

The naupliar stage represents the first larval stage and its quality is a direct reflection of broodstock condition, because at this stage there is still a strong dependence on the nutrients transferred from the female. Besides nutrition, also broodstock age, environment (e.g. temperature), health status and genetics have an influence on nauplii quality. How nauplii condition or nauplii "quality" relates to performance during further larval stages is barely described in literature, but most hatcheries do can attempt to predict larviculture performance in order to stock larval rearing tanks yes or no, by looking at nauplii deformities or nauplii color.

At the INVE Tropical Aquaculture Research Center (ITARC) in Thailand, a set of parameters is routinely measured on nauplii and zoea 1 stages and used to calculate a Nauplii Quality Index (NQI) (Table 1). In the NQI formula, a weight factor is assigned to each parameter in accordance to its estimated predictive value for further successful larviculture.

This presentation summarizes our findings related to nauplii quality criteria and correlations with performance during the larval rearing cycle. Advantages and limitations of using a calculated Nauplii Quality Index as cut-off for larval tank stocking will be discussed.

Table 1. Parameters used to calculate a Nauplii Quality Index (NQI)

Broodstock condition		Score
Spawn size	1= <75000; 2=76000-100000; 3=100000-150000; 4 > 150000	
Spawn order	1= more than 5; 2=4; 3=3; 4= less than 3	
Days since ablation	1= more than 90d; 2=60-90d; 3=30-60d; 4= less than 30d	
Egg hatching	1= below 50%; 2=50-64%; 3=65-84%; 4=85-100%	
Nauplii stage determination		
Stage by Morphology	1= nauplii1; 2=nauplii2; 3=nauplii3; 4=nauplii4; 5=nauplii5	
Nauplii Quality (stage N4-N5)		
Deformities	1= more than 5%; 2=5-4%; 3=3-2%; 4=1-0%	
Phototaxism	1= not active; 2=slightly active; 3=normal; 4=very active	
Brightness	1= very pale; 2=pale; 3=medium; 4= intense	
Colour	1= gray; 2=gray-brown; 3= yellow-brown; 4= orange-red-brown	
Length	1= very small; 2=small; 3=normal; 4=big	
Survival to Zoea1	1= 0-79.9%; 2=80-83,4; 3=83,5-87.9%; 4=88-93.9%; 5=94-100%	
Nauplii Quality Index	**NQI:**	

EFFECTS OF BETAINE ON SURVIVAL RATE, GROWTH PERFORMANCE, AND THE TOTAL NUMBER OF VIBRIO IN THE HEPATOPANCREAS AND INTESTINE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Wolfgang Koppe*, Niti Chuchird, Arunothai Keetanon, Tirawat Rairat, David A. H. Sutter, Ana Gavrău

Simplyfish AS, Baldersgate 12, 4011 Stavanger, Norway; Wolfgang.koppe@simplyfish.no

Introduction

Betaine is a multifunctional nutrient which was first discovered in sugar beet juice. Betaine occurs naturally in plants, animals and microorganisms. Betaine has become a core ingredient in formulations for land animals. With three methyl groups and a bipolar structure, betaine has a broad range of functional benefits. Both functions of betaine, as an efficient methyl group donor and as an organic osmolyte, play a key role when animals are exposed to stressors. The purpose of this study was to examine how supplemental dietary betaine influenced productive traits and *Vibrio*-associated indicators in *L. vannamei*. To induce a dietary challenge, two basal feed recipes were formulated containing low and high levels of solvent extracted soybean meal (SBM).

Material & Methods

The trial was conducted at the ABRC laboratory, Kasetsart University, Thailand. SPF white shrimp PL 12 were distributed into six groups in triplicate tanks (120 PL/m^2). Two basal feeds were formulated: C1 with 3% SBM and C2 with 23% SBM. Betaine was added in its natural form derived from the product ***Actibeet (AB)*** at levels of 0.4% and 1.0%, resulting in six experimental feeds. Experimental diets were fed for 45 days. Final body weights, survival rates, *Vibrio* spp. counts in hepatopancreas and intestines were determined.

Results & Discussion

Whiteleg shrimp reacted to the type of base diet (SBM level) and to the inclusion level of betaine (none, 0.45, 1.0%). Increasing levels of betaine to both basal formulations increased growth performance and reduced the number of *Vibrio* in tissues dramatically.

It is suggested that betaine supplementation at dietary concentrations above 0.4% improves performance and supports the robustness of juvenile *Litopenæus vannamei*.

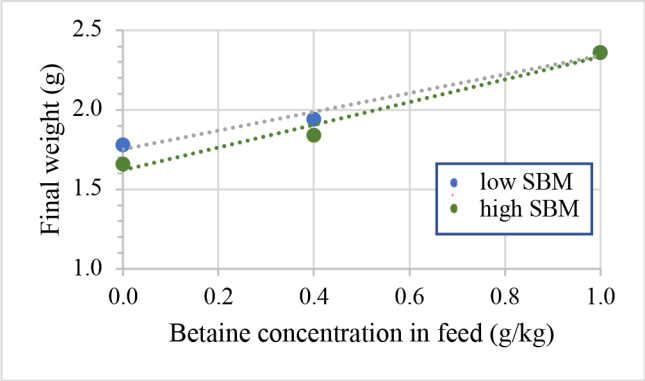


Figure 1: Average final body weight of Pacific white shrimp at 45 days of feeding with different diets.

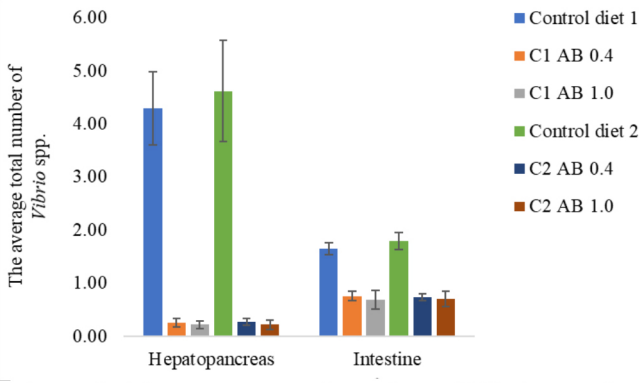


Figure 2: The average total number of Vibrio spp. in the hepatopancreas and intestine of pacific white shrimp at 45 days of feeding with different diets. Different letters indicate significant differences across means ($P < 0.05$).

AI-POWERED PASSIVE ACOUSTIC FEEDING SYSTEM FOR SHRIMPS, IMPROVING FEEDING EFFICIENCY BY UP TO 30%

Somesh Kumar*, Ivan Tay

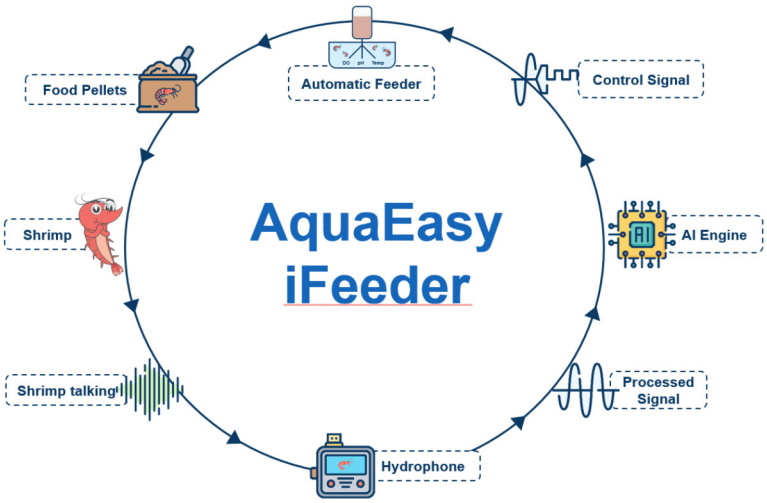
AquaEasy Pte. Ltd.
38C Jalan Pemimpin, #03-02
Singapore 577180

Inland shrimp aquaculture has been on an upswing since the last decade. There is a constant push to increase production output leading to an increase in stocking densities. The major challenge with intensive farming is to determine the most efficient feed management strategy in terms of timing and quantity of feed. Feed being the primary cost factor in shrimp production, getting this answer right becomes critical. Unlike livestock and poultry wherein visual inspection aids in optimizing the feeding process, turbid waters of a shrimp pond make it nearly impossible to see beyond a few centimeters. Too little feed will lead to growth stagnation, while too much will increase costs, foul the water, and trigger disease-related issues. So how do we solve this complex problem?

Passive acoustics monitoring (PAM) is an exceptional technology to analyze the feeding behavior of shrimp. Shrimps like other crustaceans produce several different sounds due to their movement, molting, social behavior, stress, etc. However, one sound, in particular, can be used to optimize feeding. The "click-click" sound produced during the consumption of feed by the mandibular occlusion is a clear indicator of the appetite levels of shrimp.

We have identified the unique patterns in sound signature of Vanammei shrimps from years of research and have effectively managed to reduce FCR by up to 30% under commercial shrimp farming production systems. We started experimenting in labs in Singapore and gradually expanded to commercial-scale farms in Singapore, Indonesia, and Vietnam. The technology paired with AIoT further optimizes feeding based on a variety of variables such as water quality, the behavior of shrimp, environmental conditions, and feed demand. The system tirelessly works for the success of the farmer, freeing his time to focus on other aspects of production.

The technology has a lot of potential to be further developed and leveraged for identifying patterns leading to an onset of a disease or mass molting or biomass estimation, amongst others.



ARSENIC-INDUCED HISTOPATHOLOGICAL CHANGES IN THE OVARIES OF *Heteropneustes fossilis*

Bibha Kumari*

* Department of Zoology, Magadh Mahila college, Patna University, Patna 800001, Bihar, India
bibhak136@gmail.com

An accurate method of determining the effects of chemical toxicity on reproductive health is through an examination of the histological alterations in fish organs. Thus, a study was conducted to determine the impact of various sub-lethal arsenic concentrations on the histology of the ovary of the Indian catfish, *Heteropneustes fossilis*. Adult *H. fossilis* live specimens (body weight 38–47 gm) were purchased from a nearby market and acclimated to lab conditions for two weeks in 50L plastic tanks with 15 fish each. To restrict the fish from jumping out of the tanks, wire nets were used to cover the tanks. The fish were fed commercial dry fish feed pellets at a rate of about 2%–3% of their body weight per day during the acclimatization period. Three groups of fish were created. Groups I and II were subjected to sub-lethal doses of arsenic, i.e., 4 and 8 ppm, prepared from an arsenic stock solution (Merck, Germany), while group III was kept in arsenic-free water to act as a control for 3, 6, and 15 days. Triplicate records for each group were kept.

Histo-pathological Analysis: After 3, 6, and 15 days, small pieces of ovary from each group—group I, II, and III—were taken and immediately fixed in 10% neutral buffered formalin. Routine paraffin embedding preparations were made for fixed tissues. Sections of 5-7 thick were cut from embedded tissues, which were then stained with Harris' hematoxylin and eosin stains and examined under a microscope. Fish that had their ovary exposed displayed morphological and histological changes. The ovary's exterior revealed damage to the external layer and a dark brownish green colour. It had more atretic follicles and fewer large and medium follicles, according to histopathology. Fish in the control group displayed no histopathological alterations. Additionally, it was discovered that fish exposed to higher levels of arsenic suffered greater harm.

In conclusion, the current study demonstrated that arsenic toxicity was responsible for the histo-pathological alterations in the ovary of the experimental fish. The outcomes also demonstrated a correlation between the amount of tissue damage and the arsenic concentration.

UNTARGETED FOODOMICS APPROACH TO PROFILE BIOACTIVE COMPOUNDS FROM CULTURED *Sarcodia suae* USING LIQUID CHROMATOGRAPGY-HIGH RESOLUTION MASS SPECTROMETRY

Chiu-Hui Kuo*, Ping-Ru Hsiao, Pao-Chi Liao, Chun-Hung Liu, Ming-Chang Hong, Kuohsun Chiu

Institution: National Kaohsiung University of Science and Technology (NKUST)
Institute of Aquatic Science and Technology, National Kaohsiung University of Science and Technology
Kaohsiung, Taiwan*
No. 142, Haizhuan Rd., Nanzi Dist. Kaohsiung, Taiwan
cherry201107@gmail.com

Red seaweed *Sarcodia suae* has various biological activities, but their chemical compositions have not been described in a broad spectrum. This study is to conduct a comprehensive analysis of *Sarcodia suae* metabolites using foodomics approach combining chromatography coupled with high resolution mass spectrometry (LC-HRMS) and structural-base classification for identification and screening of their potential bioactive metabolites. The seaweed samples were cultured and collected from two aquaculture systems in: (1) filtered running seawater (FR), and (2) recirculating aquaponics with tuna aquaculture (RA), respectively. The seaweed samples were dried and extracted using methanol-water, and methanol-chloroform-water extractions methods. The Q-Exactive Plus high-resolution orbitrap mass spectrometer was used to analyze compounds in both positive and negative electrospray mode and data-dependent acquisition (DDA) mode. The tandem mass spectra (MS/MS) were analyzed for chemical structure identification using ClassyFire. The number of compounds identified in two batches for the two cultured *Sarcodia suae* samples was 255 and 290, respectively. The reproducibility of two batches of extracted metabolites were 73.8 % and 74.4 % in FR and RA systems, respectively. According to structure-based classification for all identified compounds were organic acids and derivatives (25.8%), lipids and lipid-like molecules (22.1%), benzenoids (18.3%), organoheterocyclic compounds (17.1%), and other (16.6%). In conclusion, we used LC-HRMS-based foodomics approach to profile a comprehensive metabolome data and reveal differential metabolites between aquaculture systems. Bioactive potentials of extracted metabolites were discussed, and we assume that metabolites might be bioactive and can be studied and applied in food chemistry in further.

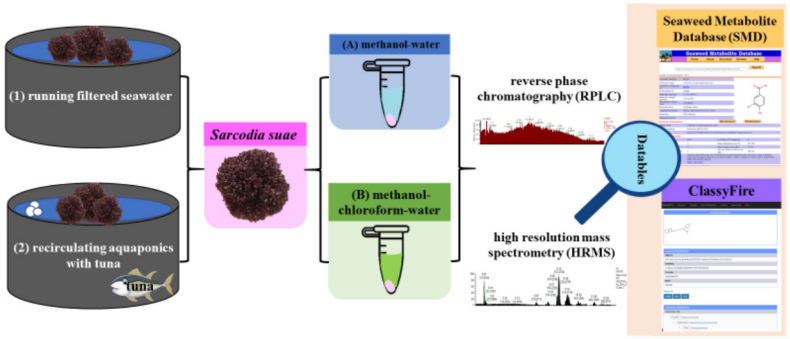


Fig 1. Flow diagram of foodomics approach to profile bioactive compounds of red seaweed *Sarcodia suae*.

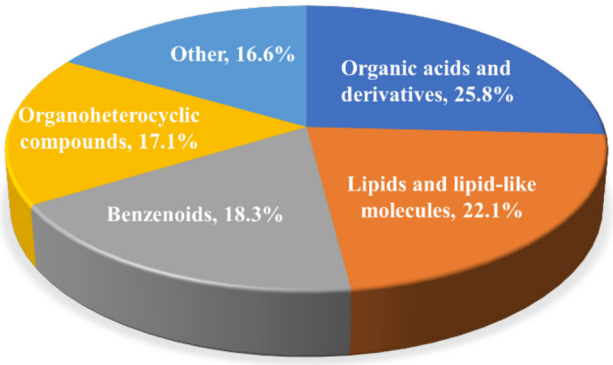


Fig 2. 兩種養殖海木耳之成長 (Growth of two types of cultivated *Sarcodia suae*)

INLUENCE OF DIFFERENT PLANT OILS AS LIPID SOURCES ON GROWTH, BLOOD TRIGLISERIDE AND FATTY ACID PROFILE IN FILLET OF CULTURED RABBITFISH, *Siganus guttatus*

Asda Laining*, Sri RH Mulyaningrum, Bunga R Tampangallo, Usman

Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE)
Ministry of Marine Affairs and Fisheries of Indonesia
Jl. Makmur Dg. Sitakka No.129, Maros 90512
South Sulawesi, INDONESIA
asdalaining@yahoo.com

The aim of this study was to evaluate the effect of using different plant oils as dietary lipid sources on growth, blood trigliseride and hepatosomatic index of golden spotted rabbitfish , *Siganus guttatus* reared in floating net cages.

The experiment was designed into Completely randomized Design consisting of four treatments with triplicates. Three different plant oils used as dietary lipid sources were tested namely soybean oil (SO), palm oil (PO) and coconut oil (CO). Fish oil (FO) was used as the conttrol diet. All lipid sources were formulated in a low fishmeal diets to contain approximately 33.6% CP and 11% lipid. Two kinds of seaweed, Sargassum and Gracillaria meal were supplemented at level of 5 and 6%, respectively as carbohydrate sources and as the binder. Three hundred juveniles of rabbitfish with initial body weight of 33.6 g were selected and randomly distributed into 12 cages of 1x1x1.5m^3 with stocking density of 16 fishes per cage. All diets were fed to fish three times a day at 0800, 1200 and 1600 to satiation. Growth sampling was performed in every four weeks by weighing individual fish. Variables observed were weight gain (WG), specific growth rate (SGR), survival rate (SR), blood trigliseride (TG), hepatosomatic index (HSI) and biochemical composition of carcass and liver of rabbitfish. All data collected were statistically analysed using one-way ANOVA using SPSS 25 version.

After 20 weeks feeding trial, the WG and SGR were significantly differents among groups. Fish fed PO diet had a significant higher SGR among groups, however it was not different from SGR of fish fed control FO diet. Similarly, WG of fish fed FO and PO diets were similar and significantly differed from other two groups. SR of fish fed CO diet was the lowest, but not significant different from other diets. There were no significant different among groups for HSI as indicated by Table 1. Data on blood TG and biochemical composition of fillet will be further presented.

TABLE 1. Growth, survival and blood TG of golden spotted rabbitfish fed different types of lipid sources

Variables	Tested diets			
	FO	SO	PO	CO
WG (%)	570.4±40.8^b	454.2±33.8^a	573.6±61.9^b	470.8±49.9^a
SGR (%/d)	1.58±0.05^b	1.43±0.05^a	1.59±0.08^b	1.45±0.07^a
SR (%)	100±0.0^a	93.8±0.0^a	95.8±3.6^a	93.8±6.3^a
HSI (%)	0.95±0.19^a	0.82±0.11^a	0.93±0.11^a	1.07±0.22^a

GROWTH, FILLET AMINO ACID PROFILES AND IGF-1 mRNA EXPRESSION OF CULTURED RABBITFISH, *Siganus guttatus* FED PLANT BASED DIET CONTAINING VARIOUS PROTEIN LEVELS

Asda Laining*, Sri R.H. Mulyaningrum, Bunga R Tampangallo, Alimuddin

Research Institute for Coastal Aquaculture and Fisheries Extention (RICAFE)
Ministry of Marine Affairs and Fisheries of Indonesia
Jl. Makmur Dg. Sitakka No.129, Maros 90512, South Sulawesi, INDONESIA
asdalaining@yahoo.com

The aim of the study was to evaluate the effect of plant based diet containing different levels of protein on growth, amino acid profile and relative mRNA expression of IGF-1 of cultured rabbitfish, *Siganus guttatus*.

Four test diets were formulated to contain different levels of protein at 25, 30, 35 and 40% designated P25, P30, P35 and P40, respectively. The major protein source was defatted soybean meal. All diets were formulated with low fishmeal at 10%. Cultured rabbitfish (G2) with initial body weight of 41.9±0.7 g were selected and randomly stocked into 12 of 1x1x1.5 m^3 cages. Fish were handly fed all the diets three times a day to satiation for 4 months.

The results showed that the specific growth rate (SGR) significantly improved as protein in diet increased up to 35%, and further decreased at 40% as illustrated by Figure 1. Based on second order polynomial regression analysis between SGR and dietary protein (r-squared 0.702), the optimum level of protein to gain the maximum growth of rabbitfish was 33.0%. Total amino acid (TAA) content in fillet was significantly higher in fish fed P30 (54.908%) compared to fish fed P25 and P40 diets. However, fillet TAA content of fish fed P30 diet did not differ from fish fed P35 diet.

The IGF-1 mRNA expression level in intestine is illustrated in Figure 2. Fish fed P35 diet had the highest IGF-1 mRNA expression level, two-fold from P30 diet, 3.6-fold from P40 diet and 5.1-fold from P25 diet.

Optimum protein level required by rabbitfish *S guttatus* was 33.0% when fed plant-based diet and cultured in floating sea cages.

The authors thank to the Australian Center for International Agricultural Research for funding the study through FIS/2016/130.

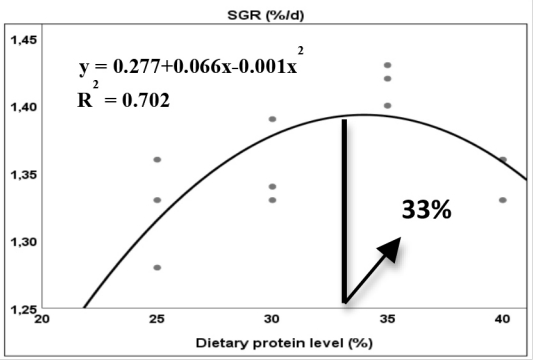


Figure 1.Second order polynomial regression analysis between SGR and dietary protein level for rabbitfish, *S.guttatus*

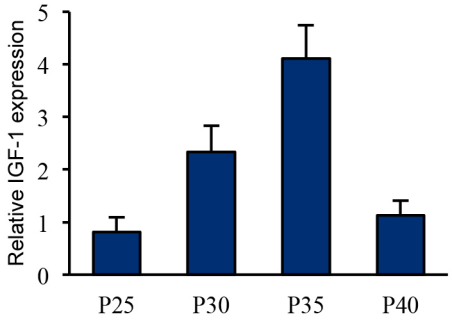


Figure 2. Relative IGF-1 mRNA expression in intestine of cultured rabbitfish, *S.guttatus* fed plant based diets containing various levels of protein

BRAZILIAN WSSV: GENOME, GENETIC VARIABILITY AND PHYLOGENY

Daniel Carlos Ferreira Lanza[1,2,3], Márcia Danielle de Araújo Dantas[1,3], Jéssica Marina de Paiva Pereira[1,3], Cayro de Macêdo Mendes[1,2]

1- Applied Molecular Biology Lab - LAPLIC, Department of Biochemistry
Federal University of Rio Grande do Norte, Natal, RN, Brazil
2 - Postgraduate Program in Bioinformatics, Federal University of Rio Grande do Norte, Natal, RN, Brazil
3 - Postgraduate Program in Biochemistry, Federal University of Rio Grande do Norte, Natal, RN, Brazil

White spot syndrome virus (WSSV) has been the cause of great economic losses in world shrimp farming. In this work the genome of a Brazilian WSSV isolate was determined from direct sequencing of total DNA extracted from an infected whiteleg shrimp, and assembled based on a chimera template approach. Improved PCR primers for WSSV genotyping were developed considering the new Brazilian sequences, and validated *in vitro*. In addition, sequences and putative proteins encoded by WSSV variable regions (ORF94, ORF75, ORF125, ORF14/15, ORF23/24) were characterized *in silico*. Comparisons between WSSV-BR and other isolates revealed that the Brazilian virus has a relatively small genome, and is very similar to isolates from Thailand and Mexico. A phylogenetic relationship using different approaches has demonstrated that these isolates share a common evolutionary history. An analysis of conflicting phylogenetic signals also considering genomes of other isolates revealed that the evolutionary history of WSSV may be related to recombination events. We observed that these events can also be traced at some level by analyzing the homologous regions in the WSSV genome. The existence of recombination events introduces a new point of view that must be considered in the evolutionary history of WSSV.

CULTURE OF SNUBNOSE POMPANO *Trachinotus blochii* IN THE RED SEA

Joseph Leopoldo Q. Laranja*, A. Mohamad, E. Pantanella, P. H. de Mello, S. Zehra, R. Saleh, A. Abulkasim, A.M. Al-Suwailem

King Abdullah University of Science and Technology, Beacon Development
23955-6900 Thuwal, Kingdom of Saudi Arabia
Joseph.Laranja@kaust.edu.sa

The snubnose pompano, *Trachinotus blochii,* is a euryhaline, pelagic, active swimming, marine tropical fish that is commonly found in the Indo-Pacific region, including the Red Sea (Froese & Pauly, 2020). This fish has been identified as a potential new species for aquaculture in Saudi Arabia, hence, baseline studies have been conducted on this species in order to determine its growth performance and production potential under the Red Sea conditions (i.e., salinity: 42 ppt). In the first experiment, baseline growth and FCR of the fish were studied by feeding the fish with locally available commercial feeds (40-46% crude protein; 9-17% crude lipid; 18-23 P/E ratio). Fish at an initial size of 209 g ABW were stocked at 6 kg ton^{-1} and were cultured for 80 days. The results showed that the fish attained 473-508 g after 80 days and gained around 99-113 g month^{1} (Avg. temperature=27°C), with FCR values around 1.59-1.89 (Table 1). The commercial diet containing 40% crude protein and 17% lipid, P/E ratio=18, showed the best growth and FCR performance. Another experiment was also conducted to determine the effects of different Red Sea temperatures (24, 28, 32°C) on the survival and growth of the fish. The results showed that survival was not affected by the different temperatures, however, the weight gain was highest at 32°C (165 g month^{-1}) but no significant difference with 28°C temperature (138 g month^{-1}). At 24°C the weight gain (67g month^{-1}) of the fish was significantly reduced by 59%. Feed intake was significantly higher at 32, followed by 28 and lowest feed intake at 24°C. FCR was significantly higher at 24°C, while 28 and 32°C showed no significant difference in their FCR value (Table 2). Based on our results and experimental conditions used, the predicted optimum temperature for growth of the fish is 32.9°C, while for FCR at 30°C. Lastly, we determined the digestibility of different protein source ingredients in the fish and used them to formulate practical diets for the snubnose pompano. The results of the digestibility experiment and the feeding experiment using the newly formulated diets will be presented at the conference. Based on our results, the snubnose pompano is considered a highly promising fish species for mariculture in the Red Sea.

Table 1. Survival, growth indices, feed intake and FCR of snubnose pompano fed different commercial diets in the Red Sea.

	Comm diet 1	Comm diet 2	Comm diet 3	Comm diet 4
Survival (%)	100	99	99	100
Initial (g)	208	210	209	207
Final (g)	489	479	473	508
Weight gain (g month^{-1})	105	101	99	113
FCR	1.68^{a}	1.74^{a,b}	1.89^{b}	1.59^{a}
Feed intake (g fish^{-1})	472	464	497	474
Final density (kg ton^{-1})	14.4	14	13.7	15.0

Values with different superscripts (^{a,b,c}) in a row are significantly different ($p<0.05$); N=3.

Table 2. Survival, growth indices, feed intake, FCR, and condition factor of snubnose pompano cultured at different Red Sea temperatures (24, 28, 32°C).

	24°C	28°C	32°C
Survival (%)	100	100	100
Initial (g)	532	532	532
Final (g)	709^{a}	900^{b}	969^{b}
Weight gain (g month^{-1})	67^{a}	138^{b}	165^{b}
FCR	2.74^{b}	2.20^{a}	2.18^{a}
Feed intake (g fish^{-1})	482^{a}	772^{b}	952^{c}
Condition factor	1.6	1.5	1.5

Values with different superscripts (^{a,b,c}) in a row are significantly different ($p<0.05$); N=3.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND ANTIBIOTIC SUSCEPTIBILITY

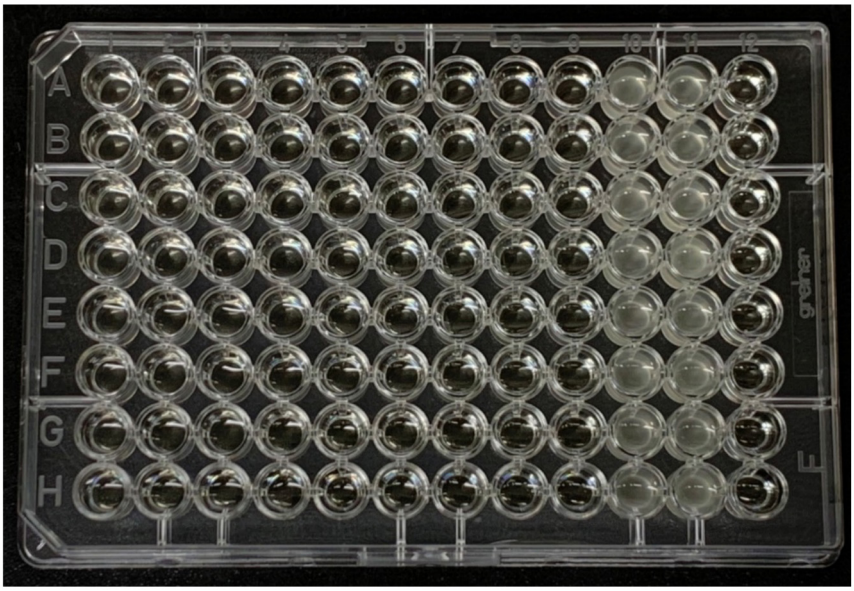
Lau Gek Khin Germaine*, Huang Yun-Tzu, Padmanabhan Saravanan, Chan Pek Sian Diana, Lee Chee Wee

Aquaculture Innovation Centre, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
Germaine_LAU@tp.edu.sg

Antimicrobial resistance (AMR) is an increasing threat globally in public health and animal health care. Emergence and dissemination of multidrug resistant bacteria resulting from overuse or abuse of antimicrobial drugs makes this situation critical. With the increased interest in intensive aquaculture to meet the needs for protein consumption for the growing global population, assessing the role of aquaculture in AMR dissemination is of paramount importance.

The minimum inhibitory concentration (MIC) assay is performed using microbroth dilution to measure, under defined test conditions, the lowest effective concentration of an antimicrobial agent that inhibits the visible growth of a bacterium of interest. Antimicrobial susceptibility testing of clinical isolates from sick aquaculture animals aid in understanding the prevalence, prediction and management of drug resistant bacteria in farming practices

Antibacterial properties of compound were assessed by microbroth dilution method using a range of concentrations 0 – 1024 μg/mL against gram negative and gram positive bacteria viz. *Vibrio parahaemolyticus* and *Streptococcus iniae*.



GUIDELINES AND RECOMMENDATIONS TO ENHANCE WATER BIOSECURITY AND PROMOTE PRODUCTION USING ULTRAVIOLET DISINFECTION

Aran Lavi*, Ytzhak Rozenberg, Michael Argaman

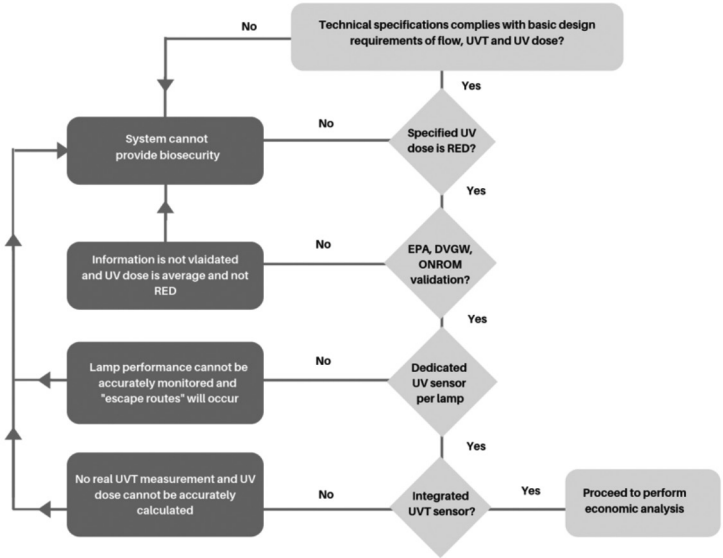
Atlantium Technologies
11 Hamelacha Street
Bet Shemesh, Israel 99100
aranl@atlantium.com

Aquaculture has been identified and welcomed as an important "remedy" to successfully address the challenges to global food security arising from climate change and intensified by the projected 2050 world population of 9.5 billion (National Research Council, 2015). However, marine and land-based aquaculture facilities are being subjected to an increase of biosecurity threats resulting from intensified production and cross pollution on the quality of their influent water. Ultraviolet (UV) disinfection is probably the safest, most effective for treatment of pathogens such as bacteria, microorganisms, and viruses.

At present, there are more than 20 different kinds of commercially available UV technologies offered into the aquaculture market, varying in performance and price. UV systems are very sensitive to water conditions, depend on proper operation of all lamps, and require routine but dedicated maintenance as control and monitoring of UV systems is difficult at best and often times, there can be no real-time indication as to the effectiveness of the UV treatment. Despite its popularity, little information is available to producers about how to qualify UV systems according to site-specific needs. Navigating the selection can be overwhelming and a systematic approach is needed. A UV system performance and cost evaluation process diagram (TABLE 1) has been developed to assist aquaculture facilities in the selection of the most appropriate UV technology to meet their application specific needs. In addition, the convoluted UV terminology needs to be simplified and clearly designated. With these tools facilities can more appropriately select a UV technology based on a defined set of parameters and requirements which will increase the chances of the UV system to provide the site with the required water biosecurity.

Following this systematic approach will aid in prevention, control, eradication of risks to life and health, and a reduction in the economic impact of diseases.

TABLE 1: UV System Performance and Cost Evaluation Process Diagram



GROWTH PERFORMANCE, HAEMATOLOGICAL PARAMETERS AND PROXIMATE COMPOSITION OF RAINBOW TROUT *Oncorhynchus mykiss* FED VARYING DIETARY LEVELS OF PROTEIN HYDROLYSATE FROM HEADS OF *Penaeus monodon* SHRIMP PROCESSING INDUSTRY

Trang Si Trung, Hich Vi Tran[1], Minh-Hoang Le[*,1], Manh Van Ngo[1]

Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu street, Nha Trang city
Khanh Hoa province, Vietnam
[1]Institute of Aquaculture, Nha Trang University, 02 Nguyen Dinh Chieu street, Nha Trang city, Khanh Hoa province, Vietnam
* Corresponding author: Minh-Hoang Le (hoanglm@ntu.edu.vn)

Fish farming is a huge industry in Vietnam, but there is a shortage of protein supplements for fish diets. A potential great source of protein supplement is the co-products of shrimp processing. The present study aimed to investigate the growth performance and haematological parameters of rainbow trout *Oncorhynchus mykiss* juveniles fed diets containing four different concentrations of shrimp head protein hydrolysates (0% (control), 1, 3 or 5% SHPH, four replicate per treatment). Rainbow trout juveniles were randomly distributed into 12 tanks at a density of 30 fish/tank and were fed twice a day at 8:00 and 17:00 for 8 weeks. Mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival (SR) were calculated. Significant differences ($p<0.05$) in MWG, SGR, FCR, PER and SR were detected between treatments, with fish fed 5% SHPH performing the best. Mean white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) and hematocrit (Hct) significantly increased as dietary protein hydrolysate increased in the diet. Protein and triglyceride concentrations in plasma of were not significantly affected by dietary treatment ($p > 0.05$). Furthermore, muscle and whole body lipid contents were higher in fish fed 3% and 5% protein hydrolysate from shrimp heads than other groups, but muscle and body protein contents in the group fed 5% were higher than other groups. Results from the present study showed that protein hydrolysate from shrimp heads has good potential for use as protein supplement in rainbow trout diets up to 5%. Supplemental dietary protein hydrolysate from shrimp heads also has potential as an immune enhancer because of an increase in WBC. These data provide a potential use for a current waste product and might serve as a model for other countries that have both warm water crustacean industries and cold water fish industries. Further studies to explore the use of protein hydrolysate from shrimp heads in fish diets at more than 5% are recommended.

IS SINGAPORE THE CENTER OF WORLD AQUACULTURE?

Le Boucher Richard*[1]

[1]Temasek Life Sciences Laboratory
Precision Aquaculture Department
1 Research Link National University of Singapore
117604, Singapore
E-mail : richard@tll.org.sg

Over the past 50 years, Asia became the main supplier of aquaculture products in the world. Positioned on the junction between the three main producing countries (China, India and Indonesia), the key geographic position of Singapore led to call it the center of aquaculture production. This statement raised the question of the actual position of aquaculture gravity center for farm production, science production and investment.

Material and methods. To answer this question, we develop a Geographical Information Systems (GIS) methodology to track the movements of the World's Aquaculture Centre of Gravity (WACG) between 1950 and 2019. Combining international data base of FAO, World Bank, lens.org and MIT, the model uses the latitudes and longitudes of 212 country centroids to build vectors in a Cartesian spherical system and to map WACG positions in a Mercator projection. Same methodology was applied to subgroups like aquatic plants, molluscs, crustaceans, diadromous and marine fish.

Results. When the WACG aimed at Singapore over the last 70 years, it is strongly attracted by Chinese centroid and, is located in the Sichuan province, 30 km North from the city of Chengdu (Figure 1). The traces movements highlighted the role of the artificial breeding discoveries in the 50's, the rise of the salmon sector in the 80's, the role of aquaculture policies in China in the 80's, the geographical diffusion of shrimp outbreaks in the 90's and the rapid growth of Indonesian farming sector. The origin of scientific publication in the aquaculture domain, since 1975, showed that science production is more homogeneously distributed between countries (China, USA, India, UK, Brazil) and located in Sverdlovsk Oblast, Russia (λ: 62.0868, ϕ: 58.9182). The growing position of China is this domain is also visible with a South-East movement over the last 4 years.

WACG methodology appeared to be a fruitful GIS approach to understand trends of aquaculture sector. The current work also emphasizes the importance of macro-economic data collection in this industry to develop more applications at national, regional and local levels to map sustainable capacity of food production.

Figure 1. Traces described by World Centres of Gravity between 1950 and 2019 for global Aquaculture production (WACG)

TRACING THE GEOGRAPHIC ORIGIN OF FARMED SHELLSFISH – PROJECT TRASEAFOOD

Miguel C. Leal*, Fernando Ricardo, Renato Mamede, Andreia Santos and Ricardo Calado

Department of Biology & CESAM & ECOMARE
University of Aveiro
Campus Universitário de Santiago
3810-193 Aveiro, Portugal
miguelcleal@ua.pt

Project TraSeafood aims to determine elemental and/or biochemical fingerprints present in farmed shellfish along the Atlantic western and south-western Iberian coast that can be used to trace their place of origin. The following species are addressed in this unprecedented effort to develop an integrative framework towards the traceability of geographic origin of seafood: *Mytilus galloprovincialis* (Mediterranean mussel), *Cerastoderma edule* (common cockle), *Ruditapes decussatus* (grooved carpet clam) and *Crassostrea gigas* (Japanese oyster). Species selection was based on market value and potential added-value that can be achieve through the certification of their geographic origin. The selection of species and sampling locations was strictly aligned with the recommendations of the project Stakeholders Steering Committee, which will speed-up knowledge transfer to the market, enhance the socio-economic benefits of the project and ensure its legacy. Elemental fingerprints will be determined through Inductively Coupled Plasma Mass Spectrometry (ICP-MS), while MS-based lipidomics, namely Hydrophilic Interaction Liquid Chromatography–Electrospray Ionization–Mass Spectrometry (HILIC–ESI–MS), will be used to determine biochemical fingerprints. Elemental and biochemical fingerprints are expected to be species-specific, unique to each farming location and thus hold the potential to be used for origin certification. Seasonal and interannual shifts in fingerprints will be investigated, as these may impair the discrimination of the place of origin. Potential shifts on these fingerprints along shelf-life will also be studied, in order to determine if and during how long post-harvesting geographic traceability can still be reliably determined.

Project TraSeafood (Tracing the geographic origin of seafood as a pathway towards the smart valorization of endogenous marine resources) (PTDC/BIA-BMA/29491/2017) is supported by FCT/MEC through national funds, and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020.

Supported by:

FCT Fundação para a Ciência e a Tecnologia
COMPETE 2020
PORTUGAL 2020
UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional

YEAST BETA-GLUCAN CONCENTRATE (SAFGLUCAN®) REDUCES TIME FOR VACCINE PROTECTIVE RESPONSE AND INCREASES SURVIVAL OF NILE TILAPIA *Oreochromis niloticus* CHALLENGED WITH *Streptococcus agalactiae*

Carlos A. G. Leal[1*], Kelly S. Sa[1], Gabriel R. R. Balloute[1], Nadege Richard[2], Otavio S. Castro[2]

[1] Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais Belo Horizonte, MG, Brazil
[2] Phileo by Lesaffre, Marcq-en-Baroeul, France
Email: carlosleal@vet.ufmg.br

Streptococcosis is widely recognized as a major infectious disease hampering tilapia production globally. Vaccines have been proven to be a sustainable and effective tool to prevent disease outbreaks, allowing farmers to achieve more stable crops. However, optimal fish protection can be affected by the type of vaccine, delivery method and due to multifactorial interaction between fish intrinsic characteristics and environmental factors.

This study evaluated the effects of tilapia feed supplementation with a yeast-based immunostimulant rich in β-1,3/1,6-Glucans (Safglucan®) on the antibody titers and survival after *S. agalactiae* challenge. Juveniles (20g ±4.7) were fed on control or supplemented diets for two weeks at 28°C ± 2.85 water temperature, prior to intraperitoneal vaccination (Aquavac Strep SA - MSD). Non vaccinated groups were injected with 0.9% saline solution. Fish were challenged by intraperitoneal injection with *S. agalactiae* (strain SA583-19) at 7- or 21-day post vaccination (DPV). Negative control group was injected with 0.1 mL of sterile BHI broth. All animals were fed on control diet with no β-glucans supplementation after challenge.

At 7-DPV, anti-*S. agalactiae* IgM ELISA results from surviving animals demonstrated an increase in antibody titers in the vaccinated groups. A more pronounced increase was observed from the 14-DPV onwards. No clear effect of the immunostimulant in the anti-body titers were observed. At 21-DPV, significant higher relative percentage survival (RPS) was observed in the vaccinated groups, with the best response on the β-glucans supplemented group. At 7-DPV, significant RPS was observed only in the group vaccinated and supplemented with β-glucans.

The supplementation of tilapia feed with Safglucan® at 1g/Kg of feed significantly reduced the time required for the initial induction of a protective immune response and increased the level of protection conferred by the vaccine against streptococcosis in the experimental infection.

Table 1. Experimental groups, average mortality rate and relative percentage survival of Nile tilapia juveniles vaccinated and challenged with *S. agalactiae* at different periods post-vaccine

Treatments	Vaccination	*S. agalactiae challenge*	Safglucan® in feed	Mortality rate (%)	Relative percentage survival (%)	P value
Control Ref	-	-	-	0	-	-
Control Neg	-	21-DPV	-	72.8	-	-
Control Pos	+	21-DPV	-	30.6	59	<0.0001
T1	-	21-DPV	1g/Kg	95	0	1
T2	+	21-DPV	1g/Kg	0	100	<0.0001
T3	-	7-DPV	-	86.4	-	-
T4	+	7-DPV	-	85	1	0.841
T5	-	7-DPV	1g/Kg	100	0	1
T6	+	7-DPV	1g/Kg	43.2	50	<0.0001

BROODSTOCK CONSTRUCTION AND DEVELOPMENT OF HIGH-QUALITY SNPS FROM OLIVE FLOUNDER, *Paralichthys olivaceus*, IN JEJU ISLAND, SOUTH KOREA

Sukkyoung Lee[1,*], Tae Hyug Jeong[1], Hyerim Yang[2], D.S. Liyanage[2], W.K.M. Omeka, Seong-Rip Oh[3], Hyung Bum Koh[3], and Jehee Lee[1,2]

1 Marine Science Institute, Jeju National University, Republic of Korea, Jeju 63333
2 Department of Marin Life Sciences, Jeju National University, Republic of Korea, Jeju 63243
3 Oceans and Fisheries Research Institute, Jeju Special Self-Governing Province, Republic of Korea, Jeju 63629
*lskshiny@hotmail.com

Aquaculture industry of olive flounder (*Paralichthys olivaceus*) is the major fisheries in Jeju island, South Korea. Nowadays farming of the flounder is depressed to come to grips with serious problems as diseases, high mortality, low-quality seeds, and price decline. To solve these issues, the breeding program was started to improve fish traits with the collection of fish from different places. Also, whole genome re-sequencing was performed to develop and analysis about single nucleotide polymorphisms (SNPs) in our broodstock.

Initially, 1286 fishes were collected from four different farms or places and analyzed the basic genomic structure by 11 microsatellite markers. From that, 100 fishes which represent the whole were selected to whole genome re-sequencing based on the phylogenetic tree. Total sequencing reads and data from 100 fishes were 24.31 Gb and 3,666.48 Gb, respectively. Average sequencing data per one fish was 36.69 Gb as 72X coverage to flounder whole genome. The read mapping rate to reference sequence was 95.32%.

As a result of variant calling, eighty percent of modification of sequencing data are SNPs and twenty percent are INDELs. Union variants were confirmed as 15,530,229. High-quality SNPs were filtered as 6,964,879 after hyper-variable filtering such as base quality SNPs, bi-allelic, genotyping rate, miner allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE). Intergenic regions, transcripts and intron regions of the data were consisted of 45.425%, 27.283% and 24.584% of high-quality SNPs, respectively.

To select suitable candidate for development of SNP chip, the specific filtering steps like MAF, linkage disequilibrium, repeat checking were performed. After that, 154,964 SNPs were selected, and they were fairly uniformly distributed in 24 chromosomes. Average length between SNPs per each chromosome were confirmed as 2,087 to 5,342. The 100 fish could be divided into 3 populations based on phylogenetic tree and PCA analysis using 155K SNPs.

Finally, it can be concluded that identified high-quality SNPs from the Jeju olive flounder broodstock will be used to analyze genotypes of important traits such as disease resistance, fast growth, thermo tolerance and shape and these data will be applied to future genomic selection.

GENOMIC CHARACTERIZATION & EXPRESSION PROFILING OF GLUTATHIONE S-TRANSFERASE ALPHA4 FROM POT-BELLIED SEAHORSE (*Hippocampus abdominalis*)

Kishanthini Nadarajapillai, Sukkyoung Lee*, Sarithaa Sellathurai and Jehee Lee

Department of Marine Life sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju self-Governing Province 63243, Republic of Korea

Glutathione S-transferase (GST) is a multifunctional protein. It is involved in phase II detoxification of chemical substances through conjugation to reduced glutathione. Further it is involved in various biological functions such as, prostaglandin D2 synthesis, fatty acid β-oxidation and signal transduction pathway. The GSTs are divided into three major families according to their localization in cell. Such as; as cytosolic, mitochondrial, and microsomal. The GSTs are further characterized into several subclasses, namely alpha (α), beta (β), delta (δ), epsilon (ε), zeta (ζ), and theta (θ) based on their substrate specificity, N-terminal amino acid sequence, antibody cross-reactivity and sensitivity to inhibitors. In this current study, we are analyzing the molecular, transcriptional roles, and substrate specificity of GST Alpha4 in *Hippocampus abdominalis* (HaGSTA4).

The cDNA sequence of *HaGSTA4* was obtained from seahorse database. *In-silico* analysis of *HaGSTA4* was conducted using various bioinformatics tools. The abiotic and biotic challenges were conducted with Poly I.C, LPS, gram-negative *Edwardsiella tarda* and gram-positive *Streptococcus iniae*. The blood was collected from immune challenged seahorses at different time points. The qPCR was carried out to determine the spatial and temporal expression of *HaTRx-2*. Further, the GSTA4 was recombinantly expressed in *Escherichia coli* BL21(DE3) and the substrate specificity check with various substrates.

The GST Alpha4 has 223 amino acids which encoded by 690 bp ORF with molecular weight of 25.7kDa. Its calculated iso electric pI is 8.47. It comprised GST_C_ super family and Thioredoxin _like superfamily. The *HaGSTA4* showed highest sequence identity (78.5%) and similarity (85.7%) with the orthologue *Parambassis ranga*. The phylogenetic analysis revealed that HaGSTA4 is closely clustered together with other fishes. According to the qPCR results, tissue distribution profile revealed that *HaGSTA4* highly expressed in kidney compared to other examined tissues (Fig.1.). In blood, *HaGSTA4* showed down regulation of most of the time points other than 6 h post injection(p.i.) when challenged with LPS and *S.iniae*. The significant upregulation of *HaGSTA4* was observed with poly I.C at 3 h, 12 h, and 24 h p.i. Further, the immune challenged by *E.tarda* showed significant up regulation at 24 h p.i. The recombinant HaGSTA4 was exhibited higher activity toward 1-chloro-2,4-dinitrobenzene (CDNB) than other substrates (18.69 μmol.min^{-1}mg^{-1}). Together, the current study suggested that *HaGSTA4* has indispensable role in immune defense regulation when pathogen attacks and xenobiotics detoxification.

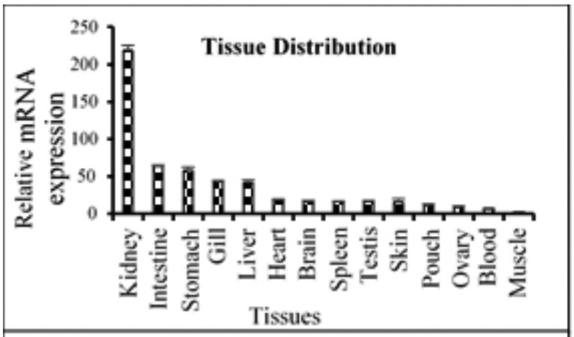


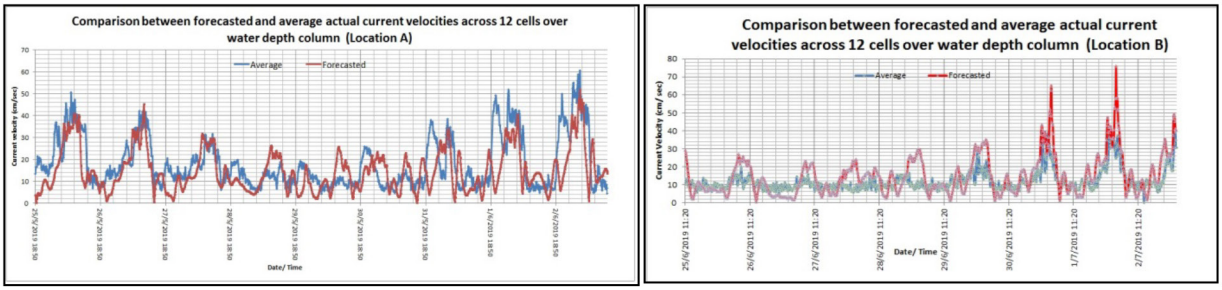
Fig.1: Tissue specific expression of HaGSTA4 from unchallenged seahorse

APPLICATION OF AANDERAA MONITORING SYSTEMS IN AN OFFSHORE SINGAPORE AQUACULTURE FARM

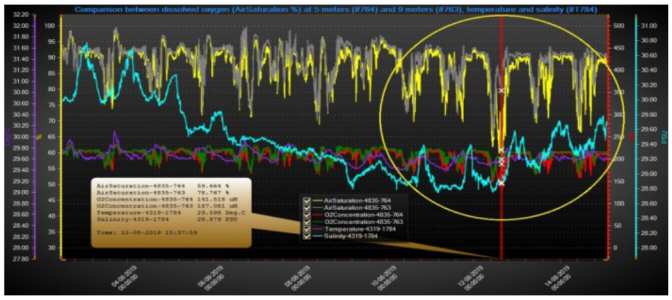
Wan Aik, Lee

Xylem Water Solutions Singapore Pte Ltd
3A International Business Park, Tower B,
#10-10/18, ICON@IBP, Singapore 609935
desmond.lee@xyleminc.com

Global marine aquaculture industry is developing rapidly to make up the shortfall in protein supply for human consumption. Knowing prevailing current and water quality conditions through continuous monitoring could actually promote a longevity profitable offshore aquaculture farm. Aanderaa has provided monitoring systems for several Norwegian offshore Salmon farms. In a Singapore Aquaculture Farm, we have deployed Aanderaa systems to monitor current and water quality conditions. Field gathered information, as opposed to forecasted models shows higher variability of current velocities within fish farm concession area. Lower Dissolved Oxygen levels are found consistently at shallower depth as compared to deeper depths. Knowing these important prevailing field conditions can be used to understand and improve fish feeding timings, sea cage layout design, working environment safety conditions for divers, fish overcrowding, fish waste dispersion dynamics and water exchange between inside and outside sea cages.



Comparison between forecasted current velocities (Red) and actual field measurements (Blue) in Locations A and B. Both show similarities in rising and decreasing trends in current velocities, especially in Location A. In Location B, the rising and decreasing trend is less manifested and forecasted values are overestimated. Lower current velocities at Location B clearly indicate the presence and arrangement of sea cages have significant impact of current velocities within the concession area.



Lower DO levels is observed at 5 meters as compared to 9 meters depth inside the cage over 2 weeks monitoring. This implies effects of overcrowding at 5 meters depth. Continuous monitoring has also detected a dip in DO levels on 12 August 2019 (Circled YELLOW). Temperature and Salinity levels do not show any sudden increase to warrant the drop in DO on 12 August 2019.

EFFECTS OF ADMINISTRATION OF VARIOUS PROBIOTICS AND PREBIOTICS ON GROWTH AND IMMUNE RESPONSES OF FLOUNDER (*Paralichthys olivaceus*)

Intan Riski Mauliasari[1], Sanaz Khosravi[1], Seong-Mok Jeong[1], Kye-Yoon Yoon[2] and Sang-Min Lee[1,*]

[1]Department of Marine Biotechnology, Gangneung-Wonju National University, Gangneung 25457, Korea
smlee@gwnu.ac.kr
[2]Biotechnology Research Center, Korean Institute of Science and Technology (KIST)
Gangneung 25451, South Korea

The present study was aimed to evaluate the possible effects of singular administration of different probiotics and prebiotic on growth performance, feed utilization, whole body composition, hematological parameters, and non-specific immune responses of juvenile olive flounder. Eleven diets supplemented with one of the following probiotic strains: *Bifidobacterium lactis*, *Lactobacillus casei*, *L. paracasei*, *L. curvatus*, *L. plantarum*, and *L. lactis* at 3×10^9 cell forming units (CFU) kg^{-1}, or one of the following prebiotics: fructooligosaccharide (FOS), inulin, β-glucan, and xylooligosaccharide (XOS), or oxytetracycline (OTC) as a most frequently used antibiotic at 5 g kg^{-1}. A diet without probiotics and prebiotics supplementation was used as a control diet. Triplicate groups of fish (15.3g/fish) were fed one of the diets to apparent satiation for 6 weeks. Results showed that fish fed the diet with probiotics or prebiotics grew better than control diet, and significantly higher growth rates were recorded in those fed on the FOS diet compared to the control diet. Fish fed the treated diets exhibited significantly lower daily feed intake than those fed the control diet. Significantly higher protein efficiency ratio was recorded in fish fed the *B. lactis*, *L. paracasei*, *L. curvatus*, *L. lactis*, FOS, inulin, β-glucan, XOS, and OTC diets than those fed the control diet or those fed the diet containing *L. casei*. Feed conversion ratio was significantly improved in fish fed on supplemented diets, except *L. casei*, compared to control diet. However, dietary treatment had no significant effects on fish carcass composition or any of the biochemical parameters measured in the present study. Significant enhancement in MPO activity was observed in fish fed the *L. curvatus* supplemented diet compared to those fed the control and *L. casei* diets. The groups fed *B. lactis*, *L. paracasei*, *L. plantarum*, *L. lactis*, FOS, inulin, β-glucan, and XOS diets exhibited significantly higher SOD level than fish fed the control diet. In summary, our findings revealed that juvenile flounders on probiotics or prebiotics supplemented diets, particularly FOS, grew faster and utilized their feed more efficiently compared with those offered an un-supplemented fish meal based control diet without any major alteration in their normal health or nutritional quality. It is also evident that most of the probiotics and/or prebiotics tested in the present study could have the potential to replace commercially available antibiotics, like OTC, in olive flounder aquaculture production.

CULTIVATION OF BRACKISH WATER CLADOCERANS AS LIVE FEED FOR MILKFISH *Chanos chanos* FRY

Alan Lee*, Fanny A. Yasumaru, Diana P. S. Chan, Lee Chee Wee

Aquaculture Innovation Centre
Temasek Polytechnic
21 Tampines Ave 1
Singapore, 529757
alanlee@tp.edu.sg

According to the Food and Agriculture Organization of the United Nations (FAO), Singapore aquaculture production in 2017 was of 5,891 tonnes, of which 2,094 tonnes was of milkfish *Chanos chanos*. Milkfish are cultured in traditional floating coastal farms at East Johor Strait, where farmers stock milkfish larvae in either canvas or fine mesh nets. Nevertheless, farmers face some challenges, such as very low survival rate in such early stages. Some farmers attempted to increase larval survival by purchasing commercially available live feed products, which increased both operation and logistical costs. Massive larvae mortality of 92 to 95% at early stages is a common issue faced by the milkfish farmers. This is mainly due to difficulty in feeding nutritionally adequate live feed and in the adequate amount, other than the issue of inadequate husbandry that also includes poor water quality. To help farmers overcome such issue, the objective of this study is to cultivate brackish water cladocerans and formulating nutritional enrichments that are low cost, easy to prepare and nutritionally adequate for milkfish larvae.

Milkfish larvae would be stocked in a low-height rectangular fibre tank within a lab at AIC facility. The simple setup stimulates a typical below-platform canvas setup at traditional fish farms. General husbandry for milkfish larvae is done in a batch cultivating method, inclusive of water changes, bottom cleaning and basic water parameter monitoring, which bears some similarities to what farmers have been practicing outdoors. The milkfish larvae are fed with cultivated and enriched zooplankton (cladocerans) twice per day, throughout all stages until metamorphosis or 1-inch long fingerling stage. The cladocerans are enriched with formulated powder feed made up of commonly available ingredients such as baker's yeast, rice bran and soybean cake. The fecundity, density and nutritive value of the cladocerans fed with the formulated feed will be measured. The growth rates, survival rates and live prey consumption rate of milkfish larvae to fingerling stage will also be measured to assess efficacy of the enriched live feed on growth performance and survival of the milkfish larvae.

IN-VITRO ASSESSMENT OF MULTISPECIES PROBIOTICS AS A POTENTIAL DISEASE CONTROL TOOL AGAINST A*eromonas hydrophila* INFECTIONS

Olivia Lee Wye Sze*, Puvaneswari Puvanasundram, Murni Marlina Abd Karim, Nur Jasmin Mohd Yaminudin

School of Applied Science, Temasek Polytechnic
21 Tampines Avenue 1, Singapore 529757
olivialee1999@hotmail.com

Probiotics has been increasingly considered as an alternative to antibiotics in combating disease outbreaks. The use of combined probiotics has been studied to possibly harbor synergistic effects that could provide better protection in aquatic species.

Three potential probionts that had conferred antagonism against *Aeromonas hydrophila* were *Bacillus amyloliquefaciens* (L9, isolated from crab), *Bacillus* sp. (A2, isolated from microalgae) and *Enterococcus hirae* (LAB3, isolated from seabass) and were combined into a probiotic mixture. The probiotic mixture was able to produce biofilm (2.441±0.346), significantly higher than *A. hydrophila* (0.578±0.124) at 24 h and showed continuous increased in production of biofilm at 48 h and 72 h. Furthermore, no hemolytic action was presented when the probiotic mixture was streaked on sheep blood agar (5%) whereas *A. hydrophila* presented α-hemolysis. The lowest concentration of potential multi-strain probiotic mixture, as determined in the minimum inhibitory concentration assay, significantly inhibited the growth of *A. hydrophila* at 10^6 CFU mL^{-1} after 24 h incubation at 10^7 CFU mL^{-1} (6.595±0.218) as compared to *A. hydrophila* only (7.247±0.061). The interaction, in the co-culture assay, between the probiotic mixture at 10^7 CFU mL^{-1} and *A. hydrophila* at 10^6 CFU mL^{-1} was 6.883±0.015 as compared to *A. hydrophila* only was 7.532±0.026, showed significant reduction of pathogen at 12 h of co-incubation. Hence, this research suggests that probiotic mixture of L9, A2 and LAB3 has the potential for further in-vivo research to confer against *A. hydrophila* infections.

DETERMINING NITRATE AND AMMONIA ABSORPTION RATE IN *Ulva sp.*, *Caulerpa sp.*, *and Graciliaria sp* TO INVESTIGATE THE FEASIBILITY OF SEAWEED CO-CULTURE IN RECIRCULATING AQUACULTURE SYSTEM

Lee. Z.* and Mei. A.

Diploma in Veterinary Technology, School of Applied Science, Temasek Polytechnic, Singapore 529757
1703725J@tp.edu.sg

Three species of seaweed, *Ulva sp., Caulerpa sp*., and *Gracilaria sp.,* were cultivated in tanks after acclimatization in an indoor culture for about 2 weeks each with calculated doses of nutrients at 2ppm representing low concentration and 8ppm as a representation of high concentration of nutrients. The nutrients absorption rate was determined through the series of nutrient trials and during the nutrient trials it showed that the *Gracilaria sp*., and Ulva sp., managed to absorb all the nutrients throughout the two weeks of the nutrient trial and showed a positive growth rate. However, the Caulerpa sp., although managed to absorb the all the nutrients for ammonia and generally did absorb nutrients throughout, slowly depleting the nutrients present in the tank, it was seen that other than the tanks with low concentration of ammonia, there was a negative growth rate for the Caulerpa sp., on average for the other three nutrient concentrations. This will then be used to determine the nutrient absorption rates of the various seaweed species and be used for the calculation of stocking density of seaweed required to implement the seaweeds into a co-culture in a recirculating aquaculture system as a bio-filter to investigate the feasibility of a co-culture. The results of this study showed that the nutrients may not be a direct factor influencing the growth rate of the seaweed species due to the amount of growth in absorption of high concentration of nutrient in Ulva sp., was observed with slower growth of the Ulva sp.

EFFECTS OF STOCKING DENSITY AND FEEDING RATE ON GROWTH PERFORMANCE OF ASIAN SEABASS *Lates calcarifer* AND DISSOLVED OXYGEN LEVELS IN RECIRCULATING AQUACULTURE SYSTEM (RAS)

Co Sin Lee*, Jessalin Ng, Weiqiang Chung, Richard Le Boucher

Temasek Life Sciences Laboratory
1 Research Link
National University of Singapore
Singapore 117604
cosin@tll.org.sg

Finding the suitable stocking density of Asian seabass reared in recirculating aquaculture system (RAS) is important in aquaculture industry, as high stocking density can bring up economic profit, but low stocking density can induce stress levels of fish in a limited space. The objectives of this study were to compare the growth performance of Asian seabass *Lates calcarifer* and dissolved oxygen (DO) levels at different stocking densities and feeding rates in RAS. The study was carried out for a period of 56 days in a new RAS at Marine Aquaculture Centre, St John's Island, Singapore. Asian seabass (51.23 ± 0.14 g) were randomly distributed in 27 tanks (1 m^3) at three initial stocking densities: low D1 (5.6 kg/m^3), medium D2 (6.7 kg/m^3), and high D3 (7.7 kg/m^3). Fishes were fed twice daily and received three different daily feeding rates (F1: 3.5%, F2: 4%, F3: 4.5%) for each stocking density. Water quality parameters were monitored, such as DO, temperature, pH, ammonia, nitrate, nitrite, salinity, oxidation-reduction potential and alkalinity. The present study showed the high stocking density group (D3) can be reared up to 33 kg/m^3 in the RAS. The stocking densities and feeding rates had no effect on survival and feed conversion ratio (FCR). However, the feeding rates affected the final body weight, weight gain and thermal growth coefficient (TGC). In high stocking density group, high feeding rate (F3) did not improve net fish yield as compared to moderate feeding rate (F2), showing there is a maximum density above which growth is limited, no matter what the feeding rate. The DO levels fluctuated throughout the study and the average of DO level was 7.58 mg/L. Low DO levels (>2 mg/L) were recorded in 5 out of 9 tanks in D3 group after feeding. Principal Component Analysis revealed that increased stocking density influenced the DO levels. This study will be useful for the practitioners to understand the good practice for stocking density in RAS.

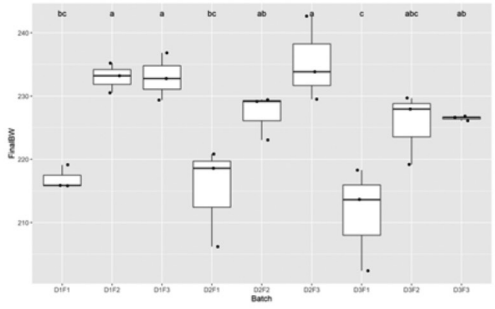


Figure 1: Final body weight of fish fed with low (F1), moderate (F2), high (F3) feeding rate in low (D1), moderate (D2) and high (D3) density in RAS.

TISSUE SPECIFIC TRANSCRIPTOME PROFILING IN STARRY FLOUNDER *Platichthys stellatus*

Hyo Bin Lee*, J. Sook Chung, Irfan Zidni, Ji Hye Yoon, Han Kyu Lim

Department of Biomedicine, Health & Life Convergence Sciences, BK21
Mokpo National University
Jeonnam, Republic of Korea
leeehyobin@gmail.com

Starry flounder, *Platichthys stellatus* is spotlighted as a valuable marine flatfish for aquaculture in Korea with a salinity and disease tolerance. One of the challenges of studying the biological and molecular mechanisms involved in the aquaculture of this species is the limited genomic information available. In this context, transcriptome sequencing is useful for identifying genes that participate in specific biological processes when genomic data is not available This study sought to establish a global transcriptome database from the tissues of the brain, head kidney, and liver of *P. stellatus*. This study aims to advance our understanding of the molecular aspects involved in energy metabolism in this species.

RNA-sequencing results yielded 5,954,872,200–14,180,685,600 bp of raw data, and 109,062,581–387,774,428 bp of high-quality filtered reads after trimming. After *de novo* assembly, 107,062–359,922 assembled contigs were obtained. Total transcripts of each tissue were classified to GO, COG, and KEGG pathways. Among the categories of KEGG pathways, three glycolysis/gluconeogenesis, insulin signaling pathway, and FoxO signaling pathway related to the energy metabolism were selected, and compared the transcripts involved in these tissues in each tissue. As a result, the brain had the highest number of transcripts among the three tissues in the categories of the insulin signaling pathway (160, 0.136%) and the FoxO signaling pathway (432, 0.366%). The head kidney had the highest number of transcripts among the three tissues in the glycolysis/gluconeogenesis category (723, 0.613%). However, when comparing the ratios belonging to each category, the liver appeared to have a relatively high ratio in glycolysis (694, 1.084%) and FoxO signaling pathways (299, 0.467%).

TABLE 1. Summary of the RNA sequencing raw data of the brain, head kidney, and liver tissues in *Platichthys stellatus*

Tissues	Sample name	Raw data			
		Read-pairs	Yield (bp)	Over_Q30-Bases (%)	Mean-Quality-Score
Brain	Br3	47,268,952	14,180,685,600	90.25	35
	Br4	45,454,515	13,636,354,500	90.15	34.95
	Br5	45,293,495	13,588,048,500	91.1	35.2
Head kidney	Ki3	43,590,097	13,077,029,100	91	35.15
	Ki4	40,258,746	12,077,623,800	90.8	35.1
	Ki5	38,554,731	11,566,419,300	90.75	35.1
Liver	Li3	18,882,011	5,664,603,300	85.75	34.05
	Li5	17,591,081	5,277,324,300	85.7	34.05
	Li6	19,849,574	5,954,872,200	85.1	33.9

TRANSCRIPTOME ANALYSIS OF STARRY FLOUNDER *Platichthys stellatus* EXPOSED TO HIGH WATER TEMPERATURE

Hyo Bin Lee*, Irfan Zidni, Youn Su Cho, Tae Min Kim, Han Kyu Lim

Department of Biomedicine, Health & Life Convergence Sciences, BK21
Mokpo National University
Jeonnam, Republic of Korea
leeehyobin@gmail.com

Global warming affects marine organisms by increasing water temperature. Starry flounder, *Platichthys stellatus*, is an economically important species widely distributed throughout the North Pacific Ocean. *P. stellatus* exhibits relatively poor thermal tolerance, which frequently suffers from mass mortality during summer in an aquaculture system. This study provides transcriptomes for profiling the gene expression responses to heat stress in the liver of *P. stellatus*, and several target genes involved in functional categories related to heat responses were identified. The experiment was conducted that moderate heat stress was applied by increasing the temperature at a constant rate (increments of 1°C per 12 h) from 16°C to 28°C.

A total of 18,774,222 raw reads in the 16°C group and 16,121,503 raw reads in the 28°C group were obtained from three libraries, and the number of clean reads was 18,254,358 in the 16°C group and 15,677,787 in 28°C group. The genes expressed differentially upregulated and downregulated in *P. stellatus* under heat stress from 16°C to 28°C were identified. Of the total DEGs, 1,196 genes were downregulated, and 1,779 genes were upregulated depending on water temperature. Among the total DEGs of 2,975, 1,944 genes were enriched with 30 GO terms (corrected $p < 0.05$), cellular process (GO:0009987, 303), metabolic process (GO:0008152, 244), and biological regulation (GO:0065007, 128) were highest enriched in biological process. In addition, cellular anatomical entity (GO:0110165, 301) was the highest classified in a cellular component, and catalytic activity (GO:0003824, 184) was the highest enriched in the category of molecular function. In the analysis of 47 KEGG pathways, signal transduction (723) belonging to the category of environmental information processing was classified with the highest number of upregulated DEGs, and the highest number of downregulated DEGs was found in global and overview maps (240) belonging to metabolism. In addition, *WASF3*, *CTSE*, *UGT*, *TLR5*, *EGR2*, *GLT25D*, *HERC4*, *DNAJB1*, *EGR1*, *FOS*, *JUNB*, *IL13RA1*, *FKBP4_5*, *FOSL2*, *MTHFD2*, *HSPB1*, and *Hsp90A* were the most differentially expressed genes between the 16°C group and 28°C group.

THE CURRENT SITUATION OF TAIWAN'S SYMBIOSIS OF GROUND-BASED FISH AND ELECTRICITY PROMOTION AND THE ENVIRONMENTAL AND SOCIAL INSPECTION MECHANISM

Ping-Lee *, Bing-Shun Huang, Hung-Ju Chiu, Wen-Kai Hsieh, Han-Yun Lee, Ming-Lung Hung

Green Energy and Environment Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan
Zoe.Lee@itri.org.tw

In order to achieve the goals of energy transformation, the Taiwan government has set specific targets for various types of renewable energy. The solar power capacity shall reach 20 gigawatts in 2025, of which 4.4 gigawatts comes from the symbiosis of ground-based fishery and electricity. To achieve this goal, it means that more than one-fourth of the fish farms in Taiwan will be covered by photovoltaic panels. This may cause some local environmental or social impacts. Therefore, the government had conducted environmental and social inspections on areas with high concentration of fish farms, expecting to take stock of issues, and remind developers to pay attention and strengthen communication to reduce the impact of solar photovoltaics on local areas.

This study focuses on the symbiosis of fish and electricity in six cities along the southwest coast of Taiwan, and inspected the local environmental ecology and socio-economic issues. The methods of environmental inspection include site surveys, ecological information mapping, interviews with ecological experts, and public consultation meetings. The socio-economic inspection mainly includes literature collection and analysis, local stakeholder interviews, expert discussions and public consultation to discover possible local problems.

The contribution of this research is to take stock of the environmental or social issues faced before development, and to classify the location so as to guide developers to choose locations with less environmental or social sensitivity. And give more opportunities for fisheries and energy transition in the process of aquaculture's adaptation to climate change.

CAST CAMBODIA: GROWING DOMESTIC FRESHWATER FISH VALUE CHAINS

Austin Leefers*

World Initiative for Soy in Human Health
American Soybean Association
St. Louis, MO, 63043
USA
aleefers@soy.org

Fresh water fish is the animal protein of choice among Cambodians. Yet, despite plentiful access to fresh water from the Mekong River, Tonle Sap lake, and rain for six months of the year, the Cambodian aquaculture sector is significantly lagging its neighbors.

The CAST Cambodia baseline study commissioned by ASA/WISHH, funded by the US Department of Agriculture (USDA), and implemented by World Fish surveyed 6 provinces and Phnom Penh municipality along with three control provinces. Analysis of this study shows the average aquaculture yield is 4.77 kg/m^2/year in pond and 44.00 kg/m^3/year in cage across species. *Pangasius* production, which accounts for over 50 percent of aquaculture production in the surveyed provinces, achieves less than 25% of the yield of similar systems in Vietnam. Fewer than 10% of Cambodian producers use commercial grade pellet feed for 100% of their fish's diet. Additionally, one hatchery produced 95% of Cambodian origin *Pangasius* fingerlings.

The CAST's total value chain approach pairs traditional agriculture technical assistance and extension services with marketing, branding, and financial training. CAST is working with the private sector to create credit products and production insurance for producers. The Cambodian Aquaculture Association has been formed in the image of US commodity groups to advocate for producers and link stakeholders throughout the production chain. Kansas State University has constructed applied research parks near Phnom Penh and Siem Reap where Cambodian technicians will train with researchers. A Cambodian Aquaculture Certificate program is being developed by Auburn University in conjunction with CAST. Finally, CAST is working with feed producers to ensure high quality fish feed is being produced in Cambodia and is available to fish farmers throughout the country.

The CAST project, funded through USDA's Food for Progress Program, aims to professionalize aquaculture production in Cambodia and create lasting institutions to ensure continual improvements well after it has closed its doors.

IMMUNOMODULATORY EFFECT OF HEAT-KILLED *Lactobacillus plantarum L-137* IN ATLANTIC SALMON (*Salmo salar*)

Peng Lei*, Byron M. M. Lange, Liv T. Mydland, and Margareth Øverland

Department of Animal and Aquaculture Sciences, Faculty of Biosciences
Norwegian University of Life Sciences
P. O. Box 5003, NO_ 1433 Ås, Norway
peng.lei@nmbu.no

To cope with multiple stress responses in fish, aquaculture sector is moving towards increased use of functional aquafeeds. Heat-killed Lactobacillus plantarum strain L-137 (HK L-137), an immunobiotic lactic acid bacterium, has been reported to have immunomodulatory effects in numerous animals, including fish such as Nile tilapia (Oreochromis niloticus) and bighead catfish (Clarias macrocephalus). Dietary supplementation of HK L-137 has also shown to improve growth performance, survival rate, and stress resistance.

The present study was conducted to evaluate the health effect of HK L-137 in salmonids by characterization of immune biomarkers in both in vitro and in vivo experiments. In vitro experiments were performed using RTgutGC, a rainbow trout intestinal epithelial cell line and SHK-1, an Atlantic salmon head kidney macrophage-like cell line. Results showed a positive effect of HK L-137 on gut barrier function as indicated by increased RTgutGC proliferation, transepithelial electrical resistance, and decreased RTgutGC monolayer permeability. HK L-137 also modulated immune responses by up-regulating expression of pro-inflammatory cytokine IL8 and TNFα in SHK-1 cel ls and decreasing the protein production of anti-inflammatory molecule Anxa1 in RTgutGC. A 9-week fish experiment with increasing dietary levels of HK L-137 were conducted (D1 0mg kg-1, D2 4mg kg-1, D3 20mg kg-1, D4 100mg kg-1, D5 2g kg-1 MacroGard®). No differences in growth performance were found among the dietary groups. However, a decrease in Anxa1 protein level in the distal intestine and an increase in non-specific IgM in the plasma were observed (Fig1), indicating an interesting immunostimulant property of HK L-137 in Atlantic salmon. 16S rDNA sequencing of gut microbiota revealed that the dominant microbial phyla are Proteobacteria, Firmicutes, and Actinobacteriota. Regarding alpha- and beta-diversity, we observed a subtle dose-response relationship. D4 had a higher richness, similar to D5, while D2 resembled the control diet.

The results from the present study suggest that HK L-137 is a promising functional additive that can improve health and welfare for Atlantic salmon.

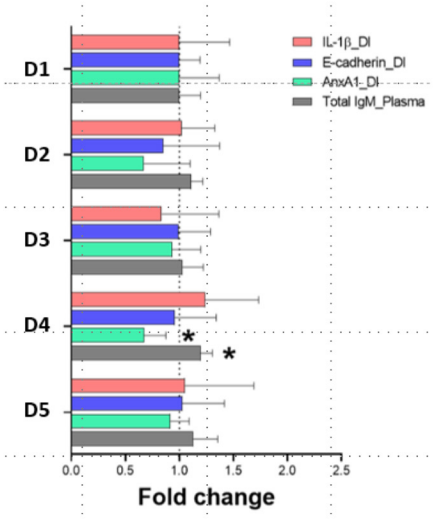


Figure 1 Immune markers in distal intestine and plasma

IN-VITRO ISOLATION AND CHARACTERIZATION OF POTENTIAL PROBIOTIC FROM GASTROINTESTINAL TRACT OF TROPICAL SPINY LOBSTER *Panulirus ornatus*

En Yao Lein*, Audrey Daning Tuzan, Mohd Tamrin Mohd Lal

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88450, Kota Kinabalu, Sabah

Email: my1921017t@student.ums.edu.my

Tropical spiny lobster *Panulirus ornatus* is one of the most prospective aquaculture species due to its high market value. Till now, methods to raise spiny lobster from eggs to adults is unavailable because of their long larval rearing stage, mass mortality, nutrient deficiency, and disease infection. One of the promising methods to overcome the spiny lobster culture obstacles is probiotics. Studies show the effectiveness of commercial probiotics and marine probiotics in spiny lobster culture. Gastrointestinal (GI) microbiota is a group of microorganisms that lives in symbiosis with the host and provides various beneficial biological functions towards the host. Thus, the study aims to screening, and characterize the potential probiotics isolated from the GI tract of spiny lobster. Bacteria strains were successfully isolated from the GI tract of *P.ornatus* on Trypticase soy agar (TSA) and De Man, Rogosa and Sharpe (MRS) agar. Bacteria count on TSA was range between 7.14 x and 4.17 x and bacteria count on MRS agar was a range between 1.81 x and 4.09 x . For screening, antimicrobial properties of isolated strains were tested against *Vibrio parahaemolyticus* and *Photobacterium damselae*. However, only 1 bacteria strain isolated on TSA shows antagonistic towards *Vibrio parahaemolyticus*, and no bacteria isolated on MRS agar shows antagonistic towards both tested pathogens. Bacteria strains and then tested for their enzymatic activities (amylase, protease, cellulase and lipase). 35 out of 50 bacteria strains tested showed positive results and could produce at least one of the enzymes. Isolated bacteria strains on TSA and MRS agar were then characterized and grouped using biochemical test kits where some of them were identified as *Shewanella putrefaciens, Stenotrophomonas maltophili, Alcaligenes faecalis, Alcaligenes xylosoxidans* and Acinetobacter with probabilities >95%. Most potential probiotic strains exhibited tolerance to high salinity (12%), acidic environment (pH 4) and alkalinity (pH 10) . 11 potential probiotic strains shows medium to high hydrophobicity and high auto-aggregation ability. 7 strains are harmless after administered to *Artemi*a nauplii with survival rate >50%. 7 potential probiotic strains will be identified.

THE INTERNATIONAL ARTEMIA AQUACULTURE CONSORTIUM (IAAC)

Yeong Yik Sung[1]*, Patrick Sorgeloos[1,2], and Jie Huang[3]

[1]Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia
[2]Lab of Aquaculture and Artemia Reference Center, Department of Animal Sciences and Aquatic Ecology Ghent University, B-9000 Gent, Belgium
[3]Network of Aquaculture Centres of Asia and Pacific (NACA), NACA PO Box 1040 Kasetsart University Post Office, Chatuchak, Bangkok 10900, Thailand

More than 40 years after the International Study on Artemia (an international interdisciplinary study of Artemia species and strains) was launched, which resulted in a lot of new knowledge and several new developments in fish and crustacean larviculture, there are several reasons to launch a new initiative to ensure a more sustainable provision of Artemia and to explore several new opportunities such as conservation of Artemia biodiversity, science-based protocols development for sustainable harvesting of wild sources, strain selection and selective breeding, propagation of improved guidelines, updated FAO Artemia manual and increased training and extension services, and many more. A meeting of Artemia experts in Kuala Lumpur in November 2019 envisaged establishing the International Artemia Aquaculture Consortium, a network of scientists, organizations, and the private sector that would collaborate in exploring opportunities. A provisional Steering Committee was formed along with members from 27 participating countries.

Following webinars on the History of Artemia Activities in Africa, Management of Artemia Resources of the Great Salt Lake, NACA Webinar on the Status of the Use of Artemia Cysts in Fish/Crustacean Hatcheries Around the World, a final consortium's SDG-aligned Artemia Aquaculture Workshop was held in September 2021 upon invitation of and in conjunction with the Global Conference on Aquaculture Millennium +20.

This new International Artemia Aquaculture Consortium (IAAC) was well received at the last FAO COFI Subcommittee on Aquaculture (SCAQ). Its structure and activities are now being finalized under the coordination of NACA who also hosts the IAAC website and prepares for the key conclusions and recommendations to be presented at the FAO COFI SCAQ meeting to be held in Mexico in 2023.

TRANSCRIPTOMIC ANALYSIS AND TIME TO HATCH VISUAL PREDICTION OF EMBRYO DEVELOPMENT IN THE ORNATE SPINY LOBSTER (*Panulirus ornatus*)

Courtney L. Lewis*, Quinn P. Fitzgibbon, Gregory G. Smith, Abigail Elizur, and Tomer Ventura

Centre for Bioinnovation
University of the Sunshine Coast
90 Sippy Downs Dr, Sippy Downs QLD 4556
courtney.lewis@research.usc.edu.au

The ornate spiny lobster, *Panulirus ornatus*, is a prime aquaculture candidate, however its complex life cycle comprised of several morphologically distinct stages presents a challenge to producing them through closed life-cycle aquaculture. Research has previously been focused on the larval and adult stages to address key issues faced by industry, resulting in a limited understanding of the morphological development, and staging of *P. ornatus* embryogenesis.

A comprehensive transcriptome comprising 11 stages across *P. ornatus* embryogenesis has been established, facilitating the characterisation of a series of key genes across embryo development. *Nanos* and *brachyury*, two gastrula-specific genes, indicate gastrulation occurs early in the embryogenesis period. Neuropeptide expression was reported across *P. ornatus* embryos, suggesting they are predominantly active later in embryogenesis as the nervous system develops.

Whilst gene expression is useful for characterising embryogenesis, this cannot be utilised to monitor live developing embryos in industry. Certain characteristic, such as eye spot development, provide a visual indicator of development. Hatch prediction models based on visual characteristics were shown to be an accurate method to predict the timing of the hatch for *P. ornatus* embryos kept at 26°C. The combination of transcriptomics and visual measures provides a basis for future characterisation and staging of *P. ornatus* embryogenesis.

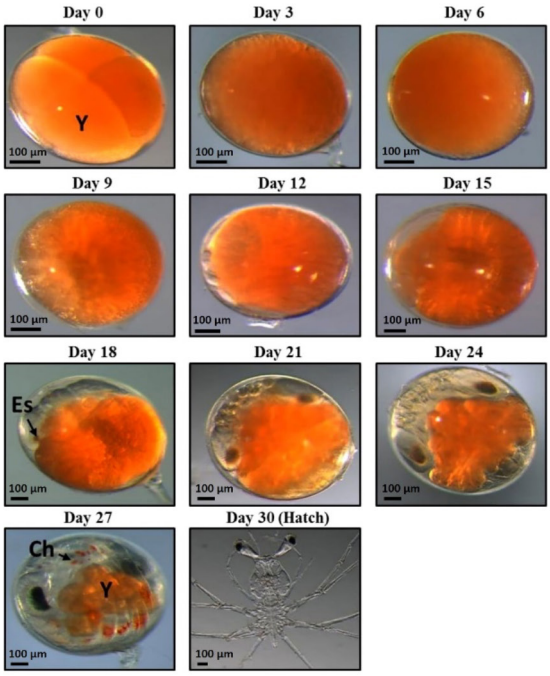


Figure 1: 11 stages of *Panulirus ornatus* development, taken for RNA extraction and transcriptome assembly.

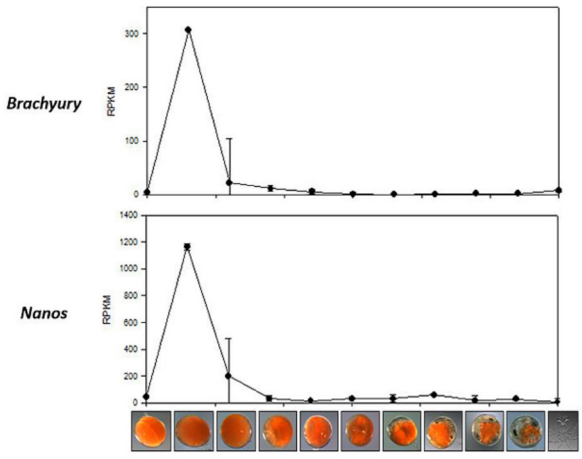


Figure 2: Expression of two gastrula specific genes, *brachyury* and *nanos* across 11 stages of embryogenesis in *Panulirus ornatus*.

MICROPLASTICS IN SEAFOOD FROM SINGAPORE LOCAL MARKETS

Haiyan LI*, Yai Foong CHEW, Lin Min LEE, Angela LI, and Dingyi Yu

National Centre for Food Science, Singapore Food Agency
TechQuest, 7 International Business Park Singapore 609919
Li_Haiyan@sfa.gov.sg

Plastic contamination has emerged as a global environmental concern. Studies carried out between 2000-2019 have estimated there are trillion individual pieces of microplastic (MPs, size less than 5 mm) in the world's oceans. Due to their small size, MPs can be ingested by many marine species, and bioaccumulated through the food chain to pose a feed and food hazard. However, the associated risks are still largely unclear as there is a lack of well-established analytical methods for identification, characterization, and quantification of MPs in food to support exposure studies for risk assessment.

In this study, we have developed and validated an analytical method that can be used to identify, size characterise and quantitate, by both number and mass, MPs that are present in seafood. Notedly, our method was able to simultaneously quantitate MPs by both number and mass, with mass being a more consistent measure for data interpretation. The analytical method was subsequently deployed for a survey of MPs in seafood sold in Singapore retail markets. Our survey results revealed that MPs were widely present in most types of seafood available in the Singapore market, e.g., mussels, clams, and fish in various shapes (pellets, fragments, or fibres in diverse polymers) and sizes (10 μm- 4000 μm), with polyethylene (PE) and polypropylene (PP) being the most abundant. While this study aimed to elucidate the presence and abundance of MPs contamination in different seafood sold in Singapore markets, our findings are largely in line with previous surveys carried out in other countries.

This study has not only revealed insights on the MPs that are present in seafood sold in Singapore retail market but also provided a model workflow that can be extended to other food types in future studies to generate data that can be used to inform the type, scale and extent of human dietary exposure to MPs from food.

IN VITRO PROFILING OF POTENTIAL FISH PROBIOTICS, *Enterococcus hirae* sp., ISOLATED FROM JADE PERCH *Scortum barcoo* AND SAFETY PROPERTIES ASSESSED USING WHOLE GENOME SEQUENCING

Wenrui Li*, Chiun Hao Lim, Yulan Wang, Patricia Lynne Conway, Say Chye Joachim Loo

NTU Institute for Health Technologies, Interdisciplinary Graduate Program
Nanyang Technological University
61 Nanyang Drive, Singapore 637335
Wenrui001@e.ntu.edu.sg

Exploiting the use of probiotics in aquaculture is one key strategy to promote fish growth while mitigating fish-associated diseases. Among the different types of probiotics, host-derived probiotics exhibit promising efficacy compared to commercial or terrestrial-derived ones as they are adapted to the host environment. This study aimed to identify and characterize potential probiotics from jade perch (*Scortum barcoo*), one of the newly farmed fish species in Singapore, to enhance its disease resistance.

Gut isolates from jade perch were screened for their ability to inhibit the growth of pathogenic *Aeromonas sobria*, *Streptococcus iniae*, and *Streptococcus agalactiae*. Several isolates that demonstrated antagonistic effects against *A. sobria* and *S. iniae* were later identified to be *Enterococcus hirae*. The pathogen inhibition efficacy of this isolate was also examined in both jade perch and tilapia gut suspensions, mimicking more complex host microbial environments. Significant clear zones were found when co-incubated with jade perch gut suspensions, whereas only minimal inhibition zones were produced in tilapia gut suspensions, suggesting the advantage of using host-derived probiotics.

The safety of the final probiotic candidate was evaluated using whole-genome sequencing. This strain did not harbor any known toxins and mobile genetic elements. Conjugation experiments confirmed that its inherent antibiotic resistance gene aac (6')-Iid was not transferable. Two classes of bacteriocins were found in the probiotic genome, which partially explained its antimicrobial activities against both Gram-positive and Gram-negative pathogens. The use of *E. hirae* as a probiotic for Jade Perch and other freshwater fish is promising as it is acclimatized to aquatic environments, safe and effective.

SEPARATION, IDENTIFICATION AND GENE EXPRESSION ANALYSIS OF PMAMP-1 FROM *Pinctada fucata martensii*

Haiying Liang*, Junjun He, Jiaping Zhu, Xiaochen Fang

Fisheries College, Guangdong Ocean University, Zhanjiang, Guangdong
zjlianghy@126.com

Pinctada fucata martensii (*P.f. Martensii*) is a bivalve mollusk, and as it lacks a specific immune system its defense against pathogens relies entirely on cellular immunity and humoral immune factors. Various diseases have occurred more frequently in recent years, resulting in increased mortality of *P.f. Martensii*. Improving the disease resistance of *P.f. Martensii* is therefore an urgent concern that needs to be addressed. In this study, multi-step high performance liquid chromatography (HPLC), combined with Mass Spectrometry (MS), was used to isolate and identify proteins with antibacterial activity from the serum of *P.f. Martensii*.

Oysters were challenged with a mixture of heat-killed Escherichia coli and Micrococcus luteus injected into the adductor muscle with a needle, and then returned to sea water. Hemolymph was collected and pretreated using the methods described by Mitta. First, plasma samples were fully eluted using a SunfireTM prep C18 column at a flow rate of 1 mL/min. Next, active fractions were loaded onto an analytical Vydac C18 RP HPLC column, eluted with acetonitrile of different gradients. The fractions with peaks were then manually collected and tested for antibacterial activity by freeze drying.

Hemolymph was collected from *P.f. Martensii*. Cells and debris were removed by centrifugation and the supernatant was loaded on to a Sunfire™ prep C18 column and eluted with a gradient of 5–60% acetonitrile, yielding the fraction having antibacterial activity. The fractions were subjected to analytical reversed phase HPLC using a Vydac C18 RP HPLC column. One of the purified proteins that eluted at approximately 29 min (Fig. 1, labelled C3) had bacteriostatic activity. The antibacterial activity of PmAMP-1 was determined using the microplate reader method. The growth of *M. luteus* (Fig. 2A) and *E. coli* (Fig. 2B) was significantly inhibited following the addition of PmAMP-1 ($p < 0.05$).

FIG. 1. Purification and identification of PmAMP-1.

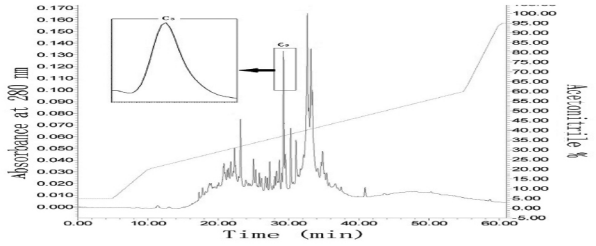
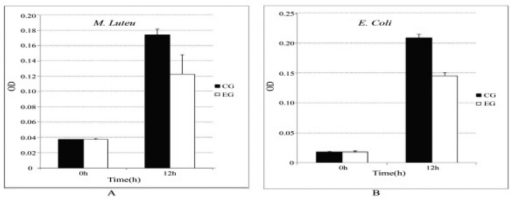


FIG. 2. Inhibition assay of PmAMP-1 for *M. luteusand* and *E. coli*. A for *M. luteusand*, B for *E.coli*, CG represent control group and EG represent experimental group.



APPLICATION OF ENDOCRINE TECHNIQUES IN FISH SPAWNING

Liang Bing, Jiang Jun Hui, Xu Qunying

Marine Aquaculture Centre
Singapore Food Agency
11 St. John's Island
Singapore 098659
LIANG_Bing@sfa.gov.sg

One of the key R&D initiatives in Singapore Food Agency's Marine Aquaculture Centre (MAC) is to close the reproductive cycles of commonly farmed fish species in Singapore. Based on MAC's previous experience, this topic summarises the factors contributing to successful spawning of different food fish in captivity. Effects of various fish related factors such as their sex maturity, nutrition and health as well as environmental or procedural related factors such as culture environment, hormone and fish handling methods will be explained. It has been shown that fish maturation and spawning are controlled by the reproductive axis, i.e. the brain-pituitary-gonad axis. The fish brain picks up signals from the environment and produces a hormone called Gonadotropin-releasing hormone (GnRH), which will stimulate the pituitary to release Follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones further stimulate the gonad to release sex hormones such as androgens and estrogens, important chemicals signifying the start of spawning activity. The poster showcases some examples of fishes that have been successfully spawned either at MAC or local fish farms.

BREEDING OF AQUACULTURE SPECIES – JADE PERCH *Scortum barcoo* AS A CASE EXAMPLE

Liang Bing, Jiang Jun Hui, Xu Qunying

Marine Aquaculture Centre
Singapore Food Agency
11 St. John's Island
Singapore 098659
LIANG_Bing@sfa.gov.sg

The Barcoo grunter, or jade perch (*Scortum barcoo*) is a freshwater finfish species native to Australia. Due to its attributes such as fast growth and good taste, the jade perch is gaining popularity as an aquaculture species in China, Malaysia and Australia. There are land-based farms in Singapore expressing interest to spawn the species locally, so as to reduce reliance on overseas fry supply. Therefore, this study was carried out to establish jade perch breeding and larviculture techniques suitable for use in Singapore's fish farms and investigate the tolerance of the jade perch to various salinities to assess the suitability of jade perch farming in coastal fish farms.

TRANSCRIPTOMIC ANALYSIS OF GONADS IN MALABAR RED SNAPPER (*Lutjanus malabaricus*) REVEALS GENES ASSOCIATED WITH REPRODUCTION

Bing Liang[1,2*], Dean R. Jerry[1,4], Xueyan Shen[1], Joyce Koh[1,3], Celestine Terence[1], Maria G. Nayfa[1,3], Vu Nguyen[1], Grace Loo[3], Shubha Vij[3], Jose A. Domingos[1]

1. Tropical Futures Institute, James Cook University Singapore, Singapore 387380, Singapore
2. Marine Aquaculture Centre, Singapore Food Agency, 52 Jurong Gateway Road
JEM Office Tower, #14-01, Singapore 608550
3. School of Applied Science, Republic Polytechnic, 9 Woodlands Avenue 9, Singapore 738964, Singapore
4. Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University
Townsville, QLD 4810, Australia
*Liang_Bing@sfa.gov.sg

Malabar red snapper is an important and high-value aquaculture species in Singapore that is attracting substantial interest and new industry investment. Despite its rising profile as an aquaculture candidate there currently is scarce knowledge and resources related to the sex determination and differentiation of the species. The production of good quality eggs consistently in a biosecure breeding facility is an important steppingstone for establishing a selective breeding program for the species which is now underway in Singapore. Related reproductive studies are needed to address the challenges in Malabar red snapper seed production such as limited success of spawning the species in closed culture systems. Herein, we report the first gonadal transcriptomes of *L. malabaricus* by sequencing testes (*n=6*) and ovaries (*n=6*) in adult Malabar red snapper using high-throughput RNA-Seq technology. After data filtering and mapping to the reference genome, a total of 27,493 genes were obtained, out of which 14,421 significant differentially expressed genes (DEGs) were identified in testis (n=7,922) and ovary (n=6,499) by comparing the gene expression in testis and ovary. The DEGs include genes previously known to be related to gonadal development and gametogenesis such as male-biased *dmrt1, spat7, sox9, and nanos2,* and female-biased *foxl2, sox3, wnt4a, esr1, and cyp19a*. The expression level of selected DEGs were verified by RT-qPCR, the result of which showed positive correlation with RNA-Seq data. This study lays the basis for further research in genes involved in sex determination and differentiation, sex control and reproduction in Malabar red snapper.

THE EFFECTS OF ENVIRONMENTAL FACTORS ON TOTAL ARSENIC BIOSORPTION IN *Sarcodia Suiae*

Mary Joy Halog Libatique*, Meng-Chou Lee, Han-Yang Yeh, Fu-Jie Jhang

Department of Aquaculture, National Taiwan Ocean University, Keelung City 20224, Taiwan
libatiquemaryjoy@yahoo.com

The present study tested the effects of environmental factors in total arsenic accumulation by *Sarcodia suiae*. *The* alga, *S. suiae* was exposed individually to a range of temperature (15, 20, and 25 °C), and LI (30, 55, and 80 μmol photons m–2 s–1) at initial arsenate [As(V)] concentration (iconc: 0, 62.5, 125, 250, and 500 μg L–1) conditions. Temperature significantly affected TAs production and maximum absorption were obtained at 15 °C, which was significantly stimulated by increasing $_i$conc. On the other hand, LI had no significant effect on TAs production, although maximum absorption was estimated in 80 μmol photons m–2 s–1. Stronger effects in morphology were observed in higher $_i$conc although the factor (temperature or LI) did affect the difference somewhat. The uptake results depended upon environmental factors (temperature, LI, and $_i$conc) that may provide important insights regarding particular culture conditions for efficient wastewater treatment processes for selective algal species. Yet, future studies are needed to further the understanding of As metabolic processes by this alga to ascertain their effectiveness if used for bioremediation.

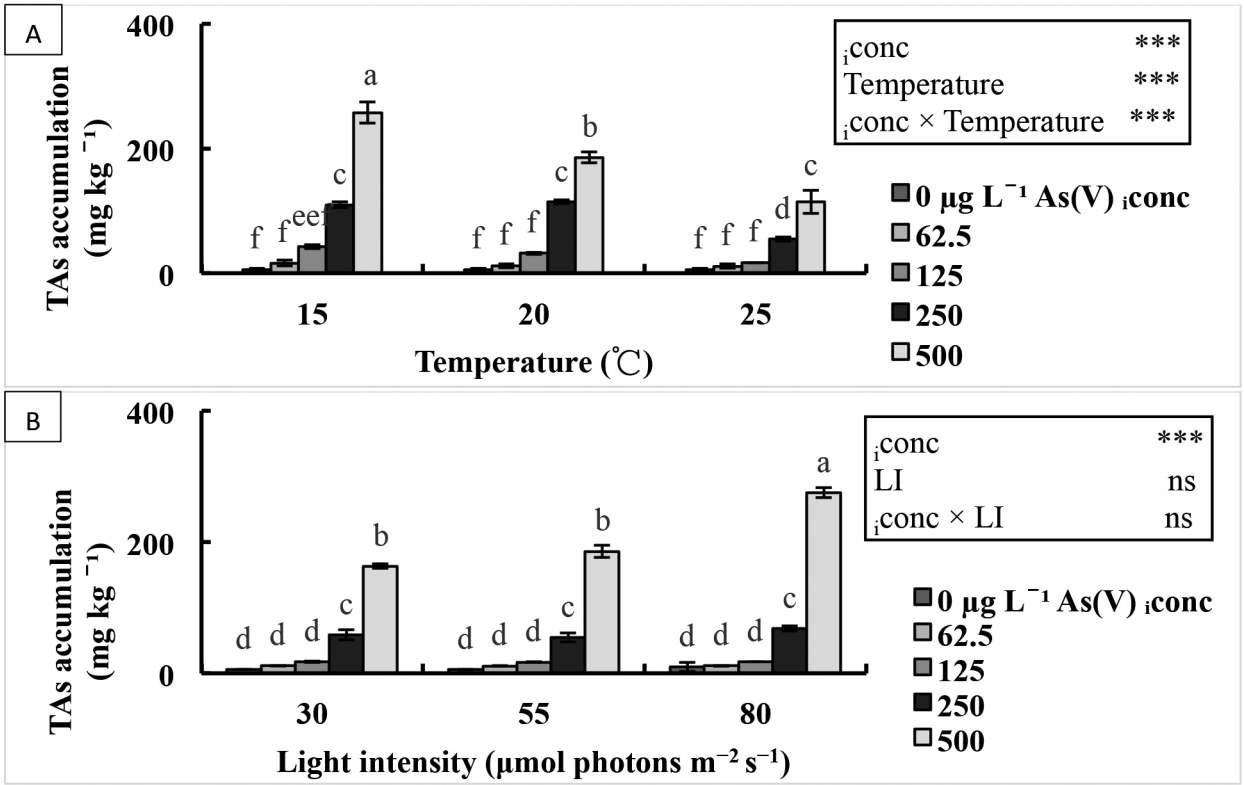


Figure 1.The effects of temperature (15, 20, and 25 °C) (A) or LI (30, 55, and 80 μmol photons m^{-2} s^{-1}) (B), $_i$conc [initial As(V) concentrations: 0, 62.5, 125, 250, and 500 μg L^{-1}] and their interactions ($_i$conc × temperature/LI) on the accumulation of TAs in *S. suiae* after seven days of culture under the indicated conditions. All data are means of three replicates, and error bars indicate one standard deviation. Different letters on bars indicate significant differences between treatments (*** $p \leq 0.001$).

WATER NUTRIENTS RECORDED OVER A ONE-YEAR OPERATION OF A MARINE LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE

Ana I. Lillebø*, Bruna Marques, Mariana Murteira, Guilherme Teixeira and Ricardo Calado

Department of Biology & CESAM & ECOMARE
University of Aveiro
Campus Universitário de Santiago
3810-193 Aveiro, Portugal
lillebo@ua.pt

Within PPS2 of project VALORMAR, water nutrients recorded on different compartments of a marine land-based integrated multi-trophic aquaculture were monitored every two weeks during a one-year operation, at sunrise, zenith and sunset. Every three months a 24-hour monitoring cycle was performed. Shifts water pH, temperature, dissolved oxygen concentration and salinity, as well as in suspended particulate matter (SPM), particulate organic matter (POM), total nitrogen (TN) and phosphorus (TP), as well as dissolved inorganic nitrogen (DIN = NOx-N + NH4-N) and dissolved inorganic phosphorus (DIP = PO4-P) were monitored. Inflowing water from a supplying coastal lagoon was sampled when entering earthen ponds stocked with gilthead seabream and seabass, as well as when latter being pumped to supply tanks stocked with different species of seaweeds (brown, red and green) and then returned to the coastal lagoon.

This study allowed an unprecedented resolution on the nutrient flow dynamics over the different compartments of the marine land-based integrated multi-trophic aquaculture surveyed, allowing to determine its carrying capacity.

The authors acknowledge the support of Rui Pereira, Bárbara Pitarma and Helena Abreu of AlgaPlus during the present study.

Supported by:

MOLECULAR CHARACTERIZATION, EXPRESSION ANALYSIS AND FUNCTIONAL INSIGHTS OF PEPTIDOGLYCAN-RECOGNITION PROTEIN SC2 (PGRP-SC2) FROM *Amphiprion clarkii*

Sarithaa sellaththurai, Chaehyeon Lim*, Kishanthini Nadarajapillai, Jehee Lee

Department of Marine Life Sciences, Jeju National University
Jeju Self-Governing Province 63243, Republic of Korea
Email: sarithaasellaththurai@gmail.com

PGRP family is widely distributed in most animal species, from insects to mammals. Structurally, all PGRPs contain a conserved peptidoglycan binding type 2 amidase domain. PGRPs can bind bacterial peptidoglycans, which exist in almost all bacterial cell walls, especially Gram-positive bacteria. Based on molecular weight, PGRPs are classified into three types, i.e. short-, intermediate-, and long- PGRPs (PGRP-S, PGRP-I, and PGRP-L, respectively). The PGRP-SC2 belongs to the short (S) super family, which are mainly secreted proteins.

Current study identified PGRP-SC2 from *Amphiprion clarkii* (AcPGRP-SC2) with an ORF of 492 bp, which encoding 164 amino acids. The molecular weight was predicted as 17.58 kDa and pI was estimated as 8.9. NCBI conserved domain search revealed PGRP super family from AcPGRP-SC2. The deduced amino acid sequence of AcPGRP-SC2 showed 97.0%, 74.3%, 71.9%, and 71.3%, identity with that of *Amphiprion ocellaris*, *Mastacembelus armatus*, *Oreochromis niloticus*, and *Maylandia zebra*, respectively. The AcPGRP-SC2 was suclustered with PGRP-SC2 of *Amphiprion ocellaris* during phylogenetic analysis. The AcPGRP-SC2 mRNA transcript was detected in all tested tissues by real-time PCR. The highest mRNA expression was observed in skin followed by the gill and stomach tissues. The expression modulations in AcPGRP-SC2 mRNA level in gill tissue was observed with all three stimulants (*Vibrio harveyi*, polyinosinic: polycytidylic, and lipopolysaccharide) during the experimental period (6 h, 12 h, 24 h, 48 h, and 72 h). Further, AcPGRP-SC2 showed binding activity different bacteria (Figure. 01).

According to the in-silico study, AcPGRP-SC2 resembled the conserved domain of the PGRP super family of previously identified PGRPs. Moreover, the pairwise alignment and phylogenetic analysis reinforcing the homology of AcPGRP-SC2 with other teleost species. The observed significant modulations in AcPGRP-SC2 mRNA level upon immune challenge and the bacterial binding activity suggested that AcPGRP-SC2 might have a potential role in host immunity.

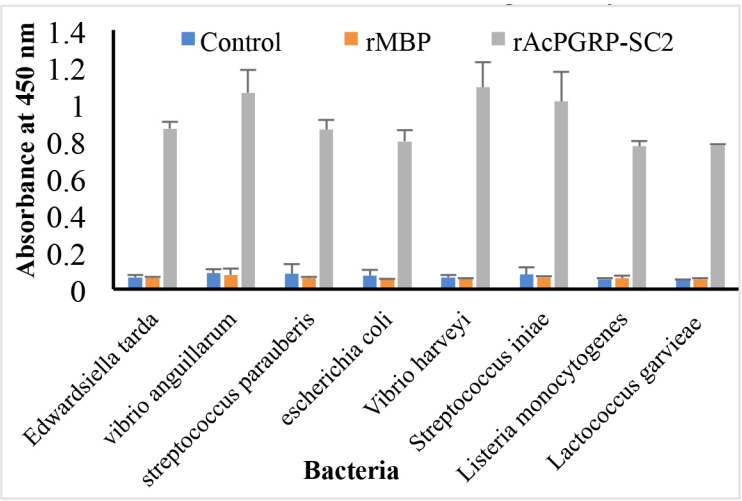


Figure. 01. Bacterial binding activity of recombinant AcPGRP-SC2 with Maltose binding protein (MBP) against eight different bacteria by ELISA.

THE STUDY OF DIFFERENT CULTURE CONDITIONS FOR THE CULTURE OF ROTIFERS, *B. rotundiformis*

Lim M.* and Loh J.

Diploma in Veterinary Technology, School of Applied Science, Temasek Polytechnic, Singapore 529757
1702816H@tp.edu.sg

Rotifers are commonly cultured in marine fish farms as live feed for fish larvae. Different methods of culturing rotifers are employed depending on the desired yield and resources the farmer is willing to put in. The aim of this experiment was to find out the most cost-effective method of culturing rotifers (*Brachionus rotundiformis*) which resulted in the highest yield in Goodview Aquarium's outdoor environment. Three trials were carried out for a duration of 10 to 12 days each. The first trial tested on the use of different feed types: activated baker's yeast (Saccharomyces cerevisiae), Live Algae (*Nannochloropsis*) and the formulated feed Selco S.parkle. The second trial tested on different feeding frequencies, at 2 times, 6 times and 12 times a day. Finally, the third trial tested the effects of water change, comparing an RAS system against 25% and 50% manual water change. From the first trial, it was found that feeding baker's yeast resulted in the highest rotifer population, at 11.5 million rotifers. For the second trial, it was revealed that feeding the rotifers twice a day yielded the highest rotifer population, at 3 million rotifers. Lastly, the third trial showed that carrying out manual water change resulted in the highest rotifer population, at about 1 million rotifers, regardless of the amount of water change. However, there were several factors present during the trials that may have affected the results, such as the contamination by ciliates and quality of the feed, causing them to be inaccurate. In conclusion, the results showed that feeding the rotifers with baker's yeast twice a day, while carrying out manual water change was the most effective way to culture rotifers in Goodview Aquarium's outdoor environment.

EFFECT OF VARIOUS FISH FEED ON THE ECONOMIC VALUE OF SELECTED ORNAMENTAL FISH

Joshua Lim*, John Chua and Andy Lim

Diploma in Veterinary Technology, School of Applied Science,
Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
1703880C@student.tp.edu.sg

In ornamental fish farms such as Sunny Aquarium, where the focus lies on the imports, exports and transhipments of ornamental fish. Furthermore, profits are largely dependent on the exports of ornamental fish. For the company to continue in the industry, the fish they export must be of good quality especially in terms of colouration, health, and long lifespans and most importantly size. This would create a higher demand of good quality fish, allowing the industry to thrive well. Hence, the food consumed is of utmost importance, so the fish can attain all these good characteristics as they grow, thus allowing the company to maximise its profits.

In Sunny Aquarium, the ornamental fish are mainly obtained by import into the farm rather than in house breeding. The fish are categorized by sizes labelled as S, SM, M, ML, L, XL in ascending order of size. XL sized fish have the highest economic value which is why a feed which promotes the fastest growth is necessary to bring in the highest amount of profit possible.

The study focused on the efficacy of different types of feeds on the growth rate of six species of ornamental fish. The growth rate was determined by measuring and recording the gain in length and weight the fishes. The fish were then categorized by size and the economic value was determined. Three pelleted feeds were used: one made up of shrimp meal, one made up of fish meal, and the third a frozen bloodworm.

THE FATE AND TRANSPORT OF MICROORGANISMS AT THE SEDIMENT-WATER INTERFACE

Presenting Author*: Elton Lim Wenxiong
Co-Authors: Stan Chan Siew Herng, Maria Yung Pui Yi, Stefan Wuertz

Bacterial pathogens are a major cause of economic losses in cultured fish production worldwide; yet the reservoirs of such pathogens in the natural environment are poorly understood. We hypothesised that microorganisms could attach to aquatic aggregates (such as marine snow) before settling to the seabed, where they may persist as viable organisms for some time. This study aimed to 1) understand the interactions of disease-causing organisms in the water column above sediments colonised by biofilms and 2) predict the fate and transport of those pathogens using flume experiments. Using 16S rRNA gene metabarcoding, we detected potential sequences from human-associated (*Mycobacterium*) and fish associated (*Tenacibaculum*) pathogens in sediments of aquaculture and non-aquaculture sites. In controlled flume studies, all the target organisms (*Tenacibaculum maritimum*, *Enterococcus faecium* and bacteriophage P22) decayed more slowly in sediments than in the open water, suggesting that sediments can act as reservoirs of aquaculture-related pathogens, potentially threatening aquatic and human health. The longer decay rates of aggregate-bound cells in both stationary microcosms and continuous-flow flume experiments revealed that marine snow could serve as vectors to facilitate microbial survival and persistence. The outcomes of this study may help to inform future aquaculture operations of their environmental impacts and improve safe aquaculture farming practices and food security.

EFFECT OF DIETARY PREBIOTICS AND PROBIOTICS ON INNATE IMMUNITY, ANTI-OXIDANT CAPACITY AND INTESTINAL MICROBIOTA OF RED SEABREAM *Pagrus major*

Jongho lim*, and Kyeong-Jun Lee

Department of Marine Life Sciences
Jeju National University, Jeju 63243
South Korea
jhlim95@jejunu.ac.kr

Prebiotics (PE) and probiotics (PO) have been being studied in various fish species as natural immune enhancers in aquaculture feeds to improve immunity and intestinal microbial flora of aquatic animals. Therefore, this study was conducted to evaluate the effects of PE or PO supplementation in diet for growing red seabream on immunity and antioxidant capacity and changes in the intestinal microbial flora.

A basal diet (Con) was formulated to meet the nutrient requirement of red seabream. Dietary starch in Con were replaced with 0.6% Bio-Mos, *Lactobacillus plantarum, Bacillus subtilis, Bacillus licheniformis* and probiotics mixture (designed as PE-BM, PO-LP, PO-BS, PO-BL and PO-Mix, respectively). Total 450 fish were stocked into 18 polypropylene tanks (400L) in triplicated groups per dietary treatment. Fish were fed ad lubitum with one of the diets two times (08:30, 18:30 h) a day for 63 days.

Phagocytic activity was not significantly affected by the supplementation. Lysozyme activity was significantly higher in all the supplemented groups than in Con group. Immunoglobulin (Ig) of PO-Mix, antiprotease (AP) of PO-BL, glutathione peroxidase activity (GPx) and superoxide dismutase activity (SOD) of PO-BS, PO-BL and PO-Mix groups were significantly higher than that of Con group. The ratio of total *Vibrio*/heterotrophic bacteria counts was significantly lower in PO-LP, PO-BL and PO-Mix groups than Con group. Therefore, dietary supplementation of PE and PO is considered to improve innate immunity and antioxidant enzyme activity and inhibit *Vibrio* bacteria in the intestine.

TABLE 1. Innate immunity and anti oxidant enzyme activity of red seabream fed the experimental diets for 63 days.

Dietary Treatments	Phagocytosis[1]	Lysozyme[2]	Ig[3]	AP[4]	GPx[5]	SOD[6]
Con	1.52±0.02^{ab}	23.2±1.30^{c}	17.0±3.69^{b}	14.0±0.57^{b}	224±18.4^{b}	30.3±4.00^{b}
PE-BM	1.64±0.11^{ab}	35.2±3.42^{a}	24.2±3.45^{ab}	15.2±0.47^{ab}	244±8.10^{ab}	43.4±8.62^{ab}
PO-LP	1.51±0.12^{b}	30.8±4.58^{ab}	22.8±2.74^{ab}	14.3±0.81^{ab}	250±9.68^{ab}	41.5±7.21^{ab}
PO-BS	1.66±0.05^{ab}	34.5±3.98^{a}	20.4±3.55^{ab}	15.3±2.49^{ab}	258±15.5^{a}	51.8±8.18^{a}
PO-BL	1.64±0.07^{ab}	29.0±3.51^{b}	22.5±4.43^{ab}	18.1±1.28^{a}	253±16.4^{a}	55.5±8.13^{a}
PO-Mix	1.67±0.09^{a}	34.0±1.83^{a}	26.4±1.83^{a}	15.3±1.67^{ab}	259±9.56^{a}	54.0±14.8^{a}

[1]Phagocytosis (% inhibition), 2Lysozyme (μg/mL), [3]Immunoglobulin (mg/mL), [4]Anti-protease (% inhibition), [5]Glutathione peroxidase activity (mU/mL), [6]Superoxide dismutase activity (% inhibition). Means in the same column with different letters were significantly different ($P < 0.05$)

VITAMIN K REQUIREMENT OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Hyunwoon Lim* and Kyeong-Jun Lee

Department of Marine Life Sciences
Jeju National University, Jeju 63243
South Korea
hwlim@jejunu.ac.kr

Among vitamin K (VK), VK_3 (menadione, 2-methyl-1, 4-naphthoquinone) is used for animal feeds in the form of menadione sodium bisulfite (MSB). It was reported that dietary VK supplementation is not necessary for Pacific white shrimp. Therefore, this study was conducted to determine VK requirement in diet for Pacific white shrimp as well as to verify whether VK supplementation is necessary or not.

Shrimp (initial mean body weight, 0.45±0.00 g) were randomly distributed in 28 acrylic tanks (20 shrimp per tank). To confirm the *in vivo* synthesis of vitamin K by intestinal bacteria, an antibiotic (tetracycline hydrochloride) was added to a basal diet (Con) and considered as Con_2. MSB was added so that the menadione content of the experimental diets was 50, 100, 200, and 400 mg/kg. The analyzed menadione content of the diets were 0 (Con and Con_2), 40.8 (M50), 113.9 (M100), 195.4 (M200) and 363.2 (M400) mg/kg. Three tanks were randomly assigned to each diet, and the shrimps were fed six times daily for 7 weeks.

At the end of the feeding trial, final body weight, weight gain and specific growth weight were significantly higher in MSB added groups than Con and Con_2 groups. Those of Con group was significantly higher than those of Con_2 group (Table 1). Feed conversion ratio was significantly higher in shrimp fed Con_2 diet than that of other groups. Protein efficiency ratio was significantly lower in Con_2 group than in all other groups. These results indicate that the addition of MSB in diet significantly affects the growth performance and feed utilization of the shrimp and that VK supplementation is necessary for the shrimp diet.

.

TABLE 1. Growth performance, feed utilization and survival of Pacific white shrimp *Litopenaeus vannamei* (initial mean body weight: 0.45±0.00g) fed the experimental diets for 7 weeks

Dietary Treatment	FBW (g)	WG (%)	SGR (%)	FCR	PER	Survival (%)
Con	6.58±0.45^{c}	1369±98.5^{c}	5.16±0.13^{b}	1.83±0.12^{ab}	1.70±0.11^{a}	97.5±2.89
Con_2	5.81±0.42^{d}	1198±94.2^{d}	4.93±0.14^{c}	1.96±0.15^{a}	1.54±0.13^{b}	100±0
M50	8.57±0.24^{a}	1822±52.3^{a}	5.68±0.05^{a}	1.72±0.06^{b}	1.71±0.06^{a}	98.8±2.50
M100	8.20±0.10^{ab}	1739±25.6^{ab}	5.60±0.03^{a}	1.72±0.05^{b}	1.72±0.05^{a}	100±0
M200	8.58±0.53^{a}	1822±116^{a}	5.68±0.11^{a}	1.73±0.07^{b}	1.70±0.07^{a}	100±0
M400	7.95±0.31^{b}	1679±70.3^{b}	5.54±0.08^{a}	1.76±0.11^{b}	1.69±0.10^{a}	98.8±2.50

CHROMIUM-L-METHIONINE COMBINATION WITH ZINC AMINO ACID COMPLEX OR SELENOMETHIONINE ON GROWTH PERFORMANCE AND ANTIOXIDATIVE ENZYMES IN RED TILAPIA *Oreochromis spp.*

Rawiwan Limwachirakhom*, Orapin Jintasataporn and Srinoy Chumkam

Department of Aquaculture, Faculty of Fisheries, Kasetsart University
Bangkok 10900, Thailand
Rawiwan.nic@gmail.com

Red Tilapia *(Oreochromis spp.)* is the freshwater fish which is important for the economic in Thailand. The production cost is wide variation adequate due to the climate and environmental conditions, resulting in sudden death in red tilapia or contagious disease. To improve the quality of live fish and immunity for preventing disease in red tilapia, dietary trace elements have a significant impact on immune functions, disease resistance, and stress free of fish. However, trace minerals come in two varieties, inorganic and organic. The organic minerals are more soluble, stable, absorbable and metabolizable than inorganic minerals. Organic minerals such as Chromium (Cr), Zinc (Zn) and Selenium (Se) are the three essential microminerals according to the important role in nutritional and physiological response in fish. The objective of this present study is to study on the effect of organic trace mineral supplementation on growth performance and immunity.

The study is assigned in CRD with 3 treatments and four replicates. Three diets supplemental with different mineral in Table 1 are applied to fish, a) Control without trace mineral supplementation, b) T1 diet of Chromium-L-Methionine at 300 ppb combination with Zinc amino acid complex at 60 ppm and c) T2 diet of Chromium-L-Methionine at 300 ppb combination with Selenomethionine at 500 ppb. Red tilapia with initial mean weight of 190± 12 gram/fish are randomly 34 fish distributed to each cage of 2 × 2 × 2.5 m per cage (totally 12 cages) in freshwater pond then the density is 15 fish/m^2. During the 8-week feeding trial, the fish are fed 3-4% of their body weight twice a day at 09.00 and 16.00. Fish are weighed every 2 weeks and then collected blood sample for study immune response. The results in Table 2 shows that red tilapia fed diets of Chromium-L-Methionine combination with Zinc amino acid complex in T1 has significantly *(P <0.05)* higher final weight, weight gain, average daily gain (ADG) and feed conversion ratio (FCR) follow by T2 which has higher growth performance compares to fish fed control diet without organic trace minerals. The antioxidative enzymes in figure 1, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and lysozyme activity, show no significantly differences *(P <0.05)*. Therefore, Chromium-L-Methionine combination with Zinc amino acid complex clearly enhances red tilapia growth performance and feed utilization.

TABLE 1. Chromium-L-Methionine, Zinc amino acid complex and Selenomethionine in experimental diets.

	Diet		
Trace minerals	Control	T_1	T_2
Chromium-L-methionine (ppb)	-	500	500
Zinc amino acid complex (ppm)	-	60	-
Selenomethionine (ppb)	-	-	300

TABLE 2. Growth performance of red tilapia fed diets with different trace minerals.

Growth performance	Diet		
	Control	T_1	T_2
Final weight (g)	547.72 ± 13.66^{b}	586.58 ± 22.91^{a}	569.49 ± 13.63^{ab}
Weight gain (g)	355.15 ± 8.60^{b}	394.07 ± 25.91^{a}	377.19 ± 18.71^{ab}
ADG (g)	6.34 ± 0.15^{b}	7.04 ± 0.46^{a}	6.74 ± 0.33^{ab}
SGR (%)	1.87 ± 0.05	1.99 ± 0.11	1.94 ± 0.11
FCR	1.55 ± 0.04^{a}	1.36 ± 0.10^{b}	1.44 ± 0.04^{b}

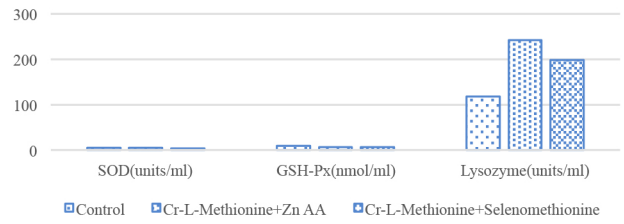


Figure 1. Superoxide dismutase, Glutathione peroxidase and Lysozyme in serum of red tilapia fed diets with different trace minerals.

DEVELOPMENT OF A COMMERCIAL-SCALE SELECTIVE BREEDING PROGRAM FOR VIETNAMESE STRIPED CATFISH (*Pangasianodon hypopthalmus*)

Curtis Lind [1*], Wagdy Mekkawy [1], Scott Cooper [1], Nigel Valentine [1], James Kijas [1], Moira Menzies [1], Nguyen Ngoc Qui[2], Nguyen Cong Can [2], Vo Minh Khoi [2], Lam Dinh [2], Harry King [1]

[1] CSIRO Agriculture and Food, Hobart, Tasmania, 7004, Australia
[2] Viet-Uc Seafood Corporation, Ben Nghe, District 1, Ho Chi Minh City, Vietnam

The striped catfish (*Pangasianodon hypopthalmus*) industry is the largest finfish aquaculture industry in Vietnam and produces over 1 million tonnes per year. The long-term sustainability and continued productivity will depend on the ongoing availability of high-quality genetics throughout the sector. To date the industry has benefitted from a government-run selective breeding program for over 15 years (Vu et al. 2019), however as the industry evolves a desire to develop commercial programs has emerged. We outline the development of a family-based selective breeding program established in 2017 and estimate the genetic parameters for growth traits (weight and length), carcass quality traits (fillet yield, red muscle content) and general survival. Founder individuals from four sources were used to establish the population through multiple spawning events. Strip-spawning was conducted twice-yearly to create full-sib families using a 2x2 mating design, whereby each male is crossed with two females and each female crossed with two males. Routine grow-out trials were conducted in lined ponds and data capture workflows developed and are described. Variance components and heritabilities for key traits were estimated using REML and an individual animal model (Harvest weight: h^2 = 0.44 ± 0.04; Red muscle %: h^2 = 0.23 ± 0.03; Fillet yield: h^2 = 0.37 ± 0.03). Genetic improvement for harvest weight was 7% per year across a four-year period using an Optimal Contributions approach for selection of broodstock and management of inbreeding accumulation. Key lessons are discussed throughout and highlight the value of data collection and management systems and the value of well-trained field staff to the overall success of the breeding program implementation. We conclude by outlining several emerging opportunities for continued improvement of the program, including the investigation of new traits and the implementation genomic selection.

SCALE DROP DISEASE VIRUS (SDDV) IN BARRAMUNDI: CURRENT UNDERSTANDING OF ITS CLINICAL PRESENTATION, PATHOLOGY, AND MANAGEMENT

Jeffrey Ling Min Than

James Cook University
jeffreyminthan.ling@my.jcu.edu.au

Barramundi or Asian Seabass (*Lates calcarifer*), is an important farmed food fish in Singapore. As the production intensifies, farmers have reported many devastating diseases. One of them is the scale drop syndrome. It is caused by Scale Drop Disease Virus (SDDV), which was recently identified as a *Megalocytivirus* of the *Iridoviridae* family. Here, we provide a review on the current understanding of SDDV in barramundi. A search was conducted on Scopus using keywords ("scale drop OR "scale loss") AND ("barramundi" OR "asian seabass" OR "*lates calcarifer*"). 11 articles were considered relevant and included. SDDV is typically characterised by scale loss. They are often accompanied by non-specific lesions, including darkened bodies, fin and tail erosions, and sometimes cloudy eyes, and red bellies. These signs could be due to other infections or poor water quality. Further histopathological investigations showed that infected fish had extensive vasculitis and associated necrosis, particularly in spleen and kidney. Currently, there is no established treatment or preventive measure that is safe and effective in controlling the infection under commercial production conditions. Recently, a recombinant MCP protein produced in E. coli vaccine showed a relative protection against mortality of 91%, when compared with the control. However, it is not yet commercialised. Researchers also attempted to develop molecular based diagnostic tests to detect SDDV that could be used for screening incoming stock. This ensures that only negatively tested stock is introduced to an aquaculture facility. This however is not yet an established practice in barramundi farms. Limitations from current studies include the lack of studies on the pathogenesis and epidemiology of SDDV. Hence, we propose to develop a SDDV disease challenge model that may be used as a useful platform for future pathological and epidemiological studies. The same model could also be extended for use in treatment and vaccine trials.

IMPROVING FISH WELFARE IN ASIA-A NEW APPROACH

Dave Little, Jimmy Turnbull, Sonia Rey Planellas, Amaya Albalat

dcl1@stir.ac.uk

This paper describes the recently initiated project 'Improving Farmed Aquatic Animal Welfare in Asia' supported by Open Philanthropy that aims to initiate transformation change in the welfare of farmed fish in Thailand and Vietnam over a two year period. The project will work with small consortia of academics and private sector in both countries to develop projects that pilot interventions leading to practical improvements to farmed fish welfare and improve the narratives and communications around the issue. These interventions will be commissioned after the project ambitions are disseminated to stakeholders in the region and an open competition for projects. Successful proposed interventions will have the potential to (i) avoid unnecessary harm and pain to the fish, (ii) help farmers to improve their efficiency and business resilience, (iii) support a sustainable environment through healthier ecosystems, cleaner waterways and conservation of local species, and, (iv) overall contribute to a healthier population through the creation of a more nutritious food source. The project will principally target species and systems that enter local markets and hence have most impact on combatting poverty and malnutrition.

THE CHARACTERIZATION AND EXPRESSION ANALYSIS OF PTSCD1 GENE IN STENOTHERMAL TROPICAL FISH TIGER BARB *Puntius tetrazona*

Lili Liu*, Hua Zhu, Rong Zhang and Xiaowen Wang

Beijing Fisheries Research Institute
Beijing Academy of Agriculture and Forestry Sciences
Beijing, China 100068
lilyinmay@163.com

The typical stenothermal tropical fish tiger barb *Puntius tetrazona*, also named as *Systomus tetrazona*, *Barbus tetrazona* or *Puntigrius tetrazona*, belongs to the family cyprinidae. Tiger barb is a popular aquarium trade fish in worldwide, originating from South-East Asia. Meanwhile tiger barb fish is an experimental fish for the study of pathogenic infection, parasitic infestation, fish diets, animal visual perception, and stomach-less teleost digestion system. The temperature window of tiger barb is relatively narrower than other tropical fish. To discover the genetic basis for improving the cold tolerance of *P. tetrazona*, we cloned the *PtScd1* cDNA sequence and analyzed the expression profile of *PtScd1* in multi-tissues under gradient cold temperature stress. The *PtScd1* gene was expected to play a vital role for the *P. tetrazona* coping with cold stress.

The results suggested that there was a highly conserved binding site for two zinc ions, or more likely a diiron center, in the dimensional structure of PtSCD1. Two ligand sites were predicted not conserved (FIGURE 1). The *PtScd1* mRNA expression was detected in adult tiger barb fish with a decreasing expression level as liver >brain >muscle >gill (FIGURE 2A). The *PtScd1* mRNA expression levels increased in multi tissues of tiger barb under cold stress (FIGURE 2B). Particularly, there was negative correlation trend between the relative expression level of *PtScd1* mRNA in brain tissue and the exposure temperature (FIGURE 2B). The study showed the tissue-specific expression of *PtScd1* mRNA in a temperature-dependent way.

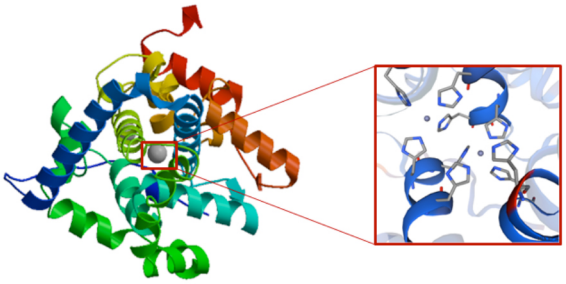


FIGURE 1. The 3D model of PtSCD1 protein.

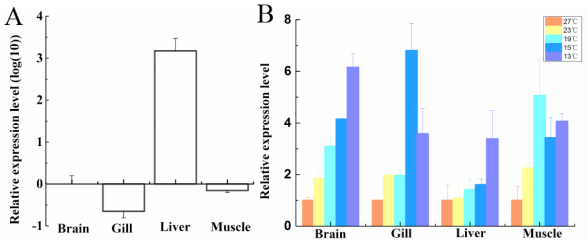


FIGURE 2. The comparative quantitative analysis of *PtScd1* mRNA levels.

CONSUMER'S CHOICE: FARM-RAISED VS WILD-HARVESTED SEAFOOD?

Chengchu Liu

University of Maryland Extension
Maryland Sea Grant College Program
Maryland, USA
cathyliu@umd.edu

The global seafood production reached about 179 million tons. Among them, aquaculture accounted for 46 percent of the total production and 52 percent of fish for human consumption. Therefore, aquaculture growth is critical to further increase seafood supply. However, aquaculture is often portrayed a poor image despite immense economic importance. Media also often report that farm-raised seafood has lower nutritional values than wild-harvest seafood. Another popular misconception is that farm-raised fish and shellfish are inferior to wild-harvested ones in terms of safety and quality. This presentation aims to convey scientific facts of nutrition values and safety attributes of farm-raised and wild-harvested seafood. Research results show that both farm-raised and wild-harvest seafood are good sources of high quality protein, healthy fats (long chain omega 3 fatty acids, Table 1), macro minerals (e.g., calcium, phosphorus, magnesium, and potassium), trace minerals (e.g., zinc, iodine, and selenium) and vitamins (e.g., vitamins A and D). For seafood safety, all regulations and safety standards are equally implemented for both wild-harvested and farm-raised seafood in the United States. Therefore, consumer's choices between farmed or wild-harvested should base on personal preference instead of concerns regarding nutrition and safety.

Table 1 Fatty Acid Compositions of Wild-harvested and Farm-raised Seafood (mg/100g raw edible portion)

	Channel Catfish		Coho Salmon		Rainbow Trout		Eastern Oyster	
	Wild	Farmed	Wild	Farmed	Wild	Farmed	Wild	Farmed
Total saturated	722	1310	1260	1816	722	1383	474	443
Total monounsaturated	844	2573	2134	3330	1129	1979	253	152
Total polyunsaturated	865	1119	1992	1861	1237	1507	528	591
20:5 ω-3 (EPA)	130	17	429	385	167	217	177	188
22:5 ω-3 (DPA)	100	15	232	0	420	516	10	0
22:6 ω-3 (DHA)	234	57	656	821	420	516	136	203

Source: USDA National Nutrient Database for Standard Reference Legacy (2021)

PILOT SCALE ASSESSMENT OF HIGH PRESSURE PROCESSING (HPP) FOR ENHANCING CRABMEAT MICROBIOLOGICAL QUALITY

Chengchu Liu, Olivia Gilstrap, Caleb Nindo, and Salina Parveen

University of Maryland Extension
Maryland Sea Grant College Program
Maryland, USA
cathyliu@umd.edu

Crab is the largest species by value harvested in the United States, with a total landing value of $650 million. Blue crab (*Callinectes sapidus*) is a species of crab native to the waters of the western Atlantic Ocean and the Gulf of Mexico. Presently the US blue crab processing industry sells crabmeat in three basic forms: fresh crabmeat, pasteurized crabmeat, and frozen crabmeat. By far "Fresh" is the most desirable form of crabmeat. However, fresh crabmeat has a limited shelf life.

In order to extend fresh crabmeat's shelf life, we investigated different packaging and HPP treatments to identify the optimal packaging and process conditions. Live blue crabs were pressure-cooked at ≥115°C for 4-6 min. Over 100 kg of crabmeat were handpicked and packed in plastic containers, then subjected to HPP treatment and stored at 4°C. Container integrity and water leakage issues were examined by observation along with weight comparison, before and after HPP treatment. The shelf life of crabmeat, with and without HPP treatments, was examined via sensory evaluations and microbiological tests. Results show that polypropylene containers sealed with OTR 10k film could stand high pressure without water leakage issues. Shelf life of the control (non-HPP crabmeat) was about 7-10 days, while HPP products could last beyond 3 weeks without significant changes in sensory characteristics (taste, texture and flavor).

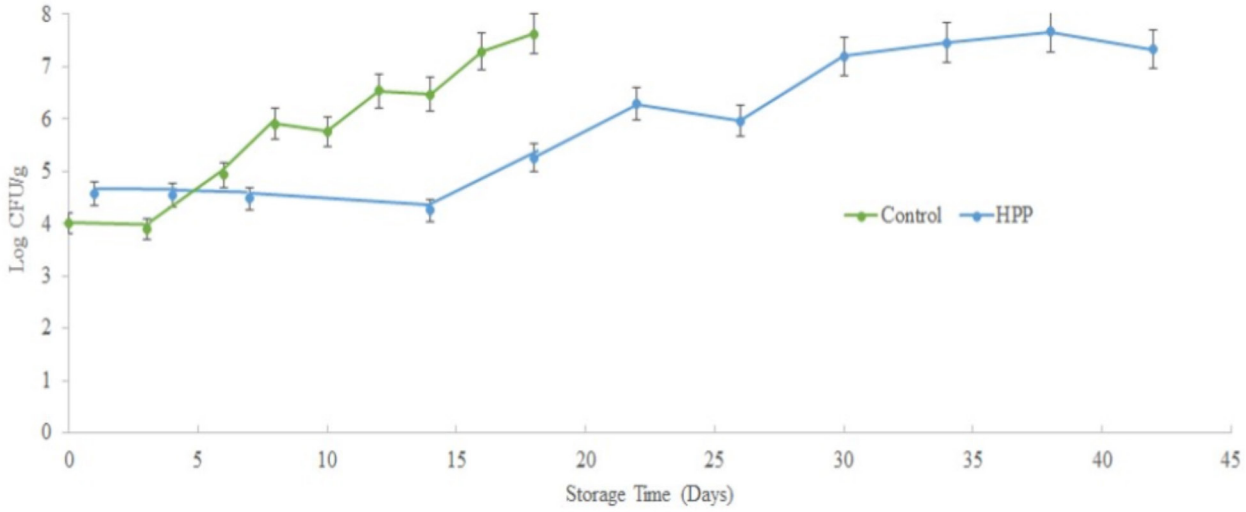


Figure 1. Aerobic Plate Counts (Log CFU/g) of Control and HPP-Treated Samples during Refrigerated Storage

IMMUNE MODULATORY ACTIVITY OF ENCAPSULATED MicroRNA MIMICS IN ZEBRAFISH

T. D. Liyanage* and Mahanama De Zoysa

College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Republic of Korea
Email: thilomaliyanage@gmail.com

MicroRNAs (miRNAs) are short (~22 nucleotides) non-coding RNAs that act as post-transcriptional regulators of mRNA. Therapeutic delivery of miRNAs has been studied in several human diseases as it associates with diverse biological processes including immune responses. However, miRNA based therapy in teleost is yet to be discovered. Therefore, we encapsulated the selected miRNA mimics into chitosan nanoparticles (CNPs) to investigate the expression of endogenous miRNAs and their role on immune modulation in zebrafish model.

Total of five miRNA mimics (miR-24, miR-146, miR-155, miR-462 and miR-734) were encapsulated with CNPs (miRNAs-CNPs). All five different miRNAs-CNPs were intraperitoneally injected (20 μg/fish) to adult zebrafish separately. CNPs and water (nuclease free) were injected as controls. Tissue samples (gills) were collected at 12, 24, and 48 hours post injections (hpi) and expression of endogenous miRNAs and specific immune regulatory genes (*il-1β*, *ifn* γ) were analyzed by qRT-PCR.

Results showed significantly upregulated ($p<0.05$) miR-24 (3.3-fold) and miR-462 (2.8-fold) expression levels at12 hpi in gills of miR-24-CNPs and miR-462-CNPs injected fish compared to the negative control. Moreover, miR-734 was significantly ($p<0.05$) upregulated at 12 (8.8-fold) and 24 (7.2-folds) hpi upon injection of miR-734-CNPs to zebrafish. At 24 hpi, miR-24, miR-146, miR-155, miR-462 and miR-734 expression had >1.5 fold of expression upon respective miRNAs-CNPs administration. Among the miRNA regulatory genes examined, *il-1β* was downregulated ($p<0.05$) in miR-146-CNPs injected fish at 12 hpi. Moreover, *ifn* γ was downregulated in miR-24, 146, 155, 462-CNPs injected fish at 24 hpi compared to control (CNPs). Overall outcome of this revealed that the delivery of encapsulated miRNAs (with CNPs), could be able to modulate the expression of endogenous miRNAs and thereby regulate the immune responses by inhibiting the expression of proinflammatory cytokines in zebrafish.

MOLECULAR DIVERSITY OF MHC CLASS I IN ASIAN SEABASS *Lates calcarifer*

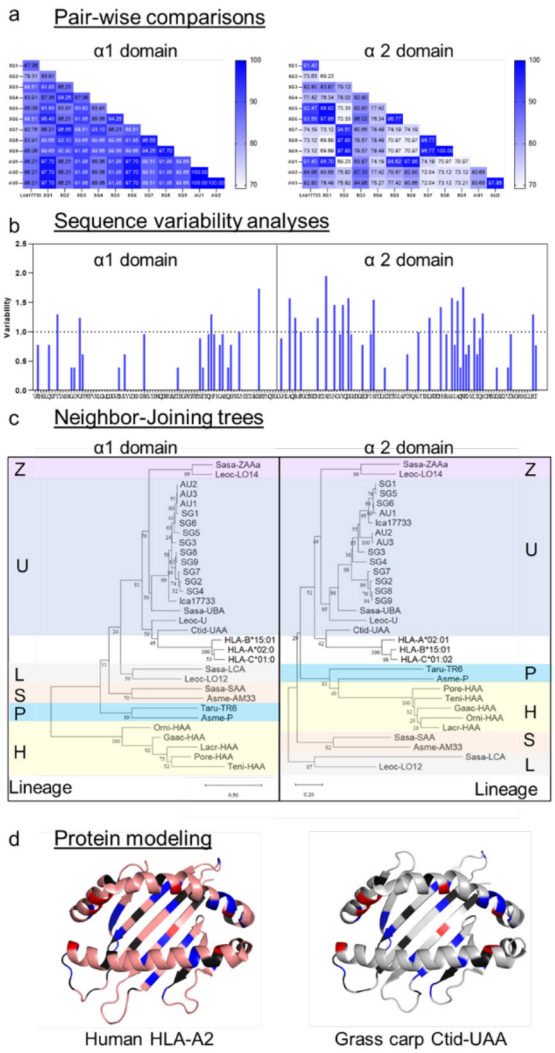
Zhixuan Loh*, Xuelu Huan, Sunita Awate, Markus Schrittwieser, Laurent Renia, and Ee Chee Ren.

Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR)
Singapore 138648.
Ken_loh_zhixuan@idlabs.a-star.edu.sg

The Asian seabass (*Lates calcarifer*) is of importance both as farmed and wild animals. However, with the emergence of infectious diseases, there is a need to understand the immune system and its diversity within the species. The highly polymorphic MHC class I (MHC-I) molecules are essential for antigen presentation to eliciate adaptive immunity. In teleost fish, six MHC-I lineages U, Z, S, L, P and H have been described, with only the U lineage being well studied at the molecular and functional level due to its peptide binding abilities similar to the classical human leukocyte antigen. Currently, other than the MHC-I sequences that were obtained from a single Asian seabass, there is no information on its diversity. Therefore, in the present study, we sequenced and characterized the peptide binding regions α1 and α2 domains of a single MHC-I gene in Asian seabass originated from Singapore and Australia.

Pairwise comparisons of the Asian seabass MHC-I α1 and α2 domain sequences show an overall similarity within Singapore and Australia -derived Asian seabass, with more conserved residues in α1 domain as compared to α2 domain (Fig. a). This is supported by variability metric (*V*) analysis where 4 polymorphic sites with $V > 1$ in the α1 domain and 16 polymorphic sites in the α2 domain were identified (Fig b). Phylogenetic tree analysis revealed that the sequences belong to the U lineage, forming a single cluster (Fig c). Mapping conserved binding residues positions (red) on human HLA-A2 and grass carp crystal structure showed a high degree of similarity (Fig d).

In conclusion, the availability of the U lineage MHC-I α1 and α2 sequences enhances the quality of MHC class I genetic information in Asian seabass and that the critical amino acid residues correspond closely to that even of humans. The sequences will provide new tools to analyse fish immune responses to pathogen infections and will be applicable in the study of the phylogeny and evolution of antigen-specific receptors.



STATUS OF MAJOR COASTAL FISHING ACTIVITIES IN THE MEKONG DELTA, VIETNAM

Nguyen Thanh Long*, Mai Viet Van, Dang Thi Phuong, Naoki Tojo and Tran Dac Dinh

College of Aquaculture and Fisheries, Can Tho University
3/2 Street, Campus II, Can Tho, Vietnam
ntlong@ctu.edu.vn

A study on the major fisheries was conducted from August 2017 to April 2018 in coastal provinces of the Mekong Delta, Vietnam. Results showed that the gillnets, trawl nets, and stow nets were the main fishing gears, and accounted for the highest number of fishing boats and yields. All the fishing gears are exploited for the whole year round. The results also indicated that the gillnets fishery is the largest scale (10.99 tons). The most effective fishing activity of gillnets fishy was the highest profit (298 million VND per year) ($p < 0.05$). However, the highest fishing yield (20.42 tons/year) was found in trawlers, but it had the lowest rate of return (0.45 times) and the trash-fish portion accounted for the highest ratio (38.4%). Although the stow nets had the highest rate of return (1.41 times), they were the lowest yield (7.17 tons/year) and a high portion of trash fish. The high proportion of trash fish may affect fisheries resources. For the sustainable development of the fisheries in the Mekong Delta, the development and management of fisheries resources should be promoted, supporting fishermen to access low-interest rates to invest in capture production, and training fishermen to use advanced fishing gear to increase their fishing efficiency.

FORMULATING FUNCTIONAL FISH FEEDS THROUGH MICROENCAPSULATION TECHNIQUES

Manish Mohatra, Kaarunya Sampathkumar, Li Ling Tan, Anyu Zhang, Matthew Tan

School of Materials Science and Engineering
Nanyang Technological University
50 Nanyang Avenue, N4.1-#02-09
Singapore 639798
joachimloo@ntu.edu.sg

As part of Singapore's drive to enhance food security, there is now a greater push to increase the productivity of our local farms, and to be less reliant on imported food. As food fish is one of the key produce in Singapore, the Singapore Food Agency (SFA) has been implementing new initiatives that would boost sustainable food fish production locally, through innovative aquaculture farming. In aquaculture, while fish feed plays a crucial role in the health, growth and development of the fish, it also accounts for over 50-70% of the total production cost. To enhance productivity, one of the key strategies is to lower feed conversion ratio through the use of micronutrients-rich, leach-free fish feeds. Such fish feeds should provide the key amino acids required for the growth of the fish, while mitigating any loss of essential nutrients to the environment or through degradation (Figure 1). Currently, the stability of amino acids in commercial feeds has always been of concern due to the leaching and consequent dissolution of these nutritional agents into the aqueous environment. In this presentation, we will highlight how innovative encapsulation techniques can be exploited for the development of micronutrient-rich, leach-free, fish feeds with the aim of improving feed conversion ratios. This micronutrients-rich encapsulation technology aims to enhance productivity by lowering feed conversion ratio, while providing a good measure of cost savings for aquaculture applications.

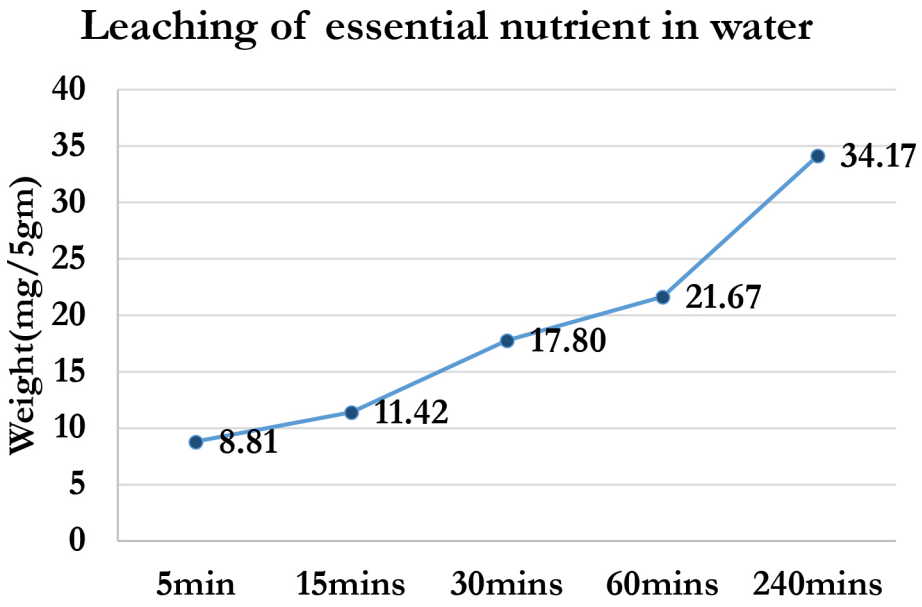


Figure 1 – Leaching of one specific essential nutrient in an aqueous environment that mimics an aquaculture farm

INFLUENCE OF DIETARY NANO-SELENIUM ON GROWTH PERFORMANCE IN PACIFIC WHITE – LEG SHRIMP

Aliya Farissa Bte Afandi, Cheng Wei Ting Enrica, Ellen Novita Tejo, Hajarratu Rahma Bte Mohammad Naim, Nursyarizah Binti Samion, Lee Co Sin, Dr Goh Chee Keong, Dr Grace Loo

Nanotechnology involves the application of materials at the nanoscale to new products or processes. It is a rapidly growing industry which is currently worth one trillion U.S. dollars of global economy value by 2015, with a significant proportion on the theme of sustainability, including food production and agriculture. There are opportunities for the fisheries and aquaculture industries to use existing nanotechnologies, and to develop new applications specific to this industry. Several applications of nanotechnology for aquaculture production are currently being developed. Recently, researchers had reported that young carp and sturgeon exhibited a faster rate of growth (30% and 24% respectively) when they were fed nanoparticles of different nano-Selenium sources (nano-Se) supplemented in basal diet. This is because nano-Se was found improving the final weight, relative gain rate, anti-oxidant status as well as Glutathione Peroxidase(GSH-Px) activities and muscle Se concentration of the young carp. Moreover, nano-Se appeared to be more effective than organic selenomethionine in increasing muscle selenium content. Similarly, the growth and performance of the experimented fishes have been assessed higher at nano- level delivery of these nutraceuticals. Selenium (Se) is an essential trace element for human and animal health. It was found to be an integral part of the glutathione peroxidase enzyme, and glutathione peroxide takes part in the cellular defence against oxidative damage of cytoplasmic structures by catalyzing the reduction of hydrogen peroxide and lipid peroxides. Other literature have reported that selenomethionine is a predominant chemical form of organic selenium in feedstuffs due to their excellent bioavailability and has been reported to have higher bioavailability for Atlantic salmon and channel catfish.

Through this project, the effects of different concentrations of Selenium nanoparticle (Nano-Se) on growth performance were studied, which were incorporated as feed additives into current commercial diets for Pacific White Leg Shrimp (*Litopenaeus vannamei*). Young *L. vannamei* were divided between duplicate 2 tanks with 20 individuals per tank, for each treatment and control group. The control group were fed only normal commercial diets without Se supplement, while treatment groups were fed supplemented diets of 0.3 and 0.5 mg Nano-selenium (Nano-Se) per kg of the same commercial feed. Animals were all fed to satiation at 5% of body weight twice daily. Each tank was sampled for live shrimps regularly, and data on Weights and Lengths of all collected shrimps were recorded at several time points, including the initial and the end of experiment, which were treated as initial weight and final weight, respectively. At the same time, shrimp survival was recorded based on individual counts in each tank. After 7 weeks of feed supplementation, statistical analysis was also carried out on the data collected.

Results from experiments showed that there were no significant differences in the lengths and weights of the *L. vannamei* shrimps between treatment groups after 7 weeks, although shrimps which were fed 0.5mg Nano-Se per kg feed were comparatively heavier than those fed the control diet. Shrimps from all treatment groups showed healthy growth over the 7 weeks of experiments, and no mortality was observed from consumption of the feed, suggesting that supplementation with Nano-Se at these concentrations was non-toxic to the shrimp. Future directions include screening and grading of shrimps for more even initial size and weights, increasing water temperature during culture, and testing the effects of higher Nano-Se supplement concentrations in commercial feed.

MICROBIAL MANIPULATION FOR SUSTAINABLE PRODUCTION OF BIO-SECURE WASTE-GROWN COPEPODS

Poh Leong Loo*, and Koh Siang Tan

Singapore Centre for Environmental Life Sciences Engineering
Nanyang Technological University
60 Nanyang Drive, SBS-01N-27 Singapore 637551
pohleong.loo@ntu.edu.sg

Thus far, sustainable production of copepods in captive is still unachievable, possibly due to the use of unsuitable feed, and the cost-effective production protocols are relatively underdeveloped. As a result, wild copepods are still widely used to raise larval fish and invertebrates in commercial hatcheries albeit their poor biosecurity. This study thus aimed to select, culture, manipulate, and engineer local microbiota to create a beneficial microbial consortium for producing bio-secure and high-quality copepods fed with waste-grown microheterotrophs.

A total of 177 indigenous marine isolates comprising bacteria, thraustochytrid protists, and yeasts had been screened for probiotic properties, bioremediation capabilities, and alternative feeds for copepods. Of which, *Bacillus subtilis* and *Alteromonas macleodii* were effective in mitigating a magnitude of *Vibrio* species, notably *V. harveyi* and *V. parahaemolyticus*. Interestingly, *B. subtilis* had better adaptation to a multitude of temperature, salinity, and pH ranges, i.e., 28°C-80°C, 5ppt-35ppt, and pH 3-11, respectively than that of *A. macleodii*. Among the selected microheterotrophs tested for bioremediation assays, *Qipengyuania flava* was highly efficient in remediating high-nutrient aquaculture wastewater. The immobilisation of *Q. flava* cells in beads had further improved their efficiency in wastewater treatment.

Irrespective of brewery wastewater (BW) or food wastewater (FW)-based microbial feeds, the marine cyclopoid copepod, *Oithona* sp. thrived well when they were fed with *Aurantiochytrium* sp. as compared to *B. subtilis*, *Rhodotorula mucilaginosa*, *Tetraselmis* sp., only autoclaved wastewater, and no feed. In addition, the inclusion of immobilised bacterial beads did not impair the growth and reproduction of *Oithona*. Regardless of bead treatments, copepods fed with FW-grown *Aurantiochytrium* registered 1.5 times greater population densities than those fed with BW-grown *Aurantiochytrium* albeit BW-grown *Aurantiochytrium* contained significantly higher protein (43.2%) and polyunsaturated fatty acids (PUFA: 31.35% of total fatty acids) ($p<0.05$) than FW-grown *Aurantiochytrium* (protein: 24.0%; PUFA: 7.14%). Irrespective of feeds, *Vibrio* counts in the *Oithona* cultures containing immobilised *B. subtilis* beads were significantly lower ($p<0.05$) than *Oithona* cultures with immobilised *Q. flava* beads or empty beads (without bacteria).

The inclusion of both *B. subtilis* and *Q. flava* beads in *Oithona* cultures fed with FW-grown *Aurantiochytrium* not only improved water quality, but also significantly reduced *Vibrio* loads ($p<0.05$) as compared to *Oithona* cultures containing either beads of *B. subtilis* or *Q. flava*. Nonetheless, copepod populations in all the bead treatments were not significantly different ($p>0.05$). In conclusion, this study has clearly demonstrated that sustainable production of bio-secure copepods fed with waste-grown microheterotrophs could be achieved through precise microbiome engineering.

QUALITY ANALYSES OF NILE TILAPIA *Oreochromis niloticus* CULTURED IN AN IN-POND RACEWAY SYSTEM (IPRS) IN PHETCHABURI PROVINCE, THAILAND

Tanakorn Lothaka*, Chuchai Kanjanamayoon, Lukas Manomaitis

U.S. Soybean Export Council (Southeast Asia) Ltd
541 Orchard Road, #11-03 Liat Towers, Singapore 238881
Korn@ct.ussec.org

The In-Pond Raceway System (IPRS) is a new technology that is valuable for the aquaculture industry as it safely produces higher fish production volumes from existing water resources. The technology was developed at Auburn University, USA, and spread across the world starting with the U.S. Soybean Export Council's (USSEC's) efforts in China before expanding globally.

A comparative demonstration was conducted from May 2020 to April 2021 at a large tilapia farm in Phetchaburi province, Thailand and compared the production capacity and fish quality produced by two different culture systems: a traditional system (earthen pond) and IPRS (three cells, each cell 220m^3). Fish (Nile tilapia – *Oreochromis niloticus*) were stocked and reached their target sizes on day 196 for the traditional pond and day 109 for one IPRS cell. Fish cultured in the IPRS cell showed significantly higher ($p < 0.05$) survival rate, average daily gain (ADG), total production, and biomass compared to a whole pond of traditional culture system (*Table 1*).

These results indicated that the IPRS technology can produce greater quantities and higher quality fish using limited water resources due to a high-technology approach paired with proper management and feeds. Hence, In-Pond Raceway System could considerably be a new aquaculture technology which provides efficient fish production ability and pays attention on environmental sustainability.

In addition, fish samples were sent to a food laboratory in Kasetsart University for flesh quality analysis (*Table 2)*. IPRS fish showed differences ($p < 0.05$) in terms of condition factor, total protein content and chewiness. These parameters are related to each other; K factor indicated a tighter fish fillet which coincided with higher protein content and better texture (flesh chewiness) found in fish from the IPRS cell. There was no Geosmin or MIB (2-Methylisoborneol, which can cause a muddy odor) detected in either system

Table 1: Tilapia Production from Traditional and IPRS Systems

Growth Parameters	Traditional Pond	IPRS Cell
Avg. BW (g/piece)	1,244±184	1,060±110
Survival Rate (%)	62.14	98.57
ADG (g/piece/day)	6.27	8.44
Total Production (kg)	12,871/pond	18,875/cell
Biomass (kg/m^3)	1.04	2.16 (85.8*)

*Calculated by a cell volume (220 m^3)

Table 2: Fish Flesh Quality Analyses

Quality Test	Traditional Pond	IPRS Cell
Condition (K) Factor	2.17±0.25^b	2.61±0.23^a
Total Protein (%)	18.73±0.14^b	19.31±0.11^a
Flesh Chewiness	106.66±6.93^b	128.30±17.08^a
Geosmin, MIB (µg/kg)	ND (< 0.01)	ND (< 0.01)

Different alphabets indicate significant differences ($p < 0.05$)

EXPLORING GONAD COLOR AND TOTAL CAROTENOIDS CONTENT IN *Paracentrotus lividus* FED WITH ISOENERGETIC DIETS OF DIFFERENT INGREDIENTS COMPOSITION

Sílvia Lourenço*, Andreia Raposo, Beatriz Cunha, Joaquina Pinheiro, Pedro M Santos, Ana S Gomes, Maria M Gil, Ana Pombo

*MARE, Marine and Environmental Sciences Centre, Polytechnic of Leiria. Av. Porto de Pesca
2520-630 Peniche – Portugal
silvia.lourenco@ipleiria.pt

The market value of the sea urchin's gonads is highly dependent of several quality parameters such as appearance, colour, texture and flavour. The use of extruded diets in the echinoculture easily promotes the high gonad yield while the other quality standards as the bright orange colour are more difficult to achieve. The gonads colour results from a combination of the carotenoid pigments present in the gonad, where the echinenone is the dominant pigment. The carotenoids content in the sea urchin gonads depends on the species, sex, gonad maturity and diet. In the present study, we evaluated the gonad colour of *Paracentrotus lividus* sea urchins fed with extruded isoenergetic diets of different ingredients for 173 days. The three diets included ingredients from algae, animal and vegetables sources respectively. The gonad colour was analysed at 103 days (T1) of trial and at the end of this at 173 days (T2) applying both semi-quantitative methods (using four levels visual scale) and quantitative methods (determining the CIELAB colour parameters). The results showed that within each sampling episode, both colour grade and CIELAB parameters were influenced by the diet and the sea urchin sex. By comparing the results between T1 and T2 differences arose, with T1 sample presenting a higher percentage of gonads with a bright orange colour when compared with the gonads analysed at T2. These results indicate that the sea urchins have a high capability to store carotenoids through time independently of the diet.

IN-VITRO ACTIVITIES OF ORGANIC ACIDS AGAINST *Tenacibaculum maritimum* AND *Streptococcus iniae* BACTERIAL PATHOGENS OF FISH SPECIES

Low Wen Jie *, Miyata Masato and Musthaq S Khader

School of Applied Science, Temasek Polytechnic
21, Tampines Avenue 1
Singapore 529757
lowwenjie09@gmail.com

In aquaculture industry, antibiotic growth promoters have used as feed additives to treat fish diseases and improve their growth. However, the continued use of antibiotics for treatment will eventually cause the development of antibiotic resistance in fishes, as well as humans when they consume the fishes. This has led the aquaculture industry to search for alternatives towards antibiotics for the treatment and growth performance of fishes. The combinations of organic acids (OAs)/salts are becoming popular for usage in the feeds of animals, poultry and aquaculture as an alternative toward antibiotics. In addition, they have shown to have antimicrobial and growth-improving properties with any known adverse effect on poultry species. Hence, this study aims to develop the effects of various food grade OAs/salts on two economically important disease-causing bacteria found in food fishes, *Tenacibaculum maritimum* and *Streptococcus iniae*. Doubling dilutions of OAs/salts or combinations of OAs/salts from sodium acetate (SA), citric acid (CA), ascorbic acid (AA), and benzoic acid (BA) were chosen to determine their efficiency in inhibiting the growth of bacteria by microtitre broth dilution method. The results revealed that AA and CA were observed to have the higher inhibitory effect at final concentrations of 50mg/ml and 25mg/ml against 10^6 cfu/ml of T.mar and S.iniae respectively, compared to various concentration of SA and BA at 20 hpi. Further, the bacterial growth curve analysis of T.mar or S.iniae inhibited by AA+CA blends with final concentration of 25mg/ml showed bacterial growth reduced significantly at 10 hpi. Therefore, the results concluded that the CA+AA blend had potentially effect and can be used to alternatives to antibiotics in the treatment of S.iniae and T.mar bacterial infections. However, further study is required to conclude OAs/salts activities in vivo efficacy test with OAs/salts incorporated feed for fish.

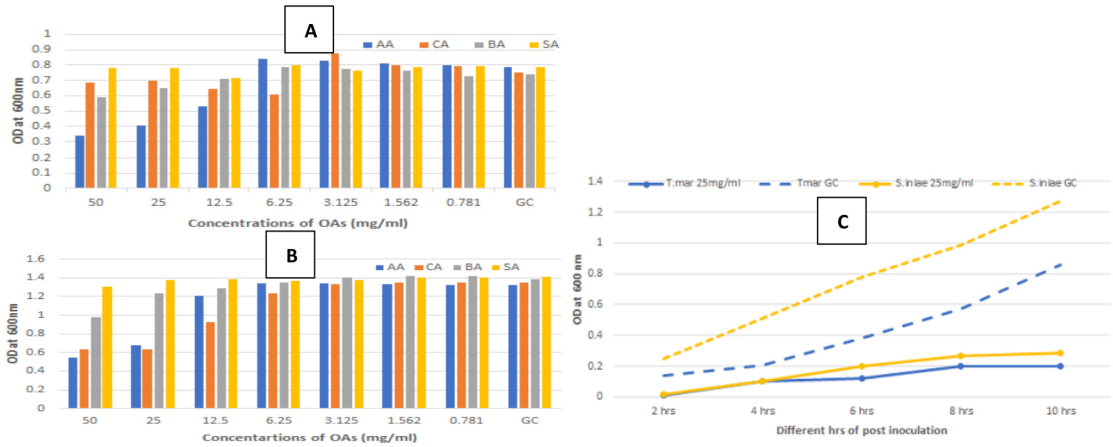


Figure A and B. Determination of MIC of different concentration of OAs/salts against (A) T.mar and (B) S.iniae by microtiter broth dilution method. Figure C. Bacterial growth curve analysis of S.inae and T.mar with 25mg/ml of AA+CA blend. AA- Ascorbic Acid; CA- Citric Acid; BA- Benzoic Acid; SA- Sodium Acetate and GC- Growth Curve (bacteria only).

AN INDICATOR OF OYSTER VITALITY BASED ON HEART RATE ACTIVITY ANALYSIS USING IMAGE PROCESSING TECHNOLOGY

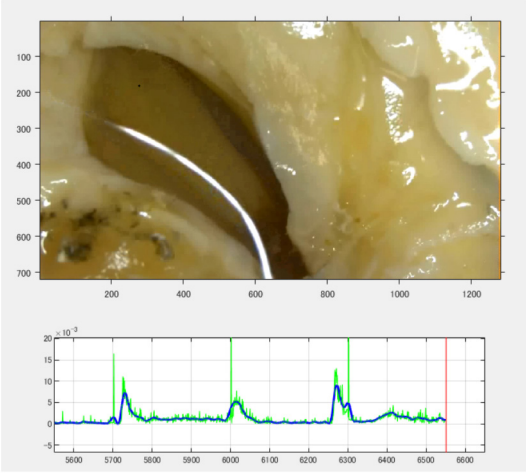
Ryosuke Bizen, Shiliang Dong, Souta Hayashi, Xin Lu*, Koichiro Kobayashi, Koichi Takaki, Chunhong Yuan

Faculty of Science and Engineering, Iwate University
Ueda 3-18-8, Morioka, Iwate, Japan
luxin@iwate-u.ac.jp

Since 2000, the opportunity to eat raw oysters with shell has been increased due to the spread of oyster bars, and the safety and freshness evaluation of oyster (*Crassostrea gigas*) are becoming more important. However, judging the vitality and freshness of oyster is technically difficult and is still kept by the experience of skilled people in actual production. Additionally, because the heart beats continuously throughout life, the corresponding beating patterns can be used as an indicator of health and stress in various organisms. In this study, we verify the relevance of heartbeat activity and vitality of oyster during storage using image processing techniques and biochemical indicators. We also examine the application to the distribution of active aquaculture products through rapid vitality and freshness judgment on site, which is difficult to handle with conventional biochemical analysis methods.

From 2021 to 2022, the oyster landed on the coast of Iwate Prefecture, Japan was stored at -1°C for 10 days with shell, and the ATP-related compounds, pH and heartbeat activities of the adductor muscle were measured over time. Here, the ATP-related compounds were measured by HPLC (Asahipak GS-320HQ, 25°C, 0.6 mL/min), and the A.E.C. value was calculated from the content. For measuring the heartbeat activity, the oyster with shell was firstly left at a temperature of 25°C until its surface temperature stabilized, then the optical microscope (Leica Digital Microscope DMS1000) was used to record the heartbeat activity after shell opening. As shown in figure, after processing the obtained videos using MATLAB, the changes in the area of the atrium and ventricle were analyzed, then the heart rate was observed and the cardiac cycle was detected. After that, the correlation between A.E.C. value, pH and heartbeat activity was considered.

The heartbeat activity tended to prolong the cardiac cycle as the stored days passed. It was suggested that the prolongation of the cardiac cycle is caused by the decline of vitality, when the A.E.C. values were 40\% or more in the early stages of storage, and the pH decreased over time from around 6.7 to around 6.3. An unusual heartbeat pattern, such as arrhythmias, was confirmed in individuals whose cardiac cycle was prolonged.



DIETARY POTASSIUM DIFORMATE IMPROVES PERFORMANCE OF BACTERIALLY CHALLENGED WHITE-LEG SHRIMP (*L. vannamei*) UNDER MEXICAN CONDITIONS

Christian Lückstädt* and Nicolas Greiffenstein

*ADDCON
06749 Bitterfeld, Germany
christian.lueckstaedt@addcon.com

Intensive production of the white leg shrimp, *Litopenaeus vannamei* (Boone 1931), in Central America and SE Asia is estimated to have reached more than 4.5 million t in 2019. Despite remarkable progress in shrimp nutrition and feed formulation, disease outbreaks in shrimp ponds can still lead to farming setbacks and increased use of antibiotics. Although state-of-the-art shrimp farm management is often practiced, severe losses have occurred, diagnosed as white feces disease (2010) and early mortality syndrome (EMS, aka acute hepatopancreatic necrosis syndrome AHPNS). EMS is a fatal disease occasionally found in farmed shrimp throughout the world, with an estimated global cost to the aquaculture industry of USD 1 billion. Both diseases are caused by *Vibrio spp*., a bacteria residing in the shrimp gut and hepatopancreas. In both cases, affected organs are ruptured, reducing feed intake and shrimp condition with massive mortalities within a few weeks after stocking. Researchers have meanwhile identified at least four strains of Early Mortality Syndrome (EMS) in shrimp farms in Latin America within the last few years. These were isolated either from the stomach of diseased shrimp or from the sediment of AHPNS-affected farms. Since dietary acidifiers are known particularly to inhibit pathogenic Gram-negative bacteria directly, they may therefore have a supporting action in suppressing the onset of the disease. This formed the impetus for a commercial trial in an affected area in Mexico, which tested the use of two different dietary acidifiers.

15 ponds on a commercial farm of approx. 6 ha each were stocked with 110,000 PL of white leg shrimp per ha. The farm had had previous issues with *Vibrio spp*., including *Vibrio parahaemolyticus* occurrence. While the control diet (containing 0.2% of an additive based mainly on citric acid, fumaric acid and phosphoric acid) was fed to shrimp in 11 ponds, the test diet in 4 ponds contained potassium diformate (KDF, 0.3%; Aquaform*, ADDCON). Shrimp were fed to satiation over the course of the day. The trial lasted for 123±8 days. Results on performance and productivity index are expressed as mean ± standard deviation. Data were subjected to statistical analysis and a significance level of 0.05 was used in all tests.

Results showed a significantly higher ($P=0.03$) number of harvested shrimp in the ponds fed with KDF (368,000 vs. 246,000). Therefore, the overall survival rate tended ($P=0.09$) to be increased in shrimp on diets containing dietary potassium diformate (54.6% vs. 39.7%). Shrimp in the KDF-fed ponds had a significantly ($P= 0.02$) lower final weight, but achieved a highly significantly ($P<0.01$) improved feed conversion (2.00 vs. 2.23). The harvested biomass per ha was numerically increased by almost 80 kg/ha when fed with KDF (900 kg vs. 822 kg). Overall, this led to an increased productivity index, based on weight gain, survival rate and FCR ($P<0.1$). by almost 30% (45.5 vs. 35.1) in shrimp fed with KDF.
Using dietary diformates therefore poses a promising nutritional alternative to reduce bacterial related losses in modern shrimp farming, even when tested against a positive control and contributes to an economically and ecologically sustainable grow-out operation.

EFFECT OF ZINC-L-SELENOMETHIONINE AND CHROMIUM-L-METHIONINE ON GROWTH PERFORMANCE OF NILE TILAPIA *Oreochromis niloticus*

Sutep Luengyotluechakul[1], Orapint Jintasataporn, Suveena Auepaiboon, Sirnoy Chumkam

[1]zinpro Animal Nutrition (Thailand) Inc, Bangkok, Thailand
Email: sutep@zinpro.com

During the culture period, many factors cause fish stress, such as stocking density, water quality, environment, etc. Mineral supplementation is one way to help enhance immunity, reducing energy allocation to stress compensation and subsequent losses in growth and muscle development, resulting in better growth and higher production efficiency. Chromium plays an important role in carbohydrate, protein, and lipid metabolism. It is a key component of glucose tolerance factor (GTF), which helps increase insulin efficiency. Selenium plays an important role in growth, antioxidant, and reproductive functions. Selenium also acts in the conversion of thyroid hormones to their active form, a key to the control of metabolic rate. This study aimed to investigate the effects of chromium-L-methionine (CrMet) and zinc-L-selenomethionine (SeMet) in the diet of juvenile Nile tilapia on growth performance.

Juvenile Nile tilapia (*Oreochromis niloticus*) were acclimated for 2 weeks in a 1000-L tank and fed a commercial diet twice daily. Following the acclimation phase, fish were fed experimental diets twice daily at 3 to 4% body weight, for 4 weeks. Basal diet contained 36% crude protein and 6% crude lipid. CrMet and SeMet were added to the basal diet to create 6 treatments:

T1 ..... No supplemental mineral
T2 ..... 0.5 ppm CrMet
T3 ..... 1.0 ppm CrMet
T4 ..... 0.3 ppm SeMet
T5 ..... 0.5 ppm CrMet + 0.3 ppm SeMet
T6 ..... 1.0 ppm CrMet + 0.3 ppm SeMet

Compared to Control, the combination of supplemental 0.5 ppm CrMet + 0.3 ppm SeMet significantly improved final body weight (FBW) and feed conversion ratio (FCR) by 11 and 14%, respectively, showing the highest potential for improved growth performance of Nile tilapia (Table 1). Selenium helps reduce stress by neutralizing excess free radicals and protecting cells from damage caused by oxidative stress. Chromium plays a key role in glucose metabolism by acting as a co- factor for insulin production and signaling pathway. Considering that most aquatic species, including farmed fish, are poor users of carbohydrates as an energy source, supplementation with Cr constitutes an interesting strategy to enhance glucose utilization, to spare dietary protein for muscle growth, increasing fillet and carcass yield. In addition, this protein sparing effect may help the aquaculture industry reduce feed costs through the utilization of less costly dietary energy sources, such as carbohydrates.

Table 1: Growth performance of Nile tilapia fed experimental diets for 4 weeks

Treatment	IBW (g/fish)	FBW (g/fish)	WG (g/fish)	ADG (g/fish/d)	FCR
T1	26.75±2.20	48.78±3.94^{a}	22.03±3.40^{a}	0.79±0.12^{a}	1.05±0.15^{b}
T2	26.75±2.05	52.68±3.49^{b}	25.93±3.75^{b}	0.93±0.13^{b}	0.90±0.11^{a}
T3	26.75±2.31	52.39±3.88^{ab}	25.64±3.69^{b}	0.92±0.13^{b}	0.93±0.15^{a}
T4	26.75±2.05	52.20±4.20^{ab}	25.45±3.61^{ab}	0.91±0.13^{ab}	0.93±0.14^{ab}
T5	26.75±2.12	54.02±3.86^{b}	27.27±3.60^{b}	0.97±0.13^{b}	0.90±0.12^{a}
T6	26.75±1.92	52.88±5.45^{b}	26.13±4.95^{b}	0.93±0.18^{b}	0.95±0.14^{ab}
P-value	1.000	0.005	0.002	0.002	0.006

CURRENT STATUS AND RECENT ADVANCES ON ABALONE AQUACULTURE IN CHINA

Xuan Luo *,Caihuan Ke, Weiwei You

College of Ocean and Earth Sciences
Xiamen University
Xiamen 361102, PR China
xluo@xmu.edu.cn

In China, the abalone has been regarded as the jewel in the seafood crown for thousands of years. The Chinese abalone industry, including seed production, formulated feed, seaweed, farming equipment, transportation, and processing, has gradually developed over the past decade. Due to the successful farming of the Pacific abalone (*H. discus hannai*) in southern China, the Pacific abalone now is the dominant farmed species in both southern and northern China, accounting for more than 95% of total abalone production. The culture of abalone in China has grown from the time of first commercial aquaculture in late 1960s to over 180,000 metric tons in 2019. Nowadays, abalone is one of the most valuable mariculture species in southern China, especially Fujian Province, and it has had substantial economic, social, and ecological impacts over the last decade. However, there are still a lot of problems and challenges, such as summer mortality, red tide and so on. Thus, we are carrying on a series of projects to develop new strains for better genetic improvement and environmental adaptation, and have received "new variety certificates" from the Chinese Ministry of Agriculture.

EFFECT OF DIETARY PHYTASE AND INOSITOL ON PERFORMANCE AND TISSUE INOSITOL IN TILAPIA *Oreochromis niloticus* FED LOW FISHMEAL DIETS

Sophie A. Hiscocks, Ingrid Lupatsch*, Gilson A. Gomes, David Terrey, Ivan Tankovski, Jack James and Michael R. Bedford

AB Vista, Marlborough, SN8 4AN, UK
Sophie.Hiscocks@abvista.com

Phytate-bound phosphorus (phytate-P) is a major anti-nutrient in plant-based aqua feeds due to its negative effect on fish growth and nutrient utilisation. Phytases (PHY) are enzymes that remove P groups from the phytate molecule, thereby making phytate-P more available to the animal and reducing the anti-nutritive effects of phytate. Applying higher PHY doses can result in the complete dephosphorylation of phytate, with the aid of intestinal alkaline phosphatase to remove the final P group, and the release of myo-inositol (MYO). Classified as a vitamin-like nutrient, MYO is often added as a dietary supplement to support fish growth. Therefore, the objectives of the current study were to determine whether high PHY doses can provide sufficient MYO and P to support growth and nutrient utilisation in tilapia.

A total of 360 male tilapia (*Oreochromis niloticus*; average weight 28g) were allocated to one of 4 diets, with 3 replicate 100L tanks per diet and 30 fish per tank. Water temperature was set at 28±1°C. Low fishmeal (5%) diets were fed to satiation (3 to 5 meals a day) over a 42-day period. Treatments were arranged in a 2 x 2 factorial, including a control diet (total P, 0.79%; phytate-P, 0.30%) and the same diet with 400 mg/kg of MYO, 5000 FTU/kg of PHY (Quantum Blue) or a combination of 400 mg/kg of MYO + 5000 FTU/kg of PHY. Data was subjected to ANOVA using the fit model platform in JMP Pro 13.0, with treatment means separated using Student's T-test. Survival was analysed using a non-parametric Wilcoxon signed-rank test. Significance accepted at P≤0.05.

Feeding phytase at 5000 FTU/kg more than doubled (P<0.001) daily weight gain of tilapia and reduced (P<0.001) FCR by 31% (Table 1). Supplementation of MYO had no effect on performance parameters.

Table 1. Growth performance

	PHY, FTU/kg		MYO, mg/kg	
	0	5000	0	400
Final weight, g/fish	55.0[b]	86.1[a]	72.0	69.0
Weight gain, g/d/fish	0.65[b]	1.39[a]	1.06	0.99
FCR, g:g	1.96[a]	1.35[b]	1.62	1.69
Survival, %	94	100	94	100

[a,b] For a specific additive, means having different superscripts differ significantly (P<0.05)

Table 2. MYO content in tissues (nmol/ g wet wt) and faeces (nmol/ g dry wt)

QB, FTU/kg	MYO, mg/kg	Muscle	Liver	Faeces
0	0	267.4[c]	256.3	61.2[b]
5000	0	488.7[a]	271.6	197.1[a]
0	400	294.6[c]	254.9	88.4[b]
5000	400	387.8[b]	266.3	351.2[a]

[a-c] Means having different superscripts differ significantly (P<0.05)

Inositol content in the muscle was significantly influenced by a QB x MYO interaction (P<0.006). Higher levels of muscle MYO in PHY diets suggest that complete dephosphorylation of dietary phytate led to the release of bioavailable MYO. Feeding MYO directly had no positive effect on muscle MYO content.

In summary, only PHY improved growth and feed efficiency of tilapia, as well as muscle MYO content. These findings suggest PHY supplementation can improve availability MYO as well as P from phytate.

SURVEY ON SPECIES COMPOSITION OF SEA CUCUMBER IN NAM DU AND TIEN HAI ISLANDS, KIEN GIANG PROVINCE, VIETNAM

Ly Van Khanh*, Nguyen Thi Ngoc Anh and Tran Tran Dac Dinh

College of Aquaculture and Fisheries
Can Tho University
Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Vietnam
lvkhanh@ctu.edu.vn

Sea cucumbers are echinoderms (Echinodermata) that are widely distributed throughout the world's oceans. They live on or near the ocean floor and are often buried beneath it, making them easily vulnerable to high levels of fishing. For centuries, sea cucumbers have been consumed as a luxury food item and used as medicine in Asian countries. In Vietnam, sea cucumbers have been widely exploited for traditional food and medicinal purposes as well as for aquariums, which has led to a rapid decline in their natural populations, thereby threatening the sustainability of sea cucumber fisheries. Kien Giang province is located in the Mekong Delta region of southwestern Vietnam. Nam Du and Tien Hai islands belong to Kien Giang's coastal water and are biodiversity hotspots that support many different important marine fisheries, including a high abundance of sea cucumbers. However, several species of sea cucumbers have been commercially harvested in these islands subsequently leading to overexploitation and a decline in the number of sea cucumber species especially high-value commercial stocks. Therefore, this investigation aimed to assess the present situation of sea cucumber species to provide useful information for planning sustainable sea cucumber management initiatives that could protect and control exploitation of sea cucumber resource in Kien Giang's coastal water.

The investigation was performed to assess the status of sea cucumber species composition in the Southwest sea of Kien Giang province. Six field trips for sampling natural sea cucumber population were conducted in Nam Du and Tien Hai Islands from March to December, 2018. It was found that fourteen species with low- and medium-value species were most commonly collected, which belong to two families (Holothuridea and Stichopodidea). Holothuridea involved three genera with eight species such as *Actinopyga echinites*. *Pearsonothuria graeffei, Holothuria atra, H. fuscogilva, H. nobilis, H. leucospilota, H. impatiens* and *H. scabra,* of which *H. leucospilota* was most dominant (27.39%) followed by *P. graeffei* (17.20%) and *H. impatiens* (9.87%), and other species accounted for less than 1%. For Stichopodidea, only one *Stichopus* genus was identified, including six species, namely, *Stichopus variegatus* occupied 24.20% and the five other species (*S. chloronotus, S. naso, S. horrens, S. hermanni* and *S. monotuberculatus*), which represented between 0.32% and 6.37%. These species was found at water depth from 2 m to 25 m. The sizes of these species in term of length varied from 9 to 47 cm and their individual weights were between 16 and 1854 g. However, the high-value species such as *H. nobilis, H. fuscogilva* and *Thelenota ananas* had been abundant in the past 10 years that were not found in the present study. This indicates these sea cucumber species were intensively exploited resulting in the drastic depletion of high-value stocks in the study area.

EFFECT OF LIGHT INTENSITY ON GROWTH AND HIGH-TEMPERATURE TOLERANCE OF JUVENILE RAINBOW TROUT *Oncorhynchus mykiss*

Shisheng Ma*, Shuanglin Dong, Yangen Zhou, Xueweijie Chen, Jianfei Yu, Yongkang Guo, and Qinfeng Gao

Fisheries College, Ocean University of China
5 Yushan Rd., Qingdao 266003, China
mashisheng@stu.ouc.edu.cn

In order to meet the demand of salmon market, Chinese scientists and entrepreneur are working on salmon mariculture far offshore in the Yellow Sea, China. Light is one of the most dominant environmental regulating the growth of salmonids. Optimal Light regulation technology is vital for far offshore salmon mariculture, especially in a full-submersible cage in China. The objective of this study is to evaluate the growth and high-temperature tolerance of juvenile rainbow trout (*Oncorhynchus mykiss*) in response to different light intensities. Five treatments were designed with different light intensities (0lx, 150lx, 300lx, 600lx and 1200lx) using LED lights as illuminant. The photoperiod was 12L:12D.

Juvenile rainbow trout (N=6; initial weight 56.90±1.01g) were fed the diets for eight weeks and subsequently underwent the high-temperature acclimation. This experiment has five treatments at a stocking density of 20 fish per tank. At the end of the 8-week period, all fish were weighted. After that, the temperature (initial 16.5 °C) was increased to 24 °C for 72 with a rate less than 1 °C per hour. Subsequently, the temperature decreased to 16.5°C for 2 weeks. On days 1, 7 and 14 after high temperature acclimation as well as at the end of the growth trial, growth hormone (GH), Insulin-like growth factor (IGF-I), melatonin (MT), stomach digestive enzymes activity, intestinal digestive enzyme activity, cortisol, superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione peroxidase (GPX) activity, lipid peroxides (LPO) and malondialdehyde (MDA), immunoglobulin M and heat shock protein 70 (HSP70) will be determined. At the end of the growth trial, there were no significant differences in final mean weight (166.45-190.67g), final biomass (3274.97-3780.91g), survival (97.50-99.17%), percent weight gain (198.20-229.92%) or FCR (0.94-0.98) (Table 1). The molecular mechanism of optimal light density on growth rainbow trout needs to be further studied.

Table 1. Effect of light intensity on growth performance of juvenile rainbow trout

Treatment	Final Biomass (g)	Final Mean Weight (g)	Weight gain (%)	FCR[1]	Survival (%)
1 (0lx)	3274.97	166.45	198.20	0.94	98.33
2 (150lx)	3590.53	183.94	222.71	0.96	97.50
3 (300lx)	3693.93	186.24	226.91	0.96	99.17
4 (600lx)	3554.44	181.79	219.72	0.98	97.50
5 (1200lx)	3780.91	190.67	229.92	0.98	99.17
PSE[2]	138.03	5.90	9.59	0.02	1.29
P value	0.14	0.07	0.18	0.46	0.80

FCR[1]: Feed conversion ratio; PSE[2]: Pool standard error

PRODUCTION OF CASH CROPS AND FISH WHILE TREATING BREWERY EFFLUENT IN CONSTRUCTED WETLANDS

Nyiko C. Mabasa*, Clifford L.W. Jones, Mark Laing

Department of Ichthyology and Fisheries Science
Rhodes University
P.O. Box 94
Grahamstown 6140
South Africa
charity.mabasa@gmail.com

Water scarcity in South Africa, and globally, presents challenges for industries. It is therefore imperative to develop responsible water use, such as recycling and reusing wastewater from food processing industries such as breweries. The Ibhayi Brewery (SAB Ltd) employs a combination of sustainable treatment processes that include anaerobic digestion, primary facultative ponds, high-rate algal ponds and constructed wetlands (CW) to treat brewery effluent on an experimental scale. The constituent concentrations of these experimentally treated effluents are within the ranges prescribed by local regulations to allow for potential downstream use in agriculture and aquaculture. However, the sodium content in this treated effluent, which originates from upstream cleaning agents and pH control at the onsite effluent treatment facility, is a constraint to the downstream use of brewery effluent. This study addressed the salt problem, by investigating potential techniques for downstream agriculture and aquaculture to mitigate the effects of salt accumulation caused by irrigation with brewery effluent.

Constructed wetlands of 15m length were planted with Swiss chard (*Beta vulgaris*), and used to treat brewery effluent after anaerobic digestion. Plant production peaked halfway down the wetland (11438±450 g/m^2) (figure 1), with reduced growth and plant health closer to the inlet and outlet.

There were no significant differences in the sodium concentration of brewery effluent treated along the CW, likely due to evapotranspiration. This was accompanied by a 95.21% decrease in ammonia from the inlet (69.93±1.49 mg/L) to the outlet (3.57±0.36 mg/L), resulting in significant improvement in water quality for downstream reuse in aquaculture. African catfish (*Clarias gariepinus*) grew in this treated brewery effluent; however, growth was moderate at 0.92% body weight gain per day, whereas the system was not suitable for Mozambique tilapia (*Oreochromis mossambicus*) since the fish did not grow (-0.98% body weight per day). While constructed wetlands remain a suitable effluent treatment solution, this technology requires additional modelling and optimization to optimize the reuse of treated brewery effluent in agriculture and aquaculture.

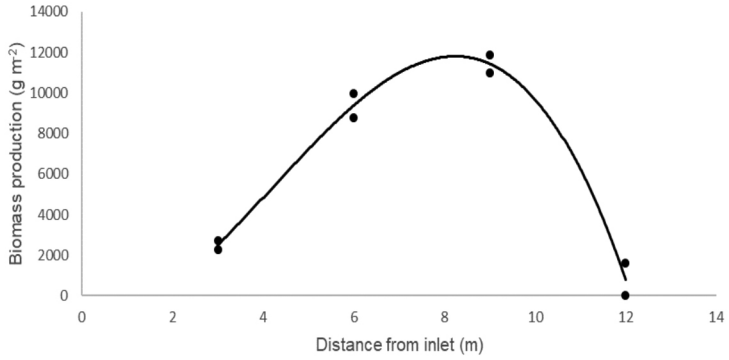


Figure 1: Biomass production in relation to distance from the effluent inlet of Swiss chard grown in two constructed wetland and irrigated with treated brewery effluent for 16 weeks

GROWTH PERFORMANCE AND HAEMO-BIOCHEMICAL PARAMETERS IN SOUTH AFRICAN DUSKY KOB A*rgyrosomus japonicus*, SCIAENIDAE OFFERED BREWER'S YEAST *Saccharomyces cerevisiae* AS A FEED ADDITIVE

Molatelo J. Madibana*, Victor Mlambo

Department of Agriculture, Forestry and Fisheries, Martin Hammerschlag way, Foreshore, Cape Town, 8001, South Africa

There is some evidence that single cell proteins (SCP) such as yeast have the potential to improve feed utilization in aquaculture fish but this has not been investigated in the economically important dusky kob (*Argyrosomus japonicus*). This study was, therefore, designed to determine the effect of graded levels of dietary inactivated brewer's yeast (Saccharomyces cerevisiae) on the growth performance and haemo-biochemical parameters of dusky kob in a six-week feeding period. Five isonitrogenous and isoenergeticdiets, consisting of three brewer's yeast-containing diets at rates of 50,150, and 300 g/kg DM (BY5P0, BY15P0, and BY30P0, respectively), a commercial dusky kob diet containing 10% probiotic mix but no brewer's yeast (BY0P1, positive control), and a commercial dusky kob diet with neither the probiotic mix nor the yeast (BY0P0, negative control), were formulated. A total of 65 fish, weighing an average of 7.02 ± 0.10 g, were randomly distributed to each of 20 replicate tanks. Each dietary treatment was randomly allocated to four tanks and offered to fish at a rate of 2.8% fish body weight per day. A total of 10 fish from each tank were randomly sampled once a week for length and weight measurements. Blood was drawn from five fish per tank (20 fish per treatment) for hematology and serum biochemical analyses at the end of the six weeks. Fish on the BY0P0 diet achieved the highest weight gain of 18.53 ± 0.69 g after 6 weeks. Growth rate was significantly reduced in the groups fed BY15P0 and BY30P0 diets compared to BY0P0, BY0P1, and BY5P0 groups. Fish fed the BY0P0 diet recorded the highest average feed conversion efficiency (FCE) of 0.22, while the BY30P0-fed group recorded the lowest FCE of 0.15. Hematocrit and alanine transaminase levels declined with increasing levels of yeast. It can be concluded that the maximum inclusion level of brewer's yeast that does not impair growth performance and health of dusky kob is 50 g/kg.

UNDERSTANDING THE EXPRESSION PATTERN OF VIPERIN IN RED-LIP MULLET (*Liza haematocheila*) UNDER DISTINCT IMMUNE STIMULI

K.P. Madushani*, K.A.S.N Shanaka, Jehee Lee

Department of Marine Life Sciences & Fish Vaccine Research Center
Jeju National University, Jeju Self-Governing Province
Republic of Korea
piyumikamadushanikaludewa@gmail.com

Viperin, an interferon inducible, endoplasmic reticulum associated gene product, which is active against RNA viral infection. Viral hemorrhagic septicemia virus (VHSV) is a negative-sense single-stranded RNA virus causing a severe loss in aquaculture farms. *L. haematocheila* which has become an economically important mugilid species in off shore aquaculture along the west coast of Korea. Even though interferon stimulated genes were suspected actions against VHSV, specific pathway or mechanisms has not discussed for red lip mullet yet. And also past studies have shown that viperin levels were boost with the presence of double stranded RNA (ds RNA).

In silico study of the mullet viperin, which is a protein contains 355 amino acids, was disclosed a SAM binding conserved domain containing CNYKCGFC sequence. Isoelectric point and the molecular weight were determined to be 7.46 and 40.66 kilo Daltons, respectively. Alignment analysis revealed that 83.5% similarity to Zebra fish (*Danio rerio*) viperin. Furthermore, phylogenetic analysis indicated that viperin has unique origin compared to other fish. When considering the tissue distribution, highest expression was revealed in blood, followed by brain. In blood of poly I.C injected samples, approximately 17-fold upregulation compared to the control of viperin was detected after 24 hours. However, non-viral immune stimuli, including, *L. garvieae* and LPS injected samples not showed any significant viperin expression.

These results agree with the ability of the viperin to express strongly with the presence of poly I: C. Tissue distribution pattern may reveal by the strengthening viperin expression in vital organs similar to brain may beneficial for the fish. Challenge experiment reveal capability of poly I:C to solely enhance the expression, thus with the presence of virus RNA may also leads to a strong expression of viperin. However, more studies may require to fully characterize this unique protein in fish.

POLY (ADP-RIBOSE) POLYMERASE (PARP) DOMAINS MAY HAVE ANTIVIRAL ACTIVITY: A STUDY ON GIG-2 PROTEINS IN ROCKFISH (*Sebastes schlegelii*)

K.A.S.N Shanaka, K.P Madushani*, Jehee Lee

Department of Marine Life Sciences & Fish Vaccine Research Center
Jeju National University
Republic of Korea
shanaka@jejunu.ac.kr

When considering the virus defence, contemporary findings suggesting availability of number of distinct interferon regulated proteins. Elucidation of detailed mechanisms of these proteins were shown to be beneficial for targeting specific and efficient antiviral drugs and vaccines towards pathogenic viruses. "GCHV induced gene-2 (GiG-2)" was identified from rockfish (*Sebastes schlegelii*). As an interferon stimulated gene (ISG) confined only to the fishes and amphibians, more and more scientific curiosity has developed on this protein.

Expression patterns of two isoforms of GiG-2 namely, *SsGiG-2-1* (163 amino acids) and *SsGiG-2-2* (233) were investigated. Study in healthy rockfish fish revealed highest expression of both *SsGiG-2-1* and *SsGiG-2-2* in the gills. Organs similar to the stomach, muscles, and the liver showed the weakest expression. Immune challenge experiment disclosed the peak expression at 24 h of post injection for the poly I:C.

Strong expression under the poly I:C may reveal the probable antiviral activities of GiG-2 proteins. Sequence based domain identification revealed the availability of a PARP domain region around the N-terminal ends of both proteins. In the *SsGiG2-1,* PARP domain spanned from 27 to 104, whereas in *SsGiG2-2,* it spanned 33 to 109. Structure comparison with human PARP domain found the availability of HYE (His-Tyr-Glu) active catalytic tried at the active site of the both proteins. Conformational alterations occur at SsGiG2-1 Tyrosine-62 (Y62) or SsGiG2-2 Y66 may lead to PARP internal cavity to open and close. These facts may collectively suggest that SsGig-2-1 and 2 may having authentic and active PARP domains. These genuine domains may capable of ADP-ribosylation of proteins using nicotinamide adenine dinucleotide (NAD), which serve as the ADP-ribose donor. Ribosylation of proteins important for the virus life cycle may leads to the hypothesized antiviral activities.

DETECTING AQUATIC POLLUTION USING HISTOLOGICAL INVESTIGATIONS OF THE GILLS, LIVER, KIDNEY, AND MUSCLES OF *Oreochromis niloticus*

Sana Shahid[1], Tayyaba Sultana[1], Salma Sultana[1], Bilal Hussain[1] and Shahid Mahboob[2*]

[1]Department of Zoology, Government College, University Faisalabad-Pakistan, Faisalabad, Pakistan
[2]Department of Zoology, College of Science, P.O. Box 2455, Riyadh 11451, Saudi Arabia
Presenting author email: mushahid@ksu.edu.sa
shahidmahboob60@hotmail.com

Altitudinous levels of toxic chemicals from Faisalabad city are disposed of directly into Chenab River through Chak Bandi Main Drain (CMD). The present study aimed to determine the degree of changes in the histological architecture of the liver, gills, kidneys, and muscles of fish *Oreochromis niloticus* collected from different polluted river sites. Fish samples collected from the Faisalabad fish hatchery and upstream to CMD acted as a control. Metabolically vital organs were subjected to histopathology. Necrosis, hemorrhage, and epithelial hyperplasia were observed in the gills of fish inhabiting the River downstream to the CMD entrance. Liver tissues were found to be affected by vacuolated cytoplasm, bile duct proliferation, melanomacrophages, and necrosis. In kidney tissues, Shrinkage of the renal cortex, necrosis, and destructive renal tubules was observed. Histopathology of muscles indicates the presence of hypertrophy and swollen myofibers. In contrast, upstream specimens of fish exhibited mild tissue alterations. Histopathology of gills tissue showed vacuolization. Liver tissues indicated the presence of hypertrophy and more frequent kupffer cells than usual. The vacuolation was observed in kidney tissues. Muscle tissues expressed splitting of muscle fibers and degeneration in muscle bundles. However, sections of tissues collected from farmed fish have normal morphology and no anomalies. The histopathological assessment indicated various cellular, biochemical and histological changes in response to the contamination in the vicinity of the fish. Such alternations can be used as a monitoring tool for freshwater contamination and the whole population's health in the ecosystem.

NUTRITIONAL EVALUATION OF SOME ECONOMICALLY IMPORTANT MARINE AND FRESHWATER SHELLFISH SPECIES OF BANGLADESH

Yahia Mahmud[a*] Mohammad Moniruzzaman[a,b], Sonia Sku[a], Parvez Chowdhury[a], Mohosena Begum Tanu[a], Selina Yasmine[a], Md. Nazmul Hossen[a], Taesun Min[b] and Sungchul C. Bai[c]

[a]Bangladesh Fisheries Research Institute (BFRI), Mymensingh 2201, Bangladesh
E-mail: yahiamahmud@yahoo.com

Molluscs are the most important resources among all the seafood items in South-East Asian countries. However, very little information available on nutritional value of molluscs in these regions. In this study, we evaluated the 7 economically important species of molluscs in terms of proximate composition, amino acids profile, fatty acids profile, cholesterol and heavy metal contents in the bivalves (mussels, oysters, clams and cockles) and univalve (snail) collected from freshwater and marine environments of Bangladesh. The results of the proximate analyses revealed that significantly higher amount of crude protein contents were present in marine water oysters, clams and cockles (59.3 ± 0.3 to 75.4 ± 0.2%) than the freshwater mussels and snail (36.9 ± 0.4 to 49.6 ± 0.6%) on dry matter basis. However, carbohydrate contents were significantly higher in freshwater mussels and snail (30.2±± 0.9 to 57.3±0.2%) compared to the marine water bivalves (8.1 ± 0.4 to 20.2 ± 0.6%). Crude lipid contents were ranged from 2.5 ± 0.2 to 11.2 ± 0.1% and ash from 11.4 ± 0.1 to 16.8 ± 0.6% among the bivalves and snail species. The amino acid contents were comparatively higher in marine water bivalves than their freshwater counterparts. Saturated fatty acid contents were found to be higher in marine water bivalves than the freshwater mollusc species. The results also show that the omega-3 (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) fatty acids were comparatively higher in oysters, clams and cockles in marine water than those in freshwater mussels and snail. However, omega-6 fatty acids like linoleic acid (LA), α- linolenic acid (ALA) and arachidonic acid (ARA) were higher in freshwater mussels and snail than in the marine bivalves. The n-3/n-6 ratio were significantly higher in oysters and cockle species than the other groups of bivalves and snail. The index of atherogenicity and index of thrombogenicity of the mollusc species ranged from 0.74 ± 0.1 to 1.74 ± 0.2 and 0.5 ± 0.1 to 2.6 ± 0.2, respectively. The results show that marine water bivalves contained higher amount of potassium, sodium, iron, chlorine especially oyster species contained significantly higher iodine than the freshwater bivalves and snail. However, freshwater mussels and snail showed significantly higher amount of zinc contents than the marine bivalves. The heavy metal contents such as arsenic, chromium and mercury were absent or present in very tiny amounts among the mollusc species. Significantly higher amount of cholesterol was present in marine bivalves and freshwater snail species than the freshwater mussels. Overall, the results indicate that marine bivalves can be good sources of high quality protein and lipid especially EPA and DHA. On the other hand, freshwater mussels and snails also could be good sources of protein, LA and ARA but scarcity of EPA and DHA.

THE STATUS OF TRAWLERS AND GILLNETS (VESSELS OF <90 CV) IN KIEN GIANG PROVINCE, VIETNAM

Mai Viet Van* and Le Thi Huyen Chan

College of Aquaculture and Fisheries
Can Tho University
Can Tho City
Viet Nam

This study was conducted from June to December 2017 by interview 60 fishing households in coastal areas of Kien Giang province (30 households using of trawl nets and 30 households using of gillnets). Key research variables including characteristics of fishermen, fishing gear specifications, fishing grounds, labor and fishing seasons; productivity, financial efficiency, advantages and disadvantages of trawls and gillnets. The results show that the trawlers and gillnets have been operated whole year round in the South-West coastal areas of Vietnam. The average fishing production of the trawlers was 37,330.50 kg/boat/year with the productivity of 845.75 kg/CV/year and then was that of gillnets was 6,445.60 kg/boat/year, the productivity of 304.97 kg/CV/year. The profit of the trawlers was 1,151.4 million VND/boat/year with its benefit and cost ratio of 1.32 times which is much higher than the profit of the gillnets of 342.6 million VND/boat/year with its benefit and cost ratio of 1.01 times. Fishery resources along the coastal areas of Kien Giang province are increasingly declining. Control of fishing activities and fishing efficiency should be strengthened to ensure sustainable development of fisheries resources.

A SURVEY STUDY ON THE LEVELS OF TEN GAMMA-EMITTING RADIONUCLIDES IN THE FARMED FISH IN SINGAPORE

Lak Kuen Mak*, Jasmine Toh, Lay Peng Neo, Shoo Peng Koh, Yuansheng Wu, Sheot Harn Chan

National Centre for Food Science
Singapore Food Agency
10 Perahu Road, Singapore 718837

Currently, international food safety standards for radionuclides are established based on the context of responding to a nuclear and national emergency. However, the robust dietary exposure assessment for radionuclides should consider contributions from both post-nuclear incidents and existing background levels. There has been ongoing IAEA and FAO discussion on the need for data gathering of radionuclides levels in food commodity and drinking water during non-emergency situations, which covers both naturally occurring and human-made radionuclides. This survey aims to study the normal background levels of gamma-emitting radionuclides in fishes farmed in Singapore. The levels of 10 gamma-emitting radionuclides comprising of naturogenic (^{40}K, ^{212}Bi, ^{212}Pb, ^{214}Bi, ^{214}Pb, ^{226}Ra and ^{228}Ac) and antropogenic radionuclides (^{131}I, ^{134}Cs, ^{137}Cs) were analysed in 42 fishes collected from farms located in the East and West Johor Straits, Southern coastal and freshwater fish farms in Singapore. Fish species analysed include milkfish (9), snapper (9), grouper (7), seabass (6), tilapia (4), jade perch (2), catfish (2), pompano (1), mullet (1), and snakehead (1). The measurements were performed using high-purity germanium detector gamma spectrometer (HPGe). Amongst all the gamma-emitting radionuclides monitored, naturally occurring Potassium-40 (^{40}K), a long-lived radioisotope with half-life of 1.251×10^9 years is present in all sample. Potassium-40 has a natural abundance of 0.012%, coexisting with stable isotopes ^{39}K and ^{41}K, with a combined natural abundance of 99.988%, is part of the common minerals present in all food of animal and plant origins. The level of ^{40}K in fish ranged from 40.3 to 140 Bq kg^{-1}. None of the samples is detected with the three anthropogenic radionuclides. The annual effective dose of the gamma-emitting radionuclides analysed in this study was estimated to be about 0.01 mSv per person when considering the dietary intake of fish for an average local consumer. Based on the calculated dose, about 98.5% is contributed by the natural radionuclide, ^{40}K. Hence, the exposure to radionuclides from consumption of fish is significantly lower than the worldwide average annual effective dose of 0.29 mSv via ingestion of food and water by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 2008).

IMMUNE STIMULATION, GENE EXPRESSION AND DISEASE RESISTANCE IN NILE TILAPIA *Oreochromis niloticus* BY MARINE EXTREMOPHILIC BACTERIA

Sarah O. Makled*, Rowan O. Saad

Oceanography Department, Faculty of Science, Alexandria University, Alexandria, Egypt
Email: sarah.o.makled@alexu.edu.eg

Nile tilapia (*Oreochromis niloticus*) is one of the most important farmed fish species in the world, second only to carps. Under intensification, tilapia become more susceptible to elevated stressful conditions and disease infection, leading to high mortality rate and severe annual economic losses. The massive use of antibiotics for disease control in tilapia culture has been under public awareness and criticism. Consequently, several extremophilic bacteria have recently emerged as an ideal and promising growth promoters and disease controllers for farmed Nile tilapia. However, very few studies have considered the potential use of extremophilic bacteria as a feed additive in aquaculture. The present study was carried out to evaluate the use of a novel marine extremophilic bacteria as a dietary supplement on the growth performance, digestive enzymes activity, immune response, and the expression of immune□related genes of Nile tilapia.

Extremophilic bacteria were isolated from marine samples along the Mediterranean Sea. Bacterial isolate showed significant antimicrobial and negative hemolytic activity, with no incidence of disease symptoms, was considered as a safe additive in fish feeds. Pure safe strain was identified, mass produced and incorporated in Nile tilapia diets at four doses; 0 (T_0), 2.3 x10^7($T_{0.5}$), 4.6 x10^7 (T_1) and 9.2 x10^7 (T_2) CFU g^{-1} diet; equivalent to 0, 0.5, 1.0 and 2.0%, respectively. The diets were fed to Nile tilapia fingerlings at a daily rate of 3% of their live body weights, for 60 days. After the termination of the study, growth performance, feed utilization, digestive enzymes activity, immune responses and gene expression were assessed.

The results showed that Nile tilapia fed diets supplemented with extremophilic bacteria at 1% displayed the best growth rates, feed utilization efficiency and digestive enzymes activity. This diet (T_1 (1%)) also exhibited a significant increase in cytokines gene expression, hematological, biochemical, hepatic function activities, and immunological parameters, compared to other diets.

Acknowledgement:
This research was supported by the Academy of Scientific Research and Technology (ASRT).

IMPROVED GROWTH PERFORMANCE OF NILE TILAPIA *Oreochromis niloticus* JUVENILES USING A PHYTOGENIC FEED ADDITIVE

Alex Makol*, Karola R. Wendler, Tuyen Han, Thanh Chau and Loc H. Tran

Delacon Biotechnik GmbH, Langwiesen 24, 4209 Engerwitzdorf, Austria
Email: alex.makol@delacon.com

Nile tilapia (Oreochromis niloticus) is one of the most aquacultured fish species worldwide, mainly due to its rapid growth performance and market value. However, as occurs for other farmed species, its growth performance, feed efficiency and disease resistance are usually challenged by stressful situations derived from common culture practices. Phytogenic feed additives (PFA) have been demonstrated to be potential tools to improve production performance.

The present study was conducted to determine the efficacy of a new phytogenic feed additive (PFA) mixture (saponins, spices and essential oils) on Nile tilapia juveniles growth performance and feed efficiency. Two isoenergetic and isoproteic diets were formulated to contain different levels of PFA bioactives (control diet: 0 ppm, PFA diet: 30 ppm) and fed for 8 weeks to Nile tilapia juveniles (initial weight: 13.05±0.27g). The trial was conducted in a 350 L tanks outfitted with a biological filter and an aeration system, with 3 replicates by diet. Water temperature was kept at 27.9±0.6ºC, dissolved oxygen and pH were monitored daily whereas total ammonia and nitrite were measured 2 times a week. At the end of the feeding trial, feces and tissue samples were collected for nutrient digestibility and digestive enzyme activities analyses.

After 8 weeks of dietary PFA supplementation, fish fed the PFA diet presented improved weight gain and showed improved ($p<0.01$) specific growth rate (SGR) and higher ($p<0.01$) feed intake (Figure 1). Results obtained are related to the nutrient digestibility and digestive enzyme activities findings. Altogether indicating that the use of the PFA blend tested can promote growth performance of Nile tilapia juveniles.

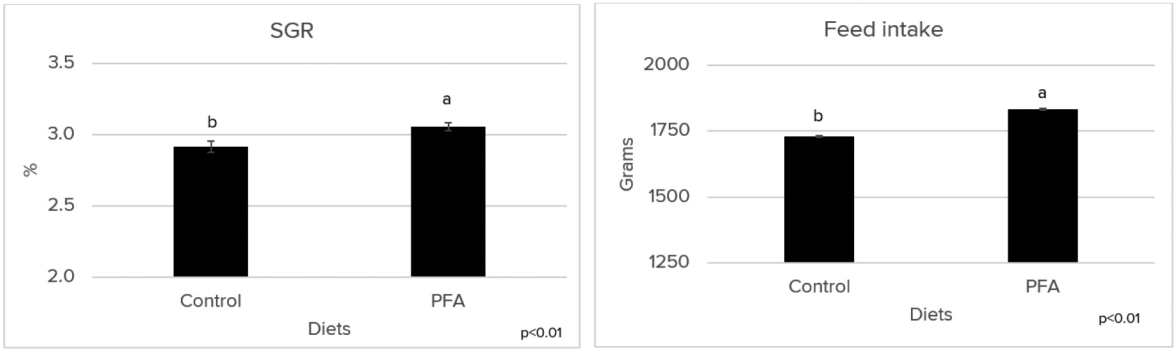


Figure 1. Growth performance parameters of Nile tilapia after 8 weeks feeding experimental diets.

REVIEW: POTENTIAL OF USING PROBIOTIC INOCULANT FOR SEAWEED SILAGE AS AN ALTERNATIVE FEED FOR AQUACULTURE

Azziza Mala[1,2]*Subha Bhassu[1,2]* Norhidayah Mohd Taufek[1]* Najiah M. Sadali[1]* and Adibi M. Nor[1]

[1]Center for Research in Biotechnology for Agriculture, 50603, Universiti Malaya, Kuala Lumpur, Malaysia
[2]Animal Genetics and Genome Evolutionary Biology Lab, Institute of Biological Sciences, 50603, Universiti Malaya, Kuala Lumpur, Malaysia
Email: azziza@um.edu.my

Aquaculture is an important sub-sector in Malaysian fisheries and plays a significant role in the national economy. Ensiling is an effective method to preserve seaweeds and should be considered one of the most sustainable sources for aquaculture feed applications. However, Seaweeds generate socio-economic benefits for tens of households, primarily in coastal communities, including numerous women empowered by seaweed cultivation. Shellfish, commercial fishermen, and finfish farmers, who are the main stockholder, will benefit by conserving seaweed as silage for aquaculture feed. Also, they can offer an opportunity to diversify a farming operation or start a new business. Despite these possibilities, there is growing concern over how collected seaweed will benefit local communities in the long run and how it will be preserved using lactic acid bacteria. The sustainability disparity between developed and developing countries, as a result of increased economic interdependence, combined with developing countries' limited management and governance capacity, is a significant challenge to the 2030 Agenda's implementation. To eliminate disparities while progressing toward the 2030 target for food security, the global community must assist developing countries. To realize the potential of seaweed silage as an alternative feed for aquaculture, the industry, governments, international organizations, scientific community, civil societies, and other stakeholders or experts must work together.

THE IMPORTANCE OF PREVENTIVE STRATEGIES IN MANAGING DISEASE RISK TO OPTIMIZE AND SUSTAIN AQUACULTURE YIELDS IN COMMERCIAL FARM SETTINGS

Martha Mamora*, Maria-Mercè Isern-Subich, Ruth Garcia, Martin Guerin, Waldo Nuez-Ortín

ADISSEO Asia Pacific Pte Ltd,
600 North Bridge Rd., Unit 15-06 to 08 Parkview Square, 188778 Singapore
martha.mamora@adisseo.com

Disease risks and outbreaks, and associated farming problems, continue to plague the reality of the shrimp farming industry, and threaten its sustainability. A major challenge is the complexity of this disease threat, as types of disease challenges and their severity vary significantly from one region or country to the other and are difficult to predict. Indeed, many contributing factors such as presence of pathogens, environmental conditions, shrimp health status, and farm biosecurity level, can influence their onset and impact. This complexity makes it difficult for providers of health solutions to propose products and approaches adapted to multiple threats and conditions, especially since most farmers tend to focus on addressing single pathogen risks, overlooking the synergetic damaging effect that multiple pathogen infections can have.

Sanacore® GM (SNGM) is a phytobiotic-based functional feed additive, with broad-spectrum health enhancing properties, widely used as a preventive tool to reduce the severity of infections in shrimp production. SNGM has demonstrated bacterial inhibitory properties which promote a healthy gut microbiota, as well as immuno-modulating properties that restore immunocompetence under production conditions.

In this study, the health status of five commercial shrimp farms in Indonesia were monitored for a period of ten months through on-farm observations. A whole-body shrimp were initially tested for AHPND, EHP, IMNV, and WSSV. Tests were carried out periodically during the culture period. Within each farm, several ponds were treated with SNGM and pond productivities were assessed and compared with control ponds. Throughout this period, coinfections with different degrees of severity were detected from stocking to harvest. The timing and duration of the disease outbreaks varied considerably between farms.

Observations on the performance of each farm are presented to illustrate the efficacy of SNGM to support shrimp in dealing with coinfections. This is a unique report showing several examples of successful application of broad-spectrum health additive under commercial conditions and confirming the importance of adopting preventive strategies to reduce the impact of disease and improve profitability.

FISH BY-PRODUCTS FROM CONVENTIONAL PROCESSING COULD BE USED FOR SUSTAINABLE AQUACULTURE

Abdullah-Al Mamun*, Shuva Bhowmik, David C. Little, Shakuntala H. Thilsted, Andrew L. Thorne-Lyman

*Department of Fisheries and Marine Science, Noakhali Science and Technology University
Noakhali-3814, Bangladesh
mamun_au22@yahoo.com

Fish is one of the key sources of high-quality food and animal protein and other essential nutrients including vitamins, micronutrients. The present study evaluated both the quantitative and qualitative of by-products arising from 14 fish and 2 crustacean farmed species in local fish markets and household levels in Bangladesh. Utilizing waste fish by-products could be a key component of a circular economy policy that minimizes the environmental impact and value chain.

The results showed 9-18% by-products (derived during cleaning process), and 3 to 19% plate waste (derived during consumption) derived from fish species, whereas shrimp and prawn generated 50-66% by-products and of plate waste. Among the by-products, shrimp, and prawn heads (36-38%), and fish viscera (2-11%) contributed the large volume followed by fish gill, fin, and scale.

The estimated by-products from the fish production in 2019 of 14 fish species total 2,81,161-5,65,332 MT on a wet weight (ww) basis, which could contribute 6-13% fish meal development for sustainable

aquaculture in Bangladesh, with a value of approximately 32-65 million USD that render to help the economy. Other nutrients from by-products, including as fat, calcium, iron, zinc, and chitosan, can be used for human consumption, livestock feed, and the food industry.

Successful exploitation of fish and crustacean by-products will provide a nutritionally enriched fish by-product meal for future aquaculture growth in a sustainable way. Current fish consumption is estimating the live total weight however filleting and plate waste correction factors should be considered to estimate the per capita fish consumption.

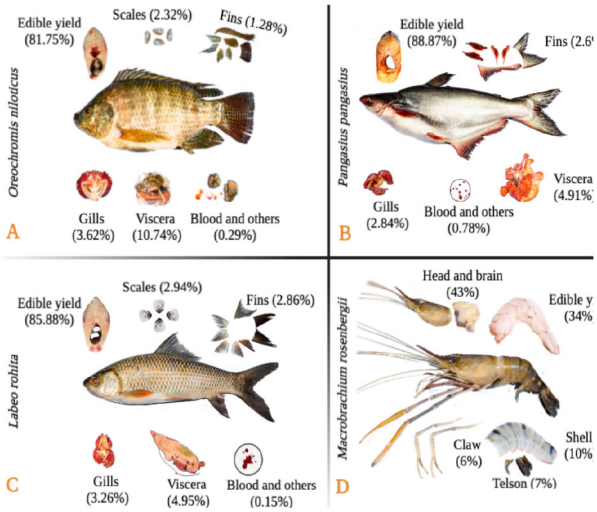


Figure 1. Total quantifying in % of by-products of *Oreochromis niloticus* (A), *Pangasius pangasius* (B), *Labeo rohita* (C), and *Macrobrachium rosenbergii* (D)

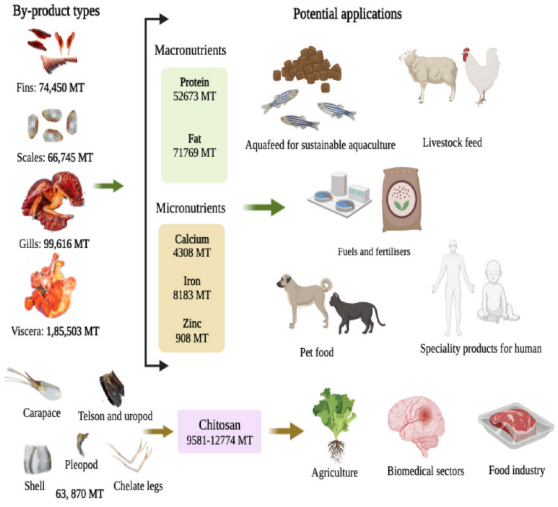


Figure 2. By-product types, production volume, macro- and micro-nutrient amount, and potential applications

Artemia INDIVIDUAL AND POPULATION LEVEL ACCLIMATION TO SALINITY INCREASES IN THE GREAT SALT LAKE, UTAH, USA

Brad T. Marden*, Phil D. Brown, Thomas Bosteels

Great Salt Lake Brine Shrimp Cooperative, Inc.
Ogden, UT 84401
bmarden@gsla.us

The ongoing drought in the western USA has resulted in continual declines in watershed inputs to the Great Salt Lake (GSL), leading to increasing salinity and lowered volume of the GSL. The decline in volume and increased salinity has had multiple consequences on the GSL ecosystem with aquatic taxa and dependent avian species exhibiting stress related impacts whereas some organisms such as the resident *Artemia* are demonstrating greater resiliency to salinity changes. Physiological changes such as alterations in per capita reproductive output, reduced size, mobilization of biochemical moderators of osmotic stress, shifts in dormancy termination, and alterations in temporal reproductive cycles illuminate the influence of increasing salinity on the *Artemia* population.

Within the broad scope of physiological transitions among the resident *Artemia* there are indications of adaptive responses that have afforded population level effective responses. Reproductive output has shifted temporally from the onset of cold inclement weather in fall to maximal production during late spring and early summer via exploitation of available algal resources. Termination of dormancy among *Artemia* cysts exhibited an alteration with dormancy ceasing in early summer thus significantly shortening the duration of diapause. Total population level reproductive output has resulted in densities of cysts similar to, or greater than, years exhibiting more 'favorable' conditions. Concerns remain about the effect of reduced volume on the GSL ecosystem, but presently the *Artemia* population is exhibiting resilience.

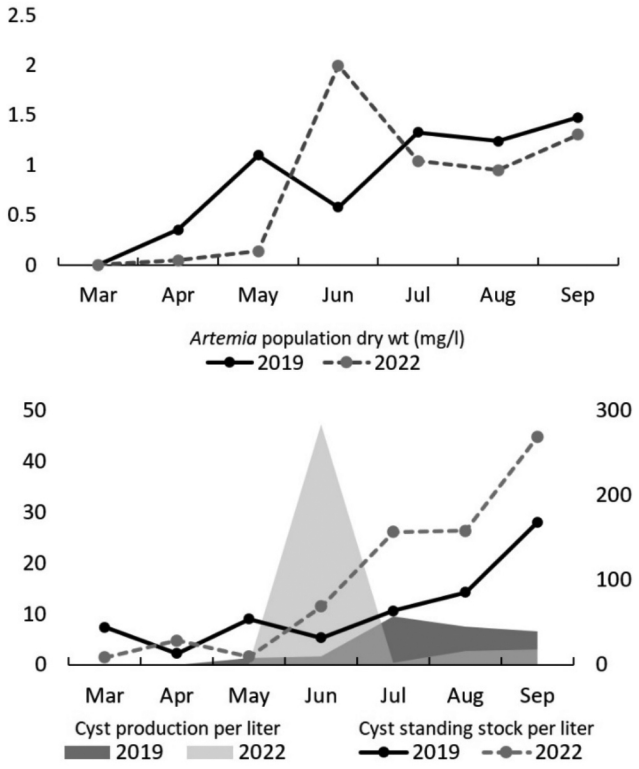


Figure 1. Later population development (top) and earlier maximal cyst production (bottom) in the high salinity year 2022 compared to a year of lower salinity (2019)

DIETARY OLEIC ACID SUPPLEMENTATION DECREASES FEED-INTAKE IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES

Nicole Martins*[1,2], Aires Oliva-Teles, Helena Peres, Carolina Castro

*nicole.pires@fc.up.pt
[1]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, Edifício FC4, 4169-007 Porto, Portugal
[2]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto Terminal de Cruzeiros do Porto de Leixões. Avenida General Norton de Matos, S/N, 289 4450-208- Matosinhos, Portugal

Regulation of feed intake (FI) and lipid deposition in fish is of extreme relevance for aquaculture, either from economic, environmental and consumer perspectives. Indeed, feeding represents more than 50% of operating costs in intensive fish farming. Thus, reducing FI may have a high economic impact and also contribute to reducing the environmental impact associated with feeding. Further, reducing muscle lipid deposition will also have considerable implications on fillet quality to consumers.

Oleic acid (OA) is a monounsaturated long-chain fatty acid, highly present in several vegetable oils. In mammals, increasing OA intake has been linked to weight gain and lipid deposition control by interfering with FI (Hammad et al., 2016). Despite its interesting potential role, the potential use of OA as an additive in aquafeeds has been almost neglected until now, except for a recent study indicating that intraperitoneal administration of OA inhibited FI in rainbow trout (Librán-Pérez et al., 2014).

Thus, the aim of this study was to evaluate the effect of OA supplementation at different dietary lipid levels on appetite regulation and fatty acid-sensing mechanisms in European seabass (Dicentrarchus labrax). For that purpose, six isoproteic (45% crude protein) with two different dietary lipids levels (16 and 22%) were formulated with fishmeal and cod liver oil as protein and lipid sources and supplemented with 0, 1 or 2% of OA. Triplicate groups of European sea bass juveniles (21.4g) were fed with these diets. At the end of growth trial, final body weight, weight gain, and daily growth index were not affected either by dietary lipid level or OA supplementation. Diet supplementation with OA was associated with a reduction of FI without major impacts on the growth performance of the animals. This finding is of very high practical interest, as improvements in feed efficiency may result in significant reductions of waste outputs and production costs of fish farming. The putative beneficial effects that dietary OA may have on fish flesh quality in terms of lipid content are being analyzed as well as mechanisms involved in FI regulation at central peripheral levels. A new abstract containing data of neuropeptides expression and fatty acid-sensing mechanisms will be submitted soon.

Acknowledgments:
Present study was funded by FEDER-Operational Programme Competitiveness and Internationalization and FCT under the project SPO3 (ref. POCI-01-0145-FEDER-030377) and by Programa Operacional Mar2020, Portugal 2020 under the project InovFeed (ref. MAR-02.01.01-FEAMP-0111)

Reference:

Hammad, S., Pu, S., Jones, P.J., 2016. Current Evidence Supporting the Link Between Dietary Fatty Acids and Cardiovascular Disease. Lipids 51, 507–517. https://doi.org/10.1007/s11745-015-4113-x

Librán-Pérez, M., Otero-Rodiño, C., López-Patiño, M.A., Míguez, J.M., Soengas, J.L., 2014. Central administration of oleate or octanoate activates hypothalamic fatty acid sensing and inhibits food intake in rainbow trout. Physiol. Behav. 129, 272–279. https://doi.org/10.1016/j.physbeh.2014.02.061

GENERATING MONOCLONAL ANTIBODIES AGAINST IgM OF COMMERCIAL FISH SPECIES

Masato Miyata*, Jean Loi, Syed Khader Syed Musthaq, Padmanabhan Saravanan, Chan Pek Sian Diana, Lee Chee Wee.

Centre for Aquaculture and Veterinary Science, School of Applied Science, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
mmiyata@tp.edu.sg

Blood sampling routine of farmed fish being popular to investigate fish health status to forecast and prevent disease outbreak. Enzyme linked immunosorbent assay (ELISA) is the typical assay to monitor fish health status by serum titer elevation after exposure to pathogen. Secondary antibody against fish IgM is the key component of the ELISA. Existing secondary antibodies for fish IgM in market are however, provided for each fish species, and low titer in general as such 40-100 times dilution to use while anti mammal IgM antibodies work with 5,000 – 10,000 times dilution.

In this study, we generated several secondary antibodies against fish IgM. Firstly, seabass IgM and grouper IgM were purified by chromatography (Superdex® 200 / ÄKTA avant). Subsequently, mice were immunized with purified grouper IgM to generate over 10^3 hybridoma clones, and 83 of positive hybridoma cells were chosen by ELISA with purified grouper IgM. Supernatant of the 83 positive cells were tested further against un-purified serum from seabass, grouper, tilapia and bovine serum albumin (BSA: negative control), to evaluate versatility to different fish species and specificity to fish IgM. Eight (8) clones gave high titer with fish serum but minimum titer with BSA. Two (2) clones out of the 8 were specific to grouper serum only. Another 2 clones react with both seabass and grouper serum but not react with tilapia serum. Rest 4 clones showed broad spectre by giving signal to seabass, grouper and tilapia serum. Western blot of grouper serum revealed that our monoclonal antibodies bind IgM. Selected secondary antibodies evaluated in ELISA with iridovirus as an antigen. Significant difference of serums from iridovirus challenged fish (represents disease) and iridovirus naïve fish (as healthy fish). Results above indicate that, we managed to raise monoclonal antibody against fish IgM. Further specifications and preparations towards product launch are undergoing.

A QUALITATIVE ANALYSIS OF THE AQUAPONIC SYSTEM PRODUCTS

Nassrin Mashaii*; Farhad Rajabipour; Homayoun Hosseinzadeh sahafi; Mahmoud Hafezieh; Alireza Ghaedi

Agricultural, Research, Education and Extension Organization, Iranian Fisheries Science Research Organization
National Research Center of Saline Water Aquatics
Yazd, 100km Bafq Road, National Research Center of Saline Water Aquatics, POBox:159

Nassrinmashaii@yahoo.com

In the present study, the quality of tilapia and plant products produced in the aquaponic system including mint, peppermint, pennyroyal, green basil, purple basil, fodder beet, Swiss chard, various cultivars of lettuce, watercress, celery, and tomato have been studied. General composition, fatty acid profile and sensory evaluation of tilapia fillet were investigated. In plant products, general composition, nitrite, nitrate, proline, chlorophyll and some minerals were measured. The results showed that the products of aquaponic system have good quality for consumption.

The aquaponic system includes hydroponic system and recycling aquaculture in which the wastes and metabolites produced by farmed fish are removed from the environment through nitration and absorbed by plants. In this system, water productivity without dependence on soil and agricultural lands happen simultaneously with healthy fish/plant products throughout the year in conditions in accordance with environmental considerations. In the present study, the quality of tilapia and plant products produced in the aquaponic system including mint, peppermint, pennyroyal, green basil, purple basil, fodder beet, Swiss chard, various cultivars of lettuce, watercress, celery, and tomato have been investigated. Results of sensory evaluation of the cooked fillet showed high score for sensory parameters. In all plant products, the amount of nitrite and nitrate were considerably lower than permissible limit of consumption. Peppermint, fodder beet, Swiss chard, watercress and celery had the lowest proline content, while peppermint, fodder beet, Swiss chard were also richer in chlorophyll. Higher levels of proline were obtained in lettuce and basil, indicating more severe environmental stress conditions for them. The amount of protein and iron in plant products were high, however calcium and potassium were a few lower than the desired amount.

USE OF SUPPLEMENTAL AMINO ACIDS IN REDUCING DIETARY FISH MEAL, CRUDE PROTEIN LEVEL AND IMPROVING DIETARY PROTEIN UTILIZATION IN THE GROWER PHASE OF WHITELEG SHRIMP

Karthik Masagounder*, Tran Thi Thanh Hien, Tran Le Cam Tu, Nguyen Van Tien, Chuong Ngo Tien, Le Quoc Viet, Tran Nguyen Duy Khoa, Tran Ngoc Hai, and Pham Minh Duc

*Evonik Operations GmbH, Hanau-Wolfgang, Germany
email: karthik.masagounder@evonik.com

Commercial shrimp feed in the grower phase typically contains 38-42% crude protein (CP). Meeting the amino acid (AA) requirements entirely by intact protein sources would not only result in excess amount dietary protein but also very costly. Formulating diets following ideal protein concept and using supplemental sources to meet the limiting AA are increasingly practiced as this strategy allows spare the dietary protein sources such as fish meal and thus, dietary protein content. The objective of this study was to evaluate the performance of whiteleg shrimp to varying levels of dietary protein balanced for amino acid profile using supplemental amino acids. In this regard, two trials were conducted, one in the tanks and the other in the commercial ponds in Vietnam.

For the tank study, a positive control diet containing high dietary protein level (40%), mimicking the industry standard diet with 14% fish meal level, was formulated. A set of treatment diets containing 38%, 36%, 34% and 32% crude protein levels was formulated by reducing the inclusion of intact protein sources such as fish meal and wheat gluten meal, while meeting our recommended amino acid levels using supplemental sources including AQUAVI® Met-Met, L-Lys and L-Thr. With this approach, the formulation cost of feed was gradually reduced with the increasing protein reduction, up to 20% between D40 and D32. Four tanks (800 L water volume) were randomly allotted to each dietary treatment. Each tank was stocked with 80 shrimp of 2.5 g mean weight (± 0.04 g, SD). Shrimp were fed with the respective diets to apparent satiation, 4 times daily over 56 days. Final body weight, growth rate, feed intake, FCR and survival did not differ among dietary treatments (Table 1). Net protein utilization (protein gain x 100 / protein intake) showed significant improvement in shrimp fed with the diet containing 34%CP versus those fed with the diets containing 36-40% CP

In the pond trial, the PC diet (40% CP) was compared with the 34% CP diet. In addition, two commercial diets with their CP level matching the PC diet were included. Each diet was fed to four randomly selected ponds (1000-1500 m^2 area) and each pond was stocked with PL12-stage shrimp with a mean stocking density of ~60/m^2. During the first 30 days, shrimp were fed with a common commercial control diet, followed by their respective treatment diets 3 times daily until 72-79 days of total production period. Results showed no significant differences among treatments in terms of body weight, FCR and yield. However, Evonik diets of both PC and the low protein diets showed 20% increase in the growth compared with the commercial diets. Economic analysis showed 34% CP diet was 10-48% more profitable compared with the PC diet and the commercial diets. Results overall indicate that shrimp do not need more than 34% crude protein in grower phase, when the dietary amino acids are balanced. Studies overall demonstrate the benefits of low protein diet (34% CP) in producing more profitable and sustainable shrimp farming.

Table 1. Growth performance and feed utilization of shrimp fed the experimental diets over an 8-week experimental period.

Diet	Final body weight (g)	SGR (%/day)	Feed intake (g)	FCR	Net protein utilization %	Survival %
D40	17.67	3.47	17.83	1.18	42.0 b	97.2
D38	17.84	3.50	17.94	1.17	42.0 b	96.9
D36	17.30	3.43	18.24	1.24	41.3 b	95.3
D34	18.19	3.56	18.24	1.16	46.3 a	95.3
D32	17.47	3.48	18.21	1.22	45.3 ab	95.3
SEM	0.36	0.04	0.41	0.03	1.27	1.12
P-value	0.51	0.35	0.92	0.36	0.04	0.56

SGR = specific growth rate; FCR = feed conversion ratio; SEM, standard error of the means
*Duncan mean separation was used

EFFECTS OF *Bacillus* BASED PROBIOTICS VIA FEED AND WATER APPLICATION ON THE PERFORMANCE OF SHRIMP UNDER ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) CHALLENGE CONDITIONS

Karthik Masagounder*, Nguyen Van Tien, Pyae Phyo Hein, Thanh Chau, Giang Vo, and Loc Tran

*Evonik Operations GmbH, Hanau-Wolfgang, Germany
email: karthik.masagounder@evonik.com

Acute Hepatopancreatic Necrosis Disease (AHPND), commonly known as Early Mortality Syndrome (EMS) is known to be caused by specific strains of *Vibrio parahaemolyticus*. This specific disease has severely affected the shrimp aquaculture industry across world. Among the several health additives developed, probiotics have been increasingly used in aquaculture to improve shrimp health as well as improve water quality by removing toxic metabolites. The objective of this trial was to evaluate the effectiveness of two Bacillus based probiotics on the disease tolerance of whiteleg shrimp when challenged with EMS- causing *V. parahaemolyticus*. *Bacillus amyloliquefaciens* CECT 5940 based product (Ecobiol®, 2E9 CFU/g) via feed application and *B. subtilis* based multi-strain product (AQUAVI® Pro-Pond, 1.5E10 CFU/g.) via water application were used for the experiment.

The trial comprised 7 groups including i) Negative control without EMS challenge (NC), ii) positive control challenged with EMS-causing *V. parahaemolyticus* (PC), iii) PC group treated with antibiotic (Cefotaxime via feed at 0.2% after the challenge (iv) PC group treated with probiotics (Ecobiol) via feed at 0.1% (v) PC group treated with probiotics (Ecobiol) via feed at 0.2% (vi) PC group treated with probiotics (Pro-Pond) via water at 0.4 ppm and (vii) PC group treated with probiotics via feed (Ecobiol) at 0.1% and via water (Pro-Pond) at 0.4 ppm. Water probiotics was applied once in every three days before the challenge and daily after the challenge whereas feed probiotic was applied daily throughout the experiment. Each treatment had 6 replicate tanks and each tank was initially stocked with 31 shrimps (0.57 ± 0.01 g, mean ± SD) at the density of 310 shrimps/m3. Shrimps were fed with the experimental diets and the water probiotic was also applied to the relevant treatment tanks for 21 days. On day 22 of the trial, shrimps in all groups except the NC were challenged with *V. parahaemolyticus* strain (LA-37) at the dose of 1E5 cfu/mL (LD50-96h) according to the calibration test results. Shrimp were observed for survival for next 10 days, i.e., 10-days post challenge period. After 12 h of the challenge, the first mortality by the AHPND was recorded and the mortality rate in the PC reached 50% after 108 hpc. At the end of the trial, the combined treatment of feed and water probiotics (0.1% Ecobiol + 0.4 ppm Pro-Pond) resulted in the highest survivability (61.6 ±8.4%), after the NC group which showed a survival rate of 95.45 ± 0.13%, both the treatments differed significantly from the PC. 0.2% Ecobiol® on feed also showed a relatively high survival rate (51.31±16.42%) and was the second highest among the treatment groups but not significantly different from the PC. According to the immunological assays, both of the test products, Ecobiol and Pro-pond, had improved immune response of shrimp. Among the treatments, the combined treatment (0.1% Ecobiol + 0.4 ppm Pro-Pond) showed the best immune response. Based on the results, combination of feed-based *B. amyloliquefaciens* CECT 5940 and water-based multi-strain *B. subtilis* products would be an effective strategy to improve the heath-status of whiteleg shrimp and protect against EMS-causing *V. parahaemolyticus*.

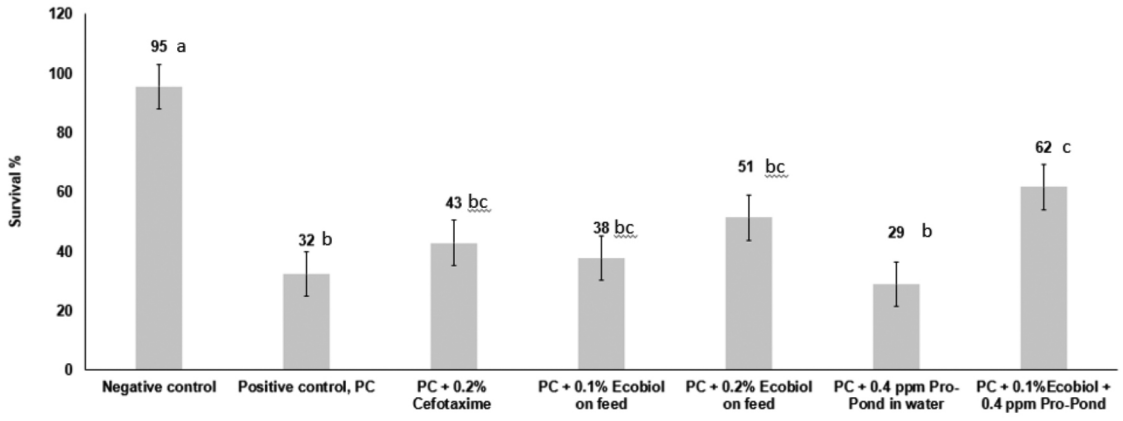


Fig 1. Effects of treatments on the survival (mean ± SE) of shrimp infected with EMS-causing *V. parahaemolyticus*

UNDERSTANDING THE EFFICACY OF DIFFERENT METHIONINE SOURCES USED IN COMMERCIAL AQUA FEEDS

Karthik Masagounder*, Sarah He and Nguyen Van Tien

*Evonik Operations GmbH, Hanau-Wolfgang, Germany
email: karthik.masagounder@evonik.com

Methionine (Met) is a key amino acid that cannot be synthesized and must be provided in the diet of aquaculture species. It is a precursor of various metabolites and is involved in several biochemical and physiological systems, including protein synthesis, antioxidant defense, immune response, bile salt synthesis, etc. Today's commercial aqua feeds are predominantly based on plant protein and so Met is often the first limiting amino acid. Met deficiency can be avoided by adding Met rich ingredients such as fish meal, but this approach increases the overall protein content, feed cost and finally increases the amount of ammonia excreted into the water. A more economical, healthy, and environmentally friendly option is to supplement the feed with the right amount and source of Met. Met supplements are commercially available in four forms: DL-Met, L-Met, DL-MHA (methionine hydroxyl analogue) and DL-Methionyl DL-Methionine (Met-Met dipeptide of DL-Met). DL-MHA is not an amino acid and therefore animal needs to convert to Met before being able to use them for protein synthesis. There have been studies on the bioefficacy of Met sources in various fish species. Overall, studies show that DL-MHA is not as efficient as other Met sources on an equimolar basis while DL-Met and L-Met performed equally well. Recent studies in Nile tilapia, rainbow trout and common carp provided physiological evidences in terms of transporter, absorption, and bioconversion elucidating why D-MHA is not as efficient as other Met sources. On the other hand, Met-Met showed significantly better performance relative to DL-Met in shrimp because of its low leaching and better availability. This was further supported by physiological data from recent studies. In summary, using the right Met value determined in these studies is a critical step when selecting a Met source in feed formulations.

EFFICACY OF A MULTI-STRAIN BACILLUS SUBTILIS BASED PROBIOTICS IN CONTROLLING AMMONIA AND NITRITE NITROGEN WASTES IN COMMERCIAL SHRIMP PONDS

Karthik Masagounder, Sarah He and Chen Shijun

*Evonik Operations GmbH, Hanau-Wolfgang, Germany
email: karthik.masagounder@evonik.com

Ammonia is the principal nitrogen waste product excreted by shrimp. Ammonia from the hemolymph (blood) of shrimp is largely excreted into the water across the gills via diffusion process in the form of un-ionized ammonia. Probiotics are increasingly used in aquaculture to control disease, improve water quality, and enhance the health status of fish and shrimp. Pro-Pond is composed of three B. subtilis strains that were selected based on their ammonia and nitrite removal capabilities under a wide range of salinities. The study evaluated the efficacy of AQUAVI® Pro-Pond on the nitrogen removal efficacy, in commercial shrimp ponds located in the Guangdong province of China. Three earthen ponds at a stocking density of ~80 shrimp/m2 were allotted to the Pro-Pond treatment. The average culture period of the three ponds lasted for 108 days (97, 112, and 115 days for the three ponds). Three other earthen ponds (average area 3,557 m2 or 5.3 mu) were assigned to commercial control and the average culture period lasted 102 days. The three ponds (average area 2,668 m2 or 4 mu) allotted for Pro-Pond group were treated with Pro-Pond at 11.3 times per pond on an average (34 times in total for the three ponds) at a dose of 0.25 ppm or 2.5 kg per ha during each application over the whole culture period (108 days). On the other hand, the three commercial control ponds were treated with four different commercial products at 10.7 times per pond on an average (32 times in total for the three ponds) over a 102-day culture period. These four commercial products were based on probiotics (Bacillus, nitrifying bacteria, Enterococcus faecalis), yeast and/or enzyme mix. Results showed that Pro-Pond was marginally better than the commercial treatment in controlling the NH3-N level (0.45 mg/l versus 0.53 mg/l; p-value = 0.10, t-test). There were no differences between the two groups on the nitrite level. Although multiple products were applied in the control ponds by the farmer, *Bacillus subtilis* alone was sufficient to control nitrogen waste in the commercial ponds. At the end of culture, the average harvest yield of shrimp was 505 kg/mu for the control group and 535 kg/mu for the B. subtilis group (6% higher). Results demonstrate that *Bacillus subtilis* based multi-strain probiotics can be effective in controlling nitrogen wastes and improving production in commercial shrimp farms

EFFECTS OF FIRST FEEDING ON THE SURVIVAL, GROWTH AND BEHAVIOUR OF LARVAE AMUR CATFISH, *Silurus asotus* IN TROPICAL REGION

Siti Umey Syahirah Mat Nawang*, Ching Fui Fui, and Senoo Shigeharu

Borneo Marine Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400, Kota Kinabalu
Sabah, Malaysia
umeysyahirah8896@gmail.com

The effects of first feeding on the survival, growth and behaviour of larvae Amur catfish, *Silurus asotus* in tropical region were examined under controlled conditions. The total length, yolk sac volume, yolk sac absorption time, nutritional transition period (NTP) and behaviour of the larvae were observed. Larval first feeding time was indicated when the eyes were pigmented, the mouth had opened with a functional lower jaw, the intestine had peristaltic movement and the anus was opened. Larval first feed intake was observed at 30 hour after hatching (hAH) with total length 7.26 ± 0.24 mm at 27.2 ± 1.0 °C. Larval first fed at 30 hAH with yolk sac volume, 0.33 ± 0.08 mm^3 and the yolk sac absorption completely at 60 hAH (yolk sac volume, 0.10 ± 0.01 mm^3) with larval total length, 8.32 ± 0.51 mm. Larvae was feed with combination of live feed, rotifer (*Brachionus plicatilis*) and formulated pellet (Otohime, Nisshin Maru, Japan). The NTP of the Amur catfish larvae was 30 hours. The survival of Amur catfish larvae was 98.0 ± 0.6% at first feeding (30 hAH) and decreased to 90.0 ± 0.7% (60 hAH). At first feeding, larvae continuously moved at the bottom of the tank and sometimes swam vertically and larvae have active swimming at this stage. Hence, larvae swam mostly at the bottom of the tank and sometimes in the middle and surface layers at 60 hAH.

MICROPLASTICS ISOLATED FROM THE BLOOD CLAM *Andara granosa* AT A LOCAL MARKET AT SELANGOR, MALAYSIA

Nurzafirah Mazlan*, Naveen Kumar Naidu, Nor Ashikin Sopian, Safaa Najah Saud, Muhammad Shirwan Abdullah Sani, Muhammad Nor Afdall Nazahuddin

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS 88400 Kota Kinabalu, Sabah, Malaysia
nurzafirah@ums.edu.my

Due to continual mass production and gradual breakdown, plastic pollution has been a growing environmental concern in recent years. Microplastics with a diameter of 5 mm to 1 m are swallowed by marine animals and later consumed by humans in the form of seafood. The aim of this research was to evaluate microplastics isolated from *Anadara granosa* samples found in the local market in Shah Alam. The soft tissue of *Anadara granosa* were dissected and digested using NaOH. Microplastics were isolated, filtered and identified through microscopic examination based on the color, shape and size. The chemical composition of microplastics were further analyzed by FTIR to identify the functional group of polymers. A total of 420 microplastics were found in *Anadara granosa*. Threads were the majority of microplastics observed in terms of shapes and colors. The size range within 0.5 to 1 and 1 to 2 μm were the highest abundance observed. There were one identified polymer type of microplastics obtained through FTIR, which is polymethyl methacrylate (PMMA) in *Anadara granosa*. Results revealed that there was presence of microplastics pollution in the marine animal. Further research can be done on the toxicity effects of these microplastics towards human upon consumption of *Anadara granosa* as seafood.

AN OVERVIEW OF THE NEED AND METHODOLOGIES FOR AQUACULTURE TRACEABILITY

Debashish Mazumder*, Fatimah Md.Yusoff, Aziz Arshad, Karthik Gopi and Jesmond Sammut

*Australian Nuclear Science and Technology Organisation (ANSTO)
Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
dma@ansto.gov.au

The global population is predicted to increase to 10 billion, which will increase the amount of food required to sustain the quality of human life. Seafood is one of the most important tradable commodities globally, and food safety and quality have become increasingly important world-wide, not only in terms of protecting the health of the consumer and ensuring food security, but also to meet certification and quality requirements for international trade. The consumption of seafood has been steadily increasing over the past decade. Consequently, capture fisheries are currently in a state of decline, with most of them being fished to capacity. The increase in population will only add to the current pressure on capture fisheries, therefore, setting the stage for aquaculture to fill the gap and relieve capture fisheries. According to the Food and Agriculture Organisation (FAO) by 2021 more than half of the fish consumed globally will be a product of aquaculture. However, the growth of aquaculture has been sullied by concerns over its environmental and social impacts. There have been several reported cases where certain aquaculture products contained toxins, hormones, antibiotics or other contaminants, adding to the pubic scrutiny of aquaculture. This raises questions about the effects of aquaculture produce on public health and safety. Therefore, in order to ensure that consumers are confident in purchasing aquaculture produce, there needs to be reliable methods of tracing aquaculture produce back to its origin. This will allow for aquaculture to expand safely in order to meet the growing demand from consumers around the globe, while allowing producers to meet certification and regulatory requirements.

This presentation will examine the significance of aquaculture traceability as well as the different methodologies available to regulatory bodies in order to determine the provenance of seafood. Through developing the current methodologies, regulatory bodies and other authorities can apply these techniques in order to protect the industry as well as consumers from fraudulent practices.

NOVEL PROVENANCE TECHNOLOGY TO COMBAT FRAUD IN THE SUPPLY CHAIN

Debashish Mazumder*; Jesmond Sammut, Patricia Gadd, Karthik Gopi; Jasmin Martino; Jagoda Crawford; Carol Tadros; and Neil Saintilan

*Australia's Nuclear Science and Technology Organisation (ANSTO)
Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
dma@ansto.gov.au

Aquaculture is one of the fastest-growing food production sectors in the world contributing to 47% of seafood production. Asian countries play a significant role in aquaculture production which is fundamental to regional economic growth. Asian countries are exporters and importers of aquaculture products. The complex market supply chains can and do enable fraudulent activities which affect profitability and sustainable development. Seafood provenance refers to whether seafood is produced sustainably and properly labelled, specifically referring to its geographic origin and production methods. Aquaculture is emerging as one of the major food production sectors. Accordingly, businesses and consumers have shown a growing interest in knowing where their seafood comes from. Seafood provenance, therefore, is immensely important not only to ensure public health and environmental biosecurity, but also for the sustainability of the aquaculture industry as it is linked to the livelihood of millions of people.

Australia's Nuclear Science and Technology Organisation (ANSTO), in collaboration with universities and industry collaborators in Australia, developed a suite of provenance technologies that analyse the intrinsic isotopic and elemental fingerprints of seafood. The technology can accurately determine the origin of aquaculture products with over 85% accuracy. This technology has two versions. Firstly, a lab-based fingerprinting technology provides a detailed analysis of the isotopic and elemental fingerprints of seafood. Secondly, a portable scanning method relies on handheld X-ray fluorescence (XRF) scanning to generate a profile of the elemental fingerprint in a seafood sample in real time, and a powerful digital tool that will help the seafood industry combat food fraud and identify mislabelled seafood products easily, allowing wholesalers, retailers and consumers to make informed decisions for their purchase.

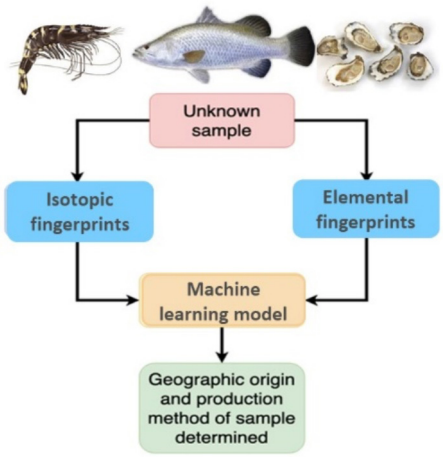


FIGURE: Seafood Provenance Technology

OCCURRENCE, ANTIMICROBIAL RESISTANCE AND VIRULENCE FACTORS OF *Vibrio* SPECIES ISOLATED FROM CULTURED SHRIMP IN PENINSULAR MALAYSIA

Md Yasin Ina-Salwany[a,b*], Wan Omar Haifa-Haryani[a], Mohd Zamri-Saad[a], Mohammad Noor Amal Azmai[a,b] and I. Natrah[a,b]

[a]Aquatic Animal Health and Therapeutics Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
[b]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia

Antibiotics are widely used in shrimp farming to prevent or treat disease outbreaks. Unfortunately, overuse of antibiotics leads to antimicrobial resistance (AMR) in the aquaticenvironment. Moreover, it is important to understand the current state of AMR strains, particularly in Malaysia. Thus, the study aims to provide a comprehensive database on theoccurrence of *Vibrio* spp. as well as the distribution of virulence genes, antibiotic resistance, and the plasmid profiles of *Vibrio* spp. isolated from cultured shrimp in Peninsular Malaysia.

Briefly, a total of 210 cultured shrimp werecollected from the seven farms in Peninsular Malaysia. The findings revealedthat 225 presumptive *Vibrio* isolates weresuccessfully isolated from the cultured shrimp which were then subjected tobiochemical tests and molecular detection using the *pyrH* gene analysis. Eventually, 13 different *Vibrio* spp. were successfully isolated and characterized (Figure 1). The isolates were then analyzedwith virulence genes; eight of the eleven virulence genes were detected, including *pirA*, *pirB*, *tlh*, *flaC*, *toxR*, *chiA*, *luxR*, and *hlyA*. Other virulence genes, suchas *tdh*, *trh*, and *ctxA*, were not detected in any isolates. The findings revealed that, *chiA* (89%) was the most commonvirulence gene found, while the leastcommon was *hlyA* (1%). Three *Vibrio* strains were chosen for the pathogenicity test based on virulence gene analysis. The results revealed that *V. parahaemolyticus* strain S12-3 was found to be more virulent compared to *V. campbellii*strain S10-4 and *V. rotiferanius* strain S24- 4.

Meanwhile, antibiotic susceptibility profiles revealed that all isolates were resistant to penicillin G (100%), but susceptible to norfloxacin (96%).84% of *Vibrio* spp.exhibited a multiple antibioticresistance (MAR) index of more than 0.2 while 16% of the isolates with MAR of lessthan 0.2. A total of 125 isolates harbored plasmids with molecular weights between 1.0 and above 10 kb, were detected among the resistant isolates. In conclusion, the current findingswill be useful for policy-making and planning in aquaculture development as well as in the management of shrimp farming.

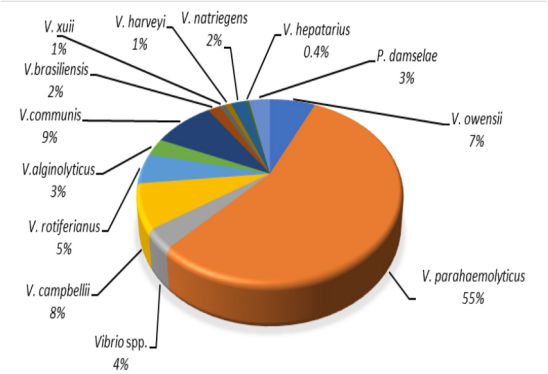


Figure 1: Occurrence of *Vibrio* spp. from cultured shrimp in Peninsular Malaysia

EXTRACTION OF ORGANIC ACIDS FROM BIOFLOC AND ITS APPLICATION IN *Penaeus vannamei*

M.Menaga*, C. Mohana Sundari, S.Felix, M.Charulatha & S. Srividya

Assistant Professor
Incharge,Advanced Research Farm Facility
Dr.MGR.Fisheries College & Research Institute
TNJFU-Madhavaram Campus
Madhavaram Milk Colony
Chennai-51

The present study aimed to evaluate the effect of organic acids on the growth and digestive performance of *Penaeus vannamei*. The organic acids were extracted from the biofloc culture water was confirmed using GC-MS and were supplemented in the commercial feed at the rate of 2%. The treatment group includes a) control (C) b) biofloc (T1) and c) diet supplemented with organic acids (T2). The animals (Initial weight- 1.08g) were stocked at a density of 500/m^3 in 100L FRP tanks in triplicates for 60 days. Water quality parameters were maintained under optimum conditions and the enzyme parameters such as Lipase, Amylase and Protease were analysed fortnightly. The final weight of the experimental groups was as follows: C- 3.27, T1- 4.14 and T2- 4.65g. The highest weight gain was found in the animals fed with organic acids. Apparent digestibility of the shrimp was found to be higher in T2(C-80.96%, T1-73.92%, and T2-83.43%). Lipase, Amylase and protease activity was higher in T2 and T3 than control at the end of the culture trial. The current study proved the positive effect of supplementing organic acids in the growth and digestive performance of *P. vannamei*.

PIGMENTATION OF THE BACTERIA ISOLATED FROM BIOFLOC PONDS AND ITS COLOUR ENHANCEMENT EFFECT IN *Xiphophorus hellerii*

M.Menaga*, S.Felix, M.Charulatha & C.Mohana Sundari

Assistant Professor
Incharge,Advanced Research Farm Facility
Dr.MGR.Fisheries College & Research Institute
TNJFU-Madhavaram Campus
Madhavaram Milk Colony
Chennai-51

A 30 days trial was conducted to evaluate the colour enhancement in sword tail through the supplementation of pigment produced from the bacteria in their diets. Sword tail fishes (1.2 ± 0.01g) were stocked in 70 litre crates at the rate of 20nos / crates in triplicates. Fishes fed with normal diet served as control (C) and diet supplemented with pigment (50mg/kg) isolated from the bacteria *Exiguobacterium profundum* (T1), *Chryseobacterium joostei* (T2), *Staphylococcus pasteuri* (T3), *Staphylococcus arlettae* (T4) served as treatments. The characteristic features of the pigments isolated from the four different bacteria were checked for antibacterial, total antioxidant activity and the reductive ability. *E. profundum* showed highest reductive (0.131) and antioxidant ability (406.5µg) whereas *S. arletta* and *C. joostei* showed higher antimicrobial activity (zone of inhibition – 30mm) against *Aeromonas hydrophila*. Significant difference was observed in water quality parameters except temperature between the experimental groups. Growth parameters showed significant difference between control and treatment. Highest carotenoid concentration (22.8µg/g) was found in fishes fed with pigments extracted from *Staphylococcus pasteuri* compared to other experimental groups. The present study proved the incorporation of bacterial pigments in the diets of sword tail enhanced the total carotenoid concentration.

RELATIVE PERFORMANCE OF BIOFLOC BASED PROBIOTIC ISOLATES WITH A COMMERCIAL PROBIOTICS IN *Penaeus vannamei*

Menaga Meenakshisundaram

Department of Aquaculture
Tamil Nadu Dr.J.Jayalalithaa Fisheries University
Nagapattinam

Comparison of the growth performance of *Penaeus vannamei* was ascertained by supplementing the probiotics from commercial and biofloc system incorporated through feed. Post larvae of shrimp (0.045 ± 0.005g) were stocked at a density of 500/m^3 in FRP tanks (500L) in triplicates for a period of 60days. A total of 40 bacterial strains were isolated from previous biofloc culture trials were tested for its antimicrobial activity against the pathogen *Vibrio parahaemolyticus*. Out of which, *Klebsiella pneumoniae, Bacillus megaterium, Exiguobacterium profundum, Pseudomonas balearica* and *Pseudomonas stutzeri* showed higher antimicrobial activity. The treatment group includes Biofloc (C), commercial probiotic (T1), isolated probiotic (T2), Biofloc + commercial probiotic (T3) and Biofloc + isolated probiotic (T4) in triplicates. Distellary spentwash was used as a carbon source for biofloc development and maintenance. The probiotic concentration of $1x10^9$ cfu/g were supplemented throughout the trial. The recorded water quality parameters (pH, alkalinity, Calcium and Magnesium) were observed to be significant among the experimental groups ($P \leq 0.05$). Highest weight gain (2.43g), SGR, PER and lower FCR values were recorded in T4. Lowest values of total *Vibrio* was found in T3. Histology analysis revealed that there was a mild increase in the B and R cell vacuoles in hepatopancreas of T1 and T4 whereas mild degeneration was found in intestine of T1 and T2.Microbiome analysis of the shrimp gut revealed that *Proteobacteria* was found to be the most abundant phylum in all the experimental groups. *P. balearica*, *K.pneumoniae*,*P. stutzeri* and *E.profundum* were found to be present in the gut of C whereas *P. balearica*, *K. pneumonia* and *P.stutzeri* in the gut of T2 and T4.The results proved that the probiotics isolated from biofloc colonized in shrimp gut would pave a promising role in aquaculture.

MICRODYNAMICS OF *EXSITU* BIOFLOC USING DISTILLERY SPENTWASH AS CARBON SOURCE

Menaga Meenakshisundaram

Department of Aquaculture
Tamil Nadu Dr.J.Jayalalithaa Fisheries University
Nagapattinam
mena.fishcos@gmail.com

The bacterial community characterization of *exsitu* biofloc was carried out to ascertain the dynamics and community structure of the microbiome. The biofloc was developed in 50tonne raceways for a period of 30 days. Distillery spent wash as carbon source was used for maintaining the C: N ratio at 10:1. The harvested biofloc powder was analysed for the mineral profile using MS analysis. Metagenomic profile of the floc was done through shotgun sequencing using Illumina NextSeq500 platform. High quality data of 3.31 GB was obtained consisting 489,136 genes. The kingdom level abundance was found in the order of 62.47% of bacteria, 36.77% of unclassified, 0.4% eukarya, 0.32% of archaea, and 0.04% of viruses. At the generic level composition, *Proteobacteria* and *Leptolyngbya sp*. were the most abundant genus identified. Gene ontology using KEGG and SEED classification revealed predominantly carbohydrate metabolism associated genes. The present study is first of its kind to study the microbial composition in *exsitu* biofloc and to identify the functional attributes of the microbiome in the biofloc system.

ESSENTIAL FATTY ACID REQUIREMENTS IN TROPICAL AND COLD-WATER MARINE FISH LARVAE AND JUVENILES

Sahar Mejri[1], Réjean Tremblay[2], Céline Audet[2], Paul. S. Wills[1], Martin Riche[1]

1 Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 US1 North, Fort Pierce, 34946, FL, USA
2 Institut des Science de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines
Rimouski, G5l 2Z9, Qc, Canada

To improve survival at early developmental stages (larvae and juveniles) of captive fish species, essential nutrients (i.e., essential fatty acids [EFA]) need to be identified. The physiological needs are likely to be different among species, particularly among those using different thermal habitats, because lipids are largely used to maintain cell membrane integrity (homeoviscous adaptation) in fishes. This review paper will focus on currently published research and the main results from our laboratories regarding optimum qualitative EFA requirements during larval and early juvenile stages in a warm-water marine species, the Florida pompano (*Trachinotus carolinus*), and a cold-water marine species, the winter flounder (*Pseudopleuronectes americanus*). To identify the qualitative optimal EFA requirements, we calculated the ratio of certain fatty acids (FA) in larval or early juvenile tissues to total FA present in the diet. This ratio indicates whether a specific FA from prey is selectively incorporated by larvae and juveniles. Overall, we found that young larvae from both cold- and warm-water species have greater demands for n-3 and n-6 highly unsaturated fatty acids (HUFA) than do larvae at weaning stages. However, the qualitative EFA requirements of the cold-water species at all early developmental stages were higher than those of the warm-water species. Enriched rotifer diets provided satisfactory amounts of omega 3 and omega 6 in Florida pompano, with small selective retention for docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA), suggesting a potential minor diet deficiency in these EFA. There were higher deficiencies in the cold-water species fed enriched rotifers, as demonstrated by the higher selective retentions of all EFA (DHA, EPA, and ARA), with the exception of larvae fed with copepods. The physiological needs in EFA for juvenile development seemed to be better met for both species when they were fed micro pellets. From the beginning of settlement and in young juveniles, qualitative values of 12% DHA, 10% EPA, 5% ARA, and 40% PUFA of total FA seem to be required for winter flounder juvenile development. In Florida pompano, these requirements could be met until larger juvenile stages, with 15% DHA, 3% EPA, 2% ARA, 2% DPA, and total PUFA below 30% of total FA. This review was done to aid future research aiming to develop nutritionally balanced microdiets or live-prey enrichment diets to satisfy the physiological requirements of captive tropical and cold-water marine fish species.

A COMPREHENSIVE BIOCHEMICAL CHARACTERIZATION OF SETTLEMENT STAGE LEPTOCEPHALUS LARVAE OF BONEFISH (*Albula vulpes*)

Uribe, V.[1], Wills, P. S[1]., Shenker, J. M[2]., Adams, A.J.[1,3], Mejri, S.[1],

[1]Florida Atlantic University, Harbor Branch Oceanographic Institute
5600 US 1 North
Fort Pierce, FL 34946
Corresponding author: Victoria Uribe; vuribe2017@fau.edu
3 Bonefish & Tarpon Trust, 2937 SW 27th Avenue, Suite 203, Miami, FL 33133

Little is known about early development of the near-threatened bonefish (Albula vulpes), a member of superorder Elopomorpha. Members of Elopomorpha are partially defined by their synapomorphic leptocephalus larval stage, for which the nutritional requirements are not well understood. Characterizing the nutritional profile, including major nutrients (such as lipids) used for energetic processes, can help to gain a better understanding of the nutritional requirements for leptocephalus larvae.

A total of 24 settlement stage A. vulpes leptocephalus larvae were collected at Long Caye Island, Belize. Samples were used to determine various biochemical characteristics including lipid class, fatty acid (FA), and glycosaminoglycans (GAGs) compositions.

Each of these biochemical components play a role in early developmental processes such as cellular membrane formation and are crucial for healthy development. Biochemical characteristics of settlement stage A. vulpes leptocephalus are presented in this study for the first time. The dominant lipid classes and fatty acids detected in these samples were consistent with prior studies using closely related species like the Japanese eel, indicating possible similarities in diets at this stage.

These results can be utilized to decide which dietary components are essential for growth and survivorship of captive A. vulpes larvae. In the future, similar analyses can be applied to other species that share the leptocephalus life stage to determine if nutritional requirements at this stage of development are unique to this species. The findings in this study will also help to facilitate the establishment of adequate aquaculture systems for captive bonefish, ultimately leading to improved management strategies for wild bonefish habitats.

ADVANCES IN GENOMIC ANALYSIS OF PATHOGENIC *Yersinia ruckeri* AND *Aeromonas sobria* ISOLATED OF PERUVIAN RAINBOW TROUT

Fernando Mesías V.*, Luis Llanco, Carmen Hurtado, Tania Rodríguez, Diego Gerpe, Jesús Romalde, and Enrique Serrano-Martínez

Laboratorio de Parasitología Veterinaria
Universidad Peruana Cayetano Heredia
Av. Honorio Delgado 430- SMP- Lima- Perú
fernando.mesias@upch.pe

Yersinia ruckeri and *Aeromonas sobria* frequently affect rainbow trout production in Peru, mostly causing haemorrhages in different organs, by expressing virulence factors such as hemolysins, cytotoxins, proteases, among others. Additionally, constant outbreaks of both pathogens in peruvian fish farms could be, despite applying antibiotics for their control, associated with the development of antimicrobial resistance in these pathogens. Although virulence and antimicrobial resistance factors have been investigated in *Y. ruckeri* and *A. sobria* in some countries, these studies are scarce in Peru. Therefore, the aim of the present study was to identify genes related to virulence and antimicrobial resistance in *Y. ruckeri* and *A. sobria* isolated from rainbow trout (*Oncorhynchus mykiss*) from Lima, Peru.

Yersinia ruckeri strain FMV-22 and *Aeromonas sobria* CHT-30 were isolated from diseased rainbow trout raised in Canta- Lima, which presented frequent haemorrhagic septicaemia despite receiving antibiotic treatments. These strains were grown in trypticase soy agar at 25 °C for 24 h, and confirmed by PCR. Genomic DNA of both strains were extracted and then they were sequenced using an Illumina MiSeq sequencer. Both SPAdes and QUAST used default parameters to perform genome assembly and to evaluate the assembly, respectively. The resulting genomes consists of 108 contigs (3 843 148 bp) with G+C content of 47.45% for FMV-22 strain, and 343 contigs (4 912 126 bp) with G+C content of 57.67% for CHT-30 strain. The N50 contig sizes were 152,665 bp for FMV-22 strain, and 151,159 bp for CHT-30. ABRIcate was used to screen antimicrobial resistance and virulence genes (Table 1) in FMV-22 and CHT-30 strains, using CARD, VFDB, ARGANNOT and RESFINDER databases. Our results will contribute to expand our knowledge about the pathogenesis and antimicrobial resistance of both bacteria that significantly affect peruvian rainbow trout production.

Table 1. Virulence and antimicrobial genes of FMV-22 and CHT-30 strains

Genes	*Yersinia ruckeri* FMV-22	*Aeromonas sobria* CHT-30
Virulence	flhD, flhC, cheR, cheB, cheY, cheZ, flhA, flgB, flgC, flgH, fliN, fliM, fliG, fliT, fliS, fliA, fliZ, ompA	*pil*T, int
Antimicrobial resistance	CRP	*aad*A, *dfr*A1, *sul*1, *tet*E, *cat*AII, *bla*OXA-12, *amp*S, *tet*A, *bla*CEPH-A3, *qac*E

SEMI-BATCH CULTIVATION OF *Chlorella sorokiniana* AS FISH SUPPLEMENT

M. N. Metsoviti[1], A. Papadopoulou[1], A. Mpesios[1], N. Gougoulias[2], G. Papapolymerou[1*]

[1]Department of Environmental Studies, University of Thessaly, Gaiopolis Campus, Larisa, Greece
*papapoly@uth.gr
[2] Department of Agrotechnology, University of Thessaly, Gaiopolis Campus Larisa, Greece

Introduction

Microalgae are used in aquaculture in fish nutrition, replacing other ingredients of the feeds, such as fishmeal and fish oil. Different microalgal species seem to be suitable alternatives due to their high protein or lipid content. The aim of this study was to investigate the nutrient content of the biomass of *Chlorella sorokiniana,* grown heterotrophically, after the lipids were extracted for use in biodiesel production. The remaining biomass was for its nutrient content. Carbon in the form of glycerol was added in a semi-batch mode during the cultivation experiments.

Materials and Methods

C. sorokiniana was cultivated in four glass bioreactors (BR) of 40L capacity with the composition of the culture medium according to SAG (2009). In all four bioreactors the same amount of glycerol (800 mL) was added but, glycerol was added in different ways in the 4 different bioreactors. The semi-batch mode of addition was 2 times x 400 mL, 4 times x 200 mL for the first two bioreactors and 10 times x 80 mL for the 3rd and 4th but, in the 4th bioreactor 27.7 mg L^{-1} additional nitrogen was added in the middle of the experiment. So, 80.5 mg L^{-1} Nitrogen were added in the first BR and 108.2 mg L^{-1} in the 4th. The temperature of the cultivations was 30 ± 0.5 °C and the pH was 7 ± 0.3.

Results and discussion

As far as the nutrient content of the biomass of *C. sorokiniana* produced, it was found that it was dependent on the mode of addition of glycerol. After the lipid extraction of the biomass for biodiesel production, the % nutrient composition of the remaining biomass was determined by NIR spectroscopy (Table 1). Before the lipid extraction from the biomass of *C. sorokiniana*, the protein content varied between about 20 % and about 25%, while the lipid content varied from about 31 % to about 48% in the four biomass samples obtained from the experiments. Due to its high protein content the remaining biomass of *C. sorokiniana* can be incorporated as a fish feed supplement.

Acknowledgements

The study was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE - INNOVATE (project code: T1EDK-01580).

Table 1. Nutrient content (%) of *C. sorokiniana* in 4 different cultivation modes after lipid extraction

Mode of Glycerine Addition	Protein(%)	Lipid (%)	Ash (%)	Fiber(%)	Moisture(%)	NFE*(%)
2 x 400 mL	54.6±1.5^{a}	3.4±0.4^{a}	11.9±0.8^{a}	3.9±0.1^{d}	2.1±0.1^{a}	24.2±0.5^{c}
4 x 200 mL	49.7±1.3^{b}	3.1±0.5^{a}	10.8±0.9^{a}	4.7±0.3^{c}	2.4±0.0^{a}	29.3±0.4^{b}
10 x 80 mL	36.8±1.1^{d}	3.6±0.2^{a}	12.4±0.7^{a}	6.3±0.2^{a}	2.6±0.1^{a}	38.3±0.8^{a}
10 x 80 mL + + 27.7 mg/L N	40.5±1.0^{c}	1.9±0.1^{b}	11.7±0.4^{a}	5.4±0.8^{b}	2.4±0.2^{a}	38.1±0.9^{a}

* NFE = Nitrogen Free Extractables

COMPASSION IN WORLD FARMING: CONSIDERING FISH WELFARE IN THE FUTURE OF AQUACULTURE

Dr. Kate Mintram*, Dr. Myriam Vanderzwalmen, Dr. Tracey Jones

Compassion in World Farming
River Court, Mill Ln
Godalming GU7 1EZ
United Kingdom
Katie.Mintram@ciwf.org

Aquaculture is the fastest growing food-producing sector in the world; but as the industry continues to expand at a rapid rate, fish welfare is often overlooked. There is a substantial, and growing, body of evidence which shows that fishes are sentient, and we have a moral obligation to consider their welfare; particularly when the numbers of individuals involved is astonishingly large.

Compassion in World Farming (CIWF) is recognised as the leading international farm animal welfare charity. In 2018, the global campaign #RethinkFish was launched in order to raise public awareness around fish sentience and to provide technical resources to companies to guide them in adopting humane rearing and slaughter practises. CIWFs Food Business team engage positively with companies throughout the supply chain to promote tangible improvements to farm animal welfare. We support companies by providing technical and strategic advice (e.g. on the production of animal welfare policies), hosting and facilitating collaborative projects and events, and recognising good practise through our Good Farm Animal Welfare Awards and by publishing case studies. NGOs can also become a valuable partner for researchers by helping them access collaborations and funding opportunities. Moreover, through our close contact with stakeholders, we can help researchers identify key research gaps that industry, consumers, governments and other related groups require in order to implement better welfare policies.

This poster will give an overview of CIWFs recommendations for improving fish welfare in aquaculture for five key fish species and give an overview of the support that we provide to companies. We will also highlight research gaps which are currently hindering the uptake of higher welfare systems. A case study industry example of a high welfare fish farm which has been awarded by CIWFs Good Farm Animal Welfare Awards will also be presented.

ANALYSING MICROPLASTICS IN WATERS AND SEDIMENTS AROUND FARMS IN URBANIZED AREAS

Huajuan Mo, Su Ting Ng, Nur Shoofiyah, Cheong Yan Ong, Nur Sara, Shumin Yu, Boon Seng Kong, Niranjala Perera, Raymond Ong, Wei Chern Foong, Umid Joshi, Guillaume Drillet

Huajuan Mo (PhD)
SGS Testing & Control Services Singapore Pte Ltd
30, Boon Lay Way, #03-01
Singapore 609957
HuaJuan.Mo@sgs.com

The capabilities to monitor for the presence of microplastics in aquaculture in view of evaluating sources and protecting the industry from potential negative impacts reside in the implementation of methods which can be implemented at large scale. Comparability of results across studies is a necessity but is proven is difficult because different methods are used globally, and the focus of published studies often differ.

We used a combination of FTIR-ATR and microFTIR to evaluate the presence of microplastic potentially impacting aquaculture productions. To ensure comparability of our results with other published studies we made use of the freeware siMPle which is implemented in large number of organizations worldwide and allows to speed up the time spent for image analysis. We validated the development of our methods and include an evaluation of the recovery rates of microplastics through the process of density separation using polytungstate and oxidation using H2O2 in view of preparing samples for microFTIR analysis.

A REVIEW: IMPACT OF ELEVATED TEMPERATURE IN MARINE FISH LARVAE

Mohamad Nor Syafizan Mohamad*, Shigeharu Senoo and Ching Fui Fui

Borneo Marine Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400
Kota Kinabalu, Sabah
Malaysia
syfznmhmd@gmail.com

Aquaculture plays a key role in provision of food security and livelihoods of millions of people for their social, economic and nutritional benefits. Yet, climate change has been identified a major threat to its sustainability since fish production is highly influenced by environmental factors particularly temperature. With the predictable rise of seawater temperature, marine fish are struggled to adapt in warmer temperature and acidified seawater. Unlike other marine organisms, little attention has been given to understand the impact of elevated temperature on the development of marine fish larvae. Marine fish larvae in particular are very vulnerable during the first stages of development and have strict requirements for biotic and abiotic conditions to survive, develop and grow appropriately. This paper reviews scientific evidences on tolerance of marine fish larvae on elevated temperature towards growth and behaviour and other aspects. It makes serious attempt to summarize the relevant records published on specific research questions to improve understanding of the diverse evidences, and to be able to identify gaps in knowledge. This has helped in articulating the present state of research and defining topics for future studies on the elevated temperature effects on marine fish larvae. From the analysis of published data it is obvious that the impacts of elevated temperature on marine fish larvae give negative impacts on aquaculture industry, thus the review helps in providing knowledge and awareness which could help in mitigate the impact of elevated temperature on marine fish larvae.

EFFICACY OF A FEED-BASED VACCINE AGAINST *Vibrio harveyi* IN ASIAN SEABASS, *Lates calcarifer*

Aslah Mohamad*, Ina-Salwany Md. Yasin, Mohammad Noor Azmai Amal, and Mohd Zamri-Saad

Laboratory of Marine Biotechnology, Institute of Bioscience
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
aslahumt@gmail.com

Vibrio harveyi is an important aquaculture pathogen that causes vibriosis and infects a large number of fish species. Vibriosis is regarded as an epizootic, described as a global outbreak of communicable disease temporarily occurring in limited geographical areas. Vaccination is one of the alternatives to overcome the disease outbreaks and was reported to be more effective and safer to human and environment than antibiotics. Thus, in this study, a newly developed feed-based killed *V. harveyi* vaccine was used to study the immune response and efficacy of this kind of vaccine in Asian seabass.

A total of 270 Asian seabass, *Lates calcarifer* of 15.8 ± 2.6 g were separated into 2 groups, in triplicate. Fish of Group 1 was not vaccinated and Group 2 was vaccinated with feed-based killed *Vibrio harveyi* vaccine. Vaccinations were done on days 0 and 14 orally using the feed-based bacterin vaccine at 4% bodyweight. At week 4, 30 fishes from each group were challenged with live *V. harveyi* and the survival rate was determined. Samples of serum for antibody study were collected at 7-day intervals throughout the 6-week study period. Following vaccination by the feed-based vaccine, IgM antibody levels showed significant ($p<0.05$) increase in serum as early as week 2 and peaked at week 6 (Figure 1). This resulted in a significantly higher protection ($p<0.05$) following challenge with 2.3 x 10^7 CFU mL^{-1} of live *V. harveyi* than unvaccinated groups (Table 1). This feed-based vaccine offers an opportunity for a comprehensive immunization regime.

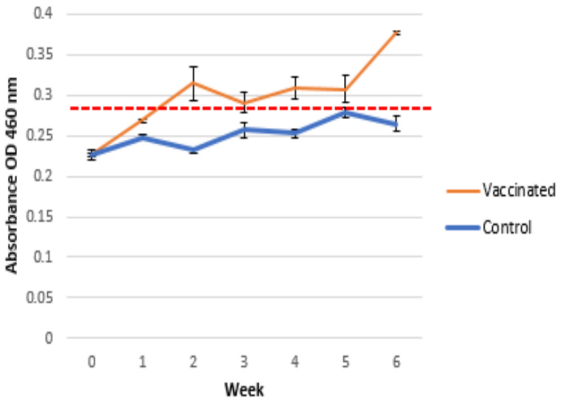


FIGURE 1. Serum antibody (IgM) responses against *V. harveyi* following feed-based monovalent vaccination.

TABLE 1. RPS evaluated base on the mortality data from fish groups.

Group	Number of fish	Mortality	Survival rate (%)	RPS
Vaccinated	30	8	73	73[b]
Control	30	30	0	0[a]

EFFECTS OF ELEVATED TEMPERATURE ON NUTRITIONAL CONDITION OF ASIAN SEABASS (*Lates calcarifer*) LARVAE

Mohamad Nor Syafizan, Norfazreena Mohd Faudzi, Ching Fui Fui, Shigeharu Senoo

Borneo Marine Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400, Kota Kinabalu
Sabah, Malaysia
Syfznmhmd@gmail.com

Aquaculture has been responsible for increasing global fish supplies and providing an essential protein source for human consumption. Climate change identified as a major threat to the aquaculture sector's sustainability. Because of the elevated temperature, fish larvae may expend excessive metabolic rate. It will lead to malfunctioned of sensory organs and maladaptive behavioural changes. Impact of elevated temperature on Asian seabass juvenile has not been documented and its consequences is unknown in aquaculture industry.

The effect of elevated temperature on the nutritional condition of Asian seabass, *Lates calcarifer* larvae were examined under controlled conditions. From 1dAH to 15dAH, the survival, average number of rotifers in the larval gut, and morphometric changes of larvae reared at five different temperatures (28,29,30,32, and 34°C) were compared. 25 of 7L sampling tanks were designed to resemble five different temperature treatments, and 180 tails were stocked per tank. Rotifer, Brachionus plicatilis sp., and cultivated Nannochloropsis oculata were fed at densities of 30 individual L and 0.5 x 10 cells ml, respectively, for the initial feeding. Statistical analysis was performed using SPSS 27.0 software (SPSS incorportion, Chicago, IL, USA) and a significance level of $P<0.05$ was applied. One-way-ANNOVA was performed to compare average rotifer number in gut and each body proportion examined. When a significant difference was found, a post hoc test using Duncen was performed to ascertain any significant differences between treatment means.

The survival percentage of larvae reared in 28, 29 and 32°C (78.70±4.79,76.85±3.94 and 73.70±4.63%) was significantly higher than ($P<0.05$) compared to 32 and 34°C (58.33±5.00 and 0%). Different number of rotifer intake was observed as early at 3dAH which show higher temperature 32°C(8.0±0.8) and 34°C(10.0±2.2) treatments was significantly higher compared to lower temperature treatments 28°C(3.0±0.8), 29°C (4.0±1.4) and 30°C (4.0±1.6). However no significant difference was detected ($P<0.05$) between all treatment on 11 and 13 dAH.
All morphometric measurements, body length (BL), body height (BH), pectoral height (PH), gut height (GH), eye height (EH) and head height (HH) on each body proportion of larvae reared in 28°C, 29°C, 30°C had increased gradually as they developed, while larvae reared in 32°C and 34°C experienced rapid growth in early larval stage and has slower development upon 10 dAH.

This study concludes water temperature can affects the larval nutritional condition that is important to maximize the larval survival and early stage of larvae growth.

PHYLOGENETIC ANALYSIS OF GONADOTROPIN-RELEASING HORMONE (GnRH) GENES OF TROPICAL CATFISHES, *Pangasius nasutus* AND *Hemibagrus nemurus*

Zarirah Zulperi*, Amirah Syafiqah Zamri, Fatin Nabilah Mohamad Sahadan, Yuzine Esa

Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia
zarirah@upm.edu.my

Pangasius nasutus (patin buah) and *Hemibagrus nemurus* (river catfish) are among the most popular tropical catfish species due to its flesh quality and nutritional values. Department of Fisheries Malaysia reported the production of *H. nemurus* and *P. nasutus* in 2021 were approximately 1,337.68 and 16.49 tonnes, which only covered 1.26% and 0.29% of the whole freshwater fish production in Malaysia. The production of *H. nemurus* could not fulfill the market demand since hatcheries encountered problems in induced spawning, especially the failure of female brood stock to undergo final oocyte maturation, preventing its ability to spawn. Meanwhile, the availability of *P. nasutus* is still depending on wild-captured, which led to its low population in their natural habitat. Study of gonadotropin-releasing hormone (GnRH), an essential hormone for reproduction, is important to understand its molecular properties in regulating gametogenesis in catfishes. This study involved cloning and characterization of a complete open reading frame (ORF) of GnRH genes, GnRH-1 and GnRH-2. Full-length cDNA encoding GnRH-1 and GnRH-2 genes were detected at 312 bp and 376 bp, respectively. Multiple sequence alignment showed GnRH-1 and GnRH-2 of *P. nasutus* and *H. nemurus* shared highest similarities (>95% values) to other catfish species; channel catfish (*Ictalurus punctatus*) and African catfish (*Clarias garienpinus*). Phylogenetic tree showed both species grouped in a similar cluster to Siluriformes followed by Cypriniformes. This is the first study of GnRH genes in *P. nasutus* and *H. nemurus*, thus the study should contribute as an initial step for a development of a better reproductive strategy in catfish species to increase their production in the industry.

PHYLOGENETIC ANALYSIS OF GONADOTROPIN-RELEASING HORMONE (GnRH) GENES OF TROPICAL CATFISHES, *Pangasius nasutus* AND *Hemibagrus nemurus*

Zarirah Zulperi[a,b,*], Fatin Nabilah Mohamad Sahadan[a], Amirah Syafiqah Zamri[b], Yuzine Esa[b]

[a]Aquatic Animal Health and Therapeutics Laboratory
Institute of Bioscience
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia

[b]Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia
zarirah@upm.edu.my

Pangasius nasutus (patin buah) and *Hemibagrus nemurus* (river catfish) are among the most popular tropical catfish species due to its flesh quality and nutritional values. Department of Fisheries Malaysia reported the production of *H. nemurus* and *P. nasutus* in 2021 were approximately 1,337.68 and 16.49 tonnes, which only covered 1.26% and 0.29% of the whole freshwater fish production in Malaysia. The production of *H. nemurus* could not fulfill the market demand since hatcheries encountered problems in induced spawning, especially the failure of female brood stock to undergo final oocyte maturation, preventing its ability to spawn. Meanwhile, the availability of *P. nasutus* is still depending on wild-captured, which led to its low population in their natural habitat. Study of gonadotropin-releasing hormone (GnRH), an essential hormone for reproduction, is important to understand its molecular properties in regulating gametogenesis in catfishes. This study involved cloning and characterization of a complete open reading frame (ORF) of GnRH genes, GnRH-1 and GnRH-2. Full-length cDNA encoding GnRH-1 and GnRH-2 genes were detected at 312 bp and 376 bp, respectively. Multiple sequence alignment showed GnRH-1 and GnRH-2 of *P. nasutus* and *H. nemurus* shared highest similarities (>95% values) to other catfish species; channel catfish (*Ictalurus punctatus*) and African catfish (*Clarias garienpinus*). Phylogenetic tree showed both species grouped in a similar cluster to Siluriformes followed by Cypriniformes. This is the first study of GnRH genes in *P. nasutus* and *H. nemurus*, thus the study should contribute as an initial step for a development of a better reproductive strategy in catfish species to increase their production in the industry.

HARVESTING MICROALGAE USING NON-TOXIC FLOCCULATING AGENT

Sitti Raehanah Muhamad Shaleh and Wan Arina Azrinor Yamin

Borneo Marine Research Institute, Universiti Malaysia Sabah
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
sittirae@ums.edu.my

Flocculation is a method of harvesting microalgae by separating cells from the culture medium using flocculating agents. The potential of soybean protein extract (SBPE) as a microalgal harvesting flocculant was investigated on *Chaetoceros gracilis* using factors such as pH, SBPE concentration, and culture density with a settling time of 20 minutes. The effect of the maximum condition (pH and SBPE) on *Nannochloropsis* sp. was also investigated. The results showed maximum flocculation of 41% at pH10 and SBPE dose of 60 ppm. While the cell density of OD 0.6 had the highest flocculation efficiency (19.93%) among all the cell densities tested. A maximum of 60% flocculation efficiency was recorded in *Nannochloropsis* sp. when the same condition (pH and SBPE dose) was applied. Flocculating microalgae using protein extract is not only eco-friendly but also fast and efficient in producing microalgae biomass and was strongly recommended for aquaculture uses.

UV-Vis INTENSITY MANIPULATION FOR GROWTH OF AQUATIC PLANTS

Rosmin Elsa Mohan*#, Jennifer Tanadi#, Khoo Eng Huat#

Institute of High Performance Computing, Agency for Science Technology and Research (A*STAR), 1 Fusionopolis Way, #16-16 Connexis, Singapore 138632
khooeh@ihpc.a-star.edu.sg
# Authors have equal contribution

The field of aquatic cultivation for species such as seaweed and microalgae is booming especially in line with the rising demand. Recent reports show new methods to increase the overall production using lighting technologies [1]. The advantages for the use of LEDs in microalgae cultures is increased biomass growth in addition to enhancement of protein and carbohydrate content. Variation in temperature, light and nutrients can affect the growth patterns [2]. Such changes in spectral irradiance can be beneficial in designing LEDs in the Visible (VIS), UV and infrared (IR) wavelengths. In this work, some preliminary studies for light intensity control in the UV and VIS regions are presented. The increase in solar irradiance across visible range from 420-680 nm has been reported to be beneficial to specific microalgae species such as *C.vulgaris* [3]. In another recent article, the response of different algae species to visible light intensity has been elucidated [4]. A latest review (published on August 22 2022) highlights that ultraviolet-visible (UV-Vis) intensities promotes protection and repair mechanisms to be induced in microalgae [5].The 365 nm wavelength in the UV-A regime has been reported to induce amino acid components in certain microalgae such as *Nitzschia closterium,* which can further be translated to other useful products using microalgae.

Modification of the transmission spectrum has potential to increase biomass and nutrition content. It has been reported that with a modified spectrum the quality of the aquatic plants grown using the same amount of light leads to more efficient and optimal use of energy [6]. We present some preliminary results for design of UV-Vis gratings that could act as a modeling reference for the design of tunable gratings for optimizing light irradiance. This could be beneficial for investigating energy efficient case studies for aquatic species pertaining to varying intensities across specific wavelengths.

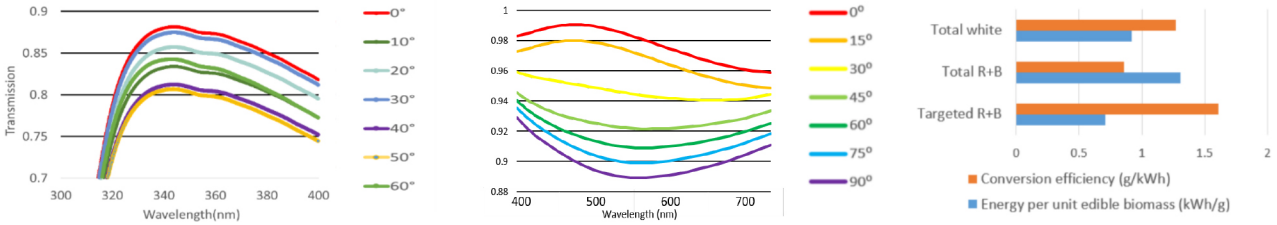


Figure 1: Tuning transmittance across UV-vis wavelengths using gratings with change in incident angle (a) using blaze gratings for UV-A transmittance (b) using zig-zag gratings for VIS range. About 10% change in light intensity has been achieved using the grating structures. (c) Relationship between total energy per unit edible biomass and conversion efficiency subjected to three different lighting treatments (adapted from [7])

(Continued on next page)

References

1. Global Energy & Environment Research Team at Frost & Sullivan , Report by The Growth Pipeline ™ Company, June 2022
Study backs benefits of LED lighting in microalgae cultivation, Article, The Fish Site, May 2022
2. Metsoviti, M. N., Papapolymerou, G., Karapanagiotidis, I. T., & Katsoulas, N. (2019). Effect of light intensity and quality on growth rate and composition of Chlorella vulgaris. Plants, 9(1), 31.
3. Bialevich, V., Zachleder, V., & Bišová, K. (2022). The Effect of Variable Light Source and Light Intensity on the Growth of Three Algal Species. Cells, 11(8), 1293.
4. Araújo, R.G.; AlcantarRivera, B.; Meléndez-Sánchez, E.R.; Martínez-Prado, M.A.; SosaHernández, J.E.; Iqbal, H.M.N.; Parra-Saldivar, R.; Martínez-Ruiz, M. Effects of UV and UV-vis Irradiation on the Production of Microalgae and Macroalgae: New Alternatives to Produce Photobioprotectors and Biomedical Compounds. Molecules 2022, 27, 5334. https://doi.org/ 10.3390/molecules27165334
5. Gómez, Celina & Izzo, Luigi Gennaro. (2018). Increasing efficiency of crop production with LEDs. AIMS Agriculture and Food. 3. 135-153. 10.3934/agrfood.2018.2.135.
6. Poulet, L., Massa, G.D., Morrow, R.C., Bourget, C.M., Wheeler, R.M., Mitchell, C.A. (2014). Significant reduction in energy for plant-growth lighting in space using targeted LED lighting and spectral manipulation. Life Sci. Space Res. 2, 43–53. 10.1016/j.lssr.2014.06.002.

GENDER EQUALITY A GROWTH ENGINE FOR THE AQUACULTURE ECONOMY

Marie Christine Monfort

President of WSI www.wsi-asso.org
International Organisation for Women in the Seafood Industry, Paris, France
womeninseafood@wsi-asso.orgI

Round table
The increasing demand for gender equality has generated significant momentum and the aquaculture industry is no exception. It has been evidenced in the dedicated literature and experienced in many businesses that gender equality and women empowerment (SDG5) brings social and economic positive transformations to the corporates and institutions which adopt them. There are a full component of sustainability. Yet the aquaculture industry has not yet explored these avenue for growth.

It would be a première to bring this complex and often misunderstood issue to a first class aquaculture meeting. Its objectives include:
• Present what gender equality means for a business in general and specifically to aquaculture stakeholders,
• Ask aquaculture senior decision makers to share their experience,
• Allow the audience to understand what is at stake including in the area of recruitment.

Speakers
• CFO of one Large scale aquaculture corporate, Asia
• President of aquaculture organisation, Asia
• President of WSI
• Gender expert in aquaculture & Fisheries, India

Moderator
• Ernst & Young Singapore, Partner, gender specialist

Duration of the event: 50 minutes

ASSESSMENT OF FEED BASED BIVALENT VACCINE AGAINST STREPTOCOCCOSIS AND MOTILE AEROMONAD SEPTICEMIA IN HYBRID RED TILAPIA *Oreochromis* spp.

Md. Shirajum Monir*, Ina-Salwany Md. Yasin, Md Sabri bin Mohd Yusoff, Zarirah binti Mohamed Zulperi and Hasliza binti Abu Hassim

Department of Aquaculture
Faculty of Agriculture & Laboratory of Marine Biotechnology
Institute of Bioscience
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia
monir_bau22@yahoo.com

Streptococcosis and Motile Aeromonad Septicemia (MAS) are important diseases of tilapia (*Oreochromis* spp.), and causes huge economic losses throughout the world. Streptococcal and aeromonad diseases have proven to be very difficult to control with the use of antibiotics. Hence, the aims of the study were to evaluate the systemic and mucosal immunity of the newly developed feed-based bivalent of killed (*Streptococcus iniae* and *Aeromonas hydrophila*) vaccine, and efficacy by intraperitoneal (i.p.) challenges in red tilapia.

A total of 1,050 red tilapia, each 61.23 ± 4.95g were divided into 5 groups with triplicates. Five groups of tilapia were vaccinated orally with bivalent (combined with *S. iniae* and *A. hydrophila*) vaccine sprayed on feed (BS group), divalent vaccine incorporated in feed (BF group), monovalent *S. iniae* and *A. hydrophila* vaccines incorporated separately in feed as MS group and MA group, respectively, and unvaccinated as a control group. During the experiment, serum, mucus and gut lavage fluid were collected to evaluate the antibody levels via indirect ELISA. The results showed that hybrid red tilapia immunized with bivalent formulate (BF group) achieved a strong and significantly ($P<0.05$) higher IgM responses in serum, gut and mucus samples than the control group. In this study, a relative percent survival (RPS) of 80.00% (i.p. challenged with *S. iniae*), 76.67±4.71% (challenged with *A. hydrophila*) and 76.67 ± 4.71% (co-infection) were also observed in bivalent formulate group, which were significantly higher ($P < 0.05$) than the other groups. Results show that feed-based bivalent vaccination is an efficacious treatment for the prevention of Streptococcosis and MAS outbreaks throughout the tilapia culture period

RESIDUAL POTENTIAL AND GENE EXPRESSION CHANGES IN TISSUE OF ZEBRAFISH *Danio rerio* EXPOSED TO POLYESTER

Hye-na MOON*, Jin NAMGUNG, Ki-hyuk KIM, Ju-hyeon JANG, and In-kyu YEO

Jeju National University
Jeju-si, Jeju Special Self-Governing Province, 63243, Republic of Korea
hyena0712@jejunu.ac.kr

Microfibers are microplastic with a diameter of 5 mm or less, including polyester, nylon, rayon and acrylic. It has been reported that microfibers are the main cause of global marine pollution. Microfibers were found to be discharged into the soil, beaches and marine sediments without being eliminated in the domestic sewage treatment process. In this study, when zebrafish were exposed to microfibers using polyester, the residual potential of tissue in zebrafish was evaluated and the expression changes of antioxidant and stress-related genes were analyzed.

The zebrafish *Danio rerio* were exposed to polyester by feed and breeding water. The feed for exposure to polyester was prepared by adding 0.1% of polyester to the base of commercial feed. And 0.1% polyester was added to the fish tank to condition the microfibers to flow into the water for experiments in breeding water. The exposure experiments were carried out for 5 days, and sampling was performed after 5 days. The gills and intestine were collected and observed using a stereomicroscope. The gene expression levels of antioxidant-related genes such as *sod*, *cat*, and *gsh* were analyzed using liver. In the brain, *tph* involved in the synthesis of the neurotransmitter serotonin and the corticoid receptors *gr* and *mr* were analyzed by the Real-time PCR.

As a result of the evaluation of the microfiber residual in tissues by exposure to feed, it was confirmed that the amount of microfiber remaining in tissues increased as the number of times exposed to microfibers through the feeding process increased. Microfibers were detected in intestinal tissue and feces, even when exposed to polyester in breeding water. The expression levels of genes in the liver and brain were lower in the polyester-exposed groups than in the control group. Therefore, it is considered that polyester, which is a microplastic, can remain in the digestive tract through the feeding process and breeding water condition of fish, and has the potential to affect oxidative stress and inflammatory responses in the body. Microfibers alone can have a detrimental effect on fish, but they can also combine with toxic chemicals, such as heavy metals, present in the water. Therefore, it is estimated that the ingestion of microfibers by fish may cause problems such as gastrointestinal damage and endocrine disorders. Through the results of this study, it is possible to understand the toxic potential of microfibers on fish. In the future, we intend to investigate the effect of microfibers on the endocrine system of fish.

PHYSIOLOGICAL REACTIONS OF GILTHEAD SEA BREAM (*Sparus aurata*) TO AN *Amyloodinium ocellatum* OUTBREAK - A HOLISTIC APPROACH

Márcio Moreira*, Denise Schrama, Benjamín Costas, Marcelino Herrera, Marisa Barata, Diogo Teixeira, Pedro Pousão-Ferreira, Florbela Soares, Pedro Rodrigues

CCMAR – Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, PORTUGAL; IPMA - Portuguese Institute for the Ocean and Atmosphere, EPPO - Aquaculture Research Station, Av. Parque Natural da Ria Formosa s/n, 8700-194 Olhão, PORTUGAL
marcio.moreira@ipma.pt

Amyloodiniosis, a parasitic disease caused by the dinoflagellate *Amyloodinium ocellatum*, affects more than one hundred species of brackish and marine aquacultured fish, causing fast and asymptomatic outbreaks with high morbidity and mortality. Fish mortalities are usually attributed to anoxia and osmoregulatory impairment. However, information regarding host physiological responses to amyloodiniosis is scarce. In this experimental trial, we propose to study the evolution of the infestation by *A. ocellatum* in gilthead sea bream.

For that purpose, we performed an experiment with two treatments (RAS systems, tanks in triplicate per sampling point, 24ºC, 100%, O_2 N=5, fish weight: ±130g, fish density: 5kg/m^3): a Control with naïve gilthead seabream juveniles non exposed to the parasite, and a treatment with naïve gilthead seabream juveniles exposed to 4000 *A. ocellatum* dinospores/mL. Tanks were sampled at 0h, 5h and when the fish start dying (36 hours after infestation). We analyzed the fish parasite load, bacterial secondary infections, fish indexes (CI, SSI, HSI), haematological analysis, osmoregulation and stress metabolites, histopathological analysis of spleen, liver and gill, immunological analysis in plasma, that will provide data to support a 2-DIGE proteomic analysis to plasma, liver, spleen and gill of gilthead sea bream.

Observation of fresh gill mounts showed an absence of parasites in the control, and the infested fish presented more than 10000 *A. ocellatum* trophonts per gill arch (Fig. 1). Regarding the physiological reactions, first results indicate a decrease in the SSI and in blood thrombocyte levels, a higher level of cortisol and a 75% incidence of secondary bacterial infections at 36 hours in infested fish. Histopathological analysis indicates several changes in spleen and gill of fish infested with *A. ocellatum*. Immunological and proteomic analysis are still being performed.

These data indicate that the physiological reactions of gilthead sea bream to an *A. ocellatum* outbreak is far more complex than the usually described in the literature.

This research was funded by project SAÚDE&AQUA (MAR-02.05.01-FEAMP-0009). M. Moreira was supported by an FCT grant (SFRH/BD/118601/2016). This study also received Portuguese national funds from FCT - Foundation for Science and Technology through project UID/Multi/04326/2019.

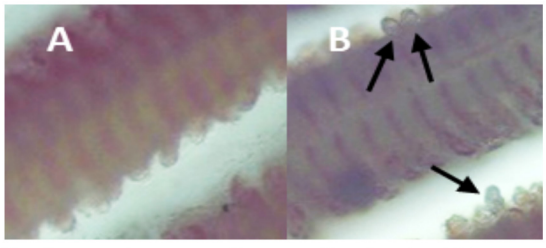


Fig.1 – Image from Control (A) and Infested (B) gilthead sea bream gill (100x amp.) at 36 hours after infestation.

SOLUBLE MANNO-OLIGOSACCHARIDES FOR PATHOGEN CONTROL IN AQUACULTURE

Richard Morgan*, Selva Kumar Rasiah, Bradley A. Saville

GreenSage Prebiotics Inc.
richard@greensageinnovations.com

Infections caused by *Vibrio spp*., *Tenacibaculum spp*. and *P. salmonis* have led to billions of dollars in losses in the aquaculture industry, yet there are few options to prevent or manage these infections in shrimp, salmon, and other aquaculture species.

Manno-oligosaccharides (MOS) are widely recognized for their ability to control pathogens such as *Salmonella spp*. and *E. coli* in the poultry and livestock industries, but are not widely used in aquaculture. GreenSage Prebiotics (GSP) has developed a high purity, soluble MOS targeted at key pathogens impacting aquaculture, animal, and human health.

GSP has conducted *in vitro* and *in vivo* trials to evaluate its novel MOS product (GSPMOS).
In vitro trials to determine the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of GSP-MOS versus *Vibrio parahaemolyticus* and *Tenacibaculum maritimum* demonstrated the effectiveness of GSP-MOS and the superiority of GSP-MOS versus low purity, insoluble yeast-based MOS products (Table 1). GSP-MOS also exhibited significant inhibition of *P. salmonis* in an Atlantic Salmon Kidney cell assay.

Additional *in vitro* studies have demonstrated efficacy of GSP-MOS vs. *Salmonella enteritidis, C. perfringens* and *E. coli*, and enhanced growth of beneficial microbes, including probiotics such as *Lactobacillus spp*., which can collectively support the digestive and immune systems.

An *in vivo Vibrio parahaemolyticus* challenge trial was completed in shrimp, following two weeks of consumption of feed containing GSP-MOS. Shrimp consuming 0.25 wt% GSP-MOS had 100% survival following a *Vibrio* challenge, compared to 42% survival in shrimp that did not receive MOS (Figure 1).

This presentation will discuss the benefits of high purity, soluble MOS as a feed additive for control of pathogens, and the corresponding impacts on aquaculture and animal health.

Pathogen	MIC (mg/mL)		MLC (mg/mL)	
	GSP MOS	Yeast MOS	GSP MOS	Yeast MOS
Vibrio parahaemolyticus	5.55	ND	50	ND
Tenacibaculum maritimum	16.67	50	50	50

ND : No detectable effect

Table 1: MIC and MLC of MOS Products

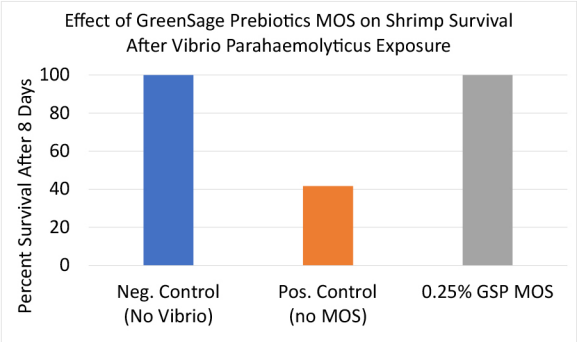


Figure 1: Impact of GSP-MOS on Survival After Vibrio Challenge

SCALING KELP FARMING AS A NATURE-BASED SOLUTION: CONSERVATION ORGANIZATIONS ROLE IN INDUSTRY ADVANCEMENT AND ADDRESSING RISK

Bailey Moritz

WWF- US, Aquaculture
1250 24th St NW, Washington, DC 20037
bailey.moritz@wwfus.org

Seaweed is on track to become the fourth largest crop globally, outpacing potato production by 2051. This continued expansion raises pushback from ocean stakeholders, often centering on concerns of environmental impacts in new communities where farms are being introduced, such as marine mammal entanglement and invasive species. Meanwhile, research continues to bolster widely recognized ecosystem services of kelp farming activities, including uptake of excess nutrients, mitigation of ocean acidification, and habitat creation. Farms also contribute to carbon sequestration, and harvested biomass can be used in value chains that offset global resource use.

Environmental NGOs recognizing the contribution of kelp farming to effectively restore ecosystems and address societal challenges as a nature-based solution have a key role to play in improving public buy-in for the industry and driving the conversation on mitigating risk.

WWF-US has implemented a strategy to reduce barriers for the responsible expansion of the seaweed industry in underdeveloped seaweed farming regions, namely the North Atlantic and Eastern Pacific. Partner projects have results on new tools to address whale entanglement fears, understand how coastal communities respond to different messaging around seaweed farms, and quantifying the benefits of farming seaweed adjacent to mussels.

This presentation will discuss the important role that conservation organizations can play in advancing growth of the global seaweed farming industry and expansion into new regions, highlighting examples of innovative work to address commonly cited risks and influencing investment funding into critical sectors of the industry. In conversation with local communities, there is room for farms to scale up, a necessary step to achieve meaningful climate impacts.

HAEMATO-BIOCHEMIAL ASSESSMENT OF BRACHYURAN CRABS AS A BIOMARKER OF ENVIRONMENTAL POLLUTION IN A TROPICAL MANGROVE SWAMP

Rasheed O. Moruf and Aderonke O. Lawal-Are

Department of Fisheries and Aquaculture,
Bayero University, Kano, Kano State- Nigeria
romoruf.faq@buk.edu.ng, +234 8022429983

Haematological profiles provide information about the health status of local populations. The haematological and biochemical parameters of the brachyuran crabs, *Goniopsis pelii, Sersama huzardii* and *Uca tangerii* from the mangrove area of a polluted creek in Nigeria were examined using standard methods. The total haemocyte count was similar for both *G. pelli* (2150±56.69 mL) and *S. huzardii* (1975.00±74.46 mL). haemocyte sedimentation rate was lowest in *G. pelli* (1.50±0.71 mm/hr) and highest in *U. tangeri* (5.70±0.82 mm/hr). Significant differences were however recorded for granulocyte, agranulocyte and monocyte with *G. pelli* having the highest sub-population of granulocyte (33.00±1.41 %) but lowest agranulocyte (67.00±1.41 %). A non-significantly higher sub-population of monocytes was recorded for *S. huzardii* (2.50±2.12 %) compared to *U. tangeri* (1.00±0.12 %). In the mean values of the serum biochemicals, *U. tangeri* showed lowest values for all except aspartate aminotransferase (22.8 U/L) and Urea (32.48 mg/dl). *G. pelli* showed highest values for serum protein (14.36mg/dl), Globulin (67.49 mg/dl) and phosphatase alkaline phosphatase (34.50 U/L). This study gave an indication of stress on the health status of the brachyuran crabs, hence the need for periodic monitoring of the ecosystem.

Samples of three brachyuran crabs species were collected along the mangrove area lining the Lagos Lagoon (6°26'-6°37'N and 3°23'-4°20'E). They were acclimatized for 48hrs and fed with sampling location mud rich organic matter until further analysis. Haemolymph was drawn with a 23G syringe from the juncture between the bases of ischium of the fifth walking leg. The haemolymph was collected into a syringe flushed with 1mL of anticoagulant (0.3 M NaCl, 0.1 M glucose, 30 mM Sodium citrate and 26 mM Citric acid), transferred into a 5mL lithium heparin bottle kept in an ice chest and haemolymph of crabs were analyzed immediately for haematological and biochemical indices using an improved Neubaeur heamocytometer according to methods described by Blaxhall and Daisley (1973) and Coles (1986) respectively. Data were subjected to Duncan Multiple Range Test at $p<0.05$.

The swamp indeed showed signs of environmental stress, which eventually pose devastating effect on the health status of the ecosystem engineers (i.e brachyuran crabs). Therefore, the need for periodic monitoring and enforcement of environmental laws.

Table 1: Body weight and Haematic Indices of brachyuran crabs of a tropical mangrove swamp in Nigeria

Haematic Indices	*Goniopsis pelli*	*Sersama huzardii*	*Uca tangeri*
Body weight (g)	24±2.11^a	16.15±15.19^a	8.8±0.50^b
Packed Cell Volume (%)	2.50±0.71^a	2.50±0.71^a	2.75±0.00^a
Total Haemocyte Count (mL)	2150±56.69^a	1975±74.46^a	1272±97.00^b
Sedimentation Rate (mm/hr)	1.50±0.71^a	4.00±1.41^b	5.70±0.82^b
Granulocyte (%)	33.00±1.41^a	11.00±1.41^b	10.00±5.12^b
Agranulocyte (%)	67.00±1.41^a	86.50±0.71^b	71.00±1.23^b
Monocyte (%)	0.00±0.00^a	2.50±2.12^b	1.00±0.12^b

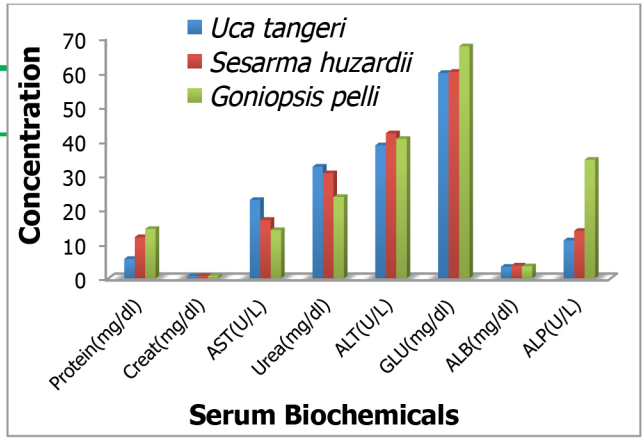


Figure 1: Serum biochemical profile of the brachyuran crabs of a tropical mangrove swamp in Nigeria

DEVELOPMENT AND TESTING OF A MODULAR, HIGH EFFICIENCY, WHALE SAFE MACROALGAE CULTIVATION SYSTEM FOR EXPOSED AND OFFSHORE ENVIRONMENTS

Zach Moscicki*, Pete Lynn, Toby Dewhurst, Beth Zotter, Michael Chambers, Jon Pompa, Melissa Landon, Igor Tsukrov, Michael MacNicoll, Noah MacAdam, Rob Swift, Louis Gitelman

School of Marine Science and Ocean Engineering
The University of New Hampshire
Durham, NH 03824
zachary.moscicki@unh.edu

We report the design and performance of an advanced macroalgae cultivation system for offshore and exposed ocean environments. The field test was completed in the Atlantic Ocean in 2022 off the coast of Maine, USA. The project is funded by the US Department of Energy's ARPA-E program to develop technologies that can enable large-scale macroalgae cultivation in deep water. Multiple new technologies were deployed in the field test, including new structural materials, components, and robotic anchoring with diverse applications in commercial offshore infrastructure. With two seasons of field tests completed, project team members are using collected data to evaluate the cultivation structure and component technology performance, improve upon those designs and to better inform the concept's economic feasibility. The desired scales of production, harsh offshore environment, and low-cost requirements for offshore macroalgae aquaculture present a challenging engineering design problem. As the technology development effort has unfolded, we have identified routes towards an economically feasible system by integrating: synergies between infrastructural components, scaling efficiencies, structural resilience in overlapping modularity, and strategic agronomic processes. Our system sets the typical hectare scale longline farm within the context of massive (square kilometers scale) arrays of semi-independent farm "modules" supported by a lattice of novel multi-line moorings capable of increasing structural efficiency and resilience through distributed accommodation of hydrodynamic loads. ROVs designed to install the anchors enable low-cost deep-water deployments. Wave actuated tethered hydrofoil upwellers integrated into the mooring system harness ambient renewable wave energy to elevate deep cold nutrient rich seawater enabling conditions capable of extending temperate macroalgae growth seasons. Fiberglass rods replace conventional ropes as mooring lines and growth substrate to reduce the risk of marine mammal entanglement (a major barrier to permitting in US waters). Through ongoing validation efforts: numerical modelling, component testing, and pilot scale deployments, we will demonstrate how our systems and component technologies could support industrial scale macroalgae cultivation in offshore waters.

FROM DRIED AND SMOKED FISH TO VALUE ADDED FISH PRODUCTS; A CASE OF LAKE TURKANA, KENYA

Chege Moses Chege

Kenya Fisheries services, Kenya
moschege@yahoo.com

With over 48 species of fish in Lake Turkana (largetst desert lake in the world) and underexploited waters, fishing is filling empty pockets in Kenya's poorest region in Arid and Semi arid rigion. Dried, smoked and frozen fish value is much lower than any value added product from the fish catch. For this reason fishermen and fish dependants at Lake Turkana have not fully financially benefited from this main activity in the region.

The author used both primary and secondary materials and methods to assess the state and gives rccommendations for better earning in the future. A small structured questionnaire was used for 20 fishermen and 10 fish traders from one Beach management (BMU) unit called Loiyangalani for this purpose. Further, as a certified fisheries office in this lake, the author has first hand information from day to day engament with the fishermen, boat owners and fish traders.

The study shows that 75% of fishermen ad traders support the idea of value addition as the best alternative compared to preserved and frozen fish. This study recommends supporting all the BMUs in the Lake with equipment for value addition such as fish mincing machines, cooller boxes, labelling and packaging materials.

THE INFLUENCE OF REARING TEMPERATURES AND LIVE FEEDS ON WHTE MUSCLE DEVELOPMENT IN GREATER AMBERJACK

Katerina A. Moutou*, Andreas Tsipourlianos† Rafael Angelakopoulos†, Alexia Fitsili, Nikos Mitrizakis, Nikos Papandroulakis

Laboratory of Genetics, Comparative and Evolutionary Biology
Department of Biochemistry and Biotechnology
University of Thessaly, Biopolis
41500 Larissa, Greece
kmoutou@bio.uth.gr
†These authors contributed equally

Greater amberjack *(Seriola dumerili)* is a very promising candidate for the diversification of the Mediterranean aquaculture due to high growth rates, consumer appreciation and commercial value. However, high growth dispersion at hatchery stages and unsynchronized development is a major drawback in intensifying juvenile production. Temperature is a key factor in promoting fish development and growth with strong epigenetic effects. Feeding at early stages is another challenging factor given the lack of a fully functioning digestive system. In this study, we investigated the influence of different rearing temperatures and different live feeds, on the process and variation of myogenesis at early development in greater amberjack.

Greater amberjack eggs were subjected to a 2X2 rearing experiment; two different rearing temperatures (20 ºC vs 24 ºC) were combined with two different types of live feeds [copepods (C) vs rotifers (R)] to form four experimental groups (20C, 20R, 24C, 24R). Larvae samples were collected at three developmental stages; notochord flexion (FL), end of larva rearing (ELR) and middle metamorphosis (MM). Larvae were subjected to histological analysis and the total cross-sectional area (TCSA) of one epaxial quadrant of white muscle was measured. In addition, the expression of *mylpfa* (myosin light chain 2a, hypertrophy), *mylpfb* (myosin light chain 2b, hyperplasia), *myog* (myogenin, coordination of myogenesis) that mark crucial mechanisms of muscle development and growth, was quantified by real-time PCR.

The combined results from histological and gene expression analyses indicate that temperature is a major factor with 24ºC favoring a higher pace of white muscle development in comparison with 20ºC. Within 24ºC, the type of live feed used resulted in different phenotypes at MM with rotifers supporting longer larvae with smaller TCSA as compared with copepods. A higher development pace was accompanied by a higher variation in gene expression levels that were not accompanied by higher variation in phenotypic traits, i.e. TL and TCSA. The shift from larvae to juveniles is dependent on the proper deployment of hyperplastic and hypertrophic processes during the early phases of muscle development. Based on *mylpfa*/*mylpfb* expression levels, it appears that hyperplasia dominates the white muscle development up to metamorphosis and it is strongly affected by rearing temperature.

«This research was Co-financed by Greece and the European Union, European Maritime and Fisheries Fund in the context of the implementation of the Greek Operational Programme for Fisheries, Priority Axis “Innovation in Aquaculture”, Project title “ Investigation of size variability in reared juveniles of greater amberjack towards improved production and husbandry practices” MIS 5010923.

MYCOTOXINS IN AQUACULTURE: OCCURRENCE IN FEED MATERIALS, EFFECTS ON AQUATIC SPECIES AND STRATEGIES TO COUNTERACT THEM

Nguyen Ngoc Diem, Roy Rosen, Timothy Jenkins, Ines Taschl and Ursula Hofstetter, Michele Muccio*

*DSM Nutritional Products Asia Pacific. 30 Pasir Panjang Road #13-31 Mapletree Business City, Singapore 117440
Email: michele.muccio@dsm.com

Mycotoxins are toxic secondary metabolites produced by certain fungi that contaminate a wide variety of grains and other commodities used in animal and human production. Mycotoxins prevalence changes among different regions, although global trade and climate change played a role in homogenizing the contamination profile worldwide. Aquatic species can be quite sensitive to mycotoxins, especially due to the increasing tendency to include plant-derived meals in aquatic diets. Since 2004 Biomin (DSM) has conducted a survey for the main mycotoxins, namely aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin (T-2), fumonisins (FUM) and ochratoxin A (OTA). In January to September 2022, *Fusarium* mycotoxins were the most prevalent in Asia: FUM, ZEN and DON were detected in 87%, 76% and 73% of samples analyzed, respectively. Alfa remains a concern in Asia as it was detected in 29% of samples analyzed, at average concentrations that are above safety guidelines. OTA and T-2 were detected in 30% and 12% of samples respectively. Concerning the effects of mycotoxins in aquatic species, those are dependent on the species, mode of action of mycotoxins, inclusion rates of contaminated material and duration of exposure. The most common effect observed on aquatic species are reduced growth and increase susceptibility to diseases due to the immunosuppressive effects of mycotoxins. Other effects include histopathological changes, hepatic damages and reproductive issues. Mycotoxins undertake synergistic interactions, thus the simultaneous presence of those secondary metabolites in the same sample, increases the overall toxicity. Synergism is well documented for terrestrial animals, but only a few studies are available in aquaculture. When it comes to mycotoxin counteraction, multiple solutions are available on the market, but only a very limited amount of those is backed up by a scientifically proven mode of action. Binders represent the most common product group, but their efficacy is limited to aflatoxins in the best case and definitely cannot support against other mycotoxins such as trichothecenes, ZEN and FUM. Enzymatic degradation of mycotoxins is the most effective way to completely detoxify mycotoxins in the gastrointestinal tract of the animal, but only a very limited number of enzymes is currently available on the market, with proven mode of action and guaranteed safety. DSM has developed several solutions for mycotoxin deactivation in aquaculture, to cover all the industry needs. Those products range from combinations of EU authorized binders and enzymes, to single ingredient solutions, with proven identity, safety and efficacy according to the strict conditions imposed by the European Food and Safety Authority (EFSA) and the EU itself. Currently, DSM is the only company existing on the market with five EU registrations in mycotoxin risk management. The DSM Mycotoxin Survey is the largest and most cited survey program worldwide and it is the current reference point for mycotoxin contamination assessment among the industry and the scientific community.

FEEDING AND DIET PREPARATION FOR LARVAE AND JUVENILE SEA CUCUMBER *Holothuria scabra*

Sitti Raehanah Muhamad Shaleh*, Wan Arina Azrinor Yamin, Noor Adzlina Abidin, Ching Fui Fui and Rafidah Othman

Borneo Marine Research Institute, Universiti Malaysia Sabah
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
sittirae@ums.edu.my

Sea cucumber *Holothuria scabra* or commonly known as sandfish is high in protein, low fat and contains bioactive compounds which exhibit antimicrobial and anticancer properties. The high demand for sandfish that has led to over-exploitation worldwide has urged for the farming of this species. Seed production of sandfish in the hatcheries is being developed to accommodate aquaculture needs as well as for stock enhancement programme. In the hatchery, artificial breeding using several techniques including heat shock and diet stimulant techniques has been well established. However, larvae and juvenile rearing remain the major constrain in the production due to very low survival rates. To establish the rearing technique, appropriate diet and proper feeding schedule were investigated. At the larval stage, *Nannochloropsis* sp. and *Chaetoceros calcitrans* were given for higher growth and faster metamorphosis from auricularia into doliolaria stage. For the early juvenile stage, diatom flocs were found to be more efficiently digested compared to *Navicula* sp. a benthic diatom. Feeding trials revealed that juvenile sandfish fed with 30% diatom flocs and 70% sea mud had higher weight gain and growth rate. These findings are very important for the establishment of sandfish production.

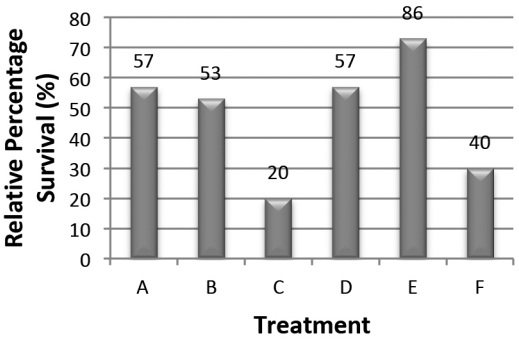
SURVIVAL RATE OF TIGER SHRIMP *Penaeus monodon* ON THE USE OF FRACTIONATION RESULT OF SEVERAL TYPES OF MANGROVE CHALLENGED WITH WHITE SPOT SYNDROME VIRUS (WSSV)

Muliani, Nurbaya, Ince Ayu, and Muharijadi Atmomarsono

Research Institute For Barckishwater Aquaculture
and Fisheries Extension, Maros South Sualwesi
mulianim@yahoo.com

Indonesia is a country rich in natural resources include mangrove forest resource, both quantitatively (area) and qualitatively (diverse type),thus it is possible to assess its use as an alternative to treat disease in shrimp farming, including WSSV disease treatment. Result of previous study showed that several types of mangrove originated from some pond areas in South Sulawesi are potential as anti-bacterial producer for treating Vibriosis disease and anti-WSSV disease. This experiment was aimed to determine the survival rate of tiger shrimp on the use of fractionation of several types of mangrovechallenged with White Spot Syndrome Virus (WSSV).

Plastic container of 40 L volume was filled with 30 L of sea waterat salinity of 28 ppt which had been disinfected stocked with 10 tiger shrimpswith size of 5-7 g/ind Challenge test of mangrove extract with WSSV was done by mixing 5 µL of WSSV suspension with 10 µL of mangrove extract solution (500 mg/100 mL of NTE buffer). The mix wasthen incubated at 29 °C for 3 hours and then given to tiger shrimp as intramuscular injection.The experimental design used was Completely Randomized Design with treatments; A). butanol fraction of *Sonneratia alba*; B). butanol fraction of *Sonneratia caseolaris*; C). butanol fraction of *Sonneratia lanceolata*; D). butanol fraction of *Bruguiera gymnorrhiza;*E).diethylether fraction of *S. alba*; F). diethyl ether fraction *of Bruguiera gymnorrhiza*; G). Control (shrimps injected with WSSV suspension without mangrove extract. Each treatment was repeated 3 (three) times and tiger shrimp were rearedfor 10 days. Observations of tiger shrimp mortality were performed daily, while Total Hemocyte Count (THC), Differential Hemocyte Count (DHC), ProPO values and WSSV infection were observed at the end of the study. The results showed that over 50% of tiger shrimp relative survival was obtained by treatment that used butanol fraction of *S.alba*, butanol fraction of *S.caseolaris*, butanol fractionof *B. gymnorrhiza,* and diethylether fractionof *S. alba (*Figure 1). These experiments showed that the four fractions of mangrove effectively increased the survival of tiger shrimp. The highest average survival rate of tiger shrimp was obtained by treatment that used diethyl ether fraction of *S. alba,* whilethe lowest was found in positive control, and both treatmentswere significantly different (*P <0.05*).The result indicated that diethylether fraction of *S. alba*was found to bethemost potential fraction to control WSSV disease in tiger shrimp*.*



Notes: **A=** ***Buthanol Fractiont of S. Alba,*** **B=** ***Buthanol Fractiont of S. caseolaris***
C= ***Buthanol Fractiont of S. lanceolata***
D=***Buthanol Fractiont of B. gymnorrhiza***
E= ***Dietyleter Fraction of S. lalba***
F=***Dietyleter Fractiont of B. gymnorrhiza***

Figure 1. Relative Percentage Survival of Tiger Shrimp *Penaeus monodon* after 10 days challenge test with WSSV and mangrove extract

ASSESSMENT AND MAPPING OF WATER QUALITY IN PANGASIUS CULTURE AREAS OF AN GIANG AND CAN THO PROVINCES, VIETNAM

Fridah M. Gacheri * and Vu Ngoc Ut

College of Aquaculture and Fisheries
Campus 2, Can Tho University
3/2 street, Can Tho City
Vietnam
Tel. (+84 292) 3872098; Fax. (84710) 3838 474
Website: http://www.ctu.edu.vn
fgacherimutea@gmail.com

The striped catfish (*Pangasianodon hypophthalmus*) (Sauvage), also commonly known as tra catfish or sutchi catfish, is a freshwater species known to have originated from the Mekong Delta in the South of Vietnam. The export of this product has significantly contributed to the country's production therefore improving the socio-economic sectors of the Mekong Delta. Nevertheless, this aquaculture sector has faced a lot of problems whereby water quality is a main challenge that has been threatening the success of pangasius culture, thus calling for frequent monitoring and managing of water quality in the pangasius culture areas.

This study aims at using the physico-chemical method as the monitoring approach for water quality surveillance in An Giang and Can Tho provinces where aquaculture is intensely developed. In addition, there will be zoning and mapping of water quality using Geographic Information System (GIS) which will involve creation of vector maps based on each water quality variable and the location of the pangasius culture areas using Global Positioning System in the identification of latitudes and longitudes, followed by combination of each variable from monitoring in correspondence with the identification of locations in order to create feature maps that will show a map of specific variables using ArcGIS 10.2. The classification parameters will be performed based on the national standards of Vietnam. This study will help in providing a proper managerial instrument for sustainable aquaculture development in the Vietnam.

IN VITRO AND *IN VIVO* EXPRESSION OF VIRULENCE GENES IN ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) CAUSING STRAINS IN RELATION TO THEIR VIRULENCE TOWARDS *Penaeus vannamei* (BOONE, 1031)

Sarmila Muthukrishnan*, I.Natrah, Mohamed Shariff, Ina-Salwany M.Y, Fatimah Md Yusoff

Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
sarmila_muthukrishnan@yahoo.com

Acute hepatopancreatic necrosis disease (AHPND) recently escalate into panzootic, leading to severe losses in shrimp industries. Three types of quorum sensing (QS) signal molecules namely N-acyl-homoserine lactone (AHL) (**Figure 1**), Autoinducer-2 (AI-2) and Cholerae autoinducer-1-like (CAI-1) molecules were detected in cell-free fluid from AHPND causing strains. The *LuxR* and *LuxS* genes screening were positive for all the AHPND positive *Vibrio* strains.

In vivo expression of AHPND virulence genes (*pirA* and *pirB*), quorum sensing regulator *luxR* and virulence regulator *toxR* in *V. parahaemolyticus* strain BpShHep31 and *V. harveyi* strain BpShHep24, causing AHPND were further investigated. A significant ($P < 0.05$) increase in the expression levels of the quorum-sensing master regulator *luxR* were detected when compared with the control shrimp (unchallenged group). There was also a substantial difference in *pirA*, *pirB* and *toxR* expressions in the challenged shrimps compared to unchallenged group. The expression of AHPND virulence genes (*pirA* and *pirB*), *luxR* and *toxR* peaked at 36 h post challenged at 20-30-, 41-59- and 25-39-fold respectively. Furthermore, expression levels of *pirA, pirB, luxR* and *toxR* positively correlate with the mortality rate of shrimp in the challenged group.

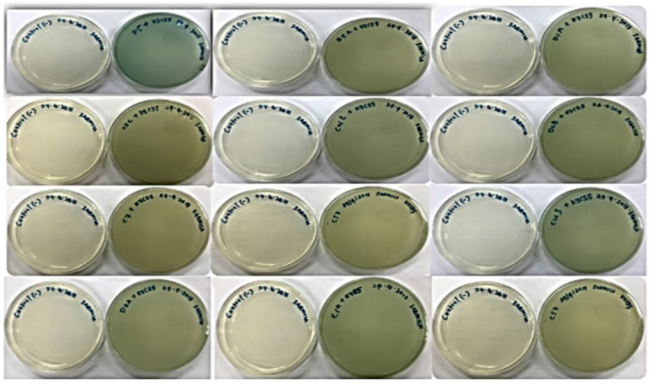


Figure 1: Screening of long-chain AHL in 12 AHPND positive strains using *Ag. tumefaciens*, KYC55 biosensor. Left plate: Control plates (*Ag. tumefaciens*, KYC55 +X-Gal); Right plate: Treatment plates (*Ag. tumefaciens*, KYC55 + AHPND strains + X-Gal).

APPLICATION OF AQUA ECTOGON-284® AS A GROWTH AND HEALTH PROMOTER IN WHITELEG SHRIMP *Litopenaeus vannamei* POST-LARVAE, AND RESISTANCE TO *Vibrio parahaemolyticus* (AHPND STRAINS)

[1]Sarmila Muthukrishnan* and Wee Wen Chen[2]

[1]Aquatic Animal Health and Therapeutics Laboratory. AquaHealth, Institute of Bioscience. Universiti Putra Malaysia 43400 UPM Serdang, Selangor. Malaysia

Corresponding author: sarmila_muthukrishnan@yahoo.com
[2]Envitgro Pte. Ltd.
w.c.wee@envitgro.com
www.livestockasiapacific.com

Diallyl polysulphide family has been established and incorporated into a patented PST-22 technology, with 22 sulphide chains. Aqua Ectogon-284® is a product which contains PST-22. It is not only not only works to repel parasites, but it can also inhibit bacterial and fungal. Aqua Ectogon-284® offers non-species specific protections, which allow it to be used in all aquaculture species production.

In the aquaculture industry, Whiteleg shrimp *Litopenaeus vannamei* is the most important species group cultured worldwide in terms of international trade. At the moment, there is no therapeutic against AHPND and the use of antibiotics is not feasible due to the presence of potential residues in commodity shrimp, development of drug resistant bacteria, and other environmental issues. That brings us to come out with this study to evaluate the Aqua Ectogon-284® dietary supplementation as a growth promoter and protective effect in *L. vannamei* post-larvae.

After 30 days of feeding trial, the shrimps were sampled and subjected to challenge trial against Acute Hepatopancreatic Necrosis Disease (AHPND) positive *Vibrio parahaemolyticus*. To confirm the result is producible, shrimps were sampled at the end of challenged test for the transcriptomic study.

We observed that 1g Ectogon/kg fed shrimp demonstrated increment of twice the weight of the control group in the final weight analysis under normal culture condition. For the challenged test study, the shrimp fed with 1g Ectogon/kg demonstrated highest survival rate, 95% compared to the positive control, 50%. Interestingly, dietary supplementation of 1g Ectogon-284 changed the expression level of some immune related gene and virulence related gene in the shrimp under the presence of *Vibrio parahaemolyticus* Acute hepatopancreatic necrosis disease (VP_{AHPND}). A total of 4031 genes were expressed shrimp fed with 1g Ectogon-284/kg whereas only 380 genes were expressed in the control group when challenged with *V. parahaemolyticus*.

This feeding study had proven the efficacy of Aqua Ectogon-284 as a growth and health promoter. At the same time, it reduced the impact of vibriosis to shrimp.

DIETARY FISH OIL AND CAMELINA OIL INFLUENCES LIPID CLASS COMPOSITION OF JUVENILE *red sea bream*?

*Kumbukani Mzengereza and Manabu Ishikawa

Kagoshima University, Shimoarata 4-50-20, Kagoshima 890-0056, Japan
kumbumzenge@gmail.com

Camelina sativa, or false flax, is an annual winter plant in the Brassicacea, or mustard family. Camelina sativa originated from Southeast Europe and southwest Asia. Cultivation trial of camelina sativa reported in Hokkaido, Japan (Fujita, *et al*.,2013), as it grows well in frost and thaw-freezing condition under minimum inputs. The present study aimed at evaluating the potential of camelina oil as lipid source in aqua feeds, a feeding trial was conducted by feeding juvenile red sea bream (15g average initial weight) an isolipidic and isonitrogenus diet formulated by gradually replacing fish oil with camelina oil. Growth performance, fatty acids, lipid classes, proximate composition and blood function in tissues and diets were evaluated after 56 days.

The results showed that the substitution of fish oil (FO) with camelina oil diets CO50 and CO90 significantly ($P > 0.05$) decreased red sea bream growth compared to control, with the highest final body weight (FBW), weight gain (WG), feed intake (FI) and recorded for (CO0) group. Hepatosomatic index (HSI), numerically increased with concomitant camelina oil addition. Total protein, blood glucose and triglyceride showed significant differences ($P<0.05$) among all fish groups, lipid class results show that total lipids (TL) and neutral lipids (NL) increased significantly ($P<0.05$) with increase in dietary camelina oils and conversely there was an observed decreasing tendency with addition of dietary camelina oil in liver polar lipids (PL). There was a marked significant ($P<0.05$) increase in the levels of triglycerides (TG) in liver polar lipids with additional dietary camelina oil and a corresponding decrease in Phosphatidylcholine (PC), Phosphatidylinositol (PI) and Phosphatidylethanolamine(PE). Levels of Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) decreased in liver polar lipids (PL) but there was observed increase in alpha-linoleic, (ALA), linoleic acids (LA) in both liver polar lipids (PL) and neutral lipids (NL)**:** Findings suggest that up to 30% dietary camelina oil replacement of fish oil without negative effects on growth performance, lipid class and blood function of red sea bream.

Liver lipid class composition (*g/100g*)					
	CO0	CO10	CO30	CO50	CO90
Total lipid	36.6 ± 3.2^{a}	36.1 ± 1.5^{a}	41.2 ± 3.2^{b}	44 ± 2.5^{b}	48.8 ± 2.5^{c}
Neutral lipids	21.1 ± 1.7^{a}	22.1 ± 1.1^{a}	22 ± 6.9^{a}	26.5 ± 1.1^{b}	33.2 ± 1.9^{c}
Polar lipids	15.5 ± 1.0^{a}	13 ± 1.5^{a}	13 ± 0.8^{a}	11.3 ± 2.1^{c}	8.0 ± 3.2^{d}

SOCIO ECONOMIC PROFILE AND CONSTRAINTS FACED BY THE FRESHWATER FISH CULTURE IN MANIPUR, NORTHEAST INDIA

N.K. Sonanla*, T. Umamaheshwari

Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Thoothukudi – 628 008, Tamil Nadu, India
Email ID: sonanngakang123@gmail.com

The present study was taken up to carry out the critical analysis and marketing efficiency of freshwater fish culture in Manipur during the period 2019-20. The study was undertaken with the objectives of documenting the socio-economic profile of the fish farmers and the constraints in the fish culture activity in Manipur. Based on the envisaged research objectives, the study was undertaken in three districts viz., Ukhrul, Imphal West and Bishnupur, where the fish culture activity and Phumdi fish farming were predominant. A total of 90 freshwater fish farmers were interviewed personally by using a pre-tested interview schedule as per the research objectives and documented the first-hand information. Tools of analyses include tabular, percentage and constraints analyses and the collected data were statistically tested in SPSS software.

The results revealed that freshwater fish culture activity was highly dominated by males (96.7%) with an average farming experience of 12 years. Community-wise, 70 per cent of the fish farmers belonged to SC and ST category and among the surveyed respondents, fishery was found to be the primary occupation for 88.9 per cent of the fish farmers, followed by other occupations (8.9%) and Agriculture (2.2%). The income distribution pattern clearly showed that 96.7 per cent of the fish farmers were realizing the income of above 80,000/-through the primary occupation. The general characteristics of the surveyed fish farms revealed that all the fish farmers (100%) were undertaking their fish culture activity during summer following semi-intensive farming practice in an area up to 2 ha. The constraints faced by the fish farmers were also identified and ranked using Garrett ranking technique. Among the identified production, resource, marketing, social and extension constraints, absence of technically skilled personnel (63.93), lack of government support (62.63), low farm gate price for fish (49.80), inappropriate use of available resources (53.91) and lack of knowledge in fish culture (58.47), respectively were reported as the most significant constraints by the surveyed fish farmers in Manipur state. Based on the output of the study, certain suggestions for the development of freshwater fish culture activity in Manipur state were drawn and discussed.

GENETIC CHARACTERISTICS OF AYU *Pecoglossus altivelis* CAUGHT BY DIFFERENT TWO METHODS FISHING AND CAST NET, BY MEANS OF MICROSATELLITE DNA MARKERS

Masamichi Nakajima*, Akinobu Tan, Wataru Teramoto, Tadahiro Sohtome

Graduate School of Agricultural Science, Tohoku University,
Sendai 980-0845, Japan

Ayu *Plecoglossus altivelis* is one of the most popular fish species not only as food, but also as fishing target in Japan. "Tomozuri" is popular capture method of Ayu. "Tomozuri" is the method to catch Ayu used decoy, and this method also use the aggression of territory formed Ayu. This territory formed Ayut is required as artificial seed population for release. It is assumed that two genetic and environmental factors are affecting to the formation of territory in Ayu. But, the details of genetic control of territory formation in Ayu are unknown. In this study, genetic characteristics of recaptured Ayu by fishing and cast net were compared.

Two Ayu strains were used in this study. One is originated from Tsuruta Lake in Kagoshima Prefecture (Southern part of Japan), and the other is originated from Ani River in Akita Prefecture (Northern part of Japan). These strains have been maintained 4 to 5 generations in private farms and the seeds for release were produced at autumn in 2017, released to Oda River in Fukushima Prefecture at May, and recaptured at August in 2018. Tomoduri (Fish) and cast (Cast) were used for recapture. The individuals caught by Fish were assumed as the territory formed type, and the individuals caught by cast net were assumed as the non-territory type. Fifteen microsatellite DNA markers were used in this study. Genetic variability was measured by average heterozygosity, and population differences were compered by F_{ST} and PCA analysis.

Average heterozygosity in the Cast groups indicated higher value compared with the Fish groups in both Akita and Kagoshima strain. Cast group was indicated similar genetic characteristics between Akita and Kagoshima, and same phenomenon was observed in Fish group. PCA analysis represented it clearly (Fig.). These results suggest that the genetic convergence in Fish group and existence of genetic factors for the formation of territory in Ayu.

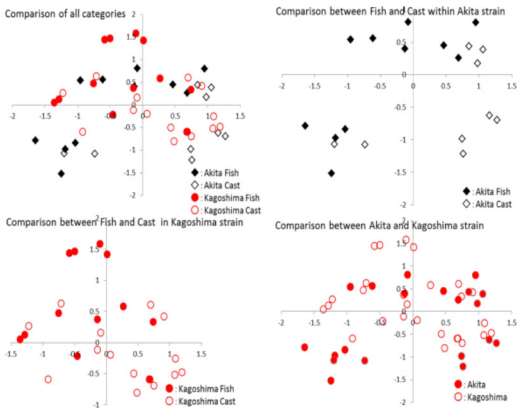


Fig. Comparisons of genetic characteristics among categories of strains and catch metohd

EFFECTS OF ANTIFOULANTS ON THE FORMATION OF MARINE BIOFOULING COMMUNITIES MONITORED IN JANGMOK USING ENVIRONMENTAL DNA METABARCODING APPROACH

Sang-Eun Nam*, Sung-Ah Kim, Jee-Hyun Jung, Min Chul Jang, and Jae-Sung Rhee

Incheon National University
Incheon, Republic of Korea
E-mail: se_nam2@inu.ac.kr

Biofouling is the colonization of various aquatic organisms (e.g., bacteria, algae, barnacles, mussels, etc.) on the submerged surface of ship hulls and artificial surfaces of mariculture equipment and floating platforms, causing major economic loss and environmental impacts. However, most studies have focused on bacteria. Here, we evaluated the usefulness of eDNA metabarcoding platform for monitoring of biofouling animals. To monitor seasonal variation of biofouling animals, animal attachment on the four plates equipped at the cage aquaculture of Jangmok. Metabarcoding approach targeting the cytochrome c oxidase subunit 1 (COI) gene was employed to investigate invertebrate and specific Urochordata communities on the plate or seawater for comparison. We identified experimentally that eDNA analyses increased detection of species by more than 90% compared to conventional PCR method. Especially, we obtained all of the Phylum level that detected from PCR. The eDNA approach was very useful to identify undetectable animals when we directly compare the samples with conventional PCR method. Relatively high percentage of unclassified taxa are remained to be resolved with further study phylum-specific primer sets. Taken together, these results can contribute to understand seasonal blooming of biofouling animals and suggest usefulness of eDNA metabarcoding approach.

THE MATURITY LEVEL MONITORING OF LARGEHEAD HAIRTAIL *Trichiurus japonicus* IN JEJU ISLAND FROM KOREA

Jin NAMGUNG [b*], Hye-na MOON[a], Ki-hyuk KIM[a], Juhyeon JANG[c], and In-kyu YEO[c]

A Department of Major of Aquatic Life Medicine, Jeju National University, Jeju 63243, Republic of Korea
b) Education & Research Group for Future Strategy of Aquatic Life Industry, Jeju National University
Jeju 63243, Republic of Korea
c) Department of Major of Aquatic Life Science, Jeju National University, Jeju 63243, Republic of Korea
E-mail: jinichu@jejunu.ac.kr

Largehead hairtail, *Trichiurus japonicus*, are one of the important commercial fish species in East Asia such as Korea, China, and Japan. Jeju Island, located in the southernmost part of Korea, has abundant food resources and a warm water temperature, forming a major fishery ground for largehead hairtail. However, due to overfishing, largehead hairtail resources have decreased by more than 50% compared to the 2000s in Korea. The fish reproductive cycle provides basic knowledge for such as establishing a closed season to prevent overfishing and next year resource management. In this study, we were monitoring maturity level of largehead hairtail caught in Jeju Island during the main spawning season.

The fish samples were purchased at fish market in harbor located in Jeju Island. The maturity was classified according to total length and anal length. The monthly maturity level investigated from April to August. The maturity level was determined based on the gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K) and absolute fecundity.

As a result, the mature female largehead hairtail maintained a higher maturity level from April than immature individuals and they began to mature more rapidly from May, showing the highest fecundity. The maturity level of mature females was maintained at a higher level until August. Therefore, some individuals had already finished spawning in August. In the case of immature females and males, they have a low maturity level until July. However, some immature females have ovulated eggs in the abdomen and most of the immature males showed high HIS levels in August.

These monitoring results suggested that mature females continue to participate during the spawning season, and they are expected to spawn eggs once or several times. The immature female and male maturation started in August, however, in some cases, fast maturing individuals may participate in spawning quickly. In the future, continuous this monitoring, we will get more detailed information about the maturity level and spawning patterns of largehead hairtail. Furthermore, we will be able to understand through this information their life history more deeply.

DEFINING THE NEED FOR SULPHUR AMINO ACIDS AND RELATED COMPOUNDS FOR GIANT GROUPER *Epinephelus lanceolatus*

Leo Nankervis*, Caroline L. Candebat, Adrien F. Marc, Thibault E. Eddie, Grace Butler

*Leo.nankervis@jcu.edu.au
Centre for Sustainable Tropical Fisheries and Aquaculture & College of Science and Engineering
James Cook University, Townsville, QLD 4811, Australia

Giant grouper (*Epinephelus lanceolatus*) is an economically important yet under-researched species, still reliant on 'trash fish' or aquafeeds that do not meet its nutritional requirements. As a key step towards formulating a species-specific aquafeed for giant grouper, we have sought to define appropriate dietary levels of methionine, sulphur amino acids and related compounds. This nutrient complex is often limiting in feed formulation and underestimation of their interactive requirement values is a major limitation in generically formulated marine fish feed. Interactions between related metabolites and the formulation considerations surrounding these interactions will be discussed.

ANALYSIS OF FOOD SECURITY STATUS OF FISH MARKETERS AND THE PROFITABILITY OF FISH PROCESSING AND MARKETING IN HADEJIA FISH MARKETS, JIGAWA STATE NIGERIA

*Nafisat N,. Nasir, M.A., Usman H.

*Binyaminu Usman Polytechnic, Hadejia Jigawa State, Nigeria
*icecool2235@gmail.com

The study analyze the profitability of fish Processing and Marketing in Hadejia Fish Markets of Jigawa State and the food security status of the marketers. It examined marketing channels, and problems during processing and marketing of fish. Multistage sampling technique was used to select 55 fish processors and 110 fish marketers. Primary data were collected using a questionnaire. The analytical tools used include food consumption score, descriptive statistics, Net Processing Income and Net Marketing Margin The mean age of the processors in the study area was 28 years while the mean years' experience is 8 years. The food security status of the marketers were acceptable while that of the processors were at the borderline. Fish is processed manually using traditional smoking kiln. There are no women processors or marketers at Hadejia market, women processors process fish from their various homes in Hadejia environs. Processed fish supply were mainly from neighboring state markets mainly Borno and Yobe State and taken to Hadejia market, and sold to other states like Enugu, Anambra, Delta, Rivers, Lagos and Benue. Processing of Fish is very profitable considering a Net Processing Income of ₦7,663.32 and ₦5.23 was derived from every naira invested. Despite the profitability of enterprise, certain constraints were faced by the marketers and processors, include insufficient fund to expand the business and lack of standard measure and poor drainage system at the market. The study therefore recommends the need for standard measurement (kg/price), NGO's to recognize the contributions of fish processing and marketing to the food nutrition and need for proper hygiene at the market, and include it as part of the programs targeted at food security and nutrition.

THE USE OF NOVEL INGREDIENTS IN LARVAL FEEDS FOR THE WHITE SHRIMP *Litopenaeus vannamei*

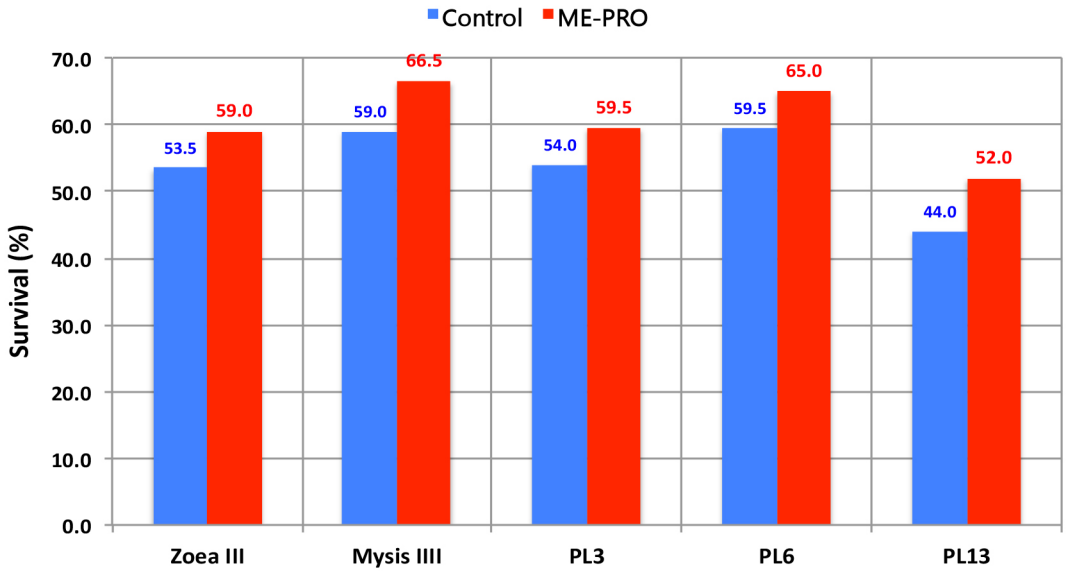
Sergio F. Nates* and Napoleon Araujo

Prairie Aquatech - R&D Center
705 32nd Ave S
Brookings, SD 57006 USA
Sergio@PrairieAquaTech.com

Supply of fishmeal from wild capture fisheries, long the staple protein ingredient for high quality aquaculture feeds, cannot expand further and is insufficient to meet the growing feed protein needs of the global aquaculture industry. Additional renewable and sustainable protein alternatives are needed. On the other hand, the limiting nutritional factors in plant proteins have been well characterized but some plant-derived ingredients, such as soy protein concentrates and fermented soybean meal (FSM) have show to perform better, which provide us new insights into FM replacement.

Fermentation improves the nutritive value of feed ingredients and it has been demonstrated that using FSM as a protein source in aquatic animal diets made a positive effect on growth performance. In addition, the short-chain peptides and free amino acids produced as a result of fermentation along with nucleotides confer excellent attractability and palatability properties to FSM's.

In the production of shrimp post larvae, feeding is one of the most important aspects. Moreover, the feeding protocols of larvae in commercial shrimp hatcheries include a wide range of balanced feed and nutritional supplements, particularly in the early stages. The results of this study showed that *L. vannamei* larvae fed with feeds containing a novel fermented soy (ME-PRO®) had the highest growth and survival among treatments (Figure 1).



OVERVIEW OF LARVAL FEED TECHNOLOGIES FOR REARING WHITE SHRIMP *Litopenaeus vannamei*

Sergio F. Nates*, Brandon White, Daniel F. Villamar+

*Prairie Aquatech - R&D Center 705 32nd Ave S
Brookings, SD 57006 USA
Sergio@PrairieAquaTech.com

In the production of shrimp post larvae, feeding and nutrition are critically important considerations. Moreover, feeding protocols of larvae in commercial shrimp hatcheries typically include a combination of a wide range of manufactured feeds, nutritional supplements, and live food organisms particularly in the early stages.

Conventional aquatic larval feeds are provided in a dry form as powdered products that are hydrated and dispersed directly into rearing tank water or flaked products, which require wet sieving to reduce particle size before administering to rearing tanks. Different processing systems have been employed to produce larval feeds including flaking, extrusion, marumerization (spheronization following cold extrusion), particle assisted rotational agglomeration and various microencapsulation techniques. In addition, a wide range of artificial feeds have been manufactured using various microencapsulation technologies. Generally, microencapsulation comprises preparation of payload and wall materials, incorporation of payload nutrients into wall material, and solidification. The size of microcapsules varies from about 1 to 1000 μm (equivalent spherical diameter) depending upon the technique and type of payload materials used. Other methods applied in the production of dietary microcapsules include interfacial polymerization, spray freeze-drying, formation of micelle and liposomes, complex coacervation and supercritical fluid base techniques among others.

Regardless of processing method, high-quality larval feeds should possess the following characteristics: high physical stability in water, reduced / controlled leaching of nutrient compounds, high digestibility and bioavailability of nutrients, adequate suspension in aerated larval rearing tanks, cost-effectiveness, and long shelf life. Superior and novel larval feeds can also contain protein hydrolysates, either or both of a vegetable and animal protein origin. These feeds can also include soybean peptides with reduced phytic acid and related glycoproteins obtained through aerobic fermentation; carnosine bioactive agents; benevolent microbes including those selected for biological control of disease and bioremediation; enzymes such as proteases and lipases; seafood by-products; natural bioactive compounds including phytochemicals, phenolic oils, organic acids, sources of hydroxy-polyunsaturated fatty acids present in fungi; and microbial biomass of algae and yeasts. Emerging ingredients include fish oil substitutes, novel emulsifiers, insect meals, seaweeds, micro-encapsulated bacteriophages, fungi-based proteins and bacteria/fungal cell-wall glucans and particular oligonucleotides.

We will discuss novel approaches to larval feed formulation, potential impacts on water quality and performance of larval shrimp populations in the hatchery.

MAY AMINO ACIDS SUPPLEMENTATION IMPROVE RESILIENCE AGAINST CHALLENGING CONDITIONS IN FISH LARVAE?

C. Navarro-Guillén*, I. Jerez-Cepa, A. Lopes, S. Engrola

Centre of Marine Sciences (CCMAR), *Campus* de Gambelas, Faro, Portugal
*E-mail: cpguillen@ualg.pt

Aquaculture industry is facing many evolving sustainability challenges. Improving feeding protocols and fish digestive efficiency will help the sector to attain a higher productivity, concomitant with a lower environmental impact. Therefore, discovering new methods to improve gastrointestinal maturation alongside with higher digestive efficiency at early stages without compromising survival and fitness represents a very promising avenue in fish nutrition research. Nutritional programming refers to a nutritional intervention during the early phases of development that will imprint an individual physiological memory resulting in long-term effects on growth and physiological function. Since digestive capacity is key to resilience of fish populations, applying this novel concept to the fish production provides numerous possibilities for improving adaptive responses to fish to challenging conditions.

Nutritional research has demonstrated that dietary protein and amino acids (AA) a play fundamental role in the overall fish digestive capacity and consequently growth performance. The objective of this work was to assess *in ovo* AA supplementation as long-term modulator of larval intestinal maturation and metabolic capacity at optimum (28ºC) and challenging temperature (32ºC). The AA supplementation was performed at zebrafish embryonic stage, using the sonophoresis technique. The experimental setup was, control (CTRL, no supplementation), amino acid glutamine (GLN) or arginine (ARG) supplementation. The experiment lasted until 40 days post-fertilization. Growth performance and digestive and metabolic enzymes activities were analysed to evaluate the larval nutrition-induced metabolic plasticity and the effects on fish resilience to challenging conditions.

The preliminary results showed that fish survival was not affected either by the sonophoresis technique or rearing temperature ($p>0.05$). Growth performance was affected by both, temperature and AA treatment. Overall, 28ºC-fish showed higher final dry weight (DW) than 32ºC-fish, reaching the maximum DW in fish supplemented with glutamine and reared at 28ºC. On the other hand, arginine supplementation statistically improved DW at 32ºC, suggesting a better metabolic adaptation to cope with higher temperatures. This study shows that sonophoresis is a good technique to incorporate amino acids in fish eggs. Amino acid supplementation at embryonic stage was able to promote fish performance at later developmental stage and, specifically, results suggest that *in ovo* arginine supplementation might improve fish resilience to challenging conditions.

Acknowledgments: The present study was supported by projects ALG-01-0145-FEDER-029151 “PROLAR – Early metabolic programming in fish through nutritional modulation”, and UID/Multi/04326/2019 financed by the FCT (Portugal). Sofia Engrola acknowledge a FCT investigator grant (IF/00482/2014/CP1217/CT0005) funded by the European Social Fund, the Operational Programme Human Potential and FCT.

PIPETTE AND PAPER: COMBINING MOLECULAR AND GENEALOGICAL METHODS TO ASSESS A NILE TILAPIA *Oreochromis niloticus* BREEDING PROGRAM

Maria G. Nayfa*, David B. Jones, Curtis E. Lind, John A.H. Benzie, Dean R. Jerry, and Kyall R. Zenger

Centre for Sustainable Tropical Fisheries and Aquaculture
College of Science and Engineering, James Cook University
Townsville, QLD, 4811 Australia
maria.nayfa@jcu.edu.au

Traditionally, genealogical data has been utilized to monitor inbreeding rates, relatedness, and co-ancestry within selective breeding programs. Errors within genealogical records are common and have been shown to be as high as 15% in terrestrial selective breeding programs. Less information is available on aquatic breeding programs. The Abbassa strain (AS) is a product of a selective breeding program of Nile tilapia (*Oreochromis niloticus*) that has been managed solely based on genealogical data. To assess the accuracy of recorded genealogical data within the AS, firstly, stringently filtered genome-wide SNPs (1,040) were used to assign parentage and 6,163 SNPs used to determine the level of genetic diversity, the pedigree genetic structure and the number of families present within this line. Inbreeding coefficients and founder contributions were calculated from two founding events for 11 generations of the AS using corrected pedigree records based on molecular data. Pedigree error rates in AS were found to be 45.5% on average. This high pedigree error rate is likely to have contributed to the low levels of genetic gain (3.8-7.0%) per generation observed within the AS, but did not appear to have a major effect on overall inbreeding levels. An assessment of founder contribution to the AS revealed that only 34 founders comprise over 84.3% of available genetic material within the AS in addition to only 27 unique family line identified based on genotyping 19.0-81.2% of generations 9-11. This indicates that founder contribution has been eroded within the AS, and that optimal founder contribution should be taken into consideration in future management strategies for the AS to ensure that the maximum amount of genetic diversity possible is preserved. This study highlights the importance of using molecular data to ensure that genealogical records are accurate so that aquatic selective breeding programs can maximize both the retention of genetic diversity and genetic gain.

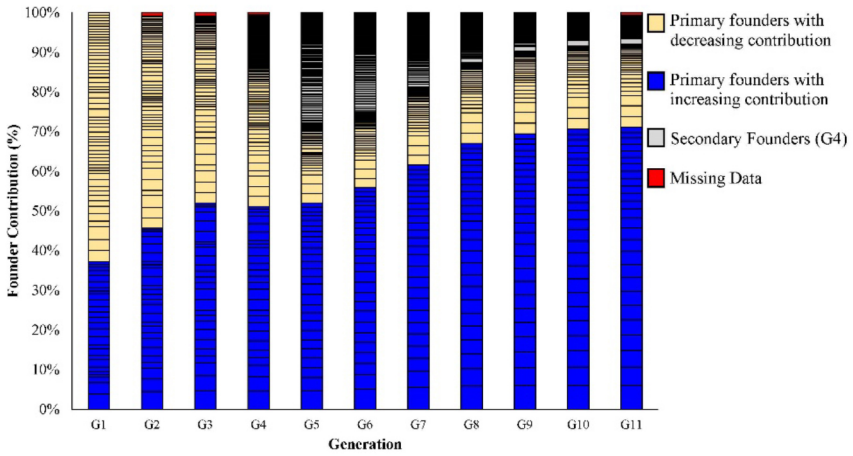


Fig. 1 Genome contributions of founders over 11 generations based on corrected pedigree records.

IMPACT OF VARIOUS DIETARY PROTEIN LEVELS ON THE SURVIVAL, GROWTH AND NUTRIENTS PROFILE OF *Channa marulius* FRY

Sadia Nazir[1*], Noor Khan[1] and Sheeza Bano[1]

[1]Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore-Pakistan
Email:sadianazir824@gmail.com

A three months experimental trial was conducted to find out the protein requirement of *Channa marulius* fry reared in 12 hapa's installed in earthen pond. Each hapa was stocked @ 15 *C. marulius* fry. The average initial weight and length for each treatment was 1.25±0.05g and 4.75±0.11cm respectively. The fish was fed with four formulated diets containing 40%, 45%, 50% and 55% dietary crude protein levels at 100% of their body weight for the first month followed by 70%, 60%, 50% and 40% on weekly basis in second and 40% in the third month. Results revealed that the highest net weight gain (154.72 g), biomass (173.72 g), SGR (2.20) and survival rate (93 %) of fish fry were recorded in 55% (CP) diet. The proximate analysis of whole body revealed that ash contents, crude protein and dry matter were found significantly ($p < 0.05$) higher in T4 followed by T3 than T2 and T1, respectively, whereas in terms of crude fat and moisture there was no significant difference ($p > 0.05$) observed among treatments. The amino acid composition of fish revealed non- significant difference ($p > 0.05$) for cysteine, methionine, aspartic acid, tyrosine and ornithine among treatments while threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, phenylalanine, histidine, lysine arginine and proline were found significantly ($p < 0.05$) higher in T2 and T4 compared to T1 and T3, respectively. It is concluded that a diet of 55% CP showed better growth performance and nutrients profile compared to other treatments for *Channa marulius* fry.

EFFECTS OF FEED ON THE GROWTH AND BEHAVIOURAL PERFORMANCE OF THE EARLY STAGES OF GREEN-AND-BLACK POISON DART FROG (*Dendrobates auratus*) AND BLUE POISON DART FROG (*Dendrobates tinctorius azureus*)

Neo Jie Ning* and Amanda Leong Suk Teng

School of Applied Science, Temasek Polytechnic
21 Tampines Avenue 1, Singapore 529757
xneojieningx@gmail.com

Dendrobatidae is a group of small, brightly coloured poison dart frogs that are becoming popular animals to be kept in public zoos, aquariums and household pets. To reduce reliance on wild ornamental trade, culture of poison dart frogs was carried out. This study aims to determine the effects of diets on the growth and behavioural performance of the tadpoles of Green-and-Black Poison Dart Frog (*Dendrobates auratus*) and Blue Poison Dart Frog (*Dendrobates tinctorius azureus*).

Dendrobates auratus and *D. tinctorius azureus* tadpoles were subjected to different diet treatments - formulated diet (Tetrabits fish pellets) and natural diet (thawed bloodworms). During the sampling period, their total body length of the tadpoles of two species was measured using an image analysis software (ImageJ) so to determine their growth rate. A diet preference study using these two diets was also conducted. Results showed that tadpoles of both species fed with formulated feed had a faster growth rate. However, when given a choice, they preferred natural diet over formulated diet. It can be concluded that formulated diet is more optimal for the growth of these poison frog tadpoles but is less preferred as compared to natural diet. To maximise frog culture, formulated feed could be provided to boost growth rate whereas natural diet could be given as maintenance diet.

DIETARY MANNAN OLIGOSACCHARIDE (MOS) ENHANCED SURVIVAL OF WHITE SHRIMP *Penaeus vannamei* AGAINST ACUTE HEPATOPANCREATIC NECROSIS DISEASE

Wing-Keong Ng*, Joao F.A. Koch, Nadira-Afina Ismail, and Kok-Onn Kwong

Fish Nutrition Laboratory, School of Biological Sciences
Universiti Sains Malaysia, Penang 11800, Malaysia
wkng@usm.my

In recent years, an emerging bacterial disease named Acute Hepatopancreatic Necrosis Disease (AHPND) has caused devastating impacts on the global shrimp industry where shrimp mortalities from 40 to 100% during the first 35 days after stocking shrimp fry in grow-out ponds were encountered. AHPND is now known to be caused by pathogenic strains of *Vibrio* bacteria that produces PirAB-like toxins. It has been reported that shrimp exposure to AHPND-causing bacteria is through the oral route, ingested into the digestive tract where the bacteria initially colonized the stomach and then releases the binary toxins to damage the hepatopancreas. Numerous reports revealed that *Vibrio parahaemolyticus* strains causing AHPND has now shown antibiotic resistance. The use of antibiotics should be avoided in shrimp farming. A new generation of functional aquafeeds containing additives such as prebiotics should be explored as a potential alternative way to enhance shrimp growth and resistance to AHPND.

A feeding trial was conducted to evaluate the efficacy of a highly solubilized MOS product (HyperGen™ , Biorigin, Brazil) on *Penaeus vannamei* postlarvae growth, health and disease resistance to AHPND. The addition of 0.2% or 0.4% MOS did not improve growth but disease resistance was significantly enhanced (Figure 1) after only two weeks of feeding, irrespective of dietary MOS level. *Vibrio* counts in the hepatopancreas of shrimp fed MOS-added diets were significantly lower compared to positive control. The total cultivable bacteria counts showed a similar trend. Histopathology signs of the hepato-pancreas of infected shrimp fed MOS-added diets showed less damage compared to the positive control group.

Prebiotics in shrimp feeds imparted hepatopancreatic protective properties and enhanced shrimp survival during AHPND infection. MOS can be used as functional feed additives in shrimp feeds for disease mitigation.

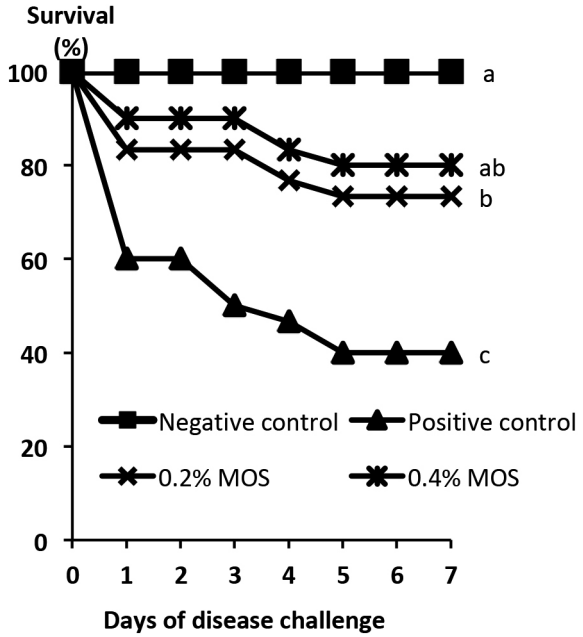


FIGURE 1. The addition of 0.2% or 0.4% MOS significantly ($P<0.05$) enhanced resistance of *P. vannamei* against AHPND. % survival was doubled when MOS was included in the feed compared to the positive

THE EFFECT OF PROBIOTICS ON CORAL RESILIENCE, GROWTH AND COLORATION UNDER PH AND TEMPERATURE STRESSES

Nicole Ng Xuan Teng* and Goh Hui Shan

School of Applied Science, Temasek Polytechnic
21 Tampines Avenue 1, Singapore 529757
nicoleng0807@gmail.com

Coral reefs are experiencing deterioration at a rapid rate due to global warming and ocean acidification caused by CO_2 emissions from human activity. While long term palliative actions focusing on minimizing global changes and CO_2 emissions are at top priority, curative actions such as microbiome manipulation was discovered to have positive effects on corals exposed to heat stressors in terms of mitigating bleaching. The possibility of probiotic supplementation to corals being a solution to coral reef resilience and sustainability when exposed to environmental stressors like heat and low pH is the focus of this study.

A supplement, *Nitribiotic* , containing probiotic bacteria strains Bacillus subtilis, Nitrobacteria, Saccharomyces, Lactobacillus, Bacteries pourpres was dosed in one of two replicated tanks containing the same corals and one of the four environments ; control , heat, low pH as well as low pH and heat combined to stimulate the natural environment .These bacterial strains work in synergy to provide a probiotic effect for marine organisms, improving their immune system for better growth. Photo samples of bleaching percentage, growth data via measurements were obtained at week 0, 6 and 12 of the study to determine effects of supplementation in relation to stressors and compare coral responses to the stressors while other water parameters were kept similar to prevent irregularities in results. It was observed that corals, especially the SPS (small polyp stony) corals without probiotic supplementation started bleaching at 20% and 50% in heat stress and heat stress with addition of low pH tanks at week 3 of the study while the ones with supplementation did not experience bleaching.

Overall, the probiotic supplementation of *Nitribiotic* had the most positive effects on growth, resilience and coloration on corals exposed to heat stress whereas the corals exposed to heat with low pH had less significant positive effects. Lastly, corals with supplementation and exposed to low pH had no significant positive effects.

STUDY OF NITROGEN REMOVAL IN RECIRCULATING AQUACULTURE SYSTEM (RAS) USING NTROGEN ELIMINATION BIOLOGICAL PROCESS

Ng Sky*, Chan Kit Lun, and Khoo Chia Loon

Diploma in Veterinary Technology, School of Applied Science
Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
skyng0208@gmail.com

Recirculating aquaculture system (RAS) is used to filter out organic waste (fish waste) in aquaculture systems. The function of RAS is to convert harmful waste compounds into less toxic, converting ammonia to nitrite and then nitrite to nitrate through nitrification process. If ammonia left untreated in water causes toxic to fish and the nitrifying bacteria helps to oxidize ammonia to nitrate (lesser toxic compound) can then be safely accumulated. Nitrogen elimination bacteria comes in further reduce nitrate to nitrogen gas to be discharged into the atmosphere with no harm.

The discharge of fish waste to the environment and the constant need of manpower to dispose of wastewater from farms has been a huge hassle. Using a RAS system consisting of a SBBR and Anammox chamber, this experiment aims to collect data on nitrifying and denitrifying trends of sludge in the Spinning bed bio-film reactor (SBBR) and Anammox chamber to create an optimal RAS system. Even with minimal water changes the experimental tank showed optimal water conditions in the aquaculture system, which is mostly due to the nitrification of the SBBR and Dissimilatory nitrate reduction to ammonium (DNRA) process of the Anammox chamber. This proves that the new configuration of the RAS is effective in conserving water and reduces the amount of manpower needed in fish culture.

EFFECTS OF DIFFERENT DENSITY AND FEED ON LARVAL QUALITY OF GOLDEN TREVALLY *Gnathanodon speciosus* DURING THE STAGE OF FEED CONVERSION

Ngo Thanh Cam*, Chau Bich Lien, Nguyen Duc Toan, Le Minh Hoang, Ngo Van Manh

Institute of Aquaculture, Nha Trang University
No 2 Nguyen Dinh Chieu Street, Nha Trang City, Vietnam
*Email: cam.nt.60ntts@ntu.edu.vn

The objective of this study was to evaluate the quality of golden trevally larvae through the appropriate density and type of commercial feed at the stage of feed conversion. The experiments on the effect of density and type of commercial feed on the quality of golden trevally during the feed conversion period were conducted in six experimental treatments with a combination of three types of feed (B1, 2/3, Cavias). at two densities (210 and 420 fish/tank). Each treatment was repeated three times for a total of 18 experimental units. Larval quality such as growth, survival rate, respiratory rate and salinity shock were evaluated after four weeks. The results showed that the growth and survival rate decreased gradually, but the respiratory rate and mortality increased with each type of food such as B1, 2/3 and Cavias, respectively. Golden trevally larvae cultured at density of 210 fish/tank had greater growth and survival rate than fish cultured at density of 420 fish/tank. In conclusion, the combination of feed type and density showed that the density of 210 fish/tank with feed type of B1 gave the best larval quality in the present study.

STUDY OF ENERGY UTILISATION EFFICIENCY IN EARLY JUVENILE MALABAR RED SNAPPER (*Lutjanus Malabaricus*)

Si Yan Ngoh* and Katheline Hua

Tropical Futures Institute,
James Cook University,
149 Sims Drive,
Singapore 387380
shawn.ngoh@my.jcu.edu.au

This study aimed to assess the growth and energy partitioning and the effect of dietary lipid versus carbohydrate on energy utilisation efficiency in early juvenile Malabar red snapper. Quadruplets groups of fish (initial weight 57.8g fish^{-1}) were fed two isonitrogenous diets with high fat (HF: 15.2g/100g DM) and low carbohydrate or low fat (LF: 6.6g/100g DM) and high carbohydrate content and at different feeding levels (25%, 50%, 75% and 100% apparent satiation) for 56 days. There was a linear response in fish body weight gain and energy deposition to the feeding levels of both diets. Higher efficiency of energy for growth (k_g) at 0.673 versus 0.503 was estimated for diet HF than LF, indicating juvenile Malabar red snapper utilised dietary lipid more efficiently than dietary carbohydrate. The maintenance energy requirement of red snapper was estimated to be 78 kJ/kg BW$^{0.8}$/day. Results from this study can contribute to the development of optimized and cost-effective feeds for Malabar red snapper.

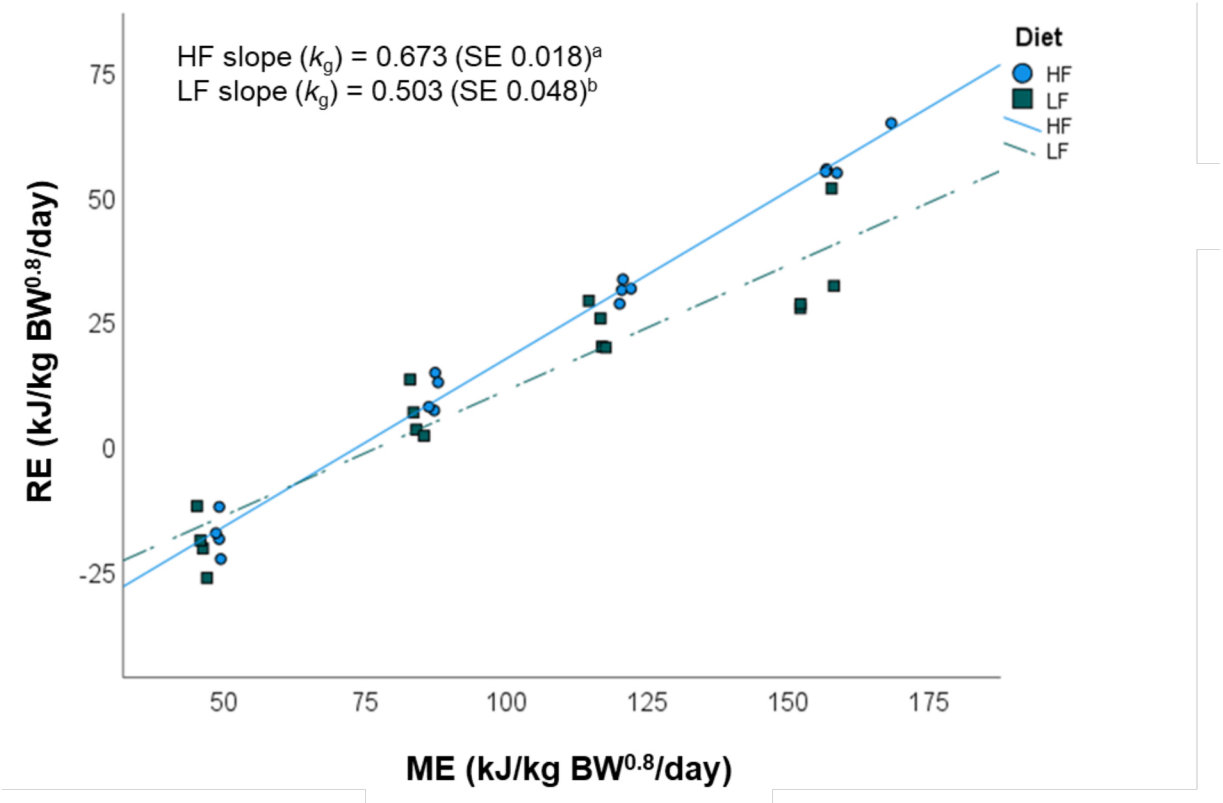


Figure 1. Relationship between retained energy (RE) and metabolizable energy intake (ME) for Malabar red snapper fed either HF or LF diets. HF: RE = 0.673 (SE 0.018) ME – 50.096 (SE 2.053) (R^2 0.99); LF:RE = 0.503 (SE 0.048) ME – 39.374 (SE 5.167) (R^2 = 0.88). Linear regression slopes lacking a common letter differ significantly ($p<0.05$).

Bacillus subtilis DSM33018 MODULATES SURVIVAL, PATHOGEN ATTACHMENT ABILITY AND IMMUNE GENE EXPRESSION OF *Vibrio* INFECTED *Artemia*

Nguyen Ngoc Diem*, Jutta Kesselring, Peter Bossier

Laboratory of Aquaculture & Artemia Reference Centre, Ghent University, Coupure Links 653, 9000 Gent, Belgium
Branch of Biomin VN Ltd at Binh Duong, No.6, Street 20, Vietnam Singapore IIA, IZ, Vinh Tan Ward
Tan Uyen District, Binh Duong Province, Vietnam
Ngocdiem.nguyen@ugent.be
Diem.nguyen-ngoc@dsm.com

The use of probiotics in animal husbandry is an alternative solution to promote animal health and disease prevention and becomes more and more popular. In addition, *Bacillus* is amongst the most common used and intensively studied as probiotic in aquaculture. Although, the precise mechanisms of Bacillus probiotic action are not well documented and remain largely unknown. The abundant of microbiota which presents naturally in the culture system or test animal often interferes the results. Here, we aim to provide a comprehensive understanding about interaction among probiotic *Bacillus subtilis* DSM33018, the host-brine shrimp *Artemia franciscana* larvae, and the pathogen *Vibrio campbellii* in a gnotobiotic system. The strain DSM33018 significantly increased the survival of brine shrimp challenged with *Vibrio campbelii* (survival rate of 77±8% with *Bacillus* supplement compared to 24±2% without *Bacillus* supplement). No growth suppression of *V.campbellii* in the cultured water was observed, although *Bacillus* reduced the amount of attached *Vibrio* in *Artemia* intestinal tract ($1.2x10^5$ cells/20animals in *Bacillus* treatment compared to $3.4x10^6$ cells/20animals in treatment without *Bacillus*). Interestingly, mRNA expression of gene encoding antioxidant enzyme Superoxidase dismutase (SOD), antimicrobial enzyme Phenoloxidase (ProPO) increased significantly in treatment with Bacillus supplementation. We also found out that *Bacillus* administration triggered some shrimp innate related genes leading to improve the early innate responses of the host. In conclusion, our findings showed that *Bacillus* protected the brine shrimp from *Vibrio* infection by reducing the attached *V.campbellii* in the shrimp intestinal tract and enhancing shrimp innate immunity, especially antioxidant capacity for Vibriosis resistance.

MOTIV – AN INNOVATIVE INGREDIENT SERVED FOR A FUNCTIONAL SHRIMP FEED

Nguyen Duy Hoa, PhD.

Global Technical Director.
Empyreal Products, Cargill Inc.
Email: Duyhoa_nguyen@cargill.com

Organic acid has played an important role in enhancing the gut health for several animals, including the livestock and aquatic animals. However, from the field it has showed the commercial products in the market, mainly a combination of formic acid, propionic acid and acetic acid has not worked well for shrimp so far while raw materials, such as soybean meal; fermented with Lactobacillus sp. has been practiced both at farm level and commercially added in commercial shrimp feeds for gut-health benefits. Recently, Cargill has developed a fermented corn protein product, namely "MOTIV" with several trials, testing in the indoor culture system and grow-out farms has resulted in very promising shrimp performances.

MOTIV was first done in the US in an indoor test to compare with a non-fermented corn protein as the control without organic acid adding and with organic acid adding at the same organic-acid level and 3 times of the organic acid level. The results showed to increase upto 19.2% in weight gain and decreased in 16.3% in feed conversion rate for the MOTIV diet vs the control without organic acid adding and it was also better than the control added with the same level of organic acid or 3 times of organic acid adding. Results also showed poor performances in the treatment of adding too much organic acid vs the diet with lower level of organic acid.

A second trial was done in Vietnam and Taiwan with fishmeal replacement by the MOTIV at 6%, 9%, and 12% inclusion of the MOTIV in the diets at medium stress condition (80 PL/m^2) and high stress culture condition (300 PL/m^2). Results showed NO significant differences ($P>0.05$) in the MOTIV diets vs the fishmeal control diet in terms of weight gain, feed conversion rate, and survival in medium stress condition while significant differences in survival rates ($P<0.05$) were recorded between the MOTIV diets (6% and 12%) vs the fishmeal-diet control in high stress culture system.

Commercial trial with a big feed mill in Ecuador for fishmeal replacement with 10 earthen ponds (5 ponds for Motiv diets and 5 ponds for commercial feeds) in around 110 days also resulted in 9.22% of weight gain increasing and decreasing in18.3% of feed conversion rate. Commercial trials in several shrimp feed ponds in Vietnam will also be updated soon.

In conclusion, the MOTIV trails has showed a very positive performances for shrimp in terms of significant improvement of the feed conversion rate, weight gain as well as survivability in high stress condition; therefore, is a promising fermented corn protein for shrimp feed industry to look for a sustainable growth.

ISOLATION OF *Flavobacterium inkyongense* FROM STRIPPED CATFISH (*Pangasianodon hypophthalmus*) IN A DISEASE OUTBREAK

Du N Nguyen*, Lan T Ma, Cuong V. Doan

Email: ngocduaqua@gmail.com
Research Institute for Aquaculture No.2
Ho Chi Minh city. Vietnam

The study report high mortality of stripped catfish fry associated with *Flavobacterium inkyongense* with signs similarly to columnaris disease. Experimental challenges of fish with the isolate showed the clinical signs as in natural outbreak. Mucus was dislodged from the skin of posterior body, beginning from tail base, resulted in discolor of the body part. In some cases mucus accumulated on tail part as cloudy or fungus-like patches, subsequently the loss of epidermis and fish tail. Immersion challenged fish was exposed with 5 x 10^3 CFU/mL to fish weighing about 0.4 to 0.5 grams (under 30 day old) and intraperitoneal challenged fish received 5 x 10^3 CFU/fish in fish from 2 to 3 grams gave high mortalities (up to 100%) within 24 to 48 hours after infection. Light yellow colonies grew on Tryptone Yeast Extract Salts (TYES) agar media after 2 days incubating at 30º C. Bacteria is gram-negative, thin, and long, measuring from 5 - 7μm. The high level identify of its 16S rRNA gene sequence identified the strain as *Flavobacterium inkyongense*. Fresh smears of mucus and gill had lot of thin and long bacteria. Histological analysis revealed the bacteria on the skin of lethal fish. To the best of our knowledge, this is the first report of *F. inkyongense* infection and causing disease outbreak in stripped catfish.

CHEMICAL USE IN INTENSIVE WHITE-LEG SHRIMP AQUACULTURE IN TRA VINH PROVINCE, VIETNAM

Nguyen Quoc Thinh [*] and Tran Minh Phu

[1] College of Aquaculture and Fisheries, Can Tho University, Vietnam
nqthinh@ctu.edu.vn

The aims of this study were to investigate the chemical use and disease occurrence in white leg shrimp (*Litopenaeus vannamei*) aquaculture in Vietnam. The study was based on visiting and interviewing 60 white leg shrimp farms in Duyen Hai and Cau Ngang Districts, Trà Vinh Province. Results showed that common diseases were white feces disease occurred in earthen pond and supper intensive system with pond liner with the same ratio of 56.6%. The hepatopancreas disease appeared differently in earthen pond and supper intensive system with pond liner with the ratio of 40% and 3.3%, respectively. The common used antibiotics were cotrim (23.3%), amoxicillin (20%) and ciprofloxacin (13.3%). Farmers used probiotic in both feeding as digestive enhancement and applying in pond water as water quality improvement. Common species presented in probiotic were *Bacillus subtilis*, *B. licheniformis* and *B. megaterium*. It is necessary to provide training to shrimp farmers to enhance knowledge on effectiveness use of chemicals, and to guarantee the food safety for the final product.

DETERMINATION OF FEEDING ABILITY AND OXYGEN CONSUMPTION ON LARVAE OF GOLDEN TREVALLY *Gnathanodon speciosus* THROUGH DIFFERENT AGES

Nguyen Duc Toan*, Ngo Thanh Cam, Chau Bich Lien, Le Minh Hoang, Ngo Van Manh, Hoang Thi Thanh

Institute of Aquaculture, Nha Trang University, No 2 Nguyen Dinh Chieu Street, Nha Trang City, Vietnam
*Email: toan.nd.60ntts@ntu.edu.vn

The objective of this study was to evaluate the feeding ability and oxygen consumption of larvae of golden trevally *Gnathanodon speciosus* at different ages. Experiments were determined at the age of 10, 13, 16, 19 days after hatching. Each day of age was collected 60 fish at the three different tanks (30 fish for determining the feeding ability, 30 fish remain for determining the amount of oxygen consumption). Feeding ability was determined by the ability to capture Artemia during 5 minutes. The oxygen consumption of fish larvae was determined by closed bottle for 10 minutes. Experimental results showed that the feeding ability and the amount of oxygen consumption changed via different ages. The feeding ability of fish larvae increased with age (10, 13, 16, 19 days) as (0.8333, 1,0000, 1,556, 2,000 Artemia/individual/5 min), respectively. Meanwhile, the oxygen consumption of fish larvae also increases with age. Therefore, it is advisable to increase the amount of food and oxygen content for good growth of larvae following the age of golden trevally larvae.

EFFECTS OF TEMPERATURE AND SALINITY ON LARVAL QUALITY OF GOLDEN TREVALLY *Gnathanodon speciosus*

Nguyen Tan Khang*, Ho Thanh Tuyen, Ngo Van Manh, Le Minh Hoang, Mai Nhu Thuy

Institute of Aquaculture, Nha Trang University, No 2 Nguyen Dinh Chieu Street, Nha Trang City, Vietnam
*Email: khang.nt.60ntts@ntu.edu.vn

The objective of the present study was to evaluate the effects of temperature and salinity on the quality of larvae of golden trevally *Gnathanodon speciosus*. The experiment was carried out in three temperatures (30, 32, 34°C) combined with four salinities (15, 20, 25, 30 ppt). Each treatment was repeated five times. The quality of larvae included growth, survival rate, feeding ability and respiration was determined after two weeks of experiment. The results showed that the growth, survival, feeding ability as well as respiration of golden trevally larvae were the most affected by the combined treatment of 34°C with 20 ppt. Meanwhile, treatment at the temperature of 30°C combined with salinity of 30 ppt gave the best survival, growth, feeding ability and respiration. If the temperature was increased to 32°C, the quality of golden trevally larvae tends to decrease and decrease sharply if the temperature was increased to 34°C. Moreover, high temperature combined with low salinity strongly affects the quality of golden trevally larvae. Therefore, the quality of golden trevally larvae will be reduced if they culture at low salinity conditions combined with high temperature.

CONTROLLING THE UNCONTROLLABLE IN A VUCA WORLD

Nguyen Van Tien* and Pradeep Krishnan

Evonik Viet Nam LLC
Ho Chi Minh city, Viet Nam
Email: vantien.nguyen@evonik.com

Few industries feel the impact of today's "VUCA" world - volatility, uncertainty, complexity, and ambiguity - as much as animal and aquaculture farming. From the immediate pressures farmers face in growing aquaculture and meeting consumers' changing needs to the accelerated adoption of technology and long-term concerns about environmental impact, the aquaculture industry is grappling every day with incredibly complex questions in a rapidly evolving industry. In recent times, the global aquaculture sector and the feed industry is confronted with dramatically increased costs of raw materials and energy. In aquaculture production, more than half of the total costs is contributed by the feed cost. Thus, the focus must be on assuring an efficient usage of the feed by the targeted species. This takes into consideration the application of the latest concepts of aquaculture nutrition; formulating diets on basis of digestible amino acids and modern systems of energy evaluation which can be summarized as efforts to achieve precise nutrition. Digital tools and technologies are being adopted by the industry to complement and help collect the necessary data to optimize the whole system of aquaculture production. On the other end, it is also necessary to have good knowledge about the nutritional quality of the feed ingredients in use and the final feed to raise healthy animals starting from the breeding operation and subsequently good quality protein source from those targeted species, with maximum efficiency and profitability. Aquaculture nutrition is a complex science and approximations are a necessary evil, particularly in the business of feeding aquatic species. Practicing aquaculture nutritionists have a dollar value or a growth response cut-off expectations for the feeding recommendations that are followed. To gain consensus on these cutoffs, combining analytics and production data is the practical way followed from an industry perspective while optimizing the productivity of the farming operation. The analytical options for quality control of feed ingredients and finished feeds are gaining even more importance if alternative feed ingredients or sources of feed ingredients shall be used while navigating a VUCA world. Analytics for precision management usually falls under four levels of classification: I) technique, II) data interpretation, III) integration of information, and IV) decision making. Moving forward analytical and data management tools that address the integration of all the above levers is of critical importance for the precision feeding of aquaculture. There is no single action that will improve production efficiency, modernize business operations and meet consumer expectations. However, a system solutions approach could offer a holistic and sustainable value proposition for aquaculture production. By combining products and services, and leveraging digitalization opportunities, in cooperation with high-value consultancy and a deep understanding of species-specific requirements, we as an industry can turn science-based, efficient, and sustainable nutrition, and precision aquaculture farming into value for end consumers. In summary, the overarching goal of the aquaculture industry remains the same as it has throughout human history irrespective of the external forces: to feed the growing global population most affordably. What is becoming increasingly important is the quality of products feeding the world and the sustainability of the practices used to do it.

STATUS OF MAJOR COASTAL FISHING ACTIVITIES IN THE MEKONG DELTA, VIETNAM

Nguyen Thanh Long*, Mai Viet Van, Dang Thi Phuong, Naoki Tojo and Tran Dac Dinh

College of Aquaculture and Fisheries
Can Tho University
Can Tho City
Viet Nam

A study on the major fisheries was conducted from August 2017 to April 2018 in coastal provinces of the Mekong Delta, Vietnam. Results showed that the gillnets, trawl nets, and stow nets were the main fishing gears, accounted for the highest number of fishing boats and yields. All the fishing gears exploited for the whole year-round. The results also indicated that gillnets fishery is the largest scale (10.99 tons). The most effective fishing activity of gillnets fishy was the highest profit (298 million VND per year) ($p < 0.05$). However, the highest fishing yield (20.42 tons/year) was found in trawler but it had the lowest rate of return (0.45 times) and trash-fish portion accounted the highest ratio (38.4%). Although the stow nets had highest rate of return (1.41 times), but they were lowest yield (7.17 tons/year) and high portion of trash-fish. The high proportion of trash-fish may affect to fisheries resources. For the sustainable development of the fisheries in the Mekong Delta, the development and management of fisheries resources should be promoted, supporting fishermen to access low interest rates to invest in capture production, and training fishermen to use advanced fishing gear to increase their fishing efficiency.

THE ROLE OF RED SEAWEED *Gracilaria tenuistipitata* ON WATER QUALITY AND PERFORMANCES OF WHITE LEG SHRIMP *Litopenaeus vannamei* POSTLARVAE REARED IN INTEGRATION SYSTEM

Nguyen Thi Ngoc Anh*, Bui Nguyen Thu An, Lam My Lan and Tran Ngoc Hai

College of Aquaculture and Fisheries
Can Tho University
Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Vietnam.
ntnanh@ctu.edu.vn

The white leg shrimp (*Litopenaeus vannamei*) is one of the most important aquaculture species, accounting for 80% of global shrimp productivity due to its wide salinity tolerance, low protein requirements, fast growth rate, and high resistance to poor environmental conditions. This species has been farmed and established in several countries throughout Asia, including Vietnam, and is playing a significant role in shrimp aquaculture production. There is evidence from many countries that intensive shrimp farming has caused environmental deterioration that represents a major threat to the health of farmed shrimp due to the potential for disease outbreak, which can result in significant economic losses. The current study aims to assess the combined effects of different stocking densities of white leg shrimp *L. vannamei* postlarvae and red seaweed *Gracilaria tenuistipitata* on water quality as well as the performance of shrimps against *Vibrio parahaemolyticus* challenge under laboratory conditions.

A 30-day experiment was conducted to evaluate the effects of integrating different densities of white leg shrimp *L. vannamei* postlarvae (PL) and red seaweed *G. tenuistipitata* on water quality and shrimp performance in the nursery phase. A 3×4 factorial experiment with three levels of shrimp density (1,000; 2,000, and 3,000 PL m^{-3}) and four levels of red seaweed density (0, 1.0, 1.5, and 2.0 kg m^{-3}) was randomly designed in triplicate. Shrimp PL12 (initial weight of 4.27±0.28 mg) and red seaweed was stocked in 150-L tanks at a salinity of 15 g L^{-1}.

The results indicated that the integration of shrimp PL and red seaweed significantly reduced the nitrogen and phosphorus contents in the culture tanks and improved the survival and growth rate of shrimp, although not significantly. A significant interaction effect ($p<0.01$) between shrimp and seaweed densities was only observed for shrimp production. Particularly, higher stocking density resulted in lower growth performance but enhanced production. Applying stocking densities of 1,000 and 2,000 PL m^{-3} reared shrimp that were bigger in size, while a density of 3,000 PL m^{-3} obtained the highest production output in the integrated system. Following the 30-day growth trial, shrimp quality was examined through a bacterial challenge test using pathogenic *Vibrio parahaemolyticus* on the shrimp groups previously reared at a density of 3,000 PL m^{-3} and integrated with different amounts of seaweed. After 14 days of immersion challenge, the cumulative mortality in the control group was significantly higher than that of other groups. This result suggests that the presence of *G. tenuistipitata* improved the antibacterial activity of *L. vannamei* against *V. parahaemolyticus* during the nursery phase.

IN VIVO Edwadrsiella tarda PHAGE (ETP-1) THERAPY EFFICACY UPON TWO MODES OF PHAGE ADMINISTRATION

Chamilani Nikapitiya[1,*], Jehee Lee[2] and Mahanama De Zoysa[1]

[1]College of Veterinary Medicine, Chungnam National University, Yuseongu, Daejeon 34134 Rep. of Korea
[2]Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province, 63243, Rep. of Korea
[*]Email: chamilani14@gmail.com

Phage therapy is a biologically friendly strategy for controlling bacterial pathogens in aquaculture as an alternative to antibiotics and rapid spread of multidrug resistant pathogens. For efficient phage therapy, phage needs to reach target organs and/or tissues of animals in adequate amounts at right time during bacteria infection. Previously, we isolated and characterized multidrug resistant *Edwardsiella tarda* infecting phage ETP-1 from farm seawater. In this study, we investigated ETP-1 dispersion and persistency efficiency following two modes of ETP-1 administrations, namely (1) intra peritoneal (i.p.) injection and (2) continuous ETP-1 exposure for 12 days to zebrafish at 10^5,10^8 and 10^{11} PFU/mL. In addition, biocontrol efficacy of ETP-1 was tested and transcriptional responses were examined for selected immune genes in kidney and gut tissues at 1, 7, 10 and 12 day post exposure (dpe) to determine safety in concern of phage therapy.

Results showed that ETP-1 disseminated to all the tissues as quickly as day 1 by two modes of phage administration. In i. p. injected tissues, persistency was decreased in different levels with the time, while in bath exposure (10^{11} PFU/mL), persistency looked consistent throughout the experiment. Moreover, among immune genes (*il1-β*, *tnf-α*, *il-6*, *il-10, cxcl-8a and sod-1*), no significant differences ($P<.05$) in the mRNA expression were observed in ETP-1-treated and control groups, except *il1-β* and *sod-1* at 1 dpe in kidney. In the gut, except *il1-β* and *cxcl-8a*, other genes were down regulated at day 1, but with the time, levels were became basal or slightly higher (except *il1-β*) than the controls of respective time points. This indicates, even though, the ETP-1induced some cytokine genes at certain time points, it did not show adverse apparent immune stimulation in host, and use of phage at 10^{11} PFU/mL was safe. In the zebrafish that bath exposed to ETP-1 for 12 days, and simultaneously challenged with *E. tarda* (1.08×10^5 CFU fish^{-1}), the survival rate was higher in ETP-1 exposed fish (68%) than the control (18%) until 4 days post challenge. In summary, our data indicate that even the absence of host bacteria as a phage carrier, ETP-1 could transport to the neighboring internal organs. Moreover, results suggest that during systemic bacterial infection stage, phage therapy may work efficiently to kill *E. tarda* cells, which internalize in those organs.

THE INHIBITING EFFECT OF DANTROLENE SODIUM ON THE HARDENING OF FRESH SCALLOP ADDUCTOR MUSCLE

Yabin Niu*, Shiliang Dong, Jianjun Xu, Xin Lu, Naoto Shimakage, Kuniaki Sasaki, Yuanyong Tian, Tetsuro Yamashita, Chunhong Yuan

United Graduate School of Agricultural Sciences, Iwate University
Ueda 3-18-8, Morioka, Iwate, Japan
u3220006@iwate-u.ac.jp

Yesso scallop (*Mizuhopecten yessoensis*) is one kind of economic fishery product in Japan. Adductor muscle is the main edible part of scallops, which has a high nutritive value. It has great popularity in making sushi and sashimi using both fresh and frozen-thawed scallop adductor muscle as raw materials in Japan. Nevertheless, people prefer to eat fresh adductor muscle because the quality of frozen-thawed adductor muscle could decline rapidly. However, the hardening of fresh scallop adductor muscle during storage is a major problem. The structure of the sarcoplasmic reticulum (SR) may be damaged with prolonged storage, thereby releasing internal Ca^{2+} into the cytoplasm, ultimately leading to muscle contraction. Dantrolene sodium (DS) has been reported to inhibit muscle contraction. However, DS is mostly used in the medical field, and there is no report that DS inhibits the hardening of aquatic products. The present study was conducted to examine the inhibiting effect of DS on the hardening of fresh scallop adductor muscle.

The fresh-shucked scallop adductor muscle (n = 60) was divided into three groups: control (non-immersion), seawater (immersed for 1 h), and DS group (immersed for 1 h). All the groups were stored at 4 °C for 7 days. The photos were taken each day to calculate the hardening rate. The determination of pH value and ATP-related compounds was taken to indicate the changes in freshness. Scanning electron microscope (SEM) was used to compare the changes in microstructure of scallop adductor muscle before and after hardening. The SR was also extracted and observed by transmission electron microscope (TEM).

When the adductor muscle in control group showed signs of hardening on day 2, there was almost no change in the DS group compared with the initial. When the hardening rate in control group reached 33.3% on day 4, only one adductor muscle in the DS group was completely hardened, which may be due to individual differences. The results indicated that dantrolene sodium has an inhibiting effect on the hardening of scallop adductor muscle. This study provides ideas for exploring the delay of scallop adductor muscle hardening.

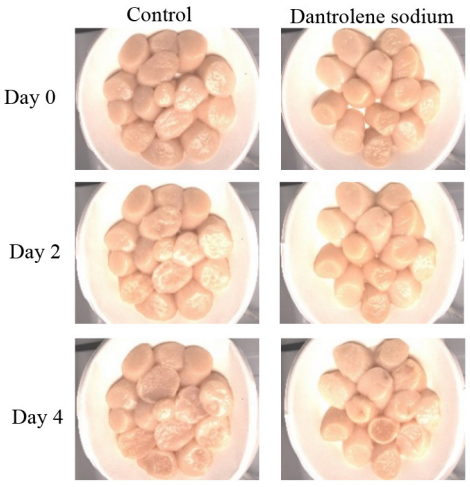


Fig.1. Effects of dantrolene sodium on the appearance of scallop adductor muscle during storage at 4°C

INFLUENCES OF THE DESIGN AND MANUFACTURING OF VARIOUS BIOCARRIERS ON THEIR PERFORMANCE IN MOVING BED BIOFILM REACTORS (MBBRs) IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

William Nowell*, Giovanni M. Cusimano, Jui-Che Chiang, Natalie E. Panasiak, Michele Gallo, Shane A. Hunter, Dannie O'Brien

AquaBioTech Group, Research Development and Innovation, Naggar Street, Targa Gap, Mosta, Malta
Email: whn@aquabt.com

For optimal water quality management in RAS, a primary design concern is to provide the biofilter capacity required to control the total ammonia-nitrogen (TAN) concentration in the culture tanks. An optimal biocarrier will have a short start-up time- the time between when the biocarrier is added to the system and when the bacterial population can support the welfare of the needed biomass of aquatic species to be produced- and a high total ammonia nitrogen (TAN) removal rate during operation. The most challenging of these start-ups are in cold water systems in a new system, where there is no existing 'mature' biomedia for inoculation of the biological community and lower metabolic activity at low temerature. Experiments assessed the start-up time of five different designs of biocarriers in 14°C fresh water and, once nitrifying activity was established, their TAN removal capacity.

Five biocarriers of different designs, specific surface area (SSA) and protected surface area (PSA), from 5 different suppliers were compared. SSA varied between 700 and 5500 and materials included virgin and recycled polyethylene, HDPE, and polypropylene. Experiments were carried out in 300L round tanks with an operational volume of 200L and maintained at 14°C. Each biocarrier type was stocked at 50% of the operational volume (0.1m^3 per tank) in triplicate and hydrated for 48 hours before the trial start. Aeration was set at 20L/min/tank using blowers in the tanks. Following initial dosing to start biological activity in the tanks (NH_4CL (3.8g); $NaHCO_3$ (43g); $NaNO_2$ (0.4g); Na_2HPO_4 (0.08g) per tank) tanks were "fed" with calculated quantities of NH_4Cl and $NaHCO_3$ according to observed conversion rates, to promote ongoing nitrifying activity. Over 10 weeks, DO; temperature; salinity; pH; KH; NH_4; NO_2; NO_3 data were collected at regular intervals using either probes (DO, temperature, salinity) or standard drop test kits, easily available to aquaculture producers, and with spectrophotometric titration.

There was variation between hydration time required for the different biocarriers. Faster hydration times were related to faster start-up times. Start-up of bacterial development differed between biocarriers (2 weeks to 4 weeks) as did cumulative TAN conversion performance after start-up (week 4-10). Maximum daily conversion rates reflected the cumulative trend. Consumption of bicarbonate was found to be proportional to conversion of TAN and was higher than the typical values considered for a mature system, likely due to increasing conversion demand during the start-up phase. Start-up time and TAN conversion performance were not found to be correlated with SSA, contrary to common industry assumptions. Additionally, PSA was not well related to rates of TAN conversion. Degassing affecting CO_2 levels; SSA vs. PSA; mixing rates; and the shape of holding tanks may have a greater influence than either SSA or PSA alone. Selection of biocarrier for optimal cold start-up of RAS systems appears to be more complex than selecting that with the greatest SSA. Users should consider their production method and harvest strategy (e.g., continuous, batch all in/all out) when considering optimal biocarrier selection.

DIETARY EVALUATION OF SINGLE CELL PROTEIN IN DIETS FOR RAINBOW TROUT

N.W. Thorringer, E. Servet, Ignasi Sanahuja, and E. Ntokou*

*Unibio
Langebjerg 1, 4000 Roskilde, Denmark
En@unibiogroup.com

As the world population rises, the demand for protein-rich foods increases. The aquacultural sector can provide protein-rich food for the increasing world population. Several studies have tried to find sustainable and renewable protein sources to substitute fish meal (FM) in fish feed, however, some ingredients as vegetable protein and industrial by-products might have a poor nutritional composition and be inadequate in essential components (Zamani et al., 2020). The aim of this study was therefore to determine the optimal level of FM replacement within compound diets for juvenile rainbow trout (*Oncorhynchus mykiss*) by single cell protein (Uniprotein Aqua, Unibio).

Five diets were included in the study with a control diet (D1) formulated based on commercial feed for rainbow trout. The four other dietary treatments were formulated by replacing FM with SCP (Uniprotein Aqua, Table 1). All diets were isoproteic (42% crude protein), isolipidic (21% crude fat) and isoenergetic (22.1 MJ/kg) produced by Sparos Lda. (Portugal). Trout (n=720) were individually measured and randomly distributed in 24 tanks (500 L) attached to an IRTAmarTM unit for mechanical, biological filtration and UV treatment, after a two-week acclimation at the Institute of Agrifood research and Technology (IRTA, Spain). The dietary treatments were provided twice a day (0800 and 1300) through automatic feeders (Arvo-tec T drum 2000, Arvotec, Huutokosk, Finland) and feed residuals were collected after two hours to determine daily feed intake. The body weight (BW) and standard length (SL) of the trout were measured in the beginning and end of the trial (83 days). The initial BW was 11.3±0.2 g (mean±SD) and standard length 9.0±0.1 cm. Histological analysis of the liver and foregut-middle intestine was also collected and examined under a light microscope.

The optimal level of FM replacement with Uniprotein Aqua was estimated by quadratic regression to 41% (R^2=0.57, P<0.05). The highest somatic growth was estimated for diet 3 compared with diet 5 (Table 1). No differences in total feed intake or feed conversion ratio (FCR) were measured despite the lowest FCR was determined for diet 3 (Table 1). No differences were found between dietary treatments for histology of the liver or intestine. For the liver no melanomacrophage centers or lymphocyte infiltration were found indicating healthy liver conditions for all diets. For the intestine, no inflammatory disorders or enteritis were recorded for any of the dietary treatments.

From this study it can be concluded that the optimal level for replacing FM with Uniprotein Aqua was 41% without affecting performance, growth, or histology of the liver and intestine.

Ref: Zamani, A., Khajavi, M., Nazarpak, M.H., Gisbert, E. 2020. Evaluation of a Bacterial Single-Cell Protein in Compound Diets for Rainbow Trout (Oncorhynchus mykiss) Fry as an Alternative Protein Source. Animals. 10:1676.

Table 1. Effect of fish meal replacement (FMr, %) with Uniprotein Aqua on total feed intake (TFI, g), end body weight (BW, g), feed conversion ratio (FCR, g feed/g BW gain), and specific growth rate (SGR, % BW/day) given in mean±SD.

Diet	D1	D2	D3	D4	D5
FM	0	25	50	75	100
TFI	4171±125	4231±135	4264±93	4176±118	4166±120
End BW	$191.3±8.4^{ab}$	$197.9±2.0^{ab}$	$201.2±6.1^{a}$	$192.1±9.7^{ab}$	$183.4±4.5^{b}$
FCR	0.81±0.01	0.81±0.02	0.79±0.02	0.84±0.02	0.83±0.02
SGR	$3.41±0.04^{ab}$	$3.45±0.02^{a}$	$3.47±0.04^{a}$	$3.41±0.06^{ab}$	$3.36±0.01^{b}$

EVALUATION OF DIETARY BENFOTIAMINE ON GROWTH AND IMMUNE RESPONSE OF *Penaeus monodon* POST LARVAE

Arnel G. Nudalo*, Barry Leonard M. Tumbokon, Augusto E. Serrano, Jr.

1. Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas Miagao 5023 Iloilo, Philippines
2. National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas Miagao 5023 Iloilo, Philippines
agnudalo@up.edu.ph

Normally, shrimps are not well adapted to high amounts of dietary carbohydrate and results in poor growth performance. Thiamine (vitamin B1) plays a key role in the conversion of carbohydrates into energy. Benfotiamine is a lipid-soluble derivative of thiamine and is absorbed up to 3.6 times more than the water-soluble forms of the vitamin. The present study aims to evaluate the effects of high carbohydrate diet alone or supplemented with benfotiamine on growth and immune response of *Penaeus monodon* post larvae.

An attractability test and two feeding trials were conducted- the first feeding trial aimed to compare the effects of a normal dietary carbohydrate level (C) with those of a high carbohydrate diet (HC) and with those of a high carbohydrate diet supplemented with 0.02 g/kg benfotiamine (HCB). At the termination of the feeding trial, the shrimps were subjected to sublethal ammonia toxicity test to determine the effects of the diets on the immune response of the experimental shrimps. The second feeding trial aimed to determine the optimum inclusion level of benfotiamine at 0.02, 0.04, 0.06 and 0.08 g/kg.

In conclusion, benfotiamine enhances growth performance and immune response at an optimum inclusion of 0.04 g/kg.

Fig. 4 **showed the result of the second feeding trial. The diet containing 0.04 g/kg benfotiamine exhibited significantly higher final average body weight, weight gain, specific growth rate and feed intake than those fed the diet containing 0.02 g/kg but was not significantly different from those fed diets at 0.06 or 0.08 g/kg.**

Treatments	IABW (g)	FABW final (g)	SGR (%)	WG (g)	FI (g)	FCR (g)	Surv (%)
Control	0.01	0.23 ±0.00^{b}	5.44 ±0.03^{b}	0.22 ±0.00^{b}	0.39 ±0.0^{b}	1.8 ±0.00^{b}	99.99
HC	0.01	0.15 ± 0.00^{c}	4.63 ±0.04^{c}	0.14 ±0.00^{c}	0.26 ±0.0^{c}	1.9 ±0.03^{a}	99.99
HCB	0.01	0.45 ± 0.00^{a}	6.56 ±0.02^{a}	0.44 ±0.01^{a}	0.52 ±0.0^{a}	1.2 ±0.00^{c}	99.99
Treatments	**IABW (g)**	**FABW final (g)**	**SGR (%)**	**WG (g)**	**FI (g)**	**FCR (g)**	**Surv (%)**
Control	0.01	0.23 ±0.00^{b}	5.44 ±0.03^{b}	0.22 ±0.00^{b}	0.39 ±0.0^{b}	1.8 ±0.00^{b}	99.99
HC	0.01	0.15 ± 0.00^{c}	4.63 ±0.04^{c}	0.14 ±0.00^{c}	0.26 ±0.0^{c}	1.9 ±0.03^{a}	99.99
HCB	0.01	0.45 ± 0.00^{a}	6.56 ±0.02^{a}	0.44 ±0.01^{a}	0.52 ±0.0^{a}	1.2 ±0.00^{c}	99.99
Treatments	**IABW (g)**	**FABW final (g)**	**SGR (%)**	**WG (g)**	**FI (g)**	**FCR (g)**	**Surv (%)**
Control	0.01	0.23 ±0.00^{b}	5.44 ±0.03^{b}	0.22 ±0.00^{b}	0.39 ±0.0^{b}	1.8 ±0.00^{b}	99.99
HC	0.01	0.15 ± 0.00^{c}	4.63 ±0.04^{c}	0.14 ±0.00^{c}	0.26 ±0.0^{c}	1.9 ±0.03^{a}	99.99
HCB	0.01	0.45 ± 0.00^{a}	6.56 ±0.02^{a}	0.44 ±0.01^{a}	0.52 ±0.0^{a}	1.2 ±0.00^{c}	99.99

Table 1. Composition of experimental diets to determine the effects of high carbohydrates supplemented with benfotiamine .

Feed Ingredients	Control (g)	HC (g)	HCB (g)
Shrimp (Acetes) meal	200.0	200.0	200.0
Soybean meal	200.0	200.0	200.0
Corn/wheat starch	130.0	200.0	200.0
Cod liver oil	40.0	40.0	40.0
CMC	104.8	34.8	34.78
Lecithin – Soy (70%)	5.0	5.0	5.0
Trace mineral premix	10.0	10.0	10.0
Vitamin premix	10.0	10.0	10.0
Peruvian Fishmeal	300.0	300	30(
BHT	0.2	0.2	0.2
Benfotiamine	0.0	0.0	0.0
TOTAL	1000.0	1000.0	1000.0

Table 2. Determined proximate composition (dry weight basis)

	C (%)	HC (%)	HCB (%)
Moisture	7.44	6.71	6.67
Crude Protein	47.50	47.09	46.30
Crude Lipid	8.31	9.80	9.43
Crude Fiber	2.52	2.28	2.34
Ash	13.18	14.75	14.13

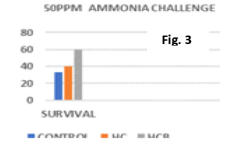


Fig. 3 **showed the results of the 3 days ammonia challenge 0f 50 ppm. The shrimps fed with diet HCB exhibited significantly the highest survival rate of 60% while those fed with diet C and HC exhibited lower survival rates of 33% and 40%, respectively, and were not significantly different from each other.**

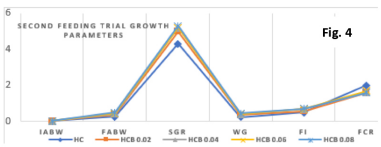


Fig.1 **showed that the HCB diet elicited significantly the highest attractability after 5 min of feed placement in the chamber.**

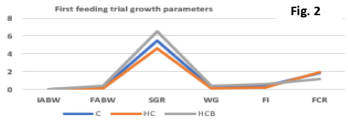


Fig. 2. **For the first feeding trial that lasted for 60 days, results showed that shrimps fed with HCB 0.02 g/kg exhibited significantly the highest growth among all groups in terms of final average body weight, weight gain, and specific growth rate and feed intake.**

PREVENTIVE STRATEGIES BASED ON HEALTH PROMOTING FEED ADDITIVES TO REDUCE THE IMPACT OF DISEASE

Waldo G. Nuez-Ortín*, Maria Mercè Isern-Subich

Adisseo
Hoogveld 93
9200 Dendermonde,
Belgium
waldo.nuezortin@adisseo.com

Functional feed additives are increasingly important to support the aquaculture industry in achieving more cost-efficient feeds and in reducing the risk and severity of disease. More specifically, health-promoting additives have proved to be efficient tools to reinforce prevention strategies against major bacterial diseases in fish and shrimp.

The present research evaluated different cost-efficient supplementation strategies to reduce the impact of relevant diseases in tilapia and shrimp production. In shrimp, reductions in mortalities under *V. parahaemolyticus* infection challenge were significantly improved by 60%, and attributed to antimicrobial effects of the additive and reduced damage of hepatopancreas (Figure 1). In tilapia, mortalities were reduced by 32 and 17% under infection challenges with *Francisella noatunensis* and *Streptococcus agalactiae*, respectively, and attributed to antimicrobial effects and enhanced immunecompetence.

The efficacy of preventive and cost-efficient doses of specific health additives to reduce the likelihood and severity of disease, and to ensure growth recovery post-outbreak, is here further proved.

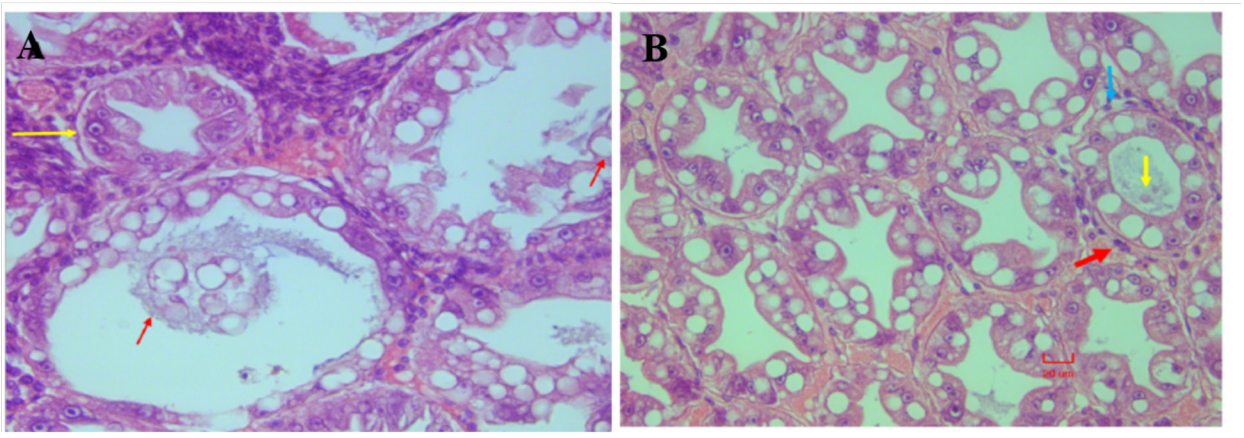


Figure 1.A) Cellular detachment of hepatopancratic tubules with infiltration of haemolyph. B) Normal hepatopancreatic tubules with infiltration of haemolymph.

ASTAXANTHIN KRILL OIL IMPROVES THE GROWTH OF JUVENILE *Litopenaeus vannamei* RAISED UNDER OSMOTIC STRESS IN AN ENCLOSED-TANK CULTURE SYSTEM

Alberto J.P. Nunes, Lucas Alves Rufino, Simone Sales Pinheiro, Lena Burri

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará, Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081, Brazil
alberto.nunes@ufc.br

This work evaluated if dietary supplementation of astaxanthin krill oil (QRILL™ AstaOmega Oil, Aker Biomarine Antarctic AS, Norway) with or without soybean oil to a grower shrimp feed enhances the growth performance of *L. vannamei* farmed under osmotic stress. Shrimp of 1.08 ± 0.11 g body weight (BW) were reared for 74 days under 135 animals/m^2 in 50 outdoor tanks of 1 m^3. Half of the tanks operated enclosed by a milky-colored sheeting and the remaining fully exposed. Shrimp were fed a 38% crude protein feed top-coated with astaxanthin krill oil (K) and/or soybean oil (S) at 0K-3S, 1K-2S, 2K-1S, and 3K-0S% of the diet, as fed basis, respectively.

In exposed tanks, mean salinity reached 31 ± 6 g/L compared to 36 ± 4 g/L in enclosed tanks. Shrimp survival was similarly high in both rearing systems (90.6 ± 3.8 and 89.2 ± 5.3% in exposed and enclosed tanks, respectively (Table 1) and was unaffected by oil supplementation ($P > 0.05$). Shrimp raised in the exposed tanks achieved a significantly higher final BW, weekly growth, gained yield, apparent feed intake (AFI) and a lower FCR (feed conversion ratio) compared to animals in the enclosed tanks. As a result of top-coating feed for the exposed tanks with the 1K-2S oil mix, the highest shrimp final BW (14.03 ± 0.52 g) and yield (1,515 ± 40 g/m^2) could be achieved when compared to the enclosed rearing system and all the other diets. Under longer exposure periods to hypersalinity in enclosed tanks, a minimum of 2K-1S was required to maximize BW (10.80 ± 0.63g) when compared to the other enclosed diet groups.

EFFECT OF GRADED-LEVELS OF DIETARY ASTAXANTHIN KRILL OIL TO THE GROWTH PERFORMANCE OF NURSERY-REARED *Litopenaeus vannamei*

Alberto J.P. Nunes, Hassan Sabry Neto, Lena Burri

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará
Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081, Brazil
alberto.nunes@ufc.br

The study consisted of two separate nursery trials in which post-larval (PL) *L. vannamei* were fed graded dietary levels of astaxanthin krill oil (QRILL™ AstaOmega Oil, Aker Biomarine Antarctic AS, Norway). In the first trial (trial #01), 142,245 PLs of 3.6 ± 0.3 mg body weight (BW) were stocked under 2.4 PLs/L (3,556 ± 31 PLs/tank) in forty 1-m^3 outdoor tanks and raised for 51 days. Shrimp were fed 20 times day and night with diets containing 0 (AKO0), 1.00 (AKO1), 3.00 (AKO3), and 5.00% (AKO5) astaxanthin krill oil (AKO) included at the cost of soybean oil (SBO) and soy lecithin oil (SLO). Diets were formulated to contain 18.00% salmon meal, along with 4.45% of poultry viscera and bone meal, 20.39% soybean meal (SBM), 10.00% wheat gluten meal (WGM), and 8.00% soy protein concentrate (SPC). After harvest, a stress test was carried out to evaluate shrimp resistance to a sudden and acute change to water salinity and temperature. In trial #02, 65,588 PLs of 2.5 ± 0.3 mg BW (1,252 ± 29 PLs/tank) were raised in fifty 0.5-m^3 indoor tanks for 42 days. Shrimp were fed 8 times a day exclusively in feeding trays. A negative control (AKO0KM0) diet was formulated without any AKO. It contained 29.80% salmon meal combined with 18.98% SBM, 7.00% SPC and 5.00% WGM. Four other diets were prepared by partly replacing salmon meal for 8.00% of a high-protein krill meal (KM, QRILL™ Aqua High Protein, Aker Biomarine Antarctic AS, Norway), including a positive control (AKO0KM8) without AKO. Other diets were formulated with 3.00 (AKO3KM8), 5.00, and 7.00% AKO added at the cost of SLO and SBO. In trial #01, no statistical effect from the inclusion of AKO was observed on final shrimp survival (84.0 ± 16.8%), gained yield (1,568 ± 181 g/m^3), daily growth (14.2 ± 2.8 mg/day), apparent feed intake (AFI, 1.12 ± 0.07 g of feed/shrimp) and feed conversion ratio (FCR, 1.70 ± 0.16). However, shrimp fed KO5 achieved a statistically higher final BW (857 ± 140 mg) in comparison to all other diets. In trial #02, shrimp final survival (91.8 ± 14.2%), gained yield (1,611 ± 181 g/m^3), daily growth (14.2 ± 1.8 g/m^3) and FCR (0.89 ± 0.09) were not significantly affected by dietary treatment. There was significant effect of AKO over AFI, starting at 3% dietary inclusion. Shrimp fed diets containing AKO significantly enhanced final BW starting at 3% dietary inclusion (AKO3KM8, 629 ± 76 mg), but not beyond this level. Shrimp fed diets with 5 and 7% AKO (613 ± 81 and 612 ± 105 mg, respectively) did not improve BW compared to 3% or to the diet with KM only (KO0KM8, 583 ± 55 mg). There was no positive effect over shrimp final BW as a result of the inclusion of 8% KM since it did not differ from shrimp fed the control diet (AKO0KM0, 567 ± 65 mg). In the stress test, when water salinity dropped from 39 g/L to 0, shrimp survival showed a clear trend towards higher survival due to the progressive increase in the dietary inclusion of AKO. A minimum of 1% AKO (AKO1, 80.0 ± 10.00%) was sufficient to promote an enhanced survival compared to 0% AKO (66.7 ± 15.28%). However, at 5% (AKO5), no shrimp mortality was recorded after 30 min. of exposure. Also, a drop in water temperature from 27.9 to 19.2 ± 0.9°C resulted in a statistically higher survival in shrimp that had been fed a minimum of 3% AKO (91.7 ± 5.16%) compared to without (AKO, 66.77 ± 10.33%) after 30 min. of exposure. This study has shown that post-larval *L. vannamei* fed nursery diets containing 5% AKO or 3% AKO in combination with 8% KM achieves a significantly higher BW at harvest. A minimum of 3% AKO is required to promote a greater resistance to an acute osmotic and thermal stress.

ASTAXANTHIN KRILL OIL IMPROVES THE GROWTH OF JUVENILE *Litopenaeus vannamei* RAISED UNDER OSMOTIC STRESS IN AN ENCLOSED-TANK CULTURE SYSTEM

Alberto J.P. Nunes, Lucas Alves Rufino, Simone Sales Pinheiro, Lena Burri

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará
Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081, Brazil
alberto.nunes@ufc.br

This work evaluated if dietary supplementation of astaxanthin krill oil (QRILL™ AstaOmega Oil, Aker Biomarine Antarctic AS, Norway) with or without soybean oil to a grower shrimp feed enhances the growth performance of *L. vannamei* farmed under osmotic stress. Shrimp of 1.08 ± 0.11 g body weight (BW) were reared for 74 days under 135 animals/m^2 in 50 outdoor tanks of 1 m^3. Half of the tanks operated enclosed by a milky-colored sheeting and the remaining fully exposed. Shrimp were fed a 38% crude protein feed top-coated with astaxanthin krill oil (K) and/or soybean oil (S) at 0K-3S, 1K-2S, 2K-1S, and 3K-0S% of the diet, as fed basis, respectively.

In exposed tanks, mean salinity reached 31 ± 6 g/L compared to 36 ± 4 g/L in enclosed tanks. Shrimp survival was similarly high in both rearing systems (90.6 ± 3.8 and 89.2 ± 5.3% in exposed and enclosed tanks, respectively (Table 1) and was unaffected by oil supplementation ($P > 0.05$). Shrimp raised in the exposed tanks achieved a significantly higher final BW, weekly growth, gained yield, apparent feed intake (AFI) and a lower FCR (feed conversion ratio) compared to animals in the enclosed tanks. As a result of top-coating feed for the exposed tanks with the 1K-2S oil mix, the highest shrimp final BW (14.03 ± 0.52 g) and yield (1,515 ± 40 g/m^2) could be achieved when compared to the enclosed rearing system and all the other diets. Under longer exposure periods to hypersalinity in enclosed tanks, a minimum of 2K-1S was required to maximize BW (10.80 ± 0.63g) when compared to the other enclosed diet groups.

Table 1. Growth performance of *L. vannamei* reared in enclosed versus exposed 1-m^3 tanks for 74 days with 135 animals/m^2. Shrimp were fed the same commercial feed but supplemented with different combinations (%) of astaxanthin krill oil (K) and soybean oil (S). Data obtained from five tanks are expressed as mean ± standard deviation. Lowercase letters indicate differences between diets within the same rearing system ($P < 0.05$). Capital letters refer to statistical differences between rearing systems within the same dietary treatment ($P < 0.05$).

System/Diet[1]	Survival (%)	Growth (g/week)	Final body weight (g)	Gained yield (g/m^2)	AFI[2] (g/shrimp)	FCR[3]
Exposed						
CT	88.0 ± 3.4	1.06 ± 0.15 A	12.26 ± 1.56 aA	1,261 ± 141 aA	15.0 ± 0.9 A	1.56 ± 0.08 aA
0K-3S	92.7 ± 1.7	1.08 ± 0.06 A	12.56 ± 0.63 bA	1,375 ± 51 abA	15.2 ± 0.26 A	1.44 ± 0.04 bA
1K-2S	90.5 ± 2.4	1.23 ± 0.05 A	14.03 ± 0.52 cA	1,515 ± 40 bA	15.8 ± 0.25 A	1.36 ± 0.04 bA
2K-1S	89.3 ± 5.8	1.22 ± 0.09 A	13.95 ± 1.00 cA	1,478 ± 62 bA	16.1 ± 0.44 A	1.42 ± 0.07 bA
3K-0S	93.4 ± 1.9	1.11 ± 0.10 A	12.82 ± 1.04 bA	1,418 ± 105 bA	15.4 ± 0.61 A	1.42 ± 0.06 bA
Mean ± SD	90.6 ± 3.8	1.14 ± 0.11	-	-	15.6 ± 0.7	-
Enclosed						
CT	88.3 ± 3.3	0.74 ± 0.07 aB	8.87 ± 0.75 aB	880 ± 91 aB	13.44 ± 0.5 aB	2.01± 0.18 aB
0K-3S	88.0 ± 3.0	0.81 ± 0.06 abB	9.64 ± 0.66 cB	964 ± 68 abB	13.88 ± 0.3 abB	1.88 ± 0.12 abB
1K-2S	91.4 ± 6.8	0.88 ± 0.11 bcB	10.37 ± 1.20 dB	1,087 ± 105 cB	14.24 ± 0.6 bB	1.72 ± 0.13 bB
2K-1S	84.2 ± 6.8	0.92 ± 0.06 cB	10.80 ± 0.63 eB	1,042 ± 61 bcB	14.34 ± 0.4 bB	1.80 ± 0.08 bB
3K-0S	93.4 ± 3.7	0.76 ± 0.05 ab	9.13 ± 0.51 bB	970 ± 73 abcB	13.33 ± 0.6 aB	1.80 ± 0.09 bB
Mean ± SD	89.2 ± 5.3	-	-	-	-	-
Two-Way ANOVA	Survival	Growth	Final body weight	Gained yield	AFI	FCR
System	ns	<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Diet	ns	<0.0001	<0.0001	< 0.0001	0.001	< 0.0001
System x diet	ns	ns	< 0.0001	ns	ns	ns

[1]Dietary inclusion of astaxanthin krill oil (K) and soybean oil (S; % of the diet, as is)
[2]Apparent feed intake
[3]Feed conversion ratio

EFFECT OF GRADED-LEVELS OF DIETARY ASTAXANTHIN KRILL OIL TO THE GROWTH PERFORMANCE OF NURSERY-REARED *Litopenaeus vannamei*

Alberto J.P. Nunes, Hassan Sabry Neto, Lena Burri

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará
Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081, Brazil
alberto.nunes@ufc.br

The study consisted of two separate nursery trials in which post-larval (PL) *L. vannamei* were fed graded dietary levels of astaxanthin krill oil (QRILL™ AstaOmega Oil, Aker Biomarine Antarctic AS, Norway). In the first trial (trial #01), 142,245 PLs of 3.6 ± 0.3 mg body weight (BW) were stocked under 2.4 PLs/L (3,556 ± 31 PLs/tank) in forty 1-m^3 outdoor tanks and raised for 51 days. Shrimp were fed 20 times day and night with diets containing 0 (AKO0), 1.00 (AKO1), 3.00 (AKO3), and 5.00% (AKO5) astaxanthin krill oil (AKO) included at the cost of soybean oil (SBO) and soy lecithin oil (SLO). Diets were formulated to contain 18.00% salmon meal, along with 4.45% of poultry viscera and bone meal, 20.39% soybean meal (SBM), 10.00% wheat gluten meal (WGM), and 8.00% soy protein concentrate (SPC). After harvest, a stress test was carried out to evaluate shrimp resistance to a sudden and acute change to water salinity and temperature. In trial #02, 65,588 PLs of 2.5 ± 0.3 mg BW (1,252 ± 29 PLs/tank) were raised in fifty 0.5-m^3 indoor tanks for 42 days. Shrimp were fed 8 times a day exclusively in feeding trays. A negative control (AKO0KM0) diet was formulated without any AKO. It contained 29.80% salmon meal combined with 18.98% SBM, 7.00% SPC and 5.00% WGM. Four other diets were prepared by partly replacing salmon meal for 8.00% of a high-protein krill meal (KM, QRILL™ Aqua High Protein, Aker Biomarine Antarctic AS, Norway), including a positive control (AKO0KM8) without AKO. Other diets were formulated with 3.00 (AKO3KM8), 5.00, and 7.00% AKO added at the cost of SLO and SBO. In trial #01, no statistical effect from the inclusion of AKO was observed on final shrimp survival (84.0 ± 16.8%), gained yield (1,568 ± 181 g/m^3), daily growth (14.2 ± 2.8 mg/day), apparent feed intake (AFI, 1.12 ± 0.07 g of feed/shrimp) and feed conversion ratio (FCR, 1.70 ± 0.16). However, shrimp fed KO5 achieved a statistically higher final BW (857 ± 140 mg) in comparison to all other diets. In trial #02, shrimp final survival (91.8 ± 14.2%), gained yield (1,611 ± 181 g/m^3), daily growth (14.2 ± 1.8 g/m^3) and FCR (0.89 ± 0.09) were not significantly affected by dietary treatment. There was significant effect of AKO over AFI, starting at 3% dietary inclusion. Shrimp fed diets containing AKO significantly enhanced final BW starting at 3% dietary inclusion (AKO3KM8, 629 ± 76 mg), but not beyond this level. Shrimp fed diets with 5 and 7% AKO (613 ± 81 and 612 ± 105 mg, respectively) did not improve BW compared to 3% or to the diet with KM only (KO0KM8, 583 ± 55 mg). There was no positive effect over shrimp final BW as a result of the inclusion of 8% KM since it did not differ from shrimp fed the control diet (AKO0KM0, 567 ± 65 mg). In the stress test, when water salinity dropped from 39 g/L to 0, shrimp survival showed a clear trend towards higher survival due to the progressive increase in the dietary inclusion of AKO. A minimum of 1% AKO (AKO1, 80.0 ± 10.00%) was sufficient to promote an enhanced survival compared to 0% AKO (66.7 ± 15.28%). However, at 5% (AKO5), no shrimp mortality was recorded after 30 min. of exposure. Also, a drop in water temperature from 27.9 to 19.2 ± 0.9°C resulted in a statistically higher survival in shrimp that had been fed a minimum of 3% AKO (91.7 ± 5.16%) compared to without (AKO, 66.77 ± 10.33%) after 30 min. of exposure. This study has shown that post-larval *L. vannamei* fed nursery diets containing 5% AKO or 3% AKO in combination with 8% KM achieves a significantly higher BW at harvest. A minimum of 3% AKO is required to promote a greater resistance to an acute osmotic and thermal stress.

FORMULATION OF COST-EFFECTIVE AND HIGH-PERFORMANCE FEEDS FOR *Litopenaeus vannamei* WITH LOW FISH MEAL LEVELS

Alberto J.P. Nunes, Lise Lotte Dalen, Geronimo Leonardi, Lena Burri

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará
Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081, Brazil
alberto.nunes@ufc.br

Protein ingredients comprise more than 50% of a grower shrimp feed formula and carry the highest economical cost. Feed mills need to rely on a continuous and constant availability of high protein ingredients obtained mainly from capture fisheries, agriculture and animal rendering. Historically, fish meal has been the protein of choice due to its high bioavailability of nutrients and ability to stimulate feeding activity in marine shrimp. However, there is a growing move in the industry in the strategic and segmented use of fish meal in accordance to production stage (i.e. broodstock, larval, starter, and grower feeds) and intensification level. Over the past 30 years, the use of fish meal in shrimp feeds has drastically reduced from more than 25% in the 1990´s to an average of 12% or less in the past decade. There is also an ongoing shift from the use of fish meal made from forage fish to cheaper and more locally available sources of fish meal. The most common practice to reduce fish meal usage has been to rely on terrestrial plant protein by-products derived from agriculture, such as meals made from soybean, canola, corn and wheat. Feed mills have also relied on proteins supplied by rendering facilities, which convert inedible animal by-products obtained from the slaughtering or processing of poultry, swine, and cattle into meals. The major drawback on relying on these proteins is related to their lower bioavailability of nutrients, lack of long-chain n-3 polyunsaturated fatty acids, and poor attractability and palatability. Their advantages include a greater availability and more accessible prices compared to fish meal. From a nutrient-base formulation, fish meal can be replaced, partially or completely, in a cost-effective way with alternative feed proteins, as long as proper attention is given to the dietary level and digestibility of key nutrients. This makes the formulation process more complex and time-consuming as crude and digestibility levels of energy, protein, EAAs and total lipids from each raw material need to be estimated. However, if shrimp growth performance and feed conversion ratio (FCR) still deteriorate, then this can be the result of other factors, including poor feed attractiveness and palatability. Fish meal-challenged diets appear to slow down shrimp feeding responses, increase feed wastage and FCR. Thus, there has been a growing use of feeding effectors in commercial shrimp feeds with the corresponding reduction in the dietary inclusion of fish meal. In the recent literature, krill meal has been the most studied feeding effector for shrimp. Krill meal has been recognized as one of the strongest feed attractant and palatability enhancers for penaeid shrimp. This present work summarizes the steps towards formulation of low fish meal feeds for juvenile *L. vannamei*.

Rhizophora mucronata'S EXTRACT FOR BACTERICIDE OF TIGER SHRIMP DISEASE

Nurhidayah*, Muliani, and Muharijadi Atmomarsono

Research Institute for Brackishwater Aquaculture and Fisheries Extension
Jl. Makmur Dg. Sitakka 129, Maros, Sul-Sel .90511
Telp. (04110 371544; Fax (0411) 371545
e-mail: nurhidayahjabir@gmail.com

Tiger shrimp diseases have been occured in Indonesian brackishwater ponds since two decades and considered as one of the factors causing mass mortality of the cultured shrimp and making big loss for the farmers. *Vibrio harveyi* is considered as the main causative agent of this disease. Natural substance is proposed to kill or inhibit the growth of pathogenic vibrios. In order to know the potency of mangrove *Rhizophora mucronata* as bactericide for *V. harveyi*, research had been conducted at the Research Institute for Coastal Aquaculture from February to May 2013. This research consists of several steps, i.e. 1) collection of plant; 2) drying of plant; 3) making powder of plant; 4) extraction of plant/herb; 5) qualitative bioassay; and 6) quantitative bioassay.

The results showed that qualitatively all part of the *R. mucronata* collected from Bone regency had activity againts *V. harveyi*, but the leaf part of this mangrove collected from Maros regency did not have this activity, and neither the leaf or bark part of this mangrove collected from Pangkep regency. The values of *Minimum Inhibition Concentration* (MIC) on *V. harveyi* were ranged between 1-10.000 mg/L depended on which part of the plant and the origin of the mangrove. The root part of the plant had higher activity againts *V. harveyi* than other parts of the plant. *Rhizophora mucronata* collected from Bone regency had the highest activity againts *V. harveyi* than that collected from Maros and Pangkep regencies.

Mangrove	Bone	Maros	Pangkep
Leaf	1,000	>10.000	>10.000
Flower	100	(-)	(-)
Fruit	100	10,000	10,000
F. cover	1	10,000	10,000
Bark	100	100	>10.000
Root	1	10,000	10,000

Minimum Inhibition Concentration (MIC) of R*hizophora mucronata* collected from different regencies on bactericide againts

OPTIMAL TEMPERATURE DEPENDANT GROWTH CHARACTERISTICS OF MATURE FRESHWATER CRAYFISH MARRON *Cherax cainii*

Brett O'Brien* and Le Ma

Aquatic Solutions
Pickering Brook, Perth Western Australia
Aquasoul@bigpond.com

Growth characteristics of marron in aquaculture ponds during the six-month warm period of Spring/Summer, has been documented for the first time. The changes in the population distribution immediately prior to marketing are examined and a population approach to growth hypothesised. The optimum growth of marron at low stocking density is recorded for the first time, which permits comparisons to the growth rates achieved during commercial production. The importance of aligning management to favourable temperatures over one summer is highlighted. The initial average weight of marron in five replicate ponds was 152.3g and at harvest after six months was 252.8g. Three growth habits were delineated including no moult approximately 10% of the population, one moult approximately 40% of the population with an average weight of 220g and two moults approximately 50% of the population with an average weight of 280g. Typically, males grew faster than females.

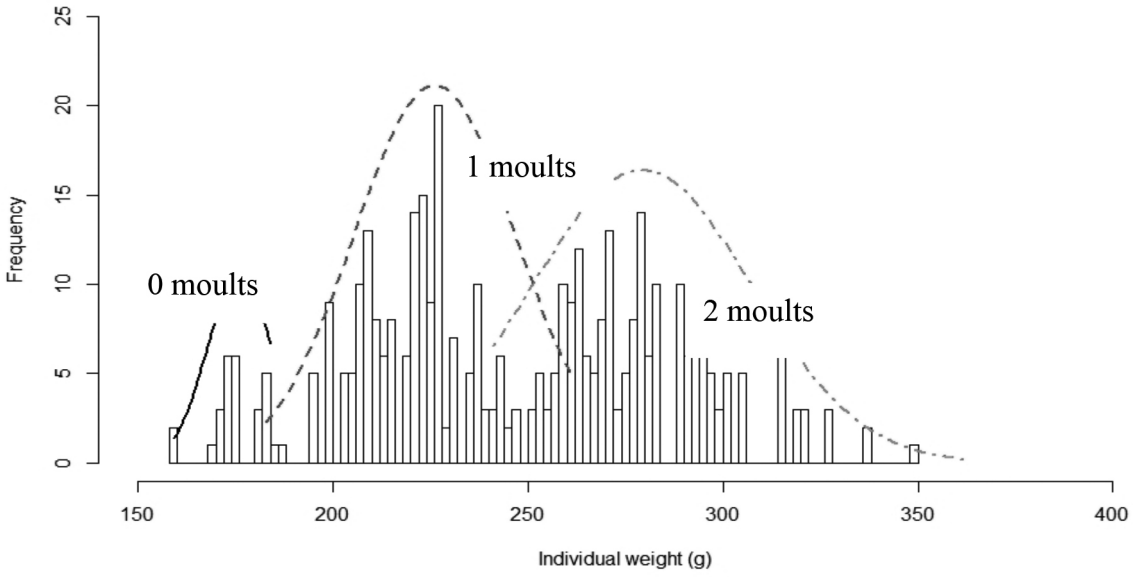


Figure 2. Distribution of the harvested marron weight after 6 months of growth indicating the 3 moult responses.

INTRODUCTION OF TILAPIA AQUACULTURE IN LAKE LANAO: AN IMMEDIATE LIVELIHOOD SUPPORT TO VICTIMS OF MARAWI SEIGE IN LANAO DEL SUR, PHILIPPINES

Ramjie Y. Odin*, Mario Corado

College of Fisheries
Mindanao State University – Maguindanao
Dalican, Datu Odin Sinsuat, Maguindanao, Philippines 9601
ramjieodin@gmail.com

As a response to the devastating result of the armed conflict between the Philippines government forces and members of the Maute Group which forced thousands of families to flee Marawi City and its nearby municipalities in Lanao del Sur in 2017 resulting to a serious threat to food security in the affected areas, the Food and Agriculture Organization of the United Nations (FAO-UN) put forth efforts and resources to procure inputs for the immediate restoration of agricultural livelihoods and food security of affected population. The FAO project team's coordination with government partners and local NGO partners led to the identification of the farmers and fisherfolks affected by the Marawi conflict. The FAO provided technical assistance and fisheries inputs for the recovery and restoration of the livelihoods of the affected fisherfolks. Most of these fisherfolks are residents of the municipalities of Marantao and Ditsaan-Ramain in Lanao del Sur.

The fisherfolks in Marantao were given five (5) fish cage units while those in Ditsaan-Ramain were given six (6) fish cage units and six (6) fish pen units. The fish cage and pen units were constructed with sufficient quantity of bamboo poles, plastic drum floaters, concrete sinkers, and quality fish netting materials. Each fish cage or pen unit was also provided with ten-thousand (10,000) sex-reversed male tilapia fingerlings and seventy-two (72) bags of tilapia feeds; which sums up to 200,000 tilapia fingerlings and 1,224 bags of feeds. One-hundred (100) sets of fish gill nets and fish pots were also provided to the fisherfolks. The distribution of all inputs to the fisherfolk beneficiaries was completed in December 31, 2018.

This paper presents the project management strategies employed, main findings and conclusions, and recommendations noted during the conduct and execution of the fisheries component of this livelihood support project.

OPTIMIZING EARTHWORM POWDER AS SUPPLIMENTARY SOURCE OF PROTEIN-LYSINE IN FISH FEED FORMULATION FOR AFRICAN CATFISH *Clarias gariepinus* FINGERLINGS

Olatayo Ogunbanwo[*a, b], Sola Babalola[b], Folajuwon Fakunmoju[b], Adebayo Asafa[b], Murisiku Onigemo[b] and Babajide Faleti[b]

[a]Faculty of Environment, School of Geography, University of Leeds, UK
[b]School of Agriculture, Department of Fisheries Technology
Lagos State Polytechnic, Ikorodu
tayogunbanwo@yahoo.com & ogunbanwo.o @mylaspotech.edu.ng

Lysine is one of the most important essential amino acid in fish feeds needed for optimum growth of fish. The combination of different feedstuff with suitable components of lysine can help to boost lysine content in fish feeds. Sources of lysine for fish feeds includes soybean waste, leucaena leucocephala leaves, fish meal and earthworm powder. The goal of this investigation is to enhance lysine content in fish feeds by incorporating different ratio of each chosen feed constituents. The investigation was conducted using central composite design (CCD) as statistical tool. The optimal lysine content was obtained at 25.00 %, 20.88 %, 10.00 % and 20.00 % of earthworm powder, soybean waste, leucaena leucocephala leaves and fish meal, respectively. The lysine content in formulated fish feeds was significantly affected by the composition of earthworm powder and fish meal in fish feed formulation. Earthworm powder and fish meal have contributed the largest portion of lysine in fish feed due to high lysine content which were 4.48 % w/w and 3.60 % w/w, respectively. The optimized fish feed shows high lysine content of 23.39 % w/w which doubles the lysine content in commercial fish feed (11.21 % w/w). The composition of fish feed obtained from this study can be used as guidelines for formulation of high lysine fish feeds for African catfish fingerlings.

SOCIO-ECONOMIC CHARACTERISTICS OF FISH FARMERS AND AQUACULTURE DEVELOPMENT PRACTICES IN IBI LOCAL GOVERNMENT AREA OF TARABA STATE, NIGERIA

Joshua, B. Ogunremi, Flourizel Igbani, Dauda, K. Abdullahi and Catherine, Y. Shetur

Federal University Wukari
Taraba State, Nigeria
jogunremi@gmail.com

Nigeria as a nation is endowed with good natural resources which can enhance aquaculture development to a greater extent; however, the reverse is the case. Aquaculture benefits are numerous; it fulfills protein demand of the country, helps prevent food insecurity, creates jobs for the unemployed, profitable, generate income for individuals and can attract foreign exchange. Despite advantages of aquaculture and various programmes of the Government on aquaculture the expected increase in production is minimal. The study was conducted to determine the socio-economic characteristics of fish farmers and aquaculture development practices in Ibi Local Government Area of Taraba State, Nigeria.

A sampling frame was drawn from the list of 740 fish farmers registered with the Department of Fisheries and Forestry of Ibi Local Government Area, Taraba State. A multistage stratified design was used for selecting the respondents for the study. The first stage involved stratification of the Local Government into Districts (Sarkin Kudu, Dampar and Ibi). The second stage was selection based on the prevalence of fish farmers in the districts. The third stage involved random selection of 30% fish farmers from each district to obtain a sample size of 222 respondents. Data from the study were collected through structured questionnaire and scheduled interview administered to the respondents. Data were analyzed using descriptive statistics and inferential statistics. Descriptive statistics used were frequency distribution and percentages while inferential statistics was non parametric and Chi square analysis to test hypothesis at $p<0.05$ significant level.

Table 1 showed that 98.7% of the respondents stocked improved fish seed, test cropping 38.3%, water aeration 38.3%, pond liming 32.4%. Table 2 indicated that there is no significant relationship between the socio-economic characteristics of respondents.

Table1: Level of Aquaculture development practices in Ibi Local Government Area.

Aquaculture development practices	Responses		Assym. Sig. (2 Tailed)
	Yes	No	
Use of Improved feeds	29.80	70.20	0.020*
Stocking of improved fish seed	98.70	1.30	0.013*
Test cropping	38.30	61.70	0.041*
Pond fertilization	31.90	68.10	0.043*
Liming	32.40	67.60	0.041*
Water aeration	38.30	61.70	0.024*

Key: * No significant relationship between the variables

Table 2: Chi Square test relationship between social-economic characteristic of respondents and aquaculture development practices

Aquaculture development practices	Value	Df	Assym. Sig. (2 Tailed)	Decision
Use of Improved feeds	1.140	1	0.286	NS
Stocking of improved fish seed	1.989	3	0.575	NS
Test cropping	5.064	8	0.752	NS
Pond fertilization	3.619	4	0.460	NS
Liming	3.025	4	0.554	NS
Water aeration	3.315	4	0.256	NS

EFFICACY OF *Ocimum gratissimum* (SCENT LEAF) POWDER AS ANAESTHETIC AND ITS EFFECT ON THE HAEMATOLOGY OF *Clarias gariepinus* JUVENILES

Irom B. Okey*

Department of Fisheries and Aquatic Science, Cross River University of Technology Calabar, Nigeria
piusbass@yahoo.com
romokey@crutech.edu.ng

Intensive nature of aquaculture have subjected farmed fish to a number of stressors due to handling proceedures and transportation from hatcheries to final stages. These stressors have most often been responsible for high mortalities recorded, hence hindering the growth of fish farming. Anaesthetics are used in aquaculture, fisheries and biological researches, as a way to minimize hypermotility during handling and transportation to reduce stress and mortality. Previous studies have reported the use of some plant materials to anaesthetized various fish species including *C. gariepinus* This studies aimed to investigates the efficacy of *Ocimum gratissimum* as anaethetic and determine its effects on some haematological parameters of *C. gariepinus* juveniles.

Fresh leafs of *O. gratissimum* were obtained from within the university campus, identified, air dry for 5 days and blended to powder. The experimental treatment were prepared from a stock solution of 2g in 10 litres of water (200mg/l) into five concentrations (0, 50, 100, 150 and 200mg/l) in 20 litres of water. Stages of induction and recovery were monitored and recorded using a stop watch. Blood was ollected by severing the caudal peduncle into Ethylene Diamine Tetra acetic Acids (EDTA) for the analysis of various hemaetological parameters using standard methods.

The result revealed that *O. gratissimum* caused anaesthesia which was concentration dependent. Induction time (min) reduced with increase in concentration while recovery increases as induction time reduces (Table 1 and Figure 1).

Haematological parameters showed some slight changes exspecially at higher concentration however some were not significant ($p > 0.05$). the mean values of red blood cells, haemoglobin, pack cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, basophile, eosinophile and neutrphile were decreasing with increased concentration of clove powder. Others such as white blood cells, platelets and lymphocytes increased with concentration (Tables 2 and 3). Fish exposed to 150mg/l cause induction shows that RBC, Hb and PCV were not significantly ($P > 0.05$) from those exposed to 50mg/l which were however not different from the control (0.0mg/).

The observed behavioural changes including initial hypermotility, hyperventilation, loss of equilibrium and no reaction to handling suggest the fish were immobilised (anaesthetized) by the plant material. This was in line with the reports of several researchers who used anaesthetics on fish during handling and transportation Slight changes in the haematological parameters reported in this study corroborates with the studies of many other researchers who have use plant materials as anaesthetic Minimal changes in RBC, Hb and PCV recorded on fish exposed to 150mg/l which were not different ($P > 0.05$) from the control but induces anaesthesia in 6.22 mins shows it is an ideal concentration. However, researchers have reported a 48 hours reversal to the haematological paramters of fish exposed to clove powder. Further research will be required to investigate the effects of *Ocimum* of the biochemical paramters of *C. gariepinus*.

(Continued on next page)

Table 1: Induction and recovery time (min) of of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Concentration (mg/l)	Induction		Recovery	
	Induction 1	Induction 2	Recovery 1	Recovery 2
0	-	-	-	-
50	26.61 ± 2.34	-	-	2.82 ± 2.44
100	11.12 ± 1.54	15.26 ± 0.81	1.26 ± 1.38	4.43 ± 0.36
150	4.16 ± 2.36	6.08 ± 0.34	2.35 ± 2.14	6.76 ± 2.18
200	1.24 ± 4.56	3.22 ± 1.26	4.81 ± 0.19	11.67 ± 1.44
Mean with the same superscript are not significantly different at p< 0.05, Induction1 (loss of equilibrium), induction2 (deep anaethesia), recovery 1 (regain equilibrium), recovery2 (normal swimming).				

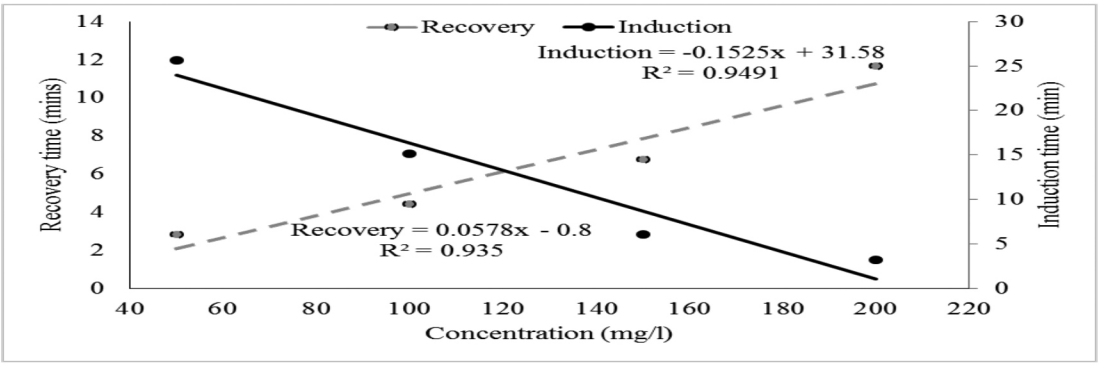


Figure 1: Relationship between Induction, Recovery time (mins) and Concentration (mg/l) of *O. gratissimum*

Table 2: The mean values of selected haematological indices of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Conc. (mg/l)	Haematological parameter							
	RBC (10^{12} cells/L)	WBC (10^{9}cells/L)	Hb (g/l)	Plt	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/l)
0.00	8.34 ± 1.05^{a}	30.95 ± 1.79^{c}	10.34 ± 0.70^{a}	58.77 ± 1.07^{c}	38.05 ± 3.20^{a}	$66.30\pm 1.92c$	21.17 ± 1.92^{c}	30.84 ± 1.28^{a}
50.00	7.06 ± 0.08^{ab}	33.12 ± 4.30^{c}	9.26 ± 0.34^{ab}	61.82 ± 1.71^{bc}	33.97 ± 1.58^{ab}	$67.88\pm2.49bc$	23.38 ± 0.58^{bc}	28.38 ± 1.98^{ab}
100.00	6.82 ± 0.70^{ab}	42.47 ± 0.87^{b}	8.86 ± 0.19^{bc}	70.63 ± 1.46^{b}	30.32 ± 1.27^{bc}	78.69 ± 5.73^{c}	24.60 ± 0.65^{b}	27.87 ± 2.22^{a}
150.00	5.93 ± 0.90^{bc}	50.07 ± 1.41^{a}	7.99 ± 0.54^{bc}	77.00 ± 3.05^{ab}	28.64 ± 0.34^{bc}	93.76 ± 4.80^{a}	26.44 ± 0.82^{ab}	26.59 ± 0.78^{a}
200.00	4.80 ± 0.33^{c}	52.87 ± 2.36^{a}	7.31 ± 0.80^{c}	98.14 ± 4.93^{a}	26.17 ± 1.34^{c}	102.52 ± 4.53^{a}	28.70 ± 1.28^{a}	25.31 ± 1.08^{c}
Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, PCV = packed cell volume , RBC= red blood cell Hb = haemoglobin, MCV =mean cell volume MCH= mean cell haemoglobin MCHC= mean haemoglobin concentration, WBC =white blood cell, Plt = platelet..								

Table 3: The mean values of selected Differential white blood cell counts of *Clarias gariepinus* juveniles' exposure to *Ocimum gratissimum* powder anaesthetic for 30min.

Conc. (mg/l)	Differential white blood cell count (%)				
	Neut	Lymp	Baso	Mono	Eosin
0	14.71 ± 1.92^{a}	56.25 ± 1.40^{b}	4.88 ± 0.20^{a}	5.99 ± 0.32^{b}	8.07 ± 0.17^{a}
50	13.74 ± 1.48^{a}	58.02 ± 1.49^{ab}	4.74 ± 0.36^{a}	7.01 ± 0.14^{b}	7.66 ± 0.44^{ab}
100	12.18 ± 0.60^{bc}	59.40 ± 1.78^{ab}	4.31 ± 0.31^{a}	7.92 ± 0.53^{b}	6.61 ± 0.36^{ab}
150	12.11 ± 0.83^{bc}	59.82 ± 1.42^{ab}	3.95 ± 0.10^{ab}	9.08 ± 0.05^{ab}	5.98 ± 0.48^{ab}
200	10.79 ± 0.31^{c}	65.44 ± 1.91^{a}	3.07 ± 0.77^{b}	10.27 ± 0.85^{a}	4.14 ± 0.42^{b}
Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, Neut = neutrophil Lymp= lymphocytes Baso = basophil, Mono = monophil, Eosin = eosinophil,					

PROGENIES OF *Pangasianodon hypophthalmus* × *Clarias gariepinus* PRODUCE MORPHOTYPES WITH DIFFERENT PERFORMANCE CHARACTERISTICS

*OKOMODA V. T.,[1,2,3] KOH I.C.C.,[2] HASSAN A.,[2] AMORNSAKUN T.[3] AND **SHAHREZA M.S.[2,4]

[1]Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria
[2]School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Malaysia
[3]Institute of Tropical Aquaculture and Fisheries (AQUATROP), Universiti Malaysia Terengganu, Malaysia
[4]Department of Technology and Industries, Prince of Songkla University, Thailand
* Corresponding authors:
*e-mail: okomodavictor@yahoo.com, phone no: +2348033319959
**e-mail: shahreza@umt.edu.my, phone no: +60192867794

This study was designed to evaluate the culture potential of the offspring from the novel crosses between Asian catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) and African catfish *Clarias gariepinus* (Burchell, 1822). ♀*C. gariepinus* × ♂*P. hypophthalmus* (Clariothalmus) gave better hatchability than ♀*P. hypophthalmus* × ♂*C. gariepinus* (Pangapinus), however, both were lower than the pure crosses.

Based on the phenotypic characters, a larger proportion of the offspring of Clariothalmus was Panga-like, while only 12% were Clarias-like. However, all offspring of Pangapinus were all Panga-like. The Panga-like Clariothalmus exhibited shared features from both parents hence much more phenotypic divergence from both parents. The remain 12% of the Clariothalmus offspring (Clarias-like) and all the Pangapinus progenies (Panga-like) on the other hand were indistinguishable from the maternal parent.

After sixteen weeks of culture, the Clarias-like Clariothalmus offspring had better growth than the other crosses including the pure sibs. However, cannibalism and aggressive behaviour were higher in this group of fish compared to others. Hence, culturing Panga-like Clariothalmus separately from the Clarias-like progenies may be a management practice of interest in the commercial production of the hybrids.

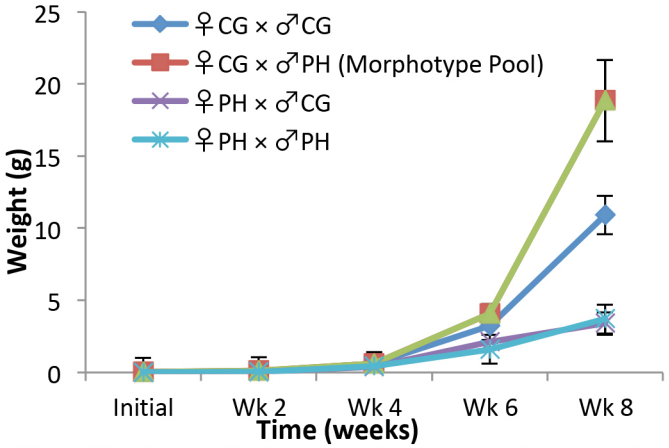


Fig 1: Weekly growth of progenies from pure and reciprocal crosses of *P. hypophthalmus* and *C. gariepinus*.

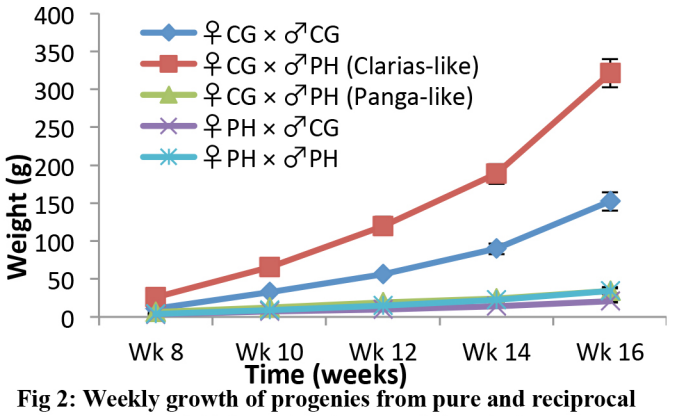


Fig 2: Weekly growth of progenies from pure and reciprocal crosses of *P. hypophthalmus* and *C.gariepinus*.

EVALUATION OF PARASITOFAUNA OF FOUR ECONOMICALLY IMPORTANT FISH SPECIES (*Synodontis budgetti, Chrysichthys nigrodigitatus*, *Clarias anguillaris* AND *Heterobranchus bidorsalis*) FROM RIVER OKPOKWU, APA, NIGERIA

*[1]Omeji, S., [2]Adadu, M.O. and [1]Obande, R.A.

[1]Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University
P.M.B. 2373, Makurdi, Benue State, Nigeria
[2]Federal College of Freshwater Fisheries Technology Baga, PMB 1060, Maiduguri, Borno State, Nigeria
*Correspondent author's: dromejisam@gmail.com

The parasitic fauna of four economically important fish species comprising of *Synodontis budgetti, Chrysichthys nigrodigitatus*, *Clarias anguillaris* and *Heterobranchus bidorsalis* from River Okpokwu, Apa, Nigeria, was evaluated. A total of 161 fish samples comprising of 57 *Synodontis budgetti,* 48 *Chrysichthys nigrodigitatus*, 24 *Clarias anguillaris* and 32 *Heterobranchus bidorsalis* were randomly purchased and subjected to parasitological examination. The overall parasite prevalence of all the fish species was 36.60% out of which *S. budgetti*, *C. nigrodigitatus, C. anguillaris* and *H. bidorsalis* accounted for 14.30%, 11.20%, 1.90% and 9.30%, respectively. However, the prevalence of infection by each fish species were 40.40%, 37.50%, 12.50% and 46.90% for *S. budgetti, C. nigrodigitatus, C. anguillaris* and *H. bidorsalis,* respectively. There was no significant difference in parasite prevalence for fish species ($p>0.05$) though, *S. budgetti* had the highest prevalence of infection (14.70%) with *C. anguillaris* recording the least (1.90%). Also, while the highest prevalence of infection was recorded for nematode (17.40%), the least (3.70%) each was recorded for trematode and copepode, respectively. Of the parasitic taxa, nematode was the most abundant (56.50%) and had the highest prevalence (17.40%). The parasites recovered from the fish species belonged to a species of acanthocephala (*Neoechinorhynchus spp*.), copepode (*Argulus spp*.) trematode (*Clinostomum complanatum*), 2 species of cestode (*Ligula intestinalis* and *Diphillobothrium latum*) and 4 species of nematode (*Capillaria philippinensis, Eustrongylides tubifex, Camallanus spp*. and *Procamallanus laevionchus*). Out of the total number (292) of parasites recovered from the infested body parts of the fish, intestine had the highest percentage parasite load (62.30%) and prevalence (29.80) while the gill had the least 16(5.50%) parasite load and prevalence of 3.10. Generally, the prevalence of parasites (43.00%), percentage parasite recovered (56.50%), mean abundance (2.60) and mean intensity (5.90) were higher for the female fish samples compared to the males with the prevalence of parasites, percentage parasite recovered, mean abundance and mean intensity of (32.00%), (43.50%), (1.30) and (4.10), respectively. However, there was no significant difference ($p>0.05$) by sex in the prevalence of parasites of all the fish species. The size class related prevalence showed an increase in parasitic infections (24.60%) and (20.80%) in bigger samples of *S. budgetti* and *C. nigrodigitatus*) in the length groups of 13.00 to 15.90cm and 19.00 – 22.90cm while the least (5.30%) and (6.30%) were recorded for smaller samples of *S. budgetti* and *C. nigrodigitatus* in the length groups of 10.00 to 12.90cm and 15.00 – 18.90cm, respectively. However, while the highest prevalence (8.30%) and (21.90%) were recorded for smaller samples of *C. anguillaris* and *H. bidorsalis* in the length groups of 10.00 to 20.90cm and 13.00 – 21.90cm, the least (4.20%) and (3.10%) were recorded in length groups of 41.00 to 50.90cm and 39.00 – 54.90cm for bigger samples of *C. anguillaris* and *H. bidorsalis*, respectively.

MODELLING SUITABILITY OF ERO RESERVOIR AND ITS ENVIRONS FOR AQUACULTURE DEVELOPMENT IN EKITI STATE, NIGERIA

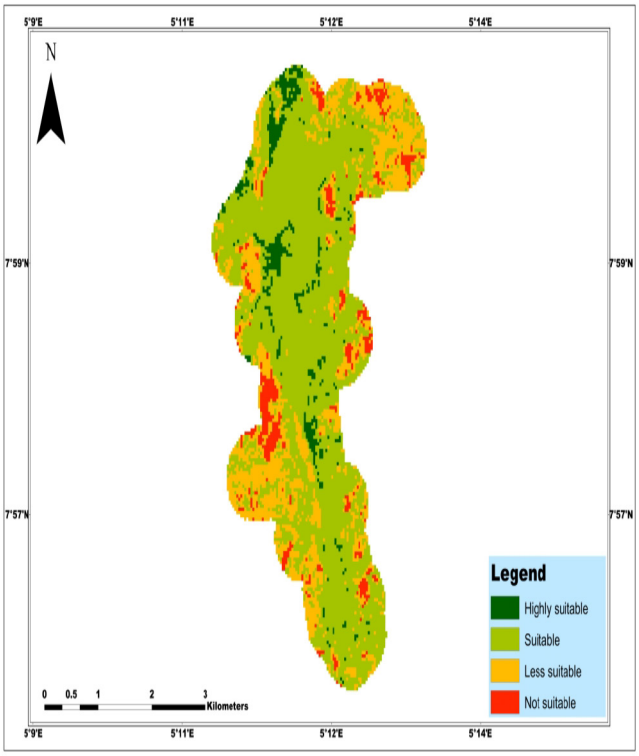
Omobepade, B. P*, Omoju, O. J, Nuhu, H, Olaniyi, O. E and Akande, O.S

Department of Fisheries and Aquaculture, Federal University Oye-Ekiti, Nigeria

Reservoirs in Nigeria are primarily constructed for public water supply and characterized by artisanal fishery. Most of the reservoirs are surrounded by a large expanse of land that could be assessed for fish culture. However, utilising these areas of land for successful and sustainable aquaculture operations require assessing different soil properties, environmental and climatological factors (soil, hydrology, topography, drainage and climate) that could guide in site selection. Hence, Landsat 8 OLI satellite datasets and Shuttle Radar Topography Mission were acquired from the Global Land Cover Facility to determine the land use type, coverage and the digital elevation model respectively within a 500m buffer. The soil profile was dug at three different locations to a depth of 1.5m and soil were collected at various horizons (0 – 37.5 cm, 38 – 75.5 cm, 76 – 112.5 cm and 113 – 150 cm) and analysed for physiochemical properties. Hydrological, soil, land use/land cover, vegetation, and elevation parameters were subjected to an analytical hierarchical process to develop a suitability map for aquaculture development in the study area according to standard methods.

Results indicated that 20.99 %, 47.74 %, 12.96 %, 6.03 % of the buffered area was water body, light vegetation, dense vegetation and built up areas respectively. The soil in the study areas was classified as Lixisols (which covered lesser land mass (11.63%) while Nitisols covered the higher land mass (88.37%). There were no significant differences in the water holding capacity of the soil collected from the three different locations. Soil taken from the upper slope had the highest water holding capacity of (40.54 ± 3.90%) while those taken from the middle slope had the lowest (34.24 ± 5.40%) water holding capacity.

Suitability model for the study area ((Figure 1) showed that land mass unsuitable for aquaculture development represents 6.07% (89.73 hectares) of the study area, 359.88 hectares (24.35%) were less suitable, and 950.76 hectares (64.33%) were suitable while 77.63 hectares (5.25%) were highly suitable. Despite the large sparse of the reservoir and it's environed belonging to the suitable classes. Land unsuitable for aquaculture could be attributed to the presence of hills, rocks, undesirable soil parameters, high slopes and dense vegetation. Hence, individuals and corporate bodies could invest in the suitable areas for aquaculture development, which at the long run will increase the fish availability, create employment, improve livelihood and enhance the economic profile of the State.

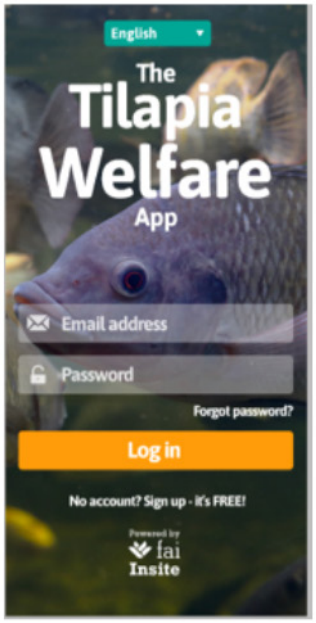


DEFINING AND IMPLEMENTING A TILAPIA WELFARE ASSESSMENT PROTOCOL IN ASIA

Ralf Onken, Murilo Henrique Quintiliano

FAI Farms Ltd
The Barn, Wytham,Oxford, OX2 8QJ. United Kingdom
www.faifarms.com

The aquaculture industry, especially in the Global South, is at a similar stage of development as the pig and poultry sectors were 30 to 40 years ago. The sector's drive is to develop production systems that remove the animal from its environment and possible disease challenges, rather than meeting the animal's health and welfare needs. This initiative aims to bring fish welfare assessment efforts closer to the welfare assessments used for many terrestrial farmed animals and with that, improve aquaculture production processes and industry engagement, toward better Fish Welfare, focused on a win-win situation for farmers, processors, retailers and food brands and also the consumers by:



- Create awareness of tilapia, carp and shrimp welfare
- Standardize data collection for tilapia, carp and shrimp welfare
- Engage farmers with best practices
- Promote transparency with culturing practices
- Engage the Tilapia, Carp and Shrimp industry stakeholders in best welfare practices
- Engage as many farmers and other stakeholders as possible under the animal welfare scope.

The farm is where most actions of welfare improvement can be performed. With that in mind, FAI is developing processes,methods and tools with several institutions and organizations in Brazil, China and Thailand.

Technological Advances

1. Develop and deploy specie specific scientific proved and industry accepted indicators, for Tilapia, Carp and Shrimp allowing Farmers to apply better fish husbandry at the farm level.
2. Develop a user-friendly self-assessment app tool to facilitate scaling the on farm self-assessment allowing farmers to constantly benchmark their own production both internally and with others and seek knowledge (online and in person), to secure optimal productivity farm level routine.
3. Secure engagement and best practices promotion through the industry and processors by allowing users to report on their developments in terms of positive welfare processes and actions and secure better yields and quality.
4. By secure and independent flow of information, Food brands and retailers can provide more transparency to their clients on how and under what conditions their food has been produced.

Based on https://www.fao.org/3/cc0407en/cc0407en.pdf

EFFECT OF ETHANOL-EXTRACT OF *Monodora myristica* ON GROWTH PERFORMANCE, GUT ABSORPTION AREA, SERUM BIOCHEMISTRY AND HAEMATOLOGICAL INDICES OF *Clarias gariepinus Fingerlings*

[1]Orisasona, O*., [2]Adesina, I. A., [1]Joseph, T. P and [1]Olanrewaju, A. A

[1]Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria
[2]Department of Aquaculture and Fisheries Management, University of Ilorin, Nigeria
*osasonagbenga@gmail.com

Intensive aquaculture characterized by the use of synthetic chemicals as growth promoters and immune boosters impacts negatively on the aquatic environment and also raises human health concerns, hence a need for organic alternatives. This study investigates the effect of extract of *Monodora myristica* as a growth promoter, immune booster and gut area modifier in *Clarias gariepinus*. Crude extract was obtained from finely ground dry seeds of *Monodora myristica* using a standard procedure. A 40% crude protein diet was formulated and the crude extract was added at 0.0 (M0), 0.5 (M1), 1.0 (M2), 1.5 (M3), and 2.0% (M4). Diets were fed to triplicate groups of *Clarias gariepinus* (2.32g ± 0.03g) randomly allotted to fifteen plastic tanks at twenty fish per tank for 84 days. Growth and nutrient conversion did not vary significantly ($p>0.05$) across treatments. Increased villi width and cryptal width were recorded in fish fed *Monodora myristica*. Extract caused a significant increase ($p<0.05$) in the recorded values for Packed cell volume, hemoglobin and platelets this group. Anti-oxidative and immune responses were improved with *M. myristica* inclusion in diets as total protein, glutathione peroxidase, glutathione and glutathione S-transferase significantly increased ($p < 0.05$). This study showed improved absorption area and enhanced immune system with the inclusion of *M. myristica* extract in the diet of *Clarias gariepinus*.

THE EFFECT OF DENSITY ON SEX DIFFERENTIATION, SEXUAL DIMORPHISM, STRESS, AND RELATED GENE EXPRESSION IN YELLOW PERCH

Rafidah Othman*, Han-Ping Wang, Hiam Elabd, Dingkun Xie, Hong Yao, Paul O'Bryant and Dean Rapp

Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
rafidaho@ums.edu.my

A 180-day experiment was conducted to evaluate the effects of density on sex differentiation, sexual dimorphism, cortisol level, and stress related gene expression. Yellow perch, *Perca flavescens*, with initial mean body weight of 0.03 ± 0.001 g were reared in three different stocking densities: 1, 2, and 4 fish/L, termed as low (LD), moderate (MD), and high (HD) density, respectively, in a flow-through tank system. Results showed no significant differences in sex ratio in all density groups compared to normal population 1:1, and sexual size dimorphism (SSD) appeared when male and female were as small as the mean size reaching 11.5 cm and 12.3 cm in TL or 13.2g and 16.9g in body weight (BW), respectively. This female-biased sexual growth dimorphism was more pronounced in LD, although it was observed across all density groups. A significantly higher condition factor (K) of females than males in the LD group, and significantly higher R values of LD and MD than HD with the length/weight (L/W) linear relationships in females, were observed. Parallelly, fish reared in LD showed significantly higher mean body weight than those in the MD and HD groups, but there were no significant differences between the MD and HD. Similar results were also observed in all the other parameters of weight gain, specific growth rate (SGR), condition factor (K), and survival. These findings suggested that high density not only affected growth itself, but also affected SSD, growth trajectory or body shape, and general wellbeing in fish, especially in females. There were no significant differences in gonadosomatic index (GSI) and viscerosomatic index (VSI) among all the density groups; however, the hepatosomatic index (HSI) of LD was significantly higher than MD and HD, suggesting high density affected liver reserves or functions. Physiologically, plasma cortisol level was significantly highest in the LD among all groups, followed by MD, and lowest in HD. At the molecular level, the expression of the 70-kDa heat shock protein (*Hsp70)*, glutathione peroxidase (*GPx),* and superoxide dismutase (*SOD)* genes involved in cellular stress were significantly upregulated in the HD group. The most significantly downregulated expression of these genes was consistently observed in the MD when compared to the LD and HD groups. In conclusion, increasing density induced chronic stress in yellow perch without affecting sex differentiation, but negatively affected expression of stress-related genes and mobilization of liver reserve, resulting in poorer wellbeing and reduced SSD, growth, and survival.

COMPARATIVE STUDIES OF THE GENETIC DIVERSITY OF SELECTED WILD AND CULTURED POPULATIONS OF *Oreochromis niloticus* FROM IKORODU AND NEW BUSSA, NIGERIA

Oyawoye Temitope Olalekan*

Department of Fisheries and Aquaculture
Federal University Oye-Ekiti
Oye-Ekiti
Nigeria
temitope.oyawoye@fuoye.edu.ng

Genomes of selected wild and cultured populations of *Oreochromis niloticus* from New Bussa and Ikorodu were comparatively assessed in this study. This was with a view to determining whether certain important genetic principles are being monitored in the cultivation of the fish species and also in the selection of broodstocks in the respective farms.

Twenty specimens of *O. niloticus* were obtained from the Kainji Lake and Usman farms at New Bussa, Niger State and also Majidun River and Toga farms at Lagos state. Twenty six morphometric characters and 9 meristic counts were taken on each specimen. The morphometric and meristic data were subjected to principal component analysis (PCA). DNA samples were extracted from caudal fin clips of the fishes using Cetyltrimethylammonium bromide (CTAB) based procedure. The purified DNA was analysed in a Multigene Gradient thermocycler using 8 microsatellite markers. The amplicons generated were subjected to fluorescent genotyping and the corresponding allele scored. The data obtained were used to determine genetic parameters such as inbreeding coefficient, observed and expected heterozygosity, allelic richness, effective number of allele and Nei genetic distance.

The average expected heterozygosities for Kainji Lake, Usman farms, Majidun River and Toga farms were 0.7488, 0.7043, 0.5953 and 0.4533, respectively. The heterozygousities of the two populations from the wild deviated from Hardy-Weinberg equilibrium (HWE) while the heterozygosities of the cultured populations were within HWE. On the average, three populations (Kainji Lake, Usman farms and Majidun River) were inbred while one population (Toga farms) was outbred. The analysis of genetic distance among the populations showed two major clusters. One cluster comprised Kainji Lake and Usman farms while the second cluster comprised Majidun River and Toga farms. However, Kainji Lake and Usman farms were significantly differentiated while Majidun River and Toga farms were weakly differentiated using morphometric characters. The wild populations largely overlapped while the cultured populations were significantly differentiated. The measures of differentiation by morphometric characters were found to be different from molecular characters.

The study concluded that the wild population of *Oreochromis niloticus* from New Bussa was a suitable source of Nile Tilapia broodstocks for aquaculture.

EVALUATION OF MERCURY RISKS IN FARMED SEA BREAM, SEA BASS, AND MUSSEL SEASONALLY

Özkan Özden[1]*, Nuray Erkan[2], Hande Doğruyol[2], Ortaboy, S[3], İdil Can Tunçelli[1]

[1] Istanbul University, Faculty of Aquatic Sciences, Dept. of Seafood Processing, 34134, Türkiye
[2] Istanbul University, Faculty of Aquatic Sciences, Dept. of Food Safety, 34134, Türkiye
[3] Istanbul University Cerrahpaşa, Faculty of Engineering, Dept. of Chemistry, 34190, Türkiye

*Correspondence: ozden@istanbul.edu.tr

Fish and shellfish expose to mercury (Hg) from environmental sources and anthropogenic activities. Methylmercury is the highly toxic form of Hg and along with Hg, this form can cause severe health problems harming organs and systems in the body at all ages. Sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*), and mussel (*Mytilus galloprovincialis*) are the most exported species in addition to being consumed frequently in the country. According to 2021 aquaculture data from the Turkish Statistical Institute, the total production rates of sea bream, sea bass, and mussel were 133476, 155151, and 4585 tonnes, respectively. Since Hg can be transmitted to farmed fish from the aquaculture environment or through feed, periodic sampling enables reducing contamination risks. The aim of this study is to investigate the control and presence of mercury risk levels originating from fish farms.

Sea bream, sea bass, and mussel samples were collected from 5 different regions throughout 2020-2021. Fall, winter, spring, and summer samplings were carried out from 6 different fish farms and 2 different mussel farms. Total of 51 samples were analyzed. The Hg analyzes were performed with NIC MA-3SOLO Analyzer (Nippon Inst. Co., Japan) by using the Direct Thermal Decomposition - Gold Amalgamation - Cold Vapor Atomic Absorption Spectroscopy (CVAAS) technique.

The minimum and maximum Hg values were 0.014-0.049 ppm for sea bream, 0.015-0.057 ppm for sea bass, and 0.006-0.013 ppm for mussel samples. The Hg levels of mussels among all samples were the lowest in all seasons. According to European Commission Regulations, the trace metal limit of Hg is 0.5 mg kg^{-1} (ppm) in fishery products. In this study, none of the samples exceeded the limit value determined for Hg. It was revealed that consuming farmed fish and mussels did not pose a risk in terms of food safety for mercury-related health problems. Yet, continuous monitoring studies are of great importance in determining the relationship between aquaculture products and toxic metals.

FEEDING ECOLOGY OF DEEP-WATER ARABIAN RED SHRIMP, *Aristeus alcocki* RAMADAN, 1938 (DECAPODA: PENAEOIDEA: ARISTEIDAE): FISHERIES MANAGEMENT PERSPECTIVE

Purushothaman Paramasivam[1,2,3] **&** Rakha Devi Chakraborty[1]·

[1]Crustacean Fisheries Division, Central Marine Fisheries Research Institute
Ernakulam North P.O., P.B. No. 1603, Cochin-682018, Kerala, India
[2]PMFGR Centre, ICAR-National Bureau of Fish Genetic Resources CMFRI Campus
Post Box No. 1603 Ernakulam North P.O.,Kochi–682 018, Kerala, India
[3]Correspondence; E-mail: purushothgene@gmail.com

Arabian red shrimp, *Aristeus alcocki* Ramadan, 1938 constitutes an economically important decapod in the southern coast of India. The caught occurred more than 2,122 tons per year. The diet content in relation to the sex, season, maturity, and size group of the species were investigated using 1,519 samples collected from the Southwest coast of India. The diet of *A. alcocki* consisted of 71 different prey categories predominantly smaller crustaceans (e.g. decapods, amphipods euphausiids), foraminiferans (Rotaliida, and Miliolida), molluscs (bivalves, gastropods, and cephalopods), polychaetes, and bryozoans. Feeding pattern was examined using Amundsen graphical method, which indicated *A. alcocki* exhibited with varying degree of specialization and generalization on different prey. There was slight seasonal variation relation to major prey items which might be due to the high environmental fluctuations of the deep waters and other biological processes. Females tend to be an effective predator than males considering stomach fullness and food quality. Parameter such as population characteristics, somatic and gonadal development might have attributed to this variation.The results of the present study indicate the quantitative criteria for the determination of major prey taxa which in turn emphasizes the need for the development of spatial management plans of the species.

OPPORTUNITIES IN ORAL VACCINES FOR SUSTAINABLE FINFISH AQUACULTURE

Padmanabhan Saravanan*, Huang Yun-Tzu, Lau Gek Khin Germaine, Deborah Chua, Fanny Ayumi Yasumaru, Chan Pek Sian Diana, Lee Chee Wee

Aquaculture Innovation Centre, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
saravanan_padmanabhan@tp.edu.sg

World aquaculture contribution to high quality protein supply to the growing population is steadily on the rise. This has demanded a shift in aquaculture practice to intensify industrial aquaculture. Infectious diseases are of major concern in aquaculture production globally, and effective disease management is a key to sustainability. Increasing array of emerging, re-emerging, and zoonoses diseases in aquaculture industry not limiting to anthropogenic activities on site is of significance. A multifaceted approach in controlling disease problem and animal health management is required to address high economic losses and safety alarms. Development of efficacious and safe oral vaccines poses several challenges and on the other hand has opened immense opportunities for biotechnological approach to provide solutions to combat viral and bacterial diseases in finfish aquaculture.

Aquaculture Innovation Centre (AIC) have developed capability in multivalent oral vaccine development and conduct of animal disease challenge in determining the specific immunity and protective efficacy of vaccines and therapeutics. The team has earlier developed a bivalent oral vaccine using microalgae as a vehicle for fish iridoviral disease and demonstrated 35% protection against 80% lethal dose (LD_{80}) challenge dose of the iridovirus strain.

MICROBIAL SCP AS A SUSTAINABLE PROTEIN SOURCE IN AQUAFEEDS

Sreedevi P*, Ravindra B, Ravikumar G, Ezhil S, Sadasivam J Kaushik

String Bio Private Limited, Nagasandra, Bangalore -560073, India.
padmanabhans@stringbio.com
subbiane@stringbio.com

The aquaculture industry with its rapid growth trajectory requires a stable supply of a protein source that is optimal and sustainable, ensuring growth, health and well-being of farmed animals. The stagnating production of fishmeal (FM), price volatility, environmental impact of reduction fisheries and growing scrutiny on the conversion of food grade fish to fish meal and fish oil and discussions over "fish-in fish-out ratios" are issues of concern.

String Bio's microbial protein offers a good alternative to fishmeal and is manufactured in a sustainable way by leveraging the String Integrated Methane Platform (SIMP). SIMP is an IP protected technology that enables sustainable manufacturing of ingredients for everyday living from methane. Bacteria are advantageous due to their high growth rates and efficient conversion of substrates to proteins. Methanotrophic bacteria utilize methane as a carbon and energy source. The efficient energy utilization of the methanotrophic bacteria in the conversion of methane to protein reduces greenhouse gas emission concomitantly to a substantial amount.

String Bio's microbial protein-based products were tested in various inclusions in the growth trials of pacific white shrimp[1,2], *Penaeus vannamei* for 12-weeks, rainbow trout[3], *Oncorhynchus mykiss* for 9-weeks and yellowtail kingfish[4], *Seriola lalandi* for 35-days. In all these trials, there was significant increase in body weight gain of animals that were fed with String Bio's microbial protein as a FM replacement. The digestibility of diets in these species was also better than the control diets. Histological observations of the gastrointestinal tract, liver and kidney did not show any differences between dietary treatments in rainbow trout. The relative gene expression of growth-related genes such as *GHR*, *IGF-1* and *IGF-2* in the SCP fed rainbow trout were higher than control fed fishes. Similarly, relative expression of the immune related genes such as lysozyme and toll-like receptor in the SCP fed animals was upregulated, when compared to control fed shrimps. These studies clearly validate String's microbial protein as a sustainable, alternative protein ingredient for the various groups of species such as crustaceans, salmonids or marine finfish.

[1]Felix N et al., Evaluation of single cell protein on the growth performance, digestibility and immune gene expression of Pacific white shrimp, *Penaeus vannamei*.

[2]Nederlof M et al., (2021). Effect of different types of single cell protein on feed intake, growth, body composition and digestibility of whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture Europe 2021 - Funchal, Madeira, Portugal.

[3]Rajesh M et al., (2022). Evaluation of a novel methanotroph bacteria meal grown on natural gas as fish meal substitute in rainbow trout, *Oncorhynchus mykiss*. Aquaculture Research, 53, 2159-2174. https://doi.org/10.1111/are.15735

[4]Pilmer L W et al., (2022). Using dietary additives to improve palatability of diets containing single-cell protein from methanotrophic bacteria in yellowtail kingfish (*Seriola lalandi*) diets. Aquaculture Research. https://doi.org/10.1111/are.15986

CULTURE OF ASIAN SEABASS *Lates calcarifer* IN THE RED SEA

Edoardo Pantanella*, JLQ Laranja, A. Mohamed, P. H. de Mello, S. Zehra, R. Saleh, A. Abulkasim, A. Al Shaikhi, A.M. Al-Suwailem

King Abdullah University of Science and Technology, Beacon Development, 23955-6900 Thuwal, Kingdom of Saudi Arabia
*edoardo.pantanella@kaust.edu.sa

Asian seabass or barramundi *Lates calcarifer* is considered a warm water species because the fish can grow better at higher temperatures (30-33°C) (Katersky & Carter, 2005). This fish has been cultured in the Red Sea, Saudi Arabia, where the country has a recent aquaculture production of around 18303 tons in live-weight (FAO, 2022). In this research, we conducted several experiments to determine the growth and production potential of barramundi under the Red Sea conditions (i.e., salinity=42 ppt.) In the baseline growth experiments, our results showed that from 60 g ABW, the fish attained 183-211 g after 78 days with weight gain of around 45-57 g month^{-1} and feed conversion ratio (FCR) values of around 1.14-1.42. In another trial using bigger size fish (initial: 454 g ABW), the fish attained 903-972 g after 77 days and gained 177-200 g month^{-1} with FCR values of around 1.41-1.55. Furthermore, a digestibility trial for the different protein and lipid source ingredients was also conducted to formulate practical diets for the barramundi. Our digestibility trial showed that barramundi has a higher protein digestibility coefficient in wheat gluten meal (0.95), soybean meal (0.94), fishmeal (0.92), and corn gluten meal (0.87). For lipid source ingredients, a higher digestibility coefficient value was observed in poultry oil (0.99), sardine oil (0.95), and rapeseed oil (0.77). From these ingredients, practical diets were then formulated as follows: Diet 1: contains 44% crude protein and 20% lipid; P/E ratio: 20 (Diet 2): contains 40% crude protein, and 24% lipid; P/E ratio 18. These diets were then tested in separate tank trials using smaller and bigger size barramundi. In the first trial using smaller fish, the fish were fed with the practical diet 1 as compared to a commercial diet for 80 days. The results showed that diet 1 attained a significantly higher final ABW (382 g), growth gain (77 g month^{-1}), and protein efficiency ratio (PER) (2.12), and a significantly lower FCR value (1.16) as compared to the commercial feed (ABW_{final}=343 g; growth gain= 63 g month^{-1}; PER=1.26; FCR=1.71), suggesting that the practical diet 1 showed better performance than the reference diet. In the bigger fish (initial: 652 g ABW), no significant differences in final ABW (1021-1.032 g ABW) and weight gain (137-143 g month^{-1}) were observed between the different diets tested, however, the FCR value was significantly lower in diet 1 (1.56) as compared to diet 2 (1.89) and the commercial diet (1.78), while PER was significantly higher in diet 1 (1.58) and diet 2 (1.45) as compared to the commercial diet (1.21). Finally, a cage trial in the Red Sea was also conducted to determine the growth and production performance of barramundi fed the practical diet. The results of the cage trial in the Red Sea will be presented at the conference. In conclusion, barramundi can grow well in the Red Sea conditions with FCR values showing commercially viable results.

EFFECT OF STOCKING DENSITY ON HEMATOLOGICAL, BIOCHEMICAL PROFILES AND PRODUCTIVE PARAMETERS OF JUVENILE *Arapaima gigas*

Paredes López D*, Robles Huaynate R., Rebaza C. and Delgado Ramirez, J.

*Department of Animal Science, Universidad Nacional Agraria de la Selva
Carretera Central Km 1.21 Po Box 156, Tingo María, Perú
E-mail: daniel.paredes@unas.edu.pe

At inadequate stocking density, fish generally show low growth, cortisol hypersecretion, hyperglycemia and other biochemical changes. For the Amazonian species, among them, *Arapaima gigas,* potential for intensive stocking has not yet been determined. The objective was to contribute for improving A. gigas production by evaluating the effect of stocking density on hematological, biochemical profile and the productive parameters of juvenile *A. gigas*. One hundred and eighty, twelve months old, *A. gigas* with a live weight of 2.37 kg were distributed into densities of 106.65 kg/m^3 (T1), 142.2 kg/m^3 (T2) and 177.75 kg/m^3 (T3) with three replicates per treatment and with a water circulation of 7 L/min. They were fed with a diet of 45% raw protein during four months, at a rate of 3% live weight. Blood samples were taken at thirteen, fourteen and fifteen months old. Hematological profiles, glucose, total protein, Albumin, aspartate transaminase (AST), alanine transaminase (ALT) and urea, were determined with a DIALAB DTN 405 spectrophotometer and 530 nm filters**.** Final weight (FW), size increase (SI), weight gain (WG), Feed consumption (FC), feed conversion rate (FCR) and volume of biomase (VB) were also evaluated.

Erythrocytes count (ERI) diminished with the increase in stocking density ($P<0.01$), nonetheless, total leukocytes (LEU), hematocrit (HTO) and hemoglobin (HB), increased as the stocking density increased ($p<0.05$) (Table 1). Albumin, aspartate transaminase (AST), alanine transaminase (ALT) and urea diminished with the greater stocking density ($p<0.01$).

(Table 2). FC diminished and VB increased in relation to the increase in stocking density ($p<0.01$) (Table 3). *Arapaima gigas* showed an adaptive sanguineous response to the effects of the greater stocking density.

Table 1: Hematological profiles of juvenile *Arapaima gigas* at different stocking densities.

Profiles / Densities	LEU (10^3/µl)	ERIT (10^6/µl)	HTO (%)	HB (g/dl)
106.65 kg m^{-3}	11.55 b	1.89 a	32.8b	10.13 b
142.2 kg m^{-3}	9.16 b	1.58 b	34.4 a	10.34 b
177.75 kg m^{-3}	14.19 a	1.55 b	36.6 a	11.41 a

Table 2: Biochemical profiles of juveniles *Arapaima gigas* at different stocking densities.

Profiles / Densities	GLUCOSE (g/dl)	PROTEIN (g/dl)	ALBUMIN (g/dl)	AST (UI/L)	ALT (UI/L)	UREA (g/L)
106.65 kg m^{-3}	31.04	2.89	1.36 ab	20.65 ab	16.61 a	8.49 a
142.2 kg m^{-3}	29.43	2.76	1.41 a	22.56 a	14.00 b	6.48 c
177.75 kg m^{-3}	28.55	2.76	1.31 b	19.47 b	14.61 ab	7.11 b

Table 3: Productive parameters of juvenile *Arapaima gigas* at different stocking densities.

Parameters / Densities	FW (Kg)	SI (cm)	WG (Kg)	FC (Kg)	FCR	VB (Kg)
118.50 kg/m^3	4.22	11.66	1.85	2.48 a	1.39	189.90c
149.31 kg/m^3	4.16	11.42	1.79	2.42 a	1.36	249.60b
196.71 kg/m^3	4.08	9.57	1.71	2.06 b	1.22	306.00a

a, b: Different letter between lines denotes statistical difference ($p\leq0.05$)

CRYOPRESERVATION OF MICROALGAE FOR THE AQUACULTURE INDUSTRY

*Estefania Paredes, Arantxa Martinez, Damian Costas

Marine Biological Resources Functional Preservation Service, Centro de Investigaciones Marinas, Universidade de Vigo, Illa de Toralla 36331, Coruxo, Vigo, Spain
eparedes@uvigo.es

Cryopreservation and storage at cryogenic temperatures is the only suitable form of stable long-term storage of live biological samples. Marine microalgae cryopreservation has extensively developed during the last decades, to provide an alternative to the continuous culture of microalgae strains, saving time, space and increasing the possible amount of stains stored without the morphological and physiological modifications of a continuous culture. We targeted a group of species used in aquaculture that might be present in different aquaculture facilities (either in research institutions or private companies) worldwide. We have studied cryopreservation protocols for *Tisochrysis lutea, Phaedoctylum tricornutum, Rhodomonas lens, Nanochloropsis gaditana, Tetraselmis suecica, Cylindrotheca closterium, Chaetoceros neogracile,* and *Synechoccocus sp*. using simple methodologies. It is possible to cryopreserve most of them with a good post-thaw outcome in terms of growth, morphology and physiology, although generating a vigorous culture from a cryopreserved sample takes longer than directly from a fresh culture. All the aforementioned species were cryopreserved using 15% (v/v) Dimethyl sulfoxide as cryoprotecting agent and using either a very cheap passive freezer Cryopreservation technology could play an important role production of primary cultures, genetic management of broodstock and conservation of aquatic resources. Moving a step further, cryopreservation could play a very interesting role as a way of preserving secondary cultures like microalgae or zooplankton that are intensively used during peak times of production but the rest of the year should either be maintained or bought new every season. Cryopreservation could also be used to maintain a back-up copy of interesting strains to guarantee the continuity of supply of a certain strain.

Funding source: European Blue Biobank (EBB), EAPA_501/2016, funded by Interreg Atlantic Area (EU)

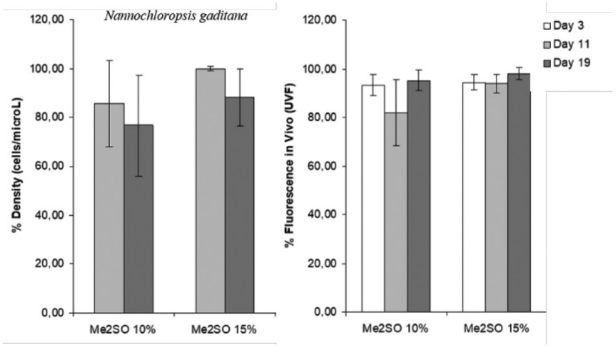


Figure 1.- Shows an example of post-thaw results obtained after cryopreservation on *Nanochloropsis gaditana*. Evaluation of post-thaw fitness was done as culture density and percentage

FROM THE OCEAN TO THE FREEZER AND BACK: MUSSEL CRYOPRESERVATION

*E. Paredes, P. Heres, J. Troncoso

*Estefania Paredes, Arantxa Martinez, Damian Costas
Marine Biological Resources Functional Preservation Service, Centro de Investigaciones Marinas
Universidade de Vigo, Illa de Toralla 36331, Coruxo, Vigo, Spain
eparedes@uvigo.es

The global production of blue mussel (*Mytilus galloprovincialis*) has a significant role on the marine aquaculture industry; hence it is one of the most cultivated mollusk worldwide (FAO, 2018). However, several hazards affect the culture and the seasonal supply of mussel seed, which sets up an increasing interest on the development of inland seed production without seasonal limitation and safe of global variations. Cryopreservation can provide the stable storage of living resource of mollusk early development stages throughout the year and the possibility of selection of genetic lines according to production issues. Here, cryopreservation long term effects were analyzed on cryopreserved mussel larvae and their capacity for competent seed production using two different larval stages and protocols. Mature blue mussels from Galicia (NW Spain) were spawned and cells were incubated to produce 72h-old D-larvae for these long-term experiments where larvae were incubated post-thaw in the lab on a full larval rearing until reaching the juvenile stage. Then those with higher survival rates were settled into culture ropes and moved into a mussel raft for a year-round monitored incubation (Fig. 1). The study of the cryopreservation long term effects is essential to achieve the totally implementation of cryopreservation for Aquaculture and fish management.

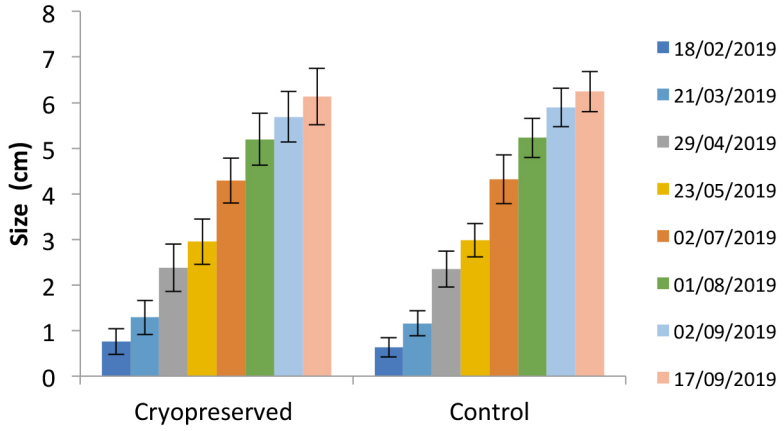


Figure 1.- Shows the average size of mussels settled on ropes from cryopreserved larvae Vs. from control Larvae along the time. This research has been funded by Assemble+, grant from the European Union's Horizon 2020 research and innovation programme (No. 730984).

APPLICATION OF dsRNA VP15-WSSV TO INCREASE SURVIVAL RATE AND RENSPONSE IMMUNES OF TIGER SHRIMP *Penaeus monodon* BY ORAL VACCINATION

Andi Parenrengi*, Andi Tenriulo, Bunga Rante Tampangallo, Agus Nawang, and Samuel Lante

Research Institute for Brackishwater Aquaculture and Fisheries Extension
Maros, Sulawesi Selatan, Indonesia, 90511
Agency for Marine and Fisheries Research and Human Resources
andi_parenrengi@hotmail.com

White spot syndrome virus (WSSV) is responsible for severe economic losses in tiger shrimp *Penaeus monodon* culture worldwide. Several studies have been carried out to control WSSV, however, there is no efficient strategy to control this serious disease. RNA interference (RNAi) has recently emerged as a powerful tool for specific gene silencing in antiviral therapy. The efficiency of RNAi in shrimp was demonstrated by *in-vivo* injection of dsRNA, however, its application by oral vaccination is very limited. The present study was conducted to assess the effect of dsRNA VP15-WSSV to survival rate and response immunes of tiger shrimp by oral vaccination.

A gene encoding VP15 was isolated from WSSV infecting tiger shrimp and cloned into the L4440 vector containing the T7 promoter. The gene construct of T7-VP15 was transformed to *Escherichia coli* DH5α and grew in LB media for mass production. The recombinant bacteria were in-activated using heat-killed bacteria method by immersion. The shrimp (25.2±4.2 g in weight) were screened for the presence of WSSV by PCR to get healthy shrimp for the experiment. For the oral vaccination treatments, commercial shrimp pellet 0.02 g were mixed with 10^8 cell of bacteria containing T7-VP15 and coated with fish oil to prevent dispersion (A), pellet coated with fish oil only (without dsRNA) as a positive control (B), and pellet without dsRNA and fish oil as negative control (C). The feed was incubated on ice for 15 min followed by room temperature incubation for 30 min to allow absorption. After feeding for 15 days, the shrimp was challenged with WSSV by muscular injection. Survival rate (SR) was observed every day and response immunes of total haemocyte count (THC) and prophenoloxidase (proPO) were measured at before and 1, 3, 5 days of post challenge (dpc).

Survival rate of tiger shrimp for all treatments sharply dropped until the 3rd dpc and the 4th dpc the dsRNA application treatment was started to exhibit the higher SR, compare to both treatments (Figure 1). Tiger shrimp fed with pellet coated with dsRNA had significantly (P<0.05) higher SR and THC compared with. both control treatments, but not significantly (P>0.05) for proPO (Table 1). The results indicate that application oral vaccination of dsRNA appears to increase SR and response immune of tiger shrimp.

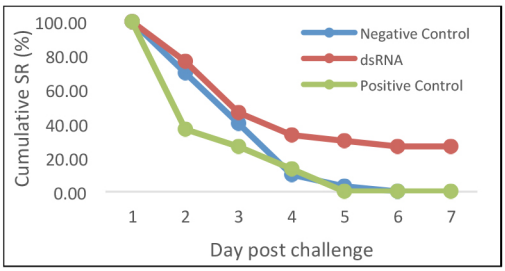


FIGURE 1. Cumulative survival rate of tiger shrimp fed with dsRNA by oral vaccination after challenge test

TABLE 1. Survival rate on the 5th dpc, average of survival rate and proPO of tiger shrimp feed with pellet coated with dsRNA. Means in a row with different letters were significantly different (P<0.05)

	Treatment		
	A	B	C
SR (%)	30.0[a]	0.0[b]	3.3[b]
THC (10^4 cell/mL)	1,981[a]	1,086[b]	1,305[b]
proPO	0.072[a]	0.076[a]	0.109[a]

SEXUAL MATURATION OF STARRY FLOUNDER, *Platichthys stellatus* AND HYBRID, *P. stellatus* ♀ × *Kareius bicoloratus* ♂ TRIPLOID MALES

Jungyeol Park[a], Jihye Yoon[b], Junwook Hur[c], Hankyu Lim[a]*

[a]Department of Marine and Fisheries Resources, Mokpo National University
Jeonnam 58554
Republic of Korea
limhk@mokpo.ac.kr
[b]Department of Biomedicine, Health & Life Convergence Sciences, BK21
Mokpo National Universityß
[c]Faculty of Marine Applied Biosciences, Kunsan National University

The production of sterile fish via triploid induction is an effective method for preventing the unauthorized duplication of breeds developed through breeding. It can also help prevent genetic contamination or ecological problems caused by the escape of farmed fish from the natural world. Moreover, triploids are utilized in fishery resource management to genetically separate native populations from artificially produced resources and to prevent the settlement of invasive species. In addition to these benefits, it is also possible to reduce male aggression that appears when sexual characteristics are expressed and to prevent problems that may occur when fish in breeding escape to the natural world.

In this study, we examined sexual maturation in diploid and triploid starry flounder, and diploid and triploid hybrids. Changes in gonad tissues and the gonadosomatic index (GSI) were evaluated from December to April, and sperm activity and volume were measured for a total of 12 weeks, from early February to late April. Male GSI was significantly lower in triploid than in diploid fish, and decreased sharply in March in the diploid hybrid, and in April in the diploid starry flounder.

Analyses of gonad tissue showed that spermatozoa were first observed in December in the diploid hybrid, and in January in the diploid starry flounder. Spermatozoa were observed in triploid fish beginning in March, with a rapid progression then seen in April, indicating a shorter spawning season in triploid than in diploid fish. Sperm volume and fertility were evaluated using sperm collected from each experimental fish. Sperm volume was > 3-fold higher in diploid than triploid fish. Sperm activity in triploid fish was < 50% that in diploid fish. To evaluate sperm fertility, we performed artificial insemination on diploid fish eggs, and observed the floating eggs. Artificial egg fertilization using the sperm of triploid fish was unsuccessful.

EFFECT OF WATER TEMPERATURE, SALINITY AND ANAESTHETIC OF OLIVE FLOUNDER, *Paralichthys olivaceus* TO LIVE TRANSPORTATION

Jungyeol Park[a], Hyonsob Han[a], Hankyu Lim[b], Junwook Hur[a*]

[a]Department of Aquaculture and Aquatic Science, Kunsan National University, Republic of Korea
junwhur@kunsan.ac.kr
[b]Department of Marine and Fisheries Resources, Mokpo National University, Republic of Korea

Acute stress response (cortisol, glucose, Na^+, K^+, Cl^-, Total protein, ALT, AST, hematocrit, red blood cell count, hemoglobin amount, MCV, MCH and MCHC) and changes in physiological conditions according to the salt and density of the breeding water (Survival rate, growth, feed efficiency and obesity, etc.) were investigated. Plasma cortisol concentration in the stress response of acute salinity change of flounder raised with low salinity was from 4.4±1.9 ng/ml at the start of the experiment to 3.7±1.9 ng/ml at 3 hours and 2.8±1.1 ng/ml at 24 hours for 35‰ fish. While there was no difference, the 0‰ sphere showed no difference at 2.0±0.1 ng/ml at 3 hours, but showed the highest value at 47.9±18.6 ng/ml at 24 hours. The Na+ concentration did not show a difference from 166.8±10.7 mEq/L at the start of the experiment to 164.5±2.5 mEq/L in the 0‰ sphere at 3 hours, but decreased significantly to 149.0±9.4 mEq/L at 24 hours. The 35‰ sphere showed no difference from the start of the experiment.

The AST value was 26.3±11.4 IU/L at 24 hours in the 0‰, which was about 2 times higher than 12.0±2.2 IU/L at the start of the experiment. In the stress response of low salinity to low water temperature and anesthesia transport, the cortisol concentration before transport increased from 2.4±0.1 ng/mL to 16.7±12.8 ng/mL in CWT+35‰, but there was no significant difference, whereas NWT+35‰. The sphere increased to 47.9±19.8 ng/ml, showing a difference. The NWT+15‰ group increased significantly to 43.5±13.9 ng/ml and the CWT+15‰ group increased to 26.1±8.3. The NWT+15‰+Anes. group increased to 61.7±3.3 ng/ml, and the CWT+15‰+Anes. group showed the highest value at 86.1±19.0 ng/ml. The K^+ concentration slightly increased from 3.1±0.0 mEq/L before transport to 4.5±1.1 mEq/L in the NWT+15‰ group, showing no difference, and significantly increased in all other experimental groups.

THE EFFECTS OF WATER TEMPERATURE CHANGES ON OXYGEN CONSUMPTION AND HEMATOLOGICAL FACTORS OF OLIVE FLOUNDER, *Paralichthys olivaceus*

Jungyeol Park[a], Hyonsob Han[a], Hankyu Lim[b], Junwook Hur[a*]

[a]Department of Aquaculture and Aquatic Science, Kunsan National University, Republic of Korea
junwhur@kunsan.ac.kr
[b]Department of Marine and Fisheries Resources, Mokpo National University, Republic of Korea

Water temperature (WT) is a major environmental factor of metabolic rate in fishes, and directly affects food intake, ammonia excretion, oxygen consumption, growth, and survival. Thus, the purpose of this study was to investigate the change of oxygen consumption (OC) and hematological response of olive flounder, *Paralichthys olivaceus* on WT changes. In Exp. I, WT increased from 20°C to 29°C within 18 hours, and then maintained at 29°C for 96 hours. WT decreased from 29°C to 20°C within 18 hours, and maintained at 20°C for 24 hours. In Exp. II, WT decreased from 20°C to 11°C within 18 hours, and maintained at 11°C for 96 hours. WT increased from 11°C to 20°C within 18 hours, and maintained at 20°C for 24 hours. The Exp. III maintained that the Exp. I and II was consecutively.

In Exp. I, the oxygen consumption increased from 116.7 mg O_2 $kg^{-1}hr^{-1}$ to 317.5 mg O_2 $kg^{-1}hr^{-1}$ with increasing WT. After, OC decreased to 130.3 mg O_2 $kg^{-1}hr^{-1}$ with decreasing WT. In Exp. II, the OC decreased from 96.5 mg O_2 $kg^{-1}hr^{-1}$ to 71.3 mg O_2 $kg^{-1}hr^{-1}$ with decreasing WT, and increased to 96.7 mg O_2 $kg^{-1}hr^{-1}$ when WT was increased to 20°C and maintained during 24 hours. In Exp. III, the OC, when the WT was maintained at 20°C for 48 hours, was found to be 117.1 mg O_2 $kg^{-1}hr^{-1}$. OC was 226.1 mg O_2 $kg^{-1}hr^{-1}$ during 3 days at 29°C, and was decreased to 108.4 mg O_2 $kg^{-1}hr^{-1}$ during 3 days maintenance of WT to 20°C. When WT was decreased to 11°C, and maintained for 3 days, OC was decreased to 54.4 mg O_2 $kg^{-1}hr^{-1}$. After, OC, when WT was kept for 48 hours after increased to 20°C, was increased 110.2 mg O_2 $kg^{-1}hr^{-1}$. In Exp. I, cortisol, glucose and AST (aspartate aminotransferase) values of olive flounder were increased with increasing WT. In Exp. II, Cl^-, osmolality, AST and ALT (alanine aminotransferase) values were significantly changed during experimental period. Glucose value was increased, and cortisol values was decreased with decreasing WT. But cortisol and glucose values were no significant differences between the initial and the final of experiment. In Exp. III, hematocrit, cortisol, glucose and AST were significantly increased, and Cl^-, osmolality and ALT were significantly decreased during experimental period. The other parameters were not significantly changed. Exp. III is likely more stressful environment to olive flounder than Exp. I and Exp. II. The results of our study would be useful for evaluating current culture procedures of olive flounder, and also for developing techniques to minimize stress during aquaculture.

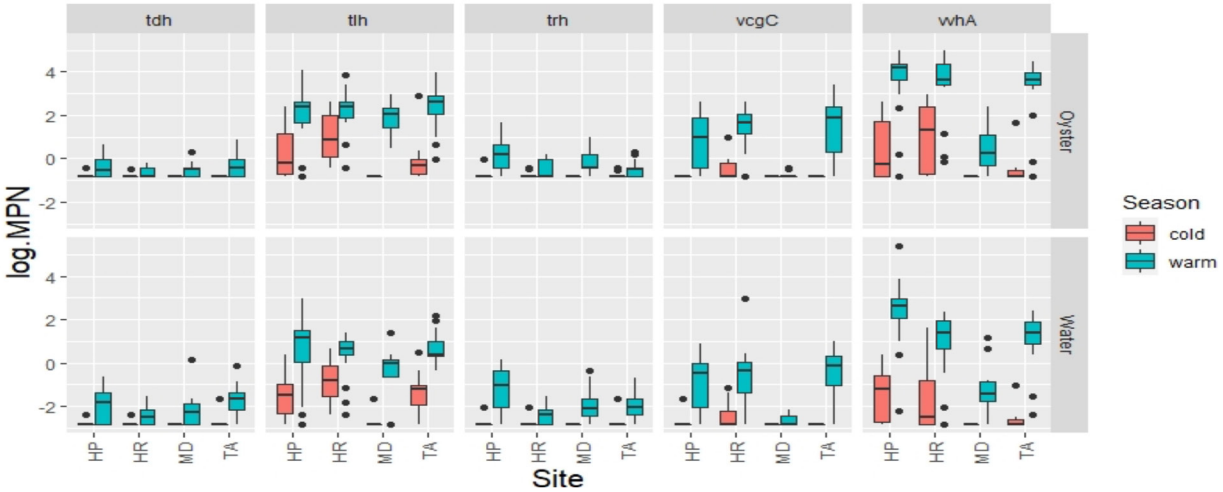
VIBRIOS IN FARMED OYSTERS *Crassostrea virginica* IN RELATION TO SEASON AND DIFFERENT FARMS ACROSS THE CHESAPEAKE AND MARYLAND COASTAL BAYS

Salina Parveen*, Esam Almuhaideb, John Bowers, Tahirah Johnson, and Gary Richards

Department of Agriculture, Food and Resource Sciences
University of Maryland Eastern Shore
Princess Anne, MD, 21853
sparveen@umes.edu

Oyster farming is one of the most important marine aquaculture practices that contributes to the Chesapeake and the Maryland Coastal Bays habitat and ecosystem. Illness caused by *Vibrio parahaemolyticus* (*Vp*) and *V. vulnificus* (*Vv*) can be contracted from consuming raw or undercooked oysters. Oyster-*Vibrio* associated outbreaks, specifically in the summer, emphasize the importance of aquaculture risk assessment. Also, it causes economic burden to the oyster farming industry. The aim of this study was to assess the effects of season on the prevalence of total and pathogenic *Vp* and *Vv* in oyster and seawater samples using MPN-qPCR. Oyster (n=108) and seawater (n=112) samples were collected from three farming sites in the Chesapeake Bay (CB) and one site in the Maryland Coastal Bays (MCB), USA from June 2019-October 2021. Weather conditions and physico-chemical parameters of the water were recorded per sampling event. MPN-qPCR was performed to quantify total and pathogenic *Vp* (*tlh*, *tdh*, *trh* genes) and *Vv* (*vvh*A, *vcg*C genes). Positivity rates for oyster and water samples were 88 and 92% for *tlh*, 81 and 80% for *tdh*, 37 and 48% for *trh*, 45 and 54% for *vvh*A, and 54 and 56% for *vcg*C, respectively.

The detection frequency of total *Vp* (*tlh*$^+$) and pathogenic *Vv* (*vcg*C$^+$) from oyster and water was significantly lower in MCB than in CB farms in the colder and warmer months, respectively. Moreover, *Vp* was more abundant than *Vv* in the MCB. During the warmer months, the average log MPN of *Vp* (*tlh*$^+$) in the oysters from the CB farms was significantly lower than *Vv* (*vvh*A$^+$). However, the average log MPN of *Vp* (1.9) was significantly higher than *Vv* (0.5) in the oysters from MCB. The average log MPN of *Vp* (1.5) and *Vv* (2.1) in oyster were higher than in water. These results indicate that *Vp* and *Vv* were more prevalent in the CB than the MCB.



Boxplot of the LogMPN (g/mL) in relation to sample type and season.
-CB farms: Horn Point (HP), Honga River (HR), Tangier (TA) -MCB farm: Maryland Coastal Bay (MD)

IMPROVEMENTS IN REPRODUCTIVE PERFORMANCE OF FEMALE *Macrobrachium rosenbergii*, (De Man) SELECTED FOR IMPROVED HARVEST WEIGHT IN INDIA

Gunamaya Patra[1&2]*, Bindu R. Pillai[1], Pramod K. Sahoo[1], Swagatika Mohanty & Namita K. Naik

[1]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India, 75100 2
[2]Department of Fisheries, Ministry of Fisheries, Animal Husbandry & Dairying, Government of India Krishi Bhawan, New Delhi -110011
*Email:gunamayapatra@gmail.com

Giant freshwater prawn *Macrobrachium rosenbergii* is an important freshwater crustacean species widely cultured in several south and Southeast Asian countries and fetches higher prices compared to freshwater fishes. Aquaculture production of this species reportedly declined in several countries including India. In order to develop a faster-growing strain of the species, ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, Odisha, India has started a family-based selective breeding programme of *M. rosenbergii* in collaboration with WorldFish, Malaysia. The base population for selection was established by using a complete three-by-three diallel cross among geographically distant populations of *M. rosenbergii* in India. The present study reports the performance of selected reproductive parameters of females of 3rd and 4th generations of selectively bred *M. rosenbergii*. Egg quality parameters such as fecundity, egg clutch weight, egg somatic index, egg wet and dry weight, and egg diameter were studied in 30 females from 3rd generation and 32 females from 4th generation females. Among the parameters studied, egg clutch weight was significantly higher in 4th generation (5.91±1.82 g) of selectively bred *M. rosenbergii* compared to 3rd generation (4.16±1.13g).). The number of eggs per female, however, showed wide variations within and between generations. The mean body weight and total length of 4th generation berried females were higher (40.5±3.17 g; 157.96±11.18 mm) compared to that of 3rd generation berried females (31.55±6.28 g; 149.93±10.12 mm). Body traits and egg quality parameters (mean±SD) of 3rd and 4th generation of selectively bred *M. rosenbergii* berried female (n=number of replicates) depicted at Table 1. The results indicated the presence of a correlated positive response in female reproductive parameters in *M. rosenbergii* selected for higher body weight.

Table 1: Body traits and egg quality parameters (mean±SD) of 3rd and 4th generation of selectively bred *M. rosenbergii* berried female (n=number of replicates)

Parameters	3rd Generation (n=30)	4th Generation (n=32)
Body Weight (g)	31.55±6.28[a]	40.5±3.17[b]
Total Length (mm)	149.9±10.12 [a]	157.9±11.18 [b]
Carapace length (mm)	37.7±3.87 [a]	44.25±10.37 [b]
Standard length (mm)	91.7±6.80 [a]	100.46±6.46 [b]
Egg clutch wet weight (g)	4.16±1.13 [a]	5.91±1.82 [b]
Egg clutch dry weight (g)	1.63±0.59 [a]	2.40±0.82 [b]
Egg somatic index	13.097±1.185 [a]	13.194±1.205 [a]
Egg wet weight (µg)	110.28±7.16 [a]	102.79±5.62 [b]
Egg dry weight (µg)	42.16±4.33	41.49±4.39
Egg Moisture content (%)	68.11±8.83 [b]	61.3±6.62 [a]
Egg diameter (mm)	0.600±0.01 [a]	0.609±0.02 [a]
Eggs/Female (nos)	38,239±11960 [a]	58,066±19744 [b]
Fecundity(eggs g^{-1} female)	1,193±145 [a]	1,289±158 [b]

MIXTURE OF TRACE MINERAL AMINO ACID COMPLEX SUPPLEMENTATION IN LOW FISHMEAL WITH HIGH SOYBEAN MEAL DIET FOR WHITE SHRIMP (*Litopenaeus vannamei*): EFFECT ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND DIGESTIVE ENZYME ACTIVITY

Kanin Patrachotpakinkul*, Orapin Jintasataporn, Srinoy Schumkam and Cinzia Boggino

Nutrition and Aquafeed Laboratory of Aquaculture Department
Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand
E-mail: kanin.pa@ku.th

Fish meal is a mineral-rich raw material used as a protein source in animal diets. Due to unsustainable fishery practices resulting in insufficient quantities of fish meal production for the aquafeed industry means other protein sources need to be used. Trace minerals are added to the feed to avoid deficiency because of their importance in enzyme modulation by acting as cofactors and activators. Therefore, this research study was conducted to evaluate the effect of trace mineral concentration in white shrimp, *Litopenaeus vannamei*, diets by focusing on growth performance, digestive enzyme activity, and the immune response. The trial was assigned in CRD with 3 treatments, each with 5 replicates. The diets were formulated to achieve 36% crude protein and 5% crude fat using the same fishmeal levels of 7.5%, soybean meal levels of 40% and varying mixture of trace mineral amino acid complex (Zinpro Corporation, USA) concentration 1x and 2x, compared to diet of fishmeal 15% with trace mineral amino acid complex (Zinpro Corporation, USA) concentration 1x that was the setting for the control group. All diets were fed to shrimps for 8 weeks. The results under normal conditions showed no significant differences ($P>0.5$) between the groups in terms of final body weight, total production, weight gain, average daily gain, specific growth rate, feed conversion ratio, protein efficiency ratio, and survival rate. Final body weight ranged between 16.49 ± 0.43 g to 17.00 ± 0.80 g. Average daily gain ranged between 0.27 ± 0.01 g/shrimp/day to 0.28 ± 0.01 g/shrimp/day. The survival rate ranged between 86.7 ± 8.58 % to 92.7 ± 4.18 %. In addition, the immune response such as total hemocyte count and hemolymph protein showed no significant differences between the groups ($P>0.5$) whereas phenoloxidase of shrimp fed fishmeal 7.5% both mineral amino acid complex 1x and 2x showed higher values than shrimp fed fishmeal 15% ($P<0.5$). For antioxidant enzyme activity, lysozyme and superoxide dismutase showed no significant difference between the groups ($P>0.5$). Interestingly, protease and lipase activity in shrimp hepatopancreas have an increasing value when top-up mineral amino acid complex concentration 1x and 2x in the diet of fishmeal 7.5%. The energy reserve in terms of glycogen of shrimp fed diet of fishmeal 7.5% with mineral amino acid complex 2x showed the highest value ($P<0.5$). These results demonstrate that a mixture of trace mineral amino acid complex improves the nutrient digestion, absorption, and accumulation in white shrimp, *Litopenaeus vannamei*, in a diet with fishmeal levels of 7.5% without any adverse effect on growth performance, immune response, and antioxidant enzyme activity. Therefore, supplemental mixture of trace mineral amino acid complex at concentration 1x and 2x in a low fish meal / high soybean meal diet can improve protease and lipase activity and promote glycogen deposition in shrimp hepatopancreas.

MIXTURE OF TRACE MINERAL AMINO ACID COMPLEX SUPPLEMENTATION IN LOW FISHMEAL WITH HIGH SOYBEAN MEAL DIET FOR WHITE SHRIMP (*Litopenaeus vannamei*): EFFECT ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND DIGESTIVE ENZYME ACTIVITY

Kanin Patrachotpakinkul*, Orapin Jintasataporn, Srinoy Schumkam and Cinzia Boggino

Nutrition and Aquafeed Laboratory of Aquaculture Department
Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand
E-mail: kanin.pa@ku.th

Fish meal is a mineral-rich raw material used as a protein source in animal diets. Due to unsustainable fishery practices resulting in insufficient quantities of fish meal production for the aquafeed industry means other protein sources need to be used. Trace minerals are added to the feed to avoid deficiency because of their importance in enzyme modulation by acting as cofactors and activators. Therefore, this research study was conducted to evaluate the effect of trace mineral concentration in white shrimp, *Litopenaeus vannamei*, diets by focusing on growth performance, digestive enzyme activity, and the immune response. The trial was assigned in CRD with 3 treatments, each with 5 replicates. The diets were formulated to achieve 36% crude protein and 5% crude fat using the same fishmeal levels of 7.5%, soybean meal levels of 40% and varying mixture of trace mineral amino acid complex (Zinpro Corporation, USA) concentration 1x and 2x, compared to diet of fishmeal 15% with trace mineral amino acid complex (Zinpro Corporation, USA) concentration 1x that was the setting for the control group. All diets were fed to shrimps for 8 weeks. The results under normal conditions showed no significant differences ($P>0.5$) between the groups in terms of final body weight, total production, weight gain, average daily gain, specific growth rate, feed conversion ratio, protein efficiency ratio, and survival rate. Final body weight ranged between 16.49 ± 0.43 g to 17.00 ± 0.80 g. Average daily gain ranged between 0.27 ± 0.01 g/shrimp/day to 0.28 ± 0.01 g/shrimp/day. The survival rate ranged between 86.7 ± 8.58 % to 92.7 ± 4.18 %. In addition, the immune response such as total hemocyte count and hemolymph protein showed no significant differences between the groups ($P>0.5$) whereas phenoloxidase of shrimp fed fishmeal 7.5% both mineral amino acid complex 1x and 2x showed higher values than shrimp fed fishmeal 15% ($P<0.5$). For antioxidant enzyme activity, lysozyme and superoxide dismutase showed no significant difference between the groups ($P>0.5$). Interestingly, protease and lipase activity in shrimp hepatopancreas have an increasing value when top-up mineral amino acid complex concentration 1x and 2x in the diet of fishmeal 7.5%. The energy reserve in terms of glycogen of shrimp fed diet of fishmeal 7.5% with mineral amino acid complex 2x showed the highest value ($P<0.5$). These results demonstrate that a mixture of trace mineral amino acid complex improves the nutrient digestion, absorption, and accumulation in white shrimp, *Litopenaeus vannamei*, in a diet with fishmeal levels of 7.5% without any adverse effect on growth performance, immune response, and antioxidant enzyme activity. Therefore, supplemental mixture of trace mineral amino acid complex at concentration 1x and 2x in a low fish meal / high soybean meal diet can improve protease and lipase activity and promote glycogen deposition in shrimp hepatopancreas.

CHARACTERISATION OF *Edwardsiella ictaluri* RECOVERED FROM CLINICAL DISEASE OUTBREAKS IN STRIPED CATFISH *Pangasianodon hypophthalmus* FARMS IN VIETNAM OVER A TWENTY-YEAR PERIOD

Christopher Payne*, Kathryn Grace, Vo Hong Phuong, Le Hong Phuoc, and Margaret Crumlish

Institute of Aquaculture, University of Stirling, Stirling, United Kingdom
Email: c.j.payne1@stir.ac.uk

Edwardsiella ictaluri, the causative agent of bacillary necrosis of *Pangasius* (BNP), has been a significant challenge to the farmed catfish industry in Vietnam since it was first reported over two decades ago. However, little is known about the current heterogeneity of *E. ictaluri* strains circulating striped catfish (*Pangasianodon hypophthalmus*) farms in Vietnam and how these populations have changed over time. Therefore, the aim of this study was to characterise *E. ictaluri* strains associated with BNP in farmed striped catfish in Vietnam over a twenty-year period.

A total of 203 strains were investigated in this study, which were recovered from clinical disease outbreaks across six Mekong Delta provinces in Vietnam between 2001 and 2021. Bacterial strains were identified using primary identification methods and their biochemical profile was characterised using the commercial API20E® system. In addition, their sensitivity to six antibiotic compounds used in global aquaculture was profiled using the Kirby-Bauer disk diffusion method. Then, a subset (n = 83) of bacterial strains were further typed by pulse-field gel electrophoresis methods and screened for the presence of eight virulence genes by standard PCR.

In general, *E. ictaluri* strains were found to be homogenous in their biochemical profiles (Table 1), irrespective of geographical location or year of recovery. Antibiotic resistance was found to increase over time, with high-level, multidrug resistance, associated with strains recovered between 2018 and 2021. Pulse-field gel electrophoresis differentiated selected strains into 16 pulsotypes. Furthermore, strains recovered between 2001 and 2011 were found to cluster distinctly from those recovered after 2017. Screening of virulence genes using PCR methods revealed six conserved virulence mechanisms in selected strains, however differences were noted in the presence of *eseJ* and *traD*, associated with the type IV and IV secretion systems, respectively. Continued surveillance of *E. ictaluri* in Vietnam will be important to inform biosecurity strategies and aid in the development of effective vaccines against BNP in striped catfish.

TABLE 1. Morphology and biochemical characteristics of *Edwardsiella ictaluri* isolated from clinical disease outbreaks in farmed striped catfish (*Pangasianodon hypophthalmus*) in Vietnam between 2001 and 2021.

Characteristic	Profile	
	I	II
Gram	Negative	Negative
Morphology	Rods	Rods
Motility	-	-
Oxidase	-	-
ONPG	-	-
Arginine dihydrolase	-	-
Lysine decarboxylase	+	+
Ornithine decarboxylase	-	-
Citrate utilisation	-	+
H2S production	-	-
Urease	-	-
TDA	-	-
Voges-Proskauer	-	-
Gelatin	-	-
Acid Production		
D-glucose	+	+
D-mannitol	-	-
Inositol	-	-
D-sorbitol	-	-
L-rhamnose	-	-
D-sucrose	-	-
D-melibiose	-	-
Amygdalin	-	-
L-arabinose	-	-
n strains	186 (92%)	17 (8%)

SOCIO-ECONOMIC STATUS OF FISHING COMMUNITIES IN BANGSAMORO”

Naima G. Pendi, Esmael L. Abas, Husna T. Lumapenet

The study evaluated the socioeconomic condition of fishermen in the coastal municipalities in the Bangsamoro. The respondents of the study were the beneficiaries of the Integrated Livelihood Assistance Program for the fishing communities. The variables included in this study were the demographic profile of the respondents, the technical assistance and livelihood assistance programs of the Bureau of Fisheries and Aquatic Resources. Frequency count, percent, and mean had been used for the description of the variables of the study, likewise, correlation and regression analysis was used to determine relationship of the variables of the study.

Results revealed that the mean age of the respondents is 42.64 year old; having a family size of 6.39; have 21.79 years for the mean years in fishing; and had an average monthly income of P5, 414.70. As to technical assistance, the fisherfolks were seldom given with technical assistance program. Meanwhile, livelihood assistance was given with moderate support in alleviating their socio-economic condition and development of the fisherfolks. It was also found out that the fisherfolks have somewhat positive knowledge learned, skills acquired and attitudes towards the assistance programs in alleviating their socio-economic condition and development. Meanwhile, it was also posted that there is a significant correlation between the knowledge, skills acquired and attitudes of the fisherfolk and the extent of technical and livelihood assistance programs. Moreover, the knowledge learned, skills acquired and attitudes of the fisherfolks significantly influence their fishing activities.

Assistance programs should be strengthened in implementing to the fisherfolks. Regular monitoring and evaluation of assistance programs should be done to have the impact of the programs in alleviating the socio-economic conditions of the fisherfolks. Similar study should be conducted to attain a more comprehensive result.

DECARBONISING OFFSHORE AQUACULTURE OPERATIONS

I. Penesis*, E. Gray, N. Abdussamie, E. Wilson, and A. Pichard

Blue Economy CRC-Co Ltd, Maritime Way, Newnham TAS 7248, Australia
*Corresponding author's email: irene.penesis@blueeconomycrc.com.au

With an Exclusive Economic Zone of 10 million square kilometres, Australia has enormous potential to use its oceans to sustainably increase aquaculture production. However, to realize this potential these industries must move offshore into more exposed high-energy operating environments. This move will involve the development of new more robust structures, technologies and production systems that are environmentally sustainable and socially responsible. The success of the offshore aquaculture industry will depend upon availability and cost-effectiveness of appropriate sustainable energy technologies to meet their energy needs.

The energy-intensive resource (power, freshwater, fuel, and potentially heat and oxygen) demands of the aquaculture industry span a range of farming operations, including for example, lighting, auxiliary pumps, feeding, aeration, desalination, net cleaning, refrigeration, monitoring, firefighting mostly done through support barges and service vessel operations. Different aquaculture operators will have alternative optimal power solutions to meet their energy requirements.

Below are some key observations on developments associated with decarbonising offshore aquaculture systems:

- Offshore renewable energy (ORE) technologies have the potential to provide an alternative, sustainable, at-sea energy source for the offshore aquaculture, and export surplus energy as electricity and green hydrogen, while the offshore aquaculture has the potential to provide a market for offshore renewable energy technologies, subsequently aiding the advancement of both industries.
- Offshore aquaculture is a likely candidate for co-location because their operations use a large range of systems and equipment that demands continuous power supply to maximise their operability.
- Hybrid offshore energy solutions (e.g., solar PV and tidal energy, solar PV and wave energy, hydrogen and wave energy, green hydrogen, and offshore wind, etc.) have the potential to increase the use of ORE technologies for aquaculture operations by offering more reliable and clean ways to supply power, replacing the current reliance on fossil fuels.
- The maritime industry is responsible for producing 2.5% of Global Greenhouse Gas emissions. The replacement of fossil fuel with green ammonia or green methanol for aquaculture service vessels is seen as a particularly promising option because the fuel can be made using clean electricity – such as solar or wind power – and burned without emitting any greenhouse gases. Several nations including Australia and Singapore have announced intentions to move toward a zero-emission shipping industry.
- Integrating offshore renewable energy technologies with offshore aquaculture systems requires several pre-development activities including techno-economic analysis, engineering design, and optimization to ensure a good fit and a smooth energy transition with the aquaculture energy system and the application/user needs.

This presentation will provide an overview of research undertaken by the Blue Economy CRC Offshore Renewable Energy Systems research program, including examples of energy requirements for different aquaculture operations and opportunities for decarbonising offshore aquaculture systems.

MICROBIAL AQUAFEED PRODUCTION WITH OLEAGINOUS YEASTS CULTIVATED ON WASTE LIPIDS

Eric Charles Peterson*[a,b], Rowanne Siao [a], Gi Gi Chua [a], Coleen Toledo [a], Megan Teo[a], Naazneen Sofeo [a], Aaron Thong [a] Christian Hermansen [a]

A Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science Technology and Research (A*STAR), Singapore
B Institut National de la Recherche Scientifique – Eau Terre Environnement (INRS-ETE) Québec (Québec), Canada

eric_peterson@sifbi.a-star.edu.sg
eric.peterson@inrs.ca

The use of lipid sidestreams from food manufacturing has been overlooked as a feedstock for the production of single cell protein (SCP), which is a promising approach for the production of marine-free aquafeeds. While some studies have demonstrated that oleaginous yeasts can accumulate microbial lipids when cultivated with oils as a substrate, little to no work has been done to characterize the use of these organisms to convert waste oils to single cell protein. Here, surprisingly robust growth is shown by two oleaginous yeasts (*Yarrowia lipolytica* and *Rhodosporidium toruloides*) cultivated on waste lipids from industrial food processing. With final cell dry weight values higher than 40 g/L, strong productivity (3.3 g/L·Hr), and a remarkable conversion of substrate to biomass, these results demonstrate that waste lipids are a high energy feedstock for SCP production. This biomass is rich not only in protein, but also microbial lipids, with 25% lipid content that is more than 50% polyunsaturated fatty acids. While some work has investigated the ability of oleaginous yeasts to accumulate microbial lipids, the results here suggest a mixed method for production of both protein and lipids on such high energy feedstocks represents a promising alternative approach for generating high quality microbial aquafeed ingredients. Thus, this sidestream represents an attractive whole-cell ingredient for use as an aquafeed, or separate purified protein and lipids for food applications, without the need for genetic modification.

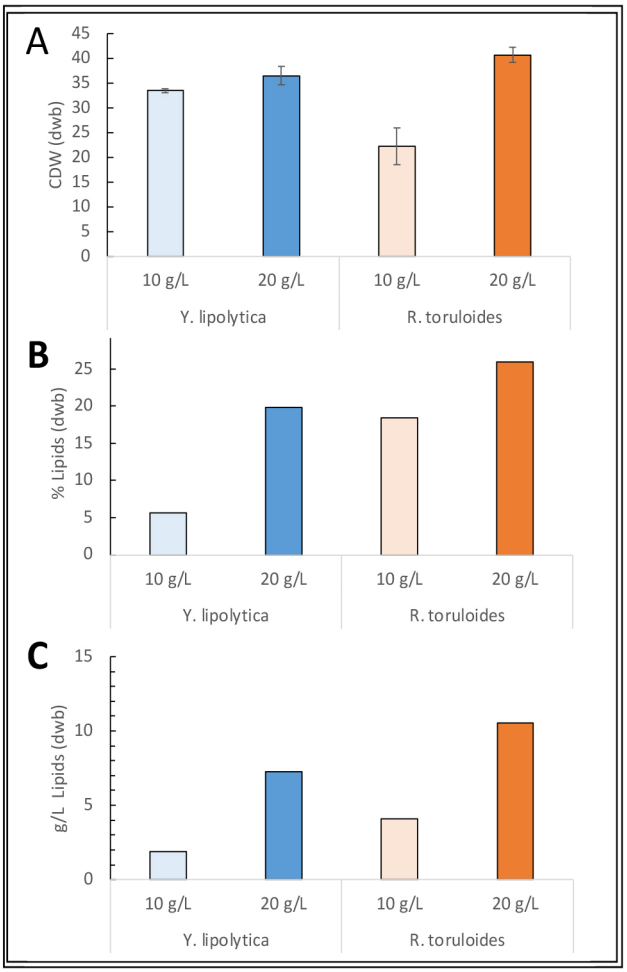


Figure 1) Cultivation of *Y. lipolytica* and *R. toruloides* on two different substrate loadings of free fatty acid waste. A) Cell Dry Weight (CDW). B) Lipids content in SCP on dry weight basis. C) Lipids content in broth.

THE POTENTIAL FOR AQUACULTURE DEVELOPMENT IN THE BRITISH VIRGIN ISLANDS

Clive Petrovic

Econcerns, Ltd., Road Town, British Virgin Islands
clivep@surfbvi.com

Located in the northeast Caribbean, The British Virgin Islands offer excellent opportunities for the development of aquaculture projects. The combination of clean water and optimal oceanographic conditions create an environment rarely found in developed countries and ideal for a variety of farming activities. A government committed to promoting the Blue Economy, stimulating free enterprise and empowering the local population provides the necessary ingredients for aquaculture development. The National Aquaculture Policy will guide all development with clear and transparent regulations that encourage free enterprise and success of approved projects. A community college with a Marine Centre containing wet lab, analytical laboratory and ancillary services can support the needs of most marine projects. Current training in marine studies can be quickly adapted to ensure a well-prepared work force ready to support any aquaculture related activity.

The natural environment is central to any aquaculture development. Clean, clear ocean water sweeps through the Virgin Islands from the equatorial currents with no up-current sources of pollution. A general east to west current averaging two knots assures good flow and exchange. The archipelago of approximately 60 islands, cays and off-lying rocks rest on a shallow underwater plateau with depths ranging from 20 to 60 metres. The distribution of islands creates a variety of ecological conditions for different kinds of projects. The sand substrate on most of the bank reduces potential conflicts with coral reefs or other sensitive habitats. The combinations of physical features create opportunities for a variety of farming projects.

The government is committed to promoting the Blue Economy and has invested in appropriate infrastructure. Thus, aquaculture projects that are sustainable and environmentally responsible are encouraged with policies and regulations that favour entrepreneurs and long-term development. Proposals for aquaculture projects will be guided by the National Aquaculture Policy that is grounded in sound economic development.

Aquaculture development is supported by a vibrant local economy and skilled workforce. The community college offers training in a variety of marine fields that can immediately supply needs of this industry. Inherent flexibility allows for the addition of courses and programmes to target specific demands of a diverse and growing aquaculture industry. The combination of existing local entrepreneurs and rapidly adapting workforce can support any new project.

The British Virgin Islands encourage investment in aquaculture projects that incorporate emerging technologies and are environmentally aware, economically sustainable and socially responsible. Both governmental policy and local workforce are resilient and able to respond rapidly to the demands of this growing industry. Good planning and implementation will assure the British Virgin Islands will be a leader in the future growth of the world aquaculture industry.

WATER USE EFFICIENCY, A KEY DRIVING FACTOR FOR FUTURE DEVELOPMENT OF WHITELEG SHRIMP *Litopenaeus vannamei* FARMING

Minh Anh Pham, Truong Ngoc Thao, Thanh Phong Chau, Marc Campet, Tran Huynh Cuong

ADM Aqua RD Center
Nha Be District, Ho Chi Minh City
Vietnam
Anhminh.pham@adm.com

Intensification has become common practices in many whiteleg shrimp (*L. vannamei*) producing countries, and farming systems are evolving very fast from very simple systems to complicated ones which can be equipped with sophisticated water treatment, water quality auto-monitoring system, auto feeders ect… But the success is still limited. In our study, it clearly demonstrated that an increase of stocking density of shrimps with improper water treatment method can quickly deteriorate water quality and consequently lead to very high mortality and destroy the production (Fig. 1). To maintain water quality, shrimp farmers start increasing water exchange. In some cases, water exchange rate can exceed 10 m3 of water per 1 kg of shrimps after 90 days of culture. Increasing water exchange do not only increase production cost but increase the risk of pathogen outbreak and ruin ambient environment. This presentation will introduce an innovative solution developed by ADM which can help to reduce water exchange, increase water use efficiency and minimize production cost.

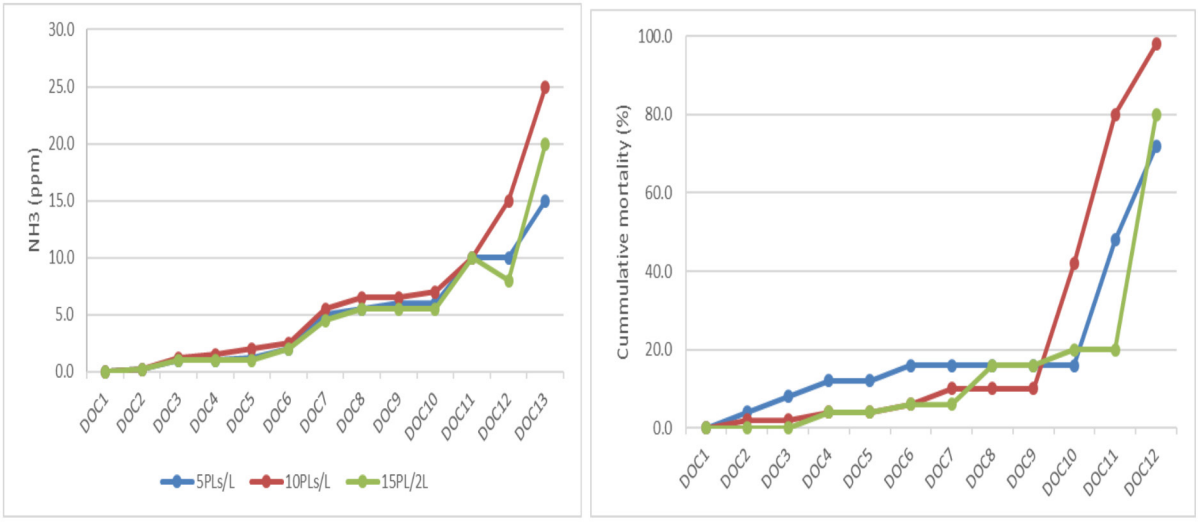


Fig.1. NH3 level and cumulative mortality at different stocking densities

ANTIBIOTIC USE IN STRIPED CATFISH (*Pangasianodon hypophthalmus*) AQUACULTURE IN VIETNAM

Pham Quang Vinh*, Nguyen Thanh Phuong, Anders Dalsgaard, Tran Minh Phu

[1] College of Aquaculture and Fisheries, Can Tho University, Vietnam
phamquangvinh@ymail.com
tmphu@ctu.edu.vn

Striped Pangasius catfish (*Pangasianodon hypophthalmus*) dominates finfish aquaculture production in Vietnam. Farmed mainly in the provinces of Dong Thap, Can Tho and An Giang in the Mekong Delta, this export oriented industry grew rapidly from 93 thousand tons in 2000 to 1.2 million tons in 2012; a levels of production that was maintained in 2018. The combination of intensive production in open farming systems, i.e. influent and effluent water sources are frequently shared and directly used by several adjacent grow-out farms has been linked to frequent disease outbreaks and high mortality rates, mainly of bacterial aetiology. Bacillary Necrosis of Pangasius (BNP) and Motile Aeromonad Septicaemia (MAS) were the most common and economically the most serious diseases experienced by nearly all catfish farmers. Small-scale grow-out farmers reported 1 to 10 episodes per crop whilst large-scale farmers reported 1 to 5 episodes per crop (mean 3.04). Antimicrobial treatments for 5-7 days are common practice. Amoxicillin, doxycycline, florfenicol and mixture of sulfamethoxazole and trimethoprim are used to treat BNP and MAS.

Thus, innovative approaches include investigating the effectiveness of the mode of application of the vaccine (i.e. immersion instead of manual injection), the alternative replacement of antimicrobials with immune stimulants and the development of fish health management approaches at a regional level (a zonal approach) are needed. In order to enhance the effectiveness of antimicrobials used in disease treatment, antimicrobial therapy for striped catfish should be rationalized. This should be done by evaluating and building up an antimicrobial susceptibility database, and by investigating the pharmacokinetics and pharmacodynamics of common antimicrobials used in striped catfish, e.g. by experimentation and/or by developing modelling approaches that allow extrapolations between different production practices and treatment regimes.

PUBLIC ATTITUDES TOWARDS LOW TROPHIC AQUACULTURE SPECIES PRODUCTS: A CHOICE EXPERIMENT ANALYSIS OF THE COUNTRIES BORDERING THE ATLANTIC OCEAN

Thuy T. T. Pham*, Sveinn Agnarsson

UiT The Arctic University of Norway, University of Tromsø
Hansine Hansens veg 18, 9019 Tromsø
Email: thanh.thuy@uit.no

Aquaculture plays a key role in the supply of high-quality seafood. However, European (EU) aquaculture contributes only approximately 10% of the global seafood production, while 65% of seafood consumed in the EU is currently imported. The production base of EU aquaculture is currently relatively narrow. Although EU aquaculture includes more than 70 different species, only five species cultured in the Atlantic Ocean region make up 90% of its cultivated fish production. This shows that EU aquaculture has a potential for expansion and diversification. If EU aquaculture is successful, it could make a considerable contribution to the local and regional coastal economies, as well as to the provision fish-based nutrition to European and export markets.

In response to that necessity, the EU Horizon AquaVitae project aims at increasing production in and around the Atlantic region by developing new and emerging low trophic species, and by optimizing production in existing aquaculture value chains under the principle of circular economy. The study is a part of this project, with the main goal to examine whether the circular products developed in AquaVitae are accepted by society in general.

Particularly, this study attempts to investigate consumers' willingness to pay towards low trophic species products. The analysis is done based on a survey of 1213 consumers from four countries bordering the Atlantic Ocean. Choice experiment is employed to examine whether the public is willing to pay a premium for "nutritional value and sustainably farmed" low trophic species products.

There has been substantial research undertaken on consumers' willingness to pay towards conventional aquaculture products using choice experiment. Yet, empirical evidence towards low trophic species aquaculture products is almost non-existent. This study attempts to fill that gap, while at the same time taking advantage of the data richness of the survey to analyse consumer attitudes toward not just one buts multiple species and explore divergence of consumers' willingness to pay across the Atlantic region.

The experiment demonstrates that the public has a willingness to pay 51% above ordinary price for a high nutrient value and 40% for environmental sustainability in low trophic species farming but not for products that are socially responsible. Also, the consumer surplus increases as we move to product scenarios with higher nutritional value, environmentally friendly, and socially responsible. All scenarios with either no certification of environmentally friendly or low or medium nutritional value have low consumer surplus, whereas the consumer surplus for all scenarios with products of high environmental awareness and high nutritional value is substantial (% WTP above the ordinary price). Hence, the single most important attribute that affects the consumers' welfare is high nutritional value, while the change in socially responsible products and products that does have medium nutritional value are both of minor importance.

REGULATORY ASPECTS OF CLIMATE CHANGE ADAPTATION IN AQUACULTURE- LESSONS FROM NORWAY, GREECE AND SPAIN

Thuy Thi Thanh Pham*, Michaela Aschan, Eirik Mikkelsen, Ann-Magnhild Solås, Rosa Chapela Perez, Nikos Papandroulakis

* UiT The Arctic University of Norway, Tromsø, Norway
Email: thanh.thuy@uit.no

The implementation of adaptation measures in aquaculture is a new and complex task with multiple facests, influenced by factors differing from site to site. This paper assesses how the regulatory frameworks for marine aquaculture sector hinder or support the implementation of adaptation measures to climate change. Three following cases are focused: Norwegian salmon aquaculture, Greek E. seabass and meagre aquaculture, and Spanish mussel aquaculture. Second, we applied a step-wise approach for the evaluation. In step (1), we present a summary of risks, opportunities and climate adaptation measures that are identified for aquaculture sector, focusing on the three cases. In step (2), we review existing regulatory legal acts and regulations for aquaculture, specifically for the climate change context. In step (3), the existing regulatory tools are mapped onto the identified adaptation measures of each case study to assess the extent to which the current legal framework accommodates the adaptation measures. The findings show that, the initiatives and regulations regarding climate change are rather varied among the countries, and thus regulatory challenges are varied as well. In Norway, the regulations are relatively comprehensive and detailed as the industry has had the opportunity to develop since the early 1970s. Therefore, legislation does not lack any major relevant content or scope. In Greece, a long list of acts and regulations already exist to promote sustainable growth of the aquaculture industry over the last few decades. There is no specific national legislation for adaptation to climate change; however. This is because the major challenges for the Greek industry relate to spatial organization, licensing, new technologies and aspects of animal welfare and environment, which are not limited to climate change. In Spain, there is a complete amount of national and regional regulations and rules for mussel aquaculture operation. Nevertheless, significant legal ambiguity relating to measures and tools for adaptation to climate change still exists. In fact, because of all these constrains, it is a challenge to continue developing aquaculture site selection and planning suitable zones for aquaculture in order to guarantee the legal certainty to promoters and investors.

ANTIMICROBIAL USE PRACTICES TO CONTROL BACTERIAL DISEASES IN VIETNAMESE STRIPED CATFISH AQUACULTURE

Pham Quang Vinh, Nguyen Quoc Thinh, Dang Thi Hoang Oanh, Anders Dalsgaard, Masashi Maita, Tran Minh Phu*

College of Aquaculture and Fisheries, Can Tho University
3/2 Street, Campus II, Can Tho, Vietnam
tmphu@ctu.edu.vn

Striped catfish (*Pangasianodon hypophthalmus*) is the dominant aquacultured species in the Mekong Delta and Vietnam exports frozen fillets worldwide. Bacillary Necrosis of Pangasius (*Edwardsiella ictulari*) and hemorrhagic disease (*Aeromonas hydropila*) are the main diseases in striped catfish and responsible for a widespread use of antimicrobials (AMU). This study was conducted to better understand AMU practices of striped catfish farmers with a long-term aim to suggest better disease prevention and control measures with reduced needs for AMU. A total of 90 small- (45 < 3 ha size) and large-scale (45 > 3 ha size) striped catfish farm owners were questionnaire interviewed in An Giang, Dong Thap and Can Tho provinces to obtained information about their fish health management practices, in particular chemical and antimicrobial usage. Farmers experienced between 2 and 10 disease outbreaks mainly during the first three-month's growth period. Most farmers (90-100%) used between one to five different classes of antimicrobials for disease treatment. Nine to 15 different classes of antimicrobials were used by farmers to prepare their medicated feed sometimes in combination (e.g. doxycycline/amoxicillin). Small-scale farmers more often administered antimicrobial treatment and used a higher number of different antimicrobial classes compared to large-scale farmers. The most commonly used antimicrobials irrespective of farm size were doxycycline (44.4-61.1%%) and amoxicillin (33.3-53.3%). Both small- and large-scale farmers reported the use of enrofloxacin despite this drug being banned for use in aquaculture in 2012. Several farmers experienced that antimicrobial treatment was not effective and they therefore had to use different antimicrobials. There is an urgent need to monitor and communicate to farmers the antimicrobial susceptibility of the two main bacterial pathogens in striped catfish so effective treatment guidelines can be established. Also, studies are needed to obtain information about challenges and problems with applying the commercial vaccine that protects against the two bacterial diseases so this and other future vaccines will be used more widely by striped catfish farmers and thereby significantly reducing the need to use antimicrobials.

NUTRITION BASED STRATEGIES TO IMPROVE THE YELLOWTAIL KINGFISH (*Seriola lalandi*) AQUACULTURE INDUSTRY IN AUSTRALIA

Igor Pirozzi*, Marina Rubio Benito, Caroline Candebat, Basseer Codabaccus, Chinh T.M. Dam, Abigail Elizur, Barney Hines, Angela Liu, Michael Salini, Jesmond Sammut, Cedric Simon, Richard Smullen, Luke Vandenberg, Tomer Ventura, Mark Booth

NSW Department of Primary Industries. Port Stephens Fisheries Institute
Taylors Beach Rd, Taylors Beach NSW 2316. Australia
Igor.pirozzi@dpi.nsw.gov.au

Yellowtail Kingfish (YTK; *Seriola lalandi*) aquaculture in Australia is considered an emerging industry that presents the most significant potential for growth of the developing aquaculture industries, with some estimates predicting that Australian production of YTK could increase to 34,000 tonnes worth $440 million over the next 10 years. However to grow the industry key bottlenecks still remain an impediment particularly with regard to nutrition, feeds and feeding strategies. Here we present a synopsis of some of the key research findings of a multi-year collaborative R&D program investigating the nutritional requirements, diet development, and feeding strategies for YTK. Key outcomes include quantifying the requirements for the essential nutrients such as choline, taurine, methionine and cysteine, the comprehensive assessment of a suite of dietary raw materials to facilitate cost effective feed formulations, successfully reducing fishmeal in diets by over 70%, refining bioenergetic growth models to improve predictive accuracy and identifying feeding strategies to reduce FCR's and improve cost benefits. Taken overall, the application of the programs results will help towards the development of more cost effective, sustainable feeds and feed management strategies ultimately improving farm productivity by reducing operating costs and improving sustainability.

EFFECT OF TEMPERATURE AND FEEDING ON HERMATYPIC CORALS OF SINGAPORE: PHYSIOLOGY, GROWTH AND RECOVERY

Po, Q. H.*, Hickey, V., Ow Yong W. L. and Tanzil, J.

Diploma in Veterinary Technology, School of Applied Science, Temasek Polytechnic, Singapore 529757
1700635D@tp.edu.sg

Episodic bleaching events primarily driven by climate-induced ocean warming have been increasing in frequency in recent years, bringing about longer lasting periods of anomalously warmer ocean temperatures known as Marine Heatwaves (MHW). This in combination with steadily climbing ocean temperatures compromises the existence of coral reef ecosystems worldwide. Singapore's coral reefs also face multiple external stressors from increasing urbanization and development. With climate change exacerbating further stress on local corals, it has become increasingly important to understand the physiological impact of sudden temperature stress on reef-building corals and their recovery post-stress to optimize coral management efforts. Previous studies have suggested heterotrophic plasticity as a coping mechanism to temperature stress, where reef-building corals increase feeding post stress to compensate for their daily energetic needs. This study explores the effect of prolonged temperature stress on hermatypic coral fitness, and the effect of an increased food availability on post-temperature stress recovery. Three species of Hermatypic corals were subjected to higher temperatures and proxies of coral fitness such as photo physiology, mortality and growth were recorded. After which, selected fragments were supplemented with *Artemia* nauplii daily for 2 weeks and monitored. Visual observations of recovery were noted earlier for the supplemented tanks and physiological measurements showed a general decline in coral fitness post-heat stress with improvement post recovery, though the performance of supplemented fragments did not reflect any observable positive trend. This suggests that heterotrophic plasticity may not be applicable to all species, corresponding with previous studies and highlighting a research gap worth looking into.

Holothuria polii FROM THE CENTRAL MEDITERRANEAN: NUTRITIONAL TRAITS

Ermelinda Prato*, Isabella Parlapiano, Giovanni Fanelli, Francesca Biandolino

CNR-IRSA, Water Research Institute, Via Roma 3, Taranto, Italy
*E-mail: linda.prato@iamc.cnr.it

Sea cucumber (Echinodermata: Holothuroidea) represents an important seafood whose consumption is becoming popular around the world because of their bioactive compounds (polyunsaturated fatty acids, essential amino acids, minerals, proteins, etc.), which have many human health benefits.

The increasing market demand and retail prices of up to USD 300–500 kg-1 (dried), has led to an exploitation often indiscriminant and excessive with a reduction of sea cucumber stocks. Although information on food composition is essential for market exchange and for consumer protection, only a few studies refer to the nutritional profile of sea cucumbers.

In this study, the nutritional properties of body wall and gonad of *Holothuria polii* male and female collected from central Mediterranean Sea, were analysed. Specimens of *H. polii* were collected from an area of Mar Grande in Taranto (Ionian Sea, southern Italy) by scuba diving, during October-November 2019.

Holothuria polii FROM THE CENTRAL MEDITERRANEAN: NUTRITIONAL TRAITS

Ermelinda Prato*, Isabella Parlapiano, Giovanni Fanelli, Francesca Biandolino

CNR-IRSA, Water Research Institute, Via Roma 3, Taranto, Italy
*E-mail: linda.prato@iamc.cnr.it

Sea cucumber (Echinodermata: Holothuroidea) represents an important seafood whose consumption is becoming popular around the world because of their bioactive compounds (polyunsaturated fatty acids, essential amino acids, minerals, proteins, etc.), which have many human health benefits.

The increasing market demand and retail prices of up to USD 300–500 kg-1 (dried), has led to an exploitation often indiscriminant and excessive with a reduction of sea cucumber stocks. Although information on food composition is essential for market exchange and for consumer protection, only a few studies refer to the nutritional profile of sea cucumbers.

In this study, the nutritional properties of body wall and gonad of *Holothuria polii* male and female collected from central Mediterranean Sea, were analysed. Specimens of *H. polii* were collected from an area of Mar Grande in Taranto (Ionian Sea, southern Italy) by scuba diving, during October-November 2019.

Table 1. Proximate composition of *Holothuria polii*

	Body wall		Gonad	
	Female	Male	Female	Male
Moisture (%)	82.20 ± 0.4	81.85 ± 0.6	80.51 ± 0.5	83.32 ± 1.0
Ash (%)	4.32 ± 0.3	4.27 ± 0.4	5.17 ± 0.3	4.15 ± 0.5
Lipid (g/100g dw)	3.68 ± 0.0	3.45 ± 0.0	18.53 ± 0.3	6.57 ± 0.1
Protein (g/100g dw)	32.35 ± 1.3	33.77 ± 1.1	62.42 ± 2.6	54.56 ± 1.5

Table 2. Main fatty acids of *H. polii*

Fatty acids mg/100g	Sex	Body wall	Gonad
C16:0	female	266.06 ± 65.3	2462.25 ± 165.3
	male	241.57 ± 27.1	285.59 ± 58.8
C18:0	female	231.31 ± 45.7	1201.93 ± 115.1
	male	223.82 ± 15.4	693.97 ± 45.6
C16:1	female	185.14 ± 20.2	2889.60 ± 38.1
	male	220.13 ± 24.5	217.08 ± 10.5
C18:1n9c	female	102.91 ± 12.3	1012.39 ± 76.0
	male	119.84 ± 12.1	453.03 ± 78.0
C18:3n3	female	296.54 ± 59.4	736.46 ± 96.8
	male	253.87 ± 34.7	329.35 ± 25.0
C20:4n6	female	588.41 ± 53.1	1337.74 ± 71.4
	male	538.39 ± 42.3	850.19 ± 10.0
C20:5n3	female	323.49 ± 38.8	1656.90 ± 154.6
	male	273.94 ± 33.1	847.98 ± 20.1
C22:6n3	female	47.19 ± 6.6	133.36 ± 16.7
	male	41.55 ± 8.0	100.91 ± 25.9

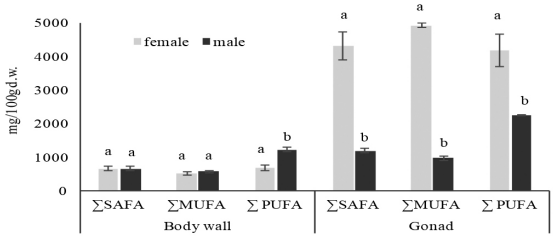


Fig. 1. Sum of SAFA, MUFA and PUFA of *H. polii*

Table 3. Amino acid profile of *H.polii*

Aminoacids	Sex	Body wall	Gonad
Isoleucine (Ile)	female	7.08 ± 0.48	5.14 ± 0.41
	male	6.68 ± 0.87	10.72 ± 0.64
Leucine (Leu)	female	17.17 ± 0.62	22.72 ± 1.75
	male	11.78 ± 0.74	15.66 ± 0.87
Lysine (Lys)	female	16.22 ± 0.48	9.51 ± 0.62
	male	13.47 ± 0.77	37.69 ± 3.21
Methionine (Met)	female	5.91 ± 0.56	2.81 ± 0.35
	male	5.92 ± 0.56	5.14 ± 0.40
Cysteine (Cys)	female	5.04 ± 0.76	1.41 ± 0.26
	male	3.99 ± 0.43	0.91 ± 0.05
Phenylalanine (Phe)	female	7.91 ± 0.68	5.07 ± 0.65
	male	6.47 ± 0.60	8.25 ± 0.43
Tyrosine (Tyr)	female	7.40 ± 0.62	7.76 ± 0.69
	male	7.06 ± 0.36	7.85 ± 0.84
Threonine (Thr)	female	11.15 ± 0.57	6.83 ± 0.54
	male	10.36 ± 0.75	11.92 ± 0.65
Valine (Val)	female	7.97 ± 0.84	6.27 ± 0.64
	male	6.86 ± 0.44	11.17 ± 0.78
Histidine (His)	female	3.49 ± 0.57	2.80 ± 0.50
	male	3.16 ± 0.25	5.12 ± 0.47
Aspartic acid (Asp)	female	5.17 ± 0.44	6.07 ± 0.83
	male	3.78 ± 0.25	15.63 ± 1.09
Arginine (Arg)	female	15.75 ± 0.69	8.72 ± 0.89
	male	13.28 ± 0.83	21.21 ± 1.42
Serine (Ser)	female	9.55 ± 0.87	5.68 ± 0.98
	male	8.26 ± 1.00	10.07 ± 0.45
Glutamic acid (Glu)	female	16.45 ± 1.25	9.80 ± 1.01
	male	10.41 ± 0.90	29.88 ± 1.76
Proline (Pro)	female	15.79 ± 0.98	6.40 ± 0.31
	male	12.84 ± 0.89	22.63 ± 1.90
Glycine (Gly)	female	20.69 ± 1.19	6.00 ± 0.24
	male	16.49 ± 0.92	28.30 ± 1.19
Alanine (Ala)	female	11.50 ± 1.00	4.58 ± 0.39
	male	11.31 ± 0.61	9.51 ± 0.07

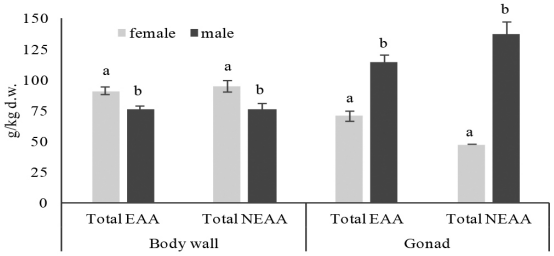


Fig. 2. Sum of Essential and Non essential AAs of *H. polii*

Therefore *H. polii* could be take into account for its potential culture.

IMPACT OF GLYPHOSATE ON FATTY ACIDS AND LIPID NUTRITIONAL QUALITY INDICES OF *Mytilus galloprovincialis* CULTURED IN MEDITERRANEAN SEA UNDER SEAWATER WARMING SCENARIO

Francesca Biandolino[a], Isabella Parlapiano[a,] Asia Grattagliano[b], Ermelinda Prato[a]*

a. CNR-IRSA, Water Research Institute, Via Roma 3, Taranto, Italy
b. Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata"
Via della Ricerca Scientifica, 1 –Roma, Italy;
*E-mail: linda.prato@irsa.cnr.it

Glyphosate (Gly) is one of the most widely used herbicide globally for weed control, and it has been detected in different aquatic ecosystems adjacent to intensive agricultural areas. In addition to emerging chemicals, organisms are simultaneously exposed to many environmental stressors. Rising water temperatures associated with global climate change represent the main concern for worldwide scientists and politicians. In the present study, the Mediterranean mussel (*Mytilus galloprovincialis*) was selected as the main European species in aquaculture production. Moreover, due to its exceptional nutritional value, it is commonly included in human diet. Increasing water temperatures may negatively affect the quality of the mussels measured by the fatty acid composition (FAs). Alteration in FAs is a sensitive early warning bio-indicator of stress, and can be considered a valuable biomarker in assessing the effects of environmental stressors on aquatic organisms. So, the main goals of this study were to determine the FAs composition and the related lipid nutritional quality indices (LNQI) in *M. galloprovincialis* collected from a mussel farm in the central Mediterranean Sea.

They were exposed for 4 days at 1 mg/L Gly at 20°C and at 28°C in 10 L tanks with 10 mussels each.

The results indicate that both rising temperature and Gly exposure affected the FAs composition and LNQI Mussel always exhibited a dominance of saturated (SFA) and polyunsaturated (PUFA). Also, omega-3 PUFAs (n-3), eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) showed a decrease according to temperature and Gly effect. All LNQI indicated a decline in lipid quality of *M. galloprovincialis* under these stressors.

This study highlights the importance of FAs as valuable biomarkers to predict the impacts of combined stressors on nutritional quality of *M. galloprovincialis*.

Table 1. Fatty acids (%) of M. *galloprovincialis* in control (CTR) and Gly-exposed groups at 20 and 28°C.

	20°C		28°C	
FAs	Ctr	Gly 1 mg/L	Ctr	Gly 1 mg/L
C14:0	6.52 ± 1.28	6.86 ± 0.30	7.74 ± 1.32	6.85 ± 0.27
C15:0	1.59 ± 0.18	1.43 ± 0.12	1.20 ± 0.18	1.21 ± 0.12
C16:0	28.52 ± 2.99	31.75 ± 0.57	30.86 ± 3.27	27.08 ± 1.37
C17:0	1.72 ± 0.25^{a}	2.17 ± 0.13^{c}	1.61 ± 0.03^{a}	1.52 ± 0.08^{a}
C18:0	6.30 ± 0.35^{c}	6.68 ± 0.18^{d}	5.43 ± 0.29^{b}	4.48 ± 0.21^{a}
∑SFA	44.65 ± 2.97^{b}	48.90 ± 1.02^{c}	46.85 ± 4.00^{b}	41.16 ± 1.55^{a}
C14:1	2.25 ± 0.19^{b}	4.36 ± 0.34^{c}	0.91 ± 0.12^{a}	2.09 ± 0.32^{b}
C15:1	2.36 ± 0.38^{b}	3.54 ± 0.46^{c}	1.48 ± 0.32^{a}	2.10 ± 0.11^{ab}
C16:1	6.30 ± 0.80^{a}	6.12 ± 0.38^{a}	8.09 ± 0.61^{b}	8.73 ± 0.26^{b}
C17:1	0.59 ± 0.12^{a}	0.58 ± 0.10^{a}	0.81 ± 0.07^{b}	0.86 ± 0.11^{b}
C18:1n7	3.17 ± 0.06^{a}	3.40 ± 0.16^{b}	3.04 ± 0.01^{a}	2.98 ± 0.09^{a}
C18:1n9c	1.18 ± 0.36^{a}	1.91 ± 0.35^{a}	4.57 ± 1.51^{b}	5.34 ± 0.91^{c}
C20:1n9	1.98 ± 0.25^{b}	1.52 ± 0.03^{a}	1.70 ± 0.21^{ab}	2.18 ± 0.13^{c}
∑MUFA	17.83 ± 0.53^{a}	21.44 ± 0.51^{b}	20.61 ± 1.10^{b}	24.30 ± 0.66^{c}
C18:2n6c	2.28 ± 0.06^{b}	2.18 ± 0.10^{ab}	2.13 ± 0.09^{a}	2.37 ± 0.07^{c}
C18:3n3	4.10 ± 0.37^{b}	3.69 ± 0.46^{a}	3.98 ± 0.08^{ab}	4.58 ± 0.25^{c}
C18:4n3	2.05 ± 0.31	1.74 ± 0.08	2.16 ± 0.08	1.77 ± 0.15
C22:0 + 20:3n6	0.54 ± 0.10^{b}	0.40 ± 0.02^{ab}	0.32 ± 0.02^{a}	0.66 ± 0.13^{c}
C20:4n6	3.24 ± 0.18^{b}	2.76 ± 0.16^{ab}	2.58 ± 0.27^{a}	3.01 ± 0.06^{ab}
C20:5n3 (EPA)	14.58 ± 1.11^{c}	12.02 ± 0.74^{a}	12.99 ± 1.49^{b}	13.39 ± 0.43^{b}
C22:5n3	0.68 ± 0.11	0.50 ± 0.03	0.60 ± 0.10	0.66 ± 0.07
C22:6n3 (DHA)	10.03 ± 1.76^{c}	6.36 ± 0.85^{a}	7.77 ± 0.87^{b}	8.10 ± 0.77^{b}
∑ PUFA	37.52 ± 3.18^{b}	29.66 ± 1.01^{a}	32.54 ± 2.48^{ab}	34.54 ± 1.55^{ab}
n-3	31.45 ± 2.84^{c}	24.31 ± 1.17^{a}	27.50 ± 2.29^{ab}	28.50 ± 1.41^{b}
n-6	6.07 ± 0.33^{b}	5.34 ± 0.17^{a}	5.03 ± 0.20^{a}	6.04 ± 0.17^{b}

Table 2. LNQI of *M. galloprovincialis* in CTR and Gly-exposed at 20 and 28°C.

	20°C		28°C	
LNQI	Ctr	Gly 1 mg/L	Ctr	Gly 1 mg/L
n-3/n-6	5.17 ± 0.47^{bc}	4.56 ± 0.25^{a}	5.45 ± 0.45^{c}	4.71 ± 0.14^{ab}
PUFA/SFA	0.85 ± 0.12^{b}	0.61 ± 0.03^{a}	0.70 ± 0.11^{ab}	0.84 ± 0.10^{b}
(MUFA+PUFA) / SFA - C18:0	1.46 ± 0.26^{bc}	1.21 ± 0.05^{a}	1.30 ± 0.24^{b}	1.60 ± 0.11^{c}
UNS/SFA	1.25 ± 0.16^{bc}	1.04 ± 0.04^{a}	1.15 ± 0.16^{ab}	1.43 ± 0.09^{c}
DHA + EPA	24.62 ± 3.41b	18.38 ± 1.58a	20.76 ± 3.35a	21.49 ± 1.19^{ab}
ARA/DHA	0.33 ± 0.03^{a}	0.44 ± 0.08^{b}	0.34 ± 0.05^{a}	0.37 ± 0.03^{b}
ARA/EPA	0.22 ± 0.02^{ab}	0.23 ± 0.03^{b}	0.20 ± 0.00^{a}	0.22 ± 0.00^{ab}
DHA/EPA	0.68 ± 0.06^{b}	0.53 ± 0.04^{a}	0.59 ± 0.08^{ab}	0.60 ± 0.04^{ab}
AI	0.99 ± 0.11^{ab}	1.16 ± 0.06^{b}	1.18 ± 0.15^{b}	0.93 ± 0.07^{a}
TI	0.38 ± 0.06^{a}	0.50 ± 0.03^{b}	0.44 ± 0.04^{ab}	0.37 ± 0.03^{a}
HH	1.05 ± 0.15^{b}	0.76 ± 0.05^{a}	0.92 ± 0.14^{ab}	1.11 ± 0.09^{b}
HPI	1.04 ± 0.14^{b}	0.86 ± 0.04^{a}	0.88 ± 0.13^{ab}	1.08 ± 0.07^{b}
UI	185.85 ± 11.70^{c}	149.91 ± 6.51^{a}	162.69 ± 14.83^{ab}	175.67 ± 7.55^{bc}
FLQ	24.62 ± 2.44^{c}	18.38 ± 1.58^{a}	20.76 ± 2.35^{ab}	21.49 ± 1.19^{abc}
PI	0.88 ± 0.15^{c}	0.58 ± 0.06^{a}	0.68 ± 0.08^{ab}	0.80 ± 0.08^{c}

RAPID MICROBIAL FINGERPRINTING FOR BIOSECURITY IN AQUACULTURE

Ruben Props*, Jasmine Heyse, Celine De Maesschalck, Geert Rombaut & Nico Boon

Center for Microbial Ecology and Technology (CMET)
Department of Biochemical and Microbial Technology
Ghent University
Belgium
Ruben.Props@UGent.be

Microorganisms represent by far the most abundant life form in all aquaculture systems. Understanding the role of the aquaculture microbiome in disease demands analytical methods that enable the measurement of all microbes, and that can provide a temporally resolved view of the microbiome. Unfortunately, our knowledge on the aquaculture microbiome has largely been derived from cultivation-based methods which detect only a small fraction of the microbiome (e.g. < 0.1 - 1 %). High-resolution monitoring campaigns that unveil the structure, interactions and dynamics of the microbes in these systems are scarce, yet, they are the key to furthering our understanding of the role of the microbiome in disease outbreaks.

Here, we developed and tested a novel microbial fingerprinting technique based on flow cytometry that takes into account all bacterial cells and is both rapid (i.e. < 30 min) and reproducible (i.e. CV < 5%). We tested this method in an experimental set-up of replicate rearing tanks of shrimp (*L. vannamei*) throughout the nauplii (N5) and postlarvae (PL10) development phases. At a sampling frequency of two times per day, we observed strong tank- and development-phase dependent dynamics in microbial load and fingerprint. The microbial load increased by almost 1.5 $\log_{10}$ fold changes during the Z1 - Z3 development stages and reached a maximum of approximately 5e7 cells mL^{-1} at the M1-M3 development phases. Several tanks also showed sudden reductions in microbial load during the transition from mysis to the postlarvae stages. These changes in microbial load were accompanied by significant changes in microbial fingerprint during developmental transitions highlighting that both the physiological and taxonomic properties of the microbial community were under constant change during the shrimp development. Our findings grant additional insights into how the microbiome responds to animal development and aquaculture practices.

INTELLIGENT MICROBIAL ECOSYSTEM FOR THE SUSTAINABLE AQUACULTURE

Zizhong Qi*, Kai Zhang, Cheng Cheng, Junzhao Leng, Wen Chen, Nico Boon, Cristina Garcia Timermans, Zhenyu Yang, Yin Han

Yushan No. 5
Qingdao 266003, P. R. China
Lab of Applied Microbial Technology
College of Marine Life Sciences
Ocean University of China
Email: zizhongqi@ouc.edu.cn

Artificial intelligence is increasingly applied in aquaculture management including feeding, water quality management, counting and animal health status. Technologies such as remote sensoring, vision analysis, case-based reasoning system are being adopted and often claimed with real-time data acquisition, decision and even predictive function. However, due to the complex nature of culture system and various species, extra manually sampling and maintaining of equipment are involved and often frustrating. As microbial communities play key roles for the healthy aquaculture, there is urgent requirement for the fully automated, online monitoring and managing microbial communities. Recently, flow cytometric analysis and micro-fluidic technology are emerging as sensitive and reproducible methods to analyze the composition and the function of microbial communities. Thus, we develop an Intelligent Microbial Ecosystem (IME) for the healthy management of aquaculture. It consists of fully automated online sampling, samples processing system with the functional characteristics of microbial populations and in combination with the analysis of chemical and physical parameters. Its high-speed to obtain and process data, multiparametric data acquisition and multivariate data analysis provides intelligent advice and suggestion for the routine healthy operations. With the accumulation of multidimensional data in different aquaculture systems, we believe such system with the function of AI data analysis will contribute significantly for the sustainable aquaculture.

DEVELOPMENT OF HATCHERY TECHNOLOGY FOR RED SNAPPER *Lutjanus spp.*

Quek Wei Li, Koh Chee Boon Amos, Rudy Hidajat, He Yong, Foo Yek Seng

Marine Aquaculture Centre
Singapore Food Agency
11 St. John's Island
Singapore 098659
QUEK_Wei_Li@sfa.gov.sg

Red snapper (*Lutjanus spp*.) fingerlings are conventionally produced in extensive outdoor ponds in South East Asia and there has been limited success in using indoor tank systems, with reported low survival rates of ~1-2%. Our project aims to understand the key challenges in indoor hatchery production of red snapper and to develop a hatchery protocol for commercial-scale fry production. In our study, we had observed a significant display of shock-syndrome between day 15-25 of culture and most of the shocked larvae did not recover. The shocked larvae would struggle near the water surface and would sink to the tank bottom after some time. These larvae were observed with having overly inflated swim-bladders. This shock-syndrome would result in high mortality during the larval rearing period in indoor tank systems. Through a series of trials, we managed to overcome the shock-syndrome of red snapper larvae by adjusting the photoperiod. Apart from addressing the shock-syndrome issue, we have also refined our hatchery protocols through the use of small-sized rotifers during the first 5 days of culture; enrichment of live feeds; and culturing in a higher water temperature to speed up the larval growth. With these protocol refinements, we have managed to increase the survival rate by ten-fold, from the initial survival rate of ~1-2% to ~15-20%. We have also tested diets with natural pigment and established a fish harvesting method to achieve a crimson red appearance, thereby enhancing the marketability of cultured red snapper.

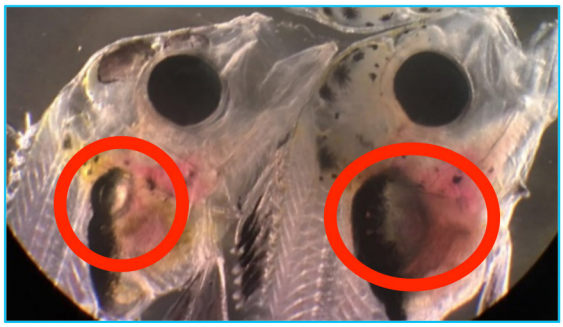


Fig 1: Comparison of swim bladder between normal larvae (left) and shocked larvae (right). Increased water temperature (by 3°C) and adjustment of photoperiod (24hr light) during the critical phase helped improve growth and reduced

DEVELOPMENT OF HATCHERY TECHNOLOGY FOR RED SNAPPER *Lutjanus spp.*

Wei Li QUEK*, Amos Koh Chee Boon, Rudy Hidajat, Yong He, Yek Seng Foo

Marine Aquaculture Centre
Singapore Food Agency
11 St John Island
Singapore 098659
QUEK_Wei_Li@sfa.gov.sg

Red snapper (*Lutjanus spp*.) fingerlings are conventionally produced in extensive outdoor ponds in South East Asia and there has been limited success in using indoor tank systems, with reported low survival rates of ~1-2%. Our project aims to understand the key challenges in indoor hatchery production of red snapper and to develop a hatchery protocol for commercial-scale fry production. In our study, we had observed a significant display of shock-syndrome between day 15-25 of culture and most of the shocked larvae did not recover. The shocked larvae would struggle near the water surface and would sink to the tank bottom after some time. These larvae were observed with having overly inflated swim-bladders. This shock-syndrome would result in high mortality during the larval rearing period in indoor tank systems. Through a series of trials, we managed to overcome the shock-syndrome of red snapper larvae by adjusting the photoperiod. Apart from addressing the shock-syndrome issue, we have also refined our hatchery protocols through the use of small-sized rotifers during the first 5 days of culture; enrichment of live feeds; and culturing in a higher water temperature to speed up the larval growth. With these protocol refinements, we have managed to increase the survival rate by ten-fold, from the initial survival rate of ~1-2% to ~15-20%.

CALIFORNIA SEAFOOD CONSUMPTION SHIFTS DURING THE COVID-19 PANDEMIC

Phoebe Racine*, Ashley Bae, Steven Gaines, Elliott Matthews, Karly Miller, Mike Weir, Matto Mildenberger

University of California Santa Barbara
2400 Bren Hall, University of California,
Santa Barbara CA 93106
pracine@bren.ucsb.edu

The pandemic has sent shockwaves through seafood consumption, with food service providers--the source of 75% of U.S. seafood sales[1]--shuttered or forced to adapt by widespread shelter-in-place orders. Seafood consumers already navigate a heavily consolidated market: just five species comprise 70% of seafood consumed, and seafood consumption in the U.S. is the lowest per capita of any industrialized nation[2]. Given the heterogeneous impacts of the COVID-19 pandemic, how will seafood sourcing and consumption change? In order to understand the COVID-19 pandemic's effect on seafood sourcing and consumption, we used Facebook quota sampling to recruit a diverse cross-section of California residents to take a series of surveys over the course of a year (n=640 remained in study for the full year).

Due to California's diverse population and varied geography, we will use fixed effects models to analyze how consumption frequency, point of sale, and diversity of species consumed was affected by the COVID-19 pandemic. We will be able to explain seafood consumption patterns by a variety of drivers, including: population density, distance from coast, and a variety of demographics (e.g. wealth, education, race, gender, household size). Our final data collection will wrap up this summer, by World Aquaculture we should have near-final results to share.

***Figure 1**: Facebook ads recruited survey participants across 4 distinct parts of California as part of our effort to get a cross-section of California seafood consumers.*

Love, D.C., Asche, F., Conrad, Z., Young, R., Harding, J., Nussbaumer, E.M., Thorne-Lyman, A.L. & Neff, R. (2020). Food Sources and Expenditures for Seafood in the United States. *Nutrients*, 12, 1810.

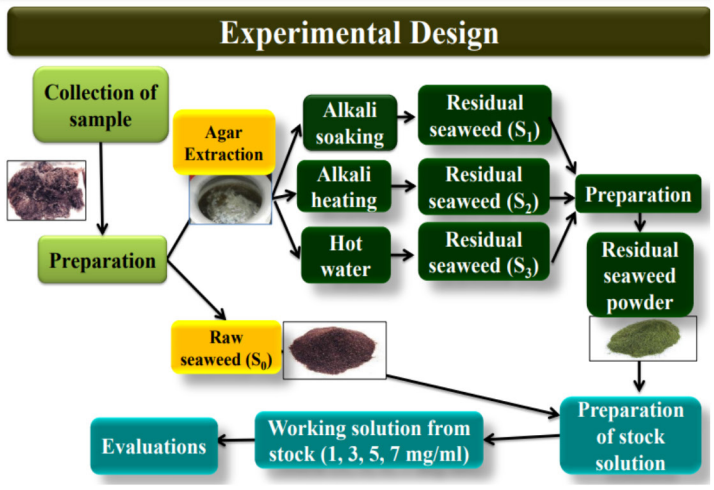
Shamshak, Gina L. et al. 2019. US seafood consumption. *Journal of the World Aquaculture Society* 50.4, pp. 715-727.

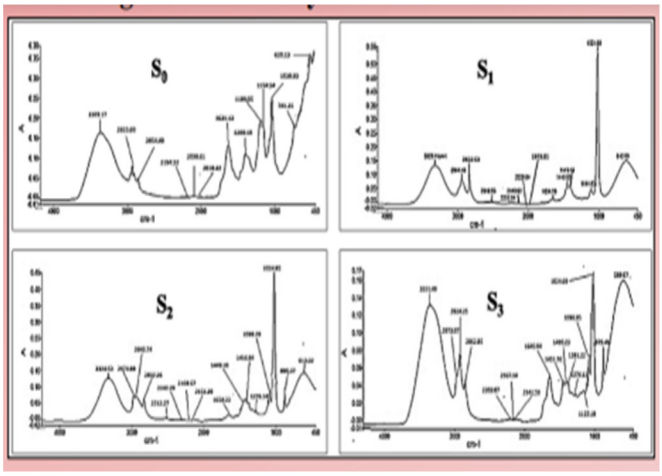
EVALUATION OF REUTILIZATION POTENTIALITIES OF *Gracilaria tenuistipitata* AFTER AGAR EXTRACTION AS A SOURCE OF PHYTOCHEMICALS AND ANTIOXIDANTS

S. M. Rafiquzzaman[1*] and Alima Akter[1]

[1]Dept. of Fisheries Biology & Aquatic Environment, Faculty of Fisheries
Bangabandhu Sheikh Mujibur Rahman Agricultural University
Salna, Gazipur-1706
Correspondence: S. M. Rafiquzzaman, rafiquzzaman@bsmrau.edu.bd

Gracilaria tenuistipitata is one of the most important widely available red seaweeds for the production of unique sulfated galactans specially agar. In industries significant volume of solid residues are created during agar extraction processes which are not handled and cause wastage of resources. Considering this issue present study was designed to explore the potential reutilization of the agar extraction residues as a source of phytochemicals and antioxidants. Agar was extracted from *Gracilaria tenuistipitata* following different methods: alkali soaking (S_1), alkali heating (S_2) and hot water extraction (S_3) worldwide used by the researchers. Phytochemical screening, fourier transform infrared (FT-IR) analysis and antioxidant activity of extracts from agar extraction residues was determined to evaluate the reutilization potentialities with comparison to raw seaweed (S_0). Results showed the presence of different phytochemicals in residues where concentration of total phenols (95.27 mg of GA/g) and flavonoids (57.46 mg of quercetin/g) was highest in methanolic extract of S_3 sample. FT-IR results confirmed the presence of functional groups of active components. S_3 sample also showed the highest antioxidant activity measured by different assays such as Phosphomolybdenum (absorbance 8.71±0.13 at 695 nm wavelength), DPPH (93.10±1.19%), ABTS (87.68±0.29%) followed by S_2>S_0>S_1, and reducing power (absorbance 3.51±0.46 at 700 nm wavelengths) followed by S_2>S_1>S_0. These results indicate that *G. tenuistipitata* residues after agar extraction can be reutilized as a significant source of natural antioxidants which could be a great contributor in circular economy and support sustainable reutilization of natural resources.





FT-IR analysis of methanolic extracts

MUSSEL BROODSTOCK MANIPULATION TO ENHANCE OFFSPRING THERMOTOLERANCE IN A WARMING OCEAN

Norman L. C. Ragg*, Joanna Copedo, Shenae Muirhead, Bridget Finnie, Gretchen E. Hofmann, Juliet Wong, Logan Kozal, Terence Leach, Nicola Hawes, Natalí Delorme

Cawthron Institute
Nelson, New Zealand
norman.ragg@cawthron.org.nz

Many temperate coastal regions are experiencing rising mean water temperatures. Aquaculturists, including mussel farmers, who currently farm stock at, or above, its thermal optimum will need to explore mitigation strategies to secure on-going production. The Greenshell mussel, *Perna canaliculus*, supports New Zealand's largest aquaculture export industry, earning $220m USD p.a.; however, production is focused in coastal regions that have experienced summer marine heatwaves since 2017. Research is therefore being undertaken to establish methods to elevate the thermal optimum and enhance overall temperature tolerance in this species.

Adult mussels from selectively-bred, full sibling families previously identified as having above- or below-average heat tolerance were acclimated to 17, 21 or 24°C water for 4 months, receiving elevated microalgal food to support gonad maturation. Mussels were induced to spawn using thermal cycling and half-sib offspring families created by crossing different 'thermotolerant' or 'thermosensitive' parent families. The fertilized eggs were then raised to D-veliger larvae (~48h old) at either 17, 21 or 24°C (n = 6). If both parents and embryos were incubated at 17°C, anecdotally considered the optimal temperature for *P. canaliculus*, an average of 63 – 77% of fertilized eggs formed viable veligers. If embryos were raised at 21 or 24°C, >80% failed to develop to the veliger stage. Conditioning 'thermosensitive' parents to elevated temperatures reduced embryo fitness at all incubation temperatures. Conversely, 'thermotolerant' parents acclimated to elevated temperatures produced correspondingly robust embryos. For example, only 5.7% of embryos incubated at 24°C survived to veliger if parents were not heat-acclimated, but this rose to 47.9% if parents had also experienced 24°C.

The results indicate that heat tolerance in the most vulnerable life stages (embryogenesis to first shell formation) can be substantially influenced by the complex interactions between genotype and parental history. Broodstock husbandry and selective breeding may therefore be key tools to support biological and commercial resilience in a changing coastal environment. On-going research now focuses on the effects of chronic heat exposure across multiple gametogenic cycles, while considering the relative importance of epigenetic effects and maternal provisioning upon offspring performance.

NUTRITION SENSITIVE ARTEMIA POND CULTURE IN BANGLADESH

Muhammad Meezanur Rahman*[1], Nguyen Van Hoa[2], Patrick Sorgeloos[3]

[1]WorldFish, Gulshan, Dhaka, Bangladesh
[2]College of Aquaculture & Fisheries, Can Tho University, Vietnam
[3]Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium
*Muhammad.Rahman@cgiar.org

Approximately, half a million people are involved in crude salt production in Bangladesh and the annual production reached 1.8 million metric tons. The salt farmers' livelihoods are vulnerable due to increased operating costs for salt production without any market expansion or innovation, unstable salt production and price, low productivity of aquaculture in the salt farms during rainy season, the seasonal unemployment and climate induced hazards, for example flood, cyclone, erratic rainfall.

The EU-supported project "Artemia4Bangladesh" aims to improve the livelihood of the salt farmers in the Cox's Bazar area by integrating their salt production with different forms of aquaculture in both the dry as well as the rainy season, with primary focus on the production of brine shrimp *Artemia* for its cysts and biomass as vital sources of food for the larviculture and nursery farming of crustaceans and fish. Apart from applications in aquaculture, *Artemia* can also be used for human consumption as it is highly nutritious in terms of protein and fatty acid content. In 2022, 20 farmers were successful in producing 1200 kg *Artemia* biomass and 13 kg of cysts in 4 ha water area in three months. Nutritional composition of *Artemia* biomass were crude protein 48.6%, crude fibre 2.4%, crude fat 6.9%, calcium 0.5%, phosphorous 0.2% on dry matter basis. *Artemia* cyst were 86% hatching rate and cyst diameter 238 μm. *Artemia* biomass were fed to *Penaeus monodon* post larvae and domesticated broodstock. Several *Artemia* recipes were developed as human food namely *Artemia* kebab, *Artemia* omelet, *Artemia* water spinach fritters. The recipes were tested among the salt farmer communities as well as aquaculture professionals. This opens a scope to apply *Artemia* as human food to tackle malnutrition particularly in the salt farmers communities. Now focus is on cyst production trials and the economic evaluation of this new integrated farming and use of *Artemia* in coastal salt ponds. Moreover, the excellent quality of cysts produced in Cox's Bazar open up a bright future not only to fill the need of local aquaculture activities but also to contribute significantly to the huge demand of cysts in the global market. Successful trials in Cox's Bazar on *Artemia* production will encourage the vast salt farming area in Bangladesh to apply the integrated *Artemia*-aquaculture-salt production system.

DEVELOPMENT OF CRYOGENIC SPERM BANK OF SILVER CARP (*Hypophthalmichthys molitrix*) AND SEED PRODUCTION IN COMMERCIAL HATCHERIES FOR BROOD BANKING

M Matiur Rahman*, M Abdur Razzak, M Rafiqul I Sarder, Mariom and Terrence R Tiersch

*Department of Fisheries Biology and Genetics
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Quality of hatchery produced seeds of the exotic species, Silver carp (*Hypophthalmichthys molitrix*) is deteriorating day by day in Bangladesh due to intergeneric hybridization with other Chinese carps resulting in failure of achieving expected production by the farmers. Therefore, development of cryogenic sperm bank of silver carp has become a demanding issue to overcome such problems. This investigation was carried out for standardization of sperm cryopreservation protocol of silver carp and production of quality seeds using cryopreserved sperm in commercial fish hatcheries. Broodstock of silver carp was developed by rearing newly imported silver carp fingerlings from China in earthen ponds using supplementary feeds. Activation of sperm motility was evaluated with different concentrations of NaCl solution (0.1% to 1.2% NaCl) among which 0.4% showed the highest motility (97.3±0.9%) and swimming duration (28.12±1.5 min). Motility of sperm was severely inhibited at 1.2% NaCl. The toxicity of cryoprotectant (DMSO and methanol) to sperm was tested at different concentrations (5%, 10%, and 15%) and with incubation time (5-40 min) with two extenders (Alsever's solution and egg-yolk citrate). Cryoprotectants at 5% and 10% concentrations produced better motility during 5- and 10-min incubation periods, respectively. Alsever's solution with 10% DMSO at 1:12 dilution (sperm: diluent) with 15 min incubation time displayed highest equilibration motility (93.3±1.2%) and post-thaw motility (91.0±0.6%) during cryogenic freezing. The quality of sperm in cryogenic sperm bank was assessed by observing post-thaw motility at 30 days interval over the twelve-month storage period and a slight decrease in motility from 82.67±1.5% to 87±1.7% was observed. Seeds of silver carp were produced through induced breeding in 10 commercial government and private hatcheries in different geographic locations using both cryopreserved sperm (treatment) and hatchery-originated fresh sperm of males (control). The average fertilization and hatching rates of eggs for cryopreserved sperm and fresh sperm were determined as 39.73% and 29.95±8.74%, and 73.16±10.49% and 59.24±9.95%, respectively. The seeds produced from both treatments and controls are separately stocked in respective hatcheries as well as in some technology adoption hatcheries and are being reared for observing growth performances. The initial sampling data revealed higher growth in cryopreserved sperm-originated seeds compared to control.

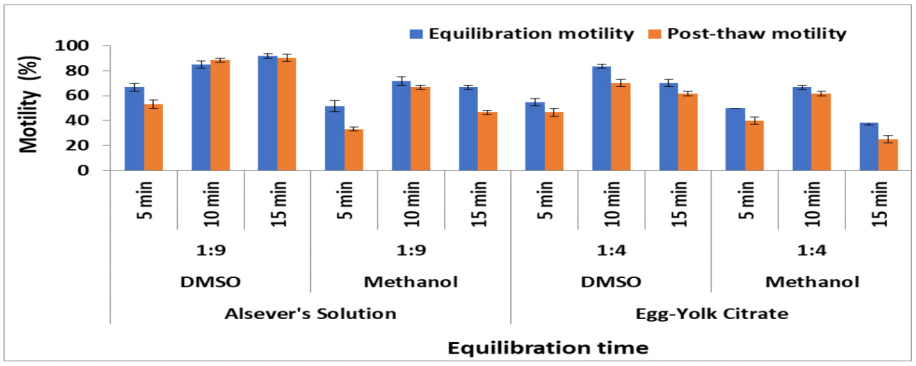


Fig. Determination of suitable equilibration time for cryopreservation of *H. molitrix* sperm

EFFECTS OF BUTYRINS ON THE GROWTH PERFORMANCE, SURVIVAL RATE, AND *Vibrio* COUNTS IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Tirawat Rairat*, Niti Chuchird, Arunothai Keetanon and Putsucha Phansawat

Department of Fishery Biology, Faculty of Fisheries, Kasetsart University
Bangkok, Thailand 10900
ffistwr@ku.ac.th

Antibiotic misuse in aquaculture causes several detrimental effects. Currently, non-antibiotic feed additives have been popularly applied in aquafeed to improve aquatic animal health and growth performance. Short-chain fatty acids, such as butyric acid, are among the most promising ones. To enhance its stability and bio-availability and decrease its unpleasant smell, butyric acid can be supplemented as butyrins (glycerol esters of butyric acid). The present study aimed to evaluate the effects of butyrins on growth performance, survival rate, and *Vibrio* spp. counts in Pacific white shrimp.

Pacific white shrimp (2-3 g) were randomly distributed into 30 tanks (40 shrimp/tank) with 200 L of water (29°C and 25-30 ppt). Ten experimental diets were formulated with different concentrations of soybean meal (SB) at 25 and 50%, and different formulations of butyrins, namely butyrins powder (BP, ProPhorce SR 130) at 0.05 and 0.10% and butyrins liquid formulations (BL, ProPhorce SR 730) at 0.03 and 0.07%. The two commercial products were obtained from Perstorp (Malmö, Sweden). These experimental diets were fed to the shrimp 4 times daily for 10 weeks. The shrimp body weight and survival rate of each group were recorded at the end of the feeding trial. At the end of growth trial, the intestinal and hepatopancreatic (HP) *Vibrio* spp. counts were also conducted using a spread plate method on TCBS agar.

Results revealed that the shrimp body weight was slightly improved by supplementation with BP and BL at both SB levels. The BP 0.10% group had highest survival rate at both 25% SB (87.50%) and 50% SB levels (62.5%), significantly ($p<0.05$) different from the control groups, which were 69.17% and 55.00%, respectively (Table 1). Regarding the *Vibrio* spp. count study, shrimp offered BP and BL supplemented diets had significantly lower ($p<0.05$) *Vibrio* spp. number in the HP, from about 8×10^3 CFU/g to about 4×10^3 CFU/g (Table 1) and the intestine, from about 8×10^2 CFU/g to about 5×10^2 CFU/g (data not shown) compared to the corresponding control groups. In conclusion, the benefit of butyrins feed additive in improving shrimp growth and survival as well as reducing *Vibrio* spp. counts in the laboratory condition were evident.

TABLE 1. The survival and hepatopancreatic (HP) *Vibrio* spp. count of Pacific white shrimp after feeding with butyrins-supplemented diets for 10 weeks

Treatment group	Survival (%)	*Vibrio* spp. in the HP (10^3 CFU/g)
SB 25% (control)	69.17±2.89^{c}	8.66±0.97^{b}
SB 25%+BP 0.05%	82.50±2.50^{b}	5.85±3.27^{a}
SB 25%+BP 0.10%	87.50±2.50^{a}	4.35±0.19^{a}
SB 25%+BL 0.03%	70.00±2.50^{c}	4.41±0.13^{a}
SB 25%+BL 0.07%	81.67±3.82^{b}	4.42±0.30^{a}
SB 50% (control)	55.00±2.50^{e}	8.71±0.38^{b}
SB 50%+BP 0.05%	58.33±1.44^{de}	4.53±0.52^{a}
SB 50%+BP 0.10%	62.50±2.50^{d}	4.51±0.86^{a}
SB 50%+BL 0.03%	55.83±0.00^{e}	4.35±0.70^{a}
SB 50%+BL 0.07%	56.67±2.89^{e}	5.23±0.87^{a}

Note: SB, soybean meal; BP, butyrins powder formulation; BL, butyrins liquid formulation

EVALUATION OF THE PRODUCTION EFFICIENCY OF BLACK TILAPIA, *Oreochromis niloticus* AND RED HYBRID TILAPIA, *Oreochromis* sp. FRIES IN TWO NURSING SYSTEMS

Farhad Rajabipour*; Nassrin Mashaii
Agricultural, Research, Education and Extension Organization, Iranian Fisheries Science Research Organization
National Research Center of Saline Water Aquatics
Yazd, 100km Bafq Road, National Research Center of Saline Water Aquatics, POBox:159

farhadrajabipour@yahoo.com

The hapa net nursing system is used for monosexing tilapia fry with the aim of increasing the efficiency of monosex fry production. In the present study, the production of SRT (Sex Reversed Tilapia) of *Oreochromis niloticus* and red hybrid *Oreochromis* sp. was investigated, using hapa net and hapa-free systems. The ranges and averages of total length and body weight, percentage of monogamy and survival of black tilapia and red hybrid tilapia fry were compared in two nursing systems.

Tilapia breeding has developed in the world and it is necessary to produce enough fry to meet the demand of breeders. The production of tilapia fries is a basic requirement for the tilapia culture industry and it is necessary to optimize the production of fries to increase the profitability of the tilapia hatcheries.

In the present study, the production fries of Nile tilapia, *Oreochromis niloticus* and hybrid red *Oreochromis* sp. was investigated, using tank and hapa systems. The results showed higher significant difference in the length and weight of fish in the hapa-free tanks, by t-test ($p < 0.05$) but much isometric fries and more survival in the hapa system. SRT were similarly 100% monosexed in both systems. Also, the means of total length, body weight and survival of hybrid red tilapia were significantly higher than Nile tilapia fries, by t-test ($p < 0.05$). Producing SRT fries in tank system with hapa tended to more isometric fries and appropriate survival, however fries produced in tanks without hapa had higher total length and body weight.

MODELLING OF INFLAMMATORY BOWEL DISEASE (IBD) IN ZEBRAFISH

D.C.Rajapaksha, S. L. Edirisinghe, Chamilani Nikapitiya and Mahanama De Zoysa

College of Veterinary Medicine, Chungnam National University, Daejeon 34134
E mail: dinurajapksha276@gmail.com

The zebrafish (*Danio rerio*) has been used to investigate pathogenesis of a variety of human diseases. Inflammatory bowel disease (IBD) is a chronic, recurrent, inflamatory disease with unclear etiology. IBD is classified into two subtypes: Crohn's disease (CD) and ulcerative colitis (UC). While UC affects the superficial layers of the intestine, CD often affects deeper layers of the bowel wall.

In this study, zebrafish model of IBD was developed using colitic grade (0.04% w/v) Dextron sodium sulfate (DSS) for 3 days. We visualized change of acidified lysosome (neutral red staining) and goblet cells (Alcian blue staining) in intestine of zebrafish larvae. Moreover, expression of known IBD markers such as mucin, pro and anti inflamatory were investigated.

DSS treatment was characterized by upregulation of *il-1β, tnfα,* hsp70 and *il-10* indicating the induction of inflammation. Down regulation of mucin 2.1 in DSS exposed larvae was concomitant with depletion of goblet cell and adherent mucin. Epithelial mucin in goblet cells and acidified lysosome were decreased with DSS (0.04% w/v) treatment. Collectively these results demonstrate that zebrafish IBD model can be applied for understanding the uncovered molecular mechanisms that lead to IBD as well as screening of therapeutic agents to control IBD.

OPTIMIZATION OF CUTTING PARAMETERS DURING THE MACHINING PROCESS USING GREY RELATION ANALYSIS

Durga Venkata Prasad Ramena

GITAM Institute of Technology
Gandhi Nagar
Rushikonda
Visakhapatnam
Andhra Pradesh 530045
India

The fundamental property of a material that determines productivity changes, machining costs, and material selection optimization in the design of mechanical parts is the machinability rating of engineering material. As a result, this work focuses on the study of surface roughness parameter (Ra), tool temperature (Tt), and work temperature (Tw) while performing Lathe on the Ti alloy, which is a relatively low machinability alloy, for various combinations of machining parameters like feed (f), depth of cut (doc), and spindle speed (N) under wet conditions using Taguchi philosophy with non-coated PVD tool inserts. A surface roughness tester and an infrared gun are used to measure roughness and temperature. For experimental design, Taguchi design of experiments (DOE) based on Orthogonal Arrays (OA) and signal-to-noise ratio (S/N ratio) is used. The generated responses are used to predict the performance and significance of machining parameter combinations in CNC lathe operations using Analysis of Variance (ANOVA). Individual response optimization is carried out using S/N ratios of the responses, and multi-response optimization is carried out using Grey Relational Analysis (GRA) for both machining conditions and better responses in wet conditions.

THE POTENTIAL OF CO-FEEDING ARTEMIA TO IMPROVE HEALTH INDICES OF *Litopenaeus vannamei* POST LARVAE IN THE NURSERY PHASE

Yathish Ramena*, Frank Martorana, Kala Mallik, Thomas Bosteels, Vikas Kumar, and Grace Ramena

Great Salt Lake Brine Shrimp Cooperative, Inc.
1750 W 2450 S
Ogden, UT, 84401, USA

With increased production, *Litopenaeus vannamei* has a number of obstacles, with a greater emphasis on larval and postlarval rearing. There are, however, very few studies attempting to understand the post-larval nursery phase of *L.vannamei,* despite the fact that these stages undergo a complex growth metamorphosis during development as a common feature of penaeids. The purpose of this study is to comprehend the impact of Artemia as a co-feed during the post-larval nursery phase when using either a premium nutrient-dense, Makay Marine MP feed or a budget friendly nutrient-low, Shrimp feed dietary options.

While Artemia is acknowledged as one of the best live foods during the larval and post-larval stages of *L. vannamei*, it is still unclear how it affects growth and health parameters during the post-larval to nursery phases. This research trial assesses three different Artemia inclusion levels, along with a premium high dense and budget friendly low-dense diet, in *L. vannamei* reared from post-larvae 12 to 25. A completely randomized design was implemented with six treatments and eight tanks per treatment. Each tank was initially stocked with 200 post-larvae. A combination of two particle feeds and three Artemia co-feeding levels (Table 1) were administered throughout the trial. All the dietary treatments were supplemented with one ppm of commercial probiotics at 500 billion CFU/g before the trial started to evaluate the effect of bacterial colonization in the gut of post-larvae at the trial conclusion through the gut microbiome. At trial conclusion, survival (%), mean body weight (g) and proximate, amino, and fatty acids in the shrimp whole body were evaluated. In addition, the gut microbiome was assessed. Relative sequence abundance of lactobacillus associated with the MP group and economy groups were analyzed.

Dietary intervention had no discernible impact on the mean body length proximate amino acid and fatty acid composition of post-larvae. Nevertheless, the shrimp tanks fed Artemia showed a higher average weight gain and survival as compared to tanks without Artemia co-feeding. A premium diet improved overall growth and survival as compared to a budget friendly diet, albeit not significantly for every treatment. The Makay Marine MP diet appeared to function as a prebiotic substrate by improving lactobacillus colonization in the gut of *L. vannamei* at PL25 with just a minimal single probiotic dosage (1ppm), which may have contributed to the improved growth and health indices obtained with the premium diet.

In general, the use of a premium diet in combination with co-feeding of 3kg of Artemia per million PL produced resulted in optimal survival and growth of the PL. To obtains similar results for weight gain with a budget friendly diet, inclusion rates of Artemia needed to be increased to a co-feeding level of 6 kg per million PL produced.

Table 1. Artemia cyst (g) requirements were estimated to produce one million *L. vannamei* postlarvae

Diet	Artemia treatments		
Mackay Marine MP	0 g	3000 g	6000 g
Budget friendly shrimp	0 g	3000 g	6000 g

INSIGHTS INTO THE INCREASED DIETARY LEVELS OF BRINE SHRIMP *Artemia franciscana* CO-FED WITH MICROPARTICLE DIETS IN THE REARING OF *Litopenaeus vannamei*

Yathish Ramena*, Femi J. Fawole, Frank Martorana, Thomas Bosteels, Krishna P. Singha, Amit K. Yadav, and Vikas Kumar

Great Salt Lake Brine Shrimp Cooperative, Inc.
1750 W 2450 S
Ogden, UT, 84401, USA

Despite the historical significance of *Artemia franciscana* as live food for many fish and shrimp larvae and efforts to co-feed live Artemia with micro-particulate feeds, the ideal co-feeding combination for early larval and post-larval stages of *Litopenaeus vannamei* remains unknown. In two independent larval experiments, six different concentrations of Artemia nauplii were investigated as a co-feed with two different commercial micro-particulate diets. In two consecutive experimental trials, postlarvae performance was assessed in terms of survival, growth, MGR, osmotic stress test, fatty acid, amino acid and whole-body composition. *L. vannamei* postlarvae were tested in two trials in which micro-particulate diets were fed to satiation and a range of Artemia feeding levels were employed.

Overall, increased Artemia inclusion levels significantly improved survival during the hatchery cycle (PL15) up to a level of 5,900 g of Artemia per million *L. vannamei* postlarvae produced. Additionally, survival further increased with increased inclusion of Artemia when continually co-feeding during extended nursery life stages up to PL 35. Biochemical analysis of body tissue and feeds were not able to offer an explanation for the improved survival rates recommending that additional research will be required to further elucidate the reasons for improved survival with increased levels of Artemia co-feeding.

EFFECT OF A LOW-FAT MEDIUM CHAIN TRIGLYCERIDE DIET ON GROWTH PARAMETERS OF SALINE-TOLERANT NILE TILAPIA *Oreochromis niloticus*

Reginna Aivel Y Rapiz*, Valeriano L Corre, Rex Ferdinand M Traifalgar, Rowena Cadiz, Fredson H Huervana, Ande Bryle Genciana, Maria Alexandra Fontanilla, Ariadne Marie Daquioag, Carmelo S del Castillo

Institute of Aquaculture, College of Fisheries and Ocean Sciences
University of the Philippines Visayas
Miagao, Iloilo, Philippines
ryrapiz@up.edu.ph

We have previously shown that a 2.5:2.5 (% over 100) ratio of soybean oil and Coconut oil is a superior replacement to a diet utilizing 5% soybean oil as the main source of lipids in a saline-tolerant Nile tilapia *Oreochromis niloticus*. In this study we explore the effects of a low-fat diet utilizing the same ratio of soybean oil to coconut oil. Three experimental diets were formulated with varying coconut oil:soybean oil ratio - Control (0:5); TrtB (2.5:2.5); and a low-fat diet TrtC (1:1). Overall growth performance and survival in response to the formulated diets were assessed after 56 days of culture. Survival rate among treatments were high and were not influenced by the different inclusion levels of coconut oil in the formulated diets. Growth performance values indicated significant differences (P>0.05) between Control and TrtB and TrtC (Figure 1).

The effect of coconut oil supplementation and low-fat diet on the expression and efficiency of growth-related genes was also investigated. Findings showed that inclusion of coconut oil in the diet produced statistically significant results on the expression of FADS2, ELOVL5, IGF, and Ghrelin genes. Body lipid composition and tissue morphology was also observed. The n-3 and n-6 levels were statistically different among treatments (P<0.05). Improved intestinal structure was observed on tilapia fed with coconut oil and growth in the dorsal muscle was observed in treatments that are enhanced by coconut oil. Overall, the low-fat diet (TrtC) seemed to exhibit similar beneficial properties as TrtB over the control diet.

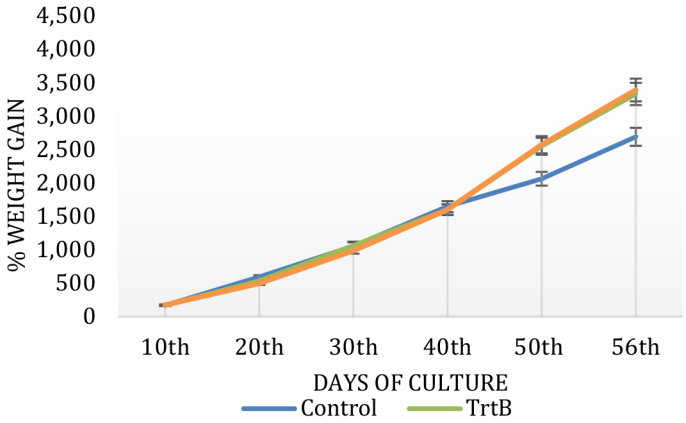


Figure 1. Percent weight gain of saline-tolerant Nile tilapia after 56 days of culture

THE HORIZONTAL INTEGRATION OF A SHELLFISH FARM IN A BROADER BUSINESS MODEL

Perry Raso*

Matunuck Oyster Farm
Wakefield, RI, 02879 USA
perryraso@gmail.com

The demand for Eastern oysters over the last two decades has increased year after year. This steady continuous growth has created opportunity not only for oyster farmers and other businesses immediately supporting the shellfish aquaculture industry, such as gear and transportation, but it has also created opportunities just outside the shellfish aquaculture market. Opportunities to reach retail markets, such as restaurants and open-air markets, with their product and integrating into establishing those market on their own.

In order to meet those markets directly Matunuck Oyster Farm has horizontally integrated in each stage of the cycle of the eastern oyster (Crassostrea virginica). Matunuck Oyster Farm was established in 2002 as a 1-acre oyster farm with one employee. Now, 20 years later, that business has expanded to five additional businesses with over 200 employees, each business having the farmed Eastern oyster being a central, integral part of the business. Each of the new businesses support the farm, and the farm supports each business, creating more financial stability for the oyster farm.

The growth of Matunuck Oyster Farm into the six other sectors has been organic and determined by company needs. In addition to selling oysters to wholesalers, we expanded by offering different products such as Bay Scallops. We then started selling oyster seed to other farmers, selling at open air markets, and established Matunuck Oyster Bar, a popular restaurant overlooking the shellfish farm. This led to the establishment of Matunuck Organic Vegetable Farm, Matunuck Marina, Matunuck Shellfish Hatchery Research and Innovation Center and most recently a retail market in our adjacent downtown area of Wakefield, Rhode Island.

Perry Raso, Founder and Owner of these businesses, will discuss synergies between each of the businesses and how each business has strengthened and added value to each other.

INCLUSION OF DRIED FISH AND SMALL FISH POWDER IN TRIBAL DIET PLAN: A POTENTIAL STRATEGY FOR ALLEVIATING ODISHA'S DUAL BURDEN OF MALNUTRITION

Baishnaba Charan Ratha[1]*, P. Arun Padiyar[1], Neetha Shenoy[1], Anu Garg[2], Rashmi Ranjan Nayak[2], Shakuntala Thilsted[3], Chadag Vishnumurthy Mohan[3], Ravishankar C.N[4], Suseela Mathew[4], R. Gopinath[5], Diptimayee Puthal[5]

[1]WorldFish, Directorate of Fisheries, Mangalabag, Cuttack, Odisha 753001, India
[2]Women and Child Development Department, Government of Odisha, Lokaseva Bhavan, Bhubaneswar – 751001, Odisha, India
[3]WorldFish, Jalan Batu Maung, Batu Maung, 11960, Bayan Lepas, Penang, Malaysia
[4]ICAR-Central Institute of Fisheries Technology, Willingdon Island, Matsyapuri P O, Kochi-682 029, Kerala, India
[5]District Administration, Mayurbhanj District, Baripada – 757001, Odisha

*Corresponding and presenting author: b.ratha@cgiar.org (B.C. Ratha)

Indigenous peoples are among the poorest and thus the most threatened segment of the world's population in terms of social, economic and environmental vulnerability. Along with other stressors, food and nutritional insecurity is still a concern in Odisha State in India. Currently, the state is dealing with burdens of undernutrition which is especially common in tribal regions. To address this issue, the Government of Odisha has launched several nutrition-specific and nutrition-sensitive schemes and interventions in recent years. One such major scheme is the Supplementary Nutrition Programme (SNP) under the Integrated Child Development Services (ICDS) which aims to improve the health and nutrition of children aged 6 months to 6 years, as well as pregnant and lactating women and adolescent girls. The government is promoting nutrition by providing hot cooked meals and take-home rations, which are supplied through about 72,000 Anganwadi Centers (Child Care Institutes) situated in each and every village of the state. With a common goal of enhancing the nutritional outcomes from aquatic foods and assisting in accelerating progress on reducing malnutrition in hard-to-reach tribal communities, WorldFish in partnership with ICAR-CIFT and with funding support from USAID, has provided technical support the Women and Child Development Department, Government of Odisha and Mayurbhanj District Administration to pilot the inclusion of dried small fish-based products in the SNP during 2021. This entailed blending of small fish powder in hot cooked meals served to children aged 3 to 6 years and addition of packaged and certified dried small fish in take-home rations for pregnant and lactating women and adolescent girls aged 11 to 18. Based on a Government approved Standard Operating Procedure (SOP), the pilot was implemented in 50 Anganwadi Centers of Kaptipada block, Mayurbhanj district for 6 months during April-September 2021. This paper describes the process followed and field results achieved from the pilot. This is a groundbreaking and unique case of incorporating fish-based products in the government ICDS meal program (SNP) to address undernutrition challenge in the state. Based on the beneficiary community feedback and acceptance of dried fish-based products in SNP, as well as an enhanced understanding congregated from the pilot, we strongly advocate adoption of this nutrition-sensitive approach at the policy level in other Indian states and thus speedily scaled to ameliorate the rampant and humongous undernutrition challenge.

EFFECT OF PROBIOTICS ON PATHOGENIC *Vibrio* BACTERIAL POPULATIONS IN CULTURE PONDS OF *Litopenaeus vannamei* AT MOPHUS BANDER, SRIKAKULAM DISTRICT, ANDHRA PRADESH, INDIA

[*]RAYAPROLU SRINIVAS

[*]Devee Biologicals Pvt Ltd, Visakhapatnam, Andhra Pradesh, India.

Corresponding author email Id: srinidbpl@gmail.com

The current study was carried out for 147 days to assess the pathogenic *Vibrio* count of *Litopenaeus vannamei* culture ponds and the influence of probiotics from Mophus Bander, Srikakulam District, Andhra Pradesh, India. Three ponds were selected, one is control and other two were experimental (Pond A and B). The physico-chemical parameters of the culture ponds were recorded by adopting standard methods. During summer crop the control ponds were harvested at 24.0 gm on 107th day and at 31.0 gm on 121st day for the year 2020 and 2021 respectively due to the incidence of *Vibriosis* disease. Where as in winter crop during 2020 the control pond was harvested at 5.5 gm on 47th day because of *Vibriosis* but in 2021 the control pond was harvested normally at 32.5 gm on 145th day, as this pond is free of *Vibriosis*. Experimental ponds were harvested normally in summer season at 30.5 gm on 124th day & 31.0 gm on 127th day during 2020 and 34.0 gm on 127th day & 35.5 gm on 127th day in the year 2021 respectively.

Where as in winter season of 2020 the experimental ponds were harvested normally at 28.0 gm on 124th day as well as on 127th day. In the year 2021 winter, the experimental ponds were harvested at 36.0 gm at 146th day and 35.5 gm at 147th day respectively. This study suggests that the probiotics are the key agents which have a great impact on the reduction of total pathogenic *Vibrio* count in culture ponds of *Litopenaeus vannamei*.

RECIRCULATING AQUACULTURE SYSTEMS INNOVATION: THE NEED FOR SMART SOLUTIONS TO ADDRESS DIVERSE FUTURE PRODUCTION NEEDS

Paola Reale*, Franck Le Gall, Nicolas Prost, Christian Jensen, Natalie Panasiak, Steven Prescott, Samuel Dupont, Maxime Paris, Giovanni Marco Cusimano, Jui-Che Chiang, Shane A. Hunter, Dannie O'Brien, Marina Garcia, Andres Acosta, Dominique Durand, Lars Ebbesson, Tamás Bardócz

*pfr@aquabt.com
AquaBioTech Group, Central complex, Targa Gap, Mosta, Malta

Introduction: Reducing production risks, increasing transparency to consumers, and ease of use solutions are the primary priorities for large aquaculture producers considering further or new investment in recirculating aquaculture (RAS) units in their production structure. Operators must focus on fine-tuning their energy, water, and feed to reduce their OpEx, often with constrains to the CapEx investment at start-up. Technological innovations to achieve better monitoring and control performance in various elements RAS, particularly in the areas of intelligent automated feeding (feeding costs can be up to 80% of production costs) and integrated farm monitoring, will have vital impacts on the operational expense and sustainability of RAS production.

Method: Innovations of the ongoing iFishIENCi Horizon 2020 project will be the next step in the future of RAS development, to ensure that the increasingly diverse needs can be met for the 1.4 million tonnes planned RAS production in the coming years. The resulting technology will improve feeding technology based on machine vision and digital twin development to reduce feed loss and improve FCR. *iBOSS* cloud connected technology for intelligent design and control of water treatment solutions, oxygen injection, fine solid removal, and disinfection (including ozone optimisation) uses water quality predictive AI modelling based on continuous data provided by in tank sensors. Camera integration coupled with a smart automated feeding system, based on modelling calculations of fish metabolism and machine vision, will allow for recognition of satiation behaviours to optimise feed delivery. The iBOSS innovation will be fully integrated with RAS production technologies to deliver *SmartRAS* to fulfil increasingly diverse RAS production and research needs.

Results and Outlook: At current status, amount of feed dispensed and feeding pattern can be user determined to suit the needs of the specific fish species in RAS production. Feed is automatically dosed throughout the day while simultaneously taking water quality into consideration. Total dispensed feed is used to predict updated growth projections daily, based on tabled FCR, and the next feeding day automatically adjusted to meet production forecast. *iBOSS* applies an adjustment factor to the feeding model based on 'Activity' calculated from smart camera observation of the fish behaviour, further adjusting the daily amount of feed automatically dispensed. The *iBOSS* AI will self-evolve throughout the remainder of the project as changes in feeding intensity, water quality, fish behaviour, and growth feedback data continuously optimise the models. *SmartRAS* equipped with the *iBOSS* integrated management system will enable more precise and accurate research with more data, shorten trial duration, as well as lowering operational costs and time to market. In this increasingly competitive and regulated industry, extrapolative modelling and prediction of challenging water quality parameters and visualisation of production data will ensure farm managers have the tools needed to reduce production issues, manage disease and mortality, reduce input costs, monitor and valorise waste products, and demonstrate sustainability and transparency of production, resulting in a highly attractive consumer product with reduced investor risk.

THE FEED THE FUTURE INNOVATION LAB FOR FISH: ACHIEVING NUTRITIONAL SECURITY THROUGH BLUE FOODS

Stephen R. Reichley*, Peter Allen, Lora Iannotti, Kathleen Ragsdale, Mary Read-Wahidi, Glenn Ricci, Joanna Springer, Elin Torrell, and Mark L. Lawrence

Feed the Future Innovation Lab for Fish
Mississippi State University Global Center for Aquatic Health and Food Security
Mississippi State, MS 39762 USA
stephen.reichley@msstate.edu

The Feed the Future Innovation Lab for Fish, funded by the U.S. Agency for International Development (USAID), aims to reduce poverty and improve nutrition, food security, and livelihoods in developing countries by supporting the sustainable development of aquaculture and fisheries systems.

The Fish Innovation Lab is one of 21 Feed the Future Innovation Labs leveraging the expertise of U.S. universities and developing country research institutions to tackle some of the world's greatest challenges in agriculture and food security. These labs harness U.S. and global expertise on a variety of food security topics – from horticulture to food security policy and beyond.

The Fish Innovation Lab focuses on applied reach in Bangladesh, Cambodia, Ghana, Kenya, Madagascar, Malawi, Nigeria, the Pacific Islands, Peru, the Philippines, and Zambia; it has 24 activities, 20 of which are currently active. The Lab's program areas include improving productivity, mitigating risk, and improving human outcomes. Additionally, the Fish Innovation Lab has four cross-cutting themes, which are incorporated into each funded project and guide the Lab's work overall. These are mainstreaming gender equity and youth inclusion, advancing human and institutional capacity development, strengthening resilience, and advancing nutrition. All the while, the Lab's theory of change (Figure 1) helps guide the activities to meet these important objectives.

One of the Fish Innovation Lab's key goals is to assess nutrition as both outcome and determinant and support research to identify interventions that optimize human health and livelihoods while sustaining aquatic ecosystems over the long term. Through research in our three areas of inquiry, we delineate and ultimately impact multiple pathways to food security and human nutrition in vulnerable groups living in low-resource households and among smallholder fisher families. It is critical to support pregnant and lactating women, infants and young children, and school-aged children with research that aims at reducing hunger and improving nutrition to decrease childhood stunting and wasting.

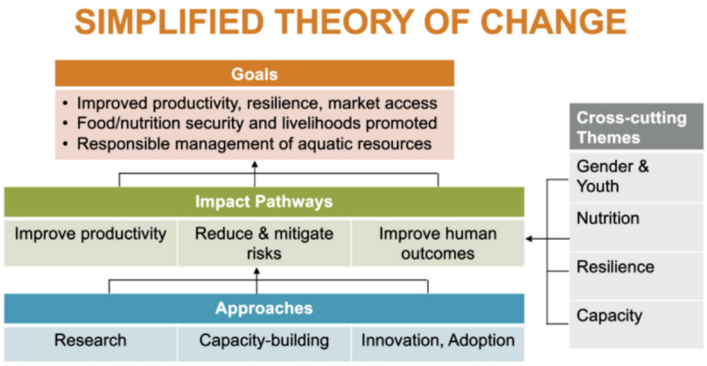


Figure 1: Fish Innovation Lab's theory of change simplified.

THE EFFECTS OF LACTIC ACID BACTERIA ON EXPRESSION OF IMMUNE RELATED GENES AND INTESTINAL FLORA IN TURBOT *Scophthalmus maximus*

Yichao Ren*, Guangxin Guo, Chao Li, Bin Xia, and Chunyan Zhao

School of Marine Science and Engineering
Qingdao Agricultural University
Qingdao 266109, China
yichaor@126.com

Turbot (*Scophthalmus maximus*) culture has become one of the most commercial flatfish species for recirculating aquaculture in north China. The rapid expansion of intensive aquaculture increased the risk of disease outbreaks. Probiotics have attracted great interest due to their positive effects on host and are considered environmentally friendly alternatives to chemicals and vaccination to prevent infectious diseases. The present study was conducted to understand the effects of lactic acid bacteria screened from fishery catch as probiotics on growth, immune response and gut microbiome of *S. maximus*.

A four week experiment was conducted in a turbot recirculating culture system. *Leuconostoc mesenteroides* HY2 strain was administrated in juvenile turbot culture water. The mRNA levels of TLR3, CXCL8 and IFIH1 in different organs were analyzed using RT-PCR technology. The structure and diversity of intestinal flora were analyzed using 16sRNA technology, in which community composition and diversity indexes (Shannon, Simpson, abundance-based coverage estimator and Chao1), principal co-ordinates analysis (PCoA) as well as cluster analysis were performed.

The specific growth rate of turbot in L treatment was significantly higher (P<0.05) than in control. The expression levels of TLR3, IL-8 and IFIH1 were almost all significantly (P<0.05) up-regulated in intestine, liver, spleen, kidney, brain and skin in L treatment after four weeks culture (Fig. 1). A total of 42 phyla in intestinal flora were identified and its composition and biomarkers showed significant difference in two groups (Fig. 2). The administration of HY2 strain was capable of improving turbot growth by enhancing the immune system. Some bacteria groups in intestine acted as biomarkers indicating the variation of the structure and diversity of intestinal flora, which may help to promote absorption and/or trigger immune function.

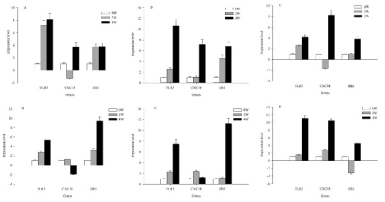
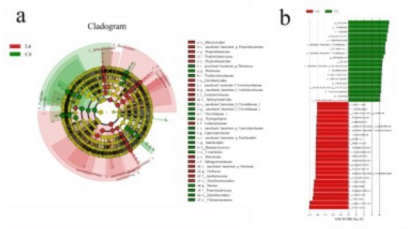


Figure 1 The expression levels of immune related genes in different organs of turbot



Figutre 2 Cladogram and histogram of the biomarkers in intestinal flora

BEHAVIOURAL ANALYSIS OF JUVENILE ATLANTIC SALMON *(Salmo salar)* IN RESPONSE TO DIFFERENT COMPOSITIONS OF INSECT-BASED AQUAFEEDS AND THE IMPLICATIONS FOR FISH WELFARE AND PRODUCTION

Sonia Rey Planellas*, Aoife Ong, Ben Clokie, Pamela Prentice, Simon Mackenzie

Institute of Aquaculture, University of Stirling, Pathfoot Building, FK9 4LA, Scotland, UK

The need to diversify novel feed ingredients for aquaculture is becoming increasingly important. Black soldier fly larvae (BSFL, *Hermetia illucens*) meal is gaining much interest due to its unparalleled efficiency at converting a wide variety of substrates into usable protein and fats. Such attributes could increase product circularity and sustainability in agreement with the Sustainable Development Goals (SDGs). To date, few studies have investigated the use and effects of the inclusion of BSFL meal in either terrestrial or aquatic farmed animal diets. This study assessed the behaviour of salmon fry (*Salmo salar*) fed on three different diets – Control diet (CD, commercial diet), de-chitinised insect meal at 2.5% inclusion (BSFL-D2.5), and de-chitinised insect meal at 25.0% inclusion (BSFL-D25). Diets were fed to naïve first feeding fry for 21 days. Growth and diet consumption was monitored every day and behaviour was recorded and analysed at the end of the trial to explore potential differences in feeding related behaviours.

An ethogram (a full suite of behaviours exhibited by fish in each treatment group) was constructed for the purpose of the study and four feeding related behaviours were selected: darting, zone-crossing, upper zone positioning and aggression. Fish were observed displaying different behaviours depending on the diet treatments (Figure 1a). Significantly more darting and zone-crossing behaviours were observed between the BSFL-D2.5 and CD group which maintained positions near the water surface to feed (Figure 1b). A similar final average weight between these two groups suggested that despite the adoption of different feeding strategies neither growth or condition was affected. Interestingly, higher darting in the BSFL-D2.5 reflected a more natural feeding strategy which may improve welfare, in addition higher overall activity may improve flesh quality in the long term. The BSFL-D25 diet displayed lower levels of feeding behaviour which translated to a lower final average weight. There were no significant differences in the levels of aggressive behaviour between the insect-meal diets and the CD groups indicating that no negative effects arise from the use of BSFL diets. Further research is required to determine whether insect-based feeds indeed elicit natural feeding behaviour in juvenile salmon and the implications on the welfare of the species.

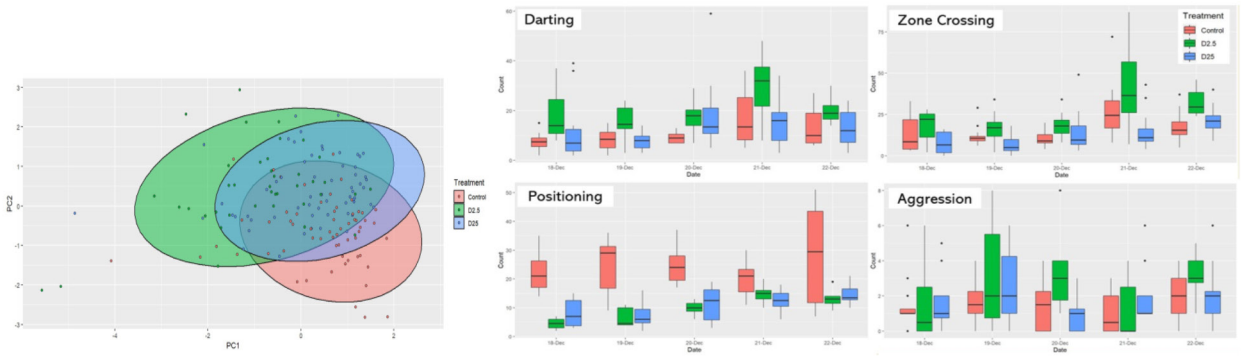


Figure 1. a) A Principal Component Analysis (PCA) plot (left), summarising the structure of the behaviour data when grouped according to treatment. b) Boxplots of each behaviour according to treatment group across the five days of analysis. Control shown in red, D2.5 shown in green, and D25 shown in blue.

CHRONIC EXPOSURE TO SUBLETHAL CONCENTRATION OF SAXITOXIN REDUCES ANTIOXIDANT ACTIVITY AND IMMUNITY IN ZEBRAFISH BUT DOES NOT AFFECT REPRODUCTIVE PARAMETERS

Md. Niamul Haque, Sang-Eun Nam, Young-Seok Han, Hyoung Sook Park and Jae-Sung Rhee*

Incheon National University
Incheon, Republic of Korea
E-mail: jsrhee@inu.ac.kr

Saxitoxin produced by dinoflagellates and cyanobacteria can be transferred to humans through intoxicated organisms such as fish, but limited research has addressed the adverse effects of this toxin on aquatic organisms. In this study, we measured the potential effects of a 90-day exposure to saxitoxin (0.1 or 1 μg·L – 1) on body weight and length, antioxidant defense system, immunity, sex hormones, and genes involved in associated key metabolic pathways in zebrafish (Danio rerio). Significant impairments in body weight and length were observed in response to 1 μg·L – 1 saxitoxin in both male and female zebrafish. A significant increase in the levels of malondialdehyde, together with decreased enzymatic activities of catalase and superoxide dismutase, was observed in fish of both sexes exposed to 1 μg·L – 1 saxitoxin, indicating the occurrence of lipid peroxidation and oxidative stress. Immune parameters such as alternative complement activity, lysozyme activity, and immunoglobulin content were also significantly reduced. However, exposure of male and female zebrafish to saxitoxin for 90 days did not significantly affect reproductive parameters such as the gonadosomatic index and plasma concentrations of vitellogenin, estradiol, and 11-keto testosterone. Transcriptional responses showed similar trends to those of the biochemical parameters, as genes involved in the antioxidant defense system and immunity were downregulated, whereas the transcription of genes related to reproductive metabolism showed no significant change upon treatment with 1 μg·L – 1 saxitoxin. Our findings indicate that long-term exposure to a sublethal concentration of saxitoxin can inhibit growth through induction of oxidative stress and immunosuppression, while the reproductive parameters of zebrafish are not a main target of this toxin at sublethal concentrations.

GOVERNANCE STRUCTURES AND PERFORMANCE INDICATORS OF TILAPIA POLES IN BRAZIL: A GLOBAL VALUE CHAIN APPROACH

Vinícius Souza Ribeiro* and Manoel Xavier Pedroza Filho

Federal Institute of Education, Science and Technology of Tocantins
Department of Natural Resources
Address: Campus Palmas - Quadra 310 Su, Lo 5, s/n - Plano Diretor Sul, Palmas – TO, Brasil, 77021-090
E-mail: vribeiro@ifto.edu.br

Tilapia is the most farmed fish in Brazil, its production continues to expand being consumed almost entirely by the domestic market. The recent drawback mechanism and environmental release laws for production in areas of the Amazon biome are two key elements in expanding and sustaining Brazil's exports. Based on the Global Value Chain (GVC) approach. This research aims to analyze two tilapia poles in Brazil with different governance structures.

First, we defined a series of indicators corresponding to the six dimensions of the GVC (i.e. input-output structure; governance; updating; local institutional context; industry stakeholders) capable of establishing a comparison between the poles. As many of the elements of GVC's analysis are essentially based on qualitative data, we use the fuzzy logic to consolidate these indicators in quantitative terms.

In the first trimester of 2019 we collected data from São Francisco Valley (Northeast) and West Paraná (South), the dataset totaled a sample of 362 production units. With complementary secondary data related to the input and processing industries, we analyze the governance structure of each pole. Finally, we calculated the indicators and aggregated them spatially.

Finally, we identified a greater proximity between the Northeast pole and the market model and the South pole with the hierarchical profile. Based on these indicators we identified some similarities and differences between the poles in different dimensions. These results open up the possibility, for example, of analyzing the causality between governance structures and performance indicators in the GVC approach.

GRADING STRATEGIES FOR MANGROVE CRABS

Mark Richards[1]

School of Chemical & Life Sciences, Nanyang Polytechnic[1]
180 Ang Mo Kio Ave 8
Singapore 569830
Mark_Richards@NYP.EDU.SG

Crustacean farmers, wholesalers, restaurant owners, and consumers can all benefit from a data-driven method to ascertain the meat quality of crustaceans. In South-East Asia's context, the mangrove crab, *Scylla serrata* is an important commercial seafood species. Mangrove crab is the basic ingredient of one of Singapore's most iconic dishes: the Chili Crab. In recent years, the supply of adult mangrove crabs has been adversely affected, both in terms of price and in quality. Soaring prices and the lack of good-quality crabs is causing supply to fall behind demand. These disruptions have placed a serious dampener on the business. It is important to be able to distinguish meaty crabs from lean crabs. The meat yield of individual crabs can vary from filled high-quality crabs to empty water-filled crabs. Only a portion of the catches, depending on season and geographical area, are of good quality. Crabs with high meat content have a higher value and can be sold live or cooked whole at restaurants. Medium quality crabs are generally limited to being used as raw material by the processing industry. A correct quality sorting of live crabs is essential to optimize this logistics. The current methods adopted by the industry to assess crabs such as "pressing carapace", "looking at shell color", "checking if claws are worn out" and "looking at moon phase to plan harvest time" are inadequate as they are very empirical. These methods often lead to errors in ascertaining whether a crab is suitable for the restaurant. A practical, portable, simple, rapid, and non-invasive technique to analyze crustacean nutritional condition has enormous potential to assist with the management of wild stocks, improve post-harvest survival and quality control, and manage brood stock as the aquaculture industry continues to expand to meet growing consumer demand. We evaluated several grading approaches for sorting Mangrove Crabs in this study. Particularly, the use of a portable Near Infrared Spectroscopy (NIRS) approach for grading mangrove crabs proved to be easy-to-use, rapid, and reliable.

EFFECTS OF INSECT MEAL AND INSECT OIL ON GROWTH PERFORMANCE, PROTEIN DIGESTIBILITY, AND RESPONSE TO CHALLENGE TEST WITH *Vibrio parahaemolyticus* IN WHITE SHRIMP (*Litopenaeus vannamei*)

Cameron S. Richards*, Romain Menard, Niti Chuchird, Piet Verstraete, and Franck Ducharne

Veolia China Holdings Limited
Hong Kong
China
E-mail: cameron.richards@veolia.com

There is a growing need to use alternative, sustainable protein ingredients in animal feeds to relieve mounting demands for traditional protein sources such as fish meal and soya meal. Insect meal is one such protein ingredient and has been shown to be a viable alternative in aquaculture feeds. This study aims to evaluate the impact of insect meal on the following parameters in white shrimp (*Litopenaeus vannamei*): 1. growth performance and indications on health (histology and microbial composition of the gut and hepatopancreas) starting with small shrimp (PL12), 2. Survival and anatomy on 1.5-2 g shrimp when challenged against *Vibrio parahaemolyticus*, and 3. protein digestibility on 3-5 g shrimp.

Five experimental groups, each with four replicates, were studied as follows: 2% Insect meal, 5% Insect meal, 10% Insect meal, 2% Insect meal + 2% Insect oil, and a control (without the inclusion of insect meal or insect oil),

After 45 days of feeding, shrimp fed 2% Insect meal +2% insect oil, and 10% Insect meal had a significantly higher average survival rate than other groups. At the same time, shrimp provided 2% Insect meal +2% Insect oil, 10% Insect meal, and 5% Insect meal had a significantly higher average body weight and lower feed conversion ratio than other groups. Regarding the immunological study, shrimp from all groups that provided insect meal had significantly higher immune responses (the total hemocyte count, phagocytosis activity, phenoloxidase activity, superoxide dismutase activity, and bactericidal activity) than the control group. Shrimp from all groups that provided insect meal had fewer *Vibrio spp* in the hepatopancreas and intestine of shrimp than other groups. Those shrimp also showed a significantly higher percentage survival and better condition of the hepatopancreas than the control group after being challenged with bacteria causing White faeces syndrome. And finally, after the 14 day digestibility trial, shrimps provided with 2% Insect meal +2% Insect oil had the best apparent digestibility of nutrients, including a percent dry matter digestibility, percent organic matter digestibility, percent protein digestibility, percent lipid digestibility, and percent nitrogen-free extract.

These results show that including insect meal up to 10% in a shrimp feed formulation can have a positive impact on growth and survival, and including both 2% insect meal and 2% insect oil improved the growth, survival and digibility in shrimp the most.

EFFECT OF PROTINOVA™ REPLACEMENT OF FISHMEAL IN THE DIETS ON GROWTH PERFORMANCE OF RAINBOW TROUT, *Oncorhynchus mykiss*, AND SURVIVAL AFTER CHALLENGE WITH *Aeromonas salmonicida* AND *Flavobacterium psychrophilum*

Andrew Richardson*, Maye Walraven, Giovanni Marco Cusimano and Simeon Deguara

Innovafeed
85, rue de Maubeuge, 75010 Paris, France
andrew.richardson@innovafeed.com

The expanding aquaculture industry is currently the major user of fish meal globally. Traditional sources of fishmeal cannot continue to sustainably meet the high demand and rapid growth rates of the aquaculture industry, whilst vegetable sources come with their own challenges, both environmental and nutritional. This has led to the continuing search for sustainable fish meal replacements. Insects, thanks to their ability to convert low-value biomass into high quality animal proteins and reintroduce them in the food chain, are emerging as an excellent natural and sustainable solution to address this challenge. ProtiNova™ is one such commercially available insect meal product, produced by Innovafeed from black soldier fly (*Hermetia illucens*) larvae, grown on sustainable co-products of the agricultural industry

A 12 week growth trial with 35g rainbow trout, *Onchorhynchus mykiss*, was carried out in fifteen 650L tanks in the RAS facilities of AquaBioTech Innovia (Malta). Five isonitrogenous and isocaloric diets containing 15, 11, 9, 7 and 5% fish meal and 0, 4, 6, 8 and 10% ProtiNova™ respectively, were tested in triplicate. Feeding was carried out to satiation and the average temperature during the trial was 16.7°C. After the growth trial, fish from treatments containing either 0 or 10% ProtiNova™ were challenged with *Aeromonas salmonicida* and *Flavobacterium psychrophilum*.

The feeding trial found that including up to 10% ProtiNova™ i.e. replacing up to 67% of fish meal, did not significantly ($p < 0.05$) affect FCR, SGR, TGC or SFR (Figure 1). Survival was at least 98% in all treatments. At the end of the trial period, there was no significant effects of ProtiNova™ inclusion level on the fish condition factor (K), hepatosomatic index (HSI) or viscerosomatic index (VSI). There were no significant differences in mortality following challenge with the two pathogens (Figure 2).

This work demonstrated that ProtiNova™ can perform as well as a high quality fishmeal in commercial trout diets.

Figure 1: Growth Performance

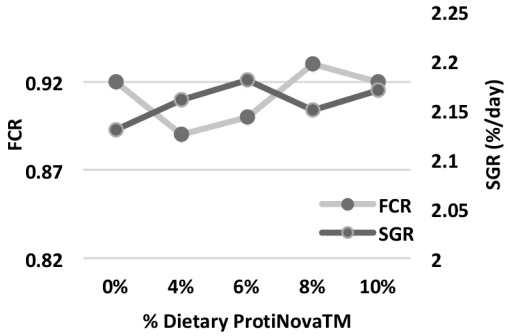
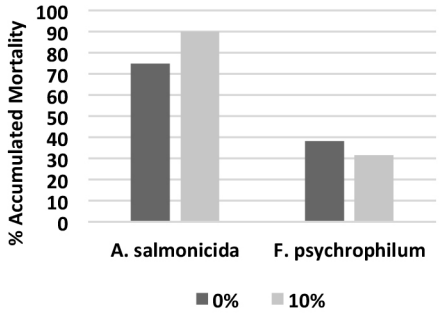


Figure 2: % Mortality after Challenge



REPLACEMENT OF FISHMEAL BY CANOLA PROTEIN CONCENTRATE IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei* DIETS ON GROWTH PERFORMANCE AND DIGESTIBILITY

William W. Riley* and Orapint Jintasataporn

Botaneco ®, Inc., Calgary, Alberta, Canada
wmriley54@yahoo.com

Canola protein concentrate (CPC: 78.9% crude protein (CP)) was added to isocaloric, isonitrogenous White Leg shrimp (*Litopenaeus vannamei)* diets as a replacement for 65% CP fishmeal at 0, 5, 10 or 20% inclusion in the final formulation. Juvenile shrimp (~2 gm) were stocked at 80 shrimp/m^3 (25 shrimp/tank) and fed 3 times daily at 3-4% bodyweight for 8 weeks. The study was carried out in 24 aquarium (4 treatments*6 replicates), each with 240 L capacity and containing 150 L of 10-15 ppt saline water, pH 7.7-8.2, DO>5 ppm, temperature 27-30° C, total ammonia <1.0 ppm. Aquarium water was batch-exchanged 15-20% every 2 days. At 8 weeks, the growth of shrimp fed 5-20% CPC was higher ($p<0.05$) than the growth of control shrimp fed 0% CPC. At 6 and 8 weeks, weight gain and specific growth rate were higher ($p<0.05$) at 10% and 20% CPC than at 0% CPC, while shrimp fed 5% CPC did not differ from the control group. Feed intake did not differ ($p>0.05$) between treatments, but the feed conversion ratios (FCRs) were lower ($p<0.05$) in 5-20% CPC-fed shrimp than in control shrimp at 2, 4, and 6 weeks. At 8 weeks, the FCRs were equivalent ($p>0.05$), as were survival rates ($p>0.05$). CPC was highly digestible: dry matter=74.10±3.55%, protein=86.00±2.70%, lipid=95.58±0.24%, calcium=40.55±15.05%, phosphorus=95.82±1.88%, energy=78.97+2.29%. The haemocyte count and hemolymph protein did not differ between treatments ($p>0.05$), whereas phenoloxidase activity was higher in shrimp fed 5-20% CPC ($p<0.05$) relative to the control. The moisture, dry matter, ash, calcium, phosphorus, lipid, and fiber contents of shrimp for all treatments were similar ($p>0.05$), while the protein content of shrimp fed 10% and 20% CPC was higher ($p<0.05$) than the protein content of the control shrimp. After 8 weeks, 10 shrimp from 3 replicates/treatment were moved to 100 L challenge test aquaria. Shrimp were subcutaneously injected with *Vibrio parahaemolyticus* (EMS) at 0.1 ml/shrimp. Shrimp survival during 7 days of infection did not differ among the 4 treatments from days 0 to 6 ($p>0.05$), but at day 7, the shrimp fed 20% CPC had a higher survival rate ($p<0.05$) than did shrimp fed 0% and 5% CPC. Hemocyte counts, hemolymph protein, and phenoloxidase activity were the same ($p<0.05$) as were the vibrio counts in the hemolymph ($p<0.05$). However, vibrio counts in the hepatopancreas and intestine were lower in shrimp fed 5-20% CPC ($p<0.05$) compared to control shrimp. CPC promoted a reduction in hepatopancreatic and intestinal vibrio counts under conditions of vibriosis due to improved health and immunity. CPC can replace fishmeal in the diet of Pacific White Leg shrimp (*Litopenaeus vannamei*) at inclusion rates from 5-20%. CPC has high protein and lipid digestibility, and it was shown to promote growth performance, immunity and disease resistance against *Vibrio parahaemolyticus* EMS at an inclusion rate as high as 20%.

BUSINESS STRATEGY FOR TOFORD PLASTIC MANUFACTURING CORPORATION TO ENTER INDONESIAN AQUACULTURE MARKET

Patih Fathin Rizal*

School of Business and Management
Institut Teknologi Bandung
Patih_rizal@sbm-itb.ac.id

Indonesian fisheries industry continues increasing over years, slightly behind China as the largest fish produces in the world. Aquaculture is the fastest growing food production sector in the world as well as one of the potential sectors in fisheries. Every year Indonesian government set higher growing rate target for fish consumption per capita around 1-2% (Figure 1). Potential area for marine aquaculture in Indonesia is around 12 million hectares, but only 0.2 million hectares being used (Table 1). For marine aquaculture, HDPE100 material is the best suited for cages raw material because of the durability, corrosion resistance, and low capital investment.

In analyzing Indonesian aquaculture market, some analysis is made such as PESTEL, Porter's 5 Forces, STP, Marketing Mix, Value Chain, and SWOT. The result showed that Indonesian aquaculture market still in the beginning phase proven by limited regulation for aquaculture, most of farmers are smallholders with simple technology and limited area, low variety of fishes being raised, lots of potential area haven't been grabbed by farmers, lots of global competitor but only few of them have presence in Indonesia market.

Based on strategic formulation TOWS and Ansoff Matrix, there are 3 solutions for Toford which are having representative agent in Indonesia, become turnkey solution provider, and building pipe factory. Representative agents in Indonesia will help Toford to take care some administrations, working on marketing projects, building up relationship and network, attending and participating in aquaculture business exhibition, expos, and conferences. Become turnkey solution provider by having new company that cooperate with other companies will have impact in medium and long term in strengthen competitive rivalry in the market. Building pipe factory aim to establish a subsidiary factory overtime in Indonesia in order to become more efficient in terms of logistics and network issues.

FIGURE 1. Fish consumption per capita in Indonesia 2015-2019

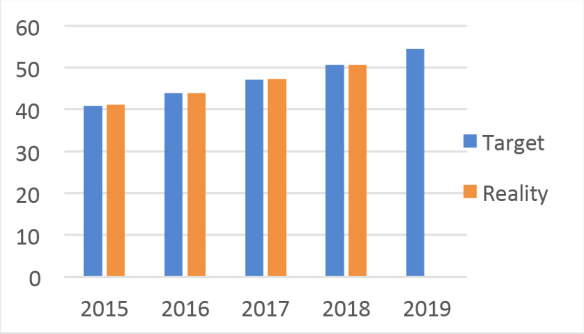


TABLE 1. Aquaculture potential area in Indonesia, 2015

No.	Type of Aquaculture	Potential Area (ha)	Usage (ha)
1	Marine Culture	12,123,383	281,474
2	Brackish water Pond	2,964,331	667,083
3	Freshwater Pond	2,830,540	30,216

UTILISING AQUACULTURE ZONE POLICIES FOR THE INTEGRATED MANAGEMENT OF SOUTH AUSTRALIAN MARINE AQUACULTURE DEVELOPMENT

Shane Roberts*, Jade Davison, Gavin Begg, Matthew Hoare, Lauren Holmes, Kate Rodda, and Mehdi Doroudi

Department of Primary Industries and Regions
Fisheries and Aquaculture Division
2 Hamra Avenue
West Beach, South Australia 5024
shane.roberts@sa.gov.au

South Australia's aquaculture industry generates nearly 50% of the State's seafood economic output, and was worth $229m in 2019/20. The *Aquaculture Act 2001* provides the State government regulatory agency, the Department of Primary Industries and Regions, with the capacity to regulate aquaculture development in South Australia. A fundamental feature of this legislative framework is the capacity to establish dedicated aquaculture zones in State waters. These zones represent a unique marine spatial planning approach for access to a shared resource; one that provides certainty to all groups, including fisheries, marine parks, tourism, shipping, and indigenous communities. The aquaculture zone process illustrates how dedicated legislation can effectively encompass integrated ocean management for the development of this industry sector.

Twelve aquaculture zone policies are prescribed in South Australia. These zone policies occupy approximately 7% of State waters. More than half (52%) of this area is allocated to aquaculture exclusion zones, where no aquaculture activity is permitted. The remaining 48% is set aside to allow aquaculture to occur, with generally 5-10% allocated at any one time.

The current review process for the *Aquaculture (Zones—Lower Eyre Peninsula) Policy 2013* will be detailed as a case study. The Policy covers one of the most diverse and in-demand aquaculture areas in South Australia, in addition to competing marine interests such as recreational, indigenous and commercial fisheries, shipping, tourism, conservation and marine parks. Permitted groups of species within the Policy includes bivalve molluscs, abalone, echinoderms, algae (seaweed), southern bluefin tuna, yellowtail kingfish, and other finfish. The Policy provides for adaptive management, and includes carrying capacity for supplementary fed species based on hydrodynamic modelling of nutrient emissions where nutrient offsets can be considered. A second case study will outline the review of the *Aquaculture (Zones—Eastern Spencer Gulf) Policy 2005*, focussing on the creation of two new intertidal aquaculture zones aimed at fostering Aboriginal and seaweed aquaculture following consultation with the Narungga Nations Aboriginal Corporation (NNAC) and the Point Pearce Aboriginal Corporation (PPAC).

The effective and efficient process undertaken to design, consult with stakeholders, finalise and implement an aquaculture zone policy, and the opportunities for aquaculture in South Australia will be described.

HATCHERYMATCH: AN AUTOMATIC MARINE FISH HATCHERY WITH INNOVATIVE WATER RECIRCULATION TECHNOLOGIES

Davide Sorella, Robert Davies, Iven Bonnici, Hugo Ortega, Jui-Che Chiang, Yulei Zhang, Vasilis Valdramidis, Shane Hunter, Freya Robinson*

AquaBioTech Group, Research Development and Innovation, Naggar Street, Targa Gap, Mosta, Malta
Email: fmr@aquabt.com

The development of marine aquaculture requires the reliable supply of fish eggs and larvae in desirable quality and quantity from land-based hatcheries, instead of relying heavily on wild-caught fry, as a majority of marine fish stocks are under pressure, fully exploited or overfished. Driven by this high pressure and demand, partners from Malta and China have come together to solve the common problems in marine hatcheries, including low controllability, high labor dependence, and compromised water quality. The HatcheryMatch project will innovate the common hatchery practices, changing from empiricism-based operation to data-based standardization, in order to achieve a reliable production of marine fry and follow the global IoT development.

The HatcheryMatch project aims to develop four new automation technologies which will improve water quality, embryonic categorization, and feeding and in turn reduce stress and losses during the hatchery phase of fish lifecycles. Prototypes for a live feed system, hydro cavitation disinfection device, tank bottom cleaning system and a digital, embryonic image analyzer are being developed. A pilot recirculating hatchery has been designed integrating these new technologies to reduce stress on marine stocks, lower labor dependence and increase seeding rate. The hatchery will be installed at facilities in Qingdao China and will be used to produce turbot for local production as well as future research activities.

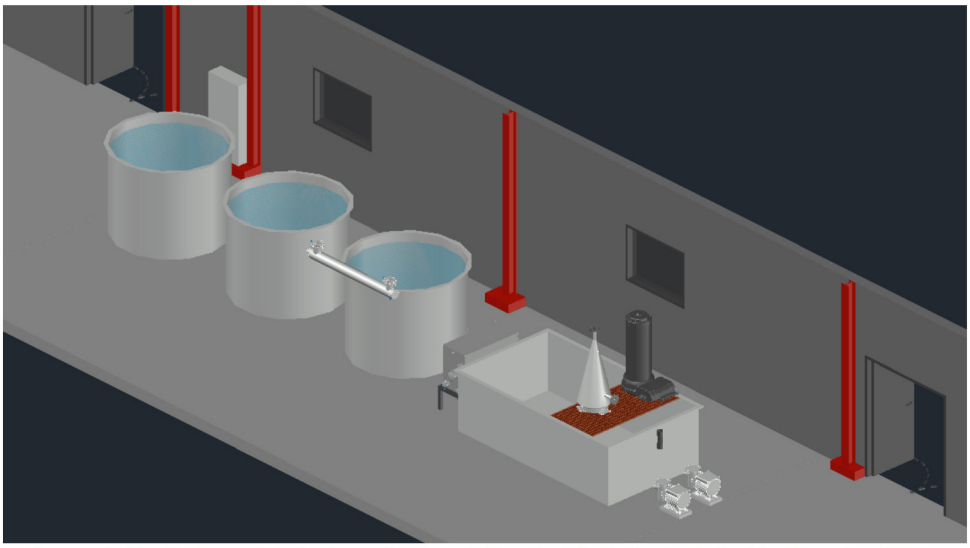


Figure 1. Preliminary 3D design of recirculating facility to be installed in Qingdao China.

COMPARATIVE PROTEOMICS ON THE EFFECT OF CREATINE SUPPLEMENTATION ON *European seabass*

Pedro M. Rodrigues*, Denise Schrama, Cláudia Raposo de Magalhães, Marco Cerqueira

University of Algarve
CCMAR, Centre of Marine Sciences
Campus de Gambelas
8005-139 Faro, Portugal
pmrodrig@ualg.pt

Considerable amounts of high-quality proteins, essential amino acids and polyunsaturated fatty acids are present in fish. Its consumption increases continuously, and aquaculture is needed to increase rapidly. Allergies to fish show prevalence's reaching 8% in case of children. A stable white muscle protein, with a low molecular weight of about 12 kDa, known as β-parvalbumin is responsible for 95% of the allergic responses in humans. This protein has an acidic isoelectric point (4.5-5.0), binds ions like calcium and magnesium and is involved in the relaxation and contraction of the muscle. Studies showed that creatine supplementation might modulate the expression of parvalbumin. The present study was conducted with the objective of lowering the expression of β-parvalbumin in fish muscle to produce a low allergenic fish species.

Twenty *European seabass* were reared in triplicate (Initial body weight of 186 ± 0,8 g) for 91 days in 500L conical tanks exposed to natural environmental and photoperiod conditions at the Ramalhete experimental station. Fish were fed twice a day till satiety with experimental diets differing in creatine concentrations (0, 2, 5 and 8%). Muscle samples were taken during sampling after a lethal dose of 2-phenoxyethanol and frozen at -80°C till further analysis. Comparative proteomics was performed to analyze parvalbumin expression in the muscle. Biochemical characterization of fish was performed during 72 hours after slaughter.

Zootechnical results show a similar growth with the different tested diets, body weight doubled during the experimental period. Comparative proteomics of the total protein extract showed a total of 17 significantly different spots, which were identified by LC-MS/MS. The parvalbumin protein shows no significant modification in its expression. Identified proteins are shown in a protein-protein interaction analysis resultant from Cytoscape (Fig. 1).

Creatine enrichment up to 8% does not seem to have an effect in the allergenic potential of fish.

This study received Portuguese national funds from FCT - Foundation for Science and Technology through project UID/Multi/04326/2019 and Mar2020 (4107IDNAD50308.18) project 16-02-01-FMP-0014 – ALLYFISH.

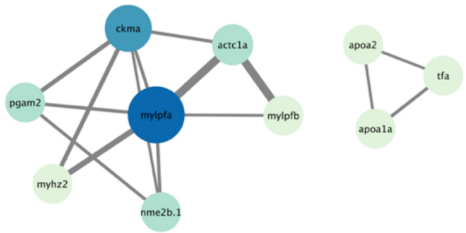


Fig.1 – Protein-protein interaction analysis of the differential proteins obtained between control and creatine supplemented groups.

LIVER MULTIOMICS ANALYSIS REVEALS REGULATION BY DIFFERENT PRO-SURVIVAL PATHWAYS IN NET-HANDLED GILTHEAD SEABREAM

Pedro M. Rodrigues*, Cláudia Raposo de Magalhães, Gavin Blackburn, Phil Whitfield, Ana Paula Farinha, Raquel Carrilho, Denise Schrama, Marco Cerqueira

Centre of Marine Sciences (CCMAR)
Universidade do Algarve, Campus de Gambelas, 8005-185, Faro, Portugal
pmrodrig@ualg.pt

Aquaculture is crucial to keep pace with the increasing fish demand, however, the industry faces its own sustainability challenges to attain such endeavor. Managing fish stress is crucial to avoid negative impacts on fish health/welfare, and ultimately, on the aquaculture productivity. In this study, an integrated proteomics and metabolomics characterization of the fish liver, a central organ during stress adaptation, was performed to provide a holistic assessment of the molecular stress response at different organizational levels. Widen our understanding into the physiological changes occurring in the fish organism during stress exposure/adaptation can leverage the industry with the scientific knowledge for forthcoming species-specific welfare assessment protocols.

Sparus aurata was exposed to a net handling challenge, along with fish reared under optimal conditions, as control. Nets were designed for the purpose, fitted inside the tanks and the fish were lifted and air-exposed for 1 min four times a week, during 1 ½ months. Extracts from liver samples were prepared for further proteomics and metabolomics analysis by LC-MS/MS. Pairwise comparisons among identified proteins and metabolites, were achieved by a student's t-test, $p < 0.05$; FDR controlled at 0.05. Integrative analysis of both data modalities was achieved by the Data Integration Analysis for Biomarker discovery using Latent components (DIABLO) algorithm.

A total of 326 proteins and 40 metabolites were differentially regulated between challenged and control fish, mostly implicated in cellular processes and metabolism related KEGG pathways, namely amino acid and carbohydrate metabolism, inflammatory response, protein folding processes, among others. A STITCH protein-metabolism interaction network revealed a coordinated response of the hepatic system to the challenge. Enrichment analysis of the variables selected by the DIABLO algorithm (Fig.1) revealed up-regulation of different pro-survival pathways such as the unfolded protein response (UPR), endocytosis, mTORC1 pathway, among others. These results shed light on the dynamics and extent of this species' metabolic reprogramming under chronic stress, supporting future studies on stress markers' discovery and fish welfare research.

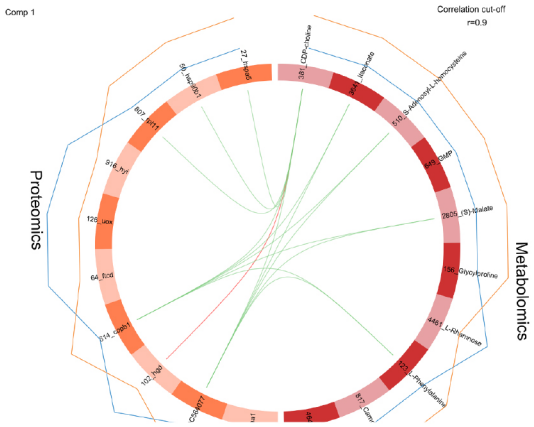


Fig.1 - Integrated proteomics and metabolomics analysis, performed with DIABLO, of the liver of gilthead seabream submitted to net-handling challenge. Circos plots representing the Pearson correlation (correlation cutoff = 0.9) between the ten most discriminatory proteins and metabolites selected by the first component of the DIABLO model.

DEVELOPMENT OF A SNP PANEL FOR KINSHIP AND PARENTAGE ANALYSIS OF TAMBAQUI (*Colossoma macropomum*)

Alexandre Rodrigues Caetano *, Noéliton Teixeira de Araújo Junior, Eduardo Sousa Varela, Michel Eduardo. B. Yamagishi, Patrícia Ianella.

Embrapa Recursos Genéticos e Biotecnologia
Brasília-DF / Brazil
alexandre.caetano@embrapa.br

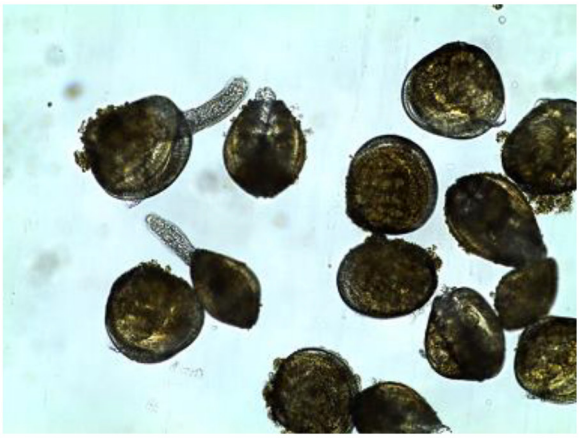
Tambaqui (*Colossoma macropomum*) is the South American freshwater fish with the highest aquaculture production levels in Brazil. Current production exceeds 150,000mt/year and accounts for close to 30% of all finfish cultured domestically. The species is at early stages of domestication, with initial efforts to stablish genetic improvement programs recently set underway. High fecundity rates (>300.000 larvae per spawning) associated with unsophisticated processes to establish and manage broodstocks have the potential to rapidly reduce effective sizes of captive populations. Therefore, practical contemporary tools to minimize inbreeding rates resulting from mattings between unknown close relatives are highly necessary. A database containing >2.5million SNPs identified in a previous high-coverage genome-wide sequencing study of pooled samples with complexity reduction for the species was analyzed to identify candidate SNPs. A total of 2,040 markers with estimated 0.45<MAF<0.5 and zero observed SNPs in flanking regions was identified. Additional filters to select 144 SNPs evenly spaced across all 27 chromosomes were applied. SNPType® assays were designed and used to test the 140 samples originally used for SNP discovery, including closely related species commonly used for hybrid production (Tambaqui=63, Pacu (*Piaractus mesopotamicus*)=23, Pirapitinga (*Piaractus brachypomus*)=48 and six known hybrids), with a Fluidigm EP1® system. Technical replicates were carried out to evaluate genotyping consistency. The best 96 markers were selected considering call rate, HWE and observed MAF. None of the tested markers were observed to be polymorphic in non-Tambaqui samples. Additional samples randomly collected from six different commercial broodstocks (n=188) were tested. Average observed MAF and Call Rate were 0.37 and 0.99, respectively. Estimated combined probability of exclusion was 9.9999999E-01. Kinship analysis of tested samples showed high levels of family-structuring within and between broodstocks. Management of broodstocks for production of fry for both grow-out, and broodstock establishment and replacement, considering obtained kinship estimates, will generate gains in productivity, minimizing losses caused by deleterious mutations and inbreeding depression.

IMPROVED SEEDSTOCK PRODUCTION OF QUAHOG (*Mercenaria mercenaria*) AND AMERICAN OYSTER (*Crassostrea virginica*) FOR REMOTE SETTING

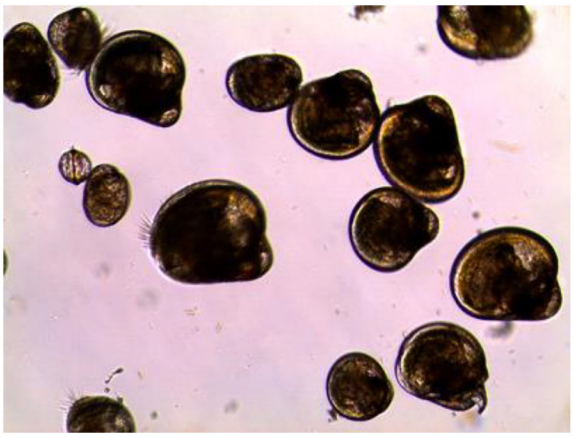
Jesse Ronquillo* and Audrie-Jo McConkey

Faculty of Trades and Technical Programs
North Island College
1565 South Dogwood Street
Campbell River, BC, Canada V9W8C1
Jesse.Ronquillo@nic.bc.ca

Quahog or hard clam (*Mercenaria mercenaria*) and American oyster (*Crassostrea virginica*) are important bivalve mollusks in North America. The demand for these species is strong but the traditional sources of supply are low and some culture sites have suffered catastrophes. In partnership with Gulf Aquaculture Association (GAA) based in Northumberland Strait, Nova Scotia, Canada, we devised less costly and innovative procedures in the spawning and larval rearing of quahog and American oyster to produce the needed seedstock. Selected species of microalgae rich in highly unsaturated fatty acids (HUFA) were mass-produced continuously in an inventive way to provide abundant and diversified source of live feeds to larvae until they reach pediveliger or eyed-larvae that were used by shellfish growers in Northumberland Strait for remote setting. About 100 million of quahog seedstock and more than 500 million pediveligers or eyed-larvae were produced for remote setting and grow-out operations of shellfish growers in Northumberland Strait. The preliminary research outcomes include: (1) Design and construction of a prototype closed-culture system for controlled quahog and oyster spawning; (2) Successful induction of broodstock spawning based on environmental manipulation among desiccation, temperature shifts, UV-filtered water stimulation, microalgal addition, and various combinations of the above; and (3) Successful development of inexpensive system for efficient and continuous culture of microalgae for feeding the clam and oyster seedstock.



Quahog pediveliger



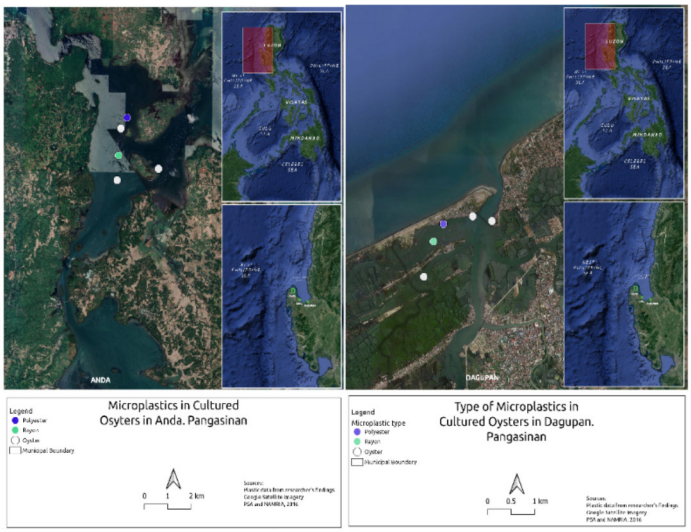
American oyster pediveliger

MICROPLASTICS IN OYSTER, SURFACE WATER AND SEDIMENT IN SELECTED MARICULTURE AREAS IN PANGASINAN, PHILIPPINES

Reginor Lyzza A. Roque[1,2], Lorenz J. Fajardo, Cesar V. Ortinero

[1]College of Fisheries, Central Luzon State University
[2]College of Fisheries, Don Mariano Marcos Memorial State University
reginorlyzzaroque@gmail.com

Microplastics have been receiving environmental concern in recent years because of the possibility of toxin being ingested by aquatic organisms. Thus this study was conducted to determine the presence of microplastics in aquaculture areas in Anda and Dagupan City, Pangasinan. It quantified and classified the types of microplastics according to polymer type, shape, size, and color. Three types of samples were collected: oyster tissue, surface water, and sediment. Samples were viewed under stereomicroscope for isolation and characterization and a confirmatory test through micro-Fourier-Transform Infrared Spectroscopy (μFTIR). Oyster samples from both Anda and Dagupan City were confirmed to contain fibers of rayon and polyester with sizes ranging from 0.90 mm to 2.21 mm with black and translucent colors, respectively. The average microplastic concentration in oyster samples from Anda is 113 ± 161 pcs/kg, and 541 ± 350 pcs/kg pcs/kg in Dagupan City. Surface water samples from Anda and Dagupan City were confirmed to contain fibers and fragments of rayon, polyester, and polypropylene with sizes ranging from 0.011 mm to 1.37 mm for fibers and 0.002 mm^2 to 1.780 mm^2 for fragments. Concentration of microplastics in surface water was highest at Dagupan City where oyster is being cultured with 77.6 ± 79.1. Surface water samples were confirmed to contain non-plastic pollutants such as cellulose and alpha-cellulose. A few sediment samples from Anda and Dagupan City were confirmed to contain fragments of rayon and low-density polyethylene with sizes ranging from 0.305 mm^2 to 1.062 mm^2. The microplastic concentrations from both areas were 1pc/kg sediments. It was concluded that while all microplastic concentrations on all sample types were relatively lower than those found in literature, microplastic pollution is still a serious concern in Anda and Dagupan City that needs to be addressed immediately to mitigate health risks and ensure the sustainable future of aquaculture in the said areas.



Type of microplastics in cultured oysters in Anda and Dagupan City, Pangasinan

BEYOND BINDING – A DEEPER LOOK INTO MYCOTOXINS MITIGATION STRATEGIES FOR AQUATIC SPECIES

Roy Rosen*, Michele Muccio, Barbara Doupovec and Dian Schatzmayr

BIOMIN Research Centre
Technopark 1, 3430
Tulln an der Donau, Austria
roy.rosen@biomin.net

Mycotoxin related pathologies in aquatic species have been constantly and globally described in scientific literature since the 1960's.

In recent years a shift towards increased use of plant origin materials and reduction of the animal components in aquatic feeds created a 'more-than-ever' risk of mycotoxicoses in aquaculture systems, which is now a globally growing and fast developing industry.

As their chemical structures vary considerably, mycotoxins cannot be classified as one group according to their mode of action, toxicology or metabolism (Gonçalves, et al., 2019). Moreover, the chemical and thermal stability of these molecules allows them to pass through the feed manufacturing procedures without reducing their toxic potential.

These characteristics together with the increasing wide range of cultured aquatic species create great challenges in development of modern mycotoxin mitigation strategies for aquaculture.

Aflatoxins (AF) were the first and most described mycotoxins in aquaculture. AF, to various extent, can be effectively adsorbed to suitable binders due to their planar molecular structure. The capacity, selectivity and specificity of the toxin binder are the main differentiating factors distinguishing these compounds from one another.

Other toxin groups cannot be bound in this manner and require different detoxification solutions such as biotransformation. Among these are trichothecenes (such as T-2 toxin and deoxynivalenol), zearalenone, and fumonisins (FUM), the last being one of the most commonly found mycotoxins.

FUM pose a major global concern, particularly in the Asian and Latin American regions where their prevalence in aquatic raw materials and compound feeds reaches levels of over 80% (Biomin , 2019). Li et al. (1994) described fumonisin B1 (FB1) as causing mortality in fish either by direct tissue damage, or by immunosuppression that results in higher sensitivity to infection. Chronic effects of FB1 include a decrease in body weight gain changes to haematological blood parameters (Matejova, et al., 2017), decreased feed consumption, feed efficiency ratio, and impaired sphingolipid metabolism.

Biotransformation by enzymes is one of the most modern forms of mycotoxin detoxification. Other biotransforming components include specific yeast and bacterial strains, all of these have been proved to effectively detoxify specific mycotoxins.

In addition to toxins binding and biotransformation, specific plant and algal bioprotective compounds and extracts designed to support immunologic and metabolic processes demonstrated abilities to restore performance parameters when combined in the anti-toxin treatment.

The ubiquity of mycotoxins and their toxic potential to animal and humans calls for an evidence-based preventive solution. Important aspects such as antibiotic reduction, animal welfare and sustainable-economic aquaculture system all can be directly improved by choosing and applying the correct mycotoxin mitigation strategy.

References

Biomin , 2019. *Biomin Mycotoxin Survey Report: H1 2019,* Getzersdorf: BIOMIN Holding GmbH.

Gonçalves, R., Muccio, M. & Mueller, A., 2019. *Mycotoxins In Aquaculture*. Sheffield: 5m Publishing Ltd.

Li , M. H., Raverty, S. A. & Robinson, E. H., 1994. Effects of Dietary Mycotoxins Produced by the Mold Fusarium moniliforme on Channel Catfish Ictalurus punctatus. *Journal of world aquaculture scoity,* 25(4), pp. 512-516.

Matejova, I. et al., 2017. Impact of Mycotoxins on Aquaculture Fish Species: A Review. *JOURNAL OF THE WORLD AQUACULTURE SOCIETY,* 48(2), pp. 186-200.

ISOLATION AND IDENTIFICATION OF AAPTAMINOID FROM *Aaptos aaptos* AND ITS POTENTIAL USE IN VIBRIOSIS PREVENTION

Rosmiati*, Andi Tenriulo, Nurhidayah, Emma Suryati, and Andi Parenrengi

Research Institute for Coastal Aquaculture and Fisheries Extention
Ministry of Marine Affairs and Fisheries, Indonesia
Jl. Makmur Dg.Sitakka No.129 Maros, South Sulawesi, Indonesia
emirosmiati@yahoo.com

Aaptos aaptos was well known to contain aaptaminoid used as natural antibacterial source without side effect. This research aims to get bioactive compounds of *Aaptos aaptos* butanol extract which were able to inhibit the growth of *Vibrio harveyi* and to evaluate the efficacy of the compound in prevention of *V.harveyi* infection.

Metabolite secondary extraction used maseration method, while isolation and identification of aaptaminoid used column chromatography and spectroscopy method as well as comparison to the published data. The antibacterial test against *V.harveyi* used agar difussion method with *paper disc*. Evaluation of the active compound in Vibriosis prevention used experimental method with 4 treatments. Each treatment used *L.vannamei* of 10 ind./aquarium. Dosages of the active compound used namely; A. 0 µg/g bb, B.0,67 µg/g bb, C. 25 µg/g bb, and D. 50 µg/g bb. Injection of 100 µL of bioactive compound at each dosage was carried out at the initial experiment and at 14 days of rearing time the tested shrimps were challenged with *V. harveyi* at the density of 10^7 CFU/mL The rearing of shrimps was continued for 7 days. Finding showed that the infection on shrimps started on 23 hours post injection of *V,harveyi* indicated by reddish colour of rostrum, body, pereopoda, pleopoda, and telson. The shrimps on the treatment C and D were recovered from the infection started on the day-4 post infection exhibited by the normal rostrum, body, pereopoda, pleopoda, and telson. The highest survival rate post infection was obtained on treatment D (50%) and that of treatment C was 25 %. In turn, shrimps on treatment B and A did not show any survival rate. It indicated that aaptaminoid at the dosage ≥25 µg/g bb can be developed as an antibacterial agent for Vibriosis prevention.

*Table 4. Antibacterial activity of compound (**1-5**) against Vibrio harveyi*

Compounds (code)	*Inhibition zone*
Aaptamine	12 ± 0.3
9- demetylaaptamine	25 ± 0.1
4-*N*-metylaaptamine	20 ± 0.1
9-demetyloksiaaptamine	24 ± 0.3
9-metoxyaaptamine	13 ± 0.1

Figure 1. *Morphology change of shrimp 1a, 2a and 3a (at infection time); Pereopoda (Ib), rostrum (2b) and telson (3b) backed to normal at 144 hours post infection.*

DOSE - RESPONSE EFFECT OF A BLEND OF ORGANIC ACIDS AND NATURE-IDENTICAL COMPOUNDS ON EUROPEAN SEABASS (*Dicentrarchus labrax* L.)

Barbara Rossi*, Jorge Dias, Andrea Piva, and Ester Grilli

*Vetagro S.p.A., via Porro 2 – 42124 Reggio Emilia (Italy)
barbara.rossi@vetagro.com

Antibiotics have been used in aquafeeds not only to mitigate infectious diseases, but also to boost growth performance. The rise of antibiotic resistance is a growing problem for aquatic pathogens as much as for terrestrial livestock farming, so there is the need to find potential alternatives. Organic acids (OA) and botanicals are widely used in terrestrial animals and they gained an increasing interest also in aquaculture, but to the best of our knowledge available data in aquaculture are very limited. The aim of the study was to determine the effect of AviPlus®Aqua (Vetagro SpA), a blend of OA (sorbic and citric acid) and nature-identical compounds (NIC, thymol and vanillin), on European seabass (*Dicentrarchus labrax* L.) growth performance.

Four hundred and eighty European seabass specimens of an average weight of 62 g were randomly distributed into 12 tanks (40 fish per tank) at SPAROS Lda (Portugal). The following 4 diets were tested in triplicate tanks: 1) control (CTR), 2) AviPlus®Aqua 1000 ppm, 3) AviPlus®Aqua 1500 ppm and 4) AviPlus®Aqua 2000 ppm. Fish were fed for 71 days and zootechnical parameters (final body weight – FBW; feed conversion ratio – FCR; specific growth rate – SGR; protein efficiency ratio – PER; feed intake - FI) were measured after 29 and 71 days. Data were analyzed with one-way ANOVA.

After 29 days of feeding, growth performance was not affected by dietary treatments. At the end of the trial (day 71), fish fed with AviPlus®Aqua at 2000 ppm showed a higher FBW than those fed the other diets (P<0.05). FCR and SGR were improved in a dose-dependent manner (Fig. 1). AviPlus®Aqua 2000 ppm group showed a significantly higher PER compared to the other diets.

In conclusion, European seabass fed a diet supplemented with 2000 ppm AviPlus®Aqua showed a significant improvement of overall zootechnical performance criteria (FBW, SGR, FCR and PER), highlighting the potential of this feed additive as promoter of growth performance in seabass farming.

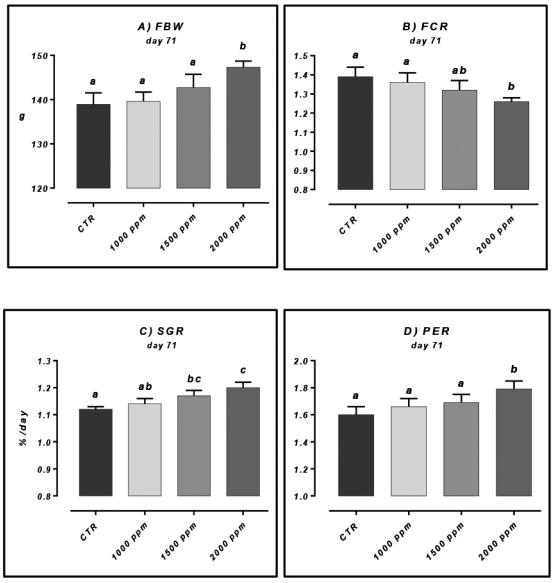


Figure 1: Effect of AviPlus®Aqua on (A) FBW, (B) FCR, (C) SGR and (D) PER in European seabass. Values are represented as mean ± SD. Different letters indicate significant difference (P < 0.05)

ANTIMICROBIAL ACTIVITY OF DIFFERENT ORGANIC ACIDS AGAINST *Vibrio* spp. *IN VITRO*

Barbara Rossi*, Maria Angéles Esteban, José María García-Beltrán, Alberto Cuesta, Andrea Piva, and Ester Grilli

*Vetagro S.p.A., via Porro 2 – 42124 Reggio Emilia (Italy)
barbara.rossi@vetagro.com

Aquaculture is a fast-growing sector and one of the main challenges is represented by disease outbreaks like Vibriosis, that is considered a significant problem for several aquatic species. In the context of an increasing demand for responsible and sustainable aquaculture, the study of compounds with antimicrobial properties alternative to antibiotics, such as organic acids (OA), is receiving a growing attention. The aim of this study was to screen *in vitro* the antimicrobial activity of 10 organic acids (OA) against 2 *Vibrio* species, *V. harveyi* and *V. anguillarum*. The OA tested were short-chain fatty acids (formic [For], propionic [Pro], butyric [But] acids), medium-chain fatty acids (hexanoic [Hex], octanoic [Oct], decanoic [Dec] and dodecanoic [Dod] acid), and others like sorbic (Sor), benzoic (Ben) and citric (Cit) acid.

V. harveyi, isolated from farmed gilthead seabream, and *V. anguillarum* (ATCC 19264) were cultured at 25°C in tryptic soy broth (TSB) supplemented with 1.5% NaCl and used as inoculums. The antimicrobial activity was evaluated after 24h incubation with microdilution method in TSB in which the substances were dissolved at the following concentrations: 7,5, 3.75, 1.88, 0.94, 0.47, 0.23, 0.12 mM for Oct, Dec and Dod and 100, 50, 25, 12.5, 6.25, 3.13 mM for the others. Control strains were incubated with TSB only. The analysis was performed on 96-wells microtiter plates with 10^5 cfu/mL and the growth inhibition was evaluated by absorbance measurement at 620 nm after incubation.

Dod showed the lowest MIC against *V. harveyi* (0.94 mM) and *V. anguillarum* (0.94 mM) followed by Oct and Dec (0.94 mM against *V. harveyi* and 1.88 mM against *V. anguillarum*). Cit decreased the growth of *V. harveyi* at 100 mM whereas no inhibition was observed for *V. anguillarum* at the tested concentrations. Pro, But and For were not effective even at the highest dose tested. Hex, Sor and Ben showed a MIC value of 50 mM against *V. harveyi* whereas they inhibited *V. anguillarum* at 12.5 mM, 25 mM and 50 mM respectively.

All the results are reported in Fig. 1. In conclusion, these findings showed the high antimicrobial power of Sor, Ben and medium-chain fatty acids against 2 *Vibrio* spp., highlighting the interest of their use in aquafeeds.

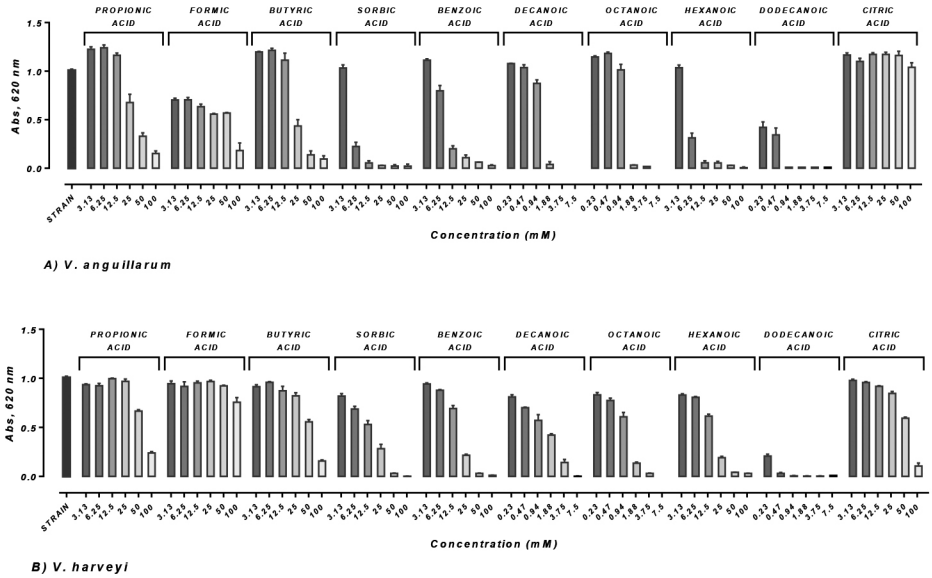


Figure 1: Growth of *V. anguillarum* (A) and *V. harveyi* (B) when cultured with the different OA.

EXPLORING THE POTENTIAL AND CONSTRAINTS OF SMALL HOLDER AQUACULTURE IN MARGINALIZED SALINE AREAS IN PAKISTAN

Cristiano Rossignoli*, Chinedu Obi, Syed Aman Ali

WorldFish, Penang, Malaysia
C.Rossignoli@cgiar.org

Aquaculture is still a relatively young activity in Pakistan, but there is immense potential for the sector to develop. Aquaculture has been identified as an alternative livelihood source for many rural farming households living in areas with brackish groundwater. Due to the arid and semi-arid climate of Pakistan, which results in scarce and irregular rainfall, several rural areas are affected by salinity and waterlogging. It is estimated that more than 6 million hectares of Pakistani soil are affected by salinity. Salinity in the soil has caused many lands to be unsuitable for growing crops, and thousands of hectares have been abandoned. As an alternative livelihood, aquaculture can be adopted on such saline lands that are not suitable for crop cultivation. However, there is still limited research and understanding of the constraints and development opportunities for small-holder saline aquaculture farmers in marginalized saline areas of Pakistan. This paper is based on a survey conducted by WorldFish and IWMI on Saline and Marine Aquaculture in Pakistan, which aims to develop a shared understanding of the potential of saline and marine aquaculture for smallholders in marginalized saline areas in Pakistan. The study focused on the Southern Punjab and Sindh Provinces due to the high levels of saline groundwater in these provinces as well as the large proportion of aquaculture farmers in these provinces.

Preliminary results show that saline aquaculture in the study area is exclusively (100%) dominated by men. This is in variance with the gender share in agriculture, where around 67.2 % of farmers are women. The pond water in the study area is highly saline having a pond average of 7.6 ms/cm conductivity. Just around a third of the farmers have nursey themselves, and almost none have a fish hatchery for breeding. Special pond management practices like species selection, liming, maintaining stocking density, and using quality fish seeds are rarely practiced, as only a third of the farmers said that they implemented some special management practices in their ponds. Around 21 % of the respondents did not apply any form of fertilization either before or after stocking the fish. Due to its high survival rate, Rohu is the most important species stocked by the farmers in the study area (84%). This is followed by Catla at 71%, Mirgel at 63%, common carp at 55%, and grass carp at 53%. Just around 12 % of the respondents stocked Singhari (catfish) and tilapia respectively, and less than 2% stocked moli seed and other local seeds. Around 88 % of the farmers are satisfied with growth parameters. In general, most of the ponds (96%) are attacked by animals including otters, rodents, etc. Almost all farmers have witnessed waterlogging or saline near their ponds. While only 28 % of farmers worry about disease outbreaks, they are more likely to worry about marketing issues. Gross income from aquaculture is low in the presence of high feed and input costs,

There are opportunities for aquaculture development as the younger generation is interested in the business. Opportunities for women to participate in aquaculture are not clear as 59 % of farmers said that it is better for women not to work in aquaculture, 31% disagree and 10% are neutral. In fact, close to 80% of the respondents said that women do not have access to aquaculture farming equally as men. Going forward, the improvement of saline aquaculture in the study area, among others will require investments and policies that will improve water availability, enhance the use of advanced techniques for measuring water quality, skill development, provision of quality seed, fertilizer, and feed at a cheaper price, and provision of a stable market rate of fish to improve earning.

BENCHMARKING TILAPIA AQUACULTURE SYSTEMS IN EGYPT: SYNERGIES, TRADE-OFFS, AND ENTRY POINTS FOR SUSTAINABLE DEVELOPMENT

Cristiano M. Rossignoli*, Kelvin M. Shikuku, Timothy Manyise, Ahmed M. Nasr-Allah, Patrik J.G. Henriksson, Rodolfo Dam Lam, Denise Lozano, Nhuong Tran, Arjen Roem, Alaa Badr, Shabaan Ashraf, Maria A. Calmet, Roberta Moruzzo, Harrison Charo-Karisa, Alexandros Gasparatos, John A.H. Benzie, Michael J. Phillips

WorldFish, Penang, Malaysia
Jalan Batu Maung, Batu Maung
11960 Bayan Lepas
Pulau Pinang, Malaysia
C.Rossignoli@cgiar.org

The growing demand for aquatic foods requires the sustainable intensification of aquaculture systems. However, policy and investment decisions for sustainable intensification of aquaculture systems are often hindered by a lack of benchmarking data related to performance of such systems. For example, whilst current interventions and research on aquaculture myopically focus on economic performance, recent sustainability research highlights that the sustainability performance cannot be univocally assessed because of multiple contradicting stakeholder perspectives and trade-offs between outcomes. Contributing to the sustainable aquaculture debate, this study uses primary surveys data from 402 aquaculture producers in Egypt, to benchmark the performance of tilapia production systems against three outcomes of aquaculture systems, namely, environmental, food security and on-farm performance. Using a combination of econometric models and inferential statistics, the study finds that while tilapia aquaculture systems and practices contribute significantly to improving aquaculture systems outcomes, trade-offs emerge both between and within these outcomes. The study generates several insights important for the sustainable intensification of aquaculture, namely; (i) investing in the collection of high-quality benchmarking data for evidence-based policy and investment decisions; (ii) supporting policies that enable investments in aquaculture systems; (iii) crafting win-win policy situations that acknowledges potential trade-offs; and (iv) the need to improve the targeting of aquaculture systems intensification interventions given the heterogeneity of outcomes between aquaculture systems.

MITIGATION OF SOYBEAN MEAL-INDUCED ENTERITIS IN RAINBOW TROUT *Oncorhynchus mykiss* USING VITAMIN D AND GLUTAMINE AS DIETARY SUPPLEMENTS

Marina M. Rubio-Benito* Nicholas Romano and Vikas Kumar

Department of Animal, Veterinary and Food Sciences, University of Idaho, Moscow, ID, 83844, USA
mrubiobenito@uidaho.edu

Soybean meal (SBM) has become an essential ingredient in fish feed formulations, reducing the aquafeed industry reliance on fishmeal (FM). However, challenges remain when using SBM as the major source of protein, especially for carnivorous species. Reduced growth, and the occurrence of intestinal inflammation, referred to as soybean meal-induced enteritis (SBMIE), are signs of limited tolerance to SBM. Formulation of functional feeds using vitamins or amino acids could help increase SBM inclusion levels in fish diets. Vitamin D (VitD), well known for its role in calcium and phosphorus homeostasis, has more recently been suggested to play a role in intestinal immunity and barrier integrity. Therefore, we hypothesized its potential role as feed additive towards SBMIE attenuation. In addition, possible synergistic effects of VitD and the non-essential amino acid glutamine (Gln), known for its protective action during intestinal inflammation, were also examined in the present study.

A 10-week feeding trial was designed to investigate these hypotheses in the carnivore rainbow trout (*Oncorhynchus mykiss*). A total of 378 fish initially weighing ~ 9.5 ± 1.0 g, were randomly distributed into 21, 65-L tanks (18 fish/tank). Seven experimental diets (42% isonitrogenous and 20% isolipidic) including a FM diet (control), a SBM30 and SBM40 diet (30 g/Kg and 40 g/Kg inclusion level, respectively); two SBM30 and two SBM40, each supplemented with either VitD or VitD-Gln (5 µg/Kg VitD or 5 µg/Kg VitD + 2 g/Kg Gln) were formulated and fed to apparent satiation.

Feed intake was recorded daily, and fish weight and tissue samples were taken at 5 and 10 weeks. Samples were collected from the distal intestine for histology and gene expression analyses of the inflammatory markers: TNF-α, IL-1β, IL-10; barrier function markers: MLCK, occludin, and claudin-12; as well as the VitD receptor. Vertebra, kidney, and muscle samples were also obtained for mineral analysis and calcium determination.

Fig. 1., shows that by week 10, growth performance was improved in terms of weight gain in the SBM30-VitD-Gln group, which also showed slightly lower FCR ($p < 0.05$). Gene expression analysis showed changes in VitD receptor and MLCK expression at 5 and 10 weeks as well as IL-1β at 10 weeks. Further analysis will help reach conclusions on the use of VitD or VitD-Gln in SBM functional feeds.

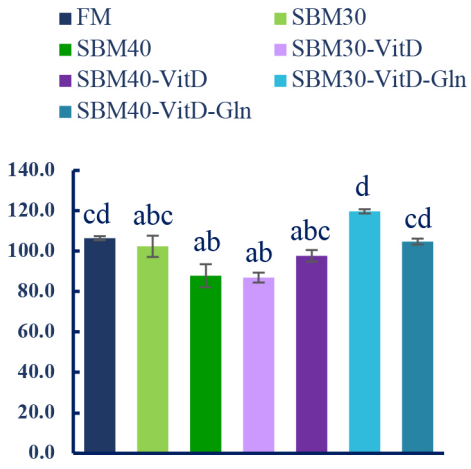


Fig. 1. Weight gain (g) of each dietary group at week 10 of the feeding trial.

THE MICROBIOTA OF WILD AND FARMED *Penaeus monodon*- HOW THE HOST IMMUNITY PLAYS ROLES IN GUT MICROBIAL ASSEMBLY

Wanilada Rungrassamee*, Pacharaporn Angthong, Tanaporn Uengwetwanit, Umaporn Uawisetwathana, Sureerat Tang, Nitsara Karoonuthaisiri, Sage Chaiyapechara, and Premruethai Supungul

National Center for Genetic Engineering and Biotechnology (BIOTEC)
113 Thailand Science Park, Phahonyothin Road
Khlong Luang, Pathum Thani, Thailand 12120
wanilada.run@biotec.or.th

Innovative farming approaches that incorporate functional feed additives to promote shrimp growth and health can play a critical role in revitalizing the shrimp industry. This approach not only allows farmers to increase throughput and production, but also minimizes the environmental footprint by reducing the use of chemicals and antibiotics in the field. The increasing importance of gut microbial populations to their host has drawn attention to prebiotics (dietary fiber to promote the growth of beneficial bacteria) and probiotics (beneficial bacteria) as promising functional feed ingredients to maintain gut homeostasis. Understanding microbial dynamics and functional roles in the shrimp microbiome will be critical for the development of such feed ingredients.

Recent advances in next generation sequencing and metabolite detection have led to various -omics-based techniques such as metagenomics (to determine bacterial identity and functional potential), transcriptomics (to determine host responses), and metabolomics (to identify active small molecules) to understand the microbial community and host-gut microbial interactions. Here, next generation sequencing analyses were used to study the gut bacterial communities in wild-caught and farmed *P. monodon*. Five phyla, Actinobacteria, Fusobacteria, Proteobacteria, Firmicutes and Bacteroidetes, were found in all shrimp from both wild and farm environments. The bacterial profiles showed similar dominant genera in wild-caught and domesticated shrimp, indicating the presence of a resident bacterial population in *P. monodon*. However, the gut bacterial profiles of wild-caught shrimp showed greater variation than those of farmed shrimp, suggesting that the environments influenced gut microbial composition. To better understand the role of host immunity in gut microbial composition, we used multidisciplinary platforms (microbiome, transcriptome, and metabolome) to determine the interactions between the gut microbiota and its animal host, *P. monodon*. RNAi suppression of Toll and IMD signaling pathways, the major immune pathways of *P. monodon*, was used as a tool to alter host immune gene expression. Suppression of Toll and IMD signaling pathways resulted in differential gene expression in hemocytes, particularly for immune-related genes encoding antimicrobial peptides, heat shock proteins, and pattern recognition proteins. Suppression of the IMD pathway had a greater impact on bacteria, genes, and metabolites in the shrimp gut. Therefore, we proposed a model representing an interaction between gut bacteria and the host immune system, in which IMD is the major immune pathway controlling and balancing commensal colonization.

EFFECT OF TRYPTOPHAN DIETARY CONTENT ON MEAGRE, *Argyrosomus regius*, JUVENILES GROWTH, MUSCLE CELLULARITY AND BEHAVIOURAL RESPONSE

Margarida Saavedra[1], Ana Vasconcelos[1], Teresa G. Pereira[1], Marisa Barata[2], Luís Conceição[3], Pedro Pousão[2] and Marta C. Soares[4]

[1]Portuguese Institute for the Sea and Atmosphere, Rua Alfredo Magalhães Ramalho, nº6, 1495-006 Lisbon, Portugal
[2]Aquaculture Research Station of IPMA (EPPO), Av. do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal
[3]Sparos Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
[4]BIOPOLIS, CIBIO-InBIO, Universidade do Porto, Vairão Portugal

The increasing importance of fish welfare in aquaculture is not only an ethical question but a chance to improve standards and quality of fish production technologies and aquaculture products. Fish kept under good welfare conditions have lower stress levels, are less prone to diseases, have better growth and food conversion rates (FCR) and have a better final flesh quality. Tryptophan is known to be linked to the stress response by enhancing fish brain serotonergic activity and can also inhibit endogenously derived behavioural performance, such as aggressiveness and cannibalism. In this study, three diets containing different contents of tryptophan: 0.2 (Trip1), 0.5 (Trip2) and 0.9 % (Trip3), were tested in triplicates in 112 days old meagre with an initial weight of 32.6 ± 3.4 g and 14.4 ± 0.5 cm length for 57 days. Although the results showed no significant differences for growth and FCR between treatments, there was a tendency to an increase of growth and decrease of FCR in meagre fed higher levels of tryptophan. This is supported by the results obtained in muscle cellularity where Trip3 had a higher fibre density, suggesting an increased fibre recruitment. In terms of fish behaviour, in the social test, meagre from all treatments took less than 7 seconds, in average, to join the shoal and in Trip1 meagre stayed closer to the shoal for 63 % of the observation time whereas in Trip3 fish seemed to spend equal amount of time away and close to the shoal. In the anxiety test, the number of horizontal and vertical swimming movements within the test tank was, in average, two times higher in Trip3 compared to Trip1, suggesting more ability to explore and lower levels of anxiety. These results suggest that tryptophan dietary levels can influence not only meagre growth pattern but, as well, its behavioural response.

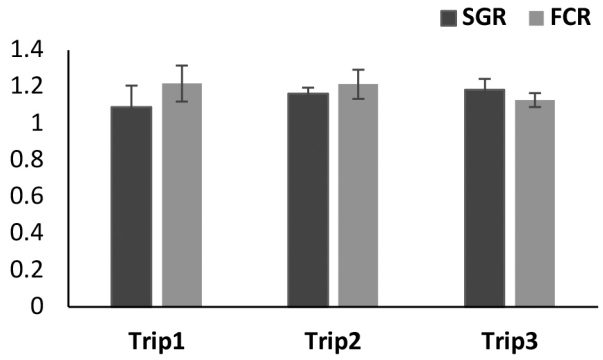


Fig. 1. Specific growth rate (SGR) and Food conversion ratio (FCR) of meagre juveniles fed a diet with 0.2 % (Trip1), 0.5 % (Trip2) and 0.9 % (Trip3) of dietary tryptophan. Values are mean and standard deviation

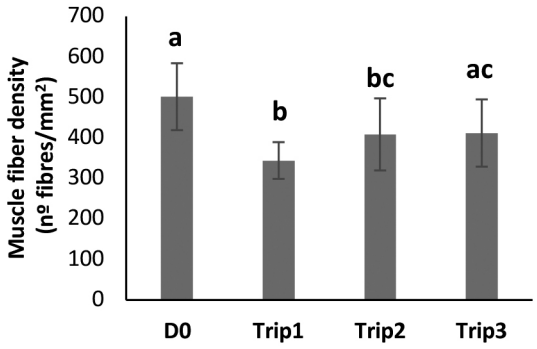


Fig. 2 . Muscle fibre density of meagre juveniles fed a diet with 0.2 % (Trip1), 0.5 % (Trip2) and 0.9 % (Trip3) of dietary tryptophan. D0- Density when the trial started. Values are mean and standard deviation

CURRENT STATUS OF EXOTIC FRESHWATER FISH PRODUCTION IN MALAYSIA

Abdulwakil Olawale Saba*, Mohammad Noor Azmai Amal, Ahmad Ismail, Syaizwan Zahmir Zulkifli

Department of Biology, Faculty of Science,
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia
sabaola@gmail.com

The introduction of invasive alien fish species has threatened various indigenous ecosystems all over the world. However, some of these fish species have contributed to food security through improved fish production. There is a general dearth of published literature on various aspects of alien freshwater fish species in Malaysia. To show the importance of alien freshwater fish species in Malaysia's culture and inland capture fisheries, this paper describes the trend in alien and native fish production by wholesale and retail value over an 11-year period (2007 to 2017).

Alien fishes dominate freshwater fish culture (93% of production) and occupies a considerable proportion of Malaysia's inland freshwater capture fisheries (41% of production). Out of the 1,317,440 metric tonnes (MT) of cultured fish produced with wholesale value of RM 7.9 billion from 2007 to 2017, about 93% (1,221,869 MT) with wholesale value of RM 6.7 billion are from species not native to Malaysia. There was an increase in the production and value of aliens between 2007 and 2017, from 63,434 MT to 98,663 MT valued at RM 70 milion to RM 103 million, respectively. A wide gap exists between the production and value of alien and native cultured fishes (Figure 1). On the contrary, native fish production dominates the inland freshwater capture fisheries sector and out of the total of 57,552 MT of fish produced with a wholesale value of RM 662 million between 2007 and 2017, alien species occupy about 41.4% (16,837 MT), with wholesale value of about 17.6% (RM 99 million), respectively. Comparing 2007 to 2017, there were slight increases in the production of alien and native fish while there was a greater increase in value for both categories, with native fishes experiencing a greater increase. Year 2013 experienced the highest alien capture fish production at 2301 MT, increasing close to native fish production which stood at 3,339 MT. As of 2017, this figure nose-dived all the way to 1,355 MT at a value of RM 11 million (Figure 2). Alien fish species are greatly important for the Malaysian economy. It is, therefore, necessary to preserve freshwater biodiversity by constantly monitoring alien fishes in the wild, and improving native fish production. This would help to control and prevent current and potential negative impacts including invasion of the native freshwater ecosystems.

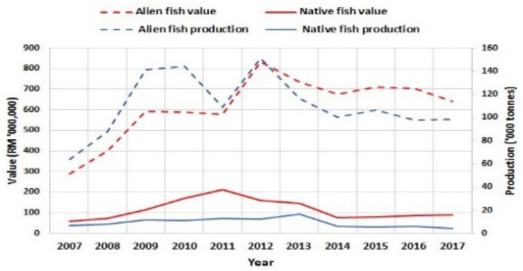


Figure 1: Trend in alien versus native freshwater aquaculture fish production by weight and wholesale value from 2007-2017

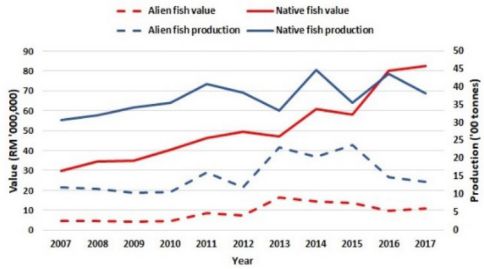


Figure 2: Trend in freshwater alien versus native inland capture fish production by weight and wholesale value from 2007-2017

INVESTIGATION OF BROOD STOKING *Aspius aspius* IN IRAN

Esmail Sadeghinejad Masouleh[1]*, Adel Hoseinjani[2], Mohaddeseh Ahmadnezhad[3], Mohammad Reza Rezaeicha[4]

1,2,3,4,Instructor in Inland Waters Aquaculture Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran
*Corresponding Author: sadeghi_E@yahoo.com

Aspius aspius is one of the important commercial fishes in The Caspian Sea which them stocks was decreased because of overfishing and loss of spawning, so, in recent years catch amount of the fish reached to 400-500 kg. The purpose of this research was to reproduce this fish for restoring its fish stocks. For this purpose 30 fishes caught in the river Arass (northwest of Iran) and transported to the pond of 2000 m^2 in 2015. The results showed that 20 fishes survived with 1^+ to 2 age and average weight 1028.1 ± 93 and 1196.7±11 gr. Also, Female fishes were larger than male fishes in average weight. The results showed that in pre-brood stock that fed with natural food Weight gain Increased was 531 g in 1^+ to 3^+age group and 541 g in 2 to 4 age group.

Table 1 Descriptive statistical analysis of age and weight of **broodstocking** of *Aspius aspius* -2015

W.G								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
3^+	6	1559.83	172.455	70.404	1378.85	1740.81	1321	1816
4	14	1737.86	117.517	31.408	1670.00	1805.71	1518	1911
Total	20	1684.45	155.825	34.843	1611.52	1757.38	1321	1911

STOCKING OF MOLA BROODSTOCK THROUGH FINGERLING TRADER: A USEFUL WAY TO SMALL INDIGENOUS FISH DISSEMINATION

Manos K. Saha*, Md. S. Mohmud, Taushik Lahiri and Benoy K. Barman

WorldFish Bangladesh
Tumpa Tower, H#7, R#27, Upashahor
Bogura, Bangladesh
m.saha@cgiar.org

Small indigenous fish species (SIS) are very rich in micronutrients. Particularly mola, *Amblypharyngodon mola* is extremely rich in vitamin A, Ca, Iron, Zinc etc. Therefore, SIS have high demand and market price in Bangladesh, India and South Asian countries. Stocking broodstock of mola in ponds and semi-closed open waters in polyculture useful to get higher production of mola and other SIS. Most of the homestead ponds are still dependent on fingerling traders locally called '*Patilwala*' for carp and other fish seed stocking. In spite of high demand of mola farming, lack of seed or broodstock of mola in the existing fish seed marketing system is the major constrain. To include mola broodfish in the existing fish seed supply channel.

A participatory research was implemented under BMGF funded IDEA project of WorldFish in northwest Bangladesh during January to August 2022. Training and practical demonstration was organized for 18 fingerling traders on mola culture system and technology, live mola collection, sorting and transportation. Baseline and end line data were collected for fingerling traders, who involved in mola trading and farmers, who purchased mola from traders.

About 40% of trained fingerling traders (07) adopted the technology and disseminated mola broodstock successfully to 33 farmers. The adopted fingerling traders further motivated about 60% fingerling traders, who didn't received training to disseminate the mola broodstock trading and through them 10 famers stock mola broodfish. About 30 sources of mola broodstock were identified in different places. 'Dhulia Beel' in Nilphamari Sadar found as a major source of mola broodfish and could be use as mola broodstock center. Lack of knowledge and training, unavailability of mola broodstock, high market price of mola broodstock were the major constrains. Fingerling traders are found as key player to promote mola with other commonly cultured fish by large number of farmers. To trained and motivate them, initiative should be taken Govt. and other development organizations.

VARIED VARIETY OF SUCCESSFUL FISH FARMING DONE IN THE CONCEPT OF FAMILY FISH FARMING IN HALDIA, WEST BENGAL

Suman Kumar Sahu1*

1 10, Birangana Block, Sahid Kshudiram nagar, PO- Hatiberia, PS- Haldia, Dist- Purba Medinipur, Pin 721657 West Bengal
*Corresponding Author E-mail: mailatsahu@gmail.com

The family and small-scale farming are inextricably linked to food security. This article evaluates the impact of the emergence of the concept of family fish farming in Haldia, West Bengal, a pilot family-farming-field-fish-school (FFFFS) program on integrated fishery management (IFM) practices related to proper resource utilization, species diversification with new species introduction, i.e., Amur common carp, Pengba, Milkfish and Pearl spot, and field-based knowledge. The practical field-based information was used to analyze and as an approach to create a comparison group similar to the FFFFS participants in observable characteristics. The results are robust across the two approaches as well as with different matching methods. It was found that the families of farmers who participated in the program achieved significant success in the IFM practices related to proper resource utilization, interest in species diversification and field-based knowledge than those from the non-participant comparison group. It was also evident that the improved knowledge about IFM practices has the potential to significantly improve productivity, fish production as well as the farmers' profit. The family farming preserves the traditional food products while contributing to a balanced diet and safeguarding the aqua biodiversity and the sustainable use of natural resources. Family farming represents an opportunity to boost local economies, especially when combined with specific policies aimed at social protection and well-being of communities.

EVALUATION OF PREBIOTICS DIETARY SUPPLEMENTATION ON GROWTH AND DISEASE RESISTANCE OF WHITE SHRIMP AGAINST ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

Nur Haziqah Salleh Huddin*, Ina Salwany and Muhammad Farhan Nazarudin

School of Applied Science, Temasek Polytechnic,
21 Tampines Avenue 1, Singapore 529757
haziqah_99@hotmail.com

With a rising demand for shrimps in the world, the industry turns to aquaculture as a substitute to wild catch shrimps to meet the consumer's demand. However, it becomes effortless for diseases such as Acute Hepatopancreas Necrosis Disease (AHPND) caused by *Vibrio parahaemolyticus* to spread due to high density cultures causing high mortalities and economic losses. The aim of this study is to evaluate the growth performance, gut microbiota and resistance to challenge with *V. parahaemolyticus* of *Peanneus vannamei*. The shrimps were fed at 5% body weight with 1.0, 2.0 and 3.0% of *Sargassum polycystum* supplemented diet. Shrimps (0.015 ± 0.001) in triplicate groups with a density of 30 shrimps per tank were fed the diets twice daily for 6 weeks and results were compared to shrimps fed with a control diet without any supplementations. At the end of the feeding trial, shrimps fed with *S. polycystum* supplemented diet has significantly higher growth of gut microbiota and a higher rate of survival compared to the control group ($P < 0.05$). However, there was no remarkable difference in growth and resistance challenge with *Vibrio parahaemolyticus* ($P > 0.05$). Overall, the use of *S. polycystum* was not useful in the improvement of growth of the shrimps however, it is effective as a prebiotic to increase the growth of gut microbiota which could lead to the reduced use of antibiotics in the shrimp industry.

FUNCTIONAL FEEDS FOR THE INHIBITION OF AQUACULTURE PATHOGENS

Kaarunya Sampathkumar*, Li Ling Tan, Mingyue Sun, Patricia Lynne Conway, Say Chye Joachim Loo

School of Materials Science and Engineering
Nanyang Technological University
50, Nanyang Avenue
Singapore 639798

There has been an increasing demand for fish production due to the expanding global population. The global aquaculture production has expanded about 527% from 1990 to 2018 [1]. Bacterial infections and disease outbreak are serious threats to the aquaculture industry which contribute to severe economic losses in the sector. An estimated total loss of more than $6 Billion USD per year is due to disease outbreak [2]. In the past 50 years, antibiotics have been the most widely used strategy to combat aquaculture related diseases. However, usage of such antibiotics is unsustainable as it can result in the development of reservoirs of drug-resistant bacteria and transferable resistance genes in fish pathogens and other bacteria in aquatic environments. This potential negative effect of residual antibiotics of aquaculture has resulted European Union, USA and China regulators to either ban or heavily restrict the usage of antibiotics as treatment for aquacultures [3]. In order to move away from antibiotics, there has been a huge impetus on developing sustainable practices to tackle aquaculture pathogens. One of the strategies widely studied for this purpose is the use of probiotics in aquaculture diet. Probiotics in fish diet have been shown to improve the growth and immunity in fishes. The mechanism of antimicrobial effect of these probiotics can be attributed to the colonization of beneficial bacterial in the fish gut, producing antimicrobial agents which compete and outweigh the growth of pathogenic bacteria. In addition to probiotics that offer disease resistance, the focus has also shifted towards using compounds extracted from natural food-based sources, generally termed as nutraceuticals, to exploit their beneficial effects into sustainable aquaculture practices. While studies of probiotics and nutraceuticals have proven their inhibition effect towards common pathogens in aquaculture, all the past studies have been focused only on using either probiotics or nutraceuticals separately. There is a dearth of studies in investigating the combined effect of probiotics and nutraceuticals against aquaculture pathogens. In light of this, this work explores the possibility of combining the use of probiotics and nutraceuticals in fish diets that could lead to potential synergistic effects between the two. Such functional diets could be used not only to combat disease outbreak but also to improve the growth performance of the diet.

1. FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en
2. Mishra, Sudhansu S. & Das, Rakesh & Swain, Priyabrat. (2019). Status of Fish Diseases in Aquaculture and assessment of economic loss due to disease.
3. Y. Shao, Y. Wang, Y. Yuan, and Y. Xie, "A systematic review on antibiotics misuse in livestock and aquaculture and regulation implications in China," *Science of The Total Environment,* vol. 798, p. 149205, 2021

FISH WELFARE IMPLICATIONS OF SEA LICE PREVENTION AND TREATMENT METHODS

Dr. Myriam Vanderzwalmen*, Dr. Kate Mintram, Dr. Tracey Jones

Compassion in World Farming
River Court, Mill Ln,
Godalming GU7 1EZ
United Kingdom
Myriam.vanderzwalmen@ciwf.org

Sea lice (*Lepeophtheirus salmonis* and *Caligus spp*.) are a common ectoparasite on farmed Atlantic salmon (*Salmo salar*). Damage from sea lice and management of the parasite cost an estimated $1.25 billion per year to the industry with Norway, Scotland and Chile being the most impacted countries globally. The free-swimming planktonic larvae disperse through the sea whereas the chalimus, pre-adult and adult stages settle on salmon and are parasitic and feed on the mucous, skin, and tissues of salmon.

The welfare of infected salmon is poor with sea lice causing injuries, reductions in osmoregulatory capability, a lower immune capacity, increased stress, reduced growth and can result in death. Many sea lice management methods are available to the industry. Research of these methods tends to focus on their effectiveness with little attention given on the welfare risks associated with sea lice management methods. Sea lice management methods can be grouped into two categories: prevention or treatment. We will review the welfare risk of common prevention and treatment methods by discussing the mortality and injury risks of each methods as well as discussing other welfare concerns associated with each management method. This welfare risk assessment will be put in the context of the methods' effectiveness and their environmental impact.

RE-CIRCULATING AQUACULTURE SYSTEMS FOR GROW-OUT SALMON: CONSIDERATIONS FOR FISH HEALTH AND WELFARE

Dr. Myriam Vanderzwalmen*, Dr. Kate Mintram, Dr. Tracey Jones

Compassion in World Farming
River Court, Mill Ln,
Godalming GU7 1EZ
United Kingdom
Myriam.vanderzwalmen@ciwf.org

As global aquaculture continues to increase, Re-circulating Aquaculture Systems (RAS) are being considered as a sustainable approach to meet consumer demand for seafood, including salmon. RAS systems are on-land, man-made fish farms which rely on technology to pump water in and out of tanks. They therefore relieve marine degradation issues associated with off-shore salmon farms, such as seabed disturbance, pesticide and antibiotic contamination, and disturbances to wild stocks from sea lice infections or escapes of farmed fish. However, due to the artificial nature of these systems, they could be detrimental for fish welfare.

Stocking density is an important determination of welfare. High welfare accreditation schemes and recommendations require stocking densities of between 10 and 15 kg/m3 (Soil Association, RSPCA, CIWF) for grow-out salmon, but the average for RAS systems is 80 kg/m3 (ISFA, 2015). Other potential welfare issues emerge from the reliance of RAS systems on technology, where technological failures can result in mass mortalities, poor water quality and disease. Their potentially high water (up 9000 litres per live salmon (ISFA, 2015)) and energy usage (1.3-6.1 kWh per kg of salmon (Bostock, 2018)) also calls into question the sustainability of these systems which will greatly vary depending on the sources of energy used, the type and source of water used and the location of the RAS facility. This poster will discuss the key health and welfare considerations associated with RAS for grow-out salmon with the aim to raise the profile of fish welfare within the RAS industry.

References:

ISFA (2015) *The evolution of land based Atlantic salmon farms*. Available at: https://www.cermaq.com/wps/wcm/connect/9144716c-37a5-4f53-8f4a-44c73396c414/ISFA_LandFarmingreport_web.pdf?MOD=AJPERES&CONVERT_TO=url&CACHEID=ROOTWORKSPACE-9144716c-37a5-4f53-8f4a-44c73396c414-lZL5WJR.

Bostock, J. (2018) *Review of Recirculation Aquaculture System Technologies and their Commercial Application Prepared for Highlands and Islands Enterprise*. Stirling, UK. Available at: https://www.hie.co.uk/media/6167/ras-study-2018-update.pdf.

RELATIONSHIP OF PHYSICOCHEMICAL PARAMETERS AND GROWTH IN THE CULTURE OF ROBALO *Centropomus viridis* IN FLOATING CAGES IN NORTHWESTERN MEXICO

Giovanni M. Giacobbe Baldini[1], Apolinar Santamaria-Miranda[2], Juan Pablo Apún Molina[2], Leonardo Ibarra Castro[3]

[1]Instituto Politecnico Nacional/CIIDIR Unidada Sinaloa, Programa de Maestria en Recursos Naturales y Medio Ambiente, Blvd. Juan de Dios Batiz Paredes 250, San Juachín Guasave Sinaloa, Mex.81101
Phone 6878729626
asantama@ipn.mx

[2] Florida University, Laboratory Whitney, 9505 Ocean Shore Blvd. St. Augustine, FL 32080-8610
Phone: 904-461-4000

Snook is considered one of the most important fisheries in Mexico due to its cultural and economic importance; nevertheless, the production potential remains to be untapped, as current commercial production is based on wild-catch temporality, distribution and abundance. For these reasons, the objective of this study was to evaluate the technical and economic viability of the Pacific White Snook *Centropomus viridis* culture in 10m diameter and 3m deep cages (235m^3). The experiment began in September 2019 when 15,000 organisms of *C. viridis* with an initial weight of 0.8±0.2g were stocked in one floating cage where they would remain for four months during the nursery stage. Once the nursery stage ended, the fish were graded and separated into three different cages. During the experiment physicochemical parameters such as dissolved oxygen, temperature, pH and salinity as well as nutrients such as ammonia, nitrites and nitrates were closely monitored, and recorded. Performance variables such as specific growth rate, daily growth rate, feed conversion ratio, survival and total biomass were evaluated. Capital expenditures and operating costs were also recorded during the entire cycle and once the cycle ended, the fish were harvested and sold.

The results of our performance variables were, for the nursery stage and 60% for the grow out stage and a total biomass produced of 973kg. Correlation between physicalchemical parameters and growth was determined through a correlation analysis and a direct correlation was found between temperature and growth.

A sensitivity analysis was used to determine economic feasibility at different scenarios and was found at a survival rate of 60%, a production of 20 metric tons and a final sales price of 10USD/kg.

Tabla 1. – Physicochemical and nutrient parameters of the 120-day pre-grow-out stage of *C. viridis* culture in floating cages.

Variables	Results
Temperature C°	21.8 ± 3.4
Salinity ups	33± 1.0
DO mg/L	4.9 ± 1.4
pH	8.8 ± 0.4
Nitrites mg/L	0.0075 ± 0.0015
Nitrates mg/L	<<
Amonium mg/L	0.079 ± 0.01

ANALYSIS OF METABOLIC VARIABLES OF WHITE SNOOK *Centropomus viridis*, CULTURE IN FLOATING CAGES, AND WILD ORGANISMS IN NORTHWESTERN MEXICO

Apolinar Santamaria-Miranda[1], Giovanni M. Giacobbe Baldini[1], Leonardo Ibarra-Castro[2]

[1] IPN-CIIDIR, Sinaloa, Blvd Juan de Dios Batiz Paredes 250, Col. San Juachin Guasave Sinaloa, México
[2] Florida University, Whynet laboratory for marine bioscience
9505 Ocean Shore Blvd. St. Augustine, FL 32080-8610

Snook is considered one of the most important fisheries in Mexico due to its cultural and economic importance; nevertheless, the production potential remains to be untapped, as current commercial production is based on wild-catch temporality, distribution and abundance. For these reasons, the objective of this study was to evaluate the biochemicals analysis of the Pacific White Snook *Centropomus viridis* culture in 10m diameter and 3m deep cages (235m^3) and wild organisms. The experiment began in September 2019 when 15,000 organisms of *C. viridis* with an initial weight of 0.8±0.2g were stocked in one floating cage where they would remain for four months during the nursery stage. During the experiment physicochemical parameters such as dissolved oxygen, temperature, pH and salinity as well as nutrients such as ammonia, nitrites and nitrates were closely monitored, and recorded. Biochemicals variables such as glucose (mg·dL-1), protein (g·dL-1), total lipids (g·dL-1) and cholesterol (mg·dL-1) were evaluated. The results of our performance variables were, Correlation between physical chemical parameters and growth was found between temperature and growth. The biochemical-blood results show us that the nutritional condition of the organisms in culture in comparison with the wild organisms. Using biochemical-blood techniques gives us the advantage of sampling broodstock in captivity without the need to sacrifice them to know their condition nutrition and state of health in which they find themselves.

Tabla 1. – Biochemical parameters of the wild and 120-day pre-grow-out stage of *C. viridis* culture in floating cages.

Biochemicals analysis	wild	grow-out	P<0.05
Glucose mg·dL-1	65.25±4.75**b**	45.76±2.19**a**	0,000002
Protein g·dL-1	4.40±0.88	3.40±0.59	NS
Total Lipids g·dL-1	0.64.8±0.06	0.67.5±0.84	NS
Cholesterol mg·dL-1	77.57±9.38**b**	32.74±6.24**a**	0,00363

MICROBIAL COMMUNITY-BASED PROTEIN FROM SOYBEAN-PROCESSING WASTEWATER AS VALUE-ADDED ALTERNATIVE FISH FEED INGREDIENT FOR SUPPORTING ASIAN SEABASS *(Lates calcarifer)* GROWTH PERFORMANCE

Ezequiel Santillan*, Fanny Yasumaru, Ramanujam Srinivasan Vethathirri, Sara Swa Thi, Hui Yi Hoon, Diana Chan Pek Sian, and Stefan Wuertz

Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University Singapore 637551, Singapore
esantillan@ntu.edu.sg

As the global demand for food increases, aquaculture is rapidly growing and is replacing capture fishery in providing protein source for meeting human nutritional needs. Existing aquafeeds using fishmeal or soybean meal as sources of protein are unlikely to support this rapid expansion under the current production systems. Single cell protein (SCP) derived from microbial community-based biomass grown on food processing wastewater could be a sustainable alternative. Here, we evaluated the use of microbial protein produced from soybean processing wastewater as partial replacement of fishmeal in the diet of juvenile Asian seabass (*Lates calcarifer*).

A 24-day feeding trial was conducted with a control fishmeal diet and a 50% fishmeal replacement with microbial community-based SCP as experimental group. Both diets met the protein, essential amino acids (except for lysine), and fat requirements for juvenile Asian sea bass. The bacterial composition of the microbial community-based SCP was dominated by *Acidipropionibacterium* and *Propioniciclava*, which have potential as probiotics and producers of valuable metabolites. Triplicate tanks were randomly set up per group, each containing 20 fish.

There was no significant difference between groups in terms of percent weight gain (Figure 1) and feed conversion ratio (FCR), specific growth rate (SGR), and percent survival of fish (Table 1). Furthermore, the experimental group had less variability in terms of weight gain and FCR as compared to the control group, suggesting that a partial fishmeal replacement diet with SCP would be a good alternative protein for use in fish feed. In conclusion, microbial community-based SCP produced from soybean processing wastewater has potential as aquaculture feed ingredient, which can help in the transition of food production systems towards a circular bioeconomy.

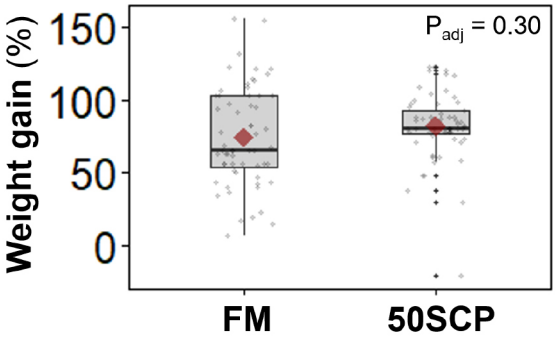


Figure 1 - Weight gain of juvenile Asian sea bass fed with a fishmeal-based control diet (FM) or an experimental diet containing 50% microbial community-based SCP (50SCP) over 24 d. Red diamonds display mean values. Grey dots represent values for each individual fish (m = 20) in each of three replicate tanks per group on day 24. Welch's t-test P-value adjusted at 5% FDR shown (n = 60).

Table 1 – Growth of juvenile Asian sea bass fed with a fishmeal (FM) control diet or an experimental diet containing 50% microbial community-based SCP (50SCP) over 24 d. Values are means ± s.d.m. (n = 3).

Parameter	FM	50SCP	P_{adj} †
Feed intake (g)	19.8 ± 1.56	16.8 ± 0.73	0.15
FCR§	1.26 ± 0.31	1.18 ± 0.03	0.69
SGR‡ (% d^{-1})	2.30 ± 0.57	2.50 ± 0.13	0.69
Survival (%)	100	100	-

† Welch's t-test P-values adjusted at 5% FDR
§ FCR = mean feed intake * mean weight $gain^{-1}$
‡ SGR = ($\ln_{\text{mean final W}} - \ln_{\text{mean initial W}}$) * trial $duration^{-1}$ *100

CRYOGENIC SPERM BANKING OF INDIAN MAJOR CARP, ROHU *Labeo rohita* AND PRODUCTION OF QUALITY SEEDS IN COMMERCIAL HATCHERIES

M Rafiqul I Sarder*, M Jahangir Alam, M Matiur Rahman, Mariom and Terrence R Tiersch

*Department of Fisheries Biology and Genetics
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

The study dealt with the standardization of sperm cryopreservation protocol of Indian Major Carp, Rohu (*Labeo rohita*) to establish a cryogenic sperm bank and production of quality seeds using cryopreserved sperm in commercial fish hatcheries. A series of experiments were conducted to achieve the goal. Quality broodstock of Rohu was developed by collecting and rearing of Halda and Padma river-origin fish in earthen ponds with supplementary feeds. Activation of sperm motility was evaluated in different gradients of NaCl solution (0.1% to 1%), and highest sperm motility of 96.0±1.0% and swimming duration of 37.46±0.8 min was observed in 0.4% NaCl solution. The toxicity of cryoprotectants (DMSO and methanol) to sperm was tested at different concentrations (5%, 10% and 15%) and incubation time (5–40 min) with two extenders (Alsever's solution and egg-yolk citrate), and found that cryoprotectants with 5% and 10% concentrations produced better motility during 5 and 10 min of incubation, but 15% concentration seemed toxic to sperm. Alsever's solution plus 10% DMSO at 1:9 dilution ratio (sperm : diluent) with 10 min equilibration time and 10°C/min cooling rate showed best equilibration motility (94.3±2.3%) and post-thaw motility (90.0±2.9%) in cryogenic freezing. Post-thaw motility of cryostored sperm remained at satisfactory level (initial 90±0% and final 81.67±0.33%) during assessment over the twelve-month storage period. During induced breeding in 11 public and private hatcheries, the average fertilization and hatching rates were estimated as 40.41±3.33% and 32.53±3.07% with cryopreserved sperm, whereas 75.22±3.85% and 63.23±4.56% with hatchery-origin fresh sperm (control) respectively. The seeds of both cryopreserved sperm-origin and controls were reared in the respective hatcheries and sampled monthly to compare their growth performances. The average final length and weight of cryopreserved sperm-origin and control fry were found as 16.76±4.11cm, 77.79±52.49g and 11.56±0.24cm, 15.28±1.19g respectively after 180 days pond rearing. Cryopreserved sperm-originated fry showed significantly ($p<0.05$) higher growth than those of fresh sperm due to introduction of new superior germplasm through artificial insemination using cryopreserved sperm collected from the cryogenic sperm bank. The results also indicate that the hatchery stocks become genetically inferior due to inbreeding, negative selection and poor broodstock management, and thus demands for improvement of quality of seeds and broodstocks through sperm cryopreservation technology.

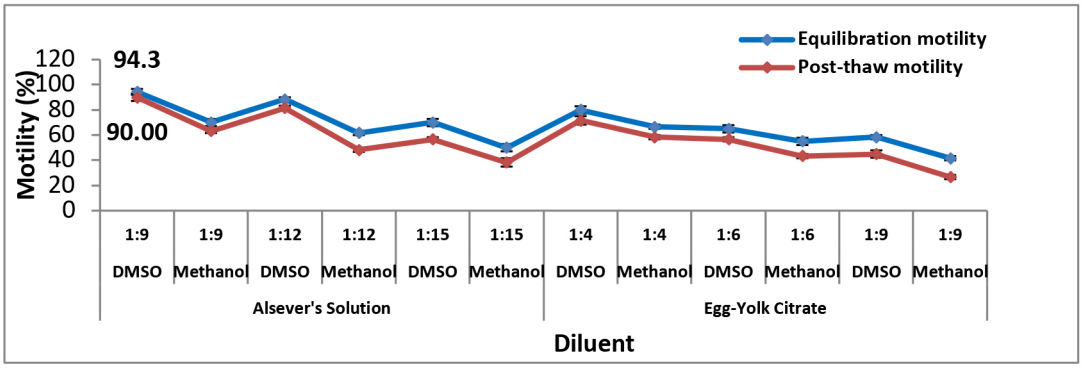


Fig. Determination of suitable dilution ratios and diluents for cryopreservation of *L. rohita* sperm

DOES MICROALGAE-DERIVED B-GLUCAN ALTER FISH SYSTEMIC IMMUNE RESPONSES?

Manuel Sardinha*, Gabriella Pereira, Bruno Reis, Luís E.C. Conceição, Benjamin Costas, Jorge Dias

SPAROS, Lda. Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
ManuelSardinha@sparos.pt

The rapid growth and intensification of the aquaculture sector had led to various constrains such as stresses and disease, which reflects on a weakened immune capacity. Researchers have prompted innovation for new solutions and methodologies aiming to improve animal immune capacity. The use of vaccines, antibiotics, and non-specific immunostimulants are three possible methods of farmed fish protection against disease. The present work aims to evaluate the efficiency of microalgae-derived ß-glucan on modulate fish systemic immune response through improved nutrition.

A benchmarking test was done with a commercial diet as control (CTRL), the same diet was supplemented with yeast-derived ß-glucan (commercial use) and microalgae-derive ß-glucan (*P. tricornutum*). Post-larvae Senegalese sole and juvenile seabream were fed the experimental diets and exposed to inactivated *Photobacterium damselae* subsp. *Piscicida*, which would trigger the host immune system to react. A panel of immune parameters were analysed, from oxidative parameters (total proteins, catalase, lipid peroxidation, total glutathione and superoxide dismutase activities) to immune parameters (lysozyme, peroxidase and bactericidal activities).

Both experimental trials presented no significant differences on growth performance and feed conversion. On the other hand, diet supplementation with microalgae-driven ß-glucan, significantly increased thrombocytes cells as well as SOD activity, in juvenile seabream. Regarding post-larvae S. sole, ß-glucan supplementation significantly increased lysozyme activity after bacteria exposure. Further analysis is underway.

β-glucan mode of action is mainly in the gut, by the cell pattern recognition receptors (PRR), leading to the activation of the host´s innate immune cells, enhancing its immune response. Further analysis on gut gene expression may lead to more detailed answer on β-glucan capacity to modulate fish immune response.

Furthermore, this work intends to develop a preliminary method to evaluate animal's short-immune response, as an alternative to the conventional challenge tests.

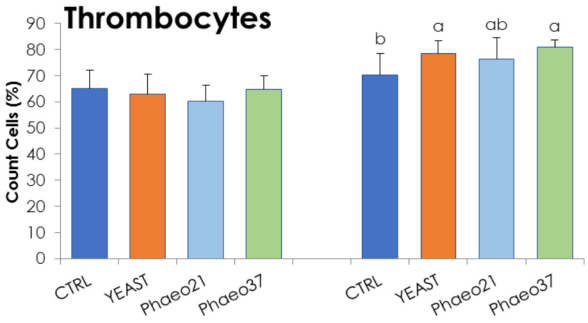


Figure 1. Thrombocytes cell count on juvenile seabream.

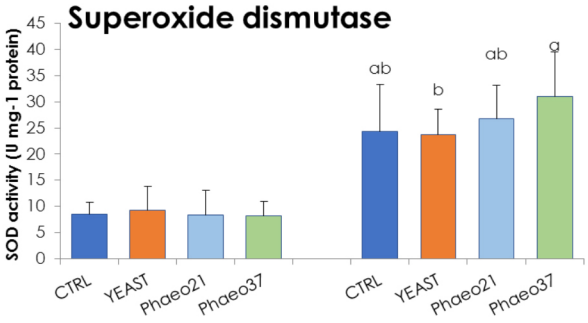


Figure 2. Liver analysis of Superoxide dismutase on juvenile seabream, fed various experimental diets.

EVALUATION OF THE NUTRITIVE VALUE OF SEAWEED PROTEIN CONCENTRATE AS A SUSTAINABLE FEED INGREDIENT IN THE DIETS OF *Penaeus monodon*

Carl John Saromines * and Rex Ferdinand Traifalgar

Camarines Norte State College/University of the Philippines Visayas
San Roque, Mercedes, Camarines Norte 4601, Philippines
carl16john@yahoo.com

To address the shortage of cheap and sustainable feed-protein ingredient for aquaculture, this study developed *Gracilariopsis heteroclada* protein concentrate (GHPC) and evaluated its nutritional value as a replacement to soybean meal protein in the diet of black tiger shrimp, *Penaeus monodon*. Five isonitrogenous and isolipidic diets were formulated replacing soybean meal protein at 0% (control), 12.5%, 25%, 50% and 75%.

Results showed that the developed GHPC has a protein, lipid, fiber and carbohydrate content of 31.11±0.12%, 1.57±0.61%, 1.87±0.31% and 24.99±0.33%, respectively. The nutritional value of GHPC was found high, exhibiting an ingredient digestibility index of 91.84 ± 0.06% and Essential Amino Acid Index (EAAI) of 0.996. Feeding trial results revealed that the overall growth performance, feed assimilation efficiency and biochemical composition of the shrimp fed with the 50% GHPC diet were similar to the control group fed with full soybean meal based-diet (Table 1). The high acceptability of this feed ingredient to *P. monodon* is attributed to its high digestibility and adequate content of essential amino acids. However, shrimps fed with replacement levels beyond 50% exhibited significant growth depression that might be due to the presence of antinutritional compounds as observed with the reduction of digestive enzyme activities (Figure 1) and histomorphological changes (B-cell enlargement) (Figure 2).

Collectively, the findings suggest that GHPC has high nutritional value and could be used as a plant protein ingredient in the diet of *P. monodon*. Utilization of GHPC is a sustainable approach to meet the growing requirements of feed-proteins in the expansion of *P. monodon* aquaculture.

Table 1. Growth performance of *P. monodon* fed with varying levels of *G. heteroclada* protein concentrate

Treatments	Parameters		
	%WTGN	SGR	PER
Control	5916.22±265.29^{b}	6.82±0.07^{b}	1.21±0.14^{b}
12.5 %	6221.89±153.43^{b}	6.91±0.04^{b}	0.95±0.04^{ab}
25 %	5920.05±398.38^{b}	6.82±0.11^{b}	1.10±0.05^{ab}
50 %	5359.01±224.21^{ab}	6.66±0.06^{b}	0.85±0.10^{ab}
75 %	3752.40±226.85^{a}	6.07±0.10^{a}	0.64±0.05^{a}

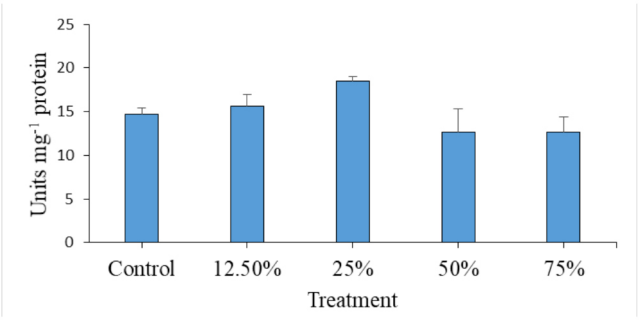


Figure 1. Protease activity of *P. monodon* fed with varying levels of GHPC as soybean meal replacement

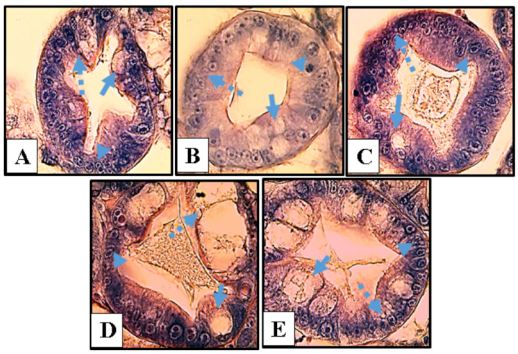


Figure 2. Transverse sections of the hepatopancreas of shrimp after 60 days feeding trial. A-Control, B-12.5% GHPC, C-25% GHPC, D- 50% GHPC, E- 75% GHPC.

GENETIC DIVERSITY OF C*hanna lucius* IN THE MEKONG DELTA, VIET NAM

Sawasawa Wilson* and Duong T. Yen

Can Tho University
College of Aquaculture and Fisheries
Campus 2, 3/2 Street, Ninh Kieu District
Can Tho City, Viet Nam
wilsonsawasawa@gmail.com

In Mekong delta, *C. lucius* is one of endangered and economically important species in Channidae family with high potential for domestication in aquaculture and postoperative medicinal applications in wound healing and reducing discomfort. In the wild, population sizes of this species have been declined due to overfishing, habitat loss and degradation because of human activities. In order to effectively conserve and manage this species, vital information on relevant population genetics is required to provide spatial conservation value of the species to prevent the species extinction. On the contrary, in spite of their economic and scientific importance to date, studies are limited on the genetic diversity of *C. lucius*. The present study was conducted to assess genetic diversity of *C.lucius* by using ISSR markers (Inter-Simple Sequence Repeats).

Samples were collected from four locations (Ca Mau, Can Tho-Hau Giang, Kien Giang and Long An) purposively, 20 to 30 individuals per location totaling to 110 individuals. Through optimization of ISSR markers, eight highly polymorphic markers were used to quantify levels of genetic diversity of different *C. lucius* populations in the Mekong delta which yielded 75 bands with the size range from 500 bp to 2000 bp. Results showed high levels of genetic diversity in the *C.lucius* four populations with unexpected heterozygosity (0.252-0.286), number of effective allele (1.428-1.477), Shannon index (0.365-0.412) and the percentage of polymorphic loci (68-77)% (table 1) . Nei's genetic distance and principal coordinates analysis (PCoA) indicated low genetic differentiation (0.021 - 0.029) with no clusters among the populations (figure1).

This study provides genetic diversity information that can be used for conservation of *C.lucius* and recommends among others control of anthropogenic activities to maintain this current genetic diversity.

TABLE 1. Genetic diversity parameters among *C.lucius* populations

Population	Polymorphism	Ne	I	
Ca Mau	68.83	1.428±0.044	0.365±0.033	(
Can Tho	74.03	1.477±0.041	0.412±0.031	(
Kien Giang	77.92	1.465±0.041	0.406±0.030	(
Long An	75.32	1.466±0.044	0.397±0.031	(
	P-value	0.275	0.308	

Ne: effective alleles, I: Shannon index, uHe: Unbias expected heteroz

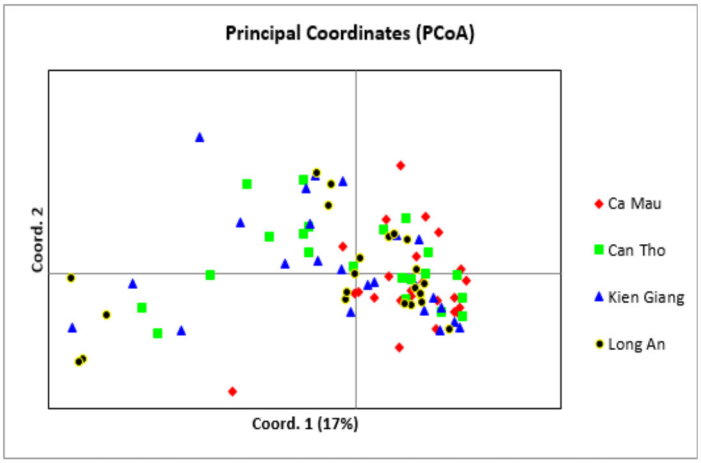


FIGURE 1. Principal Coordinates Analysis (PCoA) of *C.lucius* populations

APPLICATION OF DIRECTED BIOTICS TO CONTROL VIRAL AND BACTERIAL PATHOGENS IN AQUACULTURE

Richard Sayre*, Tatiana Vinogradova-Shah, Elena Sineva, Susan Laramore, Eugene Parsons, Diana Ballestreros, Nancy Mendoza, and Sean Flattery

Pebble Labs
100 Entrada Dr
Los Alamos, NM, USA, 87544
rsayre@pebblelabs.com

RNA interference (RNAi) mediated gene silencing of pathogen genes has been demonstrated to be an effective means to control viral replication. The challenge, however, has been how to continuously produce and deliver RNAi molecules in host animals for pathogen control. Pebble Labs has developed a platform technology called Directed Biotics that continuously produces and delivers RNAi molecules to the host using engineered symbiotic bacteria naturally associated with the host. Previously, we have demonstrated that feeding shrimp a generic RNase III-deficient *Bacillus subtilis* strain expressing dsRNA targeting essential white spot syndrome virus (WSSV) genes could substantially reduce shrimp mortality. Improved *Bacillus* strains were then selected which had the greatest efficacy for reducing pathogen numbers using RNAi. These *Bacillus* strains were then engineered to express a dsRNA targeting the silencing of an essential WSSV gene, Vp19.To test the efficacy of the Directed Biotics WSSV solution, shrimp were fed *Bacillus* expressing dsRNA-Vp19 or commercial food without bacteria for 5 days prior to a White Spot Syndrome Virus (WSSV) challenge by injection. Full protection (zero mortality) was observed at 10 days post WSSV infection for shrimp fed food pellets top-coated with *Bacillus* expressing dsRNA-Vp19 for 5 days prior to WSSV challenge. The negative control group had 85% mortality. Additionally, qPCR analysis of WSSV titer indicated there was a 3-log fold reduction in virus titer in surviving shrimp fed *Bacillus* expressing dsRNA-vp19 compared to the control group. In summary, we demonstrated that the Directed Biotics platform technology can provide full protection against WSSV challenge in the lab. Similarly, we have applied the Directed Biotics technology to control EMS (*Vibrio*) bacterial infections in shrimp. In contrast to viral control strategies, we engineered our Directed Biotics bacteria to express anti-sense RNA (asRNA) to target suppression of the *Vibrio* encoded DNA adenosine methyltransferase (*dam*) gene to reduce *Vibrio* DNA replication and toxin production. Following a *Vibrio* challenge of shrimp pre-feed our asRNA expressing bacteria, we observed an 80% reduction in toxin production and 50% survivability of shrimp challenged with a lethal dose of Vibrio. Similar applications of Directed Biotics technology for the control of viral diseases in plants has resulted in 100% suppression of viral replication demonstrating the broad efficacy of the Directed Biotics platform technology. Collectively, Directed Biotics represents a novel, cost-free means to produce and deliver targeted RNA molecules in hosts to inactivate pathogen genes with no-off target effects; potentially eliminating the need for antibiotics in the field.

EVALUATION OF ATLANTIC SALMON *Salmo salar* PERFORMANCE FOLLOWING PARTIAL REPLACEMENT OF FISH MEAL BY PORCINE HYDROLYSED PROTEIN

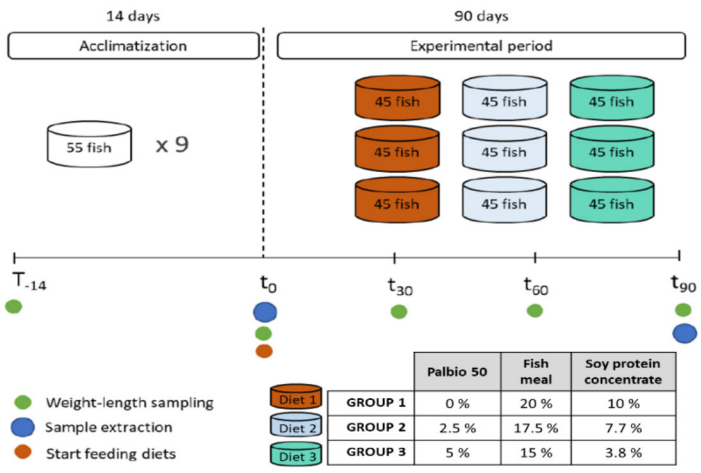
Sergi Segarra*, Nicole Acevedo, Christian Correa.

R&D Bioiberica SAU, Barcelona, Spain
ssegarra@bioiberica.com

Fish meal (FM) is a protein source commonly used in feeds for atlantic salmon (*Salmo salar*), but it is gradually becoming a more expensive and less sustainable resource. The use of alternative plant-based protein sources, especially from soy, as partial replacement for FM is currently common practice but limited due to the presence of antinutrients that negatively affect function and health of the gut of fish. Palbio 50 (Bioiberica SAU, Barcelona, Spain) is a high-quality protein source obtained from porcine intestinal mucosa as byproduct of the heparin manufacturing process, which makes it very sustainable. Palbio 50 is also a source of bioactive peptides. Its inclusion in diets for salmon could allow lower inclusion levels of plant-based protein sources, hence sparing their associated undesired effects, as well as providing economic savings. The objective of the present study was to evaluate the effects of partially replacing FM by Palbio50 on diet costs and performance parameters in salmon which are fed plant-based protein sources as alternative to FM.

Fish were allocated in 1000-L tanks and received different diets (in triplicate) as shown in Fig. 1. All diets used in the study were formulated so that they were isoprotein, isoelectrolytic and isoenergetic. In our study, including Palbio 50 allowed lower diet costs, compared to the control commercial diet. After 90 days, fish experienced a significant weight increase (863 vs 228 g; p=0.0091). Replacing FM by Palbio 50 did not affect fish performance, as there were no significant differences (p>0.05) between groups in weight, SGR, GF3, FCR and SFR (Fig. 2). No significant changes in water temperature or oxygen saturation occurred (around 12.5 ºC and 99.5 %).

In conclusion, the use of Palbio 50 in salmon diets, as partial replacement of fish meal, allows a reduction in the diet cost and leads to non-inferiority in terms of performance. Therefore, Palbio50 represents a more sustainable and inexpensive alternative protein source to FM without affecting the adequate performance of fish.



	Palbio 50	Fish meal	Soy protein concentrate
GROUP 1	0 %	20 %	10 %
GROUP 2	2.5 %	17.5 %	7.7 %
GROUP 3	5 %	15 %	3.8 %

FIGURE 1. Trial design and details of diets used in each group.

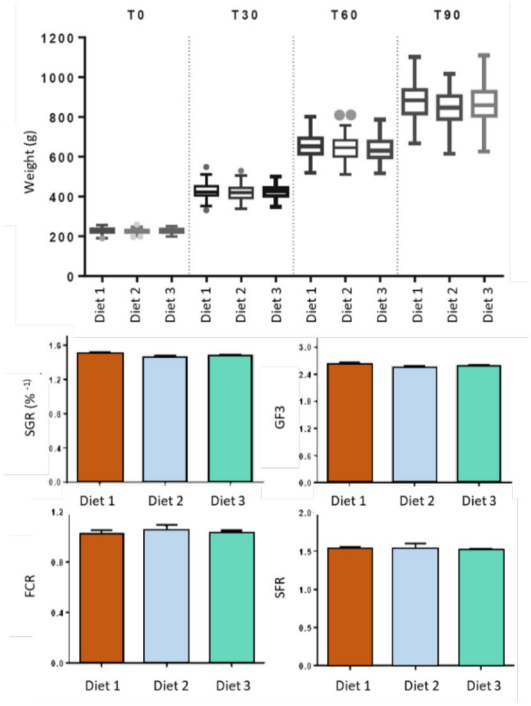


FIGURE 2. Changes in performance parameters.

DEVELOPMENT OF AN EFFECTIVE AUTOGENOUS VACCINE FOR STRIPED CATFISH (*Pangasianodon hypothalmus*) in VIETNAM

Eng Khuan Seng [1] & Tu Thanh Dung [2]

[1] Nanyang Polytechnic
School of Chemical & Life Sciences
180 Ang Mo Kio Ave 8, Singapore 569830
Seng_Eng_Khuan@nyp.edu.sg

[2] Can Tho University
Department of Aquatic Animal Pathology
College of Aquaculture and Fisheries
3/2 Street, Xuan Khanh Ward
Ninh Kieu District, Can Tho City

Vietnam is one of the largest producer of striped catfish (*Pangasianodon hypothalmus*) in the world. The contuining high demand for pangasius has led to local farmers trying to boost production capacity by increasing fish stocking density. This practice has then led to severe disease outbreaks caused by several bacteria such *Aeromonas hydrophila, Edwardsiella ictaluri* and *Flavobacterium columnare*.. which can kill up to 50-80% of fish stocks. *Edwardsiella ictaluri* causes white spot disease, while *Aeromonas hydrophila* causes septicimia and hemorrhage in catfish. Both diseases occur during the production cycle, leading to high mortalities, and may lead to great economic losses. To treat the infections, farmers often used antibiotics readily leading to the development of antibiotic resistance in the industry and the environment. As such exploring other approaches such as vaccination is required to curb the massive loss of fish due to the diseases. Although, commercial vaccines for *E. ictaluri* and *A. hydrophila* have been developed and deployed by PHARMAQ since 2013, only limited success has been reported at fish farms in Vietnam. Hence, herein, we proposed the development of autogenous fish vaccines (customised vaccines), which should be more effective in preventing the disease at a particular farm affected by the bacteria infections.

To begin autogenous vaccine development, several bacteria strains were isolated from diseased fish from a farm located in Can Tho District, Vietnam. Isolation and characterisation of the bacteria type was done using conventional microbial isolation techniques and then identified using PCR. Pathogenicity studies using the bacterial isolates were then conducted using live fish. From the results obtained, a pathogenic isolate of *Aeromonas hydrophila*, designated as AH-3 and *Edwardsiella ictaluri*, designated EI-36 was selected for the study. AH-3 was grown in Tryptic Soy Broth (TSB) medium and EI-36 was grown in Brain Heart Infusion broth (BHI) medium for 24-48h. The grown bacteria was then inactivated using formalin for 24h and sterility test was conducted to ensure that complete inactivation was achieved and no contaminants were present. These preparation results in AH-3 autogenous vaccine (AH-3v) and EI-36 autogenous vaccine (EI-36v). AH-3v and EI-36v, were then mixed in a 1:1 ratio (termed as 2 in1 vaccine) and injected into a group of juvenile catfish (10-15g). Several control groups were also incuded. After 14 day post vaccination, the fish were then challanged using either live pathogenic AH-3 or EI-36 bacteria via i.p injection, and mortality was recorded daily for 14 days post challenge. Results indicate that in the 2 in 1 vaccine group, 0% mortality was observed after AH-3 challenge compared to control (no vaccine: 90% mortality). In another 2 in 1 vaccine group, EI-36 challenge resulted in 40% mortality compared to 80% mortality in control group (no vaccine). This clearly showed that the 2 in 1 vaccine was 100% effective in preventing *Aeromonas hydrophila* infection and 50% effective in preventing *Edwardsiella ictaluri* infection in vaccinated fish. We are in the midst of revising the dose, vaccine preparation and delivery method for *E. ictaluri* & *A. hydrophila* (2 in 1) in order to achieve maximal protection. In summary, we clearly demonstrated that using autogenous vaccine route, full protection against *A. hydrophila* and 50% protection against *E. ictaluri* could be achieved.

SEAFOOD HACCP INSPECTION PROCESS BY THE UNITED STATES FOOD AND DRUG ADMNISTRATION

Stanley Serfling

US Food and Drug Administration
Center for Food Safety and Applied Nutrition
5001 Campus Drive
College Park, Maryland 20740
stanley.serfling@fda.hhs.gov

Ensuring the safety of the food supply continues to be a top priority for the United States Food and Drug Administration (FDA). To determine compliance with FDA laws and regulations, the FDA conducts seafood HACCP (Hazard Analysis Critical Control Point) inspections before these products may enter commerce in the United States. The goal of the foreign inspection program is to help ensure that foods exported to the United States are safe and meet USFDA regulatory requirements. Countries are selected based on several factors including the volume of imports, the nature of the product (high or low risk) and the violation history of the firm/country.

The components for a seafood HACCP inspection are as follows:

1. Conduct an initial interview
2. Observe facility Operations and sanitation, with a walk-thru
3. Conduct a Hazard Analysis
4. Evaluate the processor's HACCP plan
5. Assess the implementation of the firms HACCP plan
6. Assess the firms Sanitation and Good Manufacturing Practices (GMPs)
7. Review the firms Record keeping
8. Document and firm Violations (FDA from 483)
9. Conduct Close-out Interview
10. Write Establishment Inspection Report (EIR)

In addition to the international food inspection program, the FDA has a border surveillance and sampling program intended to promote cooperative relationships with foreign regulatory authorities and assist in building confidence in their seafood regulatory programs.

DATAMINING FOR AQUACULTURE PRODUCTION ANALYSIS

R. Serradeiro*, G. Zarifis, J. Leitão, S. Cardoso, H. Abreu, R. Severino, A. Nobre

RIASEARCH, Murtosa, Portugal
renataserradeiro@riasearch.pt

Aquaculture operations generate a large number of variables and data. Data science methodologies can support informed decision making. However, given the emergence of this field, farmers are still not using these techniques to improve their decisions and knowledge about their production. The objective of this presentation is to illustrate the application of data analytic methodologies in a bream farm and in a seaweed farm in the context of the Valormar project (24517 supported by Compete2020, Lisboa2020, CRESC Algarve2020, PT2020 and the EU through FEDER/ERDF). Before carrying out the relevant data analytics a famer should define specific objectives and questions to be analysed. Also, a data flow for the analysis is needed, which requires a data management system for data gathering and assimilation. Datamining is an on-going task, during which are identified new questions, variables to be collected and further analysed.

The first step to make available advance analytics to production managers is to jointly explore the data with visual datamining, in order to give visual tools for identification of drivers of growth, mortality, among other. As an example of the data exploration carried out at the fish farm, Figure 1 shows the influence of the start month of production in the fish growth per day and feed conversion ratio.

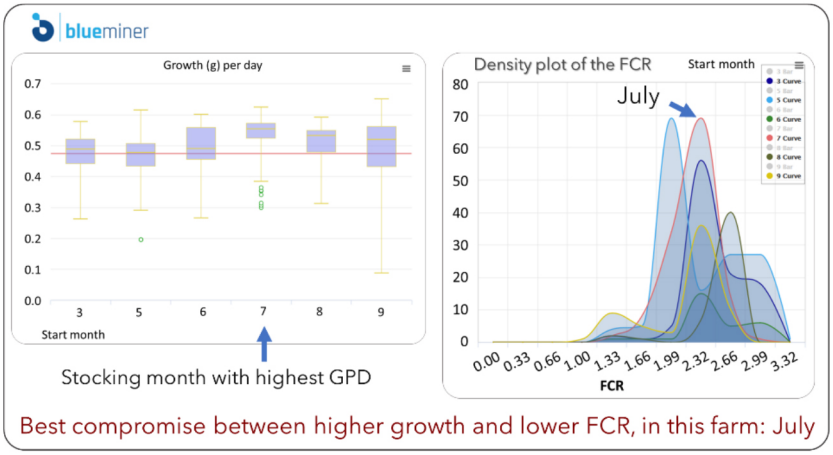


Figure 1. Influence of stocking month on bream production performance.

Machine learning algorithms were applied to create statistical models from historical data for prediction of key performance indicators (KPI) based on selected variables (predictors). Figure 2 illustrates the application of a Random Forest model to calculate the seaweed net harvest density of a given tank based on solar radiation, number of light hours, days in production and seeding density.

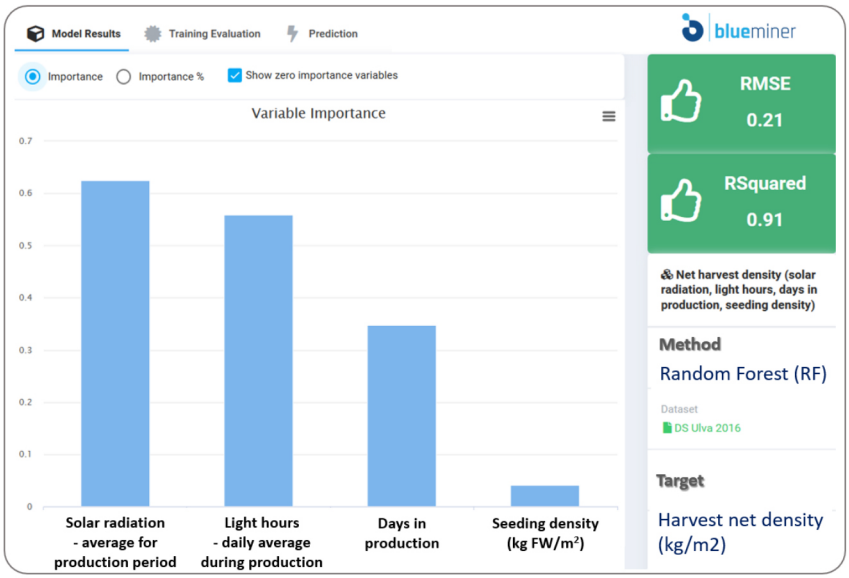


Figure 2. Variable importance for prediction of KPI at the seaweed farm.

A NEW INTERDISCIPLINARY MODELLING APPROACH TO STUDY NUTRIENT FLUXES IN AQUACULTURE AND ECOSYSTEMS

Sowdamini Sesha Prasad*, Julia L. Blanchard, Duncan D. Cameron, Chris G. Carter

Institute for Marine and Antarctic Studies
University of Tasmania
Private Bag 129, Hobart, TAS 7001
sowdamini.seshaprasad@utas.edu.au

To implement an ecosystem-approach to inform aquaculture production and management strategies, understanding the relationship between aquafeeds and aquaculture species is essential to maximise animal growth performance, quantify the flow of nutrient elements and minimise waste output. A new bridging framework, geometric stoichiometry (GS) unifies the disciplines geometric framework for nutrition (GFN) and ecological stoichiometry (ES) which share the central concept of nutrient homeostasis by extending the equations of ES to include GFN core concepts. It uses macromolecules as currencies and dietary regulation to balance nutrient deficits and excesses by the animal. In this study, we explore the feasibility of using GS approach to investigate how different formulated feed ingredients affect animal nutrient intake to maintain C:N homeostasis, growth and waste output using three opportunistically available test cases for slipper lobster *(T.australiensis*), a new species of interest for commercial aquaculture. Model outputs indicate that different crude protein sources and dietary inclusion contribute to the most variation in nutrient intake and growth performance.

GS model predicts highest nitrogenous waste output for soybean meal which has the lowest crude protein content whereas krill meal had the lowest waste output despite having the highest crude protein content, which can be attributed to the differences in absorption efficiencies. This highlights the need for targeted nutrition experiments to obtain feed and species-specific parameters to refine the slipper lobster GS model. The model will facilitate better understanding of rock lobsters' growth performance in response to feed supplied, maximise growth performance, minimise waste output and inform EAA approaches to commercial establishment of rock lobster aquaculture.

BLACK SOLDIER FLY LARVAE *Hermetia illucens* AS A PROTEIN ALTERNATIVE IN BARRAMUNDI *Lates calcarifer* AQUACULTURE DIETS

Isobel Sewell*, Gavin Partridge, Julian Partridge, Jan Hemmi

Aquaculture and Native Breeding Facility
University of Western Australia
Shenton Park, Western Australia 6008
Isobel.Sewell@research.uwa.edu.au

As food insecurity becomes a growing issue, global food sectors are forced to maximise production whilst conserving the worlds remaining resources. The aquaculture sector is one of the fastest growing protein sectors. However, many sought after species are carnivorous, with high dietary protein requirements, often delivered in the form of wild caught fish (fishmeal; FM). The current demand for fishmeal is beginning to outweigh supply, prompting the increased importance of the management of fishery resources and the progression towards a more sustainable industry from a food security perspective.

Insects are a natural part of many freshwater and marine carnivorous finfish diets. Black soldier fly larvae (*Hermetia illucens*; BSF) are of particular interest as a substitute to FM as they efficiently convert organic waste (e.g., fruit and vegetable waste) into their own biomass, resulting in high protein and fat concentrations. Current literature reports the use of BSF in several aquaculture species diets, however results are variable. This may be attributed to trials adopting either a defatted insect meal (DBSF) or a whole insect meal (WBSF). To this date, no study has directly compared the performance of the two insect meal products.

The aim of this research was to evaluate the effects of BSF on juvenile barramundi (*Lates calcarifer*) growth, well-being and feed utilisation. Eight experimental diets contained graded levels of either DBSF or WBSF as a dietary replacement to FM or plant-based protein. Each diet was randomly allocated to one of 24 tanks in a blocked design, with three tanks per diet and 24 fish (38.38 g ± 0.23) per tank. Fish were hand fed the experimental diets, once daily, for 91 days. At the conclusion of the trial, weight and fork length were recorded.

The results showed that barramundi growth performance, well-being and feed utilisation did not differ between the eight experimental diets ($P > 0.05$; Table 1). This study, therefore, demonstrates BSF suitability as a protein source. Furthermore, the results suggest that BSF can be utilised as either a defatted or whole product, and in replacement to FM.

ENCAPSULATED PROBIOTICS TO IMPROVE THE GROWTH AND HEALTH OF *Haliotis iris*

Ali Seyfoddin*, Seyedehsara Masoomi Dezfooli, Noemi Gutierrez-Maddox and Andrea Alfaro

*Drug Delivery Research Group, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand
ali.seyfoddin@aut.ac.nz

The seafood industry has been rapidly moving toward high-intensity aquaculture to prevent the exploitation of wild stocks and address ever-growing demands. However, disease outbreak is an inevitable outcome of intense aquaculture. Farmers attempts to control diseases using antimicrobial drugs which is the main cause for concerns about development of drug-resistant organisms. Probiotics and natural immunostimulants are alternatives to combat disease occurrence in a more sustainable aquaculture practice. However, application of these beneficial bioactives are complicated due to ineffective delivery methods such as immersion and injection. These methods are often inefficient due to lack of control over the stability and dosage of delivered bioactives and possible environmental contamination.

This study used encapsulation techniques to develop a controlled release system for delivery of probiotics and nutrients to black-footed abalone (*Haliotis iris*). A multi-layer bead structure was developed to immobilise three probiotic bacteria (*Vibrio* sp., *Exiguobacterium* sp. and *Enterococcus* sp.). The release of the encapsulated probiotics in seawater was measured using a fluorescence microscope. A feeding trial was performed to assess the effect of the encapsulated probiotic food on growth and health of juvenile abalone. Health parameters including reactive oxygen species (ROS) and viability of hemocytes were measured using flowcytometry. A metabolite profiling study of abalone foot muscle was carried out using gas chromatography-mass spectrometry (GC-MS).

The probiotics were successfully encapsulated in the developed beads with the encapsulation efficiency of 50-90%. The encapsulated probiotic beads showed high stability in seawater with less than 1% matrix erosion after 72 hr. Additionally, low bacterial release in seawater and high probiotic bacterial load (10^8 CFU) within the GIT of abalone demonstrated the successful delivery of viable probiotic bacteria to abalone using the developed beads. An improved growth performance observed in abalone fed with the encapsulated probiotic food compared to control animals. Flowcytometric analysis revealed lower level of ROS and high hemocyte viability (> 90%) which indicate better health parameters in encapsulated-probiotic fed abalone. Metabolomics study revealed significant variations in the profile of organic acids in encapsulated-fed abalone compared to control animals. In conclusion, this study demonstrated that encapsulation techniques can be used to design efficient carriers for delivering bioactives in aquaculture to enhance the production in a sustainable manner.

IS THE SALINITY INCREASE IN LAGOON SIVASH A PROBLEM OR OPPORTUNITY TO AQUACULTURE DEVELOPMENT?

Nickolai Shadrin*, Elena Anufriieva

A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, 2 Nakhimov Ave., 299011, Sevastopol, Russia

Lagoon Sivash, the bay of the Sea of Azov, was the world's largest hypersaline lagoon (area 2600 km2) with average salinity of 140 g L^{-1} before 1963. In the Crimea, biggest peninsula in the Black Sea was a lack of freshwater, and the area around the bay was sparsely populated. To solve this problem, in 1963–1975, the North Crimean Canal was built supplying freshwater from the Dnieper River. After this, intensive irrigated agriculture development of was becoming. Runoff waters from the irrigated fields were dumped into Bay Sivash. In the lagoon, the salinity gradually decreased, and in 1997, it was only 17 g L^{-1}. Several important fish species came from the Sea of Azov into the lagoon, their abundant populations formed, and intensive fishery developed. In April 2014, the Ukrainian government decided to stop supplying Dnieper water into the North Crimean Canal. Discharge of freshwater almost finished into the lagoon. As our study showed, salinity gradually increased up to 70–100 g L-1 in the lagoon. There are no fish in Sivash currently, and fishery died leading to collapse of fishermen community here. Irrigated agriculture was also ineffective in this area now. Most people recognized this as huge social catastrophe without any perspective. Our integrated ecosystem study in Lagoon Sivash in 2015-2019 showed that there is intensive development of several algae and animal species which are very promising for aquaculture. In the first place, Lagoon Sivash may get to be one of main habitats of *Artemia* in the world providing a significant supply of its cysts for aquaculture development worldwide. Base for this not only harvesting of cysts from wild population but pond cultivation of *Artemia*. There are all resources to develop hypersaline pond aquaculture here: hypersaline waters, large areas of salt marshes and salt dried land, and a lot of free hands looking for job. There are also other valuable biological resources in Sivash which can be successfully used (filamentous green algae, larvae of Chironomidae, Amphipoda, etc.). In new situation, hypersaline aquaculture can give new opportunities for social and economic development here.

This study was supported by the Russian Science Foundation (grant 18-16-00001).

CAN AQUAPONICS HELP AQUACULTURE?

Maryam Shafahi*

mshafahi@cpp.edu
California State Polytechnic University, Pomona, CA 91768

Aquaponics is an environmentally friendly method of food production benefiting from the integrated cycle of aquaculture and hydroponics. It uses a closed-loop water cycle with little run-off to the environment and minimum requirement for plant fertilizer. Considering the integrated nature of aquaponics and its semi-sustainable cycle, the question to answer is: can aquaponics be a solution to the problems aquaculture industry is facing? Fresh water is scarce around the globe and water pollution is an ever-increasing concern for aquaculture industry.

This research on the water efficiency of an outdoor semi-commercial aquaponics system growing lettuce and tilapia located in California State Polytechnic University at Pomona shows the annual water consumption of a raft bed system. The results can be compared to the average water discharge of a RAS system in southern California and the world. The findings can be used to analyze and evaluate whether converting RAS to aquaponics either partially or fully will be a feasible solution for a less polluting food production system.

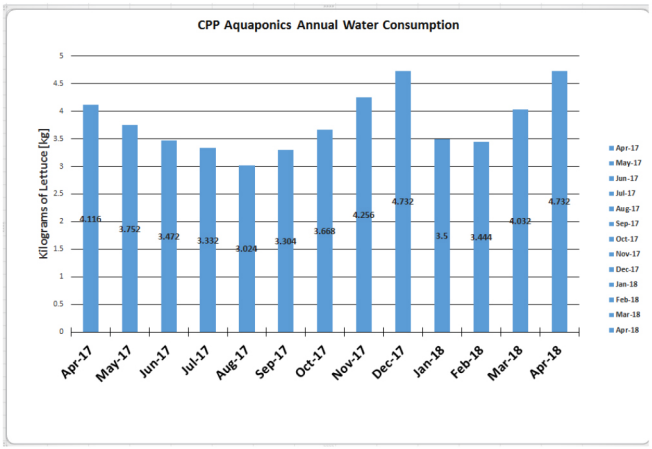


Figure 1- The annual water consumption in an outdoor aquaponics system located in California State Polytechnic University

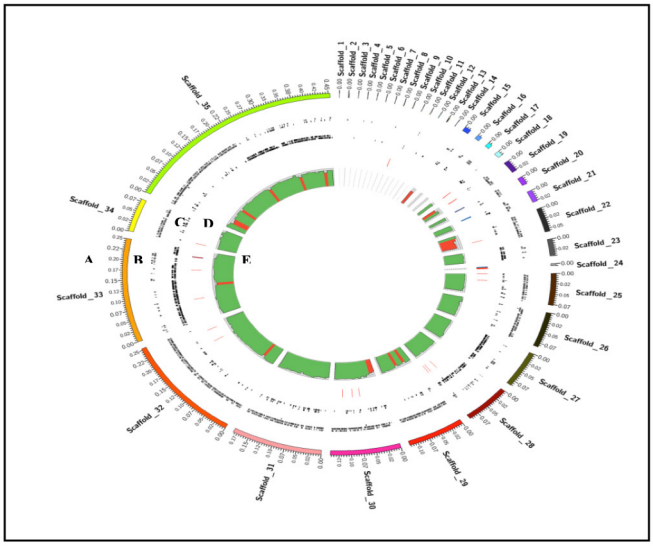
WHOLE GENOME SEQUENCE OF EMERGING ZOONOTIC BACTERIAL PATHOGEN *Lactococcus garvieae* RTCLI04 ISOLATED FROM FOOD FISH, RAINBOW TROUT (*Oncorhynchus mykiss*) IN INDIA REVEALED PRESENCE OF SEVERAL VIRULENT AND ANTIMICROBIAL RESISTANCE GENES IN ITS GENOME

Neetu Shahi* and Sumanta Kumar Mallik a

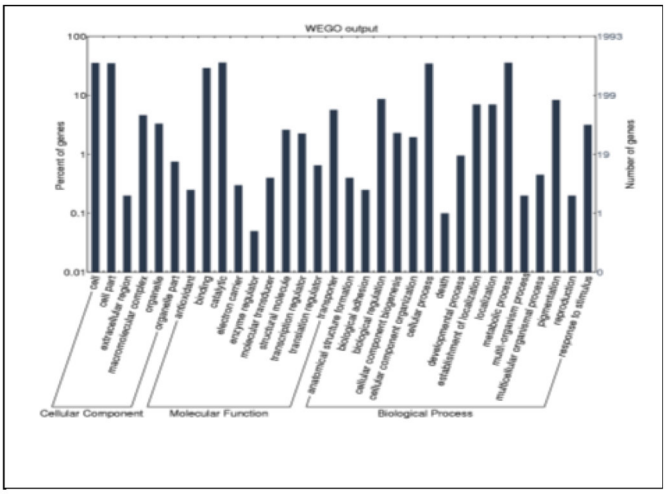
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal, 263 136, Nainital, Uttarakhand, India
*Neetu Shahi; E mail: shahineetu@rediffmail.com; neetuicar@gmail.com

Lactococcus garvieae is one of the deadliest, emerging zoonotic bacterial pathogen, causes fatal haemorrhagic septicaemia in a wide range of cultured fish species and animals, worldwide. Here, we report the whole genome sequence (WGS) of *L. garvieae* type strain RTCLI04, isolated from farmed rainbow trout, *Oncorhynchus mykiss* in India showing clinical signs of "warm water lactococcosis". The genome of *L. garvieae* RTCLI04 is a single circular chromosome of 2,054,885 base pairs (bp), which encodes 1,993 proteins and has G+C content of 39%.

The WGS analysis revealed the presence of 51 genes for tRNAs (including 2 pseudogenes), 6 genes for rRNAs (4 genes for 5s rRNA; 1 gene for 16s rRNA and 1 gene for 23s rRNA), 5 virulent domains and 28 different genetic pathways. Comparative analysis of *L. garvieae* RTCLI04 genome with two existing reference genomes, *L. garvieae* ATCC 49156 (avirulent strain) and *L. garvieae* Lg2 (virulent strain) revealed that pangenome consisted of 2,239 protein-coding genes in which 1,850 (83%) genes is core gene, 389 genes (17%) is dispensable gene and 19 genes were unique to our strain. Similar to *L. garvieae* ATCC 49156, *L. garvieae* RTCLI04 of current study lacks genomic island of 16.5 Kb capsule gene cluster. In addition, 39 virulence associated genes (VAGs) including *hly1,-2,-3*; *PavA*, *PsaA*; *eno*; LPxTG containing surface proteins 1, 2, 3 and 4; *pgm*, *sod* and 29 antimicrobial resistant genes (ARGs) including *mefE* (clindamycin), *srmB* (lincomycin), *dfrA26* (trimethoprim), *gyrB* (nalidixic acid), *arr*-3 (rifampin), *otrB* (tetracycline), *aac(6)-Ic* (tobramycin), *IrgB* (penicillin), *mecA* (oxacillin), *vanRB* (vancomycin) and *mfpA* (fluoroquinolone) were also present in the genome of *L. garvieae* RTCLI04. Our study provides new insight into understanding the virulence mechanism, AMR and development of therapeutic measures against *L. garvieae* disease outbreak in aquaculture.



Circular representation of the *L. garvieae* RTCLI04 genome



GO term for the identified genes of *L. garvieae*

ASSESSMENT OF CALCIUM CARBONATE EFFICIENCY IN ADJUSTING AMMONIA CONCENTRATION IN AQUACULTURE SYSTEMS

Pawan Kumar Sharma*, J. Stephan Sampath Kumar, V Senthil Kumar, S. Anand

Dr. M.G.R. Fisheries College and Research Institute Ponneri
Tamil Nadu Dr. J. Jaylalithaa Fisheries University, Tamil Nadu- 601204
Email- pawan@tnfu.ac.in

The experiment was conducted at Directorate for Sustainable Aquaculture Thanjavur (10° 47' 13.1964'' N and 79° 8' 16.1700'' E), Tamil Nadu Dr. J. Jayalalithaa Fisheries University, for a period of two month from 10th April 2019 to 10th June 2019. Six cemented tanks of 35 liter capacity filled with pond water were used to carry out the experiment. To increase the ammonia level the dose of urea and cow dung was calculated (0.5 gram and 50 gram respectively) for 35 liter water and applied in all the selected water tanks equally. Three treatments (T1, T2, and T3) and two replications (R1 and R2) for each treatment were performed. 5 gm dose of $CaCO_3$ for T1R1, T1R2, 10 gram for T2R1, T2R2 and 15 gram for T3R1, T3R2 were used to correct the ammonia level. The maximum ammonia and pH level observed were 1.7 ± 0.670 mg/ltr and 8.395± 0.12 respectively in tank number T2R1 while the minimum dissolved oxygen level was found 1.5 ± 0.712 mg/ltr in the same tank. The nitrite and nitrate level were remained below detectable levels in all tanks. The maximum suitable dose of $CaCO_3$ was calculated at 1428.57 Kg/ Hectare. Ammonia decreased with increasing lime concentration in the medium. It could be concluded that Liming affected positively and directly organic matter decomposition accumulated in the sediment. This is extremely important, since high concentrationsof organic matter in the sediment may result in anaerobic conditions at the soil-water interface which can lead to production of obnoxious gases.

Table No. 1 Water quality parameters before applying the lime

S.No.	T1R1	T1R2	T2R1	T2R2	T3R1	T3R2
pH	8.42± .17	8.44± .13	8.41± 0.16	8.44± 0.13	8.53±0.5	8.23± 0.12
D.O.	2.25± .61	2.27± 0.71	2.22± 0.53	2.22± 0.53	2.29±0.7	2.37± 0.59
Ammoni	1.55±0.68	1.63±0.75	1.54±0.65	1.59±0.76	1.45±0.6	1.41±0.68
Nitrite	0	0	0	0	0	0
Nitrate	0	0	0	0	0	0

Table No. 2 Water quality parameters after applying the lime

S.No.	T1R1	T1R2	T2R1	T2R2	T3R1	T3R2
pH	8.56±0.21	8.54±0.21	8.48±0.12	8.46±0.10	8.71±0.22	8.76± 0.35
D.O.	2.75±0.32	2.74±0.22	2.90±0.52	3.22±0.33	3.10±0.50	3.01± 0.48
Ammonia	0.805±0.1	0.685±0.6	0.31±0.24	0.25±0.18	0.24±0.24	0.2± 0.15
Nitrite	0.04±0.08	0.05±0.10	0.14±0.16	0.13±0.16	0.14±0.17	0.13± 0.15
Nitrate	0	0	0.04±0.09	0	0.03±0.06	0.04± 0.07

THE CULTURE OF ASIAN SEABASS *Lates calcarifer* AND FRESHWATER GAINT PRAWN *Macrobrachium rosenbergii* IN THE AQUAPONICS SYSTEMS

*JaiGopal Sharma, Ankit Yadan and Rina Chakrabarti

*Department of Biotechnology, Delhi Technological University, Delhi 110 042, India
e-mail: sharmajaigopal@yahoo.com

Aquaponics, the bio-integration of recirculating aquaculture system (RAS) and hydroponic is an eco-friendly crop production system. The Aquaponics system maximizes the production using less water compared to the traditional practices for production of fish and plants separately. The early fry of Asian seabass *Lates calcarifer* (0.456±0.003 g) and post larvae of giant freshwater prawn *Macrobrachium rosenbergii* (0.035±0.001 g) were cultured in Aquaponics System and one static water culture (contro) system. The Aquaponics system was consisted of three tanks (each 480 L) for the culture of fish/prawns and one filtration unit containing pebbles and broken earthen pots (30 cm) and plants *viz*., water spinach *Ipomea aquatica* and helancha *Enhydra fluctuans*. In the control system, three tanks (480 l each) were used for the culture of fish/prawns without plants and circulation of water. In the control system, 10% of water was replaced at weekly interval to maintain the water quality. Fish and prawns were fed with pelleted diet (protein 40%) ad libitum twice every day. The duration of culture was 90 days.

The final average weight of seabass was significantly ($P < 0.05$) higher in Aquaponics system compared to the static control system. Similarly, the average weight of prawn was significantly ($P < 0.05$) higher in the former system compared to the later. There was no significant ($P > 0.05$) difference in the moisture, protein, lipid and ash contents of seabass and prawns cultured in Aquaponics and static water systems. The amino acid composition study showed that all ten essential amino acids were present in the cultured seabass and prawns. Among essential amino acids, lysine was the dominant (19.62%) amino acid in seabass and leucine (18.17%) in prawns. The glutamic acid was the most dominant non-essential amino acid found in seabass and prawns. Among various fatty acids, n-6 polyunsaturated (n-6 PUFA) and saturated fatty (SFA) were maximum in seabass and prawns, respectively. There was 1260 L of extra water expenditure in the control system compared to the Aquaponics System due to replacement of 10% water at weekly intervals.

NEUROLOGICAL SIGNS IN GREY MULLET *Mugil cephalus* CAUSED BY *Vibrio harveyi*

Galit Sharon* and Rosa Ines Strem

Israel Oceanographic & Limnological Research Ltd., National Center for Mariculture
P.O. Box 1212, Eilat 88112, Israel

Consumption of flathead grey mullet (*Mugil cephalus*) has been growing worldwide. The National Center of Mariculture (NCM) in Eilat (Israel) is one of the major producers in the country, providing fingerlings from breading stocks held in captivity to local and foreign fish farmers. In recent years, the mullet breeding stocks and fingerlings have been showing neurological signs such as uncoordinated circular swimming and oral hemorrhages. A few days after the onset of the clinical signs, the fish die. No ecto- or endo-parasites were found. Three different cell lines were inoculated with brain extracts of clinically diseased fish and incubated at different temperatures. A cytopathic effect (CPE) was observed after several days of incubation, however, the cells recovered and CPE was no longer evident. Histological analysis indicated cavitation in the brain tissue. PCR, RT-qPCR and RNA from 24 samples processed for whole genome sequencing with MiSeQ Illumina Nextera XT using specific primers gave negative results for viral nervous necrosis (VNN) or "viral encephalopathy and retinopathy" (VER).

Bacteriological analysis showed different *Vibrio* species present in the liver, spleen and kidney. However, *Vibrio harveyi* was the bacterium most frequently isolated from brain tissue samples. PCR using a set of primers for the hemolysin and Tox R genes, regulating *Vibrio harveyi* virulence factors gene expression, showed positive results in all the brain tissue samples as well as in bacteria cultures isolates from clinically sick fish. An experimental intraperitoneal re-infection with *Vibrio harveyi* isolated from a clinically diseased fish, showed neurological signs and oral hemorrhages 5 days post infection. A comparative analysis of the 16S gene from both the bacteria used for infection and the ones isolated from brain of a clinically diseased fish showed a 100% sequence similarity. DNA and RNA samples from both bacterial isolates gave positive results for both hemolysin and Tox R genes. We therefore concluded that *Vibrio harveyi* was the cause of the neurological symptoms observed. To the best of our knowledge, this is the first report describing *Vibrio harveyi* causing neurological symptoms in grey mullets. Furthermore, we have analyzed the toxicity of the proteins secreted by *Vibrio harveyi* on cell culture and identifies those using proteomics. Different preparation using *Vibrio harveyi* were tested as potential short term vaccines against a controlled infection. *Mugil cephalus* showed 100% protection from *Vibrio harveyi* infection using these preparation and the fish immune responses were analyzed.

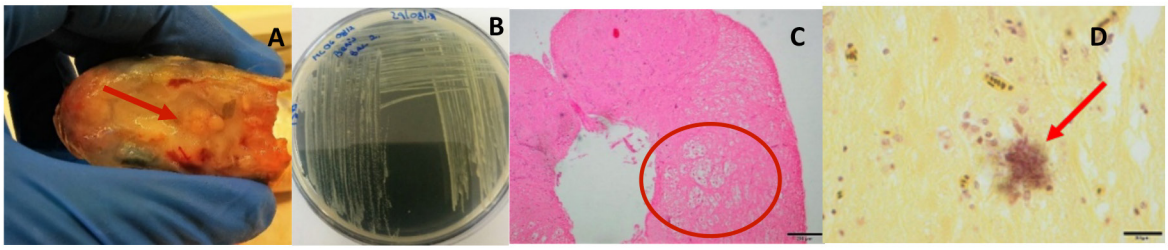


Fig 1: A) yellow viscous fluid surrounding the brain of clinically diseased mullet (arrow). B) *Vibrio harveyi* isolated on TSA agar from fish brain presenting clinical signs. C) Histological analysis of brain (H&E), red circle shows tissue damage of a clinically sick mullet. D) Histological analysis of brain (Gram stain), arrow pointing at bacteria accumulation.

LOW FISH MEAL FEEDS FOR LARGEMOUTH BASS (*Micropterus salmoides*): EFFECTS ON HEPATIC TRANSCRIPTOME PROFILES

Xueyan Shen[1*], Katheline Hua[1,2*], Anne Kristine Hansen[3], Svend Jørgen Steenfeldt[3], Dean R. Jerry[1,2]

[1]Tropical Futures Institute, James Cook University Singapore, 149 Sims Drive, 387380 Singapore
[2]Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, 1 James Cook Drive, Townsville QLD 4811, Australia
[3]Biomar AS, Bolstadvegen 24, 8430 Myre, Norway
[*]Xueyan Shen; E-mail: xueyan.shen@jcu.edu.au
Katheline Hua; Email: katheline.hua@jcu.edu.au

Sustainable largemouth bass (*Micropterus salmoides*) culture development warrants a reduction of the inclusion levels of fish meal in feeds while maintaining optimal fish growth performance and nutrient utilization. The present study aimed to elucidate whether the low level fish meal diets affect largemouth bass growth and hepatic transcriptome profiles. Four isonitrogenous and isoenergetic diets containing 5% (FM5), 15% (FM15), 25% (FM25) and 35% (FM35) of fish meal were fed to fish individuals for 8 weeks in a recirculation system. The results showed no significant differences in specific growth rate and feed efficiency among treatment groups. Comparative analysis of hepatic transcriptome showed that the primarily identified genes involved in metabolic and immune pathways were modulated significantly, with the differentially expressed genes (DEGs) involved in lipid metabolism and transport (e.g. *apoa1, fads2*, *fatp, fasn, elovl5* & *elovl6*) were mostly up-regulated in the fish fed with FM5 and FM15 diets, whereas numerous immune related genes (e.g. *il1b, tlr2, ccr9, blnk* & *cd22*) showed significant lower expression in both groups. In addition, several key antioxidant and stress related genes (*sod1*, *gpx3*, *cat*, *gst* & *oxr1*) showed elevated expression in the liver of fish fed with FM5 diet when compared to the FM35 diet. The present study suggested the potential impact of low fish meal diets on the metabolism and health of farmed juvenile largemouth bass, which will further contribute to optimal and sustainable feed formulations for the aquaculture industry of the species.

SPECIES DIVERSIFICATION THROUGH THE INTRODUCTION OF RESILIENT FISH VARIETIES: A POTENTIAL CLIMATE-SMART ADAPTATION STRATEGY FOR ODISHA, INDIA

Neetha Shenoy*, Sourabh K. Dubey, Amar Gaikwad, Arun Padiyar, Chadag V. Mohan

WorldFish, Cuttack, Odisha 753001, India
*neetashenoy09@gmail.com

Climate change has dramatic effects on the aquatic food production systems in Odisha, albeit not all elements are affected equally. Since 21% of Odisha's landmass is flood-prone, inland freshwater farming in some portions of the mainland and coastal peripheral areas are particularly vulnerable to severe inundation. Farming systems located in the western and southwestern parts of Odisha often encounters water scarcity due to prolonged summer. Meanwhile, over-dependence on a single or specific species in aquaculture is one of the major constraints that has frequently resulted in production failure by aggregating risks for farmers. Therefore, species diversification through the incorporation of hardy and stress-tolerant fish varieties into the existing aquaculture system is a climate-smart solution in the current altered scenarios. For integrated development in the fisheries sector and fish farmers, the Fisheries and Animal Resources Development Department (FARD), the government of Odisha in collaboration with WorldFish has introduced genetically improved farmed tilapia (GIFT) and Amur common carp throughout Odisha intending to diversify farm income, improve nutrition, and increasing resilience among small-scale farmers. GIFT is a genetically improved variety of Nile tilapia (*Oreochromis niloticus*) developed by WorldFish and its partners through long-term research and a selective breeding program intending to develop a faster-growing strain suitable for both small-scale and commercial aquaculture. Amur common carp (*Cyprinus carpio*) is a genetically improved strain of wild common carp of Hungarian origin developed by the Karnataka Veterinary, Animal and Fisheries Sciences University, India. GIFT and Amur common carp can be considered champion species in the light of the climate-smart farming approach. Both are the most accommodating species, able to withstand a wide range of temperature and salinity conditions. GIFT and Amur common carp can thrive in a variety of harsh environments (e.g., low dissolved oxygen, turbid water, etc.) disease resistance, and omnivorous feed habits.

As part of the centered intervention, 712,315 monosex GIFT tilapia fry were developed from a prototype GIFT hatchery at the Kausalyaganga government fish seed farm between January 2020 and March 2021, and sold to farmers from various districts in Odisha and other states across the country. The government is converting the prototype GIFT hatchery into a cutting-edge GIFT tilapia hatchery with a production capacity of 5 million seeds annually in order to meet increased demand. The FARD department is also expanding Amur common carp culture and already raised 2000 nos. of broodstocks in 2019–20. After April 2020, seed production began in all government fish seed farms, with a total of 178,000 spawns produced and sold to Odisha farmers. This strategy will have a significant impact on the purchasing and consumption patterns of poor and lower-middle-class families at the bottom of the market pyramid. These fishes are cheap sources of animal protein and high in vitamins, minerals, and micronutrients. In addition to providing income and job opportunities, it is vital for food security and healthy nutrition. Further, it could also be conceived as a possible climate-resilient adaptation plan for climatically vulnerable regions. From a sustainability standpoint, high-input monoculture must be shifted towards a low-input polyculture system with diverse resilient species. This form of farming reduces climate change susceptibility and improves feed utilization, water quality, total production, and profit. GIFT and Amur common carp are the most compatible and can be co-cultured with other fish in polyculture systems.

SUSTAINABLE AQUACULTURE TECHNOLOGIES - SUPER-INTENSIVE SHRIMP FARM IN SINGAPORE

Dr Farshad Shishehchian, CEO & Founder

Blue Aqua International
farshad.shishehchian@blueaquaint.com

Acknowledging the insecurity of future food supply, Singapore has set out goals to transform its agriculture & aquaculture technology - to resolve the pressing need to dramatically increase food production while minimizing ecological impacts.

With the scarcity of land in Singapore, the usage of technology to produce food with less space is vital in our local farms. Aquaculture Intensification - achieved through the technology transfer of water and management and nutrition of high-density culture - can be Singapore's niche in the world's marathon of feeding the world.

Besides being able to produce, concerns are raised about production practices and reducing aquaculture's environmental footprint.

This has propagated the move from traditional flow-through systems to a controlled closed system for a 'Farm to Fork' traceability - with Food safety as the main concern.

Efficient utilization of water, energy and space is vital in Singapore's economical and geographical landscape. Blue Aqua International has developed a cost-productive super-intensive culture method and farm system design capable of producing 100 tonnes of shrimp annually within a limited land space of 1.5 hectares in Singapore.

This developed modular system will be replicated in Oman, Thailand, and other countries to extend our reach into sustainable food security, job creations, and other economies of scale.

ANALYZING SOCIAL RESPONSIBILITY ON AQUACULTURE ENTERPRISES SUSTAINABILITY IN LAKE VICTORIA BASIN, KENYA

Zacharia Shitote*, Joyce Magaka, Julius Maremwa, Norman Munala, Susan Odinga

University of Eldoret, School of Business & Management Sciences, P. O. Box 1125-30100 ELDORET
zacharias2002ke@yahoo.com

Aquaculture is one of the world's fastest growing industries, and the fastest growing sector of the food production industry. As the aquaculture industry continues to rapidly grow, sustainability throughout production has increasingly become a top priority for producers. With aquaculture's growth, the sector has experienced growing pains with respect to the environment and social issues. Some of these problems have been human rights violations on fishing vessels, bad labour conditions in processing facilities, conflicts over natural resources, use of hormones and antibiotics and outcompeting smallholders as the industry grows.

The objectives of the study were; establish social issues in aquaculture enterprises, determine environmental concerns in the Lake Victoria Basin, establish challenges of social responsibility and evaluate best aquaculture practices for social responsibility on sustainability of aquaculture projects. A descriptive survey research design will be adopted for the study. Primary and secondary data was collected. Descriptive analyses and findings establish pertinent social issues Table1, environmental concerns Table 2 and challenges of social responsibility Table 3. It was established most enterprises don not engage in social and environmental responsibility practices, some issues were beyond enterprise control like economic development. Recommendations; enterprises should find new ways to contribute to social responsibility and policies to guide CSR to be formulated' to protect environment and improve the quality of life of the households in the lake basin for sustainable development.

Table 1Social Issues in Aquaculture Enterprises

	Frequency	Percentage (
Human rights violations	160	80
Bad labour conditions in Facilities	150	75
Low wages	200	100
community capacity building	40	20
Unfair sharing of resources and Benefits	60	30
Health and safety	90	45
Corruption(Extortion and Bribery)	120	60

Figure 1 Environmental Issues in Aquaculture Enterprises

Environmental Issue	Frequency	Percentage
Climate Change	100	50
Waste Pollution	164	82
Water Use	190	85
Green house Gas emission	27	13.5
Energy Use	80	40
Hormones and antibiotics use	23	12.5

Table 3 Challenges of Social Responsibility in aquaculture Enterprises

Challenge	Frequency	Percentage(%)
Use of illegal fishing gear and methods	170	85
Stork status and Management	30	15
Environmental degradation	156	78
Conflict over natural resources	187	93.5
deleplete shared resources	43	21.5

EFFECTS OF AGAR AS A PREBIOTIC ON PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Tricia J. T. Shufen,* Beryl M. Y. Chean, Nur Haziqah B. S. Huddin, Olivia W. S. Lee, Sky Ng, Sakinah Mulyana and Jason Chang

Diploma in Veterinary Technology, School of Applied Science
Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
1701284J@student.tp.edu.sg

A prebiotic is defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health." They should also 1) be resistant to gastric acids, enzymatic hydrolysis and gastrointestinal absorption 2) can be fermented by intestinal microflora and 3) selectively stimulates the growth and/or activity of beneficial intestinal bacteria required for good health. The use of prebiotics for shrimp is relatively recent and knowledge of known prebiotics for shrimp is scant. Inulin and mannoprotein, when used as prebiotics, have been shown to enhance survival rates in shrimp. In this study, agar was added to a commercial shrimp feed to assess its effects on the gut microbiome and gut histology of Pacific Whiteleg Shrimp (*Litopenaeus vannamei)*. Shrimp were fed with either agar-supplemented or unsupplemented commercial shrimp feed for 30 days and then sacrificed. Sections of the mid-gut were isolated and its contents were collected for DNA extraction, bacterial plate count and then fixed in buffered formalin, processed and stained with hematoxylin and eosin for histological examination. DNA extracted from gut content was subjected to 16S rDNA sequencing. Data revealed differences in the abundance of certain bacterial taxa between the agar-supplemented and unsupplemented groups. At the end of 30 days, shrimp fed with unsupplemented feed had a higher percentage of *Flavobacteriia* (13.5%) and *Alphaproteobacteria* (6.1%) compared to shrimp fed with agar-supplemented feed (*Flavobacteriia* 4.6%; *Alphaproteobacteria* 1%). However, shrimp on the agar-supplemented diet had a higher population of *Gammaproteobacteria* (93.3%) compared to the unsupplemented diet (75.8%). Both groups had a significant reduction in the population of *Mollicutes* by day 30 (agar-supplemented 0.2%; unsupplemented 3.1%) compared to shrimp at day 0 (18.7%). Moreover, total bacterial counts on De Man, Rogosa and Sharpe agar (MRS), selective for lactic acid bacteria, were higher in shrimp receiving the agar supplement (1.5×10^3 CFU/g mid-gut) compared to shrimp without agar supplement (5.92×10^2 CFU/g mid-gut). No differences in intestinal cell morphology could be detected from histological examination of the mid-gut. Our data suggests that supplementation of shrimp diets with agar may positively influence the population of potentially beneficial bacteria in the gut and that it has no negative effects on the cellular integrity of the gut.

CALIBRATION-FREE UNDERWATER STEREO VISION SYSTEM FOR MEASURING FISH

César Santos Silva, Flávio Rodrigues, Ricardo Aires

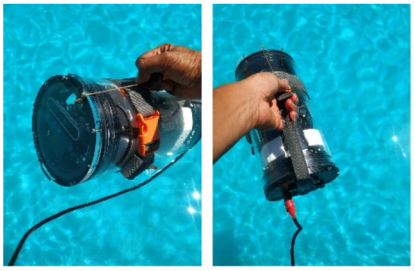
Fishmetrics, Lda, Horta, 9900-095. Azores, Portugal
Email: cesar.silva@fishmetrics.pt

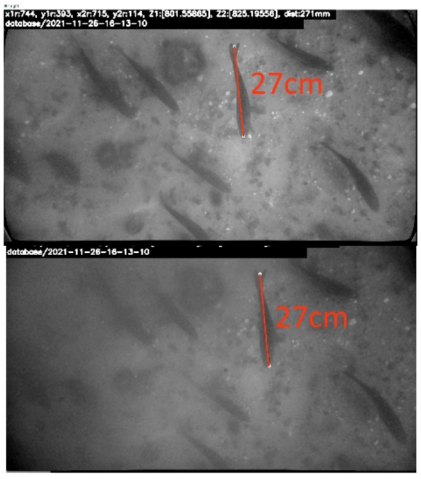
Fish length is a key parameter to assess fish stocks, it helps management and brings economic benefits in the context of aquaculture. The collection of information on the size of fish is essential in many stages of production, such as (a) determining the ideal moment of capture, (b) estimating a series of parameters at the population level and studying its evolution (volume, weight, sex or fat content); (c) stock assessment during creation; (d) classification and separation of individuals by size; (e) optimization in food distribution; (f) controlling individuals' growth; (g) forecasting market value.

To obtain length measurements, a fish ruler is usually used. A cumbersome task that, when applied to living individuals, can induce considerable stress, increasing the risk of damage or hindering their growth. Computer vision is one of the most used non-contact tools for measurement, being fast, consistent and repeatable. However, its use in aquatic environments is limited by the high cost, the difficulty of calibrating the system underwater and the complexity of implementation.

This presentation proposes a low-cost easy-to-use vision system that can obtain measurements on live fish within the aquatic environment, without the need for calibration and a demanding image analysis service. In underwater conditions, the captured images of a vision system undergo a refraction effect, as light rays cross a multiplicity of media (e.g. water, air, lens, glass). This effect increases with the angle of incidence of the light rays on the contact surface between two media. Consequently, the usual three-dimensional vision tools for a stereo camera system (in mid-air) are no longer applicable, invalidating the epipolar geometry associated with the stereo system. It becomes necessary to calibrate the system exhaustively, as the matching map is heavily distorted, a task difficulted by the need to be performed underwater.

The present work implemented a compact stereo vision system and developed an algorithm that estimates the correct length of fish, based on the variation of the angle of incidence of the light rays in the water. Given some structural conditions such as a short baseline (on the order of 100mm) and an ROI (Region of Interest of the FOV, measured in degrees) of less than 60º, the system measures fish with an error of less than 1%, using a default calibration. The short baseline allows to have a compact system and benefits the length estimation algorithm. A set of experiments were performed with real fish, working robustly for a set of orientations of the fish (even when the tail and nose are on different distances to the cameras). The photos on the left show two views of the system that integrates a computer and a stereo pair; on the right, two images captured, and a measurement of an individual are presented.





A METAGENOMICS WORKFLOW FOR RAPID DETECTION AND CHARACTERIZATION OF *Group B Streptococcus* IN FISH

Kae Hwan SIM*, Sri Harminda HARTANTYO, Jiaying HO, Vijitha MANOGARAN, Jia Qi LIM, Angela LI, and Kern Rei CHNG

National Centre for Food Science
Singapore Food Agency
TechQuest, 7 International Business Park Singapore 609919.
sim_kae_hwan@sfa.gov.sg

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a versatile animal pathogen responsible for bovine mastitis and massive fish kill. Since the 2015 outbreak in Singapore, GBS Sequence Type (ST) 283 was shown to be a foodborne pathogen that can be transmitted via consumption of contaminated freshwater fish. The findings had subsequently led to the updated policy and guidelines on the sale of ready-to-eat (RTE) raw freshwater fish dishes in Singapore and the implementation of biosurveillance and risk analysis on this pathogenic bacterium. To support rapid screening and characterization of GBS ST283 in food samples, we have investigated the application of metagenomics sequencing to facilitate simultaneous detection and subtyping of GBS in a single proposed workflow. Without the need for isolation, metagenomic analytics can potentially shorten the turnaround time (TAT) for detection and sequencing typing of GBS in food samples from approximately 12 working days using a conventional approach to around 6 working days. Toman fish samples, which were not contaminated with GBS, were artificially spiked with GBS ST283 and used to benchmark the proposed metagenomics workflow. Our preliminary results suggest that the limit of detection (LOD) for GBS associated with the proposed metagenomics workflow is approximately at 2 log colony-forming unit (CFU) per 25 gram of fish sample. Notably, we were able to show that the proposed metagenomics workflow is able to concurrently detect and sequence type the GBS strain in the fish sample without the need for bacterial isolation and purification. In addition, the proposed metagenomics workflow was also successfully applied on one toman fish sample naturally contaminated by GBS (confirmed by the conventional approach) for parallel detection and sequence typing of GBS. Our preliminary results have clearly demonstrated the potential of the proposed metagenomics analytical workflow to support rapid screening of GBS in fish to facilitate and strengthen survey efforts for biosurveillance and risk assessment. More research and extensive validation are in progress to further develop and optimize the proposed workflow.

AUGMENTATION OF NON-SPECIFIC IMMUNITY IN WALKING CATFISH *Clarias batrachus* (LINNAEUS 1758), BY DIETARY SUPPLEMENTATION OF SEEDS AND LEAVES OF *Achyranthes aspera*

Amarjeet Singh*, Rina Chakrabarti, JaiGopal Sharma, and Vungarala Hari Krishna

Aqua Research Lab
Department of Zoology
University of Delhi
Delhi, 110007, India
amar23jeet@gmail.com

The walking catfish (*Clarias batrachus*) can be easily cultured in warm climates and is inexpensive food at local grocers. In intensive aquaculture, outbreak of disease causes huge economic loss. Antibiotics, vaccines, and various immunostimulants are used to protect fish from pathogens. The applications of antibiotics and chemicals may have a negative impact on consumers. The use of medicinal plant *Achyranthes aspera* as an immunostimulant is an eco-friendly, sustainable and cost-effective approach to boost up fish immunity. *A. aspera* is commonly found as a weed in India. The study was carried out to evaluate the effects of *A. aspera* seeds-and leaves-enriched diets on the performance of *Clarias batrachus* reared in pond conditions and challenged with *Aeromonas hydrophila*.

An 85-day feeding trial was conducted in hapas (2.0 m × 1.5 m × 1.5 m) set inside the pond of ICAR-Central Institute of Fisheries Education, Rohtak Centre, Haryana, India. Fish (0.51 ± 0.032 g) were fed with 0.5% seeds (D1), 0.5% leaves (D2) and control diet (D3) without plant ingredients. After feeding, all fish (except sham control) were challenged intraperitoneally with 200 μl of virulent *Aeromonas hydrophila* (5×10^6 cells/ml). In sham control, fish were challenged with 200 μl of PBS (pH 7.4). Three replicates were used for each feeding regime.

After challenge test, mortality was recorded for 7 days and then blood and tissue samples were collected for various biochemical estimations. The average weight and specific growth rate were significantly ($P < 0.05$) higher in fish fed with D1 diet followed by D2 and D3 diets. The feed conversion ratio and cumulative mortality rate were significantly ($P < 0.05$) lower in fish fed with D1 diet followed by D2 and D3 diets. Serum lysozyme, myeloperoxidase, nitric oxide synthase and hemagglutination titer levels were significantly ($P < 0.05$) higher and thiobarbituric acid reactive substances and carbonyl protein levels were significantly ($P < 0.05$) lower in D1 diet followed by D2 and D3 diets. The genes iNOS, SOD-C, NF-kB, Bcl-2, BAX, Cytochrome-c, Caspase 9 and Caspase 3 were up-regulated in liver and head kidney of fish fed with experimental diets compared to the control one.

It is evident that *A. aspera* seeds-and leaves- enriched diets enhance the innate immunity of fish and also play an important role in apoptosis.

AQUAVITAE: NEW SPECIES, PROCESSES AND PRODUCTS CONTRIBUTING TO INCREASED PRODUCTION AND IMPROVED SUSTAINABILITY IN EMERGING LOW TROPHIC, AND EXISTING LOW AND HIGH TROPHIC AQUACULTURE VALUE CHAINS IN THE ATLANTIC; H2020 BG-08-2019 [Part C]

Philip James

Nofima
P.O. Box 6122
NO-9291, Norway
Email: Philip.james@nofima.no

AquaVitae is a new four year H2020 project with the overall objective of increasing aquaculture production in and around the Atlantic Ocean in a sustainable way by developing new and emerging low trophic species and by optimising production in existing aquaculture value chains. AquaVitae will conduct research and facilitate innovation in five value chains (VCs) selected with regard to their potential for contributing to improved, sustainable food production and their lasting, significant impacts, specifically:

VC I: Macroalgal production; New species, offshore production, and post-harvest processes
VC II: Integrated Multi-Trophic Aquaculture (IMTA); land-based and sea-based, new species and systems
VC III: New echinoderm species: Sea urchins and sea cucumbers
VC IV: Existing shellfish species: Oysters and mussels
VC V: Optimised production of selected existing finfish species; freshwater and marine

A series of cross-cutting Work Packages (WPs) will include research on biosensors, Internet of Things (IoT), product characteristics, consumer attitudes, market potential, sustainability, environmental monitoring, risk assessment, analysis of value chains, profitability, and other socioeconomic aspects.

AquaVitae will contribute to various policy dialogues and produce briefs on policy and governance issues. The AquaVitae consortium consists of 36 full partners from Europe and countries bordering the Atlantic Ocean, in addition to an Industry Reference group, a Policy Advice Group, and an External Advisory Group. AquaVitae supports extensive communication and outreach activities, employs a multi-actor approach to ensure stakeholder engagement in all phases of the project, and will set up a durable aquaculture industry and research network around the Atlantic Ocean. Industry partners are present in all case studies, and they have a special responsibility for exploitation and commercialization of the project research results and outcomes. AquaVitae will have a lasting impact on society through the introduction of new species, and through the development of new processes and products based on a circular economy / zero waste approach with improved sustainability. AquaVitae will produce Good Practice standards, facilitate industry apprenticeship and student exchange, support extensive training programs for industry, academia, and the public, and contribute to the implementation of the EU-Brazil-South Africa Belém Statement.

This presentation will highlight examples from the Case Studies within the project and the impact they will have on their respective value chains in the Atlantic.

TRANSITIONING FROM SPINY AND SLIPPER LOBSTER RESEARCH INTO COMMERCIAL PRODUCTION

Greg G. Smith*, Quinn P. Fitzgibbon, Chris G. Carter, Andrew J. Trotter, Basseer M. Codabaccus, Scott Parkinson.

Institute for Marine and Antarctic Studies (IMAS),
University of Tasmania, Private Bag 49,
Hobart, TAS, 7001, Australia.
Email: gregory.smith@utas.edu.au

Research into the propagation of spiny lobsters has been undertaken for more than 50 years with results being demonstrated experimentally for at least ten species. Early research was conducted in Japan with recent progress in Australia, America, Mexico, South Africa, Vietnam, India and Great Britain. The development of sustainable commercial processes for spiny and slipper lobster production have now been achieved in Australia at the Institute for Marine and Antarctic Studies (IMAS), University of Tasmania (UTAS). Spiny and slipper lobsters have a complex larval cycle, in the latter it is shorter, but like spiny lobsters they are vulnerable to physical damage, disease, have specific feeding behaviours and nutritional requirements. To achieve the goal of commercial production the research has focused on developing mass culture systems, manufactured larval feeds, establishment of preventive health strategies and refinement of water treatment technologies.

Working with "Ornatas Pty Ltd" our commercial partners, we are assisting with the development of a hatchery and transitioning the research into commercial production. There is a ready market for lobster seedstock in South East Asia where they are currently sourced from the wild, cultured to market size in seacages and sold primarily into China as a live product. While this is a potential market for Ornatas, they will focus on developing a growout industry in Australia. Spiny and slipper lobster growout in Australia will differ from the South East Asian model with culture undertaken in intensive onshore systems. This move onshore will be challenging due to factors including cannibalism, water quality and optimising nutritional requirements for growth and survival. The key components that will drive commercialisation in Australia will be:

1. Developing effective and efficient onshore growout systems and technology that can achieve high production outcomes with a small environmental footprint;
2. Development of economically viable manufactured growout feeds for lobsters that can perform equal or better than current fresh feed alternatives;
3. The development of better transport technologies for post-larvae and juveniles – ensuring that seedstock transported from a hatchery arrives at the growout site in optimal condition;
4. Improvements to post-larval quality of hatchery seedstock; and
5. Examining the social, environmental and economic impacts of developing a new industry.

Much of the research that will be undertaken during the next phase of the development of a sustainable onshore lobster industry will be undertaken at a research scale at the IMAS UTAS facilities with commercialisation being undertaken at Ornatas's onshore facility in Northern Australia. Production and growout of slipper lobsters commenced at the Northern site in early 2020 with experimental production of spiny lobsters due to commence during 2020, while the commercial spiny lobster hatchery in Tasmania is due for completion in 2021.

TROPICAL LOBSTERS – FROM THE RESEARCH LAB TO COMMERCIAL REALITY

Greg G. Smith*, Quinn P. Fitzgibbon, Chris G. Carter, Andrew J. Trotter, Basseer M. Codabaccus, Jennifer Blair, Scott Parkinson.

Institute for Marine and Antarctic Studies (IMAS),
University of Tasmania, Private Bag 49,
Hobart, TAS, 7001, Australia.
Email: gregory.smith@utas.edu.au

Spiny lobster propagation research has been undertaken for more than 60 years. Pioneering hatchery research was initially conducted in Japan, and most recently in Australia. The development of sustainable commercial processes for spiny lobster (*Panulirus ornatus*) production have now been achieved at the University of Tasmania's, Institute for Marine and Antarctic Studies (IMAS). This breakthrough in hatchery production has generated interest in developing commercial onshore lobster aquaculture in Australia. The research team at IMAS has partnered with Ornatas Pty Ltd to develop tools to establish the world's first onshore lobster facility.

Spiny lobsters are cultured in other regions of the world using wild seedstock and on-growing in seacages. This is not viable in Australia due to variability in seedstock settlement and strict environmental regulations. Some of the key components that will drive commercialisation include the development of growout systems, manufactured feed, improved seedstock quality and transport, and understanding of the social, environmental, and economic impacts of a developing industry. These questions are being addressed in a multi-partnered research project under the auspices of an Australian Research Council, Industrial Transformation Research Hub for Sustainable Onshore Lobster Aquaculture. To facilitate immediate commercialisation two areas require attention: the development of a sustainable manufactured feed and systems to reduce the impacts of cannibalism.

Traditionally spiny lobsters are fed fresh seafood products (Fig. 1). These feeds are popular due to low cost, ready availability, and palatability. The negative impact of these feeds is high FCR (>15), biosecurity risk, a source of pollution and disease. For an industry to flourish, a manufactured feed is needed. The feed must have a suitable nutritional profile, be water stable, palatable, have a low FCR, use economical, sustainable ingredients and be applicable to commercial production. From an experimental perspective many of these criteria have been achieved with a focus now on the commercialisation phase. The second component of commercial onshore lobster culture is the establishment of systems and methods to prevent cannibalism, especially in juveniles. Cannibalism generally occurs on moulting animals, they are readily attacked and consumed by conspecifics. Intensive video work and behavioural studies are being conducted to gain insight into ways that cannibalism can be mitigated in a commercial setting. The outcome of this work will facilitate the development of new and novel culture techniques for this and other antagonistic crustacean species.

Figure 1. Traditional fresh lobster feed products and replacement with a commercial pellet feed: 1. Mussels, 2. Squid, 3. Fish, 4. Crustaceans, 5. Pellet feed.

AN INVESTIGATION ON THE EFFECTS OF BACKGROUND COLOURS ON THE REPRODUCTIVE SUCCESS OF SEAHORSE *Hippocampus Reidi* BROODSTOCK AND THE EFFECTS OF NUTRIENTS ON JUVENILES

Sng, X.E.C.* and Tan, C.K.V.

Diploma in Veterinary Technology, School of Applied Science, Temasek Polytechnic, Singapore 529757
1701592F@tp.edu.sg

Hippocampus Reidi is a threatened seahorse species due to numerous reasons such as habitat degradation and destruction due to coastal development. Therefore, there is a need for conservation and breeding efforts to increase the population. Environment and nutrition have always played a part in reproduction success of aquatic animals and the quality of juveniles. Thus, the aim of the study was to determine the effects of background colors on the color of the *Hippocampus Reidi* broodstock, reproductive success, and the effects of nutrients on the growth and survival rates of Reidi juveniles. The broodstocks were placed in either blue colored background tanks or coral background tanks for color change observation and breeding activities. The juveniles were subjected to either fatty acid enriched artemia diet or vitamin enriched artemia diet for 13 days. The results showed that the seahorses placed in blue colored tanks were much brighter as compared to the seahorses placed in coral colored tanks, having no changes to the color at all. However, coral tank seahorse pairs showed higher reproduction success, with higher fecundity and frequency of birth. Fatty acid enriched diet showed faster growth and higher survival rates in contrast to vitamin enriched diet. It can be concluded that the background/environment does affect reproduction of *Hippocampus Reidi*, where they are more inclined to breed in their natural environment. Since fatty acid provide better development and survival rates, it is a more essential nutrient on the overall wellbeing of the Reidi juveniles. Since this research only focuses on 2 background type, there might be other backgrounds that provide higher potential in increasing reproductive success and how different percentage of fatty acid may further affect the development of Reidi juveniles. Future studies should offer greater insights into these factors and aspects to promote breeding of *Hippocampus Reidi* for conservation purpose.

NUTRIENT BASED MODELS FOR FARM PRODUCTION PREDICTION AND PRECISION AQUACULTURE

Filipe Soares*, Ana Nobre, Ricardo Severino, Luís E.C. Conceição, Tomé Silva

SPAROS, Lda. Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal
filipesoares@sparos.pt

Simulation models can support informed decision making in aquaculture by predicting the on-going production. Given the level of expert knowledge required to use complex models, such as the nutrient-based models, most organizations use simple growth models (e.g. TGC) that can predict the standard growth of the farm or cage but are of limited use for the comparison of scenarios, for instance to test different feeds virtually before field implementation. The objective of this presentation is to illustrate the application of a nutrient-based model (FEEDNETICS) in bream and bass farm, in the context of the Valormar project (24517 supported by Compete2020, Lisboa2020, CRESC Algarve2020, PT2020 and the EU through FEDER/ERDF).

The first step is to ensure the model validation for the farm which was done for 3 tanks (Figure 1). Then a dashboard with estimates was built to provide the farm managers with current and long-term predictions of relevant production site variables (Figure 2), such as fish weight, time to harvest, estimates of feed consumption, oxygen consumption and nutrient excretion, among other variables.

Furthermore, *in silico* comparisons of different feeds were carried out, indicating that, for the local temperature profile, a premium bream feed could generate savings on feeding costs of about 80 € per ton of fish produced compared with a standard feed, while reaching harvestable size one month sooner. Additionally, the scenario with premium feed would represent a decrease in generated wastes of about 10 kg of nitrogen per ton of biomass gain and a decrease of 75 kg of total solids per ton of biomass gain.

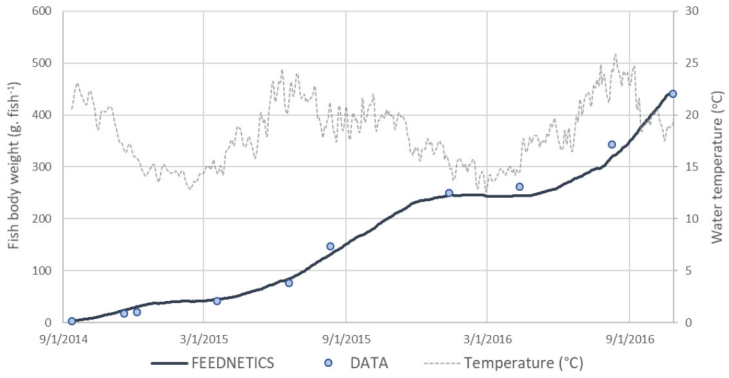


Figure 1. Example of validation plot comparing measurements (points) vs. predictions (line) for one of the three validation tanks.

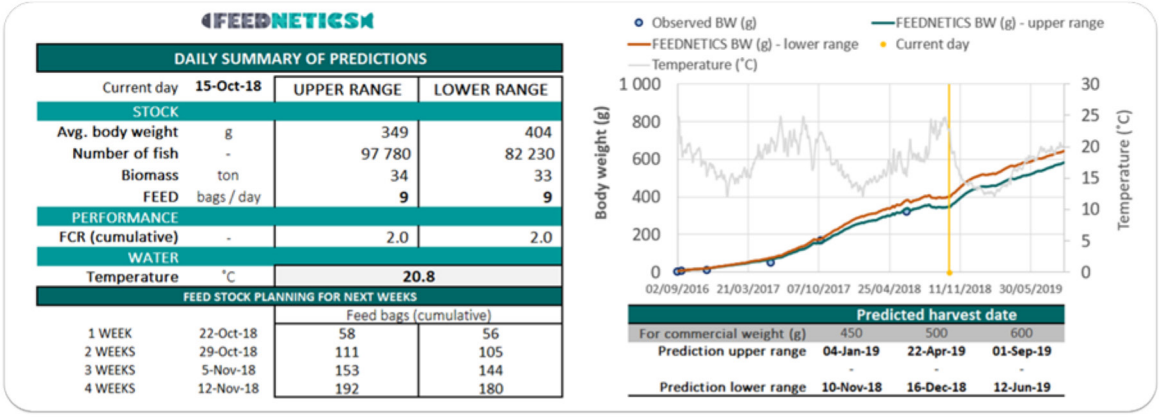


Figure 2. Dashboard with current and long-term predictions of relevant production site variables.

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THIOREDOXIN DOMAIN CONTAINING PROTEIN 5 (TXNDC5) FROM BIG-BELLY SEA HORSE (*Hippocampus abdominalis*) WITH TRANSCRIPTIONAL RESPONSE TO IMMUNE STIMULANTS

Wijamunige G. Sandamalika, Hanchang Sohn*, Anushka V. Samaraweera, and Jehee Lee

Department of Aquatic Life Medicine & Fish Vaccine Research Center
Jeju National University
Jeju Self-Governing Province, Republic of Korea
gayashanis@gmail.com

The thioredoxin domain containing protein 5 (TXNDC5), also known as resident endoplasmic reticulum 46 (Erp46); has categorized as a member of the family PDI. As a common feature of PDI family, all the members comprise one or more thioredoxin- like domain which contain CXXC signature sequence. TXNDC5 was first identified in 2003 with six splicing variants, where two of them (TXNDC5-001, TXNDC5-003) can be translated in to proteins. The full-length version of TXNDC5 bears three TXN-like domains while lacking peptide binding domain [7]. TXNDC5 mainly expressed in liver and endothelial cells and regarding its cellular location the highest expression was observed int endoplasmic reticulum (ER).

The deduced amino acid sequence of *shTXNDC5* was identified and it consisted with an open reading frame (ORF) of 1286 bp which codes a protein with 433 amino acids. The molecular weight of shTXNDC5 was predicted as 49.3 kDa with a 5.01 theoretical isoelectric point (pI). Following the in-silico predictions, shTXNDC5 consisted with three thioredoxin – like domains at the positions of 35-141, 165-270, 324-428 aa and endoplasmic reticulum (ER) retention signal at the C- terminus ($D^{430}EL^{433}$).

The spatial and temporal expression profiles of *shTXNDC5* showed its highest expression in sea horse ovary while exhibiting potential protective roles against bacterial and viral invasions, respectively. The significant changes in their expression allow us to propose possible functions of *shTXNDC5* in the innate immune system of big belly sea horse. Moreover, its thiol-disulfide, protein disulfide isomerase and mixed disulfide isomerase ability were determined by the HED, insulin disulfide reductase and the MTT assays. Altogether the outcomes of this study afford the first comprehensive report of TXNDC5 in teleost by providing sufficient evidences for the involvement of TXNDC5 in sea horse immunity.

QUANTITATIVE INVESTIGATION AND MONTORING OF *Enteromyuxm leei* (MYXOZOA; MYXOSPOREA) FROM OLIVE FLOUNDER *Paralichthys olivaceus*

Hanchang Sohn*, Chang Nam Jin, Sang Phil Shin and Jehee Lee

Department of Marine Life Sciences, Jeju National University
Jeju Self-Governing Province 63243
Republic of Korea
hcsohn2@gmail.com

Genus *Enteromyxum* comprise 3 species; *E. scophthalmi*, *E. fugu* and *E. leei*. *E. leei* is a causative agent of enteromyxosis which has a broad susceptibility against marine species. Recently, Massive morbidity and mortality was caused due to the infection of *E. leei* in olive flounder aquaculture. Real-time PCR assay targeting the 28S rDNA of *E.leei* was conducted during April to November 2018. Within 3 sampling sites, steady detection was observed from July in 2 sites. Result of *E. leei* detection had shown a difference in water-filter samples and tissue samples. In tissue samples, *E. leei* was detected after 2 months compare with the water-filter samples. Quantification method in this research is considered to provide a baseline of the infection timeline against olive flounder and development of management.

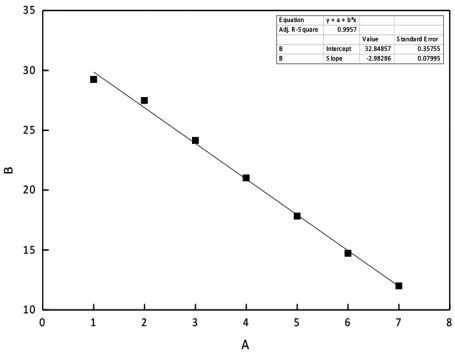


Figure 1. Constructed standard curve for this study.

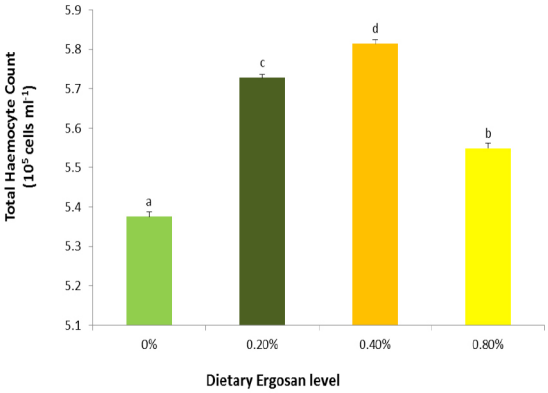
INCREASED DOSE OF ALGINIC ACID SUPPLEMENTATION CAN SUPPRESS IMMUNE RESPONSES AND RESISTANCE TO VIBRIOSIS OF JUVENILE PACIFIC WHITELEG SHRIMP *Penaeus vannamei*

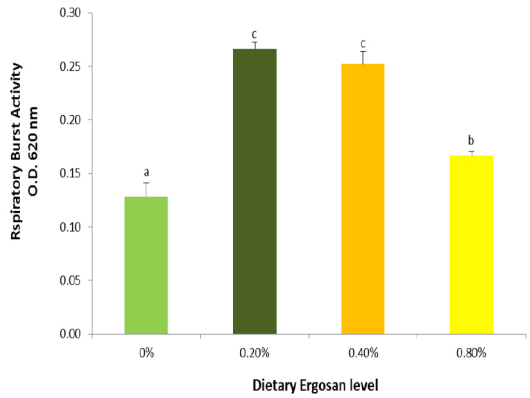
Nowena S. Solidum*, Roman C. Sanares, Karen Grace S. Andrino-Felarca and Valeriano L. Corre, Jr.

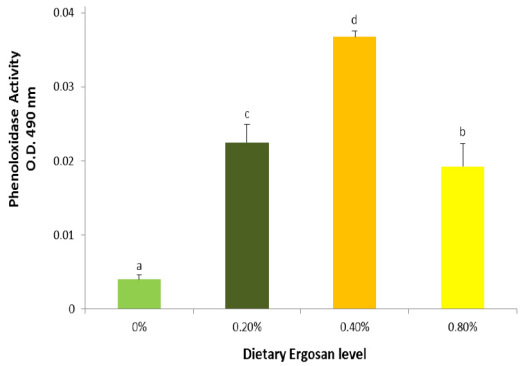
Fisheries Department
Capiz State University- Dayao Satellite College
5800 Roxas City, Capiz, Philippines
nowena_solidum@yahoo.com

A 45-day feeding trial was conducted to evaluate the effects of alginic acid (Ergosan®) supplementation in Pacific whiteleg shrimp, *Penaeus vannamei* juvenile. Shrimps (0.6 g ABW) were fed diets supplemented with different levels of alginic acid as immunostimulant. Experimental diets were formulated to contain 0.2, 0.4 and 0.8% and a control. The feeding trial was conducted in 50-L

capacity rectangular plastic container stocked with 20 shrimps each in triplicates. Growth, survival, resistance to *Vibrio parahaemolyticus*, total haemocyte count (THC), respiratory burst activity, phenoloxidase activity, and clearance efficiency were evaluated. Results showed that growth and survival were not affected by supplementation of immunostimulant. On the other hand, bacterial challenge showed 100% mortality in 0.8% alginic acid fed group though not significant with the control. Total haemocyte count, respiratory burst and phenoloxidase activity were significantly enhanced in the group supplemented with 0.4% and 0.2% of alginic acid. Immune responses of the group fed with the highest concentration (0.8%) were significantly suppressed. The same trend was obtained for the clearance efficiency. The present results demonstrated that using alginic acid less than or equal to 0.4% activates immune responses and resistance against vibriosis, otherwise overstimulation of the immune indices could cause immunosuppression.







EFFECTS OF USE OREGANO OIL AND QZ-TOSS™ ON HEALTH STATUS OF CULTURED *Oreochromus nilotucus* IN EGYPT

Soliman , M.K., Nawal ,A.M.Afar, El Lakany, H.

Dept. of Poultry and Fish Diseases, Fac. of Vet. Med. Damanhur University, Egypt
vetdrmagdy@gamail.com

In this study various health status, growth and biochemical parameters following use of oregano oil (Biorgano™ 10%Thomson Biochorporation, China) and blend of *Bacillus subtilis* (QZ-Toss^{MR} , containing 2 B cfu/ kg -QB Lab. USA) .

Six groups, in each group has 20 *O.niloticus* were kept in 3x4m concrete tank. Namely, group one as control, group 2 fed regular diet (30% protein) and QZ-Toss was added to the pond spring water , group 3 fish fed diet containing 250gm/ton Biorganoand QZ-Toss, group 4 fish as in group 3 plus addition of QZ-Toss, group 5 fish fed diet containing 500 gm/ton Bioorgano and group 6 as in group 5 plus addition of QZ-Toss.

The time of experiment was 30 days in which QZ-Toss was added in first and second weeks 2gm/m^3 and one gm/m^3 third and fourth weeks.

At the end of experiment, ammonia was measured in each pond water, total growth weight was measured and blood was collected for serum separation from each fish. Kidney and liver function enzymes were evaluated as well as some antioxidants levels were measured.

Tissue samples from liver, kidney, spleen and intestine were collected and use for expression of immune-related genes and histopathological changes.

The results revealed decreased in ammonia levels and increase in body weight in all groups compared to the control. Moreover, liver and kidney function enzymes were showed no significant difference compared to control. The both concentration of Bioorgano enhanced oxidative stress reduction but did not affect GPx antioxidant levels.

Both use of Bioorgano and QZ-Toss significantly induced the expression of cytokines and immune-related genes. Namely, IL-1B, TLR-7, MX, TL8 and TCR.

NUTRITIONAL PROFILE OF WATERMELON *Citrullus lanatus* SEED AND RIND MEAL: IMPLICATIONS FOR FISH NUTRITION

*[1]SOLOMON S. G., [1,2] OKOMODA V.T. AND [1]MIKAH J. E.

[1]Department of Fisheries and Aquaculture, College of Forestry and Fisheries, University of Agriculture, P.M.B. 2373 Makurdi, Nigeria
[2]Institute of Tropical Aquaculture and Fisheries Research (AQUATROP), Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
*Tel: +2347037275891
*e-mail: solagabriel@yahoo.co.uk

The high feeding and production cost of many Aquaculture enterprises are predicated on the use of expensive conventional feed sources in the diet. Hence, one of the ways to increase the profitability of the venture would be to identify potential cheap alternative feed ingredients with high nutritional factors that can compensate for the needs of the fish. An example of such an alternative feed ingredient is the Watermelon *Citrullus lanatus* Seed and Rind which is usually discarded as by-products during processing. This study is designed to investigate the proximate composition and Amino Acid profile of these by-products (following standard methods) with a view of its implications in aquaculture fish diet.

The result showed that the crude protein, lipid, and fibre were higher in the seed than the rind of the watermelon. While moisture, Ash and NFE content of rind were better than seeds. Similarly, amino acids such as Arginine and valine were higher than the recommended intake values for *Clarias spp* and Tilapia. The values of other amino acids were also comparable to the standard requirements previously suggested for these important fish species. It was therefore concluded that watermelon seed and rind could be used as a ready alternative energy source in the diet of tropical cultured fishes. However, further studies are needed to validate these assumptions.

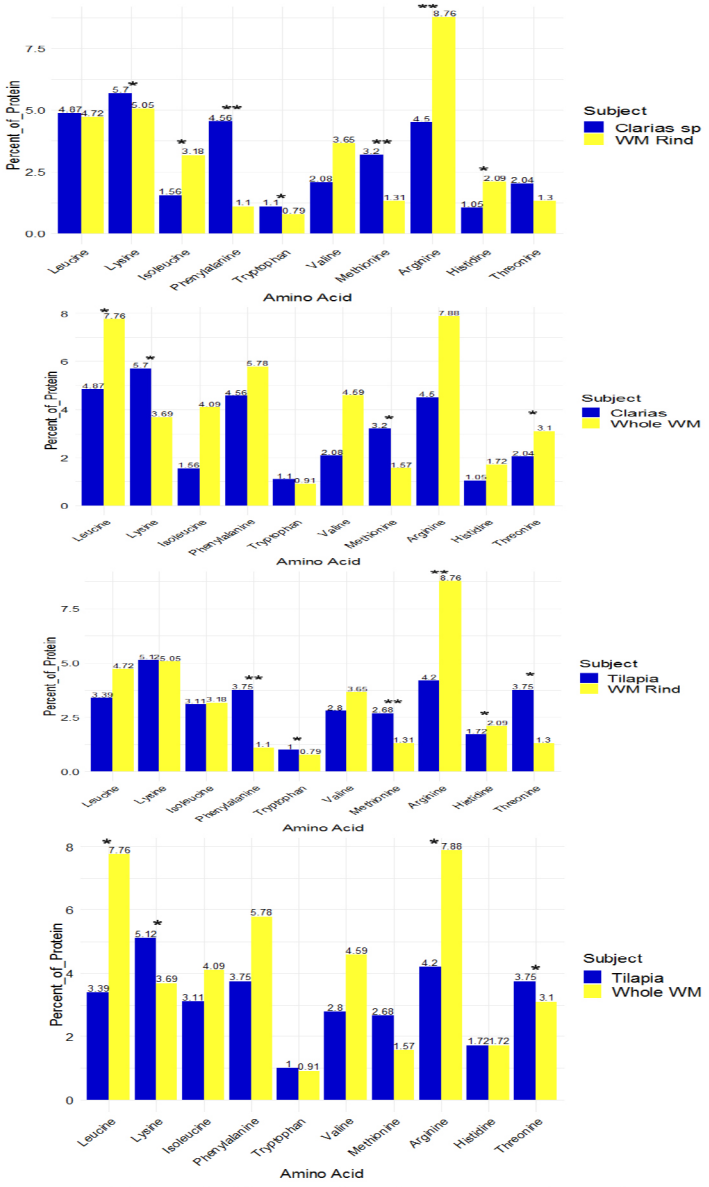


Figure 1: Comparison between amino acid requirements of *O. niloticus* and amino acid content of watermelon (p<0.01; *p<0.05).**

Table 1: proximate composition of watermelon

Parameters	Rind meal	Whole seeds meal	p-value
Crude Protein	7.77±0.05	11.97±0.11	0.001
Lipid	2.68±0.27	30.67±0.42	0.000
Ash	13.80±0.41	1.51±0.05	0.001
Crude Fibre	17.71±0.10	19.24±0.18	0.017
Moisture	19.13±0.17	11.24±0.10	0.001
NFE	38.92±0.70	25.38±0.30	0.003

INFLUENCE OF ACTUAL STOMACH AND INTESTINAL pH VALUES AND WATER TEMPERATURE ON THE ACTIVITY OF DIGESTIVE ENZYMES IN GUT OF RAINBOW TROUT *Oncorhynchus mykiss*

Mikhail M. Solovyev*, Natalia S. Pustovalova, Elena N. Kashinskaya, and Enric Gisbert

Institute of Systematic and Ecology of Animals SB RAS, Novosibirsk, 630091
yarmak85@mail.ru

The objective of this study was to evaluate the effect of water temperature, gut pH values, and postprandial time on the activity of gastric (pepsin) and pancreatic (trypsin, chymotrypsin, alpha-amylase, and lipase) enzymes. For this purpose, two hundred rainbow trouts were equally distributed among twenty four quadrangular 2000 L tanks. Then, fish were divided on two experimental groups that were kept for 20 days at 13 °C and 20 °C, respectively. All tanks were connected to a recirculation unit (IRTAmar™) equipped with mechanical, biological and UV-filters and a temperature controller. Fish were hand fed twice per day during the trial. At the end of the trial, before determination gut pH and activity of digestive enzymes, fish were fasted for 72 h. Then, fish were normally fed and they were sacrificed in 2, 5, 9, 13, 18, 26, 36, 48, 60, 84, 96, 120 hours after feeding. Fish were euthanized by a blow on the head and individually weighed and their guts removed. The values of pH were measured with a HI 8314 portable pH meter with a HI 1083 B microelectrode of 3 mm of diameter (Hanna Instruments, USA). Then, the stomachs and intestines were lyophilized and stored at -80 °C.

The activity of pepsin, trypsin, chymotrypsin, alpha-amylase, and lipase were quantified. In the present study we used “standard conditions” i.e. room temperature (25°C) and constant buffer pH (pH 2.0 for pepsin and pH 8.0 for trypsin, chymotrypsin, alpha-amylase, and lipase) and “actual conditions” i.e. actual physiological pH values in fish stomach and intestine and temperature in tanks (13 and 20 °C respectively). Moreover, for all mentioned enzymes the pH optimums were assayed as well.

The pH maximum level of pepsin activity was found at pH 2.0-2.5 whereas at pH higher than 5.0 the activity was completely absent for both groups of fish. The pH maximum level for trypsin was found at pH 9.0 and 10.0-10.5, for chymotrypsin at pH 8.0-9.0, for alpha-amylase at pH 7.5-8.0, and for lipase at pH 8.0-8.5 and 9.5 for both groups of fish. The time after feeding, actual pH values in gut, and water temperature in tanks were significantly influenced on the activity of studied enzymes (ANOSIM, $p < 0.05$). This study showed the important role of three key factors (temperature, pH, and time after feeding) on the activity of main digestive enzymes in gut of rainbow trout.

APPLICATION OF PROBIOTIC *Bacillus* IN AQUACULTURE

Mehdi Soltani[1,2*], Koushik Ghosh[3], Vikash Kumare[4], Alan J. Lymbery[1], Einar Ringø[5]

[1]Department of Aquatic Animal Health Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
msoltani@ut.ac.ir
[2]Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University, Perth, Australia
[3]Aquaculture Laboratory Department of Zoology, The University of Burdwan, Burdwan, West Bengal, India
[4]Aquaculture & Artemia Reference Center, Department of Animal Production Faculty of Bioscience Engineering Ghent University, Gent, Belgium
[5]Norwegian College of Fishery Science Faculty of Bioscience Fisheries and Economics, UiT The Arctic University of Norway, Tromsø, Norway

Bacillus as spore-forming bacteria are resistant to aggressive physico-chemical conditions showing unusual physiological characteristics that enabling them to survive under different environmental conditions such as fresh waters, marine sediments, desert sands, hot springs, and the gastrointestinal tract of finfish and shellfish. *Bacillus* bacteria can rapidly replicate and tolerate a multitude of environmental conditions, giving a wide range of beneficial effects in the aquaculture sector. Application of *Bacillus* bacteria as probiotics in feed or as bioremediatory agents of aquaculture rearing water has great potential for a sustainable aquaculture. Removal of waste products from water column, maintaining optimum water quality, and reducing stress, are parts of bacilli probiotics in aquaculture with an outcome of balancing and or enhancing of animal immuno-physiological variables and a higher aquaculture production. Probiotic bacilli can also reduce the load of pathogenic microorganisms but maintaining a higher density of beneficial bacteria in aquaculture systems. It is, however, important to know how the probiotic bacilli efficacy and potency can be affected by aquatic animal species and age, water quality and diet as well as details of bacilli mode of actions on the immune-physiological functions of aquatic animals. Also, with an increase in the potential pathogenic bacilli, their applications in form of probiotic in aquaculture required more attention. This presentation discusses the various effects of probiotics bacilli in aquaculture and future research work required.

PHYTOTHERAPY OF FISH STREPTOCOCCOSIS

Mehdi Soltani [1,2*], Hien Van Doan[3,4], Pezhman Hosseini-Shekarabi[5], Einar Ringø[6]

[1]Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
msoltani@ut.ac.ir
[2]Freshwater Fish Group and Fish Health Unit, Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute
Murdoch University, Australia
[3]Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University
Chiang Mai 50200, Thailand
[4]Innovative Agriculture Research Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[5]Department of Fisheries Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[6]Norwegian College of Fishery Science, Faculty of Bioscience, Fisheries and Economics, Udit The Arctic University of Norway, Trims, Norway

In recent decays trends have been carried to use phytotherapy as an alternative to antibiotic therapy against bacterial fish diseases. Extracts or essential oils of many different medicinal herbs and plants have been tested for their *in vitro* antibacterial activities *in vivo* bioassay against fish pathogenic *Streptococcus* species, but more attentions have been paid to *Streptococcus iniae* and *S. agalactiae* isolated from diseased fish. The *in vitro* susceptibility of *S. dysagalactiae* and *S. parauberis* strains with fish origin have been also evaluated using various medicinal herbs, but there is no report on *S. ubreis* and *S. phocae* with fish origin. As different types of extractions, the extraction fractions and essential oils have been used for their antagonistic activity by different assays, it is therefore, difficult to compare the outcome results. This presentation addresses the efficacy and potency of medicinal herbs/plants as possible alternative to antibiotics for control and treatment of streptococcal infections in fish, and discuss the gaps required future works.

HELATH STATUS OF AQUACULTURE INDUSTRY IN THE MIDDEL EAST: AN OVERVIEW OF IRAN AND IRAQ AQUACULTURE SECTORS

Mehdi Soltani1,2*; Ali Majhool Kani3

1 Department of Aquatic Animal Health Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2 Centre for Sustainable Aquatic Ecosystems, Harry Butler Institute, Murdoch University, Australia
mehdi.soltani@murdoch.edu.au
msoltani@ut.ac.ir
3Department of Pathology and Forensic Medicine, University Of Kufa, Najaf, Iraq

Iran with an average per capita of about 12 kg, at the end 2018, reached a total fisheries production of 1,262,000 tonnes that ranked the country as the 24th in the world. In the same year, the total aquaculture production increased in 489,000 tonnes, and species of carps (four species), rainbow trout, shrimp, sea bass and sea bream (cage culture), sturgeon and ornamental fish shared productions of 187, 173, 48, 14.2, 2.8, 0.25 thousand tonnes, respectively. Despite a rapid development in the aquaculture sector in during last years, the industry has been encountered with several problems and obstacles. Selective breeding of the existing commercial species is one constrain interfering the fast growth, low survival and high cost-effective for production of some species such as rainbow trout. With the development of the industry during last two decays, the aquaculture sector has been suffered from the frequent outbreaks of various infectious diseases including bacterial (streptococcosis, lactococcosis, yersiniosis), viral (VHS, IPN and IHN, WSD) and parasitic (some protozoans and monogeneans) diseases causing huge economical losses annually. Despite a value attention paid by both Veterinary and Fisheries Organizations, still most of fish farmers hesitate to use the vaccines against the current diseases.

Iraq with a short coastline with Persian gulf has faced with significant environmental changes to the coastal habitat and in the inland waters of Forat and Dejleh rivers due to dam construction and the draining of marsh areas. The aquaculture production in 2018 was about 25737 tonnes that is production of carp species in the ponds or river cages. As a results of fish farm visits and training seminars for fish farmers and also surveys by some researchers and fish farmers, the Iraqi aquaculture is suffering from various health problems mainly poor water quality and frequent outbreaks by some bacterial (Motile *Aeromonas* septicemia, carp erythrodermatitis caused by *A. salmonicida*) and viral disease probably caused by cyprinid herpesvirus, and some monogenic and protozoan parasites. This presentation gives an overview on what are the health status of Iranian and Iraqi aquaculture industries and discuss the current gaps that required further attention.

ACUTE AND MUTIGENERATIONAL EFFECTS OF ENVIRONMENTAL CONCENTRATION OF THE ANTIFOULING AGENT DICHLOFLUANID ON THE MYSID MODEL *Neomysis awatschensis*

Somyeong Lee [a] · Md. Niamul Haque [a,b] · Jae-Sung Rhee [a, b, c,*]

[a]Department of Marine Science, College of natural Sciences, Incheon National University
Incheon 22012, Republic of Korea
[b]Research Institute of Basic Sciences, Incheon National University, Incheon 22012, Republic of Korea
[c]Yellow Sea Research Institute, Incheon 22012, Republic of Korea

A broad-spectrum fungicide, dichlofluanid is widely used in antifouling paints and agricultural pesticides. Biofouling is the colonization of various organisms, including bacteria, fungi, protozoans, macroalgae, mollusks, and polychaetas attached to the surface of submerged or semi-submerged structures (e.g., ship hulls and mariculture cages). Biofouling is considered economically detrimental to ships and marine structures, as it increases weight, frictional resistance, surface corrosion, and maintenance costs and can cause significant structural damage. Exposure with dichlofluanid considerably induced oxidative imbalance, as NOEC (0.006 μg L−1 for juveniles and 0.074 μg L−1 for adults) and 1/10 LC50 values increased intracellular concentrations of malondialdehyde and glutathione, and the enzymatic activities of catalase and superoxide dismutase, whereas exposure to LC50 value decreased the values of oxidative parameters. Enzymatic activity of acetylcholinesterase decreased considerably when exposed to LC50 value. Multigenerational monitoring in response to NOEC showed a significant growth retardation with an increase in intermolt duration as well as a decrease in the number of newborn mysids from females of the third generation. Consistent exposure to environmentally relevant sublethal concentrations of dichlofluanid would be detrimental to mysid individuals and the survival of the mysid population.

MONITORING OF DISEASES IN EELS CULTURED IN KOREA

Jun-young Song*, Mun-hee Jang, Yun-Kyoung Oh, You-hee Lee, Kyoung Su Cho and Sang-Hyun Oh

Inland Aquaculture Research Center
National Institute of Fisheries Science (NIFS)
Changwon-si, 51688, Republic of Korea
jysong2012@korea.kr

Eel is the most important fish species in inland aquaculture industry in Korea. However, economic loses in eel farms have been continuously caused by mortalities of the eels occurred by various kinds of infectious diseases under the intensive culture conditions. In the study, thus, we investigated recent disease outbreak trend in mainly cultured eels in Korea, such as *Anguilla japonica*, *Anguilla bicolor* and *Anguilla marmorata* during 2018~2019. For monitoring of viral diseases in the eels, we investigated infection rates of two viruses, Japanese eel endothelial cell necrosis virus (JEECV) and anguillid herpesvirus 1 (AngHV1), by PCR. For surveying of bacterial diseases, kidney and spleen tissues from the eels were inoculated on BHI (0.5% NaCl) medium for 18~24 hours at 25°C incubator. For parasitic diseases, gills and skin mucus were observed under the microscopy.

A. japonica, *A. marmorata* and *A. bicolor* were samples from 41 farms located in various regions of Korea. JEECV and AngHV1 were detected from 49% (20 farms) and 27% (11 farms) respectively, in the 3 species of eels from most of the regions surveyed. In addition, the rate of multiple infections for these two viruses reached 17% (7 farms). From these results, it was confirmed that these two viruses were widespread in main species of eels cultured in Korea. Although detection rates of these two viruses were high, the eels examined in the study were also highly infected (55%, 25 farms) with other pathogenic agents such as *Edwardsiella anguillarum, Aeromonas hydrophila*, gill flukes, *trichodina* sp. and so on.

For investigation of viral distribution in the host tissues, we found that JEECV were 100% detected in gills, kidney and spleen, whereas AngHV1 was 100% detected in gills and pectoral fin, suggesting that gills are the most appropriate tissues for monitoring of these two viruses.

In the study, we confirmed that mortalities of eels cultured in Korea are related with not only JEECV and/or AngHV1 but also other pathogenic agents, suggesting that further investigation is needed to confirm the effects of individual or multiple infections of the pathogens on eel mortalities.

AQUACULTURE MANAGEMENT AT LANDSCAPE SCALE IN SOUTHERN CHILE

Doris Soto*, Jorge Leon-Muñoz, Carlos Molinet, Elias Pinilla, Sandra Marin, David Opazo and Yuri Soria

Interdisciplinary Center for Aquaculture Research (INCAR), Universidad de Concepción, Concepción, Chile
dorsoto@udec.cl

Planning and management of industrial aquaculture usually addresses environmental impacts of individual farms while environmental evaluations and carrying capacity studies very seldom refer to the water body or relevant ecosystem containing a farm, clusters of farms and also including other users including conservation needs. On the other hand, in most cases, the concept of carrying capacity escapes the planning and management of small scale aquaculture and often the joint impact of many small farms goes uncontrolled with negative environmental and production impacts. Yet, the evaluation of water body potential to sustain different types of aquaculture it is essential to ensure sustainability including for example implementing IMTA approaches at landscape level.

Chile is one of the top world aquaculture producers being the second largest salmon farming producer and the top farmed mussel exporter (more than 600 thousand and 300 thousand tonnes respectively in 2018). Both farming systems are often found in common fjords, and inlets along the Chilean coasts, especially in the northern Patagonia (Los Lagos Region) and there is an urgent need for integrated planning. Salmon farming generates employment and local opportunities, but also creates public concerns regarding environmental impacts from excess nutrient outputs, escapes use of antibiotics etc. On the other hand, mussel farming grows scarcely planned and many areas are overpopulated with seed collectors and fattening rafts without clearly understanding far field effects.

Through participatory processes with the national scientific community we have developed ecosystem level indicators and identified relevant water bodies (RWBs) as landscape units suitable to be managed through risk assessment based on ecosystem-level carrying capacity proxies also considering threats such as diseases, and climate change. This is consistent with the ecosystem approach to aquaculture and allows designing integrated monitoring systems that provide information on the health of a whole waterbody being used by different types of aquaculture and other uses. The initial implementation of such approach allowed to identify RWBs that are at higher eutrophication risk and where salmon farming should be reduced or even terminated. The need to maintain or even create new RWBs as conservation units also becomes extremely relevant specially in the Los Lagos Region to ensure resiliency at a large scale and also to be able to permanently assess ecosystem level effects of the farming activities. The provision of reliable and transparent information on the environmental status of an ecosystem and about its ability to adapt to shocks including climate change is essential to increase local acceptance and social integration of aquaculture. Such approach is also essential to plan and manage aquaculture integrated with other users of ecosystems and ensure the long term sustainability of de activity.

MICROALGAE, INSECT MEAL AND COOKING WATER BY-PRODUCTS AS A SUSTAINABLE ALTERNATIVE IN FEED FORMULATION

Ferreira M., Méndez D., Regueiro L., Ancosmede C., Iglesia X., Blanco B., Santiuste L., Soula M.*

ANFACO-CECOPESCA, Division of Aquaculture. Estrada Colexio Universitario 36310 – Vigo, Pontevedra (Spain)
Corresponding author: mohamed@anfaco.es

By 2030, aquaculture is expected to be the main source of fishery products in the world. This positive fact clashes with the fact that aquaculture relies heavily on fishmeal and fishmeal, soybeans and krill oil as essential ingredients for feed manufacturing, which puts significant pressure on marine and terrestrial ecosystems. In this work, sustainable alternatives to the use of traditional ingredients in the manufacture of fish feed will be assessed by evaluating the combination of three ingredients: (1) insect meal as a quality protein source, (2) microalgae biomass as a source of lipids rich in omega-3 polyunsaturated fatty acids and (3) protein and lipid fraction recovered from cooking water for tuna canning processes as new alternative.

In its first phase we focused on the optimization and production of alternative ingredients, 5 species of microalgae: *Nannochloropsis gaditana*, *Tisochrysis lutea* (CCAP 927/14), *Rhodomonas lens* (ECC030), *Isochrysis galbana* (CCAP927 / 1) and *Phaeodactylum tricornutum Bohlin* (CCAP 1055/1) and the pilot culture conditions were adjusted to get the highest quality lipid content. Lipid accumulation was higher in the stationary phase in the case of *N. gaditana*, *I. galbana* and *T. lutea*. *R. lens* and *P. tricornutum* accumulated more lipids in the exponential phase of growth. the fatty acid profile, showed that microalgae strain accumulate more PUFAs in the stationary phase except *P. tricornutum* , generally coinciding with the maximum accumulation of total lipids. *N. gaditana* accumulated mainly EPA; *I. galbana* stands out for its DHA content and *R. lens* and *T. lutea* accumulated mainly n-6 fatty acids.

Respect to tuna cooking water, the centrifugation, filtration and drying processes were optimized for the separation and concentration of the protein and lipid phases. Through the use of nanofiltration membranes, the protein was concentrated from the initial 1.5%, to 5.5%, recovering almost 80% of the protein. This concentrate was dried in a spry dryer to obtain a dry powder product with a protein content of 70-75%. Oil separated from the cooking water by centrifugation, showed similar fatty acid profile observed in a commercial fish oil.

By the other hand, we worked in the optimization of the production cycle of two species of insect: the *Acheta domesticus* and *Hermitia illucens*. In both cases, larvae were feed experiment with plant products and by-products from agriculture sector. Insect meal analysis showed a difference in terms of protein and lipid composition. *Acheta domesticus* showed a higher protein content (> 60%) and fat (<24%) with a good amino acid profile, including tyrosine, alanine and glutamic acid. Linoleic, oleic and palmitic acids.

The obtained results allowed us to make technical decisions on the formulation and the manufacturing of 4 diets for meagre (*Argyrosumus regius*) and Rainbow trout (*Oncorhynchus mykiss*) based on a substitution of fish oil and fish meal with the generated products. Fish trial will be carried on within 2020.

ALTERNFEED is funded by Fondacion Biodiversidad, the Ministry for Ecological Transition, through the pleamar Program, co-financed by the FEMP.

UNDER-DOSING WITH ANTIMICROBIALS IN AQUACULTURE: WHY DOES IT HAPPEN?

Sophie St-Hilaire[1], Hong Liu[2] ,Angus Lam[1], Edgar Leung[1], Derek Price[3], and Inez Zwetsloot[1]

[1]Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong
[2]State Key laboratory of Aquatic Animal Health at the Animal and Plant Inspection and Quarantine Technical Centre, in Shenzhen Customs District, General Administration of Customs, P. R. China
[3]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI

As global aquaculture expands and intensifies the use of antibiotics to treat bacterial diseases increases. The use of these products is controversial due to increasing numbers of reports of antibiotic-resistant pathogenic bacteria. Two of the known risk factors for antibiotic resistance are overuse and under-dosing with antimicrobials in human and animal medicine, including aquaculture. Here, we review several studies conducted by our laboratory over the last 5 years and identify reasons why under-dosing with antimicrobials occurs in aquaculture settings. First, there are data to suggest that metaphylactic applications of antimicrobials result in uneven distribution of medication in large fish populations. Second, we have found major discrepancies between the actual, active pharmaceutical ingredients in antibiotic pre-mixes and their labels for some antibiotics on the market. It is likely that under-dosing would occur if these fraudulent products are inadvertently used to treat fish. Third, often there are sick, chronically infected fish remaining in a population after the completion of an antibiotic treatment. These fish can serve as sources of infection for fish post treatment, while they are clearing the antibiotic and have subtherapeutic levels of drugs in their systems. All of these issues need to be addressed if the aquaculture industry is to reduce exposure of bacterial populations to subtherapeutic levels of antimicrobials. We offer several suggestions to ameliorate the under-dosing of aquaculture fish.

NEXT GENERATION MICROBIAL MANAGEMENT FOR SHRIMP PONDS

Benedict Standen, PhD

BIOMIN Holding GmbH
benedict.standen@biomin.net

There is more life in a shrimp pond than the animals themselves. The pond ecosystem also supports bacteria, protists, viruses, phytoplankton and zooplankton. As well as complex, a healthy pond must be in a stable state of equilibrium; any deviation from 'normal' pond conditions can have negative consequences on shrimp health and growth.

The organic matter within the pond drives many of the pond interactions. Intensive, or semi intensive, aquaculture requires a lot of organic input, primarily through the feed. Uneaten feed, feces and dead or dying phytoplankton all contribute to the overall organic loading. The accumulation and degradation of this organic matter will reduce dissolved oxygen (DO) and produce toxic metabolites such as ammonia (NH_3), nitrites (NO_2^-), nitrates (NO_3^-) and hydrogen sulfide (H_2S). In low water exchange systems, these compounds can build up and the resulting deterioration in water quality has negative effects on shrimp health. Furthermore high levels of organic matter acts as a fertilizer and can cause a phytoplankton bloom. Rapid growth of phytoplankton will further deplete oxygen reserves at night through respiration, and add to the organic matter as the cells die and start to decompose.

All these factors contribute to the contamination of water and soil, creating favorable conditions for pathogens to grow and negatively affecting shrimp health. Under these poor conditions, the shrimp will become stressed and immunocompromised, increasing the risk of infection, and subsequent disease outbreaks.

The use of beneficial bacteria, probiotics, is becoming increasingly popular as a useful and sustainable pond management tool. This biotechnology is called 'bioremediation'. An effective probiotic solution must provide multiple benefits. Specific strains can produce enzymes that reduce organic matter, whilst simultaneously facilitating the release of highly digestible nutrients. Probiotics can also aid the nitrogen cycle. Specific strains will convert toxic ammonia through to nitrate (nitrification). Although nitrates are less toxic to shrimp, its accumulation can cause problems and encourage phytoplankton growth. Therefore, different probiotic strains must be included in probiotic formulations to convert nitrates back to harmless nitrogen gas (denitrification). Furthermore, certain microbial strains are capable of reducing hydrogen sulfide, improving pond water and sediment, whilst reducing pond odor.

At the same time, probiotics must also control pathogens. Probiotic species, specifically lactic acid bacteria (LAB), produce potent antibacterial substances, such as pediocin by *Pediococcus*, providing direct antagonism to pathogens. Probiotics can also reduce pathogen abundance through competitive exclusion and may possess quorum quenching properties, disrupting bacterial communication and reducing their virulence. Furthermore, when LAB are ingested they are capable of colonizing the intestinal tract where they drive immunity, allowing the host to improve its immune response and fight pathogens using its own immune system.

Together probiotics can be used to complement good management, improving water and soil quality, reducing organic matter, controlling pathogens, improving shrimp health and survival, and ultimately increasing production. Thus, they not only ensure environmental sustainability, but also profitable sustainability.

HEPATOSOMATIC INDEX, INTESTINAL HISTOLOGY, AND DIGESTIVE ENZYMES OF JUVENILE CORAL TROUT *Plectropomus leopardus* FED SYNBIOTIC

Sudewi*, Ni Wayan Widya Astuti, Yasmina Nirmala Asih, Afifah Nasukha, Indah Mastuti, and Sari B.M. Sembiring

Institute for Mariculture Research and Fisheries Extension (IMRAFE)
Dusun Gondol, Desa Penyabangan, Kec. Gerokgak, Kab. Buleleng, Bali-Indonesia, 81155

There is common use of probiotic in larval rearing of coral trout grouper, but, its combination with prebiotic is still lack. A combination of prebiotic and probiotic in feed supplementation is known as synbiotic. This study aimed to determine hepatosomatic index, intestinal histology, and digestive enzymes of juvenile coral trout *Plectropomus leopardus* fed synbiotic.

Juvenile coral trout grouper (3.58 ± 0.22 g) were reared in fiberglass tanks (300 L), 50 fish/tank, using flow-through water system. Feeding was done at satiation, twice/day. Feeding trial was performed for 12 weeks. The formulated feed in the study was supplemented with prebiotic mannan-oligosaccharide (MOS) at 6 g/kg and a combination of probiotic bacteria at 10 g/kg of feed that consists of *Bacillus cereus* BS6, *Bacillus subtilis* BS3, and *Bacillus amiloliquefaciens* BS4. The study used Completely Randomized Design with four treatments; prebiotic, probiotic, synbiotic (pre- and probiotic), and control diet (without pre- and probiotic). Parameters observed in this study were hepatosomatic index (HSI), intestinal histology, and digestive enzymes of coral trout juvenile.

Results showed that all the treatments did not affect on HSI of the fish ($p>0.05$). HSI ranged from 0.87±0.25 to 0.97±0.34. Histological examination of the intestine revealed that prebiotic, probiotic and synbiotic supplementation in feed increased surface area of intestinal villi that indicated by the winding structure of the villi. On the other hand, villi structure of the control fish was in straight structure, as a consequence its surface area was the lowest compared to the other treatments (Figure 1). Analysis of digestive enzymes demonstrated that all treatments resulted in the equal amount of cellulose, amylose, and lipase ($p>0.05$). Protease was higher in the probiotic group thanthe control group ($p<0.05$). Level of protease was similar between prebiotic and synbiotic, and not significantly different with the control treatment.

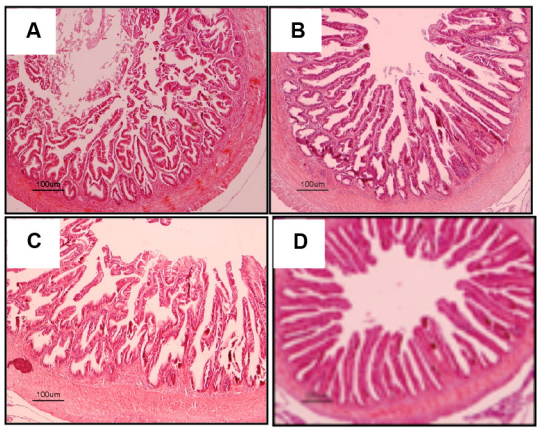


Figure 1. Histological structure of the intestine of juvenile coral trout (*Plectropomus leopardus*) fed prebiotic (A), probiotic (B), synbiotic (C), and control diet (D).

ALGAL-BACTERIAL INTERACTIONS IN RED TILAPIA CULTURE UNDER BIOFLOC TECHNOLOGY

S.Felix*, S.Srividya, M.Charulatha & C.Mohana Sundari & M.Menaga

Vice Chancellor
Tamil Nadu Dr.J.Jayalalithaa Fisheries University
Vettar River View Campus
Nagapattinam-611 002
Tamil Nadu, India
felix@tnfu.ac.in

The current research was performed to study the interaction of algae in biofloc. The experimental group consists of 4 treatments – T1- Biofloc, T2- *Chlorella vulgaris* and *Nannochloropsis sp*. (1:1), T3- Biofloc + *Chlorella vulgaris* and *Nannochloropsis sp* (1:1), T4- Biofloc + *Chlorella vulgaris* and *Nannochloropsis sp*. (2:1). The experiment is conducted in 500L FRP Tanks in Red Tilapia (*Oreochromis niloticus*) with a stocking density of 10/m^3 for a period of 60days in triplicates. Parameters such asfloc characteristics (Fortnightly), Growth parameters (fortnightly), Algal count, Total plate count and water quality (weekly once) and Sludge characteristics, Total carotenoid (TCC) in tissues and chlorophyll a, b and carotenoidin water will be analyzed at the end of the trail. Highest floc volume was found in T1 whereas higher weight gain was found in T3 where biofloc was fed along with the algae *Chlorella vulgaris* and *Nannochloropsis sp* .in the ratio-1:1. No significant difference was found in survival in all the treatments. Algal count was higher in T4 for all the experimental days observed. Reduced bacterial population was found in T2 when compared with other experimental groups. Calcium and Magnesium levels increased significantly in T3 with the days of culture period. Higher suspended solid concentration was found in T4 (360mg/L). TCC in water (0.39μg/ml) as well in tissues (37.2μg/g) was found to be higher in T3.Chlorophyll a (0.67μg/ml) and b (1.73μg/ml) was found to be higher in T4 when compared to other treatments. The results from the study iterate the fact when algae at the ratio of 1:1 added to biofloc improved the animal performance in terms of weight and carotenoid levels and proved the importance of algae in biofloc.

PROBIOTIC POTENTIAL OF BIOFLOC : A COMPARATIVE STUDY ON THE GROWTH PERFORMANCE OF *Penaeus vannamei*

S.Felix*, M.Menaga, M. Charulatha, C.Mohana Sundari & S.Srividya

Vice Chancellor
Tamil Nadu Dr.J.Jayalalithaa Fisheries University
Vettar River View Campus
Nagapattinam-611 002
Tamil Nadu, India.
mailto:felix@tnfu.ac.in

The current study was aimed to compare the growth performance of *Penaeus vannamei* by supplementing the commercial and isolated probiotics in the feed. The animals (0.045 ± 0.005g) were stocked at a density of 500/m^3 in triplicates for a 60days trial. The probiotic strains previously isolated from biofloc culture systems (*K. Pneumoniae, B. megaterium, E. profundum, P. balearica* and *P.stutzeri*) were selected based on its antimicrobial activity against the pathogens (*Aeromonas* and *Vibrio sp.)*. Suitable carbon source has been identified by inoculating these probiotic strains in five different carbon sources namely Glycerol, distillery spent wash, glucose, maltose and sucrose at the rate of 1%. Based on the probiotic growth and cell dry weight, distillery spentwash was found to perform well among the other carbon sources. The probiotic strains were inoculated in 0.5, 1.0, 2.0, 4.0 and 8.0% of distillery spentwash to identify the optimal carbon percentage. The isolates showed a better performance in terms of growth and cell dry weight when it has been inoculated with 0.5% of distillery spentwash. The treatment group includes control (C), biofloc (T1), commercial probiotic (T2), isolated probiotic (in nutrient broth) (T3), isolated probiotic (in 0.5% distillery spentwash) (T4). The commercial probiotic has been supplemented as directed whereas the isolated probiotics were supplemented at the rate of 10ml/100g of feed. Final weight of the shrimps in the treatment groups were as follows: C- 1.7, T1- 1.03, T2- 1.1, T3- 2.25 and T4- 2.47g. The highest weight gain was found in treatment group fed with isolated probiotic supplemented with 0.5% distillery spentwash as carbon source (T4).Histology analysis of the hepatopancreas of the shrimp reared under T1 and T4 revealed there was a mild increase in the B and R cell vacuoles. The present study provided an insight in the use of probiotics isolated from biofloc culture systems.

ROLE OF NUTRITIONAL HISTORY ON THE SUBSEQUENT RETENTION OF GREEN-LIPPED MUSSEL, *Perna canaliculus,* SEED ON COASTAL MUSSEL FARMS

Supono Supono* and Andrew Jeffs

University of Auckland
Auckland, New Zealand 1010
ssup441@aucklanduni.ac.nz

The global mussel aquaculture industry relies heavily on the regular supply of wild seed mussels, to initiate aquaculture production. Supplies of wild mussel seed have a lower cost than seed produced in a hatchery, but wild seed are often highly variable in quantity, size, nutritional condition, which greatly affects their performance after being seeded out onto coastal mussel farms. For example, high losses of more than 80% of seed consistently occur within the first few weeks after seeding out in New Zealand's green-lipped mussel (*Perna canaliculus*) (hereafter called GLM) industry. These losses have often been associated with the variability of nutritional condition of wild mussel seed prior to seeding.

GLM seed of 1.0 – 3.0 mm in shell length were starved for 0, 3, 6 and 9 days and then seeded onto a coastal mussel farm. Their subsequent retention on growing ropes after 2 weeks of seeding out onto the mussel farm was assessed. The nutritional condition of mussel seed both at the end of experimental starvation periods and after recovery from the farm was measured

The retention of GLM seed at the end of the experimental starvation tended to decrease with increasing periods of starvation, but was only significantly lower in mussel seed that were starved for 9 days. In contrast, the retention of mussel seed following seeding out in mussel farm was not different among mussel seed from different starvation periods (~30% across all treatments) (Figure 1).

The primary energy reserves, quantified as total carbohydrate of mussel seed decreased with increasing period of laboratory starvation treatment. Mussel seed that were starved for 9 days showed the lowest total carbohydrate content (16.5 ± 0.5 mg g tissue mass^{-1}). After seeding out, total carbohydrate content of mussel seed from all starvation treatments were at similar level of mussel seed starved for 9 days in laboratory (Figure 2).

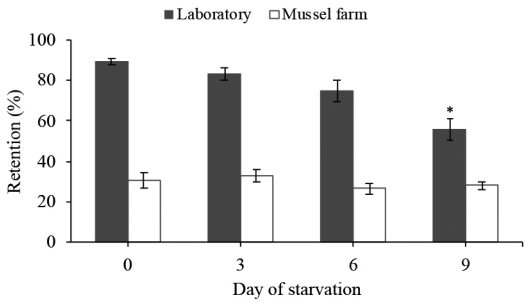


Figure 1. Mean (± SE, n=3) percentage of retention of GLM seed following different periods of experimental starvation and 14 days of seeding out onto mussel farm. Mean with * are significantly different to control treatment (0 day of starvation).

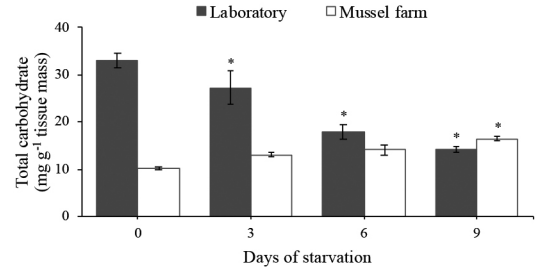


Figure 2. Mean (±SE, n=3) total

POLYMICROBIAL INFECTIONS IN TILAPIA FARMS: MANAGEMENT AND THERAPEUTICAL POTENTIAL

Win Surachetpong*

Faculty of Veterinary Medicine
Kasetsart University
Thailand
fvetwsp@ku.ac.th, win.s@ku.th

Polymicrobial infections are commonly found in tilapia farming as fish are normally exposed to rich communities of bacteria, fungi, viruses, and parasites. However, little is currently known about multiple infection patterns affecting wild and farmed tilapia. Generally, polymicrobial infection patterns involve heterogeneous infectious agents simultaneously or sequentially interacting with susceptible hosts. They can alter pathogenesis, eliciting synergistic or antagonistic effects and complex clinical symptoms when compared to single infection patterns. The outcomes of microbial interference modulate the host immune response with repercussions on the efficacy of treatments and prevention strategies. Linked to the fast growth of tilapia aquaculture and to changing environmental conditions, the study of polymicrobial disease patterns, representing a so far neglected concept, is rapidly attracting the interest of aquatic animal health scientists, and is becoming an emerging and highly researched area. The aim of this talk is to broaden our understanding of how polymicrobial infections affect the host, and how they cause disease when infecting their hosts in an array of aquatic environments and farming conditions, either in natural or artificial systems. Understanding these complex interactions will facilitate disease control and mitigation to reduce the negative impact of polymicrobial infections mainly small-scale tilapia farmers.

POTENTIAL OF *Cyperus rotundus* RHIZOMA EXTRACT AS A MOLTING STIMULANT AND DEVELOPMENT OF GONAD IN THE TIGER SHRIMP *(Penaeus mon*odon.Fab) BROODSTOCK IN HATCHERY

Emma Suryati , Andi Tenriulo, and Rosmiati

Research Institute for Brackish Aquaculture and Fisheries Extention
emmasuryati@yahoo.com

The Cyperus rotundus rhizoma is reported to have methyl fernesoate compounds which are identical to Juvenil hormone III, Juvenil hormone in crustase (methyl fernesoate) can stimulate the activity of other hormones such as ecdystetron and serotonin which play a role in the regulation of metamorposis and maturation of the male and female gonads. The objectives of the study include the isolation of the methyl fernesoate compound from cyperus rotundus rhizome, then the application of the cyperus rontundus extract to the broodstock of tiger shrimp through injection at a dose of 0.100,150, and 200 ug / g bb, and the ablation of the eye stalk as a positive control. The study was conducted using a completely randomized design (CRD) method with 5 treatments and 4 replications. To determine the frequency of molting, gonadal maturation level, number and diameter of eggs and measurement of the rate of development of vitelogenesis by PCR technique using primary vitellogenin F, R and β-Actin F, R. The observations showed that the highest molting frequency was at 150 ug / g bb treatment that was 15 times during maintenance, and the parent shrimp that had the highest gonad maturity was at 150 ug / g bb treatment as much as 75%. The results of the measurement of the rate of development of vitelogenesis through a molecular approach with PCR technique using the vitellogenin primers F, R and β-Actin F, R showed a positive development in the broodstock shrimp treated with Cyperus rontundus extract

Methyl fernesoate (JH III) plays a role in stimulating and coordinating reproduction in insects (Wyatt, 1997), contributing to the reproductive system such as vitellogenin, controlling the immune system, and the resulting embryos (Gaubard Y, 2005; Min et al., 2004; Masner et al., 2004). al., 1968). In MF crustaceans act as gonadotropins and growth, and this hormone is found in the vitellogenin of female individuals and active reproduction in males (Laufer et.al, 1993 in Enrique, et.al., 2001). Methyl Fernesoate (JH III), besides being found in crustaceans and insects, is also found in several types of plants, including in the grass of grass (Cyperus iria) (Bede et al 2000), in cananga flowers (Cananga latifolia) (Yang et 2013) and several types pine plant. So based on this information, the isolation of juvenile hormone (Methyl fernesoate) from cyperus rontundus can be used as a molting stimulant and the development of reproductive organs in tiger shrimp.

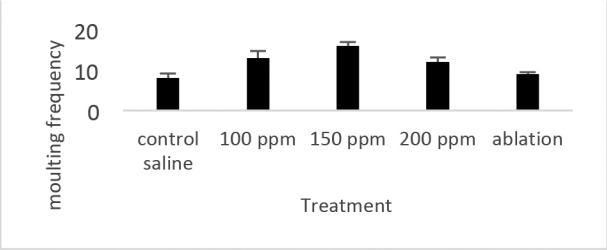


Figure 2. Frequency of molting in prospective shrimp broodstock treated with cyperus rontundus extracts

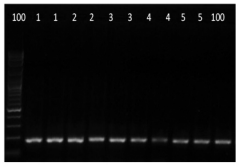


Figure 4. Results of Vitellogenin analysis in tiger shrimp broodstock treated with *Cyperus rontundus*. Note: 1 = 0 (copy control; 2 = 100 ug / g bb; 3 = 150 ug / g bb; 4 = 200 ug / g bb, and 5 = ablation of the eye stalk

MONITORING OF PARASITES IN HAEMOLYMP SHRIMP AND POND WATER LOCATION IN SOUTH SULAWESI

Endang Susianingsih*, Nurhidayah, I.A. K. Kadriah, Muliani, Bunga Rante Tampangallo

Research Institute for Coastal Aquaculture and Fisheries extension Jl
Makmur Daeng Sittaka 129 Maros, South Sulawesi, 90512, Indonesia
Telp +62411371544

One of the causes of disease in shrimp both in hatchery and pond culture is a parasite which in large quantities can reduce resistance of host even cause death in shrimp. This study aims to monitor the presence of the parasite in haemolymph shrimp and pond water from several traditional pond locations in South Sulawesi. Monitoring was conducted on tiger and white-leg shrimp both the identified disease or not. Observations on haemolymph shrimp is done by taking a sample haemolymph according to Blakxhall and Daishley (1973) procedure, followed by the calculation of total haemocyte (THC), while observations in the pond water is done directly by taking samples of pond water using sterilized bottle sample and brought to the laboratory in a cold condition. Parasite observations were made using a microscope with 40X. The monitoring results indicate the presence of parasites in haemolymp and water ponds on shrimp culture which was followed by a decrease in the amount of THC shrimp.

Table 1. The existence of Parasites and Total Haemocyte (THC) and Some Aquaculture Areas In South Sulawesi

Sampling Location	Haemolymph		Pond Water		Total Haemocyte (cell/mL)
	Number of sample	infected	Number of sample	The Existence of Parasites	
Maros	10	9	10	9	10^5 - 10^6
Barru	27	26	27	27	10^5 - 10^6
Sinjai	29	22	18	8	10^5 - 10^6

Viability of the parasite in shrimp haemolymph survive at -20 ° C for 2 days and -4oC for 3 days and at 70 ° C for 30 minutes.

Table 2. Parasite Ability in Haemolymph on Different Condition

Kind of Sample	Ability of Parasite		
Haemolymph	-20°C	-4°C	70°C
	72 Hours (live)	48 Hours (live)	30 minute (death)

Parasite prevention have been done using saponin and dolomite lime. The existence of the parasite is not found by allowing the parasite infected shrimp in ponds for two months.

ADVANCES IN SATELLITE EARTH OBSERVATION DATA ANALYTICS FOR AQUACULTURE

Jason Suwala*[1], Andy Dean[1] and Ignacio San-Miguel[1]

1 Hatfield Consultants, 200 – 850 Harbourside Drive, North Vancouver BC, V7P 0A3 jsuwala@hatfieldgroup.com

World aquaculture production continues to grow and now provides half of all fish for human consumption (FAO, 2016a), but its sustainability is being impacted by climate change, competition for space and natural resources, pollution and environmental degradation. Effective management of the sector requires appropriate and accessible data to inform management decisions. Satellite earth observation technology has developed rapidly in the past five years, with numerous advances in spatial resolution, spectral information, frequency of observation, and cost-effectiveness (open data). The information can inform aquaculture development (strategic planning, zonation and site suitability), aquaculture practice and management (inventory, status, monitoring of the environment and monitoring at the farm level), and aquaculture risk management (disaster risk management, response, recovery, monitoring and assessment). While the growing volume of satellite data represents a unique opportunity for aquaculture, it also poses a major challenge to achieve its full potential in terms of extracting valuable management information. A variety of big-data platforms currently seek to address these issues by bringing together satellite data, environmental data, and analytics processing systems. These platforms include the European Space Agency funded Coastal and Food Security Platforms and Google's Earth Engine. Each platform has a variety of benefits which are discussed.

SUSTAINABLE AQUACULTURE DEVELOPMENT FOR ENSURING THE NUTRITIONAL SECURITY IN INDIA

Dr Saroj Kumar Swain

Director
ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India
E-mail: sarojswain2008@gmail.com

Fish constitutes approximately 17 percent of the animal protein consumed by the global population. The fish production from capture fisheries (90.3 mt) has been nearly stagnant for many decades, whereas the demand for fish has increased significantly due to an increased awareness of its health benefits. Aquaculture is the fastest-growing food production sector in the world, with an estimated production of 122.6 million tonnes. Aquaculture is the only means of meeting the additional demand for fish consumption. The Asia-Pacific region has been continuing to be the major producer of fish. In 2018, aquaculturists were reported to farm about 622 species or species items including 387 finfishes, 111 molluscs, 64 crustaceans, seven frogs and reptiles, ten aquatic invertebrates and 43 aquatic plants. FAO highlights the following key strategic points for achieving the blue transformation; increase the development and adoption of sustainable aquaculture systems; ensure that aquaculture is integrated into national, regional and global development strategies and food policies; ensure that aquaculture production meets the growing demand for aquatic food and enhances inclusive livelihoods, and improve capacities at all levels to develop and adopt innovative technology and management practices for more efficient and resilient aquaculture industry. The technological landscape in the global aquaculture scenario is changing rapidly. Genomic selection, recirculating aquaculture systems, aquaponics, and novel aquafeed ingredients, rapid disease diagnostics, oral vaccines are some of the newer technological innovations that steer the aquaculture sector. From 2000-2018, aquaculture production in freshwater, brackish water and marine water increased at a compound annual growth rate of 5.7 percent, 7.7 percent and 5.2 percent respectively while total aquaculture production grew at an annual growth rate of 5.6 percent. Global food supply and per capita consumption of fish and fish products continued to increase faster than human population growth. Aquaculture is striving to innovate in order to increase production and sustainability. Fisheries and aquaculture are socio-economically important sectors to most nations in the Asia-Pacific region and most nations in the region have high rates of fish consumption, mostly sourced from aquaculture although the small island nations depend to a greater extent on capture fisheries. The aquaculture sector faces challenges including competition for land and water resources, as well as external factors such as climate change, conflict, economic uncertainties and most recently the COVID-19 pandemic. The pandemic and other stresses such as droughts and tsunamis revealed that the aquaculture industry has not engaged sufficiently in disaster preparedness. International and national mechanisms are being put in place to increase the sustainability, good governance and social license of the sector to address these challenges. The diversity of the sector, the opportunities for good jobs and commitments by governments to good governance will help the sector meet these challenges.

In India, with a population of 1.3 billion, ensuring protein security is a challenging task. The country must produce approximately 20 million tonnes of fish annually by 2030, with aquaculture accounting for 80% of total production. India has enormous water resources in the form of lakes, wetlands, ponds & tanks etc. Increasing the present average fish productivity of the water bodies from 3.5 t/ha to a level of above 5.0 t/ha is the top priority of the country. Holistic development of the natural waters also needs to be explored to reduce the stress on pond resources and ensure sustainable development. In recent years, India has implemented a number of initiatives to increase fish production via culture expansion and vertical growth in terms of resource renovation and management, system and species diversification, breed improvement, and technological interventions. A robust network of broodbanks, wider dissemination programmes, year-round production of fish seeds, alternative feed ingredients, disease surveillance and treatment to prevent and control diseases, technology impact assessment, etc are some of the key areas of intervention. Developing genetically improved varieties of fish and shellfish through integrated, cost-effective, marker-assisted selection and genomic selection programmes at the national level for prioritized species would be a game changer in boosting the productivity of the sector. Aquaculture's sustainable development is inextricably linked to policy support and appropriate strategies for technology transfer and adoption for efficient resource utilization, market linkages, and post-harvest product processing, among other factors.

RAPID ON-SITE DETECTION OF FISH IRIDOVIRAL DISEASE

Syed Khader Syed Musthaq*, Masato Miyata, Nushahidah Ali, Alden Toh, Sakinah Mulyana, Padmanabhan Saravanan, Chan Pek Sian Diana, Lee Chee Wee

Centre for Aquaculture and Veterinary Science, School of Applied Science, Temasek Polytechnic
21 Tampines Avenue 1, Singapore 529757.
musthaq@tp.edu.sg

Iridoviruses belonging to the family Iridoviridae and genus *Megalocytivirus* cause systemic disease in fishes. It responsible for high mortality of cultured marine and freshwater fishes. Current detection kits are not field deployable and often require expensive instrumentation and technical expertise to perform the assay. There is a need for a rapid, on-site and user-friendly test method to detect iridoviral pathogen in farm settings.

In the present study, optical immuno assay (OIA) method was developed to detect iridoviral infection in fish. The kit comprises of sample processing (tissue and blood) gadgets, and a disposable test device that can be deployed for on-site detection. Irido-OIA kit has the ability to detect pathogen and host response in a single test. This will help in post vaccine monitoring of fishes for their serological response/protective immunity and as well pathogen presence.

Fish samples (n=65) were analysed for the presence of iridoviruses in suspected fish samples and compared with the gold standard PCR method. The irido-OIA kit was able to detect $5x10^3$ viral particles per millilitre of processed tissue sample, with an overall sensitivity and specificity of 91.6% and ~98% respectively. Further, the performance of kit will be evaluated with more field samples. Moreover, the operational advantages of the Irido-OIA kit include field deployability, rapid detection, user friendliness with less logistic load, and visual read-out.

SMALL-SCALE SHRIMP FARMING AND COMMUNITY SUPPORTED FISHERIES IN SOUTHEAST ASIA

Brian Szuster, PhD

Department of Geography and Environment
University of Hawai‘i at Mānoa
2424 Maile Way, Saunders 445
Honolulu, Hawai’i, 96822 USA
szuster@hawaii.edu

Shrimp farming in Southeast Asia is often considered a globalization success story. The region emerged as a key area for farmed shrimp production in the 1990s, and it remains a leading producer of shrimp for export to international markets to this day. This achievement has not, however, been without cost. Small-scale shrimp farmers in Southeast Asia have suffered persistent social, economic and environmental dislocations stemming from their limited resources and influence within highly competitive global seafood markets.

Community supported fisheries represent an alternative marketing model which could potentially support small-scale producers in Southeast Asia whose viability could be threatened by large farms and the further intensification of shrimp production. This paper investigates opportunities for community supported fisheries in the region, encourages research that builds social capital in aquaculture communities, and identifies opportunities to link small-scale shrimp farming operations with local markets that seek high quality seafood produced in an environmentally sustainable and socially responsible manner.

BROODSTOCK CONDITIONING SALINITY IMPACTS SPERM QUAILTY IN EASTERN OYSTER, *Crassostrea virginica*

V. MacKenzie Tackett*, Helen R. Montague, Jim A. Stoeckel, Scott Rikard, Andrea Tarnecki, Ian A.E. Butts

School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University, Auburn, AL
vmt0009@auburn.edu

Aquaculture demands for high value products, like the Eastern oyster (*Crassostrea virginica*), continue to rise. Many factors, including eyed larval production, limit commercial oyster output. To combat this, hatcheries spawn diploid females with tetraploid males to produce triploid offspring, which in many instances exhibit faster growth than diploid oysters and maintain meat quality during the reproductive season. Despite improvements, gametes produced in hatcheries are often of low quality. While it is known that abiotic factors, like temperature and pH, influence gametogenesis in Eastern oyster the effects of salinity need to be fully identified. In *C. gigas* low salinity negatively impacted fertility, which indicates inhibition in sperm motility. To expand on these ideas, this study investigates how salinity affects Eastern oyster gametogenesis and gamete quality for triploid production. Oysters were conditioned in recirculating aquaculture systems (RAS) at 10, 20, and 30 PSU for 30 days. Temperature and light mimicked oceanic conditions in the Gulf of Mexico. RAS contained 6 ×140 L tanks, each housing 15 oysters per ploidy. Oysters were opened and sex determined by observation of their gonads under a microscope. Semen was collected from tetraploid males, sperm density was determined using hemocytometer counts while viability was evaluated by flow cytometry. Sperm velocity (VCL) and motility were evaluated by computer assisted sperm analysis. Eggs collected from diploid females were assessed for fecundity and egg size. For both sexes, lipids were extracted and analyzed by gas chromatography. Histology samples will be processed and gametogenic development assessed based on a maturity stage index.

Sperm density tended to increase at higher conditioning salinities (1.35×10^9 at 10 PSU to 1.86×10^9 sperm/mL at 30 PSU), however this trend was non-significant. At 30 and 60 s post-activation of sperm, oysters conditioned at 10 PSU had lower VCL than other salinities (Fig 1A). Sperm motility was lower for oysters conditioned at 10 PSU (Fig 1B). Conditioning salinity also affected sperm viability with lowest values observed at 30 PSU (Fig 1C). Female fecundity tended to decrease when oysters were conditioned at higher salinities (2.74×10^6 at 10 PSU to 2.41×10^6 eggs at 30 PSU), however this trend was non-significant. Results, thus far, suggest 20 PSU as a promising conditioning salinity for sperm quality. Lipid and histology analyses will provide further support for this conclusion.

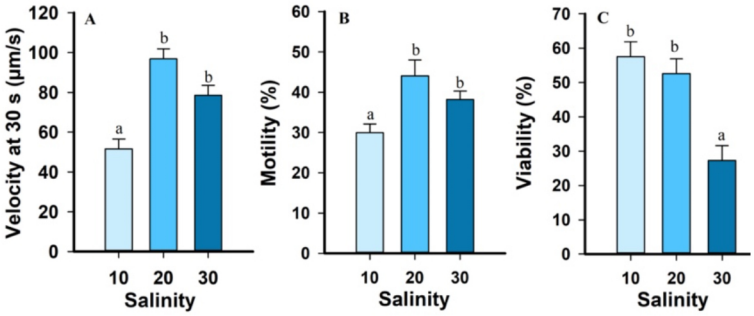


Fig. 1. Effects of conditioning salinity on sperm velocity (A), motility (B), and viability (C) in Eastern oyster tetraploid males (Crassostrea virginica).

GROWTH AND LIPOGENESIS INDICATORS OF AMAZON TAMBAQUI (*Colossoma macropomum*) FED DIFFERENT VEGETAL INGREDIENTS

Leonardo Susumu Takahashi*, Jeisson Emerson Casimiro Ferrari, Douglas de Souza Graciano, Amanda Araújo Gaspar, Thaise Mota Satiro, Hugo Henrique D'Amore Soares, Affonso Gama Pinheiro, and Gabriela Castellani Carli

São Paulo State University (Unesp)
Aquaculture Center of Unesp (Caunesp)
14884-900 - Jaboticabal, SP, Brazil
leonardo.takahashi@unesp.br

Tambaqui (*Colossoma macropomum*) is a freshwater fish species native to the Amazon and Orinoco river basins of South America. It is the most produced native species in Brazil and its total aquaculture production in 2021 was 262,370 t. The frugivorous regime of this species renders it particularly tolerant to dietary incorporation of fruits and seeds/nuts. Furthermore, carbohydrates are the least expensive dietary energy source and an interesting way to spare some dietary protein for growth. The present study evaluated the growth performance and lipogenesis indicators of juvenile tambaqui fed different energetic vegetal ingredients.

A total of 192 tambaqui (131.6 ± 1.9 g) were distributed in 24 aquaria and fed with 6 diets (4,000 kcal/kg) containing (30%): corn, sorghum, corn starch, wheat bran, rice bran and broken rice. Fish were fed for 57 days, until apparent satiation, in four meals a day. In the final biometry, the fish were weighed and measured, and blood aliquots were taken for later determination of metabolites in the blood. Then, the fish were sacrificed (eugenol, 10 g/L) for removal and weighing of visceral fat and liver. The experiment was carried out in a completely randomized design with 6 treatments and 4 replications. The results were submitted to ANOVA and Tukey's test at 5%.

No mortality was recorded. No difference on growth performance of tambaqui was observed. On the other hand, fish fed corn starch displayed an increased lipogenesis. This is probably related to the concentration and availability of glucose from the starch.

Table 1. Growth performance of tambaquis fed vegetal energetic ingredients for 57 days.

Variables	Corn	Sorghum	Starch	Wheat bran	Rice bran	Broken rice	*P value*
Final length, cm	22.7	22.6	22.8	22.6	22.0	22.9	0.38
Weight gain, g	68.5	64.7	75.4	60.6	53.1	68.1	0.08
Feed consumption, g	834.5	832.6	830.8	813.0	839.2	831.0	0.64
Food conversion	1.5	1.6	1.4	1.6	2.1	1.5	0.10

Data are mean values (n=4). Means in the same line with different letters are different by Tukey's test (P<0.05).

Table 2. Blood metabolites and somatic indexes of tambaquis fed vegetal energetic ingredients for 57 days.

Variables	Corn	Sorghum	Starch	Wheat bran	Rice bran	Broken rice	*P value*
Cholesterol, mmol/L	2.8 c	3.1 ab	3.2 a	2.9 bc	3.4 a	3.2 ab	0.002
Triglycerides, mmol/L	2.3 bc	2.4 b	3.1 a	2.1 c	2.1 c	2.3 bc	<0.001
Glucose, mmol/L	8.6	9.3	9.4	10.1	8.5	9.3	0.12
Hepatosomatic index, %	1.2 c	1.4 a	1.5 a	1.5 ab	1.4 bc	1.5 ab	0.004
Mesenteric fat index, %	0.7	0.6	0.6	0.5	0.4	0.7	0.06

Data are mean values (n=4). Means in the same line with different letters are different by Tukey's test (P<0.05).

SCALING UP THE ARTIFICIAL INDUCTION OF SPOROGENESIS IN *Saccharina latissima*

Éric Tamigneaux *, Isabelle Gendron-Lemieux, Mathilde Lemoine, Pauline Garnier and Jean-Philippe Hébert

Merinov and Cégep de la Gaspésie et des Îles
167 La Grande Allée Est, G0C1V0
Grande-Rivière (Québec), Canada
etamigneaux@cegepgim.ca

Saccharina latissima is gaining economic importance as an aquaculture crop. In eastern Canada, seeding of the culture ropes occurs at a time of the year when access to ripe kelp blades is not guaranteed because of heavy biofouling by bryozoans. A marine hatchery planned exploring the artificial induction of sorus and a series of experiments were set up to examine how to save floor space and maximise the sorus produced per tank using existing equipment. The meristem and outer wings of all fronds were discarded. Some blades were attached on a horizontal net support in a large square tank. In other fronds, the central part of the blades was cutted into short fragments which were immerged in various types of tanks with flowing seawater and aeration to keep them moving. Water temperature was controlled and white light illumination was adjusted to provide a constant short day of 8 h with an intensity of 100 μmol m^{-2} s^{-1}. In the various set-up tested, the first sorus appeared at the end of the first week and ≥50 % of the blade fragments were fertile after 28 days, whether tied on a net or moving freely in the tanks (Fig. 1). Regarding the effect of blade wet biomass in clear cylindrical tanks, it was observed that 100% of the fragments were fertile after 45 days, with 36% and 30% of the fragments surface area as dark sorus, respectively in the 2.2 g L^{-1} and 4.4 g L^{-1} treatments (Fig. 2). The same results were obtained when using the plastic bags for microalgal culture provided by the hatchery. The experiments showed that conditioning blades fragments moving freely in a raised cylindrical tank is as effective as intact blades tied on a frame in a square tank, which means significant floor space saving for kelp hatcheries. Less biofouling was observed when using the medium part of the blades at 4.4 g L^{-1}. Since some fragments were maturing faster, some culture twines could be seeded with spores at the 4th week and some at the 6th week. With this set up, one 250 L cylindrical tank produce enough sorus to seed 29 m of a 1.5 mm diam. twine if a concentration of 7 500 spores ml^{-1} is used in the seeding tank.

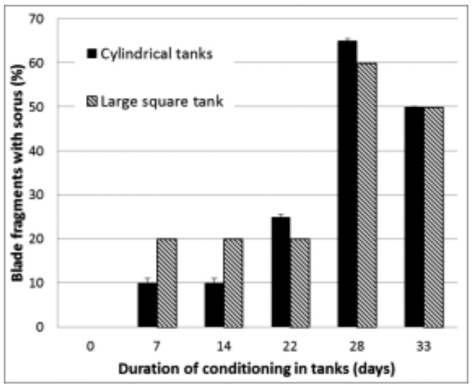


Fig. 1. Changes in fertility status of blades attached on a frame in a square tank vs moving freely in a clear cylinder tank.

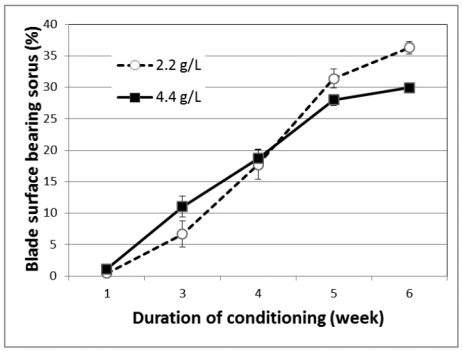


Fig. 2. Comparison of sorus surface development on blades conditioned in a clear cylinder tanks using two different wet weights per liter (average ±SE; n = 6).

EFFECTS OF DIFFERENT SALINITIES ON SURVIVAL, OSMOLALITY AND BLOOD CHARACTERISTIC OF JUVENILE RABBITFISH, *Siganus guttatus*

Bunga R Tampangallo[1*], Muhammad C Undu[2], Asda Laining[1], Ramadhan[1], Sri RH Mulyaningrum[1]

[1] Research Institute for Coastal Aquaculture and Fisheries Extention (RICAFE), MMAF
Jl. Makmur Dg. Sitakka No.129, Maros 90512, South Sulawesi, INDONESIA
[2] Polytechnic of Marine and Fisheries Jembrana
Pengambengan village, District of Negara, Jembrana Regency, 82218, Bali, INDONESIA
*bungatampangallo@yahoo.com

As a euryhaline species, rabbitfish tolerant to wide of salinity. However, it is important to determine the salinity range where rabbitfish can survive and grow normally. This study aimed to determin the effects of different salinities on growth and stress levels of juvenile rabbitfish *S. guttatus*.

The treatments were eight different salinities which were 5 ppt (A), 10 ppt (B), 15 ppt (C), 20 ppt (D), 25 ppt (E), 30 ppt (F), 35 ppt (G) and 40 ppt (H). Juvenile of rabbitfish with mean body weight of 18.85.5 g were used as tested fish. Each salinity was adjusted either by diluting seawater with freshwater or by evaporating it to obtain high salinity of 35 and 40 ppt. Twenty aquaria with capacity of 20 L were used to accomodate the 8 salinities as the treatments with triplicates. Juvenile of rabbitfish were stocked with density 5 ind./aquarium. The observed variables were body colour changes, plasma osmolality, blood leucocryte and hematocrite, survival, and growth.

After 14 days observation, the results showed that only one fish dead in G groups . Body colour of the fish did not much change from bright white yellowish to dark in C and D groups, compare to other 6 groups. Osmolality plasma increased by increasing the sanility up to 35 ppt and further decreased at the highest salinity of 40 ppt. The lowest osmolality was found in A group which was 357.7 mOsm/kg and the highest was 77.7 mOsm/kg observed in G group. Plasma osmotic rates of fish reared in 5 and 10 ppt was hiperosmotic while fish reared in 15 to 40 ppt was hypoosmotic (Table 1).

Salinity also affected the leukocrite and hematocrite where the lowest percentage of leukocrite was found in H group which was 64.09 ± 5.97% and the highest percentage was at C group (75.62 ± 10.54%) (Fig. 1).

Based on survival rate, juvenile rabbitfish can tolerate salinity of 5 to 40 ppt but the osmolality condition is approaching isotonic in 10-15 salinity.

Tabel 1. Osmolality at different salinity of *Siganus guttatus*

Salinity (ppt)	Osmolality of plasma (mOsm/kg)	Osmolality of medium (mOsm/kg)	Level of osmotic (mOsm/kg)
Fish stock	406.6±27.77	1032.5±3.54	(-) 625.9±27.77
5	357.7±4.39	183±7.2	174.7±4.0
10	360.8 ± 9.34	304.3±64.6	56.5±69.0
15	371.5±9.3	474±11.3	(-) 102.5±8.3
20	367.6±14.38	619±1.7	(-) 251.4±16.1
25	372.7±3.06	766±27.2	(-) 393.3±29.3
30	377.5±4.94	916.3±5.5	(-) 538.9±10.0
35	377.7±4.22	1074.7±3.1	(-) 697±2.4
40	375.1±4.73	1283.7±82.5	(-) 908.6±82.7

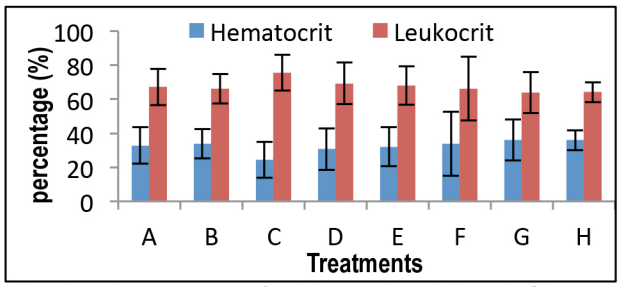


Fig. 1. Percentage of white and red blood *Siganus guttatus* at different salinity

The authors thank to the Australian Center for International Agricultural Research for finding the study through FIS/2016/130

IMPROVEMENT IN IMMUNE RESPONSE AND DISEASE RESISTANCE OF *Cyprinus carpio* AGAINST *Aeromonas hydrophila* THROUGH A DIET OF CHITIN AND CHITOSAN

Tan Jia Wen* and Liu Chun Hung

Diploma in Veterinary Technology, School of Applied Science
Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
1704007b@student.tp.edu.sg

Cyprinus carpio is one of the oldest domesticated aquatic species for food. Farmed *C. carpio* production was nearly 14 percent of the total global freshwater aquaculture production in 2002. *Aeromonas hydrophila* is one of the most common zoonotic pathogens found in the carp farms. In this study we added immunostimulants, chitin, and chitosan into the feed of *C. carpio* to enhance its immune response to other antigens. 1% chitin, 0.5% chitin, 0.5% chitosan, 1% chitosan and control were added to respective feed and 2 months feeding trial was held before the immunity was tested. Besides immune parameters such as lysozyme and respiratory burst were tested in the study, concentration of expressed mx gene in individual fish were measured using quantitative polymerase chain reaction. Blood smears were made to count the number of white blood cells and results were compared to the reference range. Challenge test with lethal dose 50 using *A. hydrophila* showed that fishes fed with 1% chitin and 1% chitosan have close to 50% of the mortality rate in the 5 days of observation period. While the rest have a mortality rate of 25% through the 5 days observation period. However, within the first 24 hours of observation, the mortality rate of fishes fed with 0.5% chitin and 0.5% chitosan was 70% less than the control. Throughout the whole feeding trial, fishes fed with 0.5% chitosan showed the highest growth rate within the set ups while fishes fed with 0.5% chitin showed the lowest growth rate followed by control.

CAPTURE FEED EFFICIENCY OF CAPTIVITY CORAL: UPTAKE OF *Artemia*-BASED FEEDS AND COMMERCIAL PELLETS BY *Zoanthus* CORALS

Tan. R. J* and Chang. O

Diploma in Veterinary Technology, School of Applied Science, Temasek Polytechnic, Singapore 529757
1700172H@tp.edu.sg

Rising sea temperatures are contributing factors of mass bleaching events in coral reefs. One such coral presents is reefs would be the *Zoanthus* corals. Such bleaching events can be prevented by increasing the stress resilience of corals and one such method is through feeding a enriched diet. Corals have been studied to be autotrophic from the symbiotic algae that grows on their polyps. However, the corals are still capable of heterotrophic behaviour by capturing prey such as artemia within water columns in the sea. Hence, in this study, the heterotrophic characteristics of *Zooanthus* were studied for further application on administering beneficial supplements in their diet to prevent bleaching. The *Zooanthus* coral undergone 3 feeding trials; Commercial pelleted feed (1), *Artemia Salina* (2) and *Artemia Salina* enriched with probiotics (3) for a total of 12 days, with each trial being fed for 4 days. The method used here was targeted feeding where the feed was pipetted directly above the polyps of the corals. The corals with closed heads after 3 min after the feed was released is recorded as receptive whereas if it is open, it is recorded as not receptiveness. The feed that was most receptive to the corals were the pelleted feed with an average of 89.15% whereas the receptiveness of *artemia salina* and *artemia salina* enriched with probiotics were averaged at 10.51% and 9.64% respectively. Overall, the feed that had a significantly higher receptiveness was the pelleted commercial feed.

NOVEL METHOD FOR ERADICATION OF MARINE LEECH *Zeylanicobdella arugamensis* IN MARINE GROUPER FARMS

Erin Chung-Wei Tan*, Sugania Vijayan, Beng-Chu Kua, Muhammad Haziq Aiman, Ann-Jie Goh

3 Little Fish Sdn. Bhd.
39-G, Blok D, 1, Jalan SS 7/26
47301 Petaling Jaya, Selangor
info@3lfish.com

Marine leeches (*Zeylanicobdella arugamensis*) are ectoparasites that can infest various cultured marine fish (e.g; groupers, snappers, seabass and pomfret). When these parasites latch onto its host, their bites causes wounds that will allow secondary infections of pathogenic bacteria and fungus leading to unsightly fish and mortality of cultured populations. BP2 is a marine leech trapping device developed to reduce the number of marine leech infestations in cage cultured marine fishes without the usage of chemicals, that is easy to apply and reduces handling stress to fish. This trial was conducted to determine the efficacy of the commercially produced devices in attracting leeches. Percentage of leech prevalence and mean intensity on the fish over time was also recorded. The trial ran for a total of 13 days with 5 days interval of 3 cycles.

Background numbers for lesions, wounds, haemorrhages, prevalence and mean intensity of leeches on each grouper were recorded by sampling 3 fishes out of each of the 8 grouper cages. To find out the effectiveness of the BP2 device, BP2 was submerged inside 4 grouper cages. BP2 was removed from the water after 5 days. The number of leeches and cocoons on the BP2 were recorded. These steps were repeated every 5 days for 2 more cycles.

The BP2 managed to harvest a total of 1,578 leeches and 52,330 cocoons in just 3 cycles of application (Table 1). After 2 cycles of BP2, the prevalence reduced from 100% (M.I = 144 leeches/body) to 83% (M.I = 9 leeches/body). And in the 3rd round, the prevalence increased to 94% (M.I = 200 leeches/body) (Table 2). Here, the M.I increased presumably due to newly hatched leeches as we noticed that the leeches collected were mostly juveniles (Figure 1).

As the marine leeches are ever present in the sea, it is important to continue applying the BP2 in the cages every 5 days to ensure that the leeches' lifecycle is disrupted, and the fishes are protected.

TABLE 1. Number of leeches and cocoons trapped on BP2 device after 3 cycles.

Application	Leeches on BP2	Cocoons on BP2
1X BP2	865	19240
2X BP2	534	26270
3X BP2	179	6820
Total	**1578**	**52330**

TABLE 2. Prevalence and mean intensity of leeches on the groupers over time.

Application	Prevalence	Mean Intensity (leeches/fish)
Before BP2	100%	144
1X BP2	100%	138
2X BP2	83%	9
3X BP2	94%	200

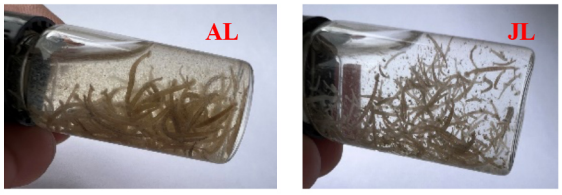


FIGURE 1. Adult Leeches (AL) vs Juvenile Leeches (JL)

DIGITAL TRANSFORMATION FOR AQUACULTURE INDUSTRIES

Jason Tan

Amazon Web Services Pte Ltd
23 Church Street #10-01 Singapore 049481
jtanct@amazon.com

Aquaculture industries traditionally use labours and experience to operate the farm. There is generally no acceptance in looking at technology to support their operation. This is especially for country where land are abundant and labor cost are manageable. This result in inconsistencies across farm in both their output and quality.

Secondly, the new generation of consumer is getting more demanding, looking at food security and environment concerns as their criteria for food selection. The expectation of traceability of the food and concept from farm to table are demanding the farmer to open up for technology to supplement their operation.

This is made more critical by the COVID situation over the past 30 months. COVID disrupts the global supply chain and result in disruption in the operation of industry. Aquaculture are not spared from the disruption. This becomes more critical for Singapore when we are aspiring to be 30% self-sufficient by 2030 for the food consumption in Singapore.

Aquaculture industries like any other industries need to explore how digital transformation can elevate the industries to meet the next decade needs for both consumer and the farm.

In this talk, we will look at how technology can be useful tools to drive both technical and economic efficiency for the aquaculture industries. We will look at some use cases where farm adopt technologies to drive the efficiency for their farm operation. We will also share some good practise in adopting technology for the aquaculture industries.

REARING OF WHITE SHRIMP *Litopenaeus vannamei* LARVAE AT DIFFERENT STOCKING DENSITIES UNDER BIOFLOC CONDITION

Chau Tai Tao*, Nguyen Van Hoa and Tran Ngoc Hai

College of Aquaculture and Fisheries
Can Tho University
cttao@ctu.edu.vn

The aim of the study is to determine the optimal stocking density in rearing larvae of white shrimp (Litopenaeus vannamei) under biofloc condition. The experiment consisted of four triplicate treatments with different stocking densities of (i) 150 larvae/L, (ii) 200 larvae/L, (iii) 250 larvae/L and (iv) 300 larvae/L. Larvae were stocked in 500-L tanks at water salinity of 30‰ with continuous aeration. Sugar was used as carbon source and added every day to rearing tanks from the larval stage of Mysis-3, with ratio of C:N of 20:1. Results showed that water quality parameters, total bacteria, Vibrio spp bacteria count and floc volume were in suitable range for larvae development. Postlarvae-12 length and survival rate were the highest at stocking density of 150 larvae/L (11.59 ± 0.16 mm; 58.7 ± 7.9%), and it was not significantly different compared to those at stocking density of 200 larvae/L (11.49 ± 0.38 mm; 55.0 ± 3.0%) ($p>0.05$). The highest yield was found at stocking density of 200 larvae/L and it was significantly different to remaining treatments (110±6 post-larvae/L) ($p<0.05$). In short, rearing white-leg shrimp larvae at stocking density of 200 larvae/L under biofloc condition presented the best practice.

A SYSTEMATIC RISK PROFILING FOR MEDITERNAREAN SEABASS AND SEABREAM FARMS

Tavornpanich Saraya*, Leandro Margarida, Alain LE Breton, Ana Muniesa, Anna Toffan, Bernardo Basurco, Dolors Furones, Eleonora Franzago, Francessco Pascoli, Hosam Saleh, Manuela Dalla Pozza, Mona Dverdal Jansen, Nadia Chérif, Panos Varvarigos, Snježana Zrnčić, Valentina Panzarin, Edgar Brun

Department of Aquatic Animal Health and Welfare
Norwegian Veterianry Institute
Oslo, Norway
*saraya.tavornpanich@vetinst.no

Aquaculture is the fastest-growing food production sector. Mediterranean marine aquaculture aims to increase its production with European seabass and Gilthead seabream being the two main species produced in this region. Biosecurity is an essential practice to minimize the risk of introduction into, establishment, and spread of pathogenic agents within a farm, allowing for an increment in production, and an improvement in animal health, welfare and public acceptance. This study aims to create a systematic scoring system to quantify the risk of pathogen introduction and spread on basis of farm management and biosecurity practices, and to develop a risk profiling for Mediterranean Seabass and Seabream farms.

A questionnaire survey on farm characteristics, production statistics, potential pathways of disease introduction and spread (i.e. introduction of life fish, water exposure, mechanical transmission with close proximity, distance independence mechanical transmission), biosecurity and management practices, vaccination, diagnostic competence and capacity, was administrated using a face-to-face interview in eight participating Mediterranean countries (Croatia, Egypt, France, Greece, Italy, Spain, Tunisia, and Turkey).

We developed a quantitative tool based on Biocheck.Ugent™ a risk-based, weighted scoring system to measure farm biosecurity. The questionnaire underwent reformulation to estimate an overall risk score by assigning individual questions into relevant subcategories of external and internal biosecurity. The risk score was then used for profiling each farm according to farm characteristics and production statistics. Results show the biosecurity scores for the different subcategories of external and internal biosecurity in land-based and sea- cages farms. Score ranges from 0 (worst scenario) to 100 (best scenario). The overall risk of pathogen introduction and spread by country indicates interesting differences in biosecurity level among the Mediterranean countries.

The quantitative scores and risk profiles received from the study would be helpful for farmers to ascertain which biosecurity measures they should apply to minimize the risk of pathogen introduction and spread both within a farm and to their neighbours.

PATTERN OF REPRODUCTIVE BIOLOGY OF SNOW TROUT, *Schizothorax richardsonii* (GRAY) INHABITING IN RIVER POONCH OF NORTH WEST HIMALAYAN REGION OF INDIA

Ishtiyaq Ahmad*, Imtiaz Ahmed and Mohd Awas

DST Sponsored Fish Nutrition Research Laboratory
Department of Zoology, University of Kashmir
Srinagar, India-190006
Safish999@gmail.com

Schizothorax richardsonii is an important genera of Schizothoracine group and is considered as important food fish of Peer Panjal region in North West-Himalaya, India. Schizothoracids represent the most dominant group of fish fauna in the snow-fed rivers of Poonch among which *S. richardsonii* has a vital role in commercial fishery. Although lot of work on the various aspects of reproductive biology of the group inhabiting in both lotic and lentic water bodies have been reported in the past from this region, however, detailed knowledge about reproductive biology of *S. richardsonii* from any geographical regions has not been reported yet. Therefore, the present study aims to characterize the reproductive biology of *S. richardsonii* inhabiting in river Poonch.

Monthly sampling was carried out from four different sites and a total of 125 specimens were collected. The specimens were transported to the laboratory for further analysis. Total length was measured using vernier caliper, while as body weight was recorded using digital electronic balance. Sex ratio was calculated on monthly basis and the results were analyzed by a χ^2 test (1:1; $P < 0.05$). For gonad collection, ventral side of the fish was cut and opened from the anterior end and gonads were carefully detached from other visceral organs and cleaned. Gonadosomatic index (GSI) was calculated by standard formula. Number of eggs and oocyte diameter were also recorded. Maturity stages and reproductive seasonality were determined by visual observation of gonads as well as by examination of monthly changes occurred in GSI. Fecundity was estimated for 24 mature females following the gravimetric method. Relationships between absolute fecundity and total length, total body weight and ovary weight were determined by using standard formula.

The results showed that GSI increased from May onwards and attained a peak in the month of October both for (8.23 ± 2.04) males and (12.42 ± 4.10) females and thereafter again an increase in GSI was recorded from January to March, indicating that fish had two spawning seasons with a peak in October and another short peak period was noted in the month of March. Based on the GSI and visual analysis six maturity stages were identified which showed that fish spawns twice in a year. Minimum ova diameter was recorded in the month of April (0.21 ± 0.09), while highest was estimated in the month of October (2.12 ± 0.80 mm). Fecundity was recorded in the range from minimum 2012.6 (*TL* = 17.8 cm) to maximum 5213.7 (*TL* = 39.2 cm). Regression analysis was used to determine the relationship between fecundity and total length (*TL*), fecundity and total body weight (*BW*) and fecundity and ovary weight (*OW*). Fecundity were correlated positively with *TL* (Log *F* = 97.65+683.1 log *TL*; $r^2 = 0.524$), *BW* (Log *F* = 9.530+226.2 log *BW*; $r^2 = 0.328$) and *OW* (Log F = 67.35+1866 *OW*; $r^2 = 0.478$). During the study period different physico-chemical parameters were also recorded.

EARLY SEX CHANGE OF ASIAN SEABASS *Lates calcarifer* FARMED IN SINGAPORE

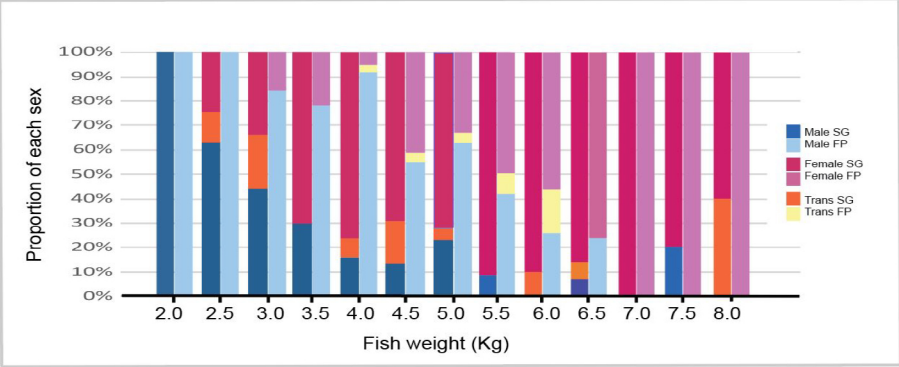
Celestine Terence*, Jose A. Domingos, Susan Gibson-Kueh, Joseph Angelo Uichanco, Yann Monteil, Loic Monteil, Quyen Q. Banh, Dean R. Jerry

Tropical Futures Institute
James Cook University Singapore
149 Sims Drive, Singapore 387380
Email: celestine.terence@jcu.edu.au

The Asian seabass, *Lates calcarifer*, is a key food fish species that has been farmed in Singapore for the past 30 years and is now receiving global attention. As production of the species intensifies, so does the need to secure steady and sustainable seedstock. As such, it is critical to understand the reproductive biology of the species. The Asian seabass is a sequential hermaphrodite whereby it first sexually develops as male, and then subsequently sex changes to female (testis to ovaries) a few years later. In Singapore, there has been anecdotal evidence that male-female sex change may be occurring much earlier than what has been recorded in Australia and Southeast Asia. This study looks into establishing a baseline of maturity rates and sex change of Asian seabass farmed in Singapore.

Fish cultured in sea cages of a commercial farm located in the Southern waters of Singapore and aged approximately 2.4 years old were individually measured (70.3 ± 7.3 cm), weighed (4.7 ± 1.4 kg) and had their gonads (n = 176) dissected for histological analyses. Results indicated that 73% were female, 19% were male and 8% were in transitional stages (both sperm and oocytes observed in gonads). In this population, sex change seemed to start after 2 kg, with the proportion of males reduced by half between 2.5 and 3 kg (55~60cm). Above 3 kg, and 5 kg, females represented 70% and 90%, respectively (Figure 1). Comparative farm studies from French Polynesia and in wild Australian stocks indicate that the farmed population in our study is sex changing into female at body weights approximately half that of what was previously known. The findings of this study are important for breeders to tailor decisions of when and at what size to select candidate *L. calcarifer* broodstock in Singaporean waters so the recommended 2:1 male-to-female spawning ratio can be successfully achieved. The exact causes for early sex change in Singaporean *L. calcarifer* are still unknown and deserve further investigation.

Figure 1. Distribution of Asian seabass, *Lates calcarifer* males, females and transitional sex type (Trans) at 500g body weight intervals* of a population farmed in Singapore (SG) and in French Polynesia (FP), adapted from Guiguen *et al*. (1994)). *"2.5" kg = 2.01 to 2.50 kg.



NUTRITIONAL INNOVATIONS IN GENETICALLY SELECTED EUROPEAN SEA BASS (*Dicentrarchus labrax*) GENOTYPES: EFFECTS OF FUNCTIONAL FEEDS ON FISH PERFORMANCE, DISEASE RESISTANCE AND GUT HEALTH

Terova G[1], Torrecillas S*[2], Serradell, A[2], Rimoldi S[1], Acosta, F[2]., Hostins, B[3]; Fontanillas, R[4], Allal, F[5], Haffray P[6], Bajek A[7], Castro P[2] and Montero D[2].

[1]Department of Biotechnology and Life Sciences, University of Insubria,Varese, Italy
[2]Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain
[3]INVE Aquaculture, Dendermonde, Belgium
[4]Skretting Aquaculture Research Centre, Stavanger, Norway
[5]MARBEC, University of Montpellier, CNRS, Ifremer, IRD, 34250 Palavas-les-Flots, France
[6]SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France
[7]Ecloserie Marine de Graveline Ichtus, Route des Enrochements, 59820 Gravelines, France
Email: silvia.torrecillas@giaqua.org

To study if selected fish genotypes can benefit from the use of functional additives in aquafeeds to guarantee a future scenario covering the limitations on the availability of FM/FO, in terms of enhancing fish growth performance, mucosal health and disease resistance, is the aim of the present study.

Two batches of European sea bass juveniles, high growth selected (HG; selected sires x selected dams) and wildtype (WT; wild sires x selected females) populations produced at MARBEC-IFREMER, were grown at the facilities of the Parque Científico-Tecnológico Marino (PCTM) at University of Las Palmas de Gran Canaria (Telde, Canary Islands, Spain). Fish were fed a "future diet", replacing totally FO by a combination of PO and DHA oil from algae origin and reducing by a 50% FM content by PM inclusion, until achieving the initial experimental size of 16 g. After that, fish were randomly distributed in 24 tanks of 500 L (34 fish/tank; 12 tanks by genotype; 19.0 ± 0.4 g) and supplemented with 3 different experimental functional additives (INVE, Belgium) top-coated on future diet as follows : (i) 2 weeks at high dose followed by (ii) 10 weeks at low dose. The functional additives used were a probiotic mixture (PROB), organic acids mixture (ORG) and a phytogenic (PHYTO). Diets were manufactured by Skretting (Skretting ARC, Norway) and top-coated by INVE (Belgium). Fish were sampled for gut integrity and functional microbiome and target immune parameters at the end of the total feeding trial. After each dosage feeding period, a pathogen challenge test (*Vibrio anguillarum*) plus stress condition (overcrowding) was applied to study the potential of additives to ameliorate the combined effect of both pathogen and stress condition.

Table 1: Initial mean weight (Wi), final mean weight (Wf), special growth rate (SGR) of bighead catfish

Treatment	Wi (g fish^{-1})	WG (g)	SGR (% day^{-1})
CT	10.2±0.29^{a}	50.9±1.65^{c}	2.98±0.03^{c}
GG	10.1±0.07^{a}	50.2±1.61^{c}	2.97±0.03^{c}
GG/HK	10.3±0.20^{a}	55.8±1.12^{b}	3.09±0.05^{b}
GG/P	10.4±0.25^{a}	54.3±1.41^{b}	3.05±0.03^{bc}
GG/P/HK	10.3±0.01^{a}	62.5±1.48^{a}	3.26±0.03^{a}

Table 2: Red blood cells (RBC), white blood cells (WBC), and the immunity of Lysozyme activity of bighead catfish

Treatments	RBC (10^6 cells mm^{-3})	WBC (10^3 cells mm^{-3})	Lysozyme (µg mL^{-1})
CT	2.14±0.09^{bc}	96.6±17.7^{b}	226±8.69^{b}
GG	2.06±0.06^{c}	103±12.3^{b}	238±11.2^{b}
GG/HK	2.24±0.03^{b}	146±7.89^{a}	278±12.8^{a}
GG/P	2.72±0.13^{a}	104±24.2^{b}	232±13.3^{b}
GG/P/HK	2.28±0.09^{b}	145±6.09^{a}	295±7.55^{a}

No effects on weight gain were detected after 2 weeks of feeding in relation to the two fixed factors evaluated: genotype origin and functional diet supplemented. After 10 weeks of low dose experimental functional additives supplementation, HG fish presented a higher weight and SGR than WT fish, presenting fish the control diet higher final weight than fish fed the PROB diet within the HG genotype group.

(Continued on next page)

In terms of gut microbiota, discriminant analysis did not show a clear separation among fish fed the future diet and fish fed the experimental additives regardless of the genetic background, however variations on specific taxa relative abundance were detected. For example, fish fed ORG diet presented higher relative abundance of Streptococcus in both genotypes, whereas for fish fed PHYTO this effect was observed for Lactobacillales order and in fish fed PROB presented lower abundance of Pseudomonas and Acinetobacter genera.

Funding: The current study was supported by the EU Horizon 2020 AquaIMPACT (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture), number: 818367.

EFFECTS OF GUAR GUM, *Lactobacillus plantarum* L-137 AND PHYTASE ON THE GROWTH PERFORMANCE AND IMMUNE RESPONSES OF BIGHEAD CATFISH *Clarias macrocephalus* IN RECIRCULATION AQUACULTURE SYSTEMS

Pham Thanh Liem*, Tran Le Cam Tu, Pham Minh Duc, Tran Thi Thanh Hien

College of Aquaculture and Fisheries
Can Tho University, Vietnam
Email: ptliem@ctu.edu.vn

This study was conducted to determine the effect of guar gum-GG, heat-killed *Lactobacillus plantarum* L-137 (HK L-137) and phytase on the growth, digestibility, and immune response of bighead catfish (*Clarias macrocephalus*), in order to contribute to formulating effective and environmentally friendly feed for bighead catfish reared in recirculation aquaculture systems (RAS). The experiment consisted of five diet treatments with and without additives: T1 (Control-CT), T2 (CT supplemented with GG at 5 g kg^{-1}), T3 (CT with GG at 5 g kg^{-1} and HKL 137 at 0.02 g kg^{-1}), T4 (CT with GG and 0.15g phytase per kg of diet) and T5 (CT with GG, HKL 137 and phytase).

The feeding trial was carried out for 56 days and all treatments were performed in triplicate. The results showed that the survival rate of the fish was not affected by diets supplemented with either guar gum (at 5 g kg^{-1}), phytase (0.15 g kg^{-1}) or HK L-137 (0.02 g kg^{-1}). Dietary guar gum (5 g kg^{-1}) addition did not diminish performance or feed digestibility and it did not improve the fecal characteristics or immune response of bighead catfish. On the other hand, inclusion of both phytase and HKL 137 in the diet not only increased growth performance and feed digestibility, but also the fecal characteristics and the immune response of bighead catfish. Neither phytase nor HK L-137 had any effect on the fecal pellets size. A supplement of 5 g guar gum, 0.15g phytase and 0.02 g HK L-137 per kg of diet appears to be suitable for raising bighead catfish *(Clarias macrocephalus)* in RAS systems.

DIETARY SUPPLEMENTATION OF SEAWEEDS MODIFY THE INNATE IMMUNITY AND GUT MICROBIOME OF RABBITFISH *Siganus fuscescens*

Valentin Thepot[1*], Michael Rimmer[1], Alexandra Campbell[2] and Nicholas Paul[1]

1 School of Science and Engineering, the University of the Sunshine Coast, Sippy Downs, QLD Australia
2 School of Health and Sport Sciences, the University of the Sunshine Coast, Sippy Downs, QLD Australia
*valentin.thepot@research.usc.edu.au

The diet of farmed fish has evolved rapidly over the past 40 years to cope with demand and key limiting resources such as fish meal. Growth and food conversion ratio were the main criteria when developing aquafeed, but recently the fish immune system and its microbiome are emerging important considerations. Seaweeds and their natural products are also emerging as a alternative source of functional ingredients in aquaculture. Here we evaluated the effects of 11 species of seaweed from three taxonomic groups (Phaeophyceae, Chlorophyceae and Rhodophyta), two commercial ingredients (sodium alginate and Hilyses®) and two microalgae previously used as functional ingredients (spirulina and *Haematococcus pluvialis*) on the innate immunity and the gut microbiome of the rabbitfish *Siganus fuscescens*.

Each functional ingredient was included at 3% by weight in a commercial fish feed and fed to the rabbitfish for 2 weeks. All 15 treatments altered the immune response and hindgut microbiome compared to the control diet. At least one seaweed from each taxonomic group led to a doubling in the activity of one immune parameter (the alternative complement system) compared to the control. Bacteria of the genus *Ruminococcus* sp. and *Akkermansia* sp. were lowest in abundance in control fish but increased with all treatments, up to 6- and 9- times respectively. Understanding how dietary inclusions shape the gut microbiome of fish and whether, in turn, the microbiome influences fish health, will be crucial for developing tailored fish feed.

RECOGNIZING THE ROLE OF WOMEN IN AQUACULTURE IN THE PHILIPPINES

Isidora P. Ticao

Finfish Hatcheries, Inc.
Lun Masla, Malapatan
Sarangani Province
Philippines
Email: ipticao@yahoo.com

"Who is this girl? Whose face is all eyes?" barked our Consultant. That was me in 1982, a mere 84lbs, 5 feet in height, fresh graduate from the University, while brushing off the algae from the *P. monodon* larval tank and barely able to carry one (1) 20liter bucket of water to splash the tank walls.

How can a female survive in male dominated Aquaculture industry in the Philippines?

Back then the statistics was 2 female (me and my friend) vs. 21 male Technicians, all working in the 1st commercial *P. monodon* production facility in the Philippines. It was very challenging and yet fun.

This year 2020, is my 38th year in Aquaculture. Managing a Fish Hatchery and overseeing 8 female and 17 male Technicians. Women are almost equal to men. Women are going up the ranks.

A journey of a lifetime that started as a Probationary Hatchery Technician of a Prawn Hatchery to become the Operations Manager of the biggest Finfish Hatchery in the Philippines.

This is my story.

USE OF NOVEL HIGH PROTEIN DISTILLERS FEED PRODUCTS, ANDVANTAGE™ 40Y AND ANDVANTAGE™ 50Y, IN PRACTICAL DIETS FOR TILAPIA, *Oreochromis niloticus*

Scott Tilton*, Julia Green, John Hagios, Callum McAree, Sidra Nazeer

The Andersons, Inc.
Colwich, KS, USA 67030
stilton@andersonsinc.com

Two technologically advanced high protein distiller feed products, ANDVantageTM 40Y and ANDVantage 50Y (HP50Y & HP40Y) were evaluated for juvenile tilapia. This experiment assessing the nutritional potential of using high protein (40 and 50%) distillers dried grain products to replace corn protein concentrate (CPC) in formulation of tilapia diets. A 10-week growth study was conducted, using nine diets formulated to contain 32% protein and 6% lipid. Each protein was included at levels (0, 5, 10, 15 and 20%) replacing CPC on protein basis, with the 0% diet serving as a common control. Juvenile tilapia (mean initial weight 5.23 ±0.20g) were evenly distributed in thirty-six, 75-L aquaria working as a recirculating system and fed twice daily to apparent satiation throughout the study. Tilapia exhibited no significant ($p>0.05$) differences in growth, FCR, survival, whole-body proximate composition, mineral composition, and hematological parameters when fed HP50Y and HP40Y supplemented diets compared to the control diet. Results from this study revealed that HP50Y and HP40Y both are good alternative protein sources and can be used up to 20% inclusion level in the diets of tilapia.

Table 1 Growth Performance of Tilapia fed increasing levels of ANDVantage 50Y over a 10-week study.

50Y Level,%	Weight gain, g	Weight gain, %	FCR	Survival
0	49.9	992	1.23	95.0
5	51.1	973	1.24	90.0
10	50.8	948	1.25	93.3
15	49.8	939	1.23	93.3
20	54.5	1056	1.22	86.7

Table 2. Growth Performance of Tilapia Fed Increasing Levels of ANDVantage 40Y over a 10-week study

40Y Level, %	Weight gain, g	Weight gain, %	FCR	Survival
0	49.9	992	1.23	95.0
5	50.9	1001	1.24	93.3
10	52.4	994	1.23	93.3
15	52.7	1006	1.19	93.3
20	53.5	1013	1.16	90.0

NUTRITIONAL EVALUATION OF AN AQUEOUS-PROCESSED CANOLA IN DIETS OF POST-SMOLT ATLANTIC SALMON *Salmo salar*

Van Pham Thi Ha To*, David Dzisiak, John Brennan, André Dumas

The Center for Aquaculture Technologies, Souris, PE, C0A 2B0, Canada
Email: vto@aquatechcenter.com

The nutritional value of graded inclusion levels of canola protein concentrate (CPC; 75.8% crude protein, 4.4% crude lipid) obtained through aqueous processing was assessed in post-smolt Atlantic salmon over a 168-day growth study and a 34-day digestibility study. In the growth study, eight experimental diets containing 0 (Diets A, E), 10% (Diets B, F), 15% (Diets C, G) and 20% (Diets D, H) CPC were randomly allocated to 24 750-liter tanks at 33 fish (228.0 ± 4.9 g) per tank. Diets A through D were formulated to mimic commercial salmon feeds in geographies where processed animal proteins (PAP) are used (Americas), whereas diets E through H included no PAP (Europe). In the digestibility study, salmon (57.3 ± 6.7g) were randomly assigned to 12 100-liter tanks (21 fish/tank) and were hand-fed for 34 days on four digestibility diets containing 0% CPC (reference diet), 70:30, 80:20 and 90:10 blend of reference diet to test ingredient to determine nutrient digestibility.

Overall, growth performances, measured using the thermal-unit growth coefficient (TGC), varied between 0.143 and 0.160, and were not significantly different among treatments (P>0.05) (Table 1). Feed conversion ratios (FCR) were ≤1.08, and although there was a significant CPC effect (P=0.003), differences were marginal. Over the 168-day study, the best TGC and FCR were obtained with salmon fed 10% CPC, regardless of formula type. However, 20% CPC was optimal for growth and FCR during the first 28 days of this salmon study. Weight gain, TGC and FCR between days 0 and 28 improved in a linear manner (P<0.01) in response to 10-20% CPC. There were no significant differences (P>0.05) in the final whole-body composition or nutrient retention among treatments. Increasing dietary inclusion of CPC from 0-20% demonstrated a positive relationship with intestinal villi length (P=0.0314) and improved histological morphology (e.g., lamina propria). The apparent digestibility coefficients were 91.1% for protein, 88.8% for lipid and 79.6-98.6% or essential amino acids in CPC. Based on these results, CPC stood as a safe and nutritious protein alternative up to 20% inclusion in salmon diet. The effect of higher inclusion level of CPC in nutrient-balanced diets deserve further studies.

Table 1. Initial body weight (IBW), final body weight (FBW), weight gain (WG), feed intake (FI), feed conversion (FCR), and thermal-unit growth coefficient (TGC) of Atlantic salmon fed experimental diets containing graded levels of canola protein concentrate (CPC) using either American (A) or European (E) formula for 168 days. Data are means ± SEM. Means within a column with no superscript in common differ significantly (P<0.05) based on two-way ANOVA followed by Tukey test.

Treatment		IBW	FBW	WG	FI	FCR	TGC	Survival (%)
Formula	CPC	(g fish^{-1})	(g fish^{-1})	(g fish^{-1})	(g fish^{-1})		[g$^{1/3}$ (ºC d^{-1})]	
Americas	0	228.2 (3.2)	972.4 (29.6)	744.2 (26.5)	779.8 (26.0)	1.05 (0.00)^{ab}	0.160 (0.004)	93.9 (0.0)
Americas	10	223.6 (2.0)	961.8 (48.5)	738.1 (46.6)	757.2 (42.3)	1.03 (0.01)^{b}	0.160 (0.006)	100.0 (0.0)
Americas	15	228.3 (1.6)	916.7 (44.6)	688.4 (43.0)	744.0 (46.0)	1.08 (0.01)^{a}	0.152 (0.006)	100.0 (0.0)
Americas	20	228.5 (0.9)	920.7 (30.2)	692.2 (31.1)	744.6 (24.6)	1.08 (0.02)^{a}	0.152 (0.004)	100.0 (0.0)
Europe	0	230.1 (6.6)	863.8 (44.9)	633.7 (38.5)	668.1 (46.5)	1.05 (0.00)^{ab}	0.143 (0.005)	89.9 (6.1)
Europe	10	227.5 (2.7)	946.5 (16.0)	718.9 (13.9)	753.1 (15.5)	1.05 (0.01)^{ab}	0.156 (0.002)	99.0 (1.0)
Europe	15	230.3 (2.0)	913.6 (19.0)	683.3 (17.6)	734.4 (14.4)	1.08 (0.01)^{a}	0.151 (0.002)	100.0 (0.0)
Europe	20	227.7 (1.6)	908.6 (16.8)	680.9 (16.3)	728.6 (12.2)	1.07 (0.01)^{ab}	0.151 (0.002)	100.0 (0.0)
Two-way ANOVA (P-value)								
Formula		0.4357	0.1630	0.1205	0.1321	0.7335	0.0604	0.6577
CPC		0.6104	0.5950	0.4777	0.8028	0.0033	0.1769	0.0004
Formula x CPC		0.8912	0.3839	0.3260	0.3035	0.4297	0.0769	0.9356

TRANSPORT PATHWAYS OF METHIONINE SOURCES IN THE INTESTINE OF RAINBOW TROUT *Oncorhynchus mykiss*

Van Pham Thi Ha To*, Karthik Masagounder, Matthew E. Loewen

Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada
Email: matthew.loewen@usask.ca

Nutritionists are constantly challenged to formulate diets that not only meet the nutritional requirement of animals but are also cost-effective and have a low environmental impact. To ensure adequate growth and health of fish, methionine (Met) supplementation using DL-methionine (DL-Met) or DL-methionine hydroxy analogue (DL-MHA) is a common practice, allowing for increased use of plant-based protein ingredients in aquafeed. Determining how much and how quickly these Met sources are transported in the fish intestine is a critical step in assessing their bioefficacy because the intestine is the most important location of nutrient absorption, and insufficient absorption in the gut would increase waste excretion. This study used radiolabeled flux assays and transporter gene expression to 1) characterize transport pathways of DL-Met and DL-MHA across *ex-vivo* intestinal segments, and 2) compare the transport kinetics between the two. Intestinal transport of DL-[^{14}C]Met and DL-[^{14}C]MHA were measured in the presence or absence of sodium at physiological apical/basal pH of 7.7/7.7, as well as various pH levels (6.0/6.0, 6.0/7.7 and 7.7/8.7) at the substrate concentration ranging from 0.2-20mM. The results demonstrated that DL-[^{14}C]Met flux was primarily driven by Na^+-dependent process, with flux rates the in the pyloric caeca (PC) and midgut (MG) regions being higher than in the hindgut (HG). On the other hand, the apical transport of DL-[^{14}C]MHA appeared to be facilitated by Na^+-requiring systems, whereas basolateral flux was governed by an H^+-independent process in PC and MG, but an H^+-dependent process in the HG. Finally, a comparison made under identical experimental conditions showed that the flux rates of DL-[^{14}C]Met were significantly greater than that of DL-[^{14}C]MHA in PC and MG, suggesting that the intestinal transport of DL-Met in trout is more efficient than its corresponding analogue (Table 1).

Table 1. Transport kinetics of Met sources in the intestine of rainbow trout.

Met source	Vmax (µmol/cm^2.hr)			Km (mM)		
	PC	**MG**	**HG**	**PC**	**MG**	**HG**
DL-Met	0.0014^a (0.0002)	0.0020^a (0.0002)	0.0006 (0.00001)	0.73 (0.10)	0.98 (0.13)	0.67 (0.14)
DL-MHA	0.0007^b (0.00001)	0.0009^b (0.00001)	ND	1.10 (0.23)	0.97 (0.14)	ND
***P*-value**	**0.002**	**<0.0001**	**-**	**0.458**	**0.941**	**-**

*PC = Pyloric caeca; MG = Midgut; HG = Hindgut; ND = not detected. Values were expressed as mean and standard error (in parenthesis).

EVALUATING INVASIVE MARBLED CRAYFISH AS A POTENTIAL LIVESTOCK FOR SUSTAINABLE AQUACULTURE

Sina Tönges[1,*], Vitor Coutinho Carneiro[1], Florian Böhl[2], and Frank Lyko[1]

[1]German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, 69120 Heidelberg, Germany
sina.toenges@dkfz.de
[2]Creavis, Evonik Operations GmbH, Rodenbacher Chaussee 4, 63457 Hanau-Wolfgang, Germany

The marbled crayfish (Procambarus virginalis) is a recently discovered freshwater crayfish species, which reproduces by apomictic parthenogenesis, resulting in a monoclonal, all-female population. The animals were widely distributed through the aquarium trade and have established numerous stable wild populations through anthropogenic releases. They are highly prevalent in Madagascar, where they have become a popular source of nutritional protein. As freshwater crayfish aquaculture in open systems is a thriving, but ecologically damaging global industry, alternatives are urgently needed. Although marbled crayfish are often branded by their invasive mode of reproduction, their overall invasiveness is not higher than for other cultured crayfish species. Furthermore, their resiliency and high adaptability provide a strong rationale for evaluating them for closed, and environmentally safe aquaculture approaches. Here we show that marbled crayfish grow to sizes and weights that are comparable to commercially farmed freshwater crayfish. Tailored feed development and laboratory testing demonstrated highly efficient feed conversion, suggesting a considerable capacity for sustainable production in closed systems. We further show that marbled crayfish meat can be readily introduced into European meals. Finally, chemical analysis of marbled crayfish exoskeletons revealed comparably high amounts of chitin, which is a valuable source for the synthesis of chitosan and bioplastics. Our results thus suggest that production of marbled crayfish in closed systems may represent a sustainable alternative for crayfish aquaculture.

We explored the suitability of marbled crayfish as a source of nutritional protein in European meals. Indeed, we found fried marbled crayfish tails (Figure 1A) to be suitable for crayfish risotto, and for appetizers (Figure 1B). Furthermore, the shell waste that was generated in this process, could be easily collected, and preserved by air-drying (Figure 1C). To determine the chitin content of marbled crayfish shell waste, we used a chemical extraction protocol. Because they represent the major source of commercially extracted chitin and because data or material from P. clarkii were not available, we used similarly processed whiteleg shrimp (L. vannamei) shells for comparisons. This revealed a significantly higher chitin content for marbled crayfish than for L. vannamei (2.60% vs. 0.85%, $p < 0.05$, and Figure 1D). These findings suggest that marbled crayfish represent a valuable source of chitin. To determine the potential of chitin extracted from marbled crayfish, we also produced biodegradable straws.

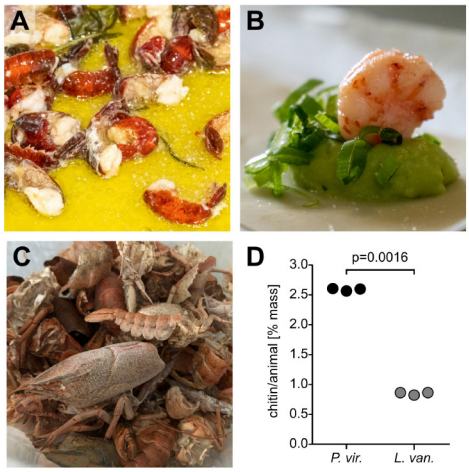


Figure 1: (A) Marbled crayfish tails with herbs, fried in butter. (B) Marbled crayfish tail meat on Avocado mousse. (C) Dried exoskeletons (shell waste) from marbled crayfish. (D) Comparison of chitin content per animal in marbled crayfish (P. vir) exoskeletons and whiteleg shrimp (L. van) shell waste. An unpaired two-tailed t-test showed that the difference between the two groups is highly significant (p = 0.0016).

PREZYGOTIC REPRODUCTIVE BARRIER DETERMINATION AMONG LABORATORY CROSSES OF THREE SPECIES OF THE GENUS *MYTILUS*

Jorge E. Toro [1*] , Xiomara Avila[1], Pablo A. Oyarzún[2] and Alex Illesca[1]

[(1)] Instituto de Ciencias Marinas Y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile
jtoro@uach.cl
[(2)] Centro de Investigaciones Marinas Quintay (CIMARQ), , Facultad de Ciencias de la Vida
Universidad Andrés Bello

The *Mytilus* genus is composed by at least 5 species, *M. californianus* (Conrad 1837), *M. edulis* (Linnaeus 1758), *M. galloprovincialis* (Lamarck 1819), *M. trossulus* (Gould, 1850) and a recently corroborated as a valid species the chilean blue mussel *M. chilensis*. The Chilean native blue mussel *M. chilensis*, is an economically important marine resource in Chile. The *M. chilensis* aquaculture began in 1943 in Chiloé Island, southern Chile, and the aquaculture production increased rapidly from 3,864 t in 1993 to 398,274 t in 2018, equivalent of 29,3% of the total Chilean aquaculture production, occupying the second place after the salmonids exotic species aquaculture, which represent a 67,7% of the total aquaculture production in Chile. Therefore, and because the relative recent description of *M. galloprovincialis and M.* in Chilean waters, there is a need to carry out basic and applied studies to evaluate their actual and potential effects on their distribution, on the biodiversity along the coast and on the fasting growing mussel (*Mytilus chilensis*) aquaculture industry. Among some of the main features of these *Mytilus* species is the well-known capability to naturally hybridize among them in locations where their distributions overlap therefore, these alien mussel species could have also the potential for natural hybridization with the native mussel *M. chilensis*.

Adult mussels were collected from: *M. chilensis* from Yaldad Bay, Chiloé, *M. galloprovincialis* from Caleta Tumbes (Central Chile) and *M. edulis platensis* from Buque Quemado (Strait of Magellan). These sites were chosen on recently published evidence of presence of each of these species. Spawning was induced and male and female gametes were collected into 150 ml cups containing filtered seawater. Factorial crosses design was used to obtain embryos from pure and hybrid fecundation. Mantle tissue from each spawened mussel was removed and stored in 96% ethanol for genotyping. The objective was to determine if pure species crosses showed differences in compassion of hybrid crosses. Analysis of variance for fecundity (%) between these two types of crosses carried out at the laboratory showed no significant differences. The results indicate that ther is no intrinsic prezygotic barriers in externally fertilizing *Mytilus* mussels. Further research is needed to evaluate the growth and viability among the laboratory crosses to assess any differences in fitness between pure and hybrids crosses.

Research founded by FONDECYT 1170194.

THE EFFECT OF HEAT TREATMENT ON THE QUALITY OF BLACK TIGER SHRIMP UNDER FROZEN STORAGE

Tran Minh Phu [*], Huynh Nhu Huynh, Huynh Thi Kim Duyen, Nguyen Le Anh Dao, Nguyen Thi Nhu Ha, Nguyen Quoc Thinh

[1] College of Aquaculture and Fisheries, Can Tho University, Vietnam
tmphu@ctu.edu.vn

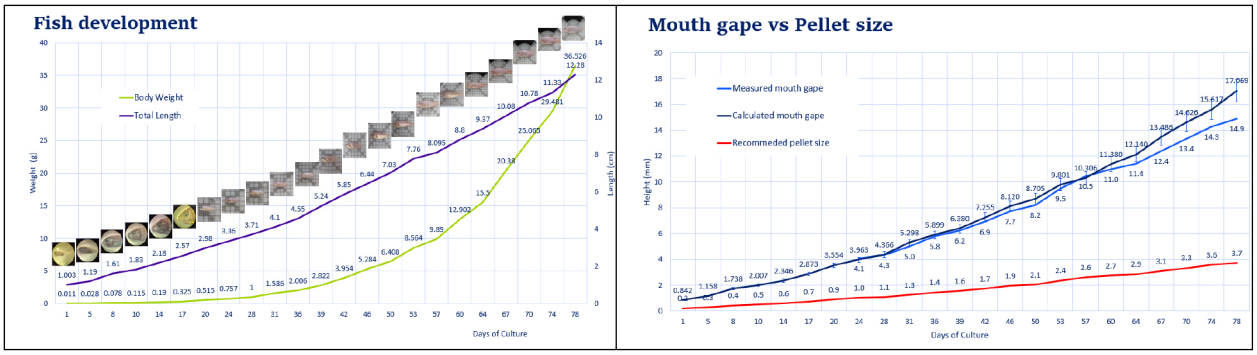
The study was conducted to investigate the effect of heat treatment on the quality of black tiger shrimp under frozen storage. The experiment consisted of two treatments: (i) soaking shrimp in cold water for 30 minutes (control treatment), (ii) soaking shrimp in cold water for 30 minutes and steamed for 5 minutes. Samples were placed into PE bag (25 shrimp/bag) sealed and stored in the freezer at the temperature of -18°C to -20°C. Samples were collected on months 0, 1, 3, 6, 9 and 12. Analyzed criteria included temperature, total viable bacteria count value, sensory property, elasticity, WHC, pH, TVB-N, PV and TBARs. The results showed that shrimp in heat treatment had significantly lower PV value and higher TBARs than control treatment. Heating shrimp before frozen storage reduced total bacteria count during frozen storage. After 12 months of frozen storage, shrimp in control treatment had the melanosis syndrome which significantly reduced sensory property of the product whereas the shrimp in heat treatment storage presented the better color. In conclusion, heating shrimp before frozen storage can prolong the shelf life of shrimp during frozen storage.

ARE YOU FEEDING THE RIGHT PELLET SIZE TO YOUR FINGERLINGS?

Henry Cuong, Tran Minh Triet, Truong Thao, Pham Minh Anh, Marc Campet

ADM Aquaculture R&D Center
Nha Be district, Ho Chi Minh, Vietnam

Feed capture in fish is based on a suction mechanism to ingest prey and feed. Then, particle size determines if it can be ingested by the fish or not depending on mouth size. Therefore, pellet size is especially important during the nursery period, notably the first time when fish larvae switch to complete feed. To identify proper pellet sizes for tilapia during the nursery period, we conducted an experiment to measure the mouth gape of red tilapia fingerlings for 78 days at ADM Aqua R&D Center. Tilapia fries of 5-day-old were stocked into 6 nursery tanks (400 L of water each) at a density of 300 fish/tank. Six fish were sampled twice per week in each tank until the fish reached 35 g. For young fish that were under 0.5 g, the mouth gape was estimated based on the linear regression equation between mouth gape (G) and body weight (W) of *O. niloticus*: ($r^2 = 0.96$) (Yonas Fessehaye et al., 2005). For fish weighing greater than 0.5 g, the mouth gape was measured with a technical ruler and we established the new linear correlation for red tilapia: ($r^2 = 0.97$). From the experiment results, we found that there is a remarkable gap between actual mouth gape and pellet sizes in current practices. More precise feeding guidelines will be discussed and proposed in our presentation.



IDENTIFICATION, CHARACTERIZATION, DEVELOPMENT OF INFECTION MODELS FOR MUSCLE NECROSIS DISEASE CAUSED BY *Vibrio* spp. ON WHITELEG SHRIMP (*Penaeus vannamei*) IN VIETNAM

Loc Tran*, Nguyen Tran, Thanh Le, Saw Yadanar, Ei Mon Ko, May Moh Moh Khin, and Phuc Hoang

ShrimpVet Laboratory
Minh Phu AquaMekong Co., LTD
Ho Chi Minh, 720371, Vietnam
thuuloc@arizona.edu

Many whiteleg shrimp farms in Vietnam have been attacked by an emerging disease which is recognized with focal white muscle on abdominal segments of dead or moribund shrimp along with significant mortality. The occurrence of the disease is commonly related to the poor management of water quality in shrimp ponds such as low dissolved oxygen, low alkalinity, high toxic gas, and algal bloom. The gross signs are similar to some infectious diseases, including both bacterial and viral agents that cause damage to the shrimp muscle. However, the histopathology of this disease was different from the diseases caused by viruses but similar to the cases caused by bacteria. Furthermore, the PCR results showed that both virus factors such as Infectious Myonecrosis Virus (IMNV), Penaeus Nodavirus (PvNV), and Covert Mortality Nodavirus (CMNV) and bacterial factors (*Vibrio harveyi*) were not detected. Therefore, the study hypothesized that this disease is caused by new bacterial agents.

Bacterial isolation, subculture, reinfection, and reisolation were carried out according to Koch's Postulates. Three consecutive challenge tests along with some microbiological, histopathological, and molecular analyses were conducted to confirm the causative agents of the disease. Based on the biochemical test, PCR, and 16s sequencing results, strains of *Vibrio* spp. were identified as the causative agents. Notably, these strains produced green colonies on the Thiosulfate-citrate-bile salts-sucrose (TCBS) media. The present study proved that these strains were able to induce symptoms of the disease via immersion route with high mortality and signs of damage exhibiting skeletal muscle necrosis as an opaque, whitish, discoloration of several abdominal segments in infected shrimps. In addition, the histopathology of infected shrimps in laboratory conditions was the same as the samples from the farm with extensive coagulative necrosis, hemocytic infiltration in skeletal muscle, and the presence of rod-shaped bacteria. Thus, the present study revealed that the muscle necrosis disease was caused by strains of *Vibrio* spp.

DEVELOPING LABORATORY INFECTION MODEL FOR WHITE FECES DISEASE IN WHITELEG SHRIMP (*Penaeus vannamei)*

Loc Tran*, Phuc Hoang, Yin Thant, Khin Khit, Saw Yadanar

Founder-CEO, ShrimpVet Lab, Minh Phu AquaMekong, Ho Chi Minh, Vietnam
thuuloc@arizona.edu

White Feces Disease (WFD) is one of the most threatening diseases in Asian countries (e.g., Thailand, Vietnam, China, India, Indonesia) since 2009 until date leading to significant losses for shrimp farmers due to high FCR, slow growth and size variation at harvest. Generally, the white fecal strings floating on the pond surface indicates the WFD outbreak, and it occurs in black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Penaeus vannamei*). Although the specific disease-causing agent has not been identified, several studies have shown that WFD can be stimulated by opportunistic *Vibrio* species and *Enterocytozoon hepatopenaei* (EHP) an association with high WFD prevalence. However, the identification of causative pathogens and the replication of WFD infection *in-vivo* are still under development. This is one of the major drawbacks in consideration of prevention strategies for the outbreaks as well as in developing curative products to promote the early recovery of shrimp after WFD infection.

The laboratory induction method for WFD has been developed at ShrimpVet Laboratory, Vietnam, and the mode of disease infection was studied. The pathogenic strains of *Vibrio* spp. from WFD affected shrimp samples from shrimp farms in Vietnam were isolated by plate culture method, and the replication of the selected bacteria and the confirmation of disease occurrence via bioassay challenges on SPF (specific pathogen free) shrimp. An EHP challenge group, by cohabitation method, and a combined challenge with EHP (cohabitation) and WFD (*per os*) were set up and studied in parallel. A negative control group was also established to identify the significance in shrimp feeding behaviour and appearance of clinical signs in WFD challenged groups. During the post-challenge, the observed symptoms of challenged shrimp included loose shell, white/yellow hepatopancreas and gut tract filled with white feces strands, similar to the gross signs from the field specimens.

The wet mount method was used to check the occurrence of aggregated transform microvilli (ATM) in shrimp gut and hepatopancreas (HP) tissues, as formation of ATM is a precursor sign of disease. However, this method solely cannot confirm the disease infection. Hence, the histological assay on the shrimp HP and gut was performed to examine the severity levels of WFD infection and to deliver the case definition for WFD. Most importantly, the re-isolation of pathogens achieved positive results by recovering the exact strain of bacteria challenged to the animals, which can induce the symptoms and effect of WFD under *in-vivo* challenged condition, in this study.

Among three challenge groups, the combined challenge was the most effective way at inducing WFD under laboratory condition. The direct course of white feces could be stimulated under the influence of *Vibrio* spp. However, the underlying conditions of EHP infection leads to a persistent occurrence of WFD in whiteleg shrimp. To minimize the impact of WFD, two comprehensive management strategies were proposed from this study. Firstly, it is important to eliminate EHP out of the shrimp ponds or shrimp stock, and secondarily, the healthy stock should be protected against bacterial infections by maintaining the optimal health and minimal stress from the environment.

NEW EMERGING DISEASE CAUSED BY A HIGHLY VIRULENT *Edwardsiella ictaluri* STRAIN IN FARMED TILAPIA, *Oreochromis* spp.

Truong Dinh Hoai*, Nguyen Thi Huong Giang, Kim Van Van, Lua Thi Dang, Ha Thanh Dong, Doan Thi Nhinh

Faculty of Fisheries
Vietnam National University of Agriculture
Hanoi 131004, Vietnam
tdhoai@vnua.edu.vn

Edwardsiella ictaluri is an emerging bacterial pathogen that affects farmed tilapia (*Oreochromis* spp.). This study reports the widespread of *E. ictaluri* in farmed tilapia in Vietnam. Among 26 disease outbreaks from nine provinces in Northern Vietnam during 2019–2021, 19 outbreaks originated from imported seeds, while outbreaks in seven farms were from domestic sources. Clinically sick fish showed the appearance of numerous white spots in visceral organs, and accumulative mortality reached 30%–65%. A total of 26 representative bacterial isolates recovered from 26 disease outbreaks were identified as *E. ictaluri* based on a combination of phenotypic tests, genus- and species-specific polymerase chain reaction assays, *16S rRNA* and *gyrB* sequencing, and phylogenetic analysis. All isolates harbored the same virulence gene profiles *esrC*$^+$, *evpC*$^+$, *ureA-C*$^+$, *eseI*$^-$, *escD*$^-$, and *virD4*$^-$. Antimicrobial susceptibility tests revealed that 80.8%–100% of isolates were multidrug resistant, with resistance to 4–8 antimicrobials in the groups of penicillin, macrolides, sulfonamides, amphenicols, and glycopeptides. The experimental challenge successfully induced disease that mimicked natural infection. The median lethal doses (LD_{50}) of the tested isolates (n = 4) were 42–61 colony forming units/fish, indicating their extremely high virulence. This emerging pathogen is established and has spread to various geographical locations, causing serious impacts on farmed tilapia in northern Vietnam. It is likely that this pathogen will continue to spread through contaminated stocks (both imported and domestic sources) and persist. Thus, increased awareness, combined with biosecurity measures and emergent vaccination programs is essential to mitigate the negative impact of this emerging disease on the tilapia farming industry.

EFFECT OF AN ESSENTIAL OIL-BASED FUNCTIONAL ADDITIVE ON REDUCTING THE IMPACT OF ECTOPARASITES AND IMPROVING GROWTH PERFORMANCE IN GROUPER (*Ephinephelus fuscoguttatus*)

Truong Dinh Hoai1*, Tran The Vinh1, Hoang Van Dung1, Dang Thi Hoa1, Mai Van Tung1, Nguyen Thi Huong Giang2, Doan Thi Nhinh1, Ruth Garcia Gomez3*

1 Faculty of Fisheries, Vietnam National University of Agriculture, Hanoi, Vietnam
2 Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi, Vietnam
3 Aquaculture Farm Care & Health ISC/APAC, Adisseo, Spain
Corresponding author: Email: hoaibts@gmail.com and ruth.garcia@adisseo.com

The present study investigated the effect on growth performance and ectoparasites prevalence in grouper (*Epinephelus fuscoguttatus*) after being fed with 0%, 0.3% and 0.7% essential oil-based functional additive supplementation (control group; medium-dose group and high-dose group). The experiment was designed with three replicates of each treatment group, each replicate located in a net pen within the same earthen pond.

Final survival rates were 91.6-92.9% and no significant differences were detected between groups. However, the functional feed addictive improved the DWG from 0.93 to1.02 and 1.06 g/day and the FCR was reduced from 1.56 to 1.45 and 1.40 for control (0%) to medium-dose (0.3%) and high-dose (0.7%) supplementation, respectively. Regarding morphometric indexes, the total length, body thickness and condition factor were significantly increased in treatment groups, while for the hepatosomatic index and the visceral somatic index no significant differences between group were detected.

Fish were naturally infected to four ectoparasites including *Trichodina* sp., *Caligus* sp., *Zeylanicobdella* sp., and *Dactylogyrus* sp. The prevalence and infection intensity of the aforementioned parasites in control group was significant higher compared to treatment groups. Red blood cell count (RBC), white blood cell count (WBC), hemoglobin level, lymphocyte count, and monocyte count were significantly increased in grouper fed with the functional additive compared with control group, while the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and thrombocytes did not show any statistically significant difference between groups. This study validates in the field that the essential oil-based functional additive has a strong positive impact on growth performance, flesh quality, immune response and significantly reduces the prevalence and impact of ectoparasites in grouper culture.

ANTIMICROBIAL USE AND RESISTANCE IN *Streptococcus agalactiae* IN TILAPIA FARMING IN NORTHERN VIETNAM

Hanh T.T.M[1,2], Van P.T[2], Nghia N.H[1], May L.T[1], Matthew, P.[3] and Dalsgaard A. [3]

[1] Centre for Environment and Disease Monitoring in Aquaculture
[2]Research Institute for Aquaculture No1
[3] International Centre for Antimicrobial Resistance Solutions
tmhanh@ria1.org

Antimicrobial resistance (AMR) is a threat to animal and human health globally. Antimicrobials are used (AMU) in aquaculture to control different bacterial-associated diseases. This study was conducted to determine AMU in tilapia aquaculture in Northern Vietnam and antimicrobial resistance in *Streptococcus agalactiae*, the main disease-causing bacterial pathogen.

A total of 78 *S. agalactiae* strains were isolated from kidney samples of diseased farmed tilapia during 2017-2021 in farms located in the provinces of Hai Duong, Bac Giang, Bac Ninh, Hoa Binh, Yen Bai, Son La and Ha Noi. The sensitivity of the strains to 7 antimicrobials were established by disc diffusion (Oxoid; Table 1). Thirty-two farm owners/managers of tilapia farms in Hai Duong, Bac Ninh and Bac Giang provinces were interviewed in 2022 using questionnaires to collect data on farm health management and antimicrobial use practices.

All tested *S. agalactiae* strains were found *S. agalactiae* is mostly resistant to 7 antimicrobials, of which were highly resistant to NOV, NEO and TMP-SMX (88.9-100%), followed by RIF, TET, ERY (57.2-77.8%) and finally DOX (33.3%) in 2021 (Table 1)

The majority of tilapia farms had used several different antimicrobial classes including products containing two antimicrobials (Table 2). There were differences in the type of antimicrobials used in the provinces. Commonly used antimicrobials included amoxicillin, trimethoprim-sulfamethoxazole and doxycycline (combined with florfenicol).

Vietnamese tilapia farmer needs guidelines for effective antimicrobial treatment and a wider take up of vaccines to prevent infections caused by *S. agalactiae*.

Table 1: Prevalence (%) of antimicrobial resistant *S. agalactiae*

Year	Prevalence % of antimicrobial resistant						
	DOX	RIF	TET	ERY	TMP-SMX	NOV	NEO
2017	0	0	0	0	0	11.1	0
2018	0	0	0	0	0	75.0	12.7
2019	25.0	20.0	25.0	40.0	57.2	100	28.6
2020	25.0	28.6	57.1	42.9	75.0	100	50.0
2021	33.3	57.2	71.4	77.8	88.9	100	100

Note: DOX (Doxycycline), RIF (Rifamycin), TET (Tetracycline), ERY (Erythromycin), TMP-SMX (Trimethoprim-sulfamethoxazole), NOV (Novobiocin), NEO (Neomycin)

Table 2: Antimicrobial used in tilapia farming at Hai Duong, Bac Ninh and Bac

Antimicrobial	Hai Duong	Bac Ninh	Bac Giang
Doxycycline	21.4	0	44.4
Florfenicol	7.1	0	11.1
Erythromycin	0	11.1	0
Amoxicillin*	14.3	0	0
Amoxicillin	**28.6**	22.2	**77.8**
Sulfamethoxazole	21.4	11.1	**66.7**
Enrofloxacin	0	11.1	11.1
Doxycycline+ Florfenicol	0	**55.6**	11.1
Amoxicillin+Sulfamethoxazole	7.1	11.1	0
Unnamed antimicrobial	35.7	0	11.1
Total	85.7	77.8	**100**

ment and a wider take up of vaccines to prevent

DEVELOPMENT OF MONOVALENT VACCINES PROTECT STRIPED CATFISH (*Pangasianodon hypophthalmus*) AGAINST *Aeromonas hydrophila* AND *Edwardsiella ictaluri*

Tu Thanh Dung*, Bui Thi Bich Hang, Eng Khuan Seng, Seah Keng Hian, Tran Thi Tuyet Hoa, Dang Thuy Mai Thy, Le Minh Khoi and Tran Ngoc Hai

College of Aquaculture and Fisheries
Can Tho University
Campus II, An Khanh, Ninh Kieu, Can Tho, Vietnam
ttdung@ctu.edu.vn

Vietnam is the world largest producer of striped catfish (*Pangasianodon hypophthalmus*). The intensification of striped catfish production has resulted in increased disease outbreaks. Developing a multivalent vaccine for prevention is the optimal option, to achieve this goal requires basic research on monovalent vaccines. The study was carried out to evaluate the effectiveness of monovalent vaccines against diseases caused by *A. hydrophila* and *E. ictaluri* bacteria on striped catfish cultured in the Mekong Delta, Vietnam. A total of 65 strains of *A. hydrophila* and 57 strains of *E. ictaluri* were isolated in striped catfish farms from the year 207-2020. Screening the highest virulence isolates were performed and the two candidate bacterial isolates: *A. hydrophila* AH3 and *E. ictaluri* 37 were selected. Lethal dose 50 (LD_{50}) values of *A. hydrophila* AH3 and *E. ictaluri* 37 strains were $4.7x0^2$ CFU/mL and $2.35x0^5$ CFU/mL, respectively. The oil-emulsion form vaccines containing formaline-killed bacteria was developed from these two strains of bacteria and evaluated for effectiveness on striped catfish in the wet-lab. The results showed that the specific antibody response to *A. hydrophila* of vaccinated fish showed at high levels (level of 6.5) after 20 days post vaccination, then reduced in day 30 (level of 4.5). Whereas, the antibody response to *E. ictaluri* were also high antibody levels (level of .0) after 0 days post vaccination, but slowly reducing after the day 30 (level of 9.5). In addition, the challenge experiments to evaluate the relative percent survival (RPS%) showed a protection rate of 93.3% in the AH3 vaccine and 80% in the EI37 vaccine.

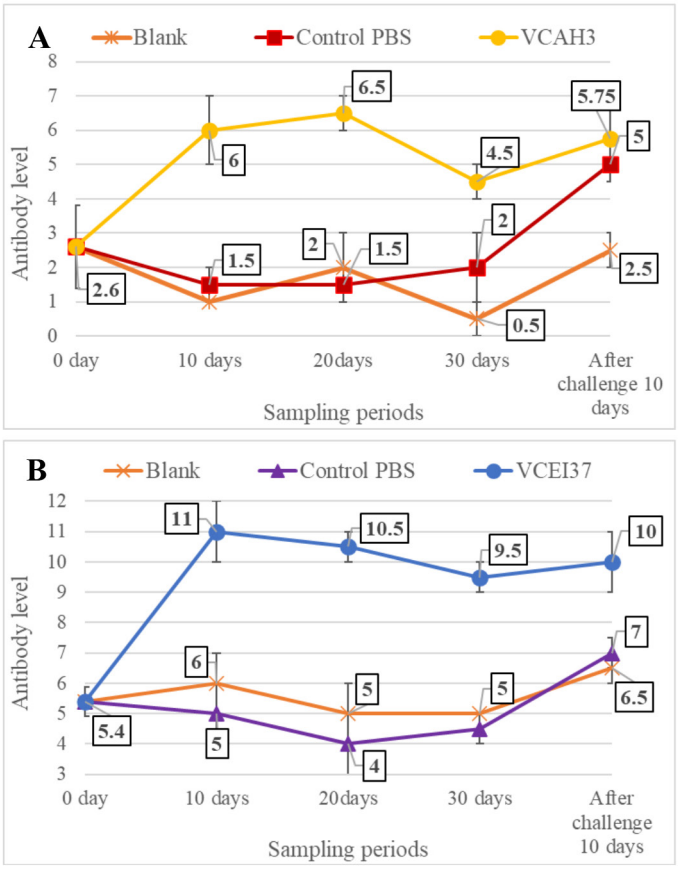


FIGURE 1. Antibody response to *A. hydrophila* (A) and *E. ictaluri* (B) of vaccinated striped catfish

SEA CUCUMBER FARMING IN TUN MUSTAPHA PARK, SABAH, MALAYSIA: CHALLENGES AND OPPORTUNITIES FOR DEVELOPING SUSTAINABLE COASTAL LIVELIHOODS

Audrey Daning Tuzan*, Nurul Ain Jais, Ben Parker, Sofia Johari, Wei-Kang Chor and Victor Andin

Borneo Marine Research Institute
Universiti Malaysia Sabah
88400, Kota Kinabalu
Sabah, Malaysia
audrey@ums.edu.my

Sea cucumbers play a vital role in sustaining the health of the marine environment. It is also a popular delicacy in South-East Asia and is used in traditional Chinese medicine. Thus, due to the increased demand and high market value, sea cucumber harvesting became a lucrative business. In Malaysia, Sabah has been reported as the largest sea cucumber producer in the past decade regarding fisheries landing and aquaculture production, with the largest producer districts, are located within Tun Mustapha Park (TMP) in Northern Sabah. Sea cucumbers have become one of the most important sources of income for the coastal communities in TMP. In TMP, sea cucumbers can be easily hand collected during low tide on the intertidal zone or by diving in the deeper waters close to the reef. Sandfish, *Holothuria scabra,* is the major farm species in TMP, where the seeds were harvested from the wild and cultured in pens until they reached marketable size. These poor natural resources-dependent coastal communities face local and global threats and engage in unsustainable practices of exploiting limited natural resources. Thus, aquaculture of sea cucumber is much needed to offset the rapidly declining resource, conserve stock biodiversity and sustain the ecological, social, and economic benefits of these high-valued marine resources. Therefore, to provide insight into the common challenges, opportunities, and recommendations of sea cucumber farming in TMP, a Strength, Weakness, Opportunities and hreat (S.W.O.T) analysis was conducted through literature reviews and social surveys involving practitioners and key stakeholders. Based on the findings, we conclude that a more systematic sea cucumber management plan involving stakeholders and related agencies should be considered. Furthermore, the coastal community farmers should be educated and trained to improve their farming techniques. Additionally, post-harvest and processing training workshops and village-level processors should be capacity-built to increase their product's quality and bargaining power needed.

BEHAVIORAL CHANGES AND GILLS HISTOPATHOLOGICAL ALTERATIONS OF RED HYBRID TILAPIA (*Oreochromis* sp.) EXPOSED TO GLYPHOSATE HERBICIDE

Abubakar Muhammad Umar*, Nur Adeela Yasid, Siti Aqlima Ahmad, Hassan Mohd Daud and Mohd Yunus Abd Shukor

Department of Biological Sciences, Faculty of Science, Gombe State University, Gombe, Nigeria
abubakar.umar1980@gmail.com

A 49 days experiment was conducted under laboratory condition to ascertain the effects of technical grade glyphosate on behaviour and histopathological conditions in the gills of red hybrid tilapia using light inverted microscope. Air gasping, erratic swimming, fin movement, mucus secretion, hemorrhages and loss of scales were observed in the exposed fish. There was no any histopathological complication observed in the gill of the control fish, but various level of alterations were seen in the gills of the fish exposed to glyphosate herbicide. These include lifting of primary lamella, congestion of secondary lamella as well as hyperplasia in both primary and secondary gill lamella and hypertrophy of secondary gill lamella. Based on the findings of this study, glyphosate herbicide exerts behavioural and histopathological changes in the gill of red hybrid tilapia, and therefore the fish is considered as good bioindicator in aquatic environment monitoring.

The lethal concentration (LC_{50}) was determined and found to be 215 mg/L, the mean water quality parameters were temperature, 26-29°C, dissolved oxygen, 5.3-9.3 mg/L, and pH, 6.5-7.3 respectively. Three (3) concentrations of glyphosate were prepared for the purpose of the experiment (0, 50, and 150 mg/L). Behavioral changes was constantly recorded at daily basis. At the end of exposure period, the gill of the fish was removed and prepared for histopathological evaluation using light inverted microscopy.

The outcome of the study revealed various behavioural changes such as air gasping, erratic swimming, fin movement, mucus secretion, hemorrhages and loss of scales were observed. There were also many histopathological alterations in the gills of the fish. There was no histopathological changes observed in the gill of the control fish (**Figure 1A**). Fish exposure to glyphosate concentration of 50 mg/L caused the lifting of primary and congestion of the secondary gill lamellae (**Figure 1B**). A further increase in glyphosate concentration to 150 mg/L resulted in hyperplasia and hypertrophy of primary and secondary gill lamella and vacuolation of the secondary gill lamellae (**Figure 1C**).

Table 1: Behavioral and physical changes of red hybrid tilapia due to glyphosate exposure

Behavioral and physical observation	Glyphosate concentration (mg/L)		
	0	50	150
Air gasping	-	+	+++
Erratic swimming	-	++	++
Fin movement	-	+	++
Mucus secretion	-	-	++
Hemorrhages	-	-	++
Loss of scale	-	-	+

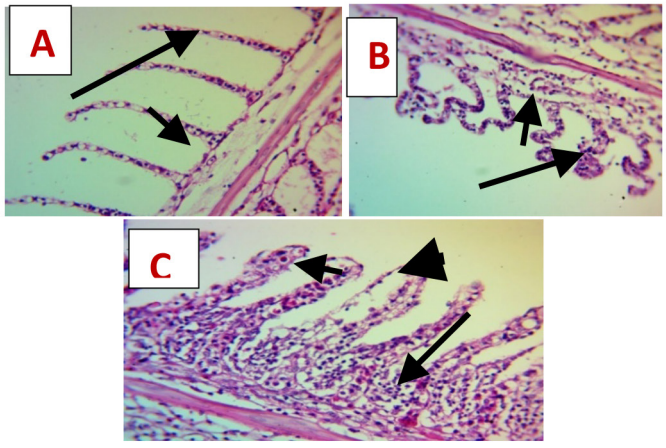


Figure 1: Micrograph of red hybrid tilapia gills exposed to glyphosate herbicide (H & E stain, 400x, scale bar=100 µm). Control group (A), fish exposed to 50 mg/L (B) and Fish exposed to 150 mg/L (C).

GROWTH, MORTALITY, RECRUITMENT AND STOCK STATUS OF THE GRAY EEL CATFISH *Plotosus canius* IN THE COASTAL WATERS OF PORT DICKSON, PENINSULAR MALAYSIA

Binta I. Usman*, Amin S. M. N. and Arshad A.

Department of Fisheries and Aquaculture
Faculty of Agriculture
Bayero University, Kano
P.M.B. 3011, Kano, Nigeria
biusman.faq@buk.edu.ng

Plotosus canius Hamilton, 1822; family plotosidae is primarily a marine catfish, but at times has been found to live in fresh or brackish waters (Riede *et al*., 2004; Ambak *et al*., 2010; Amornsakun *et al*., 2018). Literature on this fish both from national and international perspective is very scarce. Although, the status of this fish has not been assessed in the IUCN red list (IUCN, 2016), it has been declared endangered in Bangladesh and India (Mukhopadhyay, 1994; IUCN Bangladesh, 2000; Mijkherjee *et al*., 2002). Thus the present study was undertaken to examine status of *P. canius* from the coastal waters of Port Dickson, Peninsular Malaysia. The present study has been undertaken for 12 months to investigate the population structure of *P. canius* in the coastal waters of Port Dickson, Peninsular Malaysia. The growth, mortality, recruitment and relative yield per recruit of *P. canius* were examined based on monthly length-frequency data, using the FiSAT (FAO-ICLARM Stock Assessment Tools).

The estimated total mortality (Z) value was 2.73 yr^{-1} during the study period. Natural mortality (M) and fishing mortality (F) were estimated at 1.42 yr^{-1} and 1.31 yr^{-1} respectively. The value of exploitation rate (E) obtained for *P. canius* in the study was 0.48. Higher natural mortalities (M) versus fishing mortalities obtained in the present study suggested an unbalance position in the fish stock. Two major recruitment events were observed per year and the recruitment pattern was continuous (Figure 1). The findings from the analysis of the exploitation rate (E) based on the fishing mortality estimates, and from the yield-per-recruit showed that the *P. canius* fishery in the study area is slightly below the optimum level of exploitation (Figure 2). However, more research is suggested in such direction to corroborate the present findings as management for this fish is indispensable for maximum sustainable yield.

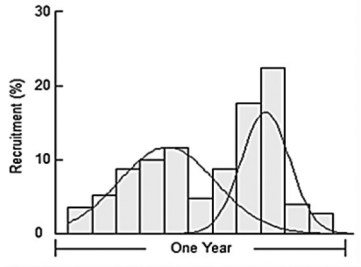


Figure 1: Recruitment pattern of *P. canius* in the coastal waters of Port Dickson, Peninsular Malaysia

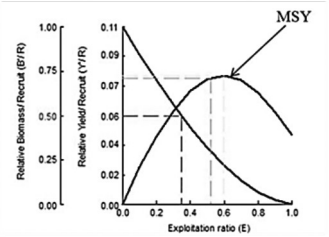


Figure 2: Yield-per-recruit and biomass-per-recruit model, showing level of yield index in *P. canius* from the coastal waters of Port Dickson, Peninsular Malaysia

DIFFERENT CONCENTRATIONS OF NITRATE (NO_3^-) FOR PRODUCING TOMATO SALADETTE *Solanum lycopersicum* Mill and CHERRY (*Solanum lycopersicum* Cerasiforme AND TILAPIA *Oreochromis niloticus* IN AQUAPONIC

2 Soto-Medina[1], Wenceslao Valenzuela-Quiñonez[1]*, Adolfo Dagoberto Armenta-Bogorquez[1], Ely Sara Lopez-Alvarez[1], Nadia Vázquez-Montoya[1] and Mariel López-Espinoza[1]

[1]Instituto Politécnico Nacional-CIIDIR Unidad Sinaloa, Boulevard Juan de Dios Batís Paredes # 250 Guasave, Sinaloa 81101, México

One alternative for producing sustainable food is aquaponic, which by recirculating effluent from aquaculture and hydroponic systems generates more organic and efficient crops, since it reduces the production of polluting effluents to make a biorational use of resources used in farming techniques and traditional aquaculture. Saladette (*Solanum lycopersicum* Mill) and cherry (*S. lycopersicum* var cerasiforme.) and the production of tilapia (*Oreochromis niloticus*) in an aquaponic system from different concentrations of nitrate. In this study, the yield of two tomato materials in different concentrations of nitrate and the effect on tilapia growth were evaluated, with the purpose of finding a concentration to provide the best conditions for the survival and healthy development of both species. In a closed system the nitrate concentration was controlled by dilution, to obtain the concentration of each treatment. In tilapia growth 4 treatments were evaluated; (TS_I: 350 mg/L, TS_{II}: 150 mg/L, TC_{III}: 150 mg/L and TC_{IV}: 150 mg/L) with 45 fish m^3. In both studies growth variables (specific growth rate, SGR), food conversion efficiency (FCE) and survival (S%) were evaluated. In agricultural experiment were evaluated 6 treatments: Saladette (TS), TS_I= 744 mg/L (control), TS_{II}: 350 mg/L; TS_{III}: 150 mg/L and Cherry TC_{IV}: 744 mg/L (control), TC_V: 350 mg/L and TC_{VI}: 150 mg/L, and; were grown 3.6 plants/m, and physiological variables (fruit weight, height and thick of stem) and production total (TP) were evaluated. Between treatments of aquaculture no significant difference ($p > 0.05$) were obtained. The growth variables were recorded as follows: mean 0.7 g/day and and FCA 1.2 and 100% survival in all treatments. Physiochemical parameters (temperature, pH and OD), recorded values within appropriate for cultivation of *O. niloticus* limits. Tomato yield significant differences were observed in both main effects. In the agricultural cultivation the best production materials tomato was presented in Saladette with a value of 65.53 t/ha, and the reduction test of nitrate was obtained at the level of 350 mg/L NO_3- with 57.73 t/ha.

INVESTIGATING THE GROWTH OF YEARLING *Rutilus kutum* IN EARTHEN PONDS

Alireza Valipour[a*], Ali Hasanpour[a], Hossein Mousapour[a], Naser Safarzadeh[a], Hossein Asgari[a], Bahman Mohammaditabar[a], Ali Alizadeh Najd[b], Danial Goroohi[b], Soheil Lesani[a]

[a] Inland Water Aquaculture Research Centre Iranian Fisheries Science Research Institute (IFSIR) Agriculture Research Education and Extension Organization (ARREO), Bandar-e Anzali, Iran
[b] Directorate general of Guilan Fisheries, Shahid Ansari Teleost Fish Restocking and Genetic Conservation Center Rasht, Iran

Rutilus kutum is one of the most important economic fishes of the southern basin and Iranian coasts of the Caspian Sea. More than 5,000 tons of this fish are caught from this area every year, and more than 8,000 fishermen are engaged in catching it in the sea. In recent years, Iranian Fisheries Science Research Institute has sought to determine the bio-technique of its breeding in earthen ponds and rearing tanks for introduction to the country's aquaculture industry and in this regard has started extensive research. In part of this research, the effect of density on the growth of this fish in earthen ponds was investigated. For this purpose, at the beginning of the spring season, at the temperature of about 14-16 °C, the broods artificially propagated, and the eggs after passing the incubation period and becoming active swimming larvae transferred to earthen ponds with a density of 1,500,000 per hectare. The fry were reared in these ponds until they reached the initial weight of about 0.897 ± 0.677 grams and were fed with special food for larvae and fingerlings (SFK) in addition to natural food. Then, they were transferred to fish breeding ponds with a density of 45,000 per hectare. The length of the rearing period in this stage was about 4 months and the fry were fed with formulated food with 43-46% protein and 10-14% fat. During the breeding period, health checks of the fish fry were carried out, the physical and chemical factors of the water, as well as the plankton and benthos organisms of the rearing ponds were measured and identified. The results showed that the weight of fry at the end of the breeding period reached an average of 17.07±1.99 and a maximum of 52 grams (fig. 1), so the yearling Kutum in the pre-breeding stage in the first year had a good growth in earthen pond.

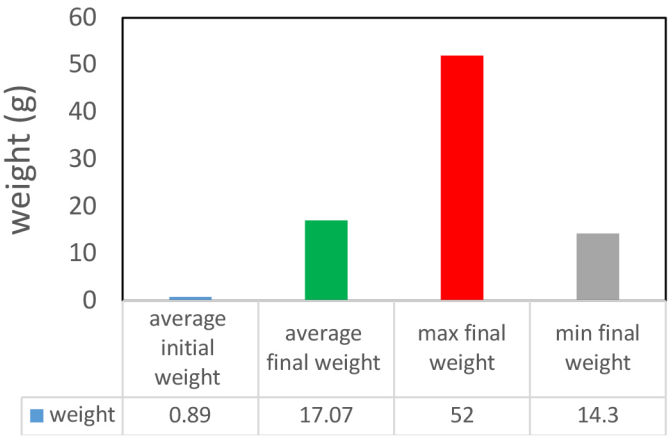


Fig 1. Weight of farmed yearling kutum in earthen pond

RED SEAWEED DERIVED BIOACTIVE INGREDIENTS IMPROVE AQUATIC ANIMAL HEALTH AND PRODUCTIVITY IN SHRIMP AND FISH

Hemanth Giri Rao Vantharam Venkata*, Sinduja Ramanan, Sri Sailaja Nori, and Shrikumar Suryanarayan

Sea6 Energy Pvt Ltd
1st Floor, C-CAMP, NCBS-TIFR
GKVK Campus, Bengaluru
Karnataka, India - 560065
hemanth@sea6energy.com

Intensive cultivation of animals reared for human consumption makes them susceptible to stresses arising from overcrowding, heat, ammonia accumulation and diseases. We have specifically identified and extracted novel biologically active compounds known as sulfated Galacto-oligosaccharides (sGOS) from red seaweeds. We administered them to commercially cultivated species of shrimp and fish to understand their effect on increasing productivity, tolerance to environmental stresses, as well as resistance to diseases in both lab and field conditions.

We find that inclusion of sGOS in the diet increases the survival of shrimp (*L. vannamei* and *P. monodon*) by at least 10%, and their resistance to diseases such as Early Mortality Syndrome (EMS), *Vibrio* infection, and White Spot Syndrome (WSSV) by at least 15%. The increased survival arises due to the immune stimulating activity of sGOS wherein it can specifically upregulate genes associated with anti-pathogen defense mechanisms. The increased survival also correlates well with increased yields of *L. vannamei* for the farmer under field conditions.

In fish, we find that sGOS increases the weight gain, specific growth rate and survival of fish (Tilapia). Overall, our results indicate that red seaweed derived sustainable feed additive rich in sGOS stimulates the immune systems of commercially important aquatic animals to sustain the stresses of intensive cultivation, while also facilitating higher productivity.

EFFECT OF COPING STYLE ON WELFARE AND PRODUCTIVE PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*)

Reynaldo Vargas*, Joseph Grajales, Alex Ríos, Reggie Guerra, Richard Mudarra, Carlos Solís

*Animal Science Department, College of Agricultural Science, University of Panama, Chiriqui 04004, Panama

The Nile tilapia (*O. niloticus*) is the most cultivated freshwater species in Panama. To modernize the aquaculture sector, it is necessary to select phenotypes that show the best productive characteristics under stressful situations. It is known that fish, like mammals, exhibit variability in their behavior when faced with stressful agents. When these behavioral differences are related to different physiological responses, the fish might have different coping styles such as proactive or reactive. Therefore, this work aimed to assess whether the behavior is associated with a differential response in variables of performance and animal welfare.

Nile tilapia males with an average weight of 15 grams, previously separated by the proactive and reactive restriction test, were housed in 350 L tanks. The tanks were equipped with a recirculation system and an auxiliary oxygenation system. The Nile tilapia were fed with the same commercial pellet feed.

The physicochemical conditions of the water were monitored daily, and the photoperiod was established at 12:12 (light-dark) hours. The treatments were carried out in duplicate, housing 10 fish of each phenotype in the same tank. Fish were challenged to different hypoxic and temperature conditions on days 35 and 49 of the trial, respectively.

Exposure to different hypoxic conditions consisted of reducing dissolved oxygen levels from 6mg/L to 3mg/L for one hour. At the end of the time, 10 fish from each treatment were individually isolated to evaluate the latency to resume feeding as the time to consume a pellet, and the opercular movements per minute as a physiological evaluation of stress response.

The temperature challenge consisted of increasing the temperature from 28 to 30 °C for one hour, evaluating the same variables described during hypoxia. The final weight and the degree of erosion of the caudal fin were determined.

Proactive fish showed lower latency than reactive fish to resume feeding during hypoxia ($p<0.001$) and when challenged under increased temperature ($p<0.01$). Reactive fish showed higher values of opercular movements ($p<0.001$), and there was no difference in caudal fin erosion between groups ($p>0.05$). Proactive fish were heavier ($p<0.001$, Figure 1) than reactive fish at the end of the trial.

These results showed that Nile tilapias separated by their coping styles have differential responses to stressful situations that can affect well-being and productive performance.

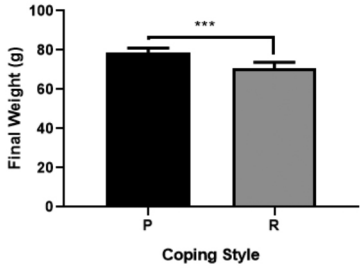


Figure 1. Mean (SD) of weight between proactive (P) and reactive (R) at the end of the trial. ***p>0.001

A PILOT SCALE MASS CULTURE PROGRAM FOR *Dunaliella salina* FOR ACHIEVING HIGHEST PRODUCTION LEVELS

Dr. V. Jayaprakas*

*President, Innovative Aquaculture Technologies Inc.
D4, Infra Hillock Apartment, Parijathom Road, Pathadipalam, Kochi-682033.
jayaprakasvasu@yahoo.in

Mass culture of algae in round tank of 500 liter capacity with internal illumination tubes were used for the study. These tanks had 5 tubes each having a length of 120 cm. in each tube 200 light emitting diodes of white light were placed which had illumination control. Three 60 watt flood LED (red) lights were also placed on the roof of the tank. The resultant illumination was 170μmol/m^{-2}. The illuminations for the tanks was fixed for 16 hours daily with low illumination during start and shut down time of a cycle. The tanks were filled with 0.5M Sea water and a marine micro-algae *Dunaliella salina* was used as a organism in this study. The tanks were fed with 10% CO_2 and 90% N2 mixture of gas for a period of 30 minutes a day with a flow rate of 3 L/min, for every hour 1.25minutes was set for dosing with micro-bubbles through a ceramic diffuser. The tanks were fed with three different modified D. salina media, Nitrate based media, Ammonia based media and fish paste based media for making highest biomass in period of 10 days. The highest biomass was recorded in fish paste based media with a $18.76x10^6$ cells/ml, a count of $15.2x\ 10^6$ cells/ml in nitrate based media and $14.89x10^6$ cells/ml in ammonia media. The total wet biomass generated were in the range of 1.97g/L, 1.6g/L, 1.56g/L for the three media respectively.

EVALUATION OF NUMBER AND TOTAL AREA OF GILL MUCOUS CELLS OF ATLANTIC SALMON (*Salmo salar*) FED DIFFERENT DIETS WITH OR WITHOUT PROBIOTICS

Ioannis Vatsos*, Nimalan Nadanasabesan, Adriána Fečkaninová, Jana Koščová, Dagmar Mudroňová, Viswanath Kiron and Mette Sørensen

Faculty of Biosciences and Aquaculture, Nord University, Postbox 1490, 8049, Bodø, Norway
ioannis.vatsos@nord.no

Over the last decades, Norwegian salmon feed industry has been replacing major portion of fishmeal and fish oil in fish feeds with plant ingredients, like protein concentrates, usually with reduced content of antinutritional factors (ANFs). Nevertheless, amino acid imbalance and presence of ANFs are still matters of concern for the industry. Furthermore, plant lipids have an unfavorable ratio of n-3 : n-6 and does not provide the recommended EPA and DHA to the fish. Hence, ingredient composition of feed may have an effect on the health of the fish. Dietary administration of probiotics can influence the mucosal immune system of the fish. Gills are the main organs for respiration but they play also an important role as first line defense of the fish. Therefore, the microscopic structure of the gills and specifically the number and size of mucous cells, is considered a health indicator of fish, which may be affected by several extrinsic factors, including feed ingredients.

A feeding trial was conducted with Atlantic salmon (mean weight of 146.97 ± 4.9g SD). The fish were fed three types of feeds with different basal diets with or without probiotics. The ingredient composition of the diets were, Diet 1: fish meal/ fish oil based, Diet 2: a commercial-like diet dominated by plant ingredients (plant : marine is 70:30), and Diet 3: a fish meal/ fish oil based diet in which soybean meal replaced 20% of the fish meal. Dietary probiotic was cultured in the laboratory and vacuum coated on the diets. Gills samples were collected from 12 fish per treatment and fixed in 4% formalin. After paraffin embedding, tissue sections of 4 μm were prepared and stained with Hematoxylin and Eosin (H&E) and Alcian Blue – Periodic Acid Schiff (AB-PAS). Microphotographs (n=50 secondary lamellae/ fish) from five different filaments per fish were acquired using a camera attached to a light microscope. Quantitative analysis of gills morphology was performed with Image J (1.52a); total mucous cell area per epithelium area (ME), and number of mucous cells per epithelium area (NE) were determined.

The results showed significant differences in ME and NE. Both parameters were significantly influenced by diets and probiotics. Fish fed Diet 2 and 3 had significantly more ME and NE compared to those fed Diet 1. Addition of probiotics to all the diets increased the ME and NE. This study revealed that the ingredient composition of diets directly influences the gill mucous cell area and number of gill mucous cells, while dietary administration of probiotics increased both the number of mucous cells per epithelium area and the total area of mucous cells per epithelium area.

NON-IONIZED AMMONIA ASSESSMENT MODEL IN *Chirostoma estor estor* INTENSIVE CULTURE USING ARTIFICIAL NEURAL NETWORKS

José Luis Vázquez Burgos*
José Juan Carbajal Hernández
Luis Pastor Sánchez Fernández

Centro de Investigación en Computación – Instituto Politécnico Nacional. Av. Juan de Dios Bátiz s/n, Nueva. Industrial Vallejo, Gustavo A. Madero, Ciudad de México, C.P. 07738, México
E-mail: luis.vb24@live.com, carbajalito@hotmail.com, lsanchez@cic.ipn.mx

The *Chirostoma estor estor*, commonly known as white fish, is an endemic species of great importance in the area of Lake Patzcuaro, located in the state of Michoacan, Mexico. This represents a large income in the local economy, unfortunately it is currently in danger of extinction due to the pollution of the lake and its excessive fishing. National institutions, through aquaculture farming, have made efforts in the conservation of this type of fish, however there is still a lack of penetration of the scientific sector in this problem. This paper presents a computational model for the evaluation of non-ionized ammonium which is highly toxic and of vital importance in aquaculture farming systems. Using an Artificial Neural Network (ANN), a relationship is established between non-ionized ammonium (NIA) and parameters such as pH, temperature and total ammonia (TAN). Data bases obtained from measurements in culture ponds and generated by similar models have been proposed to generate efficient RNA training.

The computational model is divided into four stages (figure 1), in the first a database was simulated using the model proposed by Kennet. In the second stage it has a pre-processing so that the efficiency of the ANN is not diminished by a poor selection of patterns. The third stage consists of an ANN whose topology is given by the expression , which indicates that it consists of two inputs, a number of hidden layers with neurons in each hidden layer and one output. Finally, in the last stage the value of NIA is obtained.

The lowest MSE was the criterion for the choice of ANN configuration, both in the training set and the test set, and this was obtained with the topology . The results obtained by the training of the RNA show a learning rate of 99.91% of success in the approach. In the visualization of these results it is important to underline that each parameter (temperature and pH) individually affects differently the behavior of the NIA, for which the evaluation of the RNA for each of these was carried out individually (figure 2).

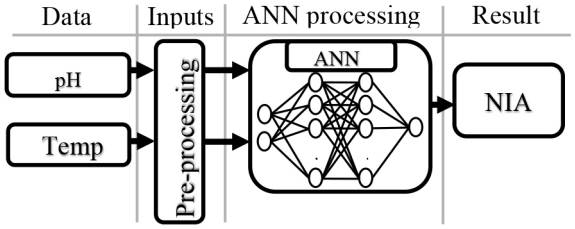


Fig. 1. Computational model for the evaluation of the NIA based on artificial neural networks.

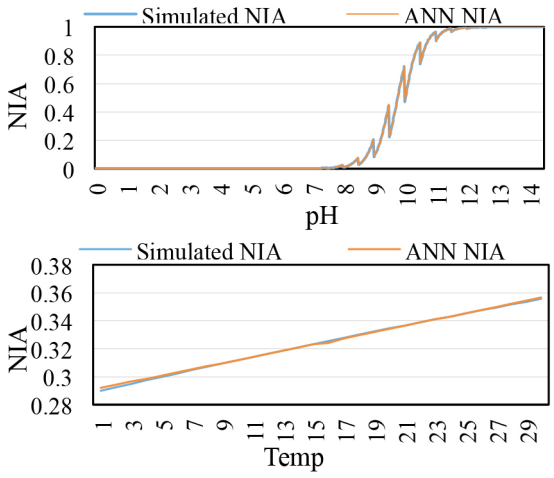


Fig. 2. Comparison of the NIA simulated with the ANN NIA, for pH and temperature.

DIAGNOSTICS FROM BASIC TO EMERGING TECHNOLOGIES: A KEY TO A HEALTHY SHRIMP AQUACULTURE INDUSTRY

Joseph Carlo V. Vergel*, Mary Beth B. Maningas, Eduardo M. Leaño, & Melba G. Bondad-Reantaso

National University Philippines
SM Baliwag Complex, Doña Remedios Trinidad, Highway
Brgy. Pagala, Baliuag, Bulacan 3006
jvvergel@nu-baliwag.edu.ph

Diagnostics plays a significant role in maintaining a healthy shrimp aquaculture industry. Some diagnostic techniques are used to screen healthy shrimps to ensure that they are not carrying any disease at sub-clinical levels by specific pathogens. This method is mostly done on stocks or shrimp populations destined for live transfer internationally. Diagnostics also determine the cause of unfavorable health or abnormalities for the recommendation of mitigating measures applicable to a certain condition which is the most recognized role of diagnostics in aquatic animal health. There are three levels of diagnostics based on Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy published by the Food and Agriculture Organization (FAO) of the United Nations and Network of Aquaculture Centers in Asia-Pacific (NACA) in 2000. Level 1 includes farm/production site observations and record keeping and health management of shrimp ponds. Level 2 includes laboratory procedures involving parasitology, histopathology, bacteriology, and mycology. Level 3 includes advanced diagnostic specialization involving virology, electron microscopy, molecular biology, and immunology. There are also available low-cost diagnostic field kits for farm or pond-side use which can be classified under Level 1. Microbiology and histology laboratories used for diagnosis of shrimp diseases can be classified under Level 2. In this study, we discuss the value of Levels I and II observations and combined with Level III to make an accurate diagnosis. Lastly, we also discuss emerging technologies on the horizon and how these technologies can be transferred to small scale shrimp producers.

WATER QUALITY MODELLING IN INTENSIVE CULTURE USING A WEIGHTED FUZZY INFERENCE SYSTEM

*Midory Esmeralda Vigueras Velázquez Luis Pastor Sánchez Fernández, Margarita Hernández Martínez, José Juan Carbajal Hernández

Corresponding author - midory.vigueras@gmail.com

This work proposes a new computational model (CWI) for water quality assessment in freshwater intensive cultured ponds, by using Weighted Fuzzy Inference Systems through a rule categorization process to preserve and help the growing and fish production. Each species unique and its requirements for habitat, environment, and nutrition must adapt to these, but there are elemental physical, chemical parameters which are necessary for coexisting and interact in any aquatic environment, as temperature, dissolved oxygen, pH, total ammonia and non-ionized ammonia. For this reason, these parameters were considered and measured.

To show the proposed index performance, a comparison of CWI index against by the National Sanitation Foundation (NSF) and the Canadian Council of Ministers of the Environment (CCME) was performed. Those are the most representative models for water assessment and can be adjusted to species. First, the NSF provides a good basis for quality assessment when weight is assigned to each parameter, and according to their importance in the ecosystem. However, the NSF score shows high results of water quality, although some parameters are not in optimal conditions. In the same way, CCME constantly shows good water quality. This is because, in its calculation, an average of the parameter set compensates the bad conditions of one parameter with the good of another. In Fig. 2, it is possible to appreciate a great similarity between CWI, NSF and CCME.

In Table 1, the obtained values in measurement 3) and 4) show that all parameters are within allowable values. The results shown by CWI indicate there is a gap of 1.24 points between 3) and 4). Due to higher temperature produces most chemical reactions, and the DO concentration decreased, the CWI had a better assessment in 4). That difference helps to explain why CWI is an alternative, suitable and reliable tool for the aquaculture of any aquatic species. Overall, the results of NSF and CCME show a good result, but the values obtained are not entirely satisfactory, because they do not contemplate the importance of each parameter. CWI was designed for that purpose, enabling the monitoring and the assessment of high-accuracy of species.

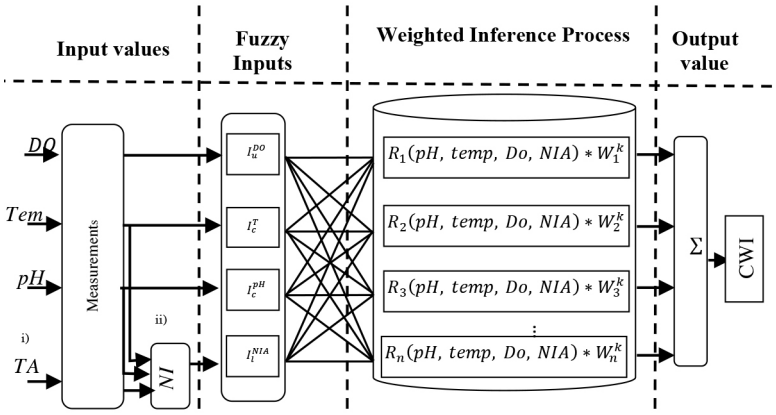


Fig. 1. TAN (i) and NIA (ii) are obtained by using the Nessler method and the correlation between pH temperature proposed. W_n^k denotes the importance weights that are computed according the rule impact in

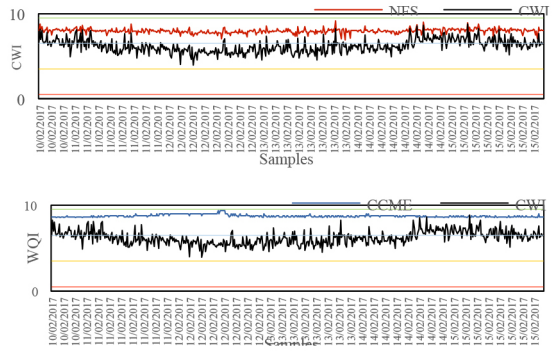


Fig. 2. a) CWI proposed vs NSF b) CWI proposed vs CCME

Date	N°	Parameter measurements				Water Quality Indexes			
		pH	DO	Temp	NIA	NFS	CCME	FIS	CWI
12/02/17	1	7.069	2.97	21.48	0.005711	6.673	6.171	4.369	2.781
13/02/17	2	7.347	4.29	20.51	0.010873	8.148	7.896	5.564	5.431
15/02/17	3	7.491	5.3	***21.48***	0.011419	7.404	7.733	6.131	5.973
15/02/17	4	7.465	5.46	***19.53***	0.011239	7.955	7.678	6.060	7.213

Table.1 Numerical comparison between water quality indexes.

DEVELOPING GENETIC RESOURCES FOR RED SNAPPER, AN IMPORTANT MARINE FOOD FISH FOR SINGAPORE

Shubha Vij, Grace Loo and Steven Fong

School of Applied Sciences (SAS), Republic Polytechnic
9 Woodlands Avenue 9, Singapore 738964
shubha_vij@rp.edu.sg

To boost local production and increase availability of food fishes, farmers require high quality genetically diverse broodstock to avoid inbreeding. Red Snapper is one of the two major farmed marine fish species in Singapore and there is good market acceptance for its meat texture and attractive red colour appearance. The supply of Red Snapper fry is mainly from out-door pond cultures but with the increasingly unpredictable weather conditions and expected longer term climate change, the future supply of Red Snapper will become unreliable and unable to support increased production of the species. Hence, to ensure a resilient supply of Red Snapper fingerlings, SFA has recently embarked on developing intensive indoor hatchery technology for sustainable production to support the farming industry. However, there are many species of Red Snappers that are morphologically similar and hence it is not easy to correctly identify the different farmed species. This would hamper any potential selective breeding efforts. In addition, there are also reports of potential Red Snapper hybridisation, which would further complicate the correct identification of species to be selected as brooders. Hence, there is a need to develop genetic resources for Red Snapper to address these problems faced in aquaculture. A multiplex genotyping platform based on novel tetranulceotide microsatellites was reported in 2006 for the Crimson Red Snapper. However, there was no follow up on this study and therefore, no proof on how applicable the resource is for the red snapper and hence there is a need to build this resource. Thus, in the present study, red snapper samples were collected from various locations across Singapore such as the Jurong port, Giant, Sheng Siong and the wet market. The nine pairs of primers reported previously as part of the microsatellite multiplex were tested individually on the genomic DNA extracted from snappers representing the different locations. The PCR products showed differences in size for some of the tested primer pairs. Based on the observed length polymorphism, it was possible to distinguish samples which were collected from locations such as Sheng Siong/ Jurong port/wet market from the ones collected from Giant. The next step would involve assessing the efficacy of the red snapper multiplex genotyping platform to distinguish snappers collected from various locations. These resources together would help in species identification and also help in evaluating the heritability of commercial traits in Red Snappers.

STARTING FROM SCRATCH: LAYING THE GENETIC GROUNDWORK FOR A NEW SELECTIVE BREEDING PROGRAM FOR MALABAR RED SNAPPER (*Lutjanus malabaricus*)

Shubha Vij*, Maria G. Nayfa, Liang Bing, Vu Nguyen, Joyce Koh, Celestine Terence, Jerryl Tan, Michael Voigtmann, Melvin Tan, Leow Ban Tat, Tim Tan, Xueyan Shen, Grace Loo, Jose Domingos, Dean Jerry

*School of Applied Science, Republic Polytechnic, 9 Woodlands Avenue 9, Singapore 738964, Singapore
shubha_vij@rp.edu.sg

For many countries, the COVID-19 pandemic highlighted their reliance on other nations for food security. Singapore responded with a government initiative to produce 30% of the country's nutritional needs by 2030, and emphasising aquaculture production. One of the fish of interest is tropical red snapper (*Lutjanus* spp.), but there are currently issues related to seedstock quality and lack of genetically improved strains. While a popular and valuable market fish (US$13/kg), little is known about Red Snapper regarding instigation of a selective breeding program, including what species is farmed in Singapore. To resolve this and build the genomic resources and quantitative parameters useful for an advanced breeding program, a project is underway to establish: species identity, a genome, a population based genetic audit, a 70K SNP array, coupled with heritability and quantitative genetic parameter estimates. DNA barcoding identified the Malabar Red Snapper (*L. malabaricus*) as Singapore's principal market species. A Singaporean sourced snapper was then used to produce the first genome of Malabar Red Snapper with an estimated genome size of 1Gb, with 24 chromosomal level scaffolds comprising 98.6% of the genome, n50 of 42,082,220 bp, and BUSCO score of 98%. This assembly was used to call SNPs for downstream genotyping-by-sequencing analyses. A population genetics study of ~1,000 fish from throughout the Indo-Pacific further unravelled the population structure and dynamics of Malabar Red Snapper. Setting this early foundation of genetic resources will help streamline establishing a new selective breeding program and optimize our capabilities for genetic prediction for improved commercial traits.

OXYGEN SUPPLEMENTATION SOLUTIONS AND THE BENEFITS IN AQUACULTURE

Andrew Villajuan*, Enrique Dacal, Xiong Zhao, Bianca Florian, Tianyan Chen, Caren Quek

Air Liquide
2 Venture Drive, #22-28, Vision Exchange, Singapore 608526
andrew.villajuan@airliquide.com

Air Liquide oxygen gas solutions Bicone, Mesoxal and Poroxal aim to provide higher dissolved oxygen (DO) levels into water in fish farms. Unlike conventional aeration systems, the levels of dissolved oxygen achieved are up to 400% which is significant gain to ensure survival of the fishes.

The advantages and benefits of the solutions to the fishes and fish farms across Air Liquide installations are presented in Table 1.

Providing efficient gas dissolution efficiency into the water enables fish farmers more productivity and competitive operational costs.

Table 1. Derived benefits from Air Liquide oxygen supplementation in fish farming

Country	Species	Solutions and Benefits
Japan	Flounder, Pufferfish	**Increased stocking density to 210%** by increasing and maintaining DO of ground seawater from 5.1-5.5 to 6 mg/L
Japan	Tiger Prawn	**Reduced mortality by 20%** by supplementing paddle wheel aerators with oxygen injection in the morning from 7 to 10 mg/L and during summer nights with DO from 1-2 to 4-5 mg/L
China	Whiteleg Shrimp	**Increased stocking density to 143%** while maintaining DO at 6-7 mg/L from air blower to oxygen
Turkey	Trout	**Increased activity w/n 30 minutes, increased appetite and better color of the fishes after 24 hours** after increasing DO from 6-8 to up to 18 mg/L
Greece	Sea bass, Sea bream	**Safe and stable DO concentration** of 9.5 - 13 mg/L, above the minimum level required for the fish and **oxygen dissolution efficiency improvement by around 30%.**
Spain	Rainbow Trout	**Year-round production is achieved,** eliminating the summer off-season production when natural water source is limited and DO is low at 4-6 mg/L by increasing the DO to 16mg/L .

WILL THE REDCLAW CRAYFISH, *Cherax quadricarinatus*, BE THE NEXT COMMERCIALLY IMPORTANT CRUSTACEAN FOR AQUACULTURE?

Humberto Villarreal*, José Naranjo-Páramo, Mayra Vargas-Mendieta

Aquaculture Program. Northwest Biological Research Center (CIBNOR). La Paz, Baja California Sur, México
e-mail: humberto04@cibnor.mx

With an increasing demand worldwide for most seafood species, over 100 new species have entered the aquaculture scene in the last 15 years. Freshwater crayfish have been recognized because of their culinary adaptability and market acceptance. However, capture volumes for several species have declined, particularly because of diseases in Europe and the USA. Production of 20-40 g crawfish, *Procambarus clarkii*, is centered in China, where there is a market for more than 1.2 million tons per year. It now represents the 5th most valuable cultured species worldwide. Now the markets are looking for larger sizes to support an increasing demand worldwide. Australian freshwater crayfishes are often considered in that regard, with Redclaw, *Cherax quadricarinatus*, at the top of the list. Since 1985, the species has been translocated to several regions in the world, to evaluate its potential for culture. In spite of this, commercial production has not met expectations. We discuss traditional commercial production techniques and their limitations, and offer new available technologies that improve harvest sizes, survival, and yield consistency, reducing production costs and improving the potential for a new thriving industry to develop.

REVISED POPULATION STRUCTURE AND EVIDENCE FOR LOCAL ADAPTATION IN AUSTRALIAN GIANT BLACK TIGER SHRIMP (*Penaeus Monodon*) USING SNP ANALYSIS

Vu N.T.T.*, Zenger K.R., Guppy J.L., Sellars M.J., Silva C.N.S., Kjeldsen S.R., and Jerry D.R.

ARC Industrial Transformation Research Hub for Advanced Prawn Breeding
Centre for Sustainable Tropical Fisheries and Aquaculture
College of Science and Engineering
James Cook University, Townsville, 4811, Australia
thithanhnga.vu1@my.jcu.edu.au

Restrictions to gene flow, genetic drift, and divergent selection associated with different environments are significant drivers of genetic differentiation. The black tiger shrimp (*Penaeus monodon*), is widely distributed throughout the Indian and Pacific Oceans including along the western, northern and eastern coastline of Australia, where it is an important aquaculture and fishery species. Understanding the genetic structure and the influence of environmental factors leading to adaptive differences among populations of this species is important for farm genetic improvement programs and sustainable fisheries management. Based on 278 individuals obtained from seven geographically disparate Australian locations, 10,624 high-quality SNP loci were used to characterize genetic diversity, population structure, genetic connectivity, and adaptive divergence. Significant population structure and differentiation were revealed among wild populations (average $F_{ST} = 0.001 – 0.107$; $p < 0.05$). Eighty-nine putatively outlier SNPs were identified to be potentially associated with environmental variables by using both population differentiation (BayeScan and PCAdapt) and environmental association (redundancy analysis and latent factor mixed model) analysis methods. Clear population structure with similar spatial patterns were observed in both neutral and outlier markers with three genetically distinct groups identified (north Queensland, Northern Territory, and Western Australia). Redundancy, partial redundancy, and multiple regression on distance matrices analyses revealed that both geographical distance and environmental factors interact to generate the structure observed across Australian *P. monodon* populations. This study provides new insights on genetic population structure of Australian *P. monodon* in the face of environmental changes, which can be used to advance sustainable fisheries management and aquaculture breeding programs.

ASSESSMENT OF WATER QUALITY ON HAU RIVER, MEKONG DELTA USING A BIOMONITORING APPROACH

Vu Ngoc Ut*, Nguyen Thi Kim Lien, Au Van Hoa, Truong Quoc Phu, and Huynh Truong Giang

College of Aquaculture and Fisheries
Can Tho University
Campus II, 3/2 street, Ninh Kieu district, Can Tho City, Vietnam
*Corresponding: vnut@ctu.edu.vn

Biomonitoring has been widely applied as one of the best approaches and tools to monitor and manage water quality in lotic systems. Macro-invertebrates including macro-benthos and aquatic insects are the major group that has been commonly used in biomonitoring. Hau River or Bassac river is the largest branch of Mekong river flowing down to the sea through the Vietnam territory and plays a vital role as water resources for aquaculture in the Mekong Delta. Monitoring water quality on Hau river is of determined factor for sustainable aquaculture. An assessment of water quality on Hau River using biomonitoring approach was therefore conducted to determine trophic conditions on Hau river for better water quality monitoring and management. The study was implemented by sampling water quality parameters and macro-invertebrates. Sampling was conducted 4 times per year (2 times in the dry season and 2 times in the rainy season) at 15 sites on the mainstream and 5 sites on the tributaries of Hau River belonging to Can Tho and An Giang provinces. Water quality parameters collected included temperature, pH, DO, BOD, COD, TAN, NO_2^-, NO_3^-, PO_4^{3-}, TSS, TN, TP. These parameters were assessed based on PCA analysis and water quality index (WQI). Macro-invertebrates composition and diversity were analyzed using biological indices such as Shannon-Weaver, Simpson and Margalef, and scoring systems including ASPT (Average score per taxon), BI (Biotic index) and FBI (Family biotic index). The results showed that WQI varied significantly among sampling sites. Bivalves, gastropods, and worms were the groups with high density and closely correlated with water quality. Organic pollution is inferred by elevated percentages of *Branchiura sowerbyi, Limnodrilus hoffmeisteri* (Oligochaeta), *Namalycastis longiciris, Tylorhynchus heterochaetus* (Polychaeta), *Clea helena, Melanoides tuberculata* (Gastropoda). Water quality on the Hau River was in a wide range of trophic conditions (ultraoligo- to eutrophic), in which eutrophic status occurred in the areas affected by aquaculture.

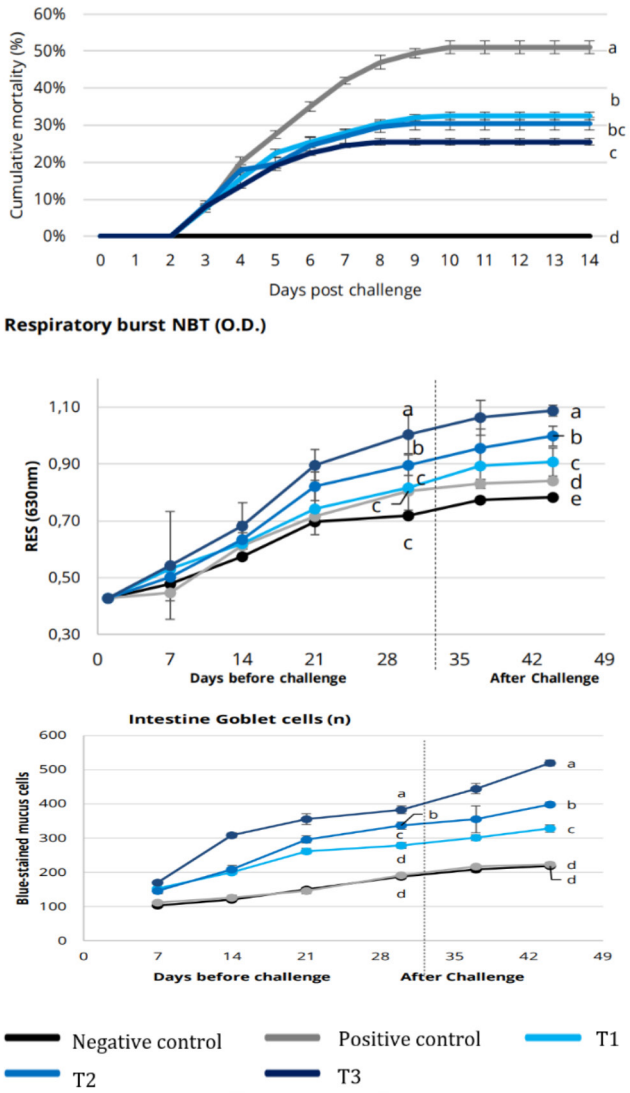
EFFECT OF EXTRACTS FROM SEAWEEDS, *Ulva spp.* AND *Solieria chordalis*, ON IMMUNE RESPONSE AND SUSCEPTIBILITY TO *Streptococcus agalactiae* IN RED TILAPIA (*Oreochromis* sp)

Vu Anh Tuan, Maria Garcia Suarez, Dang Thi Hoang Oanh

Olmix Group Representative Office
167 Luong Dinh Cua Street, Thu Duc Town, Ho Chi Minh City, Vietnam
Email: tuanvu@olmix.com

The extracts from seaweeds, *Ulva spp*. and *Solieria chordalis*, were incorporated in the diet of red tilapia (*Oreochromis sp*) juveniles of 57.3 ± 2.8 g. Two thousand fish were allocated into 20 tanks of 1000 L/each with a density of 100 fish/tank using an indoor recirculating system and implemented for 30 days. Fish were fed with 5 diets including 2 control diets (one negative control and one positive control) without seaweed extract inclusion and 3 test diets containing 3 inclusion levels of seaweed extracts including 0.1% (T1), 0.2% (T2) and 0.3% (T3). All diets are commercial feed with iso nutritive values. The seaweed extracts were mixed with 20 mL fish oil/kg and coated on top of the test diets. The control diets were top-dressed with 20 mL fish oil/kg only. After 30 days of the growth phase, the fish were submitted to a bacterial challenge phase between day 31 and day 44 with 50 fish/tank and 4 replicates. The fish were injected with a *Streptococcus agalactiae* bacterial strain at LD_{50} (0.5 x 105 CFU/fish) at day 31 and recorded clinical signs for 14 days follow post challenge. Fish body weight was recorded at day 28 and 44. Blood samples were collected from the caudal vein of 3 fish/tank at day 1, 7, 14, 21, and 30 at the pre-challenge; at day 7 and 14 post-challenge with heparin as anticoagulant. Cumulative mortality was calculated. Blood immune parameters (respiratory burst) and intestinal goblet cells (mucin producing cells) were quantified.

After 4 weeks of the growth phase, fish fed the seaweed extracts at 0.2% and 0.3% had the highest average body weight (ABW) of 152.6 g and 154.1 g, respectively, compared to control diet, 128.8 g (p<0.05). After being challenged, fish fed with the seaweed extracts showed higher average body weight (157.2 g and 159g) than that of the positive control 130.6 g and like the negative control 161.3 g (p<0.05). All fish were fed with seaweed extracts at 0.1%, 0.2% and 0.3% inclusions had significantly lower cumulative mortality (32.5%; 30.5%; 25.5%) than the positive control group, 51.0% (p<0.05). The respiratory burst increased significantly (p<0.05) as increasing the seaweed extract levels of 0.1%, 0.2%, and 0.3% and higher than fed control diets. The number of blue-stained goblet cells were significant higher in all fish fed seaweed extract groups than control groups (p<0.05). Moreover, the increasing inclusion levels of seaweed extracts stimulated linearly (p<0.05) the number of goblet cells. Fish were fed the mix of seaweed extracts at 0.3% had the highest goblet number followed by 0.2% and 0.1%. These results show the efficacy of the seaweed extract mix on reinforcing intestinal and systemic health in red tilapia meanwhile assuring growth performance.



BLUE ECONOMY: VALUING CARBON POTENTIAL IN OYSTER AQUACULTURE

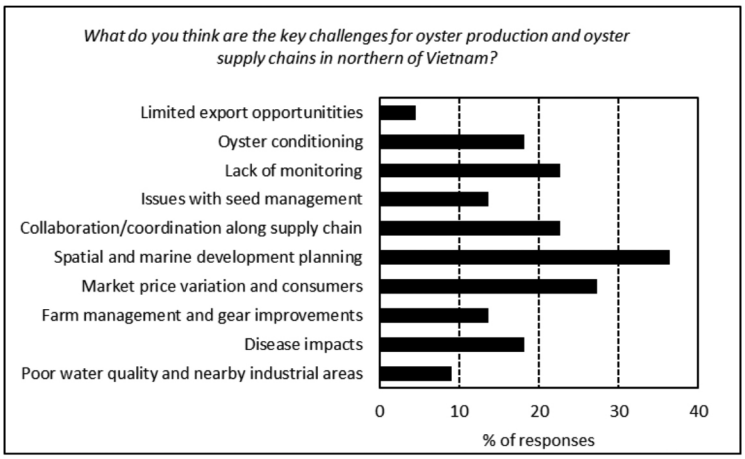
Vu Van Sang*, Cao Truong Giang, Nguyen Thi Hong Ngoc, Vu Van In, Wayne O'Connor, and Sarah C. Ugalde

Research Institute for Aquaculture No.1
Dinh Bang, Tu Son, Bac Ninh
Vietnam16352
vvsang@ria1.org

Oyster (*Crassostrea angulata*) aquaculture in Vietnam is relatively new but has proven highly successful. Conservative estimates of production are 15,000 tonnes/annum, and farming has now spread across 28 Provinces with 2,500 coastal-dwelling families benefiting from the industry (O'Connor et al., 2019). These small-scale businesses in rural Vietnam have been supported through a series of investments from the Australian Centre for International Agricultural Research (ACIAR) that have promoted profitable and sustainable use of coastal and near-shore resources. This industry has enabled diversification into other types of farming and created more jobs, more assets, more protein sources, and improved quality of life for farmers and their families, but these benefits have also come with challenges (Pierce, 2014).

In 2017, Vietnam was ranked 6th in the world for countries most affected by climate change according to Germanwatch's Global Climate Risk Index. Therefore, the sustainability and ongoing success of oyster production and aquaculture practices in Vietnam are highly vulnerable to the effects of climate change. Although the overall carbon footprint of oysters is currently under evaluation, there exists an opportunity to rapidly advance and sophisticate oyster aquaculture in Vietnam, and to explore the full economic, environmental, and social benefits it can bring.

A survey and supply chain analysis (Ugalde et al., *in press*) were conducted on the industry – from farm to plate – in northern Vietnam. The analysis identified key challenges for the new and growing industry (figure below) and areas to improve efficiency to make the supply chain more profitable. 68% of survey respondents thought that oyster shells should be sold as a by-product, and seven potential uses were identified: 1) carbon and carbon off-set schemes, 2) use in hatcheries for spat settlement, 3) mineral supplements in livestock feed, 4) soil management to reduce acidity, 5) crop and vegetable growth and vitality, 6) building foundations, road manufacture, or artificial stone, and 6) water filtration and purification. This presentation will discuss the status and challenges of the growing industry and tease apart the supply chain to show areas for improved efficiency. The potential for oyster by-products will be discussed, including the potential for carbon off-set schemes in Vietnam.



APPARENT DIGESTIBILITY OF MARINE MICROALGAE IN AQUAFEED FOR THE SHRIMP *Penaeus stylirostris*

Nelly Wabete*, Benedicte Lorgeoux, Celia Lemeu, Noemie Coulombier, Loic Le Dean, Thierry Jauffrais, Dominique Pham

Ifremer, UMR 9220 ENTROPIE (Ifremer, IRD, Univ Nouvelle-Calédonie, Univ La Réunion, CNRS)
101 Promenade Roger Laroque, 98897, Noumea, New Caledonia
Nelly.Wabete@ifremer.fr

Microalgae are considered as a source of healthful natural products in human and animal nutrition. Because of their unsustainability, fishmeal and fish oil must be reduced in aquafeed, and some microalgae strains have interesting compositions in terms of proteins and lipids both quantitatively and qualitatively owing to their amino acid or fatty acid profiles. In addition, their pigment composition and antioxidant activity make them an ingredient of choice for aquafeed. For crustaceans, their polyunsaturated fatty acids, such as EPA and DHA, and carotenoid pigments are of particular interest for broodstock feeding to improve fertility and egg quality.

To improve microalgae incorporation in aquafeeds, studies are necessary to determine their suitability in terms of nutritional composition, palatability, digestibility, growth performance, health and post-harvested quality product. The digestibility of an ingredient is one of the most important factors to consider in feed formulation. A highly digestible feed can lower production costs, reduce feed waste, and reduce the risk of eutrophication. The intrinsic characteristics of microalgae, the biomass pretreatments, and the feed preparation methods contribute to the microalgae nutrient and energy digestibility in shrimp feeding. In particular, the composition of the microalgae cell wall is known to affect its digestibility.

The aim of this study is to evaluate *in vivo* the digestibility of two tropical microalgae isolated, selected and produced in New-Caledonia, a *Prymnesiophyceae (Sp.1)* and a *Chlorodendrophyceae (Sp.2)*, as potential feed ingredients for the blue shrimps *Penaeus stylirostris*. No significant impact on shrimp survival and growth is noticed at 10 and 30% incorporation in feed. These two microalgae species have very different nutrient composition. The first one is rich in proteins and lipids and shows a significant capacity for assimilation by shrimps while the second one is less digestible by shrimps, probably due to a lower accessibility of its nutrients linked to the rigidity of its cell wall. The application of a microwave treatment on this second strain apparently improves the availability of lipids, but reduce their assimilation (Fig. 1).

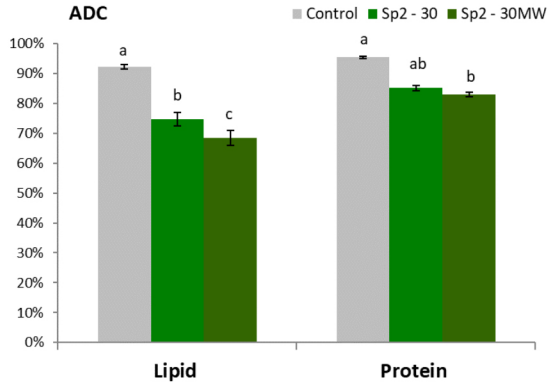


Figure 1: Apparent digestibility coefficients (ADC) of lipids and proteins of feeds containing 30% of microalgae *Sp.2* treated or not with microwave (MW) compared to Control (without microalgae).

IMPROVEMENT OF EGG CUSTARD USING MORINGA AND TURMERIC TO IMPROVE GROWTH AND SURVIVABILITY OF GIANT FRESHWATER PRAWN, *Macrobrachium Rosenbergii* LARVAE

M. Amiruddin Wahab*, Rusli N. D., Mat K. B., Syed M. Al-Amsyar., Mohd M., S. A. M. Sukri., and Hasnita C. H.

Faculty of Agro-Based Industry
Universiti Malaysia Kelantan, Jeli Campus
17600 Jeli, Kelantan
amir.f18d002f@siswa.umk.edu.my

This study aims to determine the efficiency of *M. oleifera* and *C. longa* mixed with egg custard as a booster for *M. rosenbergii* larvae. A total of 8 experimental diets; T1 (Egg custard + 1% turmeric), T3 (Egg custard + 3% turmeric), T5 (Egg custard + 5% turmeric), M1 (Egg custard + 1% moringa), M3 (Egg custard + 3% moringa), M5 (Egg custard + 5% moringa), TM1 (Egg custard + 1% moringa and 1% turmeric) were tested on day 10 of *M. rosenbergii* larvae. Egg custard was used as control diet and larvae in all treatments were fed with *Artemia sp*. during night time. Proximate analysis was done to determine crude protein, metabolize energy, lipid, ash, moisture content and crude fibre in each egg custard formulations. Feeding trial was carried out for 30 days. Stocking density was set at 30 larvae/L for each treatment. Larvae stage index (LSI), survivability, first day post larvae (PL) appear, weight and length were measured. It is expected that M5 diet contain more crude protein than other diet formulations. Present study showed that there were significant findings ($P \leq 0.05$) in all parameters except for LSI. M5 diet showed significant survivability rate (12.13±1.12%), weight (15.86±0.012mg) and length (1.06±0.081cm). However, among all treatments, the earliest day larvae turned into PL was recorded in TM1 diet. In conclusion, *M. oleifera* could potentially enhance survivability and growth of *M. rosenbergii* larvae.

Table 1: Biometric data of *M. rosenbergii* postlarvae (PL) in 8 diet formulations

TREATMENT	FIRST DAY PL APPEAR (d)	SURVIVABILITY (%)	LENGTH OF PL (mm)	WEIGHT OF PL (mg)
C	27	6.40 ± 2.57	105± 0.70	15.47± 0.012
T1	40	1.50 ± 0.93	ND	ND
T3	38	1.27± 0.90	ND	ND
T5	39	5.40 ± 0.12	ND	ND
M1	39	2.17± 0.97	ND	ND
M3	40	3.63± 1.15	ND	ND
M5	24	12.13 ± 1.12	106 ± 0.81	15.86 ± 0.012
TM1	20	7.37 ± 2.97	105 ± 0.59	15.53 ± 0.015

ND = not determined

OYSTER PRODUCTIONS UNDER OLIGOTROPHIC IN HIROSHIMA BAY, JAPAN; RESULTS OF PREY-PREDATOR NUMERICAL MODEL

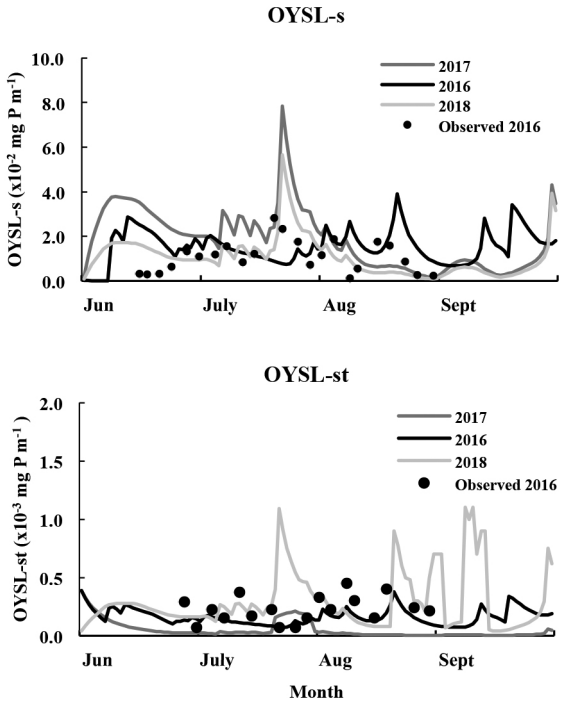
Wahyudin[1,2]* and Tamiji Yamamoto[1]

[1]Graudauate School of Biosphere Science, Hiroshima University, Japan
[2] Faculty of Marine Science and Fisheries, Raja Ali Haji Maritime University, Indonesia
E-mail: wahyudinps@umrah.ac.id

Hiroshima Bay is the top oyster production site in Japan, producing ca. 60% of the total national production. However, the production has been decreased from its peak production of 30 years ago with fluctuation in recent years. Notably, the lowest collection of seed larvae was recorded in 2017. This study aims to compare the oyster production in recent years in terms of the nutrient load into the bay.

Oyster larvae data cited from the report of Hiroshima Fisheries Promotion Center. The samples were collected from 0–5 m depth at five stations during the same season (June-August) of each year and counted the number for each size category under a microscope. The size categories are small (>90–150 μm), medium (150–210 μm), large (210–270 μm), and settling size (>300 μm). In addition to observing environmental conditions during summer (June to September) of the spawning season in 2016 to 2018, we calculated the material flow through prey-predator interactions each year using a numerical model with 25 compartments.

Under the oligotrophic condition of the bay, particularly the nutrient load was lowest in 2017. There was no significant difference in the number of small-sized oyster larvae, implying that the adult oysters were in good condition to spawn. However, the survival rate of oyster larvae during the small-sized to settling sized development was lowest in 2017. It is concluded that the primary cause of the low collection of seed larvae is insufficient food supply due to low nutrient load to the bay.



COMPARATIVE TRANSCRIPTOME ANALYSIS DURING THE SCUTICOCILIATE *Miamiensis avidus* INFECTION UNVEILS THE ANTI-PARASITIC IMMUNE DEFENSE MECHANISM IN OLIVE FLOUNDER (*Paralichthys olivaceus)*

Qiang Wan*, Hyukjae Kwon, Changnam Jin and Jehee Lee

Marine Science Institute
Jeju National University
Jeju Special Self-Governing Province, Republic of Korea
oneqiang@jejunu.ac.kr

Olive flounder (*Paralichthys olivaceus*) is one of the most economically valuable fish species in Korea. However, the olive flounder aquaculture industry is suffering massive losses due to the outbreaks of various diseases. In particular, the scuticociliatosis caused by *Miamiensis avidus* infection is responsible for more than 50% of the mortality of juvenile flounders. To develop effective scuticociliatosis treatment strategy and to understand the molecular mechanisms underlying the host defense response after parasitic infection, we performed this study to clarify the genes and pathways that are involved in the protective response of olive flounder after *Miamiensis avidus* infection via different routes.

Healthy juvenile flounders with average body weight of 30 g were experimentally infected by *Miamiensis avidus* via either intraperitoneal injection of 4 $x10^5$ cell/fish or immersion of 1 x 10^4 cell/ml for 6 hours. The skin, gill and head kidney tissue samples were collected at 0 h, 9h, 48h and 96h after infection. The total RNA of each samples was extracted, concentration/integrity determined, prepared for cDNA library and then paired-end sequenced using an Illumina HiSeq 2500 system. The raw sequencing data was processed by Trinity assembly and unigene annotation according to the Gene Ontology (GO) and BLAST analysis. Finally, over 5000 differentially expressed genes (DEGs) were determined by normalized ratio of reads between the PBS control and *Miamiensis avidus* infected fish in a tissue specific manner. Among the DEGs, a number of genes were clustered into immune related pathways, including immunoglobulin production pathway, cytokine/chemokine mediated signaling pathway, regulation of hematopoietic progenitor cell differentiation, complement and coagulation cascades pathway. In conclusion, this study uncovered the key immune genes and pathways closely related to scuticociliatosis and provide insights into the development of effective vaccine and immunostimulants to prevent the parasitic diseases in flounder aquaculture.

FOLLOWING THE INFECTION PROCESS OF VIBRIOSIS IN PACIFIC OYSTER (*Crassostrea gigas*) AND BLUE MUSSEL (*Mytilus edulis*) LARVAE USING FLUORESCENCE LABELING AND HISTOPATHOLOGY METHODS

Dongdong Wang*, Gilbert Van Stappen, Nelia Mbewe, Nancy Nevejan

Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Coupure Links 653, 9000 Gent, Belgium
E-mail: dongdong.wang@ugent.be

Pathogens, especially vibrios, are largely responsible for larval diseases in shellfish aquaculture. In order to understand how the infection process occurs in Pacific oyster (*Crassostrea gigas*) and blue mussel (*Mytilus edulis*) larvae, it is of great importance that the anatomy of the animals is well understood. In such a way, the sequence in which the internal structures are destroyed by the invading bacteria can be unraveled. In this study, three techniques were used in combination in order to have the full picture of what is happening during the infection process. After exposure of the larvae to different pathogens during challenge tests, clinical signs such as abnormal swimming behavior and destruction of the velum were observed under the inverted microscope. At the same time, by labeling *Vibrio hemicentroti* (ME09) and *V. anguillarum* (NB10) with the Green-Fluorescence Protein (GFP), we found a proliferation of the bacteria within the visceral cavity and subsequent necrosis of digestive organs, until a completed occupied body cavity. In addition, histological sectioning confirmed the damage on the level of individual organs and cells in function of exposure time. Interestingly, ME09 and NB10 had different lethality risk in Pacific oyster and blue mussel larvae but with the same progression in the infection process. This is the first time that the invasive pathways and infection dynamics of these two *Vibrio* pathogens have been investigated in Pacific oyster and blue mussel larvae. Understanding the infection process will help improve bio-control strategies and enhance the prospects of viable larviculture for oysters and mussels.

TOWARDS A DEEPER UNDERSTANDING OF THE MOLECULAR REGULATORY NETWORK ORCHESTRATING OVARY DEVELOPMENT AND MATURATION OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Wei Wang*, Jiahui Liu, Tingting Zhou, Siuming Chan

College of Fisheries, Guangdong Ocean University
Zhanjiang City, Guangdong Province, People's Republic of China
Email: wangwei.hg@outlook.com

Induction of gonad maturation and spawning is an important step in artificial propagation of shrimp, but our understanding of the molecular regulatory mechanism underlying shrimp ovary development and maturation is still very limited.

Using high-throughput sequencing platform, we analyzed the transcriptomes of five reproduction-related organs, including the eyestalk ganglion, brain, thoracic ganglion, hepatopancreas and ovary, from the Pacific white shrimp *Litopenaeus vannamei* at various stages of ovary development. A total of 48,722 unique transcripts (or unigenes) were assembled (N50=2,463 bp and average length=1,244 bp). Through Blast comparison with sequences in major databases, 18,661 unigenes were annotated. 2,974-11,999 genes were detected as differentially expressed at different ovarian stages, and 7 groups of genes were significantly enriched. By weighted gene co-expression network analysis (WGCNA), 14 distinct gene expression modules were identified. GO and KEGG analysis showed that these genes were involved in many important biological processes and pathways. In particular, genes related to CHH family neuropeptides, ecdysone signaling pathway, juvenile hormone signaling pathway, insulin signaling pathway, PI3K-Akt signaling pathway and TGF-β signaling pathway showed tissue-specific expression patterns (Fig. 1), suggesting their crucial roles in different tissues and at different development stages. Some candidate genes, including juvenile hormone pathway genes, were further investigated and their regulatory effects on vitellogenesis and ovarian growth were preliminarily confirmed.

Taken together, our study revealed the dynamic transcriptomic changes of several neuroendocrine organs during ovary maturation of *L. vannamei*, laying a foundation for further elucidation of the molecular regulatory network underpinning ovary development and maturation of shrimp.

Fig. 1

CHALLENGES AND DEVELOPMENTS IN OFFSHORE FISH PENS

C.M. Wang*, Y.I. Chu, J. Baumeister, H. Zhang, N. Abdussamie and I. Penesis

Blue Economy CRC-Co Ltd, Maritime Way, Newnham TAS 7248, Australia
*Corresponding author's email: cm.wang@uq.edu.au

Offshore fish farming has its challenges as listed in Table 1. Nevertheless, it is gaining traction for more sustainable and high-quality fish production due to larger water space, better water quality, stronger currents to dilute waste and avoid coastal ecosystem degradation often seen in nearshore fish farms.

Recently, many offshore fish pen design concepts have been proposed. Below are some key observations on offshore fish pen developments:

- Most offshore fish pen designs are at their conceptual stage.
- There are, however, some full scale proto-type pen designs been built (e.g., Ocean Farm 1 and Havfarm 1 in Norway, Shenlan 1 in China) in the last few years. So, operation of offshore fish farms is still in its infancy.
- Instead of an array of pens used in nearshore fish farms, offshore fish farms tend to have a single "mega" fish pen that can accommodate more than a million fish. These mega pens with their rigid steel frames and stiffer nets (e.g., polyethylene terephthalate) are better to withstand the more energetic environment.
- Submerged pens are also pursued as a solution for offshore fish farms as it avoids strong surface waves, algae bloom, parasites, warm surface waters during summer. Their structural strength and stiffness do not need to be overly designed as surface floating structures. Examples of submerged pens are the Atlantis in Norway and Innovasea pens in Hawaii.
- Offshore fish pens have to be equipped with remote and autonomous devices for operation, maintenance, monitoring and surveillance of feeding, venturation, lighting, cleaning, and removal of wastes and dead fish.
- Offshore fish pens, sited at a considerable distance from the shoreline, will have to tap on offshore renewable energy sources such as wind, wave and solar energies for power supply to minimize OPEX and environmental impacts due to the dependency on fossil fuels
- Integration or co-location of fish farming with other synergetic marine activities (such as renewable energy production) are desirable from an economic viewpoint. The overall infrastructure and operational procedure will no doubt be more complex, and the increased functionalities will bring more risks and require more rigorous assessments for insurance coverage. More research and developments are needed in this space.

Table1. Challenges associated with offshore fish farming

Environmental challenges	Operational challenges	Design challenges
• deep water • unknown seabed condition • exposure to harsh environment of strong waves, current and wind • climate change • uncertain environment for fish welfare and growth	• difficult working environment for workers due to the strong waves • vessel collision with fish pens • larger marine animal invasion • expensive infrastructure for offshore fish farming (e.g. utility vessels, power supply) • economic sustainability for operations (including material selection)	• lack of experience in designing mega/submerged offshore fish pens • lack of standardized and comprehensive design guidelines/codes

DEEPCHILLING: MAXIMIZING YIELD AND FRESHNESS WITH SUBZERO COLD CHAIN

Ming-Jian Wang*

Deepchill Technologies Inc.
180 Caster Avenue
Woodbridge, Ontario, Canada L4L 5Y7
mwang@deepchill.com

Seafood is a highly sensitive and perishable product. It presents major challenges in handling and maintenance of optimal conditions during processing and distribution. The current cold chain which has been established over the years does not effectively meet the requirements of today's automated processing and much broader distribution. Deepchilling (rapid cooling to and maintenance of subzero temperature) seafood can optimize the cold chain, offering significant advantages over traditional methods such as RSW or flake ice.

Studies have been conducted at various stages of the cold chain (harvesting, processing to distribution) using different species of fish such as farmed salmon, trout, cobia and yellowtail. The tests analyzed cooling rates, bacterial growth, texture after maturing and freshness and evaluated the impact on the yield, shelf life, and quality of the fish.

When salmon and yellowtail are harvested before being gutted, bacteria are quickly developing inside of the fish. Deepchilling fish immediately upon harvest minimizes bacteria growth and maintains freshness from the beginning, so fish are kept safe with an extended shelf-life of up to 5 days. Salmon, once gutted, are normally stored overnight or extra days before filleting and pin-boning. This is a critical period where bacteria growth shall be controlled, and firm texture must be maintained to prevent gaping. To go through the filleting machine efficiently, a deepchilling process shall be arranged, through which fish are kept at optimal subzero temperature and firmness. This reduces fat accumulation on the knife, allowing better operation with cleaner cuts and less gaping. As a result, the quality of fillets is significantly higher and more filleting yield can be achieved.

Yellowtail are very sensitive fish, requiring fast processing time to maintain the highest quality. Often shipped overseas, yellowtail requires extreme quality control, keeping the freshness at peak condition. Deepchilling maintains yellowtail's prime condition even during filleting, packaging, and overseas shipping. With the extremely high cooling rate and ability to consistently maintain subzero temperature, Deepchilling enables sashimi quality fish and allows distribution from as far as Japan to North America. Similarly, the deepchilling process provides an efficient method for rapid chilling of farmed cobia from a typical tropical temperature down to 0°C and reducing the rate of bacteria growth and spoilage.

The results have shown significant improvement in both quality, shelf life and yield. Deepchilling can provide a sub-zero cold chain and is a proven method in keeping fish at optimal temperatures, controls bacteria growth, freshness and extends shelf-life up to 5 days.

DEEPCHILLING: MAXIMIZING YIELD AND FRESHNESS WITH SUBZERO COLD CHAIN

Ming-Jian Wang*

Deepchill Technologies Inc.
180 Caster Avenue
Woodbridge, Ontario, Canada L4L 5Y7
mwang@deepchill.com

Seafood is a highly sensitive and perishable product. It presents major challenges in handling and maintenance of optimal conditions during processing and distribution. The current cold chain which has been established over the years does not effectively meet the requirements of today's automated processing and much broader distribution. Deepchilling (rapid cooling to and maintenance of subzero temperature) seafood can optimize the cold chain, offering significant advantages over traditional methods such as RSW or flake ice.

Studies have been conducted at various stages of the cold chain (harvesting, processing to distribution) using different species of fish such as farmed salmon, trout, cobia and yellowtail. The tests analyzed cooling rates, bacterial growth, texture after maturing and freshness and evaluated the impact on the yield, shelf life, and quality of the fish.

When salmon and yellowtail are harvested before being gutted, bacteria are quickly developing inside of the fish. Deepchilling fish immediately upon harvest minimizes bacteria growth and maintains freshness from the beginning, so fish are kept safe with an extended shelf-life of up to 5 days. Salmon, once gutted, are normally stored overnight or extra days before filleting and pin-boning. This is a critical period where bacteria growth shall be controlled, and firm texture must be maintained to prevent gaping. To go through the filleting machine efficiently, a deepchilling process shall be arranged, through which fish are kept at optimal subzero temperature and firmness. This reduces fat accumulation on the knife, allowing better operation with cleaner cuts and less gaping. As a result, the quality of fillets is significantly higher and more filleting yield can be achieved.

Yellowtail are very sensitive fish, requiring fast processing time to maintain the highest quality. Often shipped overseas, yellowtail requires extreme quality control, keeping the freshness at peak condition. Deepchilling maintains yellowtail's prime condition even during filleting, packaging, and overseas shipping. With the extremely high cooling rate and ability to consistently maintain subzero temperature, Deepchilling enables sashimi quality fish and allows distribution from as far as Japan to North America. Similarly, the deepchilling process provides an efficient method for rapid chilling of farmed cobia from a typical tropical temperature down to 0°C and reducing the rate of bacteria growth and spoilage.

The results have shown significant improvement in both quality, shelf life and yield. Deepchilling can provide a sub-zero cold chain and is a proven method in keeping fish at optimal temperatures, controls bacteria growth, freshness and extends shelf-life up to 5 days.

COMPARATIVE EFFECT OF ORGANIC AND INORGANIC SELENIUM SUPPLEMENTATION ON GROWTH PERFORMANCE, HEMATOLOGICAL VALUE AND IMMUNE STATUS IN NILE TILAPIA (*Oreochromis niloticus*)

Matthijs de Jong, Eakapol Wangkahart, Brecht Bruneel, Ludo Segers, Anut Chantiratikul

Orffa Additives BV
Minervum 7032, 4817ZL Breda, The Netherlands
jong@orffa.com

This study was carried out to investigate the effects of dietary organic and inorganic selenium (Se) on growth performance, hematological value and immune response in Nile tilapia. A total of 735 fish (initial weight 13.52±0.5g) were fed one of 7 experimental diets (in triplicate) in fiberglass tanks for 8 weeks. Organic Se (L-selenomethionine, SeMet; Excential Selenium 4000, Orffa Additives BV) and inorganic Se (sodium selenite, Na_2SeO_3) were each added to the basal diet of Nile tilapia at 1, 3, and 5 mg Se/kg. The basal diet (28% crude protein), without Se supplementation, was used as a control. The final Se concentration of the basal diet was 0.68 mg Se/kg. Organic and inorganic Se supplemented diets contained 1.78, 3.53 and 4.9 mg Se/kg and 1.75, 3.49 and 5.3 mg Se/kg, respectively. Fish were fed at 5.0% of their body weight twice a day. Parameters were assessed at the end of the rearing period. After 8 weeks, 20 fish from each treatment were challenged with intraperitoneal injection of virulent *Streptococcus agalactiae* ($1x10^7$ CFU/ml). The cumulative mortality was observed for 21 days and the relative percent survival (RPS) was calculated. Statistical analysis was performed by using one-way ANOVA and Bonferroni post hoc tests.

The results show that weight gain (WG) of fish fed SeMet at 1 mg Se/kg was significantly higher than that of fish fed basal diet ($p<0.05$) (Table 1). Lymphocytes (Table 2) were significantly ($p<0.05$) higher in fish fed SeMet (1 mg Se/kg) compared to fish fed basal diet. Alanine transaminase (ALT), aspartate transaminase (AST), creatinine, blood urea nitrogen (BUN), albumin, globulin and total protein were not significantly influenced by dietary Se supplementation (Table 2). Increasing dietary Se level, in particular in the form of SeMet, led to a decrease in serum cholesterol concentrations (Table 2). Interestingly, the innate immune response, including lysozyme, catalase, myeloperoxidase, superoxide dismutase and glutathione peroxidase activities, was significantly ($p<0.05$) increased with Se supplementation compared to the basal diet group, especially for fish fed SeMet (1 and 3 mg/Se kg), whereas malondialdehyde (MDA) in fish serum was numerically decreased for all supplementation levels (Table 3). Fish fed SeMet (1 mg Se/kg) showed the highest relative percent survival (RPS) after challenge with *S. agalactiae* (data not shown).

Table 1: Growth performance and feed utilization of fish fed experimental diets

	Basal Diet	L-selenomethionine (mg/kg)			Sodium selenite (mg/kg)			Pooled	p value		
Parameters	0.68	1.78	3.53	4.9	1.75	3.49	5.3	SEM	S	L	SxL
WG	40.36 b	53.62 a	47.98 ab	39.34 b	40.51 ab	43.07 ab	39.02 b	2.01	0.001	0.001	0.005
SGR	2.32 ab	2.70 ab	2.51 ab	2.26 b	2.28 b	2.36 ab	2.29 ab	0.12	0.695	0.717	0.003
ADG	0.67 ab	0.89 ab	0.80 ab	0.66 b	0.68 ab	0.72 ab	0.65 b	0.06	0.161	0.277	0.464
FCR	1.77 ab	1.42 a	1.54 ab	1.79 ab	1.75 ab	1.67 ab	1.82 b	0.1	0.205	0.24	0.529
VSI	8.65 b	10.32 a	9.98 ab	6.35 d	6.22 d	6.39 d	7.74 bc	0.44	.001	.001	.005
HSI	1.81	1.82	1.84	1.97	1.61	1.96	2.03	0.14	.135	.005	.064
GSI	0.66 ab	0.84 a	0.64 ab	0.67 ab	0.41 b	0.30 b	0.32 b	0.12	.073	.000	.010

Note: Values show mean, pooled SEM, n = 90; S= L-SeMet (Excential Selenium 4000) and sodium selenite (Na_2SeO_3), L=level of selenium supplementation. Values in the same row with different subscripts differ a significantly ($p < 0.05$). Weight gain (WG), specific growth rate (SGR), average daily gain (ADG), feed conversion ratio (FCR), viscerosomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI).

Table 2. Hematological and blood chemistry parameters of fish fed experimental diets

Parameters	Basal Diet	L-selenomethionine (mg/kg)			Sodium selenite (mg/kg)			Pooled	p value		
	0.68	1.78	3.53	4.9	1.75	3.49	5.3	SEM	S	L	SxL
RBC (x106 cells/mm²	2.3 a	2.0 b	2.4 a	2.3 a	2.4 a	2.4 a	2.3 a	0.1	.048	.074	.048
Hemoglobin (g/dL)	11.3 ab	9.8 c	11.9 a	10.5 ab	11.9 a	11.3 a	10.7 ab	0.54	.123	.086	.018
Haematocrit (%)	36.3 ab	33.7 b	38.3 ab	36.0 ab	43.0 a	37.5 ab	33.0 b	2.33	.384	.301	.063
MCV	158.3	166.3	158.1	160.5	176.0	151.0	140.0	9.06	.358	.039	.187
MCH	48.4 a	48.2 a	48.7 a	45.4 b	48.5 a	45.5 b	44.9 b	0.81	.048	.001	.034
MCHC	30.8 ab	29.4 ab	30.8 ab	28.6 ab	27.5 b	30.2 ab	32.1 a	1.29	.705	.165	.086
W.B.C. (cells/mm3)	2,275.5 ab	2,849.0 a	1,859.8 b	2,109.0 ab	2,109.0 ab	2,026.3 ab	1,887.0 b	108.89	.110	.024	.083
Heterophils (%)	57.0 ab	46.0 c	54.4 abc	55.5 ab	49.0 ab	56.3 ab	62.0 a	3.59	.118	.004	.706
Lymphocytes (%)	39.3 bc	49.7 a	42.6 abc	40.5 abc	48.0 ab	41.5 abc	36.0 c	3.6	.323	.009	.832
Monocytes(%)	3.3 a	2.3 b	3.0 ab	2.0 b	3.0 ab	2.3 ab	2.0 b	0.28	.925	.033	.036
ALT (U/L)	22.0 a	17.6 ab	19.67 ab	14.0 ab	16.33 ab	13.4 b	15.0 ab	2.93	.074	.070	.024
AST (U/L)	53.33 a	51.4 ab	50.67 ab	61.67 a	48.6 b	67.5 a	66.2 a	10.3	.000	.000	.000
ALP (U/L)	18.0 c	21.0 bc	24.0 ab	22.33 b	25.33 a	25.33 a	25.0 a	3.02	.383	0.921	0.924
Creatinine (mg/dl)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	>0.001	>0.001	>0.001
BUN (mg/dl)	2.0	1.0	1.3	1.3	1.7	1.0	1.3	0.27	0.626	0.783	0.215
Albumin (g/dl)	1.03 ab	1.17 ab	0.93 b	1.00 ab	1.03 ab	1.20 a	1.10 ab	0.07	0.317	0.858	0.131
Globulin (g/dl)	2.3	2.3	2.1	2.3	2.3	2.3	2.2	0.07	0.881	0.332	0.332
Total protein (g/dl)	3.33 ab	3.50 a	3.03 b	3.27 ab	3.33 ab	3.47 a	3.27 ab	0.15	0.435	0.422	0.112
Cholesterol (mg/dl)	175.67 a	153.67 ab	125.67 b	159.67 a	157.00 a	161.0 a	152.33 ab	9.31	0.075	0.967	0.029

Note: Values show mean, pooled SEM, n = 90; S= L-SeMet (Excential Selenium 4000) and sodium selenite (Na_2SeO_3), L=level of selenium supplementation. Values in the same row with different subscripts differ a significantly ($p < 0.05$). Red blood cells (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cells (W.B.C.), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN).

Table 3. Immune parameters of fish fed experimental diets

Parameters	Basal Diet	L-selenomethionine (mg/kg)			Sodium selenite (mg/kg)			Pooled	p value		
	0.68	1.78	3.53	4.9	1.75	3.49	5.3	SEM	S	L	SxL
Innate immunity											
Lysozyme activity (U/ml)	12.50 d	30.25 a	23.75 b	17.67 c	12.80 d	25.00 b	17.00 c	3.82	0.000	0.000	0.000
Catalase activity (U/ml)	6.67 d	20.00 a	13.13 bc	6.67 d	11.25 c	15.63 b	3.25 d	4.42	0.005	0.000	0.001
Myeloperoxidase (OD at 450)	0.70 d	1.16 a	1.13 ab	1.05 abc	0.83 cd	0.71 d	0.71 d	0.10	0.149	0.421	0.588
MDA (nmol/mg protein)	130.12 a	116.39 a	114.24 a	111.96 a	98.29 a	102.76 a	115.71 a	7.30	0.149	0.421	0.588
Superoxide dismutase (U/ml)	39.19 c	47.81 a	43.65 bc	42.75 bc	42.43 bc	45.66 ab	42.75 bc	2.97	0.244	0.327	0.051
Glutathione peroxidase (mU/ml)	15.13 b	38.91 a	27.23 ab	20.32 b	30.4 a	36.2 a	16.75 b	3.40	0.001	0.001	0.005
Adaptive immunity											
Immunoglobulin M (OD450)	1.38	1.93	1.83	1.77	1.58	1.87	1.77	0.05	.711	.232	.332
Immunoglobulin D (OD450)	0.31	0.43	0.41	0.39	0.35	0.42	0.39	0.18	0.16	0.32	0.22

Note: Values show mean, pooled SEM, n = 90; S= L-SeMet (Excential Selenium 4000) and sodium selenite (Na_2SeO_3), L=level of selenium supplementation. Values in the same row with different subscripts differ a significantly ($p < 0.05$). Malondialdehyde (MDA).

In conclusion, the results of this study demonstrate that the supplementation of L-selenomethionine at a dose of 1 mg Se/kg leads to a beneficial effect on growth performance, immune response and protection against *S. agalactiae* in Nile tilapia.

GUT MICROBIOTA AND RESISTOME OF FRESHWATER FISH: A CULTUROMICS APPROACH OF FOOD SECURITY

Soon Keong Wee*, Suppiah Paramalingam Sivalingam, Jaekan Wong, Mark Boon Pho Chan, Eric Peng Huat Yap

Lee Kong Chian School of Medicine
Nanyang Technological University
Clinical Sciences Building
11 Mandalay Road
Singapore 308232
wees021@e.ntu.edu.sg

Freshwater fish play an important role in global food security. The diversity of gut microbiota in fishes and their microbial communities can have an impact on the health of the fish as well as human. Antimicrobial use in aquaculture as growth enhancers or prophylaxis may disturb the balance of the gut microbiome leading to dysbiosis and cause diseases to the hosts and potentially to humans. It may also influence the antimicrobial resistance (AMR) of the gut microbiome which could threaten food security and food safety. This study aims to characterise the diversity of gut microbiota and their antimicrobial susceptibility in freshwater and marine fish. Freshwater tilapia (n = 3) and marine seabass (n = 3) were collected from a local wet market and their stomach and intestines were processed and cultured on the same day. Bacteria colonies were isolated using 10 different growth conditions and incubated aerobically and anaerobically. Bruker MALDI-TOF MS Biotyper was used to identify the species and subsequently, 14 different antibiotics was used for antimicrobial susceptibility testing by disk diffusion method. We isolated a total of 41 different bacterial species from both stomach and intestine of tilapia and seabass. We found that the composition of gut microbiota differed between freshwater and marine fish, as well as in the different gastrointestinal tract regions (e.g. stomach and intestine). In seabass, 36% of the species were *Pseudomonas* and 24% were *Aeromonas* compared to 10% and 10% in tilapia. Out of 14 antibiotics tested, *Aeromonas jandaei* isolated from tilapia was resistant to 3 carbapenem antibiotics. In addition, co-trimoxazole resistance in *Aeromonas hydrophilia* and tetracycline resistance in *Aeromonas veronii* were present in seabass. Species identification using MALDI-TOF MS has enabled us to rapidly characterise and detect the diversity of the culturable microbiota. In addition to AMR being present in farmed fish where antimicrobials maybe be used as growth factor or prophylaxis, antimicrobial resistance found in the marine seabass suggest that there may be a potential aquatic environmental reservoir of AMR. The fish pathogenic species (e.g. *Plesiomonas shigelloides* and *Aeromonas spp*.) that could also cause human diseases and presence of AMR found in our study may present a potential risk to human health and welfare. The limitations of culturomics can be overcome by current next-generation sequencing technologies which will be able to provide a more detailed microbiome profiling and resistome determination. Our preliminary results demonstrated that antimicrobial resistance in pathogenic species poses a threat to both fish health and human population. The role of gut microbiota has an impact on food safety and food security. There is a need for ongoing bio-surveillance for emerging antimicrobial resistance and to limit the use of antibiotics in aquaculture.

CORRELATION OF ENDOGENOUS AND WATER CORTISOL LEVELS AFTER ACUTE STRESS IN ASIAN SEABASS *Lates calcarifer* IN A RECIRCULATING AQUACULTURE SYSTEM

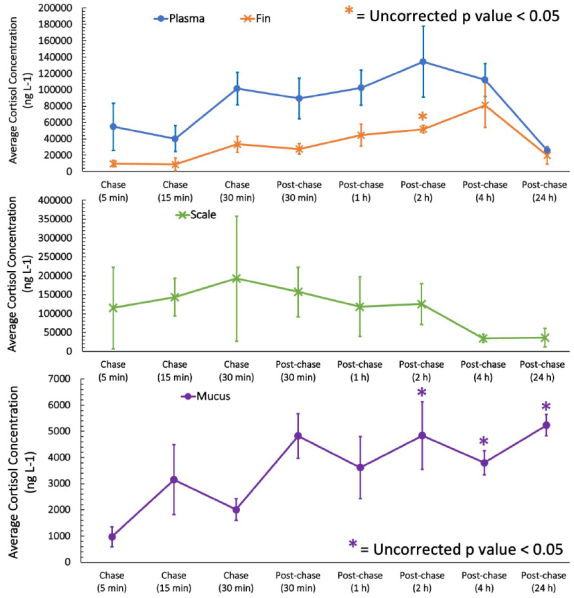
Marie Tan, Khin Moh Moh Aung, Jolin Tan, Kai Lin Chua, Gaynah Javier Doblado, Dinah Mardhiana Yusoff, Kai Xin Chua, Vindhya Chaganty, Nur Asinah binte Mohamed Salleh, Yi Long Ng, Steven Fong, Shubha Vij, Xiao Di Su, Laura Sutarlie, Caroline Lei Wee*

Institute of Molecular and Cell Biology, A*STAR, Singapore
weel@imcb.a-star.edu.sg

Stress in farmed fish is associated with poor feeding, slow growth, disease, and mortality. Hence, fish stress level monitoring is critical to improve aquaculture productivity and welfare. The stress hormone, cortisol, is a reliable indicator of fish stress; however, blood sampling is highly invasive. Alternative sampling methods, such as fin, scale, mucus, as well as cortisol released into the surrounding water, have been suggested as less-invasive means to assess fish stress. As part of this project we seek to understand the relationship between cortisol in these different biological samples, and in the water, in response to a stress challenge in Asian Sea bass (*Lates calcarifer*). Initial results using handling stress demonstrate an expected increase in plasma, fin, and mucosal cortisol peaking within 2-4 hrs. Water cortisol analysis is still ongoing. Further studies will manipulate water quality and stocking density to determine their effects on Asian Sea bass cortisol release and assess the reliability of water cortisol as a stress indicator.

A stress trial was conducted in a 9000 L recirculating system (3000 L tank) with 35 g *Lates calcarifer* at stocking densities of 3.28 kg/m^3. Water (100 ml) and biological samples from 4-5 fish were sampled during and after handling stress (chasing and air exposure) (Fig 1). Samples were tested via ELISA (Cusabio kit). In a pilot experiment, we observed a post-stress elevation of plasma, fin, and mucus cortisol, peaking between 2-4 hrs (Fig. 1). Mucus cortisol stayed significantly elevated at 24 hrs, unlike plasma and fin. Scale cortisol did not show significant changes. Finally, plasma cortisol is most strongly correlated to fin cortisol on a fish-by-fish basis (R = 0.56). Overall, we show that fin and mucus cortisol are feasible as less-invasive cortisol sampling methods. Water cortisol analysis by ELISA and HPLC is ongoing. Further investigation will be performed to optimize cortisol sampling and quantification, and to establish the relationship between water and tissue cortisol with differeny stressors, including disease, water quality, and stocking density.

Figure 1: Cortisol measurements from fish samples after acute stress



RAPID DISCOVERY OF AQUACULTURE NUTRITIONAL INTERVENTIONS FOR INTESTINAL INFLAMMATION AND MICROBIAL DYSBIOSIS VIA A QUANTITATIVE ZEBRAFISH SCREENING PLATFORM

Dinah Mardhiana Yusoff, Caroline Lei Wee*

Institute of Molecular and Cell Biology, A*STAR, Singapore
weel@imcb.a-star.edu.sg

In fish and shrimp aquaculture, diet and microbial dysbiosis as well as stress and pathogenic infection are common triggers of intestinal inflammation, leading to impaired growth and increased mortality. Probiotic or nutritional interventions have been proposed as safe and effective means to promote gut immune health and animal survival. The conserved innate immune system of the larval zebrafish makes it an excellent high-throughput model for identifying novel interventional strategies. Here, we report a rapid, semi-automated pipeline for identifying compounds that are sufficient to rescue intestinal inflammation triggered by two independent mechanisms: chemical-induced colitis and high-fat diet-induced microbial dysbiosis. As a proof-of-concept, we demonstrate that Aloe Vera extract is able to rescue both modes of inflammation, with additional screening of nutritional compounds ongoing. Overall, our platform will support the rapid discovery of novel nutritional supplements which can promote intestinal health and immunity in aquaculture.

We have developed a custom pipeline using the larval zebrafish model to rapidly screen for anti-inflammatory nutritional interventions (Fig. 1). Using a transgenic line fluorescently labeling neutrophils (*Tg(mpx:GFP)*), we subjected zebrafish either to 4-hr chemical inflammation by 2,4,6-Trinitrobenzenesulfonic acid (TNBS), or a 24-hr high-fat chicken egg yolk diet, both of which promote rapid intestinal neutrophil accumulation. We also developed a custom FIJI macro for semi-automated quantification of intestinal neutrophil numbers. Using overnight antibiotic (ABEM) treatment, we show that only the latter (diet-induced inflammation) is dependent on the gut microbiome (Fig. 2A). Consistent with reports in the literature, a 2-hr aloe vera extract feeding is sufficient to rescue both modes of inflammation (Fig. 2B), suggesting effects on convergent inflammatory pathways downstream of microbial dysbiosis. Screening of additional compounds and pre/probiotics is ongoing, along with further characterisation of the role of the microbiome in gut inflammation.

Figure 1: Schematic of Screening Pipeline using Multiple Inflammatory Stimuli

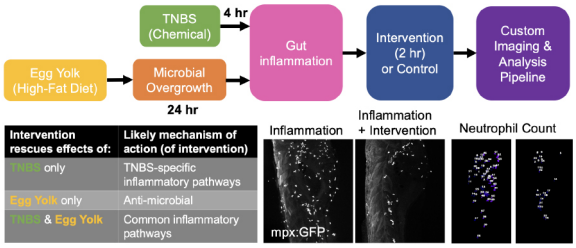
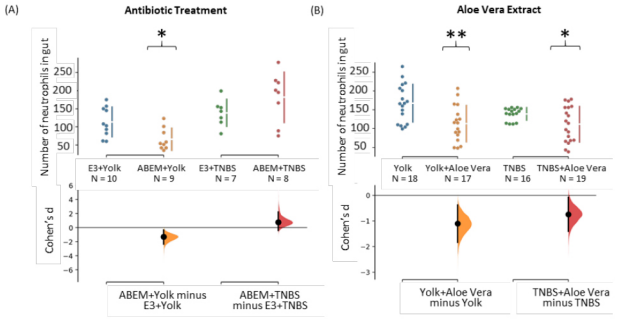


Figure 2: Effects of Aloe Vera Extract on Chemical and Diet-Induced Inflammation and Role of the Microbiome. Estimation plots show bootstrapped Cohen's d values
*= p<0.05, **=p<0.01 (Student's t-test)



HIGH QUALITY EARLY NUTRITION AS A STRATEGY FOR A PRODUCTIVE AQUACULTURE

Delphine Weissman*[1], Ivan Gavioli

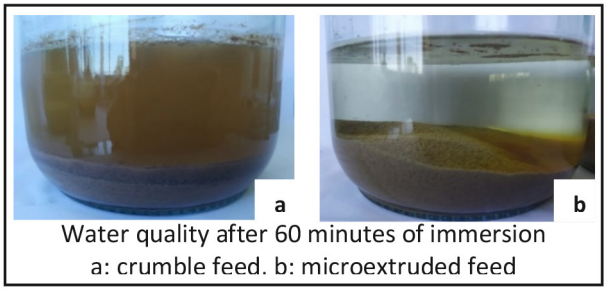
ADM, Development & Application for Aquaculture,
France
delphine.weissman@adm.com

The early stage in life is a crucial step in fish development and impacts fish quality during their whole lifespan. High-quality nutrition during the hatchery and nursery stage is part of the strategy to maximize fish performance. The ambition to improve aquaculture productivity up to harvest already starts in newly hatched fish.

Nutrition at the early stage impacts phenotypes during the entire lifespan. In addition, key developments of organs happen in early life (Fujimura and Okada, 2007). That's why it is necessary to bring the right nutrients in sufficient amounts during this phase. Muscle also starts to be built early in life and nutrition favoring a good muscle structure will promote larger body weight at harvest (Kiesslingetal et al., 2006). Even if the period remains short in comparison to an entire life cycle, the importance of a high-quality nutrition should not be minimized as it is part of the strategy to prepare the future of the fish.

In order to demonstrate this statement, an experiment was conducted on tilapia for 236 days, starting from the first feeding. Only the feed used during the first month of feeding differed. One group was fed with a micro-extruded feed produced by marumerisation technology (Group 1) and the second group was fed a standard crumble feed (Group 2) during that period. The two groups were managed the same way and fed the same feed for the rest of the trial. A huge difference in live weight was observed after the first month since live weight was 3.17g and 0.52g ($P<0.0001$) in groups 1 and 2 respectively. The feed fed during the first month not only impacted performances during this period, but also performances up to harvest. Final live weight reached 797g and 683g ($P<0.0001$) and fillet yield was 37.1% and 34.5% ($P<0.0001$) in groups 1 and 2 respectively. Survival was also strongly impacted.

The benefit of premium micro-extruded feed on fish performances during the first month can be explained by nutrition, digestibility of the feed, feed stability in water and the pellet size adapted to fish mouth size while fish are growing. The benefit observed beyond, 7 months later, might come from a better development of the fish This experiment illustrates why investment in high quality-feeds at larvae and juvenile stages is part of the strategy to improve aquaculture productivity.



Water quality after 60 minutes of immersion
a: crumble feed. b: microextruded feed

THE USE OF THE DEPOSITIONAL MODEL TROPOMOD FOR SUSTAINABLE PLANNING OF TROPICAL CAGE AQUACULTURE

Patrick White[1], Reinhold Fieler[1], Rebecca Weeks[2].

[1]Akvaplan-niva AS, Fram Center, Tromso N-9296, Norway
[2]Scottish Association of Marine Science, Oban PA37 1QA, United Kingdom

Tropomod is one of a suite of particle tracking models (including Depomod and Meramod) which simulates the dispersion of waste feed and faecal particles from aquaculture to the seabed. The model is used to predict sediment quality footprint below fish cages in order to be able to ensure that severe impact to the sediment is prevented and to optimise farm layout and production within the different aquaculture zones.

The simulation begins with particles released from a defined cage volume, the mass of particles released being dependent on husbandry data (feed ration, feed type, etc.) and numerous waste conversion coefficients (e.g. % uneaten feed, feed digestibility, etc.). Particles have settling characteristics according to their size or type (i.e. feed pellet size, trash-fish, etc.), or in the case of faecal particles, according to the main species being farmed in that aquaculture zone. Particles in deeper areas are dispersed further away from the cages. Coarse, fast settling particles (e.g. feed pellets) deposit on the seabed close to the cages, whereas finer feeds which settle more slowly disperse more widely.

Particles settle down through the water column and are advected horizontally according to local current velocity and direction. In addition, particles are dispersed more randomly using a random walk model with horizontal and vertical dispersion coefficients from drogue surveys. The particles settle on the bed and create a deposition 'footprint', the severity and extent of this footprint being useful for farm management and strategy.

Tropomod has been used in Singapore and the Philippines for planning new fish cage culture development as well as optimising existing cage culture development to ensure sustainability of development.

Where farms have closely spaced cages and poor feeding practices waste flux to the bed is high, resulting in a severe deposition footprint and high environmental impact close to the cages. Conversely, farms with good feeding practices (e.g. careful feeding, high-quality feed, etc.) and located in dispersive areas with appropriate spacing between cages, often have larger extent deposition footprints but these are less severe.

This paper will give details of the model and it's use for predicting sediment impact for locating and sizing new farms, use for locating extractive species in conjunction with cage culture (IMTA), use for optimising small scale cage culture by reducing cumulative sediment impact, identifying operational management options for reducing sediment impact, etc. based on some of the previous research project outputs in South East Asia.

OPPORTUNITIES AND CONSTRAINTS FOR LARGE SCALE CAGE CULTURE DEVELOPMENT IN SOUTHEAST ASIA

Patrick White[1], Lukas Manomaitis[2], Hsiang Pin Lan[2].

[1]Akvaplan-niva AS, Fram Center, Tromso N-9296, Norway
[2]U.S. Soybean Export Council, 541 Orchard Road, #11-03 Liat Towers, Republic of Singapore 238881

Fish cage culture has developed rapidly in South East Asia however the majority of production is undertaken in small cages located in sheltered coastal locations, shallow waters and close to supporting coastal infrastructure. This is in contract to cage culture development in other regions which is based on larger companies growing fish in larger cages in deeper more exposed locations.

Countries such as Indonesia and the Philippines have long coastlines with suitable depths relatively close to shore for large conventional floating cages where as other countries such as Thailand, Cambodia, Vietnam and Malaysia have less areas with suitable depths located close to shore but have the potential for using newly developed sinking cage technology.

Large cage technology has been developed for Salmon and Seabass and Seabream and is available for use in South East Asia. Some pioneering companies have started to use this technology but have yet to reach the production levels seen in other sectors. Storms and typhoons are a risk mainly for the strong winds and for the resultant waves and currents generated in the sea. The typhoon belt is located above 10^{o} latitude North and below 10^{o} latitude South and affects north Philippines and Vietnam and North Australia. This typhoon risk may mean that submersible cage technology will need to be used.

There are a number of potential tropical species that are suited for culture in large cages, that have hatchery production of juveniles and have a relatively well developed market demand and reasonable market price. The leading species are those that have been farmed are Japanese amberjack, Japanese seabass, Asian Seabass, Cobia and more recently the production of emerging marine species such as kingfish, snapper and pompano.

South East Asian countries also need to establish regulatory processes that clearly identify where cage culture facilities can be located and for how long. This will give Investors confidence that they have sufficient time to get profitable return on their investment.

The expansion of development of larger cages located in more exposed waters off the coast is more challenging from a technological, logistical and investment requirement viewpoint. This paper makes recommendations on how the private sector together with the regulators can establish large scale cage culture allowing South East Asian countries to continue the expansion of production in the near future.

DEVELOPING AN AMBITION FOR AQUACULTURE BEYOND 2033 – A TASMANIAN CASE STUDY

J. Whittington*, and A. Williamson

Blue Economy CRC-Co Ltd, Maritime Way, Newnham TAS 7248, Australia
*Corresponding author's email: john.whittington@blueeconomycrc.com.au

In just over three decades, salmonid farming in Tasmania has gone from a harvest of 50 tonnes in 1986 to an industry that has a production value of more than $1 billion in 2021-22. Salmonid aquaculture is now Australia's largest fishery and Tasmania's largest primary industry with several thousand people employed. Salmonid marine farms cover approximately 0.14% of Tasmania waters.

Extensive and repeated community sentiment tracking demonstrates a very high (93%) level of awareness of the industry with more than half of the population supportive of the industry (with a net support score of 37).

On the other hand, 20% of Tasmanians oppose the industry citing concerns relating to environmental protection, animal welfare and transparency. Those concerns can often be amplified through the tv, newspapers, radio and social media, which are the dominant channels by which the community receives information on the industry.

Whilst community sentiment has remained constant and positive over recent years, and consumer demand has increased, there is a growing sense that the status quo will not deliver a thriving sustainable aquaculture industry into the future.

In this presentation we will provide an overview of the current finfish aquaculture industry in Tasmania and the work the Blue Economy CRC has been undertaking to understand the values, experiences, concerns and aspirations of businesses, industries, governments, consumers, community, NGOs and researchers.

Using these insights and drawing on global megatrends, best practice farming, innovative legislative reform, contemporary leadership, and emerging technologies, we will present an ambition for aquaculture as part of a broader and integrated ocean economy, in Tasmania for beyond 2033.

The adoption of this ambition will require changes to the current way of doing things. It will require leadership and innovation from government, business, researchers and the community.

The refinement and adoption of this roadmap could see Tasmania with an ambitious sustainable aquaculture industry, balanced with nature, and integrated into the Tasmanian way of life for the decades to come.

VIRULENCE AND ANTIMICROBIAL RESISTANCE OF *Vibrio* spp. FROM PACIFIC ABALONE (*Haliotis discus hannai*) MARKETED IN KOREA

M.V. Kasun Sameera Wickramanayake*, Gang-Joon Heo

Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea
wickramanayake.mvks@gmail.com

Abalone is considered as one of the most expensive and popular seafood around the world due to its daintiness and high nutritional value. However, eating abalone as raw or undercooked may facilitate the entering of pathogenic microorganisms into the human body. This study assessed the virulence factors and antimicrobial resistance properties of *Vibrio* species isolated from Pacific abalone (*Haliotis discus hannai*) marketed in Korea. Thirty-two *Vibrio* strains were identified as below, *V. alginolyticus* (n=15), *V. diabolicus* (n=14), *V. antiquarius* (n=2) and *V. parahaemolyticus* (n=1). The neighbor-joining phylogenetic tree which was constructed by *gyrB* gene sequencing data sorted identified 32 *Vibrio* spp. isolates into 2 major clades. *V. parahaemolyticus* strain belonged to the first major clade with *V. parahaemolyticus* reference strain. *V. antiquarius, V. alginolyticus* and *V. diabolicus* strains showed scattered distribution patterns in the second major clade with their reference strains.

All isolates demonstrated DNase, lipase, phospholipase and amylase activities. Additionally, slime production (97%), gelatinase activity (94%), α-hemolysin activity(22%), β-hemolysin activity (78%) and protease production (53%) were detected. According to the virulence-related genetic profile, *toxR*, *tlh*, *tdh*, *collagenase* (*VAC*), *V. cholerae* pathogenicity island (*VPI*), *V. cholerae* toxin (*ctxAB*) and *hupO* genes were reported as 56%, 59%, 13%, 100%, 41%, 9%, and 9%, respectively. All isolates were resistant to ampicillin and 88% of the isolates were resistant against cephalothin and colistin sulfate. Twenty-one isolates (66%) showed multiple antimicrobial resistance (MAR) indices ≥ 0.2. Those MAR indexes ≥ 0.2. showed that the *Vibrio* spp. isolates are from a high-risk source where antimicrobials were frequently used. Antimicrobial resistance gene assessment could detect the presence of *blaCTX* (85%), *blaTEM* (10%), *blaSHV* (10%), *strAB* (13%) *and aphA-IAB* (22%) genes. Class 1 integron integrase gene *intI1* and gene cassette gene *qacE2* were detected in 19% of the isolates, respectively.

Not only the presence of virulence-related determinants but also the presence of multidrug resistance properties in the abalone-borne *Vibrio* isolates reveal its pathogenic potency. These Vibrios pose a high risk to consumer health and aquaculture. Thus, monitoring activities combined with food safety and aquaculture practices are recommended to prevent further dissemination of virulence and antimicrobial resistance.

EFFICACY OF ISOBUTANOL TO DECREASE TRANSPORT STRESS OF JUVENILE TROPICAL ROCK LOBSTER *Panulirus ornatus*

Jaime R. Willis*, Alice R. Harford, Quinn P. Fitzgibbon, Gregory G. Smith, Andrew G. Jeffs, and Anthony J. R. Hickey

School of Biological Sciences
University of Auckland
Auckland, New Zealand
jwil497@aucklanduni.ac.nz

The ornate tropical lobster (*Panulirus ornatus*; TRL) is in high demand, especially in live seafood markets throughout many parts of Asia where it is prized for its flavour and vivid colouration. Adult TRL are harvested from the wild and shipped live to market, and juvenile TRL captured from the wild or raised from eggs in hatcheries are transported live to provide seed stock for grow out locations. With the growth in TRL aquaculture there is a need to develop more effective methods for the live shipping of juveniles, that eliminate any mortalities. The live transport of juvenile lobsters results in stress that is primarily metabolic; i.e., elevated CO_2 and ammonia. Approaches to minimize this stress through alterations to metabolic rates has the potential to decrease mortality. Isobutanol is a low cost, naturally occurring food additive that is safe for human consumption and has shown efficacy as a transport sedative for adult TRL. This study investigated the effect of isobutanol on juvenile TRL physiology during immersion, air exposure and simulated transport to determine its efficacy for the transport of juvenile TRL.

Anaesthetic induction and subsequent recovery times were determined for 5 concentrations (25, 50, 250, 350 & 500 ppm). We found that induction time was dose dependent with the highest concentration (500 ppm) inducing the deep sedative state the fastest. Four deaths occurred following re-acclimation, 2 each at 350 and 500 ppm. CO_2 production during air exposure was monitored after exposure to isobutanol. Individuals were assigned to one of four treatment groups (control, 25, 50 or 500 ppm). After exposure to treatment for 20 minutes, animals were emersed and CO_2 production was tracked for 2 hours. CO_2 production was significantly lower with 25ppm isobutanol compared to controls at all times points (Figure 1). All individuals survived except for 2 at 500ppm.

During 20 hours of simulated transport pre-treatment with isobutanol at 25 and 50 ppm resulted in lowered haemolymph ammonia and lactate compared with untreated individuals while tissue glycogen content was defended.

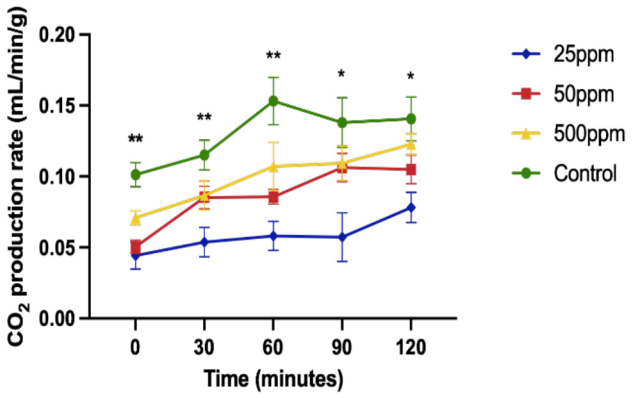


Figure 1. CO_2 production rate over time following isobutanol treatment. 25 ppm had lower CO_2 production at all time points compared to control. 50 and 500 ppm CO_2 production rate was not significantly different from each other or control and 25 ppm at most time points.

NOVEL APPROACHES TO EVALUATE PROTEIN SOURCES FOR THEIR INCLUSION IN FEED FORMULATION FOR JUVENILE SLIPPER LOBSTER *Thenus australiensis*

Andrea Wirtz*, Chris G. Carter, M. Basseer Codabaccus, Quinn P. Fitzgibbon, Debashish Mazumder, Ashley T. Townsend, Gregory G. Smith

Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania, Private Bag 49
Hobart, TAS, 7001, Australia
andrea.wirtz@utas.edu.au

A major challenge to the commercial culture of the slipper lobster, *Thenus australiensis,* is to develop a formulated feed that meets the lobster's nutritional requirements, is highly digestible, and thus can be readily utilised and assimilated. In feed formulation, identifying suitable protein sources is of primary importance. Hence, the present study used novel approaches to evaluate five commercially available protein sources for their potential inclusion in the feed formulation for juvenile slipper lobster *T. australiensis*.

In a 12-week experiment, bioavailability and assimilation proportion of five protein sources (fishmeal, krill meal, lupin meal, soybean meal and squid by-product meal) were determined using a combined approach of apparent digestibility, gastrointestinal evacuation rate, and carbon and nitrogen stable isotope analysis. Apparent crude protein digestibility varied (79.6% to 95.3%), fishmeal protein was significantly less digestible than lupin meal, squid by-product meal and soybean meal. Gastrointestinal evacuation was completed (≥ 95%) between 4 and 6 hours with no significant differences among protein sources. Overall, squid by-product meal had the best growth performance. Protein source assimilation proportion ranged from 7% for soybean meal to 32% for squid by-product meal, whereas squid by-product meal and fishmeal (29%) were the only protein sources assimilated at approximately their dietary inclusion level (30%). The present study is the first in crustaceans to examine the relationship between apparent digestibility and gastrointestinal evacuation, showing that more digestible protein sources had slower evacuation rates. The combined approaches provide a deeper insight into ingredient utilisation and help understand the lobster's ability to digest, utilise and assimilate specific ingredients.

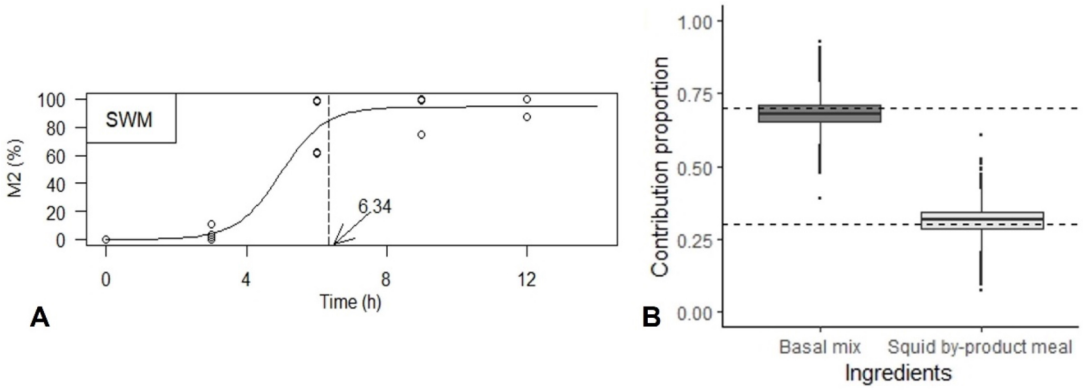


Figure 1. A: Gastrointestinal evacuation expressed as the percentage of the second marker (M2, Yb_2O_3) in the faeces of juvenile *T. australiensis* fed squid by-product meal (SWM). Completion of the gastrointestinal evacuation (—). **B:** Comparison of assimilated proportion for basal mix and squid by-product in whole-body samples of juvenile *T. australiensis*. Dashed lines indicate supplied proportion of basal mix (0.7) and protein source (0.3).

PACIFIC WHITE LEG SHRIMP (*Litopenaeus vannamei*)'s BEHAVIOUR AND OXYGEN CONSUMPTION, AND SEDIMENT OXYGEN DEMAND AT DIFFERENT SEDIMENT REDOX POTENTIAL

Wiyoto Wiyoto[1*], Sukenda Sukenda[2], Enang Harris[2], Kukuh Nirmala[2], Daniel Djokosetiyanto[2]

1. Production Technology and Management of Aquaculture, College of Vocational Studies, IPB University (Bogor Agricultural University) Kampus IPB Cilibende, Jalan Kumbang No 14, Bogor, Indonesia 16151
Email: wiyoto@apps.ipb.ac.id
2. Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University) Jalan Lingkar Kampus IPB Darmaga, Bogor, Indonesia 16680
Ph/Fax : 0251 8628755

Understanding the shrimp behavior, shrimp oxygen consumption, and sediment oxygen demand in a culture tank could contribute to the improvement of *L. vannamei* culture. The objective of the study was to evaluate the influence of sediment redox potential on the white shrimp behaviour. Shrimp with an average body weight of 7.27 ± 1.20 g were distributed in tanks containing substrate at different redox potential (+100, -106, and -210 mV). Subsequently, the vertical distribution of the shrimp were observed directly at 1 h interval. We found that the sediment redox potential did not affect the behaviour of the shrimp and the percentage of shrimp that stayed on the bottom was ranged from 72 to 81%. Shrimp oxygen consumption (OC) rate was 0.33 ± 0.06 g O_2 kg^{-1} h^{-1} . The levels of SOD in the treatment tanks were 7.78 ± 1.67 mg O_2 m^{-2} h^{-1}, 12.22 ± 0.96 mg O_2 m^{-2} h^{-1} and 27.22 ± 0.96 mg O_2 m^{-2} h^{-1} for the RP+100 mV, RP-106 mV and RP-210 mV respectively.

THE EFFECTS OF DIET ON THE GROWTH AND DEVELOPMENT IN MANGROVE CRAB *Scylla serrata* ZOEA AND CRABLETS

Wong Yee Man Cynthia*, Tung Yu Chen and Khoo Hock Lai William

Centre of Aquaculture and Veterinary Science, School of Applied Science,
Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
cynwym@tp.edu.sg

The aquaculture of mangrove crabs *Scylla* spp. has increased over the years, primarily due to the need to meet the high consumer demand and to reduce wild harvest. The price of the crab corresponds to the crab quality; hence, the health and nutrition of the early stages of crabs in culture are intensively studied for profit maximization. In this study, Zoea (Z1) were obtained during a spawning episode and were fed with SELCO®-enriched and non-enriched rotifers. The stocking density of the zoea were recorded every two days whereas the growth and development of zoea individuals were monitored daily. In midst of the study, an incidental occurrence of *Vorticella* parasitism occurred in both treatments. Although an increase in zoea mortality was observed over the days, the stocking density of parasitized zoea fed with both treatments were significantly different – lower mortality was observed in parasitized zoea fed with SELCO®-enriched rotifers. In addition, Z1 fed with SELCO®-enriched rotifers managed to metamorphose into Z2 more readily; the stocking density, however, crashed two days later after Z1 that was fed with non-enriched rotifers. In another study, crablets (C1) were fed with formulated feed and seafood. The carapace length and weight of crablets were significantly different – crablets fed with formulated feed were larger and heavier, suggesting that formulated feed provided more nutrients than seafood. Results observed from these two studies inferred the benefits of bioenrichment and the importance of nutrient availability for the optimal growth and development in the early stages of crabs.

AQUA NUTRITION FACILITY AT THE MARINE AQUACULTURE CENTRE (MAC)

Yao Xing WONG*, Yi Hang Ong, Qunying Xu, Janelle Ching, Man Yin Teo, Jia Ying Lee, Woei Chang Liew

Marine Aquaculture Centre
Singapore Food Agency
11 St John Island
Singapore 098659
WONG_Yao_Xing@sfa.gov.sg

The Singapore Food Agency's Marine Aquaculture Centre (MAC) has been spearheading the development of aquaculture technologies in Singapore to increase local fish production in a productive, sustainable, and resilient manner. The MAC team recognised that to support the sustainable increase in aquaculture production, fish feeds would need to be optimised for locally farmed species to promote healthy growth, be formulated with ingredients from sustainable sources and to improve feed conversion ratios. There were, however, no proper aquaculture nutrition facility in Singapore. Hence, the aquaculture nutrition facility, consisting of a pilot feed mill and experimental tank systems, was established at MAC.

A proper feed mill is complex as it consists of multiple processes and requires many specialised equipment for raw material handling, quality control, extrusion process, drying process and coating application. The pilot-scale feed mill at MAC was planned to replicate the feed extrusion process of a commercial feed mill and with the flexibility to produce small batches of different feed formulation in quick succession. Currently, the pilot aquaculture feed mill is equipped with state-of-the-art equipment for milling, sifting, mixing, extrusion and drying. An inventory of common raw ingredients such as fish meal, macro-nutrients and micro-nutrients are also available. The central piece of equipment is the Clextral EV25 twin-screw extruder which is designed for feed product testing and reliable scale-up production. It can produce floating and sinking feed via various processing parameters. A vacuum coater is available for inclusion of heat sensitive feed additives or attractants.

A great part of aqua nutrition research includes feeding trials to test for digestibility, FCR or other effects. Therefore, the aquaculture nutrition facility also consists of experimental tank systems ranging from 200L tanks to 1000L tanks. Each system encompasses 20 to 36 tanks. All systems are equipped with the recirculating aquaculture system (RAS) and faecal collection units for digestibility studies, ensuring accurate feeding trial results. The range of tank size and number tanks will allow aqua nutrition experiments of various scope.

TRACKING THE SOURCE OF *Perna canaliculus* MUSSEL SPAT FOR AQUACULTURE USING SHELL MICROCHEMISTRY

Wenjie Wu*, Carolyn J. Lundquist, and Andrew G. Jeffs

Institute of Marine Science
The University of Auckland
Auckland 1010, New Zealand
wwu313@aucklanduni.ac.nz

The unpredictability of wild seed supply is a severe obstacle for sustainable production in mussel aquaculture globally. An improved understanding of the spawning of wild broodstock and subsequent patterns of larval dispersal and settlement could help to greatly progress the sustainable management of wild spat resources and increase mussel spat supply. In this study, the shell microchemistry of green-lipped mussels, *Perna canaliculus*, that had recently settled on an offshore mussel farm in the Bay of Plenty, New Zealand, was used to identify their patterns of larval dispersal and likely natal locations.

The shell microchemistry profile of mussel spat settling on the offshore mussel farm were compared with those from mussels cultured *in situ* at 22 sites (~6 km apart) arranged around Bay of Plenty, New Zealand, over two consecutive months. Six elemental signatures (Li, Co, Ni, Ba, La and U) were the primary drivers for correctly reassigning shells back to their individual natal sites with an average of 58% accuracy,

ranging from 20% (near Hikuwai Beach) to 78% (on West Spat Farm). Grouping of *in situ* sites increased the mean accuracy to 75% but reduced spatial resolution to ~40 km. The microchemistry tracked ~160 spat arriving on the mussel farm back to two main natal sites near the Ōhiwa Harbour, which accounted for 85% of mussels arriving on the mussel farm.

Overall, this study evaluated the reliability of shell microchemistry for tracking mussel seed back to their source. The information from this study will assist with managing local mussel seed resources in the Bay of Plenty.

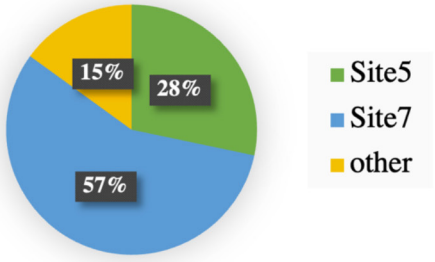


Figure 1. Proportion of natal sites for mussel spat arriving on farm.

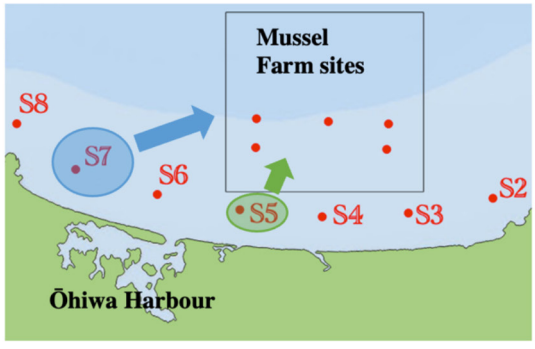


Figure 2. Natal source of mussel spat arriving at mussel farm (S2-S8 = Site2-Site8).

THE UTILITY OF LAKE STURGEON *Acispenser fulvescens* AS BIOMONITORS OF PERSISTENT CHEMICAL CONTAMINANTS IN A GREAT LAKES' US EPA AREA OF CONCERN

Jeff Wyatt DVM, MPH, DACLAM* and Dawn Dittman PhD

University of Rochester School of Medicine and Dentistry
Rochester New York USA
jeff_wyatt@urmc.rochester.edu

The lake sturgeon (*Acipenser fulvescens*), a successfully repatriated native fish of the Great Lakes, serves as both a bioindicator and biomonitor of environmental health. Serum measurements of PCBs, dioxins/furans, mirex, mercury, cadmium, nickel and silver compared between two age-matched populations of sturgeon from Rochester, New York's Embayment Area of Concern's (AOC) lower Genesee River and non-AOC Oswegatchie River demonstrate a lake-wide and not Rochester-specific source for persistent chemical contaminants responsible for fish consumption advisories.

Blood samples collected from sixty six, 9- to 10-year old lake sturgeon netted by United States Geological Survey (USGS) in summers of 2012 and 2013 were assayed by Axys Analytics (Sidney, British Columbia, CA) for PCBs, dioxins/furans, organochlorine pesticides, mercury, cadmium, nickel and silver. Morphometrics including weight, girth and length were taken on each fish. Year class was determined by a combination of techniques including fin ray assessment of growth rings, scute markings, Floy™ tags or Passive Integrated Transponder (PIT) tags. The fish were sampled from two age-matched hatchery-reared populations residing in the Genesee and Oswegatchie Rivers since their introduction as hatchery reared fingerlings in 2003 and 2004. Serum samples were lipid normalized for lipophilic contaminants.

Sturgeon serum levels of PCBs, dioxins/furans, mercury and cadmium were no different between the two study sites. The mean serum levels of mirex, nickel and silver were higher in the Genesee compared with Oswegatchie River fish.

Five thousand, hatchery reared, lake sturgeon, reintroduced to the lower Genesee River since 2003, have grown at similar rates as conspecifics in other Great Lakes' unpolluted waters. Serum assessments from these healthy, repatriated fish identified chemical contaminants detected in other studies in sera of fish-consuming anglers and families in the Great Lakes. Human sera samples were correlated with contaminated fish and lake-specific water or sediment burden in the Great Lakes (Anderson 1998, Fitzgerald 2005, Hanrahan 1999). The lake sturgeon serum concentrations comparison between the Rochester Embayment AOC and the Oswegatchie River non-AOC control site indicate no AOC-specific Beneficial Use Impairments (BUIs) for PCB congeners, dioxins/furans, mirex, mercury or cadmium. The elevated silver and nickel serum concentrations in the Genesee sturgeon are not of animal or human health concern or specific to fish consumption restrictions listed in AOC advisories.

Chemical contaminant concentrations in animal sentinels reflect environmental burden and risk of exposure to humans sharing the same ecosystem. The lake sturgeon serum assessments for chemical contaminants evaluated in this study demonstrate utility of sturgeon as time-trend biomonitors and bioindicators of ecosystem contamination.

GENOMIC SELECTION BREEDING OF LARGE YELLOW CROAKER RESISTING *Cryptocaryon irritans*

Ji Zhao, Huanqiang Bai, Qiaozhen Ke, Zhixiong Zhou, Fei Pu, Tao Zhou, Peng Xu*

College of Ocean and Earth Sciences, Xiamen University
Xiamen, Fujian, 361102, China
xupeng77@xmu.edu.cn

The large yellow croaker is one of the most important marine culture fish in China which ranks the No. 1 in marine fish species on annual production in China. *Cryptocaryon irritans* is the most harmful parasite of large yellow croaker which causes massive death and ecomonic loss in large yellow croaker aquaculture. Disease resistant breeding against *C. irritans* infection has been considered as a sustainable method to prevent disease loss.

This study aims to apply genome selection technique in *C. irritans* resistant breeding in large yellow croaker. We developed a large reference population and collected using standardized *C. irritans* challenging test. The disease-resistant phenotype was recorded as dichotomous trait, survival or death. A total of 46,125 high-quality SNPs were genotyped using ddRAD pipeline in both reference population and selective parental population. GWAS analysis was performed and the top 600 significant SNP markers was collected representing maximize the prediction accuracy of the genomic selection model. Using the best model established, the top 10% large yellow croakers in breeding population with high genomic estimated breeding value (GEBV) were selected for reproduction. The F1 offspring was then tested using the same *C. irritans* challenge test. The result showed that GS strain had survival rate of 62% after 96-hour infection of *C. irritans*, while the control strain had only 26% of survival rate. The result demonstrated the dramatic survival rate difference, indicating our genome selection approach was effective way to generate a resistant strain against *C.irritans* infection in large yellow croaker.

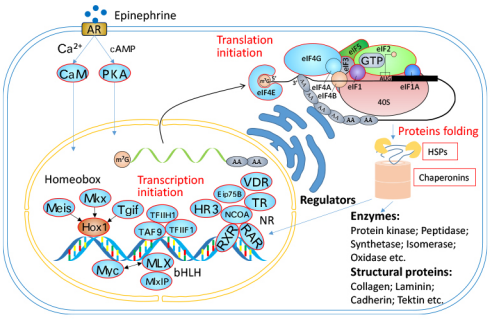
EFFECTS OF EPINEPHRINE ON GENE EXPRESSIONS DURING THE METAMORPHOSIS OF PACIFIC OYSTER *Crassostrea gigas*

Fei Xu*

Key Laboratory of Experimental Marine Biology, Center for Mega-Science
Institute of Oceanology, Chinese Academy of Sciences
Qingdao 266071, China
xufei@qdio.ac.cn

Many major marine invertebrate phyla are characterized with indirect development. These animals transit from planktonic larvae to benthic adults via settlement and metamorphosis, which has many advantages for organisms to adapt marine environment. Studying the biological process of metamorphosis is thus a key to understand the origin and evolution of indirect development. Although the mechanism of metamorphosis has been largely studied on their relationships with marine environment, microorganisms, as well as the neurohormones, little is known on the gene regulation network (GRN) during metamorphosis. We treated competent oyster pediveligers with epinephrine, which was known to be able to effectively induce oyster metamorphosis, and analyzed the dynamics of gene and proteins with transcriptomics and proteomics methods. The result indicated significant upregulation of protein synthesis system, as well as some transcription factors including Homeobox, basic helix-loop-helix and nuclear receptors. The result suggested the GRN complexity of the transition stage during oyster metamorphosis.

Figure Summary of gene responses. Shapes with red edge denote genes which was included in URGs. Abbreviations: AR, adrenergic receptor; CaM, calmodulin; PKA, cAMP-dependent protein kinase; Meis, homeobox protein Meis; Mkx, homeobox protein Mohawk; Tgif, TGFB induced factor homeobox; Hox1, homeobox 1; TFIIH1, general transcription factor IIH, polypeptide 1, 62kDa-like; TFIIF1, General transcription factor IIF subunit 1; TAF9, TATA-Box binding protein associated factor 9; bHLH, basic helix-loop-helix transcription factors; Myc, MYC proto-oncogene; MLX, MAX dimerization protein MLX; MlxIP, MLX interacting protein; NR, nuclear receptor; VDR, vitamin D receptor; THR, thyroid hormone receptor; NCOA, nuclear receptor coactivator; RXR, retinoid X receptor; RAR, retinoic acid receptor; Eip75B, Ecdysone-induced protein 75B; HR3, nuclear hormone receptor HR3; eIF, Eukaryotic translation initiation factor; HSP, heat shock protein.

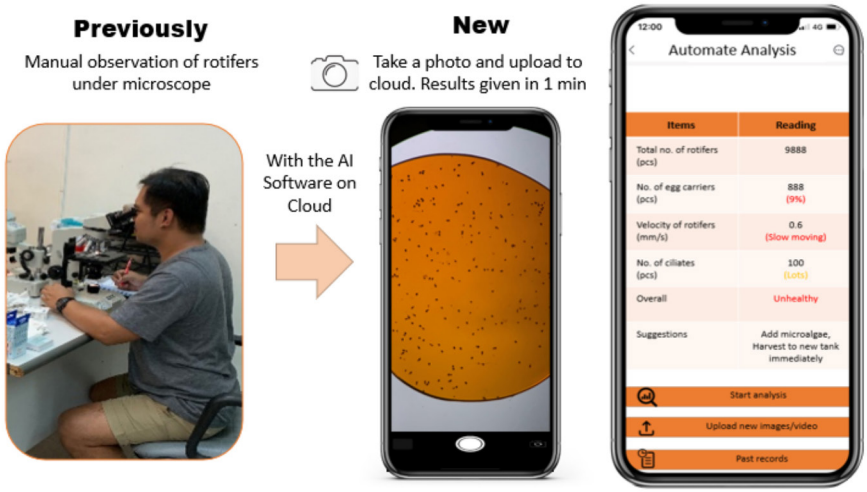


USE OF ARTIFICIAL INTELLIGENCE (AI) TO AUTOMATE ROTIFER COUNTING AND OBSERVE ROTIFER POPULATION

Xu Qunying, Tan Kai Wei, Ng Yong Kiat, Koh Chee Boon Amos, Jose Lowell, Punithavathi K Haridas, Ng Ang Seng

Marine Aquaculture Centre
Singapore Food Agency
11 St. John's Island
Singapore 098659
XU_Qunying@sfa.gov.sg

Rotifers, a type of zooplankton, are critical first feed for young marine fish larvae. A successful intensive marine fish fry production in a hatchery therefore depends heavily on a stable in-house rotifer cultivation. A lack of rotifer supply due to culture instability can result in mass mortalities of fish fry due to lack of food, and sharp reduction in hatchery production. However, ensuring rotifer culture stability is both time consuming and labour-intensive. At SFA's Marine Aquaculture Centre, staff spend at least 40 minutes daily using a microscope to count both live and dead rotifers, note rotifer movement speed, and presence of contaminants such as ciliates. Staff then analyse these observations and make decisions to implement operational actions to maintain and improve stability of rotifer cultures. The rotifer counting and assessment of culture quality require well-trained staffs, with the experience to make the right calls when the rotifer culture is unhealthy. This topic describes a prototype using Artificial Intelligence (AI) to automatically count rotifer and assess rotifer population for greater operational efficiency. With this method, end users simply use their smartphones to capture images of rotifers and upload it to a cloud whereby the online software will analyse and provide parameters and suggest operational actions to maintain the stability of rotifer cultures. Potential adoption by farms and Institutes of Higher Learning (IHLs) both locally and word widely could also result in increased industry productivity.



USING ENCAPSULATION TECHNOLOGY FOR FISH SPAWNING BY ORAL HORMONE DELIVERY IN LIEU OF MANUAL INJECTION

Qunying XU *, Woei Chang Liew, Kirby Chen, Yao Xing Wong, Janelle Ching, Yi Hang Ong, Jia Ying Lee, Yukun Yu, Manish Mahotra, Say Chye Joachim Loo

Marine Aquaculture Centre
Singapore Food Agency
11 St John Island
Singapore 098659

Nanyang Technological University
50 Nanyang Avenue
Singapore 639798

xu_qunying@sfa.gov.sg

Induced spawning of various farmed fishes has been consistently accomplished through the injection of hormone, e.g. Asian seabass, grouper, pompano etc. However, this procedure requires the catching and anaesthetizing of fish prior to injection, a process which adds handling stress and increase the risk of death to the precious brooder fishes.

Oral hormone delivery system has offered a convenient, familiar, and painless alternative to manual injections. At MAC, past studies on oral administration of LHrHa3, a type of GnRHa, had been performed on Asian seabass with some successful spawning events, however the results were inconsistent due to the rapid degradation of hormone in gastrointestinal system.

Encapsulation technology is a new method to reduce hormone degradation in fish gastrointestinal system and deliver hormone while reducing handling stress of broodstock fish.

MOLECULAR AND TRANSCRIPTIONAL ANALYSIS OF GLUTATHIONE REDUCTASE (HaGSR), AND ITS INNATE IMMUNE RESPONSES IN BIG BELLY SEAHORSE *Hippocampus abdominalis*

Anushka Vidurangi Samaraweera, Hyerim Yang*, W.M Gayashani sandamalika and Jehee Lee

Department of Marine Life Sciences & Fish Vaccine Research Center
Jeju National University
Jeju Self-Governing Province 63243, Republic of Korea.
Email: anushkavsamaraweera@gmail.com

Glutathione reductase is an antioxidant enzyme which is able to convert oxidized glutathione (GSSG) into reduced glutathione (GSH) to maintain the GSH/GSSG level of the cells during the oxidative stress. Reactive oxygen and nitrogen species such as super oxide radicals, hydroxyl radicals, hydrogen peroxide and nitrogen oxide molecules cause for the oxidative stress in most aerobic organisms. Glutathione (GSH) is the major intracellular thiol compound can find in organs such as endoplasmic reticulum, nucleus and mitochondria. In this study we characterized structural and functional features of the mitochondrial glutathione reductase gene (*HaGSR*) of seahorse.

The identified cDNA sequence of *HaGSR* was 1467 bp long and consist of 1398 bp long open reading frame (ORF). The HaGSR protein composed with 465 amino acids with molecular weight of 50.14 KDa and calculated theoretical pI of 7.63. Also, the HaGSR protein contains typical GSR structural features, including NADPH binding site (184-189 aa), FAD binding domain (14-32 aa) and glutathione binding site (45-55 aa). Glutathione reductase mitochondrial isoform 3 gene of *Fundulus heteroliticus* showed the highest 82.9 % identity and 91.1 % similarity with HaGSR. According to the phylogenetic analysis HaGSR showed close relationship with teleost fish.

Ovaries showed the highest expression for tissue specific distribution among fourteen tissues of seahorse. The mRNA expression profiles of *HaGSR* in blood and liver displayed significant inductions after the immune challenge mounted by Lipopolysaccharides (LPS), polyinosinic:polycytidylic acid (Poly I:C), *Edwardsiella tarda* and *Streptococcus iniae*. According to the results all the stimuli showed symbolic response at 48 h and 72 h post infection in both tissues. Altogether, these results suggest that the HaGSR might play an important protective role in seahorse against invading pathogens.

FISH COUNTING AND HEALTH MONITORING SYSTEM

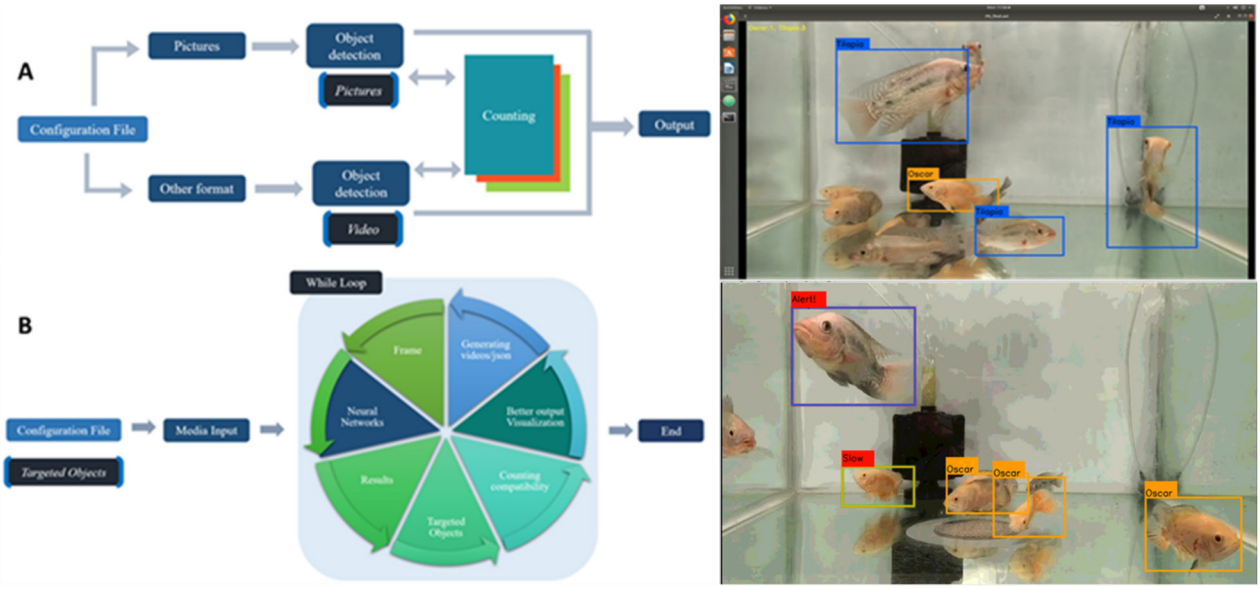
Yap Chern Nam*, William Khoo, Toh BingYan Kenneth, Osman Sophian Izdihar, Padmanabhan Saravanan, Syed Khader Syed Musthaq, Masato Miyata, Chan Pek Sian Diana, Lee Chee Wee

School of Informatics and IT, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757
cnyap@tp.edu.sg

There is a need for efficient fish detection and a tracking device to count, track and determine abnormal fish behaviour. This would potentially help fish farms for a non-invasive, less stressful, continuous monitoring of fish during quarantine and growth, for diagnostic behaviour and early intervention of disease with appropriate treatment

The team has developed a video analytics software that uses YOLOv2 (you only look once) model and the Darkflow neural network Image or live fed video is used as a media input to track the fish. The count results were stored in JavaScript Object Notation (JSON) file format and the live detection were stored in MP4 file format. The format is chosen for ease for future data manipulation. The neural network algorithm developed has successfully differentiated multiple species in tanks and able to track fish movements to identify sick fish. Improvements to be made includes expanding the training data set to increase accuracy.

The following picture depicts the overall process (A) and the coding that interacts with the neural network (B).



BATH VACCINATION OF ASIAN SEABASS FINGERLING *Latest calcarifer* WITH LIVE ATTENUATED *Vibrio harveyi* AGAINST VIBRIOSIS BY SKIN ABRASION IMMERSION CHALLENGE.

Ina-Salwany Md Yasin*, Chin Yong Kit, Nurhidayu Al-saari, Zarirah Zulperi, Mohammad Noor Amal Azmai, Annas Salleh, Santha Silvaraj, Mohamad Aslah, Lee Jing Yie, Mohd Zamri Saad

Laboratory of Marine Biotechnology (MARSLAB),
Institute of Bioscience, Universiti Putra Malaysia (UPM),
43400 Serdang, Malaysia.
salwany@upm.edu.my

Vibrio harveyi is a causative agent for vibriosis in natural wounded young marine aquaculture fish species which lead to major economic losses for aquaculture farms. Vaccination is recommended to prevent this disease. This study describes the efficacy of a live attenuated *V. harveyi* strain MVh_vhs (LAVh) as a vaccine candidate in preventing the vibriosis by wild-type *V. harveyi* (WTVh) in *Lates calcarifer*.

A total of 240 fingerlings were separated into four groups. Group 1 was environmental control that not vaccinated and was not challenged, Group 2 was vaccinated with a formalin-killed *V. harveyi* (FKVh), Group 3 was vaccinated with the LAVh before challenge and Group 4 was negative control that not vaccinated and was challenged. Bath vaccination was applied for one hour before the gills, livers, kidneys and skins of Group 1, 2 and 3 were sampled for gene expression analysis and histopathology. Skin abrasion was applied on the fish before the fish were challenged by immersion with WTVh for all groups.

The results revealed distribution of LAVh in liver and kidney resulting in mild lesions compared with environment control. Consequently, higher expressions of the Chemokine ligand 4 (CCL4) and major histocompatibility complex I (MHC I) genes was shown in the liver and skin of the fish LAVH in comparison with other groups (Figure 1). Vaccination with LAVh resulted in a significantly high rate of survival (68%) after the fingerlings after being challenged with WTVh.

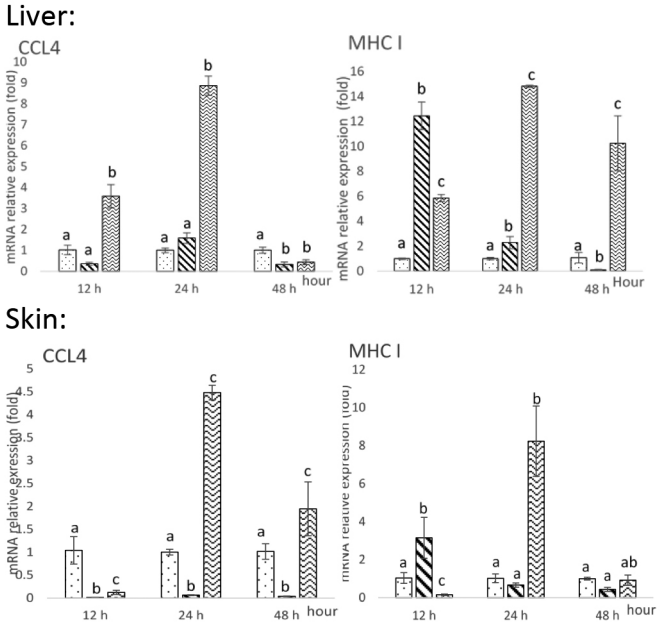


Figure 1: The mRNA expression patterns of immune related gene CCL4 and MHC I in the vaccinated fish. indicated control (Group 1); indicated FKVh (Group 2); indicated LAVh (Group 3).

GROWTH PERFORMANCE OF JUVENILE MILKFISH *Chanos chanos* FED WITH FARM-MADE PELLETED FEED

Fanny A. Yasumaru*, Diana P. S. Chan

Aquaculture Innovation Centre
Temasek Polytechnic
21 Tampines Ave 1
Singapore, 529757
fanny@tp.edu.sg

In 2017, Singapore aquaculture produced 5,891 tonnes and nearly half (2,094 tonnes) was of milkfish *Chanos chanos*, according to the Food and Agriculture Organization of the United Nations (FAO). Traditionally, milkfish are cultured in floating coastal farms in brackish water. Currently, they are fed with low cost expired confectionary, breadcrumbs or farm-made feeds. Such farm-made feeds may consist of a variety of by-products or other cheap ingredients that are locally available, such as "okara" (solid by-product of soy milk or tofu production), soybean cake (by-product of soy sauce production), chicken innards, rice bran, wheat pollard, dry noodle etc. Such practices may cause a few drawbacks, e.g., negative impact in culture water quality and slow fish growth (more than 12 months for a 500 g fish). To help farmers overcome such issue, this study aims at assessing the growth performance of juvenile milkfish fed commercial feed and pelleted feed using cheap and locally available ingredients.

Juvenile milkfish will be allocated to 300-L tanks in a recirculated water system and fed to satiation twice a day for 12 weeks. The commercial feed and the experimental diet will be assessed in triplicate. The growth performance variable to be measured are weight gain, specific growth rate, feed conversion rate, and survival. In addition to growth, feed quality parameters to be measured are water stability and carcass nutrient deposition.

FISH FEED PELLET WATER STABILITY ASSESSMENT

Fanny A. Yasumaru*, Diana P. S. Chan, Chee Wee Lee

Aquaculture Innovation Centre
Temasek Polytechnic
21 Tampines Ave 1
Singapore, 529757
fanny@tp.edu.sg

When measuring feed quality, some of the parameters to be assessed are pellet water stability, feed palatability and digestibility. Pellet stability measures the ability of a pellet to retain its physical integrity in the water until consumed by the animal. It is very important for slow-eating species, such as shrimp, who nibble on the feed, and, most importantly, to avoid water pollution. In this way, it is desirable that a more water stable, pelleted compound feed is used to feed the farmed species to avoid economical and nutritional wastage and be environmentally friendly. Water stability can be measured in different types of feeds, for example, farm-made mash feed, farm-made pelleted feed, extruded feed, commercial feed etc., by calculating dry matter loss. For such ,feed samples are immersed in water for desired time, salinity and temperature, according to animal culture conditions. Horizontal back and forth motion can be used to provide water movement and pellet agitation. After immersion, all solids are recovered by filtration, dried in oven (103 °C, 24h), cooled and weighed. Pellet stability is calculated as:

$$\text{Pellet stability} = \left(\frac{gram\ feed\ remaining}{gram\ initial\ feed}\right) \times 100$$

In this study, the objective was to compare the water stability of farm-made feed mash and extruded pellets, which are currently used in coastal milkfish *Chanos chanos* farms in Singapore. Duplicate 2-g samples of farm-made feed mash made into a ball and extruded feed were immersed in seawater (28 ppt) at 28 °C for 15 minutes. Samples were agitated with circular movements every 5 minutes. Solids were filtered and dried for dry matter loss calculation. The farm-made mash feed ball started collapsing in the first 5 minutes and totally collapsed by the end of the study. Extruded feed presented swallowing after 15 minutes in water but shape remained intact. Farm-made feed mash presented 21% dry matter loss, whereas extruded feed lost 9% of dry matter. Further to feed nutrient digestibility, pellet water stability is also very important when it comes to effect of uneaten feed in water quality. Therefore, further studies should assess the effect of feed nutrient leaching in water and the impact in the culture environment.

FEED DEVELOPMENT FOR JUVENILE MUD CRAB *Scylla serrata*

Fanny A. Yasumaru*, Diana P. S. Chan, Chee Wee Lee

Aquaculture Innovation Centre
Temasek Polytechnic
21 Tampines Ave 1
Singapore, 529757
fanny@tp.edu.sg

According to the United Nations Food and Agriculture Organization (FAO) statistics, mud crab *Scylla serrata* aquaculture production in 2017 in South East Asia was of near 100,000 tonnes, equivalent to US$ 866 million. Major producing countries in the region are Vietnam, Philippines, and Indonesia. Mud crab aquaculture is at an early development stage and the use of formulated feeds is still in its infancy. Hence, crabs relying only on artificial feeds have been facing some issues, such as slow growth, faded color, deformities and molting difficulties, possibly related to nutritional unbalance and deficiency. The development of mud crab feeds aims at promoting crab growth, as well as healthy molting, enhanced coloration and reduce deformities by making use of carotenoid pigments, phospholipids, and essential omega 3 fatty acids.

Fourteen juvenile mud crabs were individually housed in floating plastic containers in a water recirculation system and fed one of the four experimental diets: (1) commercial grouper extruded feed; (2) commercial grouper feed with beta-carotene and vitamins; (3) commercial shrimp feed; (4) gelatinized feed containing phospholipids, omega 3 fatty acids and astaxanthin. Crabs will be fed for 8 weeks and weight gain and carapace width measured. It is expected that such additives will enhance mud crab coloration, increase survival and growth.

DEVELOPMENT OF PELLETED FEEDS FOR MUD CRAB *Scylla serrata* REARED IN RECIRCULATED AQUACULTURE SYSTEM

Fanny Yasumaru*, Kenny Wong, Chen Jinhui, Ng Bo Kai, Deborah Chua, Diana Chan

Aquaculture Innovation Centre
Temasek Polytechnic
21 Tampines Avenue 1
529757, Singapore
fanny_yasumaru@tp.edu.sg

Formulated feed was developed to feed farmed mud crabs *Scylla serrata* for growth and maturation. Currently, there are no commercial feeds available in the market for mud crab farming in pelleted form. Farmers rely on other fisheries and animal by-products (e.g. low value fish, fish trimmings, mussels, shrimp, chicken offal etc.) to feed them. The formulated feeds in form of dry or semi-moist pellets can benefit the sector by providing better nutrition, consistent growth, biosecurity (reduce risks of contamination or disease outbreaks), and ease of handling. Additionally, formulated feed can also provide consistent nutrition, supporting better growth, better health, and the natural pigmentation of the crab shell.

The formulated crab feeds included nutrient rich marine ingredients, such as fishmeal, squid liver meal, krill meal, polychaete worm meal, fish oil, and lecithin, vitamin and mineral premixes, natural pigments (astaxanthin) and gelatine as binder, to supply the necessary nutrients for optimum crab growth and health. The mix were made into pellet and "worm"- or "spaghetti"-shape so that the crabs could easily grab and nibble on it. A 12-week feeding trial was conducted with juvenile mud crabs reared in a recirculated aquaculture system and individually housed in floating plastic containers to compare the growth performance of groups fed formulated feed and frozen seafood. Juvenile crabs fed with the formulated diet presented a specific growth rate (body weight gain) of 6.7% per day, compared to 3.8% of crabs fed seafood. Survival rate was 21% higher in the group of crabs fed with the formulated feed than in the seafood group. Successful moulting rate was similarly higher in the group fed with the formulated feed (86%) than in the group fed with seafood (71%). Difference in crab shell pigmentation due to feed was also observed, where crab fed with formulated feed with astaxanthin (a pigment and antioxidant) presented a brown colour carapace, whereas crabs fed with seafood presented a blue-shade carapace.

FORMULATION OF FEEDS FOR GROWTH PERFORMANCE AND COLOUR PIGMENTATION IN FLOWERHORN CICHLID AND RANCHU GOLDFISH

Fanny Yasumaru*, Bo Kai Ng, Timothy Tan, Joel Ho, Diana Chan

Aquaculture Innovation Centre
Temasek Polytechnic
21 Tampines Avenue 1
529757, Singapore
fanny_yasumaru@tp.edu.sg

An 8-week feeding trial was conducted to compared growth performance and head circumference of Flowerhorn and growth performance and pigmentation of Ranchu fed with three (A, H, S) commercial feeds, respectively. Weight and size measurement of the head of each male Flowerhorn and the belly or roundness of each male Ranchu, in addition to colour scoring were taken at the beginning and after four and eight weeks of study. Flowerhorn were individually housed in 120-L tanks and three Ranchu goldfish were housed per tank. Each commercial feed was tested in triplicate tanks. All fish were fed to satiation twice a day and uneaten feed removed, dried, and weighed to calculate the feed intake. Based on the weight and size measurement results, commercial feed S for Flowerhorn presented the best growth among the three commercial feeds. Feed S also resulted in highest growth in length, specific growth rate (SGR), weight gain, lowest feed conversion rate (FCR). Despite having lower head circumference growth than feed A, feed S has shown to have lower standard deviation in the head circumference growth. In terms of the width growth (from crown to base of fish body), feed S has shown to have the lowest growth, but again with much lower standard deviation as compared to the other two feeds. For the Ranchu, feed H presented the best growth performance among the three commercial feeds tested. Feed H has also shown to achieve the highest belly growth, length growth, body circumference growth, roundness growth, specific growth rate (SGR), weight gain and lowest feed conversion rate (FCR). Nevertheless, in terms of colour development in Ranchu, feed S has shown to achieve the highest increase in relative luminance. Based on the results of this feeding trial, feeds have been formulated using alternative feed ingredients and produced in our facilities to be tested in Flowerhorn and Ranchu aiming at growth performance and pigmentation. Feeding trials are currently under way.

HSP70 KNOCKDOWN IN THE BRINE SHRIMP *Artemia franciscana* AND THEIR TOLERANCE TO BIOTIC AND ABIOTIC CHALLENGE

Mimi Iryani Mat Taib, Saranya Sinnasamy, Kou Aishi, Tengku Sifzizul Tengku Muhammad, Yeong Yik Sung*

Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia

Heat shock protein (Hsp) accumulation enhances biotic and abiotic stress tolerance in the brine shrimp *Artemia*. Although there is evidence that Hsp70 plays a role in improved tolerance, the processes involved have yet to be elucidated, especially since the particular Hsp70 isoform conferring shrimp resistance is unknown. RNA interference gene knockdown is a promising method for studying the role of Hsp70 in stress tolerance in this crustacean species. Specifically, dsRNA capable of knocking down HSP70 was produced, purified, and injected into *Artemia*'s female brood. The ability of dsRNA to knockdown Hsp70 was confirmed by Western immunoblotting on larval protein extracts. Upon obtaining Hsp70 knockdown, animals were exposed to abiotic and biotic stressors, the latter being a pathogenic *Vibrio* challenge. The acquisition of stress tolerance in Hsp70- knockdown animals and controls was established based on the challenge test survival percentage, work that involves larval development of these animals following dsRNA injection. Further understanding of the role of Hsp70 is expected to aid in the development of strategies to protect Artemia and possibly other shrimp species from stress, with the development of Hsp70 stimulating factor offering as an excellent example.

RAPID THERMAL ADAPTATION IN PACIFIC ABALONE SUPPORTED BY GENETIC DIVERGENCE AND EPIGENETIC MODIFICATION

Weiwei You *, Huang Zekun, Xu Peng, Xuan Luo, Caihuan Ke

College of Ocean and Earth Sciences
Xiamen University
Xiamen 361102, PR China
wwyou@xmu.edu.cn

The potential of marine organisms to adapt to rapid climate changes is not well understood. The temperate species Pacific abalone *Haliotis discus hannai* has successfully adapted to subtropical climate in southern China after four generations of large-scale transplantation and breeding. We produced a chromosome-anchored assembly of its genome, which shows remarkable karyotype conservation over 516 million years, and conducted multi-omics studies on its rapid thermal adaptation. Population resequencing uncovered pronounced divergence, consistent with geographic distribution across the regional thermal gradient. Comparison of northern and southern populations identified 1429 genes under selection that may be important for thermal adaptation. Differences in physiological and transcriptomic responses demonstrated that southern and northern populations were respectively adapted to warm and cold climates. Genes related to primary metabolism, apoptosis and stress response along with DNA methylation may play crucial roles in thermal response and adaptation. Adapted populations showed reduced transcriptomic plasticity, suggesting genetic and epigenetic divergence are more important for local adaptation. These findings provide insights into rapid thermal adaptation in abalone and possible molecular mechanisms, which are critical in understanding the adaptive potential of marine molluscs and other invertebrates under global warming.

CURRENT STATUS OF MARINE TILAPIA *Oreochromis spilurus* AQUACULTURE IN THE KINGDOM OF SAUDI ARABIA

B. C. Young [1, 2*], A. A. Shaikhi [1]

[1] National Fisheries Development Program, Ministry of Environment, Water & Agriculture
Riyadh 11195, Kingdom of Saudi Arabia
[2] Taiwan International Cooperation and Development Fund, Taipei 11047, Taiwan
[*]Corresponding author
E-mail address: benjamin@nfdp.gov.sa

Introduction

Mariculture is essential to sustainable economic development and food security in Saudi Arabia. Owing to the limitations dictated by government policies and environmental restrictions, euryhaline tilapia are more suitable for aquaculture than freshwater tilapia. n 2020, Saudi Arabia manufactured approximately 99,906 tons of aquaculture; the mariculture production (including marine shrimp and finfish) was recorded at 77,264 tons, which was an increase from the previously recorded 67,833 tons in 2018. Pacific white shrimp *Litopenaeus vannamei* farming had accounted for >70% of the total mariculture production in the 2010s; Contrastly, white shrimp farming accounted for ~47% in 2020. The main mariculture finfish species grown in Saudi Arabia are Asian sea bass *Lates calcarifer*, gilt-head bream *Sparus aurata*, and Sabaki tilapia *Oreochromis spilurus*, which contributed 30,521 tons to Saudi Arabia's total fish production in 2020. Moreover, given the declining price of Asian sea bass due to overproduction and import-dependent gilt-head bream fingerlings, Sabaki tilapia aquaculture has been gaining popularity and is one of the high-valued euryhaline tilapia farming species.

Methods

A questionnaire was designed based on the s previous surveys in the industry (Young et al., 2021b). A total of 65 marine tilapia aquaculture-related personnel were sampled from all 9 primary aquaculture companies from the Ministry of Environment, Water and Agriculture in Saudi Arabia.

Results

The results show the existence of marine tilapia farming on small, medium, and industrial levels in 2019, with average farm size >10 ha and employing >5 farmworkers throughout the production cycle in polyculture systems. Most farms used significantly more amounts of commercial feed than natural feed. The culture cycle on the surveyed farms was <6 months, with the fish produced sold directly to buyers and regular customers. Most producers were not considering a job change. The primary costs involved in Sabaki tilapia aquaculture production were feed (50.06%–69.33%), labor (14.51%–20.98%), and fry (5.29%–5.81%). The medium and industrial-level farms showed advantageous profitability.

Discussion

Survey responses indicated that the primary costs of marine tilapia aquaculture in Saudi Arabia were feed, labor, and fry costs, which are consistent with studies on tilapia aquaculture in Central America, China. Similarly, a survey on tilapia aquaculture in The Philippines indicated that fry and labor were substantial costs for the tilapia industry, with interest on capital accounting for > 90% of the total cost. Unlike other leading tilapia production countries that were sold to the export market, our result indicated that Saudi Arabia tilapia product was sold to the domestic market. Most aquaculture companies in Saudi Arabia are either industrial or large-scale businesses (Young et al., 2021a). In developing countries, this is important because the scale of aquaculture operations makes a significant difference. For example, we reported that industrial-scale producers spent less on feed because they could produce feed in their own facilities. By contrast, small-scale Chinese tilapia producers were economically inefficient. Furthermore, limited environmental factors, higher-cost production facilities were more common for marine tilapia farming in Saudi Arabia.

References

Young, B.C., Alfaggeh, R.H. and AlMoutiri, I. 2021a. Growth, fry production, and cost analysis for Sabaki tilapia production systems. N Am J Aquac 83:290-293.

Young, B. C., Alfaggeh, R. H., and AlMoutiri, I., 2021b. Status and cost analysis of Sabaki tilapia farming in Saudi Arabia. Aquac Int 29: 871-878.

THE LARVICULTURE OF SNUBNOSE POMPANO *Trachinotus blochii* IN THE KINGDOM OF SAUDI ARABIA

B. C. Young [1, 2*], A. A. Shaikhi [1]

[1] National Fisheries Development Program, Ministry of Environment, Water & Agriculture
Riyadh 11195, Kingdom of Saudi Arabia
[2] Taiwan International Cooperation and Development Fund, Taipei 11047, Taiwan
[*]Corresponding author
E-mail address: benjamin@nfdp.gov.sa

Introduction

Snubnose pompano (*Trachinotus blochii*) is a new potential aquaculture species in Saudi Arabia and, as such, is of economic importance in the Red Sea region (Young et al., 2021a). However, there is a lack of information on the larviculture of the snubnose pompano in high salinity conditions outdoors. On the coastline of Jeddah, West Saudi Arabia, the salinity of seawater is 42 to 45‰ during the producing period. Based on our previous records (Young et al., 2021b), we noticed that high salinity (42‰) cause fry deformity indoors. Moreover, due to high salinity, the reproduction rate of live food reduced to lower than the rates required for culture. Therefore, outdoor systems might prove more successful in raising fry. However, there is a lack of information on the larviculture of the snubnose pompano in high salinity conditions outdoors. Therefore, this study was designed to improve our understanding of outdoor larviculture of snubnose pompano under high salinity conditions.

Methods

This study estimated the survival rate, growth performance, and cost of the larviculture of snubnose pompano outdoors at different levels of salinity (33‰, 36‰, 39‰, and 42‰) at 32.3°C–36.0°C. Experiments were conducted in triplicates with 1000 larvae per outdoor tank. The control group was maintained at salinity level of 42‰ indoors. Eighteen hours after hatching, the larvae were transferred outdoors and maintained at different salinity levels (33‰, 36‰, 39‰, and 42‰). The survival rate and growth performance of raising larvae were monitored from day 1 to day 45.

Results

Table 1. Survival rate and growth performance of snubnose pompano larvae fed on microalgae, rotifers, and 120 μm of commercial feed at different salinity levels at 32.3°C–36.0°C

Salinity	Survival rate (%) (45 dph)	Initial weight (g) (21 dph)	Final weight (g) (45 dph)	Specific growth rate (% day^{-1})
Control group 42‰ indoor	0[a]	0[a]	0[a]	0[a]
42‰ outdoor	0[a]	0[a]	0[a]	0[a]
39‰ outdoor	26.5±2.1[b]	0.14±0.02[b]	3.97±0.31[b]	8.38±0.68[b]
36‰ outdoor	24.9±1.7[b]	0.13±0.03[b]	3.89±0.27[b]	8.36±0.54[b]
33‰ outdoor	19.3±1.3[c]	0.13±0.05[b]	4.1±0.21[b]	8.82±0.42[b]

In each column, different letters indicate a significant difference ($P < 0.05$)

Discussion

Young et al. (2021[b]) reported that the survival rate of snubnose pompano larvae was 32%–36% in the indoor conditions exposed to salinity levels between 33‰–39‰. Under outdoor conditions in this study, the survival rate of snubnose pompano larvae was 19.3%–26.5%. In this study, the maximum salinity tolerated by snubnose pompano in larviculture was 39‰. Notable, Young et al. (2021b) noticed that high salinity (42‰) causes snubnose pompano fry deformity indoors. In this study, we observed that snubnose pompano fry was not shown deformity in 33‰ -39‰ outdoor, yet 42‰ still cause snubnose pompano fry deformity outdoors. Our results indicate that the larviculture of snubnose pompano is feasible in high salinity conditions in outdoors.

References

Young, B.C. and Alfaggeh, R.H. 2021a. Entirely farm-raised Snubnose pompano (*Trachinotus blochii*) under high salinity in Saudi Arabia. Isr J Aquac 73:1546070.

Young, B.C., Alfaggeh, R.H. and AlMoutiri, I. 2021b. Larviculture of Snubnose pompano under conditions of high salinity. N Am J Aquac 83:38-40.

RESPONSE OF RAINBOW TROUT *Oncorhynchus mykiss* TO DIFFERENT GAS REPLENISHER IN TANKS

Jianfei Yu, Shuanglin Dong, Yangen Zhou, Yongkang Guo, Yunwei Dong, Qinfeng Gao

Fisheries College, Ocean University of China
5 Yushan Rd., Qingdao 266003, China
yujianfei@stu.ouc.edu.cn

In order to meet the demand of salmon market, Chinese scientists and entrepreneur are working on salmon mariculture far offshore in the Yellow Sea, China. A fully submersible ocean farming cage (Deep Blue No 1) was conducted in China since 2016. However, submergence causes loss of air from the swim bladder, leading to negative buoyancy, modified swimming behaviours, and reduced growth and feed utilisation. The objective of this study is to evaluate the response of rainbow trout to the negative buoyancy and gas replenisher in tanks.

Rainbow trout (N=3; initial weight 63.84 g) were distributed to four treatments with different devices (control, mesh, dome & mesh, and airstone & mesh) at a density of 13 fish per tank over a 40-day feeding trial. The mesh in the tank was used to isolate rainbow trout from the water surface to simulate the submerged sea-cage. The gas replenisher is designed as an underwater gas domes or airstone at the bottom of tank. The meshes and gas replenishers treatments were compared with the control group to ensure that gas replenishers can effectively solve the negative buoyancy.

At the end of the trial, the final mean weight ranged between 77.69 and 90.00 g, weight gain varied from 7.09 to 40.51%, survival ranging from 76.92 to 100.00%, and FCR was between 1.33 and 2.43 (Table 1). Results of the study showed the mesh treatment had a significant effect on the growth of rainbow trout. The dome & mesh treatment can successfully eliminated the effect of the mesh. More physiological index will be further analyzed and reported.

Table 1. Effect of different equipment on initial weight, final weight and survival rate of the rainbow trout during long-term submerged culture

Trt	Final Mean Weight(g)	Final Biomass (g)	Weight Gain (%)	FCR[1]	Survival (%)
control	90.00 [a]	1170.00 [a]	40.51 [a]	1.33 [b]	100.00 [a]
mesh	70.00 [b]	700.00 [c]	7.09 [b]	2.43 [a]	76.92 [b]
Dome & mesh	86.41 [a]	1123.33 [ab]	34.52 [a]	1.53 [b]	100.00 [a]
Airstone & mesh	77.69 [b]	860.00 [bc]	26.04 [a]	1.46 [b]	84.61 [ab]
PSE	2.7805	63.2675	4.7240	0.2353	3.8467
P-value	0.0039	0.0023	0.0051	0.0376	0.0061

Note :PSE: Pool standard error. [1] FCR: Feed conversion ratio

AQUACULTURE AND THE BREEDING PROGRAM OF ASIAN SEABASS IN SINGAPORE

Gen Hua Yue[1]

[1] Temasek Life Sciences Lab, 1 Research Link, National University of Singapore, Singapore, 117604
genhua@tll.org.sg

Singapore is a small island country with limited land and sea space for agriculture and aquaculture. Foofish aquaculture production in Singapore is mainly from cage aquaculture in sea and supplied about 10% of total fish consumed. The major cultured species are Asian seabass, red snapper, golden pompanos, hybrid groupers and shrimps. Although small scale of its aquaculture, constrained by limited available land and coastal areas and high cost for aquaculture, the industry is thriving and transferring from low-tech to high-tech. With the strong support of the government for food security, aquaculture research and development are geared towards developing novel hatchery technologies, marker-assisted and genomic selection to accelerate genetic improvement, the establishment of high-tech aquaculture farms and ensuring biosecurity. In this paper, I will summarize the status of Singapore's aquaculture and its challenges and discussed the future directions. I will also talk about some details on the breeding program for the genetic improvement of Asian seabass. Using conventional breeding approaches, marker-assisted selection and genomic selection for three generations, we have substantially improved growth (> 45%), disease resistance against big belly, nodavirus and iridovirus (no breakout in our hatchery), and omega-3 contents (> 700 mg/100 g meat). We have established three elite lines, one for growth, the second for disease resistance and the third for higher omega-3 contents. Superior hybrid fingerlings have been supplied to local farms and neighbouring countries for commercial production.

COMPARISON ON GROWTH PERFORMANCE, BODY INDICES, AND FEED EFFICIENCY OF ASIAN SEABASS *Lates calcarifer* REARED IN DIFFERENT SALINITIES UNDER CONTROLLED CONDITION.

Yusof M. N.*, Ching F. F., Mohd Faudzi N., Jasni N. A., Nillian E., and Senoo S.

Borneo Marine Research Institute
Universiti Malaysia Sabah, 88400
Kota Kinabalu, Sabah
mohammed_noorfaiz_my21@iluv.ums.edu.my

Asian seabass is one of the most cultured aquaculture species particularly in Southeast Asian region due to its remarkable tolerance for a wide range of environmental fluctuation. Salinity is one of the water quality indicators that has a direct impact on the metabolism and osmoregulation of fish. Therefore, the effect of different salinities (0, 15, and 30 ppt) on growth performance, body indices and feed efficiency of Asian seabass, *Lates calcarifer* were examined in this study. The experimental fish with an initial body weight (BW) 254.50 ± 21.70 g and total length (TL) 26.18 ± 0.66 cm were used, and groups of 10 fish per tank in triplicate group were distributed in 700 litre tank with recirculation and filtration system. The experiment was conducted for 85 days. Survival, growth in BW, body weight gain (BWG), total length (TL), total length gain (TLG), specific growth rate (SGR), condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI), intraperitoneal fat (IPF), total feed intake (TFI), daily feed intake (DFI) and feed conversion ratio (FCR) were evaluated. Asian seabass reared in 15 ppt attained significantly higher BW (470.40 ± 41.16 g), TL (31.51 ± 0.81 cm) and BWG (180.57 ± 24.85 g) compared to 30 ppt. However, there are no significant difference in survival, TLG, SGR, CF, HSI, VSI, IPF, TFI, DFI and FCR. The findings in this study showed that 15 ppt is able to promote better growth performance in Asian seabass compared to other salinities.

THE EFFECT OF FISH OIL REPLACEMENT BY GRAPESEED OIL ON DIGESTIVE ENZYMES ACTIVITY IN RAINBOW TROUT *Oncorhynchous mykiss*

Abbas Zamani *, Maryam Khajavi, Aboozr Moafi , and Enric Gisbert

Fisheries Department
Faculty of Natural Resources and Environment
Malayer University
Malayer, Hamedan, Iran.
a.zamani@malayeru.ac.ir

The limited availability of fish oil (FO), as the main source of lipid in aqua-feeds, calls for the identification of alternative lipid source for the development of sustainable fish farming. In this context, the utilization of vegetal oil (VO) sources, due to their relatively low cost and stable production, has dramatically increased as an important alternative in recent years. From a nutrition point of view, VOs are rich in C18 polyunsaturated fatty acids (PUFA) such as linoleic acid (LA; 18:2n-6) and α-linolenic acid (LNA; 18:3n-3), but the main drawback of using VOs is their FA profile (a different n3:n6 ratio) in comparison to FO. However, digestive and absorptive processes such as enzymatic activities can be affected. Grape seed oil (GO) includes the unsaturated fatty acids, namely oleic acid (18:1n-9) and LA with a higher ratio of unsaturated to saturated fatty acids than animal fats and it does not contain cholesterol. Commercial diets for rainbow trout (*Oncorhynchus mykiss*) mostly contain FO as the main lipid source. Therefore, successful replacement of FO with GO would be an alternative for reducing the dependence on this ingredient and its associated costs. This work was aimed to investigate the effects of dietary increasing levels of GO replacing FO on the activity of digestive enzymes in rainbow trout.

Table 1. Feed ingredients of experimental diets

Ingredients (%)	Experimental diets				
	D1	D2	D3	D4	D5
Fish meal	46	46	46	46	46
Soybean meal	14	14	14	14	14
Meat and bone meal	13	13	13	13	13
Wheat flour	8.98	8.98	8.98	8.98	8.98
Fish oil	14	10.5	7	3.5	0
Grapeseedoil	0	3.5	7	10.5	14
Mineral premix	1	1	1	1	1
Vitamin premix	2	2	2	2	2
BHT	0.02	0.02	0.02	0.02	0.02
Toxin Binder	1	1	1	1	1

Table 2. Specific activity (mU mg protein $^{-1}$) of digestive enzymes from the pyloric caeca and intestine of rainbow trout (*O. mykiss*) fed one of the five experimental diets. Values are means ± SD (n = 3). Values without a common alphabetical letter among diets indicate a significant difference (P < .05).

Diet	Trypsin		lipase		α-amylase	
	Pyloric caeca	Intestine	Pyloric caeca	Intestine	Pyloric caeca	Intestine
D_1	46.2 ± 1.6^{a}	49.5 ± 2.1^{a}	12.2 ± 1.8^{a}	26.4 ± 1.6^{a}	337.3 ± 11^{b}	283.4 ± 9
D_2	48.2 ± 2.5^{a}	54.7 ± 1.9^{a}	24.0 ± 1.5^{b}	34.3 ± 1.3^{b}	328.5 ± 10^{b}	241.5 ± 1
D_3	80.6 ± 3.1^{b}	67.2 ± 2.5^{b}	30.1 ± 1.1^{c}	49.8 ± 1.5^{c}	256.2 ± 10^{a}	162.4 ± 1
D_4	50.1 ± 2.3^{a}	62.2 ± 2.4^{b}	28.5 ± 1.8^{cd}	32.7 ± 2.1^{b}	244.1 ± 11^{a}	144.2 ± 1
D_5	46.4 ± 1.4^{a}	50.1 ± 1.7^{a}	25.1 ± 1.6^{d}	25.2 ± 1.7^{a}	223.8 ± 7^{a}	128.2 ± 1

Fig.1 (c) Feed Intake

EFFECTS OF DIETARY *Bacillus subtilis natto* SUPPLEMENTATION ON GROWTH, OXIDATIVE STATUS, DIGESTIVE ENZYME AND IMMUNE RESPONSE OF JAPANESE FLOUNDER, *Paralichthys Olivaceus*

Yukun Zhang*, Manabu Ishikawa,
Shunsuke Koshio

The United Graduate School of Agriculture Sciences, Kagoshima University, Japan
zhangyukun672@gmail.com

In this study, *Bacillus subtilis natto* was evaluated on the growth, digestive enzyme activity, oxidative status, Intestinal bacteria, non-specific immune response and the growth-related genes' expression in the skeletal muscle of Japanese Flounder. Fish fed five different levels of B. s. natto powder at 0 (BN0), 0.01 (BN1), 0.10(BN2), 1.00(BN3) and 10.00(BN4) g/kg diet for 60 days.

The fish of BN3 and BN4 groups displayed better growth performance and specific growth rate than the BN0 groups ($P < 0.05$). *B. s. natto* supplementation significantly improved the specific activities of amylase, protease and lipase enzymes when compared to the control group ($P < 0.05$). Furthermore, hemoglobin and the nitro blue tetrazolium values up regulated significantly upon *B. s. natto*, especially in case of BN3 and BN4 diets ($P < 0.05$). Serum peroxidase activity enhanced significantly in fish fed BN2 and BN3 diets ($P < 0.05$). When compared to the control, the number of *Bacillus*, *Lactobacillus* and total bacteria in intestinal were significantly increased. The specific growth rate analysis and expressional regulation of the growth-related genes stimulated by B. s. natto suggest the potential application of B. s. natto in improving the growth performance on the Japanese Flounder. Additionally, the supplementation of *B. s. natto* in the diet of Japanese Flounder at 1.00 and 10.00 g/kg diet could improve the growth, feed utilization, health condition and immune response.

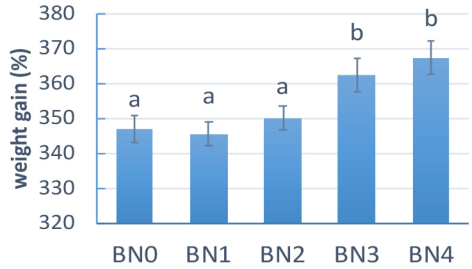


Fig.1(a) Weight gain (%)

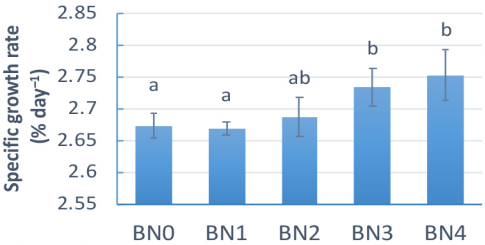


Fig.1 (b) Specific growth rate

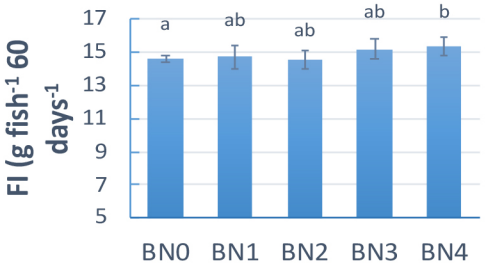


Fig.1 (c) Feed Intake

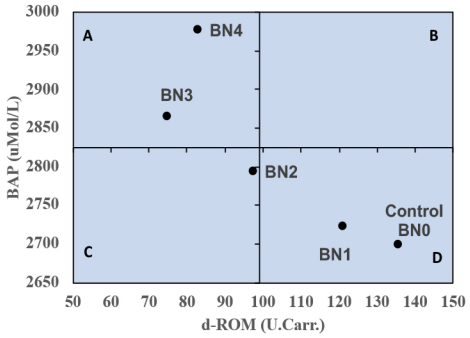
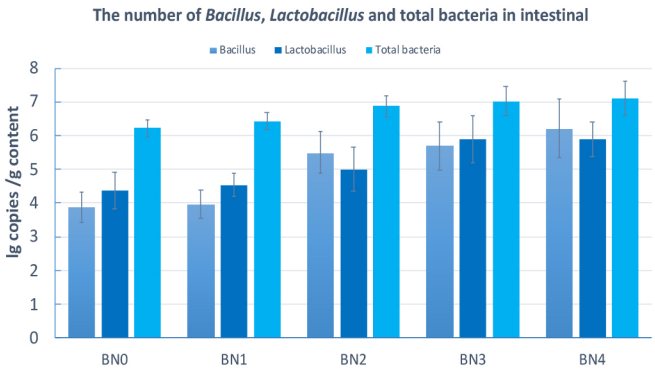


Fig. 2 Oxidative stress parameters



INVESTIGATION OF CARBON COPPER NANOPARTICLE COMPOSITE FILTER MATERIAL FOR DISINFECTING WATERBORNE FUNGUS AND BACTERIA

Ju Zhang[1], Hong Liu[2] , Qianjun Huang[1], Juncai Chan [1] and Sophie St-Hilaire[1]

[1] Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong
[2]State Key laboratory of Aquatic Animal Health at the Animal and Plant Inspection and Quarantine Technical Centre, in Shenzhen Customs District, General Administration of Customs, P. R. China

One of the biggest challenges facing the aquaculture industry in 2020 is clean water. As industries intensify it is becoming more difficult for them to control infectious disease outbreaks, which is leading to an increase in antibiotic use. Preventing the introduction of pathogens into aquaculture systems and controlling the amplification of pathogens in intensive production systems is the key to reducing infectious disease outbreaks and the use of antimicrobials. We investigated the use of a novel chemically engineered product to reduce the level of fungus and bacteria in the water. Our findings indicate that a carbon copper nanoparticle composite incorporated in standard aquarium filter material can be used to reduce pathogens in the water column. This material also appeared not be detrimental to the health of the fish in our study.

TOWARD UNDERSTANDING FACTORS ASSOCIATED WITH OUTBREAKS OF MOTILE *Aeromonas* SEPTICEMIA IN CHANNEL CATFISH (*Ictalurus punctatus*)

Dunhua Zhang*, Dehai Xu, Craig Shoemaker, and Benjamin Beck

Aquatic Animal Health Research Unit
USDA-ARS
990 Wire Road
Auburn, AL 36830
dunhua.zhang@ars.usda.gov

Since the first outbreak of motile *Aeromonas* septicemia (MAS) in farmed catfish in southeastern United States in 2009, recurring outbreaks of MAS had resulted in the loss of millions of pounds of food-size fish annually. Fish mortalities in ponds ranged from 5 to 100%. A new pathotype of *Aeromonas hydrophila*, named virulent *A. hydrophila* (vAh), was identified and attributed to the cause of the outbreak. However, information is limited as for what nourished vAh population in pond water to reach a threshold level that caused acute outbreaks of MAS in fish.

In this study, the effects of nutrients in water on proliferation of vAh and severity of MAS in channel catfish were investigated. Results of the study revealed that both nutrient-rich tryptic soy broth powder (TSBp, the microbiological growth medium) and the commercial fish feeds supported vigorous growth of vAh in water (Figure 1). By addition of 6 g TSBp or 6 g of fish feed to 15 L of water, vAh multiplied from 2.4 x 10^5 CFU/mL to approximately 4.7 x 10^8 CFU/ml and 2.0 x 10^8 CFU/mL, respectively, at 24 h post inoculation (hpi). Challenge of fish in the vAh-propagated water at 24 hpi resulted in approximately 96% and 73% mortality, respectively. Finding s of this study suggest that, when fish were under environmental stresses and had poor appetite for feed supplied in the pond, the unconsumed feed provided vAh ample nutrients to propagate instantly and rapidly, which would result in outbreaks of MAS. Methods of culture of vAh *in situ* performed in this study was also shown to be useful for mimicking vAh growth dynamics in response to nutrient status in relatively natural environment.

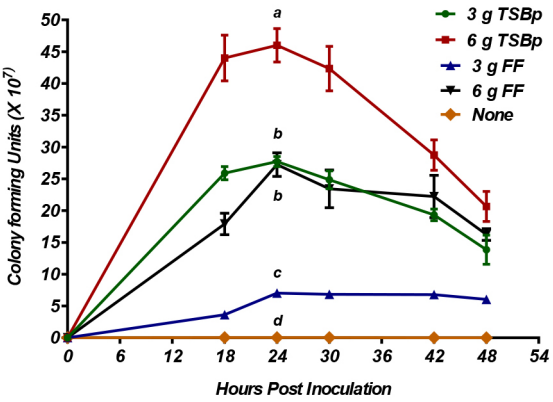


Figure 1. Growth dynamics of *A. hydrophila* in water supplemented with different media. A. hydrophila was inoculated in approximately 15 L of water held in an aquarium tank with initial concentration at 2.4 x 10^5 CFU per mL of water. Significant differences among means at 24 h post inoculation were marked with different superscript letters (p<0.05). TSBp: tryptic soy broth powder; FF: fish feed pellets; and None: nothing added

DEVELOPMENT AND TESTING OF PORTABLE MULTI PARAMETER OPTICAL WATER QUALITY DETECTOR

Xiande Zhao*, Leizi Jiao, Hongwu Tian Daming Dong

zhaoxd@nercita.org.cn
Beijing Research Center of Intelligent Equipment for Agriculture, Beijing 100097, China

The measurement processes of water quality parameters are complicated and tedious. In order to solve the problem of lacking multi-parameter and portable equipment in the field of environmental protection and aquaculture, a hand-held multi-parameter water quality detector based on optical technology was developed, which can realize the rapid measurement of COD, turbidity, chrominance and other parameters in water according to the principle of light absorption and scattering in the water sample. The detector has designed a highly integrated optical structure, easy to carry, and can realize the rapid measurement of multi-parameters in water.

EVALUATION OF FISH FEEDING INTENSITY IN AQUACULTURE USING A CONVOLUTIONAL NEURAL NETWORK AND MACHINE VISION

Chao Zhou*, Daming Xu, Lan Chen, Song Zhang, Chuanheng Sun, Xinting Yang, and Yanbo Wang

Beijing Research Center for Information Technology in Agriculture
Beijing 100097, China
Supperchao@hotmail.com, zhouc@nercita.org.cn

In aquaculture, information on fish appetite is of great significance for guiding feeding and production practices. However, most fish appetite assessment methods are inefficient and subjective. To solve these problems, in this study, an automatic method for grading fish feeding intensity based on a convolutional neural network (CNN) and machine vision is proposed to evaluate fish appetite. The specific implementation process was as follows. First, images were collected during the feeding process, and a dataset was constructed and extended using rotation, scale, and translation (RST) augmentation techniques and noise-invariant data expansion. Then, a CNN was trained on the training dataset, and the fish appetite levels were graded using the trained CNN model. Finally, the performance of the method was evaluated and compared with other quantitative and qualitative feeding intensity assessment methods. The results show that the grading accuracy reached 90%; thus, the model can be used to detect and evaluate fish appetite to guide production practices.

CHANGES IN IMMUNE FUNCTION AND INTESTINAL MICROBIOTA OF KOI CARP *Cyprinus carpio* IN RESPONSE TO DIETARY OREGANO ESSENTIAL OIL

Hua Zhu*, Rong Zhang, Xiaowen Wang, Lili Liu, and Yongchun Cao

Beijing Fisheries Research Institute
Beijing Academy of Agriculture and Forestry Sciences
Beijing, China 100068
zhuhua@bjfishery.com

Oregano essential oil (OEO) has antimicrobial, anti-inflammatory, and antioxidant properties. Thus, OEO is regarded as a promising alternative to antibiotics. In fish, dietary supplementation with OEO improves growth performance, the immunological responses, and pathogens resistance. However, few studies to date have investigate the effects of OEO on the gastrointestinal tract microbiotas of aquatic species. The GI tract supports a vast population of microorganisms that play important roles in host growth, development, and health. We hypothesized that the beneficial effects of OEO on host animals might be closely associated with the host intestinal microbiota. Thus, in this study, we assessed the effects of dietary OEO on both the host immune response and the host gut bacterial community, in order to identify interactions between the host and the gut microbiota.

A total of 300 fish were randomly assigned to one of the four treatments supplemented with OEO at a concentration of 0 (control), 500 (OEO-L), 1500 (OEO-M) or 4500 mg/kg (OEO-H). After eight-week rearing trial, blood and intestinal samples were collected to investigate the immune response and intestinal microbiota. The cumulative survival of fish was recorded for 7 days following *A. hydrophila* infection. Oral administration of OEO increased the concentration of lysozyme, complement C3 and complement C4 in serum (Fig 1). The 7-d cumulative survival was greater in the OEO- treatment groups (Fig 2). Bacterial community composition in the OEO-H group was distinct from that in the control (Fig 3).

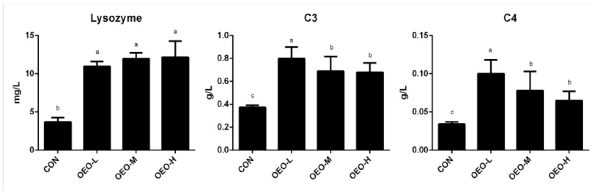


Fig 1. Serum lysozyme, complement C3 and C4.

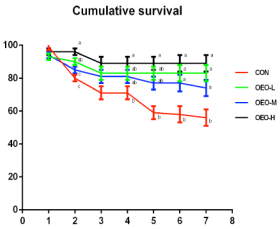


Fig 2. Survival rate

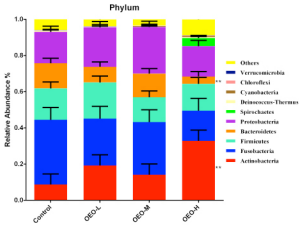


Fig 3. Relative abundance

ANTIBIOTICS AND ANTIOXIDANTS IMPROVED SPERM QUALITY OF SPOTTED HALIBUT *Verasfer variegatus* DURING IN VITRO SHORT-TERM STORAGE

Irfan Zidni*, Hyo Bin Lee, Ji Hye Yoon, Jung Yeol Park, Hyun Seok Jang, Youn Su Cho, Il Young Lee, Han Kyu Lim

Department of Biomedicine, Health & Life Convergence Sciences, BK21 Four
Mokpo National University,
Jeonnam, Republic of Korea
i.zidni@unpad.ac.id

Short-term storage of sperm is a useful method in the reproductive management of fish spermatozoa; however no information is available on spotted halibut, *Verasper variegatus* sperm. One of the important parameters in analyzing sperm quality is sperm motility. This study aimed to optimize a short-term storage protocol for spotted halibut sperm, by investigating the effects of different diluents, storage temperatures, dilution ratios, and evaluating the effect of the addition of antibiotics, and antioxidants on sperm motility and cell survival rate of spotted halibut sperm by computer-assisted sperm analysis (CASA) and Cell Counting Kit-8, respectively. All the parameters tested in this study were determined using one-way ANOVA and evaluated using a multiple range test with Duncan ($p > 0.05$).

Various diluents were evaluated, such as marine fish Ringer's solution (MFRS), Stein's solution, 300 mM sucrose, and 300 mM glucose. The spotted halibut sperm was stored at 0°C, 2°C, 4°C, and 6°C, diluted at 1:1 (sperm: diluent), 1:2, 1:4, and 1:10. Different antibiotics such as neomycin and gentamycin at concentrations of 100, 200, 400, and 800 ppm, variations of antioxidants including Mito-TEMPO (0, 25, 50, 75, 100, 125, 150, 175, and 200 μM), reduced glutathione (0, 2, 4, 6, 8 and 10 mM), and trehalose (0, 50, 100, 150, 200, 250 mM) were tested for sperm preservation. In this study, sperm motility and cell survival rate were evaluated every 5 days. The results showed that sperm motility was affected significantly by diluent, storage temperature, dilution ratio, antibiotic, and antioxidant ($P < 0.05$). The most effective condition for cold storage of spotted halibut sperm was Stein's solution as a diluent in a dilution ratio of 1:4 at 2°C in which the preserved sperm-maintained motility for 30 days. The best antibiotic concentration was 800 ppm of gentamycin and neomycin and the preserved sperm retained motility for 60 days. The combination of neomycin 800 ppm with 250 mM trehalose as antioxidant showed a spermatozoa motility> 43% for 60 days. The short-term storage protocols obtained in this study will be useful in spotted halibut hatcheries.

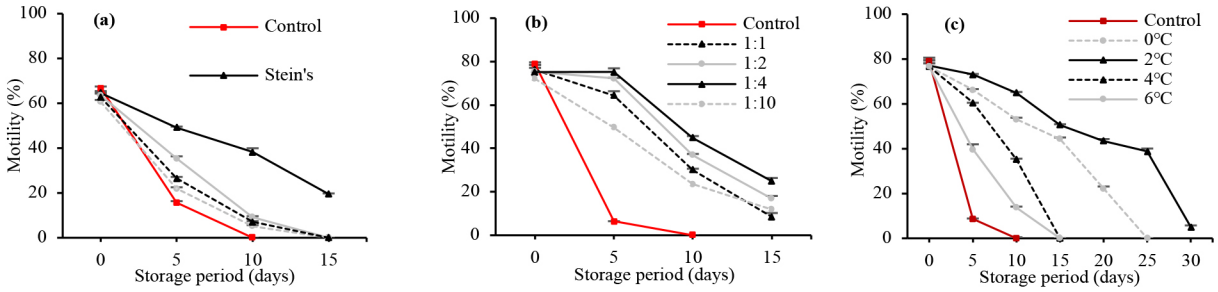


Figure 1. Changes in motility (%) of spotted halibut (*Verasper variegatus*) sperm with different treatments, (a) variation of diluents, (b) dilution ratio, and (c) storage temperature during storage. Control: fresh sperm, MFRS: Marine Fish Ringer's solution

PRODUCTION OF DRIED SEAWEED SHEET USING EDIBLE GREEN MACROALGAE, *Caulerpa macrodisca* Decaisne AND *Caulerpa lentillifera* J. Agardh (Bryopsidales, Chlorophyta)

Wahidatul Husna Zuldin*, Nur Azeera Aming and Siti Nurnilam Sari Nadiah Nain

Borneo Marine Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
wahidatul@ums.edu.my

Caulerpa macrodisca and *C. lentillifera* are type of green macroalgae species, generally consumed as fresh salad with high nutritional properties by local communities in Malaysia. The present study aimed to evaluate the potential of using *C. macrodisca* and *C. lentillifera* for dried seaweed sheet production. The proximate composition of both species were analysed before being incorporated in the dried seaweed sheet recipe formulation. Organoleptic test was performed to evaluate the potential of this species as the main ingredient. There were 50 panelists selected among students and staffs from Faculty of Food Science and Nutrition within the university. The characteristics that were evaluated for each samples were appearance, aroma, texture, taste, colour, and overall satisfaction. Three formulations incorporating both macroalgae in the dried seaweed sheet recipe were created labelled as sample A (50 % *C. lentillifera* and 50 % *C. macrodisca*), sample B (100 % *C. lentillifera*) and sample C (100% *C. macrodisca*). The proximate analysis (dry matter) showed *C. lentillifera* contained 12.47±0.06 % (moisture), 39.18±0.29 % (ash), 1.76±0.09 % (crude lipid), 11.35±0.24 % (crude protein), 23.99±0.95 % (crude fiber), 35.24±0.59 % (carbohydrate); and *C. macrodisca* 8.99±0.25 % (moisture), 34.14±1.65 % (ash), 0.78±0.09 % (crude lipid), 21.51±0.44 % (crude protein), 19.70±0.82 % (crude fiber), 34.57±2.12 % (carbohydrate). The outcome from the organoleptic test conducted showed that sample A had the highest average of overall satisfaction on the characteristics of texture, taste, and colour. However, sample C had the highest average on appearance characteristic while sample B had the highest average on aroma characteristic. Hence, this study indicated that dried seaweed sheet made using formulation sample A (combination of both species) has high potential to be commercialized as healthy snack.

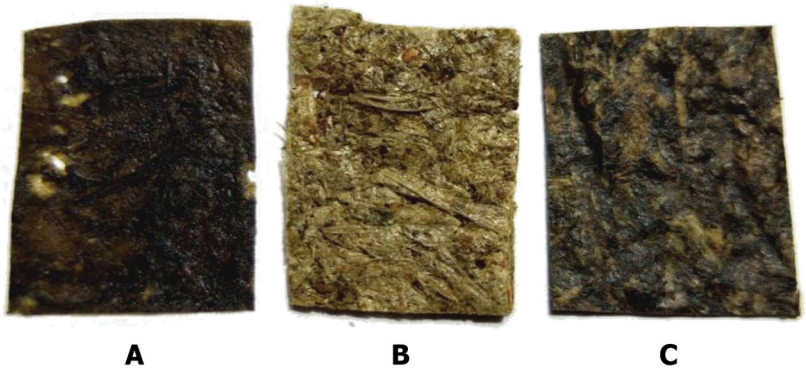


Figure 1: Final product of three types sample formulations. Sample A (50 % *C. lentillifera* and 50 % *C. macrodisca*), Sample B (100 % *C. lentillifera*) and Sample C (100% *C. macrodisca*).

ADDENDUM

ENVIRONMENTAL CONDITIONS IN THE ADRIATIC SEA WITH RESPECT TO THE DEVELOPMENT POTENTIAL OF OFFSHORE MARICULTURE

Tena Bujas*, Nikola Vladimir, Manuela Vukić, Marija Koričan, Zdenko Tonković and Vladimir Soldo

University of Zagreb, Faculty of Mechanical Engineering and Naval Architecture, I. Lucica 5, 10002 Zagreb, Croatia
tena.bujas@fsb.hr

Aquaculture is most rapidly expanding food industry globally, and nowadays practically all marine production takes place not far from the coast. However, coastal zones are becoming increasingly limiting, particularly in tourism-oriented countries like Croatia, which motivates research into use of open sea sites for mariculture production. It is likely that this direction will be associated with higher both capital and operative costs, and therefore design of offshore mariculture should be carefully addressed considering environmental conditions of the target site, technical features of the mariculture farm as well as economic indicators of a whole production chain. There is no clear definition of offshore mariculture, but in this type of activity fish farms are considered as located in deeper and less sheltered waters at some distance away from the coast (definition set by Food and Agriculture Organization of the United Nations (FAO) includes some quantitative indicators), and the cultivated fish stocks are exposed to more naturalistic living conditions with stronger environmental loads (waves & currents), more difficult mooring conditions, and more diverse nutrient flow. There are basically two types of cage structures intended for offshore aquaculture: floating or submersible, where the former ones have been most commonly used up to this stage, while the latter ones are emerging. Before developing the fish farm, important factors to consider are wind and wave conditions, currents, water depth as well as specific seawater parameters desirable for individual species. This part of the overall research within the INTEL-MARIC project deals with mapping of environmental parameters in the Adriatic Sea from the viewpoint of design of offshore floating structures to assess techno-economic viability of offshore mariculture farms, while considerations on physical (temperature, salinity, turbidity…) and chemical (pH, dissolved oxygen…) seawater parameter monitoring will be addressed next steps, Figure 1.

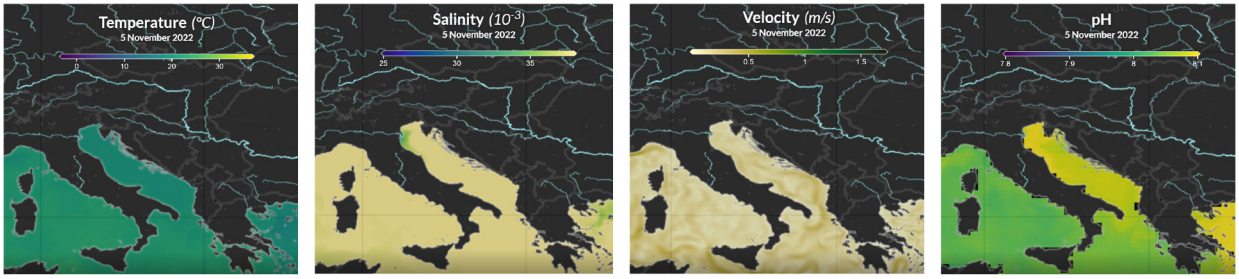


Figure 1. Predictions of selected seawater parameters (source: https://www.copernicus.eu/en/copernicus-services/marine, access: November 4, 2022)

Acknowledgement

Europska unija
Operativni program ZA POMORSTVO I RIBARSTVO
Republika Hrvatska
Sufinancirano sredstvima Europske unije iz Europskog fonda za pomorstvo i ribarstvo

This investigation has been co-funded by the European Maritime and Fisheries Fund of the European Union within the project "INTEL-MARIC", granted by the Ministry of Agriculture, Directorate of Fisheries, Republic of Croatia (Award No. UP/I-324-01/21-01/385).

PILOT PRODUCTION OF PURPLE SEA URCHINS *Paracentrotus lividius* IN SOUTH PORTUGAL (EPPO AQUACULTURE RESEARCH CENTER - IPMA)

Ana Candeias-Mendes*, João Araújo, Ivo Monteiro, Margarida Gamboa, Daniel Rodrigues, Elsa Cabrita, Florbela Soares, Narcisa M. Bandarra, Jorge Dias, Pedro Pousão-Ferreira

IPMA- Portuguese Institute for the Ocean and Atmosphere
Olhão, Portugal
ana.mendes@ipma.pt

Sea urchin gonads are a gourmet product in many parts of the globe. In recent decades there has been an increase in demand, which consequently led to overexploitation of wild populations. Therefore, sea urchin production in aquaculture is the way for a sustainable consumption of this marine resource. The species *Paracentrotus lividus*, known as purple sea urchin, occurs on the rocky bottoms of the Eastern Atlantic and Mediterranean Sea, and its production in aquaculture is still limited. The Aquaculture Research Station (EPPO) has been working since 2017 on research and optimization of *P. lividus* aquaculture. The OURIÇAQUA project aims to increase knowledge and optimize each stage of production of this echinoderm species. Sea urchin reproduction was studied in both wild animals and captivity born individuals, as well as comparison of gamete quality considering origin and generation. Breeding trials were also conducted to improve gamete preservation and fertilization techniques. In the larval stage, several trials were carried out to study and optimize factors such as culture densities and food requirements (for each larval stage) among other zootechnical parameters. Several diets composed of a variety of microalgae, from diatoms to cocolitophores, were tested. This work was accompanied with analyses of the nutritional quality of the diets and its influence on the biochemical composition of the larvae. Through this work it was possible to develop a suitable larval production protocol with a high success rate. Considering the metamorphosis and settlement of post-larvae the most complex and sensitive phase in sea urchin aquaculture, work was carried out to understand and develop strategies to increase the success of this phase, namely through the production of settlement substrates based on different biofilm. In juvenile production, trials were carried out with the main objective of developing diets that promote rapid body growth and optimize survival. Inert feeds specially formulated for juvenile sea urchins were developed and tested, the results of which were frankly encouraging, having significantly promoted the growth and development of the gonads. In partnership with commercial companies, different systems for fattening *P. lividus* were tested, in offshore and inshore systems. This phase of the work is still in progress, but preliminary results are quite positive, especially in systems installed in coastal lagoons. In conclusion, IPMA has given an important contribution to improve the knowledge of aquaculture of the purple sea urchin. Through the dissemination of these results IPMA intends to promote the production of this species regionally, nationally, and internationally, thus making this species a sustainable resource for human consumption.

Diversiaqua MAR-02.01.01-FEAMP-0175 e Ouriçaqua FA_05_2017_009

EGYPTIAN FEED PRODUCTION MARKET FOR TILAPIA AND OTHER FRESH AND SALT WATER SPECIES, GRAND FISH FEED

Joseph P. Kearns

Grand Fish Feed
33El-Moltka El-Arabi
Sheraton, Cairo
1st Floor
jkearns@grand-aqua.com

The Egyptian aquaculture market has been growing rapidly. The combination of weather, land, water and good feed ingredients have allowed for continued growth of a number of species with tilapia being the major followed by mullet with shrimp sea bass and sea bream steadily increasing. The presentation will review the steps taken insuring a quality feed for the market and how this is effectively maintained. The receipt of ingredients, the processing steps and assistance given the farmers will be reviewed which points out how and why this area has grown so successfully and will continue to do so in the future.

FINANCE PATHWAYS FOR AQUACULTURE ENTREPRENEURS: A GLOBAL ACCELERATOR APPROACH

Dylan Howell, Benedict Tan, Wayne Murphy*

HATCH Accelerator Fund Management LLC
73-4460 Queen Kaahumanu Hwy. #138 Kailua-Kona
Hawaii, 96740, USA
wayne@hatch.blue

As an aquaculture entrepreneur, it can be challenging to navigate different sources of funding to grow and scale your business. Investment in this sector has also changed and evolved in the past 5 years with more finance exploring new opportunities in the blue economies, and concurrently new ocean and aquaculture-specific funds have emerged alongside this trend.

HATCH was the first aquaculture investor globally to apply the accelerator model to develop founders and startups starting in 2018. HATCH has applied a unique methodology to select startups within the aquaculture sub-sectors to invest in. Over the last four years, there have been key successes and failures of developing startups and these will be showcased to highlight the learnings that future startups can apply to their growth plans.

Moving forward, HATCH will adapt this strategy to support the growth of the aquaculture sector through its global accelerator in 2023. As the fastest growing food sector in the world, aquaculture is an exciting space to invest in and there are big opportunities for finance to play a role.

FACILITIES AND RESEARCH LINES OF THE AQUACULTURE RESEARCH STATION OF OLHÃO (EPPO)

Pedro Pousão-Ferreira*, Ana Candeias-Mendes, Florbela Soares, Laura Ribeiro, Catarina Matias, Marisa Barata, Sara Castanho, Hugo Ferreira, João Araújo, Ana Gamboa, Ivo Monteiro, Ravi Araújo, Cátia L. Marques

IPMA - Portuguese Institute for the Ocean and Atmosphere/EPPO - Aquaculture Research Station
Avenida do Parque Natural da Ria Formosa s/n, 8700-194 Olhão, Portugal

*pedro.pousao@ipma.pt

The Portuguese Institute for the Ocean and Atmosphere (IPMA, I.P.) is a public research institute and act as a counselor to the national authorities on the sea and atmosphere. IPMA, I.P., possesses a strong cluster of competences for the ocean and marine resources related to research, carried out by different groups, particularly dedicated to aquaculture and fisheries.

The Aquaculture Research Station of Olhão (EPPO, figure 1) stands out for the unique experimental conditions on aquaculture at the national and international levels. This marine core facility is equipped to carry out production studies at every scale from bench-top laboratory work to a much larger semi-industrial level. EPPO has an area of about 7ha with more than 250 tanks, including an hatchery fully equipped for research and experimental production with different rearing circuits (for broodstock, larvae, juvenile production and research with live animals), a support building (with rooms for trophic chain production, daily routines and biological sampling), several analytical laboratories (biochemical, histological, molecular, microbiological and fish pathology), an unit for seafood packing, an area for pre-fattening (for earthen ponds and sea cages production) and 17 earthen ponds. It holds breeders of several marine fish species (e.g. meagre, gilthead seabream, seabass, Senegalese sole and sardine among others), microalgae and invertebrates as well as the know-how on the production of these species.

Production of new species, nutrition, welfare, environmentally friendly production systems and assessment of onshore and offshore and production systems for fish grow-out are some of research lines developed at EPPO (figure 2).

Acknowledgments: The research was funded by DIVERSIAQUA II (Mar2020-P02M01-0656P) and AQUARAS (Mar-02.05.01-FEAMP-0223) projects.

Figure 1 - Aerial view of the EPPO

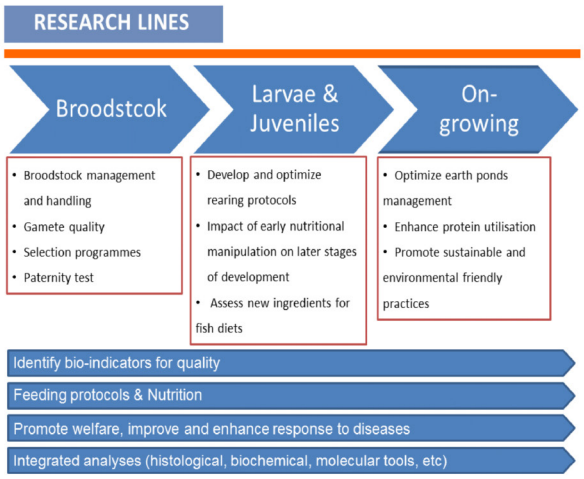


Figure 2 - Research lines developed at EPPO

APPLIED RESEARCH AND INNOVATION AT THE SERVICE OF THE AQUACULTURE INDUSTRY: THE S2AQUAcoLAB CONTRIBUTION

Pedro Pousão-Ferreira[1,2] and Cátia Lourenço-Marques[1,2]

[1] S2AQUA Collaborative Laboratory, Association for a Sustainable and Smart Aquaculture
Av. do Parque Natural da Ria Formosa s/n, 7800-194 Olhão, Portugal
[2] IPMA - Portuguese Institute for the Ocean and Atmosphere, EPPO - Aquaculture Research Station
Av. Parque Natural da Ria Formosa, s/n 8700-194 Olhão, Portugal
E-mail: pedro.pousao@s2aquacolab.pt

The S2AQUAcoLAB is a private non-profit institution, founded in 2021, whose mission is to elevate aquaculture to a new level by playing an interface role between academia, research, and industry. The coLAB performs research in production optimization, identification of health and welfare markers, climate change adaptations and development of new products for market diversification. Its members include 1 state laboratory (IPMA), 2 higher education institutions (UAlg and IPL), 2 R&D centres (CCMAR and ARDITI), 1 municipality (CMO), 1 producer's association (FORMOSA) and 9 private companies (FLATLANTIC, P. VALE da LAMA, SPAROS, NECTON, ATLANTIK FISH, P. ESPARGUEIRA, OCEANO FRESCO, RIASEARCH, DOCAPESCA). The S2AQUAcoLAB has 49 associated researchers with proven experience and countless scientific publications in the area and hired, until now, 20 highly qualified human resources. The ongoing research lines comprise: 1) Training of specialized human resources; 2) Optimization of the production of several marine organisms; 3) Analytical tools for the evaluation of bioindicators of health and welfare and development of in vitro systems; 4) Environmental monitoring and adaptation to climate change; and 6) New products, technological development, and market. The S2AQUAcoLAB and its network of partners have the infrastructure, equipment and means to pursue scientific research of excellence whilst is exceptionally well-positioned to establish the link between scientific outputs and stakeholders.

The main goal of the S2AQUAcoLAB is to carry out R&D activities with a view to innovation for sustainable and smart aquaculture. It aims to play an active role in the transfer of knowledge and technology and diversification of aquaculture products, through the development of services and partnerships for international projects and consortiums.

Acknowledgments: CCDRAlgarve - Projects ALG-05-3559-FSE-000021 and ALG-05-3559-FSE-000024

CHALLENGING THE SINGLE SPECIES STRATEGY OF THE MOST WIDELY FARMED SEAFOOD

Dr. Assaf Shechter

Co-founder & CEO
Enzootic

Shrimp, being the most widely farmed and traded seafood in the world, is totally dominated by a single commodity marine species, *L. vannamei*. This heavily fragmented farming industry is still primarily located in ecologically vulnerable coastal areas. The next two commercially important species, *P. monodon* and *M. rosenbergii*, despite their high local demand and market value are lagging far behind. Without diversity of species, the unavoidable next shrimp pandemic has the potential to decimate global supply. As wild caught from fisheries is slowly declining and is destined to disappear, demand for seafood diversity from aquaculture will grow. Sustainable freshwater alternatives, disconnected from seawater and oceans, hold a huge untapped potential to benefit from these changes and trends.

Enzootic is a fully vertically integrated shrimp biotechnology company with a proven proprietary all-female, non-GMO genetic technology that solves one of the biggest obstacles of scaling freshwater prawn (*M. rosenbergii*) production, grow-out densities. Without the aggressive territorial males in the ponds, farming densities of freshwater prawn, which are usually quite low because of the males, can be quadrupled, enabling dramatic increase in yields that for the first time justify the economics of scaling the production of this amazingly delicious sustainable alternative.

Presently, freshwater prawns are mainly produced and consumed in Asia, with local preference for larger males, either live or chilled. Like the rest of the shrimp industry, production across Asia is fragmented, mostly in small traditional operations. Enzootic has strategically decided to exploit its unique technological advantage and become fully vertically integrated, with capacity to control the quality and value of the entire production and supply chain. To realize the full value of this delicious and sustainable freshwater alternative, Enzootic has set up independent production facilities and farms in Thailand, aiming at short grow-out cycles at high densities to an average size of 38gr. The company is expecting to reach an independent annual production capacity of 1,000MT in 2024. The medium size category at harvest is selected as the ideal size for processing and exporting to premium markets, under the company's brand, Queen Prawn®. To support this strategic objective, the company has also developed a novel processing and freezing formula, which unlocks the potential of exporting this premium quality specialty seafood as a sustainable alternative to North American and European markets.