



ASIAN-PACIFIC AQUACULTURE 2017

Transforming For Market Needs

Putra World Trade Centre

July 24-27, 2017 • Kuala Lumpur, Malaysia

Hosted by: Department of Fisheries, Ministry of Agriculture & Agro-Based Industry, Malaysia



Gold Sponsor



SHENG LONG

President Reception Sponsor



FISHANCE

Session Sponsors



EVONIK
INDUSTRIES



MSD
Animal Health



nutriad
applying nature

WAS Premier Sponsors



BLUE AQUA®

sonac



zeigler
nutrition through innovation



WELCOME TO ASIAN-PACIFIC AQUACULTURE 2017



Selamat Datang and Welcome

On behalf of the Department of Fisheries, Malaysia (DOF) and the Steering Committee, it is my pleasure to welcome all participants and industry professionals to Kuala Lumpur and to the Asian Pacific Aquaculture 2017. This is the second time that DOF is hosting this leading Asian-Pacific aquaculture industry event in Kuala Lumpur. The theme of this year's APA 2017 is "Transforming for Market Needs." This is the way forward for the region's industry; market-driven production of fish and marine shrimp and in a sustainable manner.

We are very proud that the Asian Pacific Chapter of the World Aquaculture Society has again chosen Malaysia for this annual event. We hosted APA in 2009 and since then, there have been rapid developments and changes in Malaysia's aquaculture industry. Many involved in fish and shrimp farming will realize that the recent years have been challenging times, in particular with diseases. Together at this conference, I envisage that there will be active knowledge sharing to help all of us overcome these challenges. DOF together with Malaysian stakeholders look forward to an exciting exchange of knowledge and information among local and foreign participants. Of particular interest to local farmers, is the Malaysian Farmers Day where we will discuss the transformation of its aquaculture towards 2020.

DOF and the Ministry of Agriculture and Agro Based Industry have developed a transformation program for some segments of industry in Malaysia. We will require advancement of technologies and technical expertise. We welcome interactions at the conference and trade show to help us achieve our targets.

I wish you a successful conference and trade show. However, while you focus on the conference, I urge our foreign participants to also explore the hospitality, culture and beauty of Malaysia.

Thank you

DATUK HAJI ISMAIL BIN ABU HASSAN
Director General, Department Of Fisheries Malaysia

TABLE OF CONTENTS

WELCOME	2
ASIAN-PACIFIC AQUACULTURE 2017 ABSTRACTS	5
ADDENDUM	403

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

DISCLAIMER

ASIAN-PACIFIC AQUACULTURE 2017 prints abstracts in this Abstract Book exactly as they are submitted without editing or confirmation of material contained in the abstract. ASIAN-PACIFIC AQUACULTURE 2017 has no responsibility for the information contained in the abstracts. ASIAN-PACIFIC AQUACULTURE 2017 is not responsible for authors or contact information contained in the abstracts.

ASIAN-PACIFIC AQUACULTURE 2017 does not have any liability for problems or damages caused by the use of the information in the abstracts published in the Abstract Book. Any one using these abstracts needs to verify the information in the abstracts on their own before utilizing such information and will have full liability for results of using such information.



APA17
KUALA LUMPUR

ABSTRACTS

MODERN INDUSTRIAL GROUPER FARMING IN LANGKAWI, MALAYSIA

Mohd Addin Aazif

Chief Operation Officer
Fishance Berhad, Langkawi, Malaysia
No.31, 1st Floor, Baron Bandar Baru
Persiaran Putra, 07000, Kuah, Langkawi
Kedah Darul Aman

a.aazif@fishance.com

Fishance Berhad is a newly-established aquaculture business specializing in indoor hatchery and cage system fish farming. Based in Langkawi, Malaysia, the pristine waters surrounding the group of islands is ecologically suitable for farming of high value species like hybrid groupers (tiger grouper, *Epinephelus fuscoguttatus* x giant grouper, *Epinephelus lanceolatus*), cantik grouper (camouflage grouper, *Epinephelus polyphkadion* x tiger grouper, *Epinephelus fuscoguttatus*), orange spotted grouper, *Epinephelus coioides* and other aquaculture commercially-potential species.

The groupers are the major cultured species as their advantages of hybrid vigor which improved the species by strengthening the progeny, generating healthier and high resistance species as well as better yield, faster growth, high prices, improved quality, hardy and stronger breed that contributes to superior economic profitability. Fishance Berhad's commitment to the modern grouper farming is by constructing 10,000 conventional cages and HDPE round cages which will be located in Langkawi's natural, clean and strategic waters. Grouper seed production will be based on a world-class marine fish hatchery in Tuba Island which consists of brood stock rearing facilities, live feed culture area, larviculture and grow out tanks, HDPE liner earth ponds complete with an advance diagnostic disease laboratory. Grouper seed production also guarantees sustainability, either for our own use or for commercialization.

The participation of Fishance Berhad in the modern farming will definitely create a new dynamic dimension on the country's aquaculture industry.

THE EFFECT OF FEEDING PRIMEZEAT® ON HEALTH STATUS OF *Penaeus monodon* AND PERFORMANCE OF *Macrobrachium rosenbergii* BROODSTOCK

Mohammed Suhaimee A. Manaf*, Rosnani Yaakub, Nazariah Nazri and Nur Awatif Othman

Fisheries Research Institute Pulau Sayak
08500 Kota Kuala Muda, Kedah, Malaysia
suhaimee@dof.gov.my

At present, most of shrimp (*Penaeus sp.*) and prawn (*Macrobrachium rosenbergii*) hatchery operators still rely on fresh food such as squid, fish, shellfish and polychaete as feed for broodstock. However, there are risks of disease transmission such as parasites, bacteria and viruses from fresh food. Although imported shrimp broodstock maturation feeds are available, yet costly and not easy to use. Therefore, an innovative disease-free moist feed for shrimp broodstock called PrimeZEat® was developed. PrimeZEat® is available in frozen form and is very easy to use. There were two feeding trials conducted to evaluate the effect of feeding PrimeZEat® and fresh feed on the health and performance of shrimp (*P. monodon*) and prawn (*M. rosenbergii*) broodstock.

In Trial-1, a total of 30 pairs of wild tiger shrimp (*P. monodon*) broodstock with an average weight of 64.3 ± 15.2 g (male) and 103.9 ± 13.4 g (female) were stocked in six, 5MT tanks at 5 pairs/tank with the sex ratio (σ : φ) of 1:1. The initial qPCR screening found that 50% of samples were infected with IHNV, 80% with WSSV, and 60% with AHPND. Three tanks were assigned with fed fresh squid and commercial pellets (D1) compared with another three tanks with PrimeZEat® and commercial pellets. Wilcoxon comparative analysis of virus presence indicated that all tiger shrimp broodstock were found to be free from virus ($Z = -2,558$, $p = 0.011$) on Week-24 compared to Week-1. While Independent Samples T-Test analysis on the survival rate (mean \pm SD%) showed no significant difference ($P > 0.05$) between tiger shrimp broodstock fed D1 ($60.0 \pm 15.5\%$) and PrimeZEat® ($50.0 \pm 17.9\%$).

In Trial-2, a total of 120 female prawn broodstock (average weight = 28.1 ± 5.8 g) stocked with 40 male prawn broodstock (average weight = 39.6 ± 10.1 g) in four 10MT tanks with 1: 3 ratio (σ : φ) per tank. Fresh squid, cockle meat and commercial pellets (D1) and PrimeZEat® with commercial pellets were fed to prawn broodstock at a rate of 7.5%/day. The t-test analysis on the performance of prawn broodstock for two months (TABLE 1) showed no significant difference ($P > 0.05$) between prawn broodstock fed with D1 and PrimeZEat® in terms of final weight (g), survival rate (%), egg weight (g), number of naupli/broodstock, number of naupli/g broodstock and hatching rate (%).

These trials proved that PrimeZEat® had no negative impact on the health, survival rate of tiger shrimp broodstock and the performance of prawn broodstock. Consider the risk of disease, food quality and operating costs suggest that prawn or shrimp hatchery operators may change from using fresh feed to a disease-free formulated moist pellet PrimeZEat® as broodstock feed.

TABLE 1: Independent t-test analysis on the performance of prawn broodstock fed with D1 and PrimeZEat® (mean \pm SD) with t values and Sig. (2-tailed). Mean in a row with different superscripts were significantly different ($P < 0.05$).

Performance parameter	D1	PrimeZEat®	t	p
Weight (g)	28.4 ± 6.8	27.9 ± 5.4	0.14	0.890
Survival rate (%)	82.6 ± 4.8	70.3 ± 12.6	2.24	0.064
Bearing eggs (%)	76.7 ± 0.0^a	46.7 ± 11.0^b	6.71	0.001
Egg weight (g)	3.8 ± 1.0	4.1 ± 0.8	-0.41	0.689
No. of naupli/prawn	$33,876 \pm 5,827$	$31,708 \pm 8,955$	0.50	0.630
No. of naupli/g	$1,219.0 \pm 213.8$	$1,155.1 \pm 318.4$	0.41	0.692
Hatching rate (%)	65.2 ± 4.8	73.3 ± 9.4	-1.87	0.101

HISTOLOGICAL STUDIES OF THE OOGENESIS AND MATURATION OF THE PORTUNID CRAB *Portunus pelagicus* (L.) IN EGYPTIAN WATERS

F. A. AbdelRazek*; M. Ismaiel; A. Khafagy; J. Sorour; A. Attia

National institute of Oceanography and Aquaculture, Invertebrate Laboratory, Alexandria, Egypt

Email address: fatma_abdelrazek@hotmail.com

Abstract

This study is concerned with understanding the process of reproduction and the changes happening in the ovary of *Portunus pelagicus* during maturation, which would be useful for its broodstock development for hatchery purposes. Results showed that, the ovarian development stages of *P. pelagicus* females were divided into five stages: immature; early maturing; maturing; ripe and spent.

Introduction

Marine blue swimmer crab *Portunus pelagicus* is considered the most economical established invasive alien crab species in Egypt, The studies on the structural details of reproductive stages form the base knowledge to perceive the process of its reproduction, which in turn is the primary step toward developing the hatchery technology of *P. pelagicus* in Egypt.

Material and Methods:

Live female specimens of *P. pelagicus* collected from Egypt, Alexandria Mediterranean coasts during March 2015-February 2016, and dissected in order to determine the gonad development stages according to Ravi *et al.* (2013). Gonad lobes were fixed in Davidson solution, then preserved into the 70% alcohol solution, after that dehydrated and moved into cedar oil, embedded in paraffin. Sectioning was at 5 μ m, then mounted, and stained with H&E, then examined with Olympus CX 31 light microscope.

Results and Discussion:

The ovarian maturation stages during one reproductive cycle have been classified into five stages included **A- Immature stage:** Ovary comprised of three different kinds of cells; Og, Oc I and Oc II. Og mean cell diameter were about 11 μ m, they are localized in at the center of each follicle moved toward the periphery of the ovary as they grow. Oc I mean cell diameter were about 39 μ m. their cytoplasm appeared with highly distinct blue color, while Oc II mean cell diameter were about 57 μ m. The cytoplasm was blue and had eosinophilic yolk granules. Supporting follicular cells are present adjacent to Oc I & II (Fig.1.A).

B- Early maturing stage: ovaries characterized by the appearance of Oc III. Which had a mean cell diameter about 92 μ m. The cytoplasm was slightly eosinophilic. The follicular cells were also found indicating a special microenvironment for them, (Fig.1.B). **C- Maturing stage:** Oc III were predominant, as they mature to form Oc IV. The developing cells from the germinal zone invaded and formed extension of germinal zone called "germinal nests", this was also observed in the study of Ravi *et al.* (2013), (Fig.1.C). **D- Ripe stage:** Ovary was filled with Oc IV (Fig.1.D), which had a mean cell size of 156 μ m, where size increased as yolk was developed into the cytoplasm, these results are in agreement with the work of Islam *et al.* (2010) of the mud crab *Scylla paramamosain*. The cytoplasm was highly eosinophilic and granular. Lumen and germinal zone were completely absent. **E- Spent stage:** "spawning" stage, also termed as 'resting' as it was reported by (Fondo *et al.*, 2010), after which most of the completely mature and viable eggs have been released from the ovary. At this stage, the ovary had a large number of spent/resorbing oocytes. Visible degeneration of the oocytes occurs, then oogenesis is reinitiated immediately, (Fig.1.E).

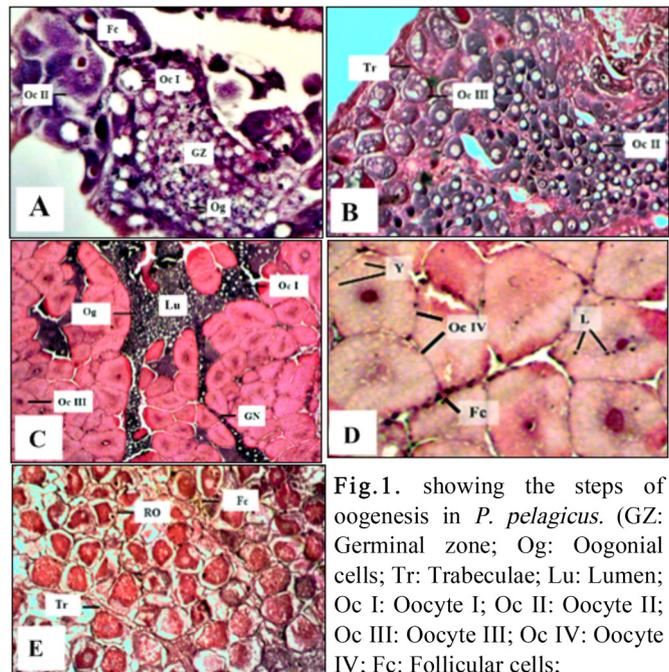


Fig.1. showing the steps of oogenesis in *P. pelagicus*. (GZ: Germinal zone; Og: Oogonial cells; Tr: Trabeculae; Lu: Lumen; Oc I: Oocyte I; Oc II: Oocyte II; Oc III: Oocyte III; Oc IV: Oocyte IV; Fc: Follicular cells; L: Lipid droplets; Y: Yolk granules; RO: Resorbing oocytes. oogenesis is reinitiated immediately, (Fig.1.E).

(Continued on next page)

References:

- 1-Fondo, E.N., Kimani, E.N. and Odongo, D.O., 2010. The status of mangrove mud crab fishery in Kenya, East Africa. *International Journal of Fisheries and Aquaculture*, 2(3), pp.079-086.
- 2-Islam, M.S., Kodama, K. and Kurokora, H. 2010. Ovarian development of the mud crab *Scylla paramamosain* in a tropical mangrove swamps, Thailand. *Journal of Scientific Research*, 2(2), pp.380-389.
- 3-Ravi, R., Manisseri, M.K., and Sanil, N.K., 2013. Ovarian maturation and oogenesis in the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae). *Acta Zoologica*, 94(3), pp. 291-299.

COMPARATIVE HISTOMORPHOLOGY OF DIGESTIVE SYSTEM AND ENZYMES IN *Johnius belangerii*

Rahim Abdi*, Saeed Hasanzadeh, Mohammadali Salarialiabadi, Abdulali Movahedinia, Zahra Basir

*Department of Marine Biology, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology

E. Mail Address: abdir@kmsu.ac.ir

Johnius belangerii have high economic value in the Persian Gulf. In this research the differences in histological and enzymatic of digestive tract in *Johnius belangerii* as a carnivore diet was studied. For this purpose, 10 fish were collected from the beach of Persian Gulf. Then, tissue samples from first and end of the esophagus , stomach and intestine along with middle of intestine were carried out. Also for enzyme samples the first and end of the stomach and intestine along with middle of the intestine were removed from the gastrointestinal tract. Rutine procedure of tissue preparation including fixation, dehydration with ethanol, clearation with xylene, blocking with paraffin and cutting with microtome as a thickness of 5 microns were done. After clearing of slides, studied by light microscope. Results showed the length of intestinal villi in the first part of the intestine was more than other parts ($p \leq 0.05$). Also intestinal mucosal cells increased from the primary to the end. There was no significant difference in histological observation. In enzyme samples the activity of the amylase that breaks down carbohydrates in food and alkaline phosphatase activity was high in other species more than *Johnius belangerii* ($p \leq 0.05$). Also lipase activity that breaks down lipids in *Johnius belangerii* was higher than from species that reported by some researchers ($p \leq 0.05$).

EFFECT OF MICROALGAE-ASSOCIATED BACTERIA ON THE GROWTH OF *Botryococcus braunii* Kützing 1849

Muhamad A.R. Ridzuan^{1,*}, Fatimah Md. Yusoff^{1,2}, Yam Sim Khaw¹, and Fatin M.I. Natrah^{1,2}

¹Laboratory of Marine Biotechnology
Institute of Bioscience
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia

²Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia
mdridzuan.rahim@gmail.com

Microalgae have high commercial value in addition to their crucial role in providing energy in the aquatic food chain. They are valuable sources of various compounds such as antioxidants, fatty acids and amino acids that can be utilized in food, nutraceutical, pharmaceutical, and aquaculture industries. Among many microalgae species, *Botryococcus braunii* is well known for its high lipid content and its potential application in renewable energy industry. However, its slow growth rate is a major hindrance to be utilized commercially. This study aimed to examine the bacterial community and its interaction with *Botryococcus braunii* in influencing the growth of latter. The *Botryococcus braunii* (UPMC-A0021) was isolated and purified from Putrajaya Lake, Malaysia, and its identity was confirmed based on its morphology (light and scanning electron microscope) and DNA sequences (PCR method). Symbiotic bacteria were isolated and identified from the microalga-growing culture. Each isolated bacteria was added into an axenic microalgae culture respectively to examine the effect of bacteria on the microalga growth. Nine different heterotrophic bacteria were living symbiotically with *B. braunii*. These bacteria were classified into four distinct phyla, Actinobacteria, α -proteobacteria, β -proteobacteria and γ -proteobacteria. Five isolated bacteria particularly *Rhizobium* sp. demonstrated synergistic relationship with *B. braunii*. The growth pattern of *B. braunii* with or without bacteria was not significantly different for 25 days. However, *B. braunii* culture with the inoculation of *Rhizobium* sp. showed significantly higher ($p < 0.05$) biomass compared to the other bacterial inoculation. This study illustrated that the symbiotic bacteria could be utilized as inoculants for microalga culture to optimize the growth and biomass production of *B. braunii*.

EFFECT OF STOCKING DENSITY ON SURVIVAL RATE OF GIANT TIGER PRAWN *Penaeus monodon* FROM MADAGASCAR IN PONDS

A.D. Rosmaria*, R, Mohd Afiq, T. Abu Bakar and M. Saberi

Brackish Water Aquaculture Research Division
81550 Gelang Patah
Johor, Malaysia

rosmaria@dof.gov.my

Production of giant tiger prawn (*P. monodon*) is one of the activity by the aquaculture industry that contribute to food security in Malaysia. In order to maximize production of *P. monodon*, effect of stocking density on survival rate of *P. monodon* originating from Madagascar was studied. The study was joint venture project between company Rentas Saga Sdn Bhd and Brackish Water Aquaculture Research Division. The study was located at Brackish Water Aquaculture Research Division, Gelang Patah (FRI Gelang Patah), Johor, Malaysia. Ponds size used for this study range from 1936 to 2170 m². *P. monodon* were cultured at three stocking densities (40, 60 and 80 prawn m⁻²) with each density conducted in triplicate. The water exchange rate were done weekly started from day 20 of culture until end of the production cycle. The feeding rate was five times/day and four times/day from 61 days of cultured until end of the production cycle. The survival rate (SR), average body weight (ABW) and feed consumption ratio (FCR) of the prawn cultured were identified at end of the cycle which up to five months for each cycle. The SR, ABW and FCR were 83.47, 88.30, 81.50%; 27.33, 26.81, 24.72g and 1.74, 1.83, 1.87 for stocking density 40, 60 and 80 prawns m⁻² respectively. No significant differences ($P>0.05$) in survival rate ($84.44\pm 6.44\%$) or average body weight (26.29 ± 1.94) were found between 40, 60 and 80 prawns m⁻² of stocking density. However, the average body weight of the prawns showed negative correlation to the stocking density of the production. The survival rate was not affected by stocking density therefore high stocking density in ponds will be effective technique to maximize production output of Giant Tiger Prawn in Malaysia.

ZOOPLANKTON BIOINDICATORS FOR TROPICAL LAKES OF DIFFERENT TROPHIC STATUS

Umi Wahidah Ahmad Dini^{1*}, Fatimah Md Yusoff¹, Ahmad Zaharin Aris² and Zati Sharip³

¹Laboratory of Marine Biotechnology, Institute of Bioscience, University Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia

²Faculty of environmental studies, University Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia

³Lake Research Unit, Centre of Water Quality and Environment, National Hydraulic Research Institute of Malaysia (NAHRIM), 43300 Seri Kembangan, Selangor, Malaysia

Presenting Author: umiwahidah2013@gmail.com

Zooplankton composition and distribution patterns in lakes are closely related to environmental factors and biotic interactions with other organisms in the aquatic food web. This study was conducted to evaluate the zooplankton species composition and biodiversity in lakes with different trophic status in order to identify potential zooplankton species as bio-indicators. Bimonthly sampling was carried out in Sembrong, Putrajaya and Subang lakes from April 2015 to February 2016. Duplicate zooplankton samples were collected with a 60- μ m mesh net using vertical hauls from about 30 cm from the bottom to the surface, and preserved with 10 % buffered formalin to produce 5 % final solution for identification. Zooplankton species assemblage of hypereutrophic Sembrong lake was dominated by a cladoceran, *Ceriodaphnia cornuta*, followed by the rotifers, *Brachionus forficula*, *B. calyciflorus*, *Trichocerca similis* and *Pompholyx complanata*. These species contributed 63.8 % of all the total zooplankton. In the mesotrophic Putrajaya lake, the dominant species was *Keratella cochlearis*, *Ptygura libera* and *Bosmina longirostris* which made up 65.5 % of the total zooplankton. Meanwhile, in the acidic mesotrophic Subang lake, *P. libera* and *Ascomorpha ecaudis* were the dominant species contributing to 82.2 % of the total species. This present study illustrated that the dominant species found in the mesotrophic and eutrophic tropical lakes were mainly small-bodied zooplankton such as rotifers and small cladocerans. More studies are needed to confirm the reliable indicators for different lake types.

THE EFFECT OF MOBILE PHONES WAVES ON THE LEVEL OF GtH II IN ANGEL FISH *Pterophyllum scalare* , BROODERS

Mohaddeseh Ahmadnezhad*¹, Mohammad Sayyad Bourani¹, Sohrab Dajandiyani¹, Alireza Valipour¹, Ali Asghar Khanipour¹, Homayoun Hosseinzadeh sahani², Hossein Khara³

*¹Inland Waters Aquaculture Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran

²Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

*m_ahmadnezhad@yahoo.com

The study was conducted to evaluate the effect of 900MHz radiofrequency – electromagnetic waves (RF-EMW) sent out from mobile phone on spawning and gonadotropin hormone II level in male and female brooders of *Pterophyllum scalare*. 54 mature male and female angelfish with weight of 6.7 ± 0.5 g and length of 76.9 ± 2.3 mm divided into three groups including control, exposed groups 1 and 2, and each group with three replicates. For 10 days, experimental 1 and 2 groups were exposed to three mobile phones 4 times a day and each time for 30 minutes. The experimental group 1 was exposed to non-calling switched-on mobile phones and the experimental group 2 was exposed to calling mode mobile phones. The concentration of GtH II measured using ELISA method. Three pair of fishes of control spawned during the test. The results indicated that in the level of GtH II of females increased significantly in exposed group 2 in compared to other groups. In males, the level of GtH II increased significantly in exposed groups 1 and 2 in compared to control group.

EFFECTS OF INTENSE INBREEDING ON GROWTH TRAITS IN TILAPIA (*Oreochromis mossambicus*)

K.M. Akinoshun^{1#}, B. Dube² & D. Brink¹

¹ Department of Animal Sciences, University of Stellenbosch, Private Bag X1, Matieland, 7602, RSA

² ARC Animal Production Institute, Private Bag X2, Irene, 0062, RSA
collalaosebikan@gmail.com

Tilapia species plays an important food security role in developing countries, but is often farmed by farmers with very little technical knowledge about breeding. When animals are kept in groups and depend on natural mating, inbreeding is inevitable, as inducing spawning by artificial means is impossible. Large amounts of inbreeding in these farming communities may reduce production efficiency. In as much as inbreeding is unavoidable, understanding its effects may aid in the decision making processes of breeding programmes, especially in developing countries. The extent of inbreeding in small-scale production systems farming with tilapia should be estimated to create awareness and alleviate the effects of inbreeding. Therefore, the objective of study was to evaluate tilapia (*Oreochromis mossambicus*) productivity under the maximum possible inbreeding conditions. Full-sib matings were conducted on *O. mossambicus* for three generations at Welgevallen experimental farm at the University of Stellenbosch. A total of 25 males and 25 females were used as the parental stock, where a 14-day spawning period was followed by three generations of full-sib mating. Measurements of body weight (BW), standard length (SL) and specific growth rate (SGR) were recorded for each of the sixteen randomly sampled fish per replicate, at two weeks growth intervals for the period of 90 days at each generation. Regression analysis was used to determine the rate of phenotypic depression per unit increase in F and analysis of variance used to establish the difference between the means. The results show that BW, SL and SGR decreased with increase in inbreeding at each generation. Average inbreeding depression for BW ranged from 8.35 to 46.57%, while the average inbreeding depression per 10% increase of inbreeding ranged from -12.42 to -18.62%. For SL, the mean inbreeding depression was 18.15 and 17.95% at G₂ and G₃, respectively. Inbreeding depression coefficients for SL were -7.2 % at F = 0.250 and -4.79% at F = 0.375 per 10% increase of inbreeding. The inbreeding depression for SGR was 21.76 and 20.34% at F = 0.250 and F = 0.375, respectively. Per ten percent increase in inbreeding, inbreeding depression coefficient was -8.70% at F = 0.250 and -5.43% at F = 0.375 for SGR. These results show the extent of inbreeding that can be expected under extreme conditions where inbreeding is not controlled, especially where technical knowledge is lacking. They indicate that inbreeding reduces the performance of *O. mossambicus*; hence such decrease in performance may be addressed by minimizing inbreeding, which can be achieved by avoiding the mating of full-sibs. Therefore, if inbreeding is not checked in the small-scale production systems, overtime production will decrease.

RECENT ADVANCES IN SILVER POMFRET (*Pampus argenteus*) CULTURE IN KUWAIT

K. Al-Abdul-Elah*, M. A. Hossain and S. Akatsu

Aquaculture Program
Environment and Life Sciences Research Center
Kuwait Institute for Scientific Research
Kuwait

This paper reports the recent advances in silver pomfret (*Pampus argenteus*) research at the Kuwait Institute for Scientific Research (KISR), Kuwait. Since last several years KISR has been trying to develop a sustainable culture technology for this commercially important species. The average hatching rates of eggs collected from wild silver pomfret were 25.6%, 44.8%, 76.7%, and 53.5.0% for the spawning seasons 2012, 2013, 2014, and 2015 respectively. The average survival rates of metamorphosed fry produced were 3.7%, 5.7%, 4.4% and 3.8% for the spawning seasons 2012, 2013, 2014, and 2015 respectively. In 2012, the captive silver pomfret broods in two tanks spawned a total of 62,712 and 66012 eggs but the eggs were unfertilized. In 2015, captive silver pomfret broods in three tanks spawned about 653×10^3 , 673×10^3 and 270×10^3 eggs. A total of 300 and 123 hatched larvae were produced from captive broods in 2015 and 2016 respectively but they did not survived beyond 56 days of metamorphosed stage. Recently dietary inclusion of compound feed and raw shrimp muscle was found very effective in improving the growth and survival of juveniles as well as the captive spawning of captive silver pomfret broods in 2015. The results of nutritional requirement studies showed that a dietary protein and lipid level of 49% and 16% respectively is optimum for better growth and feed utilization in silver pomfret. A dietary level of 196 mg kg^{-1} of vitamin E has also been found optimal for silver pomfret fingerlings. A patent for development of vaccine against parasitic protozoan was achieved and a potential probiotics bacterium was isolated, characterized and archived.

TABLE 1. Egg collected, larvae and fry produced from wild silver pomfret during 2012 to 2015

Year	No. of female used	Amount of eggs (ml) collected	Hatching rate (%)	No. of larvae produced	No. fry produced	Survival rate (%)
2012	19	729.5	25.6	93,000	2378	3.7
2013	3	75.0	44.8	18,370	1042	5.7
2014	2	205.0	76.7	104,00	4524	4.4
2015	2	169.5	53.5	23,000	872	3.8

TABLE 2. Summary of brood stock weight, eggs collected, larvae produced and hatching rate of captive silver pomfret during 2015 and 2016.

Date of hatching	Origin of brood group	Average brood weight (g)	No. of floating eggs	No. of sunken eggs	No. of hatched larvae	Hatching rate (%)
29/07/2015	Year 2013	178	1,400	89,400	300	21.4
13/06/2016	Year 2012	242	1,000	53,720	123	12.3

EFFECTS OF AUTOCLAVING, SODIUM HYDROXIDE ADDITION AND THEIR COMBINATION ON PROTEIN AND AMINO ACID CONTENTS AND PEPSIN DIGESTIBILITY OF CHICKEN FEATHERS

Ahmed Al-Sooti^{1*}, Wenresti Gallardo, Michel Claereboudt and Osman Gaafar

*Corresponding author: A. AL-Sooti, Department of Marine Science and Fisheries, College of Agricultural and Marine Sciences, Sultan Qaboos University, PO Box 34, Al-Khod 123, Muscat, Sultanate of Oman
E-mail: souti@squ.edu.om Tel.: +968 24141211 Fax: +968 24413418

Feather waste at poultry processing plants has been of interest in nutritional studies because of its high protein content. For it to be useful on feed ingredient, it must be treated to be digested by the animals. The aim of this study was to determine the effects of two types of treatments (NaOH addition and autoclaving) on the protein, amino acid contents and pepsin digestibility of chicken feathers. The first experiment consisted of the following treatments: 1) 2-h treatment with 50ml of 1.0N NaOH, 2) 12-h treatment with 50ml of 1.0N NaOH, 3) 24-h treatment with 50ml of 1.0N NaOH and 4) control which was raw feathers incubated with only 100 ml of distilled water for 24 h at 37°C. The second experiment consisted of the following: 1) raw chicken feathers autoclaved at 2.5 kgf.cm⁻², temperature of 121°C for 30 minutes; 2) raw chicken feathers soaked in 0.5% NaOH solution for 24 hours, followed by autoclaving at 2.5 kgf.cm⁻², temperature 121°C for 30 minutes. The results indicated that prolonged treatment (24 h and 12 h) with NaOH improved feather solubility but low protein retention, whereas NaOH addition followed by autoclaving resulted in high protein and amino acid contents, pepsin digestibility, and in vitro amino acid digestibility.

Table 1. Proximate composition of raw and treated feathers. Values are means and standard deviation of three replicates. Means in a column with different superscripts are significantly different ($p < 0.05$).

Sample #	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Fiber (%)	Dry Matter (%)
Raw Feathers	87.47 ^b ± 0.01	1.90 ^a ± 0.01	1.50 ^b ± 0.10	0.42 ^b ± 0.10	93.31 ^a ± 0.05
Treated Feathers (autoclaved+NaOH)	97.99 ^a ± 0.02	1.01 ^b ± 0.07	4.95 ^a ± 0.04	1.06 ^a ± 0.53	84.01 ^b ± 0.07

Table 2. Protein contents in original sample and pepsin digestibility of the chicken feathers treated with autoclave and NaOH.

Sample Name	Protein in original sample %	Pepsin digestibility %
Autoclave only-30min	98.96 ± 0.28	39.23 ^b ± 0.02
Autoclave + NaOH-30min	97.99 ± 0.02	58.01 ^a ± 0.07

EFFECTS OF CARBOHYDRATE SOURCES ON THE GROWTH, BODY COMPOSITION, INTESTINAL SHORT CHAIN FATTY ACIDS AND LIVER GLYCOGEN OF LEMON FIN BARB HYBRID

Muhammad Aliyu Sulaiman, Mohd Salleh Kamarudin* and Mohammad Fadhil Syukri Ismail

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang

*Corresponding author's email: msalleh@upm.edu.my

The requirement of dietary carbohydrate in fish and crustaceans is still controversial and not completely understood. Understanding the ability of the fish species in utilising carbohydrate is the basis for appropriate feed formulation. In the natural environment, most fish and crustaceans have limited access to carbohydrate sources which are not well adapted to the digestive system for complete metabolism at high levels of dietary carbohydrate. It is believed that the capacity to utilise different carbohydrate sources varies among fish species.

A 60-day feeding trial was conducted to determine the best dietary carbohydrate source for lemon fin barb hybrid fingerlings. Triplicate groups of fish (1.2 ± 0.05 g) were fed twice a day until apparent satiation with five isocaloric (17 kJ g^{-1}), isonitrogenous (30% protein) and isolipidic (4%) test diets containing 35% carbohydrate from corn, tapioca and sago starches, and wheat and rice flour as carbohydrate sources. At the end of the experiment, the growth, feeding efficiencies, whole body proximate composition, intestinal short chain fatty acids and liver glycogen were measured. The weight gain, specific growth rate, protein efficiency ratio feed conversion ratio and survival were unaffected by the dietary carbohydrate source ($P < 0.05$). The optimal feed conversion ratio (1.48) and the best protein efficiency ratio (Table 1) were achieved using corn and tapioca starch. The whole body lipid, protein content, carbohydrate, HIS and VSI were also unaffected by the dietary carbohydrate source. No significant difference was found in the intestinal butyric acid among treatments, although acetic acid and propionic acid were higher in fish fed the corn and tapioca starch diets, respectively. The current study showed the high ability of the hybrid to optimally utilise different carbohydrate sources.

Table 1: Growth performance and feed efficiency of lemon fin barb hybrid fed different dietary carbohydrate sources for 60 days

Parameters	Carbohydrate Source				
	Corn	Wheat	Rice	Tapioca	Sago
Initial body weight (g)	1.2±0.09 ^a	1.2±0.14 ^a	1.2±0.04 ^a	1.2±0.07 ^a	1.2±0.06 ^a
Final body weight (g)	2.82±0.09 ^a	2.81±0.07 ^a	2.80±0.11 ^a	2.81±0.10 ^a	2.80±0.12 ^a
Body weight gain (g)	1.62±0.36 ^a	1.61±0.11 ^a	1.60±0.69 ^a	1.61±0.51 ^a	1.60±0.15 ^a
Body weight gain (%)	135.04±0.11 ^a	133.85±0.7 ^a	133.33±0.8 ^a	135.02±0.8 ^a	133.33±0.12 ^a
SGR (% d ⁻¹)	2.70±0.04 ^a	2.68±0.02 ^a	2.67±0.06 ^a	2.70±0.02 ^a	2.67±0.04 ^a
FCR	1.48±0.17 ^a	1.49±0.21 ^a	1.51±0.08 ^a	1.48±0.24 ^a	1.50±0.07 ^a
PER	2.25±0.07 ^a	2.24±0.04 ^a	2.22±0.08 ^a	2.25±0.02 ^a	2.22±0.09 ^a

Values are means ± SE of three replicates. Different superscripted letters in the same row indicate significant differences ($P < 0.05$). SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency.

POPULATION PARAMETERS OF *Parapenaeopsis sculptilis* (DECAPODA: PENAEIDAE) IN THE COASTAL WATERS OF TERONG, PERAK, MALAYSIA

AMANI A. A. *, ARSHAD A., NURUL AMIN S.M.

*Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

*Email address: aazs25@yahoo.com

The growth and mortality of the *Parapenaeopsis sculptilis* (Heller, 1862), in coastal waters of Terong, Perak were estimated based on monthly length frequency data using the FiSAT software. The total lengths (TL) for males and females were 9.13(\pm 0.83) and 11.96 (\pm 2.2) cm. The von Bertalanffy growth (VBGF) parameters were estimated as asymptotic length (L_{∞}) = 17.69 cm and growth coefficient (K) = 2 yr⁻¹. The total mortality (Z), natural mortality (M) and fishing mortality (F) were found to be 6.71, 1.77 and 4.94 yr⁻¹ respectively. The exploitation (E) rate was calculated as $E = 0.74$ which is higher than optimum level of exploitation ($E = 0.50$). Based on the present data, it can be concluded that the status of the stock of *P. sculptilis* is over exploited in the coastal waters of Terong, Perak, Peninsular Malaysia.

GROWTH AND SURVIVAL OF TWO DIFFERENT SOURCE OF DOMESTICATED BLACK TIGER PRAWN *Penaeus monodon* REARED IN BRACKISH WATER POND IN JOHOR, MALAYSIA

Amatul Samahah Md Ali^{1*}, Mohd Afiq Md Razi², Yeong Kun Yong² Saberi Mawi^{1*}, Zainoddin Jamari¹

Brackish Water Research Division
Fisheries Research Institute Gelang Patah
Dept. of Fisheries Malaysia, 81550, Gelang Patah, Johor, Malaysia
*corresponding author : amatul@dof.gov.my

A joint project between Fisheries Research Institute (FRI), Dept. Fisheries Malaysia and a private company Rentas Saga Sdn. Bhd. was initiated to help enhance the local prawn industry activities. In this project we would like to compare the growth performance and survival of two different domesticated source of black tiger prawn (*Penaeus monodon*). The domesticated black tiger shrimp were from Asia Pacific family and from Madagascar family. Both specific pathogen free (SPF) stocks were obtained from local hatcheries. A few farms in Malaysia have been using both domesticated SPF stocks. However, the growth performance and survival for these two stocks have not been well-documented locally. Thus, in this study the growth performance and survival rate of Black Tiger Prawn from both domesticated stocks in brackish water ponds were recorded and evaluated. The prawns were reared in six earthen ponds of the same size (0.2 ha) located in FRI Gelang Patah, Johor, Malaysia and were carried out within the same culture period. Three pond A1, A2 and A3 were stocked with domesticated postlarvae(PL)s from Asia Pacific family and another three, B1, B2 and B3 were stocked with domesticated PLs from Madagascar family. Stocking density in each pond was standardized at 60 PL/m². Water quality parameters were monitored regularly. Commercial prawn feed were given to the cultured prawns and the ponds were harvested after 120 days of culture. The average final weight of black tiger prawn cultured in pond A1, A2 and A3 is 37.0 ±1.1 g and in pond B1, B2, and B3 is 31.2 ±1.3 g. Average specific growth rate (SGR) for pond A1, A2 and A3 is 2.78 ±0.07 %/day while for pond B1, B2 and B3 is 2.27 ±0.03 %/day. The average survival rate of PLs were 86.73 ±6.30% and 83.13 ±5.01% for Asia Pacific family and for Madagascar family respectively. Based on the SGR and survival rate data of both PLs, PLs from Asia Pacific family showed better performance. However, this study only look at the grow-out phase of the two different source of PLs, further studies need to be carried out to see the differences in terms of growth and survival at the prematuration phase and maturation phase.

PERFORMANCE OF CUSTOMIZED PROBIOTIC *Bacillus mycooides* ON MARRON *Cherax cainii* (AUSTIN, 2002) IN EARTHEN COMMERCIAL MARRON PONDS

Irfan Ambas*, Ravi Fotedar and Nicky Buller

* Department of Fishery, Faculty of Marine Science and Fishery
Hasanuddin University, JL. P Kemerdekaan km. 10 Makassar 90245, INDONESIA
E-mail address: irfanambas@yahoo.com

Today, it has been widely accepted that probiotics play a significant role in aquaculture as an ecofriendly method for disease control for sustainable aquaculture. However there has been only a few *in vivo* studies on the use of probiotics in a controlled environment. In screening a probiotic candidate, an *in vivo* test is essential as *in vivo* physiology is more complex and different from *in vitro* monoculture. No study has ever compared probiotic beneficial effects *in vitro* and *in vivo*.

The present study is validating the performance of customised host origin probiotic *Bacillus mycooides* post laboratory scale trials. To evaluate effectiveness of *B. mycooides*, a number of parameters were measured including hepatosomatic indices (Hiw), intestinal bacteria population, total haemocyte counts (THC), glutathionine peroxide (GPx) enzyme activity, survival rate and productivity of the marron pond which measured at various sampling dates.

The probiotic was supplemented to commercial marron feed (basal diet) at 10^8 CFU/mL and given to marron during eleven months feeding trial using a commercial marron ponds (± 900 m²) and stocked with 3000 marron juveniles/ponds. The probiotic feed was prepared weekly to ensure its freshness and viability, then kept in refrigerator before using.

The results suggested that THC of marron fed basal and probiotic, *B. mycooides* supplemented diets was not significantly different ($P > 0.05$) at day 90th of marron rearing, however on day 160th the THC of probiotic diet fed marron was significantly higher ($P < 0.05$) than the THC of basal diet fed marron. In the present study, the wet hepatosomatic indices (Hiw) of probiotic diet fed marron was significantly higher ($P < 0.05$) both at day 90th and day 160th of measurements (Fig. 9.2) than the Hiw of basal diet fed marron. The Hiw of basal diet fed marron was lower at day 90th than the Hiw at day 160th.

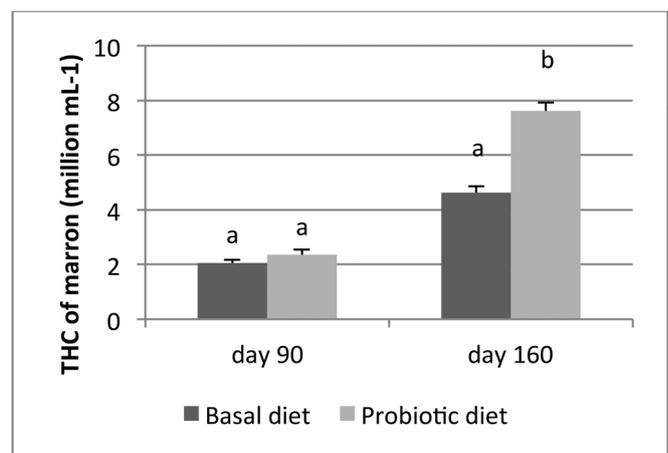


Figure 1. THC of marron fed basal and probiotic diets

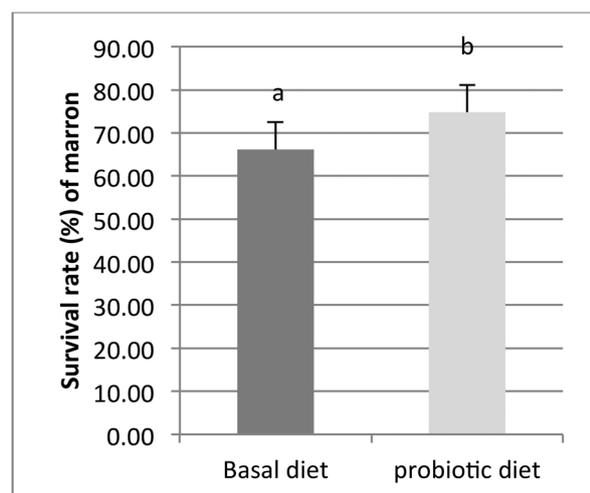


Figure 2. Survival of marron fed basal and probiotic diets

EMBRYONIC AND EARLY LARVAL DEVELOPMENT OF HYBRID BETWEEN LAMPAM ♀ (*Puntius gonionotus*) X KERAI ♂ (*Hypsibarbus wetmorei*)

S M Nurul Amin*, Mohd Hazmadi Zakaria and Aziz Arshad

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang,
Selangor, Malaysia
Email: smnabd@gmail.com

Embryonic and larval development of hybrid between Lampam ♀ (*Puntius gonionotus*) X Kerai ♂ (*Hypsibarbus wetmorei*) were investigated under captive rearing condition. The matured eggs and sperms were collected by stripping of the each brood stock. The embryonic developing stages were observed at every 10 min intervals at the first hour, 20 min intervals at the second hour, 30 min for the next hour and then hourly intervals up to hatching. When hatching was completed, the observations were continued within 2 h interval for the first day and at least every 6 h interval for the following days. Eggs of Lampam were fertilized with sperm of Kerai. The hatching of hybrid breeding *P. gonionotus* ♀ X *H. wetmorei* ♂ was occurred at 14 h after fertilization at temperature ranged from 23.0 to 25.0 °C. The larvae of *P. gonionotus* ♀ X *H. wetmorei* ♂ swam actively when the yolk sac became absent at 46 h after hatching, that were considered completed for the early larval stage. This study represents the first time description of the early development stages for hybrid of *P. gonionotus* ♀ X *H. wetmorei* ♂ in captive culture breeding. The valuable knowledge and information emerged from the designated studies will ultimately be helpful to know the basic breeding biology towards the establishment of seed production and rearing techniques of hybrid *P. gonionotus* ♀ X *H. wetmorei* ♂ for aquaculture production.

ESTROGEN -VITELLOGENIN INTERACTIONS ON *Oreochromis mossambicus* DUE TO ENDOCRINE DISRUPTING CHEMICALS COLLECTED FROM VARIOUS DRAINAGES IN VAIGAI RIVER TRIBUTARIES AROUND MADURAI, TN, INDIA AND ITS BIOREMEDIATION THROUGH ENZYME-NANOPARTICLE CONJUGATES

Chinnaiah Amutha¹ and Periasamy Subramanian²

¹Department of Animal Behaviour and Physiology
School of biological Sciences
Madurai Kamaraj University
Madurai-625021 TN, INDIA

²Department of Animal Science
School of Life Sciences
Bharathidasan University
Tiruchirappalli-620024, TN, INDIA

Endocrine disruption is one of the major life phenomena noted during the recent years. The xenobiotic chemical substance tends to act upon endocrine function as either agonist or antagonist over the organisms especially in the reproductive mechanism. This event may finally end up with a collapse of population propagation in the wild. The present study focused on the endocrine sensitivity of the fishes collected from three different contaminated regions of a non-perennial Holly river Vaigai, which flows across Madurai city. The contaminated locales are Sewage confluencing area (Point source), Liquid food Effluent area (Point source), and Agricultural runoff area (non-point source). From these three regions, the inhabiting common fishes such as *Oreochromis mossambicus* species were collected and examined for their morphological and anatomical changes, GSI, HSI, egg numbers beside Enzyme assays were carried out employing chemi-luminescent method during different seasons. The result obtained showed that the fishes of both candidate species showed feminization development at different level. Thus it was discernible that estrogens-like Endocrine disrupting chemicals' present in the river water and that may caused the catastrophe. On observing the seasonal variations, summer season showed peak values of the all the hormones analysed particularly in sewage drainage area which is a point source. Thus concluded, the point source during summer exerts maximal impact on fishes hormones and there GSI/ HSI ratio. It is our duty to find out the remedy to remove the threats of different pollution from the nature in safest ways. Bioremediation will play an important role in this aspect. Microbes can use wide range of chemicals for their metabolic need using different enzymes and immobilization of the enzymes with the help of nano particles and utilization in bioremediation may play a vital role against different toxic chemicals.

PERFORMANCE CHARACTERISTICS OF NILE TILAPIA *Oreochromis niloticus* FED DIET WITH REGULAR AND PHYTASE-TREATED CORN-DISTILLER'S DRIED GRAIN WITH SOLUBLES (DDGS) FOLLOWING AMMONIA STRESS

Isagani P. Angeles Jr.* Joden G. Quinto, Wilbur F. Dubon, and Yew-Hu Chien

Provincial Institute of Fisheries
Isabela State University-Roxas Campus
Roxas, Isabela, Philippines 3309
ipangelesjr_15@yahoo.com

This study was conducted to determine the effect of regular distiller's dried grain with solubles (R-DDGS) and phytase-treated DDGS (PT-DDGS) on growth, hematology, and survival of Nile tilapia *Oreochromis niloticus* under normal and stress condition. The fish were fed diets with 150 g kg⁻¹ R-DDGS (R150), 300 g kg⁻¹ R-DDGS (R300), 150 g kg⁻¹ PT-DDGS (P150) or 300 g kg⁻¹ PT-DDGS (P300) for 6 weeks, while a commercial diet served as the control (C).

Growth and survival were monitored periodically. After rearing, fish were exposed to ammonia stress for one week. Blood sampling was conducted after rearing, one day and seven days after ammonia stress test to evaluate the hematological parameters. WG and SGR of fish fed diet with P150 and R300 were significantly higher than the C. In addition, WG of fish fed diet with R150, R300, P150 and P300 increased by 11, 26, 41 and 22% as compared to the C, respectively. After rearing, the RBC of R150 was higher than the C; while HCT of R150 and P300 were higher than the C but comparable to R300 and P150. On the other hand, WBC, HGB and HCT of fish fed diet with DDGS were significantly higher compared to the C; while RBC of R150 and R300 were 254 and 204% higher than the C but comparable to P150 and P300 after ammonia stress.

Overall, DDGS improved growth performance and exhibited favorable hematological parameters of fish under normal and ammonia stress-condition.

DNA MICROSATELLITE-BASED EVALUATION OF GENETIC DIVERSITY OF STRAIN GI MACRO II (GENETIC IMPROVEMENT OF MACROBRACHIUM II)

Fajar Anggraeni*, Hary Krettiawan, Asep Sopian, Huria Marnis, Khairul Syahputra and Imron

Research Institute for Fish Breeding
Jalan Raya 2 Sukamandi Pantura
Patokbeusi, Subang 41263
West Java, Indonesia
anggra_xl@yahoo.com

Five microsatellite DNA loci were used to assess genetic diversity of strain GI Macro II. Strain GI Macro II has been set up through individual selection on growth character. GI Macro II has formed by four strains of prawns nature geographically came from different places, Barito, Musi, Asahan and Ciasem and strain prawns GI Macro. Overall, these strains is a strain of prawns in western Indonesia. Otherwise, GI Macro is a strain of freshwater prawn was released in 2001. The purpose of this study was to evaluated genetic diversity of GI Macro II one of strain freshwater prawn using DNA microsatellite.

Twenty five sample of freshwater prawn was extracted from swimmeret tissues using *Genejet™ Genomic DNA Purification* (Fermentas) commercial kit. Five microsatellite primers (Mbr-2, Mbr-3, Mbr-4, Mbr-5 and Mbr-9 with accession numbers DQ019864.1, DQ019865.1, DQ019866.1, DQ019867.1, and DQ019873.1) developed from *M. Rosenbergii* genomic library were used to amplify DNA samples as described by Charoentawee et al. (2006). PCR reactions were performed in 25 µl reactions which used Type-It™ Microsatellite, Qiagen. The PCR profile was initial denaturation at 95 °C for 5 min; then 30 cycles of 95 °C for 30 s, annealing temperature for 90 s, and extention 72 °C for 30 s; then final extention 1 cycle of 60 °C for 30 min. Following amplification, PCR products were fragment analyzed using qiaxcel screening gel.

Genetic characteristics of GI Macro II has obtained from analyzes using markers microsattelite presented in Table 1. Genetic parameters profile of this population such as the Hardy-Weinberg equilibrium (HW), fixation index (Fis), observed heterozygosity (Ho) and expected heterozygosity (He) all confirmed that the selection process has occurred. For example, parameter P (HW) on an ideal natural populations, ie population size is very large, the mating process occurs randomly, no migration and selection occurs, the value is not significant, which means that the population in balance genetically. Instead the **p**-value of (HW) significantly show that the population in the genetic imbalances. In this case an imbalance occurs because of the selection process. Similarly, Ho value lower than He and Fis positive values, all of which are typical of populations experiencing genetic code selection. The selection process of the growth character has been fixing some alleles resulting in lower observed heterozygosity and improve fixation index (Fis) so that is positive.

Table 1. Genetic variability at five microsatellite loci in GI Macro II population including range of alleles, total number of alleles (A), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (Fis), and P value for test of HW expectations (HW)

Locus	Range of alleles (bp)	A	H _O	H _E	F _{is}	HW
Mbr-2	305-358	9	0,57	0,87	0,35	0,00011
Mbr-3	244-290	7	0,68	0,84	0,19	0,03669
Mbr-4	234-258	3	0,39	0,45	0,13	0,00218
Mbr-5	306-328	6	0,52	0,78	0,34	0,00121
Mbr-9	256-284	6	0,41	0,79	0,49	0
Average		6,2±2,2	0,5±0,1	0,7±0,2	0,3±0,1	

EFFECTS OF NANNOCHLOROPSIS CONCENTRATION IN DIET ON GROWTH, SURVIVAL AND IL-10 PRODUCTION OF THE SEA CUCUMBER *Apostichopus japonicus*

Anisuzzaman Md*, Feng Jin, Jong- Kuk Choi, U-Cheol Jeong, Kabery Kamrunnahar, Hak Sun Yu and Seok-Joong Kang

Department of Seafood and Aquaculture Science, Gyeongsang National University, Tongyeong 53064, Korea
E-mail: anisnstu@gmail.com

Sea cucumber *Apostichopus japonicus* which has long been exploited as an important fishery resource in China, Japan and South Korea (Sloan, 1984). In recent years more and more farmers have started to feed the sea cucumbers with macroalgae to improve yield (Shi et al., 2013). Moreover, Sea cucumbers have many therapeutic effects against various diseases and have antiviral, anti-cancer, antibacterial, anti-oxidant, anti-inflammation (Bordbar et al., 2011; Guo et al., 2015). In China and Malaysia, sea cucumbers have been traditionally used for the remedy of different inflammatory diseases like asthma. IL-10 is a potent anti-inflammatory cytokine but till now, there are no reports demonstrating the effect of sea cucumber on IL-10 production. Formulated diets for sea cucumbers are commonly made of macroalgal powder mainly *Sargassum thunbergii*. However, it is difficult to satisfy demand for sea cucumber culture because this algal species is not produced commercially. So, Reducing the *S. thunbergii* content of sea cucumber feed will be one strategy to increase the sustainability of the sea cucumber culture. *Nannochloropsis oculata* algae might be an important choice. In the present study, the effects of different concentration of *Nannochloropsis oculata* algae in prepared feeds on growth, survival and anti-inflammatory cytokine IL-10 production of the sea cucumber *Apostichopus japonicus* were examined.

Experiment 1: A 60 day feeding experiment was conducted to evaluate the growth performance and survival of the sea cucumber *Apostichopus japonicus* fed on six experimental diets containing different inclusion level of *Nannochloropsis oculata* (0%, 2%, 4%, 6%, 8% and 10%) in a recirculating aquaculture system (RAS). After the feeding trial, survival was not significantly different among the dietary treatments. Specific growth rate (SGR), ingestion rate (IR), faeces production rate (FPR) and food conversion efficiency (FCE) of sea cucumber showed significant differences among different dietary treatments. SGR and FCE of sea cucumber fed diet containing 8% *Nannochloropsis oculata* algae was significantly higher than that of sea cucumber fed the other diets. The lowest IR and FPR were found when sea cucumber fed diets containing 10% *Nannochloropsis oculata* algae ($P < 0.05$). Experiment 2: To determine anti-inflammatory cytokine IL-10 production, Six-week-old female mice were fed 200 μ L of 10-fold diluted extracts in PBS and PBS only (control) every other day up to 7 times for four weeks. The results showed that IL-10 production was significantly higher in 6% *Nannochloropsis oculata* algae containing diets.



Fig. 1. Feeding of sea cucumber, *Apostichopus japonicus*

Table 1: Initial and Final wet weight (WW), dry weight (DW) of *Apostichopus japonicus* fed different test diets (mean \pm SE)

Exp. Diets	Initial WW (g)	Initial DW (g)	Final WW (g)	Final DW (g)
Diet 1	2.97 \pm 0.15	0.27	4.20 \pm 0.12	0.38
Diet 2	2.86 \pm 0.08	0.26	4.78 \pm 1.03	0.44
Diet 3	2.85 \pm 0.16	0.26	5.49 \pm 0.94	0.50
Diet 4	2.96 \pm 0.08	0.27	6.73 \pm 1.18	0.61
Diet 5	2.86 \pm 0.14	0.26	7.20 \pm 1.78	0.66
Diet 6	2.96 \pm 0.19	0.27	5.74 \pm 1.04	0.52

EFFECTS OF DIFFERENT MICROALGAE IN PREPARED FEEDS ON ANTI-INFLAMMATORY CYTOKINE, IL-10 PRODUCTION OF THE SEA CUCUMBER *Apostichopus japonicus*

Anisuzzaman Md*, Jong- Kuk Choi, Feng Jin, U-Cheol Jeong, Kabery Kamrunnahar, Hak Sun Yu and Seok-Joong Kang

Department of Seafood and Aquaculture Science, Gyeongsang National University, Tongyeong 53064, Korea
E-mail: anisnstu@gmail.com

Sea cucumber *Apostichopus japonicus* is temperate species, which has long been exploited as an important fishery resource in Russia, China, Japan and South Korea (Sloan, 1984). Market demand for this species has increased because of its aphrodisiac and curative properties. By feeding commercial feed where mostly used *S. thunbergii*, sea cucumber have high level of n-6 fatty acids and low n-3 fatty acids, balance of n-3/n-6 ratio is not good and quite low IL-10 production. But for many allergic and inflammatory diseases like asthma, ω 3 fatty acids, good balance of n-3/n-6 ratio, IL-10 production is very important. In China and Malaysia, sea cucumbers have been traditionally used for the remedy of different inflammatory diseases like asthma. Asthma is a chronic inflammatory disease and have major public health problem. IL-10 is a potent anti-inflammatory cytokine that plays a vital role for the mitigation of allergic responses. But till now, there are no reports demonstrating the effect of different algae on IL-10 production of sea cucumber. . In the present study, the effects of different algae (*Ulva prolifera*, *Undaria pinnatifida*, *Gloiopeltis furcata*, *Laminaria japonica*, *Pyropia yezoensis*) in prepared feeds on anti-inflammatory cytokine IL-10 production of the sea cucumber *Apostichopus japonicus* were examined.

Four replicate groups of sea cucumber, *Apostichopus japonicus* were fed one of the five experimental diets containing *Ulva prolifera* (UP), *Undaria pinnatifida* (UPD), *Gloiopeltis furcata* (GF), *Laminaria japonica* (LJ), *Pyropia yezoensis* (PY) for 08 weeks. After feeding trail, sea cucumbers were cut into small pieces and homogenized. 20g of samples were boiled in 20 ml distilled water for 4 hr at 100°C. After removing solid materials from the water, the boiled water was vaporized using a microwave until the mixture was reduced by 50%. Finally sea cucumber extract carried out at room temperature. After that, Six-week-old female mice were fed 200 μ L of 10-fold diluted extracts in PBS and PBS only (control) every other day up to 7 times for four weeks. The results showed that IL-10 production was significantly higher in 10% *Laminaria japonica* (LJ) algae containing diets than that of sea cucumber fed the other diets.



Fig.1. Mouse spleen is separated to determine IL-10 gene expression.

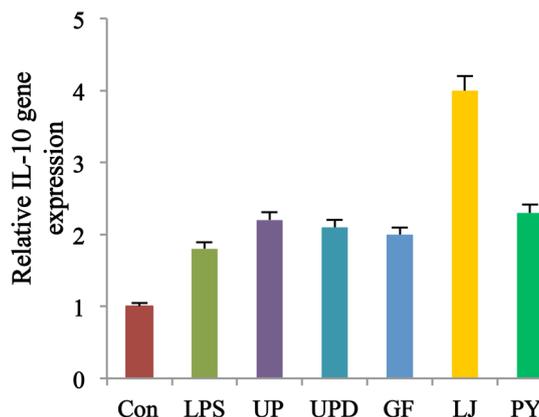


Fig.2. Sea cucumber extract-induced Treg-related cytokine gene expression

EFFECTIVENESS OF POWDER PROBIOTIC CULTURED IN SEAWATER WITH MOLASSES AGAINST VIBRIO IN WHITE SHRIMP *Litopenaeus vannamei*

Lutfi Anshory*, Bagus R Basuki, Ngurah S Yasa

National Broodstock Center for Shrimp and Mollusk
Bugbug village, Karangasem, Bali - Indonesia
lutfi.anshory@gmail.com

In the case of bacterial disease, vibriosis was the chief problem particularly in the stages of the shrimp's life history. Vibrio infection can lead to necrosis, melanisation, even mass mortality of shrimp. An alternative method to prevent it by using probiotic. Probiotic can be found in powder form because powder probiotic can make it long term to keep and stability in quality. However, some cases indicated that vibrio in ponds was still high although the powder probiotic has been applied. The objective of this study was to evaluate the effectiveness of powder probiotic cultured in seawater with molasses against vibrio.

White shrimp (*Litopenaeus vannamei*) was captive in four of 60.000 m³ ponds. Total of 0,1 ppm powder probiotic cultured in seawater with 0,2 ppm molasses as carbon source. The data analyzed was probiotic colony growth in probiotic culture, vibrio colony growth in ponds, and water quality of ponds for one month. The result of probiotic culture showed instead of probiotic colony, dominant colony growth was vibrio with 93% *Vibrio parahaemolyticus*. The seawater wasn't steril so it still contain vibrio, and probiotic was defeated. The result of vibrio colony growth in ponds showed probiotic can controlled green vibrio but still can't defeated yellow vibrio. Green vibrio can be pressed until 1x10¹ cfu/mL to 1x10² cfu/mL while yellow vibrio stood in 1x10³ cfu/mL to 1x10⁴ cfu/mL. The result of water quality of ponds was ammonia can be reduced between 0,06 ppm to 0,56 ppm but nitrite was still high. It means that activity of probiotic was not finished to nitrate.

Based on this study, it can be concluded that powder probiotic cultured in seawater with molasses still ineffective contribution to defeated against vibrio in white shrimp (*L. vannamei*), in case of seawater that used to culture was directly taken from the sea. Seawater with sterilized treatment would be recommended before applied in probiotic culture to clear it from vibrio.

MORPHOLOGICAL AND BEHAVIOURAL CHANGES OF F2 HYBRID TIGER GROUPEP *Epinephelus fuscoguttatus* x GIANT GROUPEP *E.lanceolatus* LARVAE AT EARLY STAGE

Azaharie B. Anuar*, Ching Fui Fui, and Shigeharu Senoo

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jln Ums, 88400, Kota Kinabalu Sabah
Azaharie@ums.edu.my

The F2 Hybrid Tiger Grouper *Epinephelus fuscoguttatus* x Giant Grouper *E. lanceolatus* known as Tiger GG (TGG) was produced in Fish Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. The F1 hybrid TGG had spawned naturally in captivity since February 2016. Total 25,000 fertilized eggs were kept in 1 tonne rounded fibre glass tank (temperature 28.5-29°C; Salinity 30ppt) and morphological and behavioural changes of the larvae were observed. Developments of morphological during the early larvae stage are relatively similar compared to other groupers. The development of newly hatched larvae was started with unpigmented eye, undeveloped mouth, closed anus, and the yolk sac volume decreased as the larvae grow. Cranial development and orbit pigmentation started at 12 hours after hatched (hAH). At 24 hAH, eyes lenses formed and the presence of cupula and free neuromast was observed. The eyes are fully pigmented, while the pectoral fin buds, melanophores and anus are formed at 50 hAH. Lower jaw was actively moved at 60 hAH. The swimming behaviour of the newly hatched larvae was motion less and transformed to vertical swimming at 1 day after hatched (dAH). Horizontal swimming was observed at 2 dAH and the head was moving actively as the larvae reached 3 dAH. Overall, the morphological and behavioural changes showed by the F2 hybrid TGG is as almost similar as the F1 hybrid TGG.

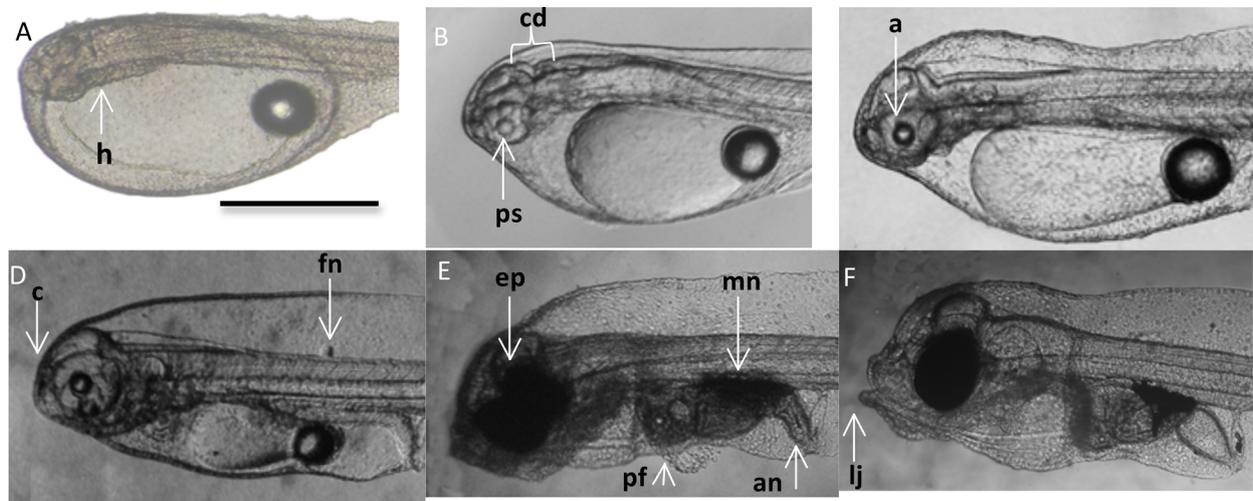


Figure 1: Larval development of F2 hybrid tiger grouper *Epinephelus fuscoguttatus* x giant grouper *E. lanceolatus*. (A) larva at 0 hour after hatch (hAH) (h: heart beating). (B) larva at 6 hAH (cd: cranial development, ps: start of orbit pigmentation). (C) Larvae at 12 hAH (a: eyes lens formed). (D) larva at 24 hAH (c: presence of cupula, fn: free neuromast). (E) larva at 50 hAH (ep: eyes fully pigmented, pf: pectoral fin, mn: melanophores formed, an: anus formed). (F) larva at 60hAH. lj: lower jaw actively moved. Scale bar 500µm.

EVALUATION OF WOOD CHARCOAL SUPPLEMENTATION IN THE DIET OF NILE TILAPIA *Oreochromis niloticus* L. REARED IN CONCRETE TANKS

Goldyn Anne G. Aquino* and Rodora M. Bartolome

College of Fisheries
Central Luzon State University
Science City of Muñoz, Nueva Ecija, Philippines 3120
goldynanneaquino@yahoo.com

Institute of Fisheries
Isabela State University-Main Campus
Echague, Isabela, Philippines 3309

This study evaluated the growth response of Nile tilapia (*Oreochromis niloticus* L.) and monitored the changes of ammonia levels of the water in tanks upon the application of wood charcoal supplementation. Wood charcoal was pulverized and added to commercial feed at 1% and 2%.

Based on the evaluation, results on growth parameters revealed that wood charcoal supplementation showed no improvement on the growth of Nile tilapia. T1 (no charcoal) had the highest average weight of 95.08 grams while T2 (1% charcoal) had the lowest with an average weight of 90.14 grams. However, ANOVA showed comparable weights of fish. Correlation analysis revealed a positive effect of receiving charcoal added supplementation on gain in weight of the Nile tilapia. Highest final mean standard length and mean total length were observed in T2 (13.5 cm) and T1 (16.65 cm). While ANOVA showed no significant difference among treatments, correlation analysis revealed that wood charcoal supplementation increased the standard length and total length of Nile tilapia.

Results on computed TAN revealed that wood charcoal supplementation can decrease the ammonia concentrations of the water. T2 frequently obtained the lowest TAN concentration during the 65th, 72nd, 100th, 107th, and 114th day; while T3 (2% charcoal) obtained the lowest during the 56th, 74th and 94th day. Correlation analysis on TAN showed that decrease in TAN was only observed from 11th day until 67th day of culture which can be attributed to the shift on the form of feed from pellet to mash form. In terms of NH₃-N, T2 obtained the lowest level of NH₃-N during the 11th, 65th, 72nd and 86th day of culture; while T3 obtained the lowest level during the 56th, 74th, 94th, 100th, and 107th day of culture. Correlation on NH₃-N revealed that decrease in NH₃-N was only observed from the 58th day until 67th day due to the shift on the form of feed from pellet to mash form.

EFFECTS OF EXOGENOUS MELATONIN AND ZINC AMINO ACID IN MALE *Clarias macrocephalus* SPERM QUALITY

Siti-Ariza, Aripin*, Orapint Jintasatporn, and Ruangvit Yoonpundh

School of Fisheries and Aquaculture Sciences
Universiti Malaysia Terengganu
21030, Terengganu, Malaysia
*siti.ariza@umt.edu.my

This study investigates the effects of combined Zinc Amino Acid (ZnAA) and melatonin treatment to the male broodstock of the Walking catfish, *Clarias macrocephalus*. Zinc is one of the essential elements that are required during physiological and developmental processes. For male fertility, zinc deficiency may lead to negative effects on seminal plasma which eventually causing infertility. In addition, melatonin is mainly involved in reproductive processes at the hypothalamus–pituitary–gonadal axis.

The gradual ZnAA levels and melatonin of Control (0 ppm ZnAA and 0 mg/kg melatonin), MZn1 (100 ppm ZnAA and 50 mg/kg melatonin) and MZn2 (200 ppm ZnAA and 50 mg/kg melatonin) in the diet mixed in isonitrogenous and isocaloric of 37% crude protein and 9.3% crude lipid was applied to 45 male catfish broodstock (88.07 ± 9.57 g). ZnAA accumulation, and sperm quality parameters were evaluated in this study. The ZnAA concentration was significantly high in serum, meat, bone and testis with the mean ranging from 8.23 – 12.60 ppm; $p = 0.003$, 5.75 – 7.22 ppm; $p = 0.038$, 35.18 – 51.96 ppm; $p = 0.001$, and 10.77 – 16.49 ppm; $p = 0.005$, respectively (Table 1). In addition, combined ZnAA and melatonin feed, treatments were significantly increased the sperm concentration, sperm motility, and lowering sperm abnormality with the mean ranging from 109.4 – 317.5 $10^6/ml$; $p = 0.037$, 13.9 – 25.5 %; $p = 0.013$, and 14.5 – 44 %; $p = 0.022$, respectively (Table 2).

The recommended treatment to enhance the *Clarias macrocephalus* male broodstock first sexual maturation is MZn2 with 50 mg/kg melatonin and 200 ppm ZnAA. The current results showed that combined ZnAA and melatonin treatment may improve the sperm quality of male *C. macrocephalus*.

Table 1: ZnAA concentration in serum, meat, liver, bone, and testis with melatonin and different ZnAA levels

Treatment	Control	MZn1	MZn2	P value
Serum (ppm)	8.23 ^b ± 2.6	9.08 ^b ± 2.3	12.60 ^a ± 1.9	0.003
Meat (ppm)	5.77 ^b ± 1.2	5.75 ^a ± 0.8	7.22 ^a ± 1.5	0.038
Liver (ppm)	18.39 ± 2.8	16.94 ± 2.6	20.94 ± 3.8	0.058
Bone (ppm)	51.96 ^a ± 2.2	49.53 ^b ± 9.2	35.18 ^b ± 2.9	0.001
Testis (ppm)	10.77 ^b ± 1.8	15.08 ^b ± 3.4	16.49 ^a ± 3.9	0.005

^{a,b} Values with different superscripts in a row differ significantly ($P < 0.05$).

Table 2: Sperm analysis for *C. macrocephalus* with melatonin and different ZnAA levels

Treatment	Control	MZn1	MZn2	P value
Sperm abnormality (%)	44 ^b ± 34	20.63 ^a ± 7	14.5 ^a ± 5	0.022
Sperm concentration ($10^6/ml$)	109.4 ^b ± 82	197.5 ^a ± 116	317.5 ^a ± 217	0.037
Live sperm rate (%)	70.6 ± 29	89.1 ± 4	90 ± 6.7	0.063

^{a,b} Values with different superscripts in a row differ significantly ($P < 0.05$).

THERAPEUTIC APPLICATIONS OF INNATE IMMUNE MOLECULES AND THEIR DERIVED PEPTIDES FROM AQUATIC ANIMALS

A. Jesu Arockiaraj*

Division of Fisheries Biotechnology & Molecular Biology
Department of Biotechnology, Faculty of Science and Humanities
SRM Universirt, Kattankulathur 603 203, Chennai, India
jesuarockiaraj.a@ktr.srmuniv.ac.in

Innate immune system is an evolutionarily conserved form of host defense that gets activated during pathogenic attack subsequently enabling a cascade of downstream effector mechanisms to fight against a broad spectrum of pathogens. In this regard, innate immune molecules have remarkable therapeutic features against pathogenic infections, and, as a consequence, researchers are targeting on those molecules for the development of therapeutics to control infections. Though the research has reached the pharmaceutical industries by developing few drugs, still understanding about those molecules remain poor, especially among aquatic candidates. Moreover, invertebrates and primitive vertebrates such as fish and shellfish are rich in these kinds of molecules since they primarily rely on innate immune molecules.

From this perspective, to understand and identify the key innate immune molecules from them, a comparative transcriptomic approach was carried out between healthy and pathogenic challenged organisms. Transcriptome analysis of freshwater organisms such as snakehead murrel, *Channa striatus* and giant prawn, *Macrobrachium rosenbergii*, revealed a wide range of innate immune molecules which are involved in varied functions such as pathogen recognition, antibacterial, antioxidant, apoptosis, protein repair and so on. In *C. striatus*, more than 60 full length immune genes have been identified with direct immune role and similarly in *M. rosenbergii*, around 55 full length immune genes were identified. Gene expression analysis revealed that the expression of the identified genes was modulated during bacterial, fungal and viral pathogens. Functional assays with recombinant proteins showed that they have the potential to be used as therapeutic agents during various pathogenic infections. Additionally, we have identified few antimicrobial peptides (AMPs) from both the organisms including naturally available and derived synthetic peptides that showed high potential to be used as antimicrobial agents to treat various pathogenic infections.

The identified AMPs function in a unique mode of action on the bacterial membrane such a way that the pathogens could not develop resistance against those AMPs. Considering the above facts, the identified potential immune molecules could be developed as therapeutic agents to treat pathogenic infections among aquatic candidates.

EXPLORATION OF OYSTER (*Crassostrea tulipa*) UTILIZATION AND ADAPTATIONS TO CLIMATE CHANGE IN THE FISHERY IN A SOUTH WESTERN WETLAND IN GHANA

Sandra Akugpoka Atindana*, Olajire Fagbola, Emmanuel Ajani, Elliot H. Alhassan and Akwasi Ampofo-Yeboah

Department of Fisheries and Aquatic Resources Management. Faculty of Renewable Natural Resources
University for Development Studies, P. O. Box TL 1882, Tamale, Ghana

*Corresponding author's E-mail: Sandybrownatindana@gmail.com

Tel: +233205163280

In Ghana, coastal fishery resources particularly shellfishes are envisaged to be heavily affected by changes in climate. Also among this group, oysters are the most vulnerable due to potential impacts from sea level rise, salinity changes and ocean acidification. The Whin estuary (longitude 1° 48W and latitude 4° 56N) is feared to be lost to climate change due to its close tie to the sea. This paper examines how oyster fishers in the Whin Estuary in western Ghana utilize fish products of *C. tulipa* and how they perceive and adapt to indicators of climate change. Mixed methods involving participatory approaches such as individual and key informant interviews and focus group discussions were used to gather data on the indigenous knowledge on the fishery and climate change. Moisture, crude protein, carbohydrate, iron, calcium, ash and moisture content were determined following the procedures of the Association of Official Analytical Chemists (AOAC). The mean percentage nutritional values determined for its meat were 43.28 ± 0.35 protein, 8.67 ± 0.24 % carbohydrate, 0.03 ± 0.001 mg of Iron, 22.98 ± 0.78 calcium, 10.89 ± 2.18 % ash and 79.03 ± 0.97 % moisture. From the focus group discussions, individual and key informant interviews, the shells are used in manufacturing of poultry feed, powder for demarcation of fields and paint for building. Kendall tau-b correlation analysis was further performed to explore the relationship between the causes of the perceived changes in climate variables in the area among the gender groups. There was a negative correlation ($r = -0.47$, $p = 0.05$) in perceived causes of changes in climate variables between men and women with men having diverse and well informed views. Women perceive decline in frequency of rains, increasing temperatures and prolonged droughts in recent times. They adapt to increasing rainfall and temperature by diversification, temporal storage for future use and use of improvised tools. Women predict changes in climate by the appearance of dark clouds and cold waves from seaward direction, morning dew and flying of wild birds in columns.

INDUCING INNATE PROTECTION IN GREEN MUSSELS BY MANIPULATION OF HEAT SHOCK TREATMENT

Nor Afiqah Aleng², Yeong Yik Sung^{1,2} and *Effendy, A.W.M.^{1,2}

¹School of Fisheries and Aquaculture Sciences

²Institute of Marine Biotechnology, Universiti Malaysia Terengganu (UMT), 21030, Kuala Terengganu, Malaysia

*Corresponding author: effendy@umt.edu.my

Small aquatic animals are known to have lack of systemic immune system in protection against pathogenic microorganism. Most of aquatic species depend on innate immunity, which are non-specific in nature and would prevent different types of pathogen in disease pathogenesis. A study was conducted to determine the non-lethal heat shock (NLHS) of *P. viridis*, and to observe if the treatment could promote the production of Hsp70, which will be then enhanced its resistance towards stresses and *V. alginolyticus* infection. The LT50 and LHT were determined to be at 42°C and 44°C, respectively, with no mortality to the animals exposed to heat shock treatment compared to the untreated group. Immunoprobings of western blots revealed augmentation of constitutive (PvHsp70-1) and inducible (PvHsp70-2) Hsp70 in tissue from adductor muscle, foot, gill and mantle of *P. viridis* exposed to 38°C for 30 min followed by 6 h recovery. Characterization by liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed that PvHsp70-1 and PvHsp70-2 respectively corresponded most closely to Hsp70 from *P. viridis* and *Mytilus galloprovincialis*. Priming of adult mussels with NLHS promoted thermotolerance and increased resistance to *V. alginolyticus*, suggesting Hsp70 functions in *P. viridis* as a molecular chaperone and as a stimulator of the immune system.

EFFECT OF SUBSTRATE AND BLACK WATER ON GROWTH PERFORMANCE AND BODY COMPOSITION OF MALAYSIAN PRAWN, *Macrobrachium rosenbergii*

Nursyafiqah Zahidah Binti Azahar*, Che Siti Nur Hanisah Che Nur Rifin, Md. Abdul Kader, Mahbuba Bulbul

School of Fisheries and Aquaculture Science, Universiti Malaysia Terengganu
T145, 21300 Kuala Terengganu, Terengganu, Malaysia
syafiqahzahidahazahar@gmail.com

An experiment was conducted to study the effects of addition of substrate and black water on growth performance, feed utilization, metabolic enzymatic activities and body composition of Malaysia prawn, *Macrobrachium rosenbergii*. Four treatments were designed with five replicates per treatment where treatment 1 (T1) was control (no substrate and clear water). Substrate and black water were used in treatment 2 (T2) and treatment 3 (T3), respectively, whereas treatment 4 (T4) was designed with both substrate and black water. The PVC pipes and plastic nets were used as substrate. On the other hand, black water was purchased commercially. Twenty units of rectangular fiberglass tanks (150L) were filled with 100 l freshwater and equipped with the aeration by using air stone. Before stocking, the tanks were prepared with the respective treatments. Homogenous sized 40 PL with mean body weight of 0.143 ± 0.003 g (mean \pm SD) was stocked in each replicate tank. The prawn was fed with commercial diet and dried anchovies with the rate of 10-20% of their body weight, three times a day for 70 days. At the end of the feeding trial, significant differences ($P > 0.05$) were not found among mean final body weight (FBW, g), weight gain (WG, g), percentage weight gain (%WG), specific growth rate (SGR, % day⁻¹) and survival (%) of prawn. The highest ($P > 0.05$) FBW of prawn was found in T3 compared to that of the other treatments. Similarly, WG, %WG and SGR were also highest in T3 followed by T2, T1 and T4, respectively. On the contrary, the survival was lowest ($P > 0.05$) in T3 and the prawn in T2 that contain substrate only, exhibited the highest survival (66.20%). The feed utilization parameters, enzymatic activities related to amino acid metabolism and body composition were also not affected by the any of the treatments. Apparent feed conversion ratio and protein efficiency ratio were better in T2, while feed intake was highest in T3. The glutamate oxaloacetic transaminase and glutamate pyruvic transaminase were highest in control (T1) compared to all the treatments. Comparatively higher values for muscle crude protein and crude lipid were found in T2 compared to those of the control. Based on the overall performances, it can be concluded that addition of substrate had beneficial effects on the performance of prawn. Although, black water improved the growth performance, however it decreased the survival which provided a setback on their utilization. Further research are warranted with different types of substrates and long term duration.

QUORUM SENSING INHIBITOR FROM ROTIFER *Brachionus plicatilis* AS BIOCONTROL AGENTS IN AQUACULTURE

Shariza A*, and Fatin M. I. Natrah

Laboratory of Marine Biotechnology
Institute of Bioscience
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia
shariza.azizan@gmail.com

Pathogenic bacteria such as *Vibrio* spp. have become a threat in aquaculture since decades ago. Its virulence has been reported to be regulated by quorum sensing (QS), a bacteria cell-to-cell communication. As the uses of antibiotic in aquaculture greatly introduce new resistant bacteria, quorum sensing inhibitor (QSI) has been discovered to be an alternative biocontrol agent. It was suggested to disrupt the bacteria communication through inhibition of QS signal molecules. The present study was conducted to isolate and characterize the QSI from live feed, rotifer *Brachionus plicatilis* as potential QS probiotic bacteria to control diseases. A series of QS signal molecule, hexanoyl homoserine lactone (HHL) enrichment was performed to isolate HHL degrading bacteria as QSI candidate. Anti-QS test and HHL degradation assay were done to screen the sample with positive anti-QS activity. Selected sample was characterized through Gram staining and endospore staining. Further molecular analysis was done through 16S rRNA amplification and the sequence was submitted to GenBank (Accession No. KU285429) prior to phylogenetic tree construction. Finally, *in vivo* experiments were done to figure out the impact of potential QSI towards aquaculture live feed; microalgae *Nannochloropsis* sp., rotifer and *Artemia* challenged with *Vibrio campbellii* BB120. Statistical analysis was performed using *t*-test.

The results showed there was one candidate species of QSI isolated from rotifer, a Gram-positive and endospore forming bacteria. The strain has the ability to inhibit QS activity and degraded 10 ppm of the HHL within 3 hour after incubation. 16S rRNA sequence and phylogenetic analysis showed it displayed highest similarity with *Bacillus* sp. and was designated as *Bacillus* sp. BpRotSA. *In vivo* experiments showed it increased growth of *Nannochloropsis* sp. and rotifer at the concentration of 10^7 cell/ml. Although it did not improve the growth performance of *Artemia*, significant ($P < 0.05$) increase of *Artemia* size was observed (TABLE 1). The outcomes showed *Bacillus* sp. BpRotSA can be a potential QSI probiotic and could improve the live feeds production.

TABLE 1. Size of *Artemia* fed with *Bacillus* sp. BpRotSA and challenged with *V. campbellii*.

Treatments	Non-challenge	Challenge
<i>Artemia</i>	1±0	0.9±0.06
<i>Artemia</i> + BpRotSA	2.67±0.16	0.93±0.07
<i>Artemia</i> + dBpRotSA	1.77±0.03	1.1±0.06

PHYLOGENETIC RELATIONSHIPS OF PANGASIIDS (TELEOSTEI: PANGASIIDAE) IN PENINSULAR MALAYSIA BASED ON MITOCHONDRIAL DNA 16S rRNA AND CYTOCHROME OXIDASE I PARTIAL GENE SEQUENCE

Haslawati Baharuddin*, Mohd-Rizman Iddid, Mohd. Zakaria-Ismail and Sekaran Muniandy

Fisheries Research Institute (Freshwater Division)
Glami Lemi, Titi, Jelebu
71650 Negeri Sembilan

The catfishes of the family Pangasiidae are important Asian catfish widely distributed throughout Asia. There are valid 28 species under four genera. However, these groupings are still unstable at the genus level even though many studies have been done to resolve this problem. Only low levels of intraspecific variations were observed. From previous studies, there is no study of the Peninsular Malaysian species' distribution which is located in the middle of the freshwater faunal distribution of South East Asia.

This study presents an analysis on two partial mitochondrial DNA genes (non-protein coding gene partial mtDNA 16S rRNA (16S) and protein-coding Cytochrome c Oxidase I. (COI)) to determine the phylogenetic relationships and genetic variation of the pangasiid catfishes in Peninsular Malaysia.

Samples were collected from Pahang and Perak Rivers using various local fishing gears and traditional methods. Specimens were identified and voucher specimens were preserved. Muscle tissues were taken for molecular analysis where DNA samples were undergo extraction, Polymerase Chain Reaction (PCR) amplification and sequencing processes. Published 16S rRNA and COI sequences were included in the analysis to get better overview of the pangasiid distribution in phylogenetic analyses. Neighbour-joining (NJ), Maximum Parsimony (MP) were run using Tree-Bisection-Regrafting Algorithm (TBR) using MEGA 6.0. Maximum Likelihood (ML) and Bayesian Inference were performed using PAUP version beta 10. Bayesian phylogeny were done using MrBayes version 3.0 implementing Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) analysis.

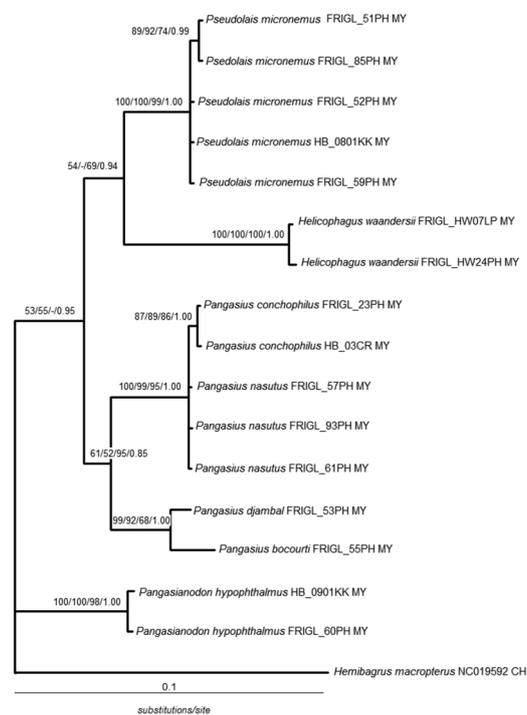


Figure 1: Bayesian phylogram inferred from concatenated 16S rRNA and COI mitochondrial DNA sequences of the pangasiids from Peninsular Malaysia. Numbers at each node indicates bootstrap values for NJ/MP/ML and Bayesian Inference, respectively.

CURRENT SUCCESS IN THE BREEDING OF INDIGENOUS FRESHWATER FISH IN PENINSULAR MALAYSIA FOR FUTURE AQUACULTURE AND STOCK ENHANCEMENT

Haslawati Baharuddin*, Jamaludin Ibrahim, Mohd Zudaidy Jaapar and Hanan Mohd Yusof

Fisheries Research Institute (Freshwater Division)
Glami Lemi, Titi, Jelebu
71650 Negeri Sembilan

The production of freshwater aquaculture species in Malaysia was dominated by the introduced species such as Tilapia, the catfishes (Clariid and Pangasiid) as well as exotic carps. Historically, the only indigenous species that was successfully bred in captivity was the Sultan Fish, Jelawat, *Leptobarbus hoevenii* tackling the high demand in the 1980s. Following this success much more breeding techniques were achieved in the 1990s for other local species including the walking catfish (*Clarias macrocephalus*), river catfish (*Hemibagrus nemurus*), Jullien's Golden Carp (*Probarbus jullieni*), Tengalan (*Puntioplites bulu*), Puyu (*Anabas testudineus*), Haruan (*Channa striata*).

It has been some time since the last documentation of the captive breeding techniques of the indigenous species by the Department of Fisheries. Following popular demand from the niche market, more species were studied and successfully bred in captivity in the recent years including Loma (*Thynnichthys thynnoides*), Kerai Kunyit (*Hypsibarbus wetmorei*), Lampam sungai (*Barbonymus schwanefeldii*), Terbul (*Osteochilus hasselti*), Kelah (*Tor tambroides*) and Patin Buah (*Pangasius nasutus*).

Table 1: Indigenous species recently bred using induced breeding techniques

No	Species	Priming	Induction Dosage (Ovaprim/kg)		Breeding processes
			♀	♂	
1	Loma, <i>Thynnichthys thynnoides</i>		0.6 ml	0.3 ml	Strip after 8-10 hours, eggs hatched after 18 hours
2	Kerai Kunyit, <i>Hypsibarbus wetmorei</i>		0.6 ml	0.3 ml	Strip after 10-12 hours, eggs hatched after 18-24 hours
3	Lampam Sungai, <i>Barbonymus schwanefeldii</i>		0.6 ml	0.3 ml	Strip after 8-10 hours, eggs hatched after 18-24 hours
4	Terbul, <i>Osteochilus hasselti</i>		0.6 ml	0.3 ml	Strip after 8 hours, eggs hatched after 18 hour
5	Kelah, <i>Tor tambroides</i>	♀ 250 IU HCG	0.3 ml	0.6 ml	HCG (24 hours), Ovaprim – strip after 24 hours, eggs hatched after 72 hours
6	Patin Buah, <i>Pangasius nasutus</i>	♀ 500 IU HCG	0.7 ml	0.4 ml	HCG (24 hours), Ovaprim – strip after 18 hours, eggs hatched after 28-30 hours

Note: HCG: Human Chronic Gonadotrophin, IU: International Unit

(Continued on next page)

No	Species	Priming	Induction Dosage (Ovaprim/kg)		Breeding processes
			♀	♂	
1	Loma, <i>Thynnichthys thynnoides</i>		0.6 ml	0.3 ml	Strip after 8-10 hours, eggs hatched after 18 hours
2	Kerai Kuningit, <i>Hypsibarbus wetmorei</i>		0.6 ml	0.3 ml	Strip after 10-12 hours, eggs hatched after 18-24 hours
3	Lampam Sungai, <i>Barbonymus schwanefeldii</i>		0.6 ml	0.3 ml	Strip after 8-10 hours, eggs hatched after 18-24 hours
4	Terbul, <i>Osteochilus hasselti</i>		0.6 ml	0.3 ml	Strip after 8 hours, eggs hatched after 18 hour
5	Kelah, <i>Tor tambroides</i>	♀ 250 IU HCG	0.3 ml	0.6 ml	HCG (24 hours) Ovaprim – strip after 24 hours, eggs hatched after 72 hours
6	Patin Buah, <i>Pangasius nasutus</i>	♀ 500 IU HCG	0.7 ml	0.4 ml	HCG (24 hours) Ovaprim – strip after 18 hours, eggs hatched after 28-30 hours

Table 1:

Note: HCG: Human Chronic Gonadotrophin, IU: International Unit

A REVIEW ON THE EFFECTS OF PROBIOTICS AS FEED ADDITIVES ON JAPANESE EEL (*Anguilla Japonica*), STARRY FLOUNDER (*Platichthys stellatus*) AND RAINBOW TROUT (*Oncorhynchus mykiss*)

Sungchul C. Bai*, Jin-hyeok Lee, Ali Hamidoghli, Seonghun Won, Seunghan Lee, Youngjin Park

Dept. of Marine Bio-materials and Aquaculture
Feeds and Foods Nutrition Research Center (FFNRC)
Pukyong Nat'l University, Busan 608-737, Rep. of Korea
Corresponding author: scbai@pknu.ac.kr

Aquaculture industry has become a vital economic activity securing protein production in several countries. Along with the fast expansion and intensification of aquaculture, emergence of large varieties of pathogenic diseases are considered limiting impediments. On the other hand, the excessive treatments of antibiotics has caused the appearance of bacterial resistance strains. In this regards, a series of experiments were conducted to investigate probiotics such as *Bacillus subtilis*, *Lactobacillus plantarum*, *Bacillus licheniformis* and multi-probiotics as feed additives. Results for the first experiment indicated that single (*B. subtilis* or *B. licheniformis*; 2×10^9 CFU kg⁻¹ diet) or multi-probiotics (commercial probiotics; 2×10^9 CFU kg⁻¹ diet) had equal beneficial effects as antibiotic replacers in terms of growth performance, non-specific immune responses and disease resistance against *Edwardsiella tarda* in starry flounder. In the second experiment on rainbow trout, also results showed same beneficial effects of single (*B. subtilis*, 0.5%) or multi-probiotics (*B. subtilis* + *B. licheniformis*, 0.5%) as antibiotic replacers on growth performance, non-specific immune responses and disease resistance. In the third experiment, a comparison was made for oral administration of *B. subtilis* (106, 107 and 108 CFU/g diet) and *L. plantarum* (106, 107 and 108 CFU/g diet). Results showed that *B. subtilis* (at 108 CFU/g diet) could have more beneficial effects on growth performance, immunological parameters, gut morphology and disease resistance (against *Vibrio anguillarum*) in Japanese eel. Therefore, according to what was mentioned, it could be concluded that probiotics have the potential as antibiotic replacers while enhancing growth performance and immunity. In addition, application of selective strains of probiotics for specific fish seems to be vital in order to maximize the effectiveness.

GROWTH AND SURVIVAL RATE OF SPS HARD CORAL *Psammocora contigua* IN AN EX-SITU NURSERY

Mohamad Saupi Ismail and Dzulfikkar Baitul Ma'mur

Fisheries Research Institute, Department of Fisheries, Malaysia, 11960 Batu Maung, Penang, Malaysia

*Corresponding author: alfiks2001@dof.gov.my

Corals face many serious threats and problems that can result in extinction in the future. Various techniques for coral propagation have been created to perpetuate this valuable heritage. This study examined the difference in fragment size of *Psammocora contigua* in an ex-situ nursery with twenty fragments each for fragment size that was less than 5cm² and fragment size of more than 9cm².

The coral donor colonies were collected from shallow sandy lagoon of about 5 m depth off Pulau Kendi, Pulau Pinang, and transported to Tunku Abdul Rahman Aquarium. The “dry” transportation technique was used according to Yates and Carson, (1992). The corals were transported in medium-sized Styrofoam box to the Aquarium. The transit time was approximately four hours. All transplanted fragments were placed in fibreglass tanks measuring 5 m x 1 m x 1 m. Coral fragments were cleaned every week to remove fouling organisms. Water quality parameters such pH, KH (Calcium hardness), NH₃, NO₃, and PO₄ were measured every month. Coral growth was measured as the change in fragment size over time.

The bigger fragment size took 120 days to grow and cover the entire 38cm² base, while the smaller fragment size took 180 days to achieve the same growth. The survival rate was 100% for both fragment sizes after a period of twelve months. Therefore, fragment size is important in efforts to propagate and manage coral species such as *Psammocora contigua* for the purpose of coral reef conservation throughout the world.

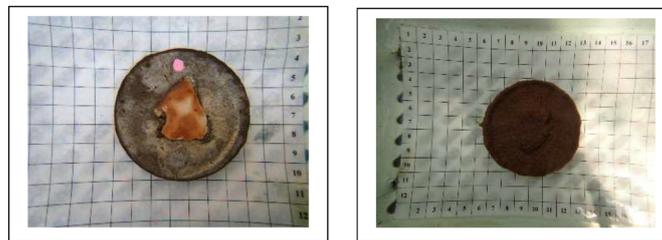


Figure 1: Coral fragments <5cm² at the beginning of propagation (left); the same fragments after 120days (right).

Table 1: Average growth of Coral fragments <5cm²

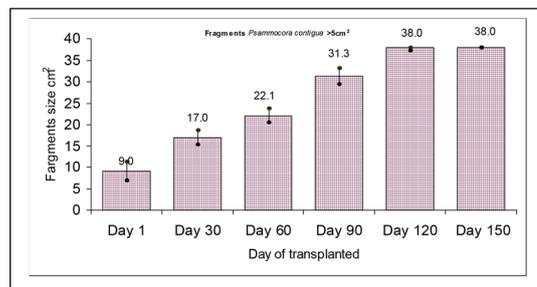
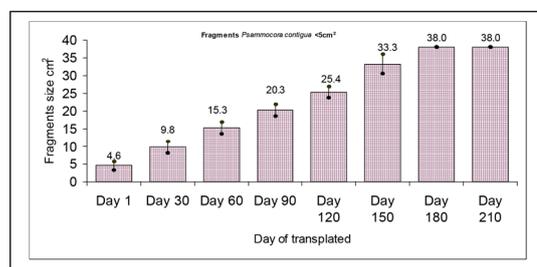


Table 2: Average growth of coral fragments >9cm².

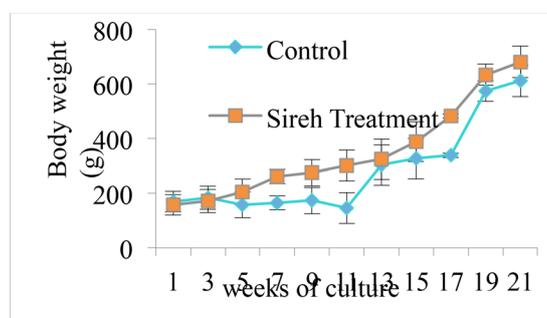


EFFICACY OF SIREH EXTRACT *Piper betel* PREVENTION TREATMENT IN PELLETTED FEED AGAINST MOTILE AEROMONAD SEPTICAEMIA (MAS) IN PATIN *Pangasius hypophthalmus* CAGE CULTURE AT PAHANG RIVER, MALAYSIA

Padilah, B.*, Rimatulhana, R., Wan Rozana, W.A.R., Siti Hawa, M.A., Shahidan, H., Fahmi S., Siti Zahrah, A., Nik Haiha, N.Y. and Zamri Saad, M.

*National Fish Health Research Centre, Batu Maung, Pulau Pinang
Corresponding author: padilahbakar@yahoo.com

Betel leaf *sireh* extract or scientifically known as *Piper betel* was used as prevention treatment against bacterial diseases in particular Motile Aeromonad Septicaemia (MAS) caused by *Aeromonas hydrophila* infection in grow out catfish *patin* (*Pangasius hypophthalmus*) culture at Pahang River. The growth performance of *patin* was measured in body weight (BW g) and total length (TL cm) every week with once a week treatment of *sireh* leaf extract at 100 mg kg⁻¹ feed. The control group is devoid of *sireh* treatment. Three sampling locations consisting of two culture sites at the upstream Pahang River (Kuala Krau, Temerloh) and one farm from the downstream river (Pangsenam, Temerloh) were chosen for this study. The fish was cultured in a cage size of 3 m x 6 m x 1 m with stocking density of 1000 fishes per cage. A total of 250 fishes were sampled from March to September 2015. Bacteria isolation from the internal organs and skin lesions were identified using API Staph, API 20 Strep, API 20E, API 20NE followed by API 50CH bacterial identification system for confirmation of *A. hydrophila*. Low bacterial prevalence was found in treated fish (29.6%) compared to control (44%). The growth performance of *patin* in the treated group was significantly better than the control ($p < 0.05$). The bacterial prevalence (%) of *Staphylococcus* sp., *Plesiomonas Shigelloides*, *Pseudomonas* sp, *Klebsiella* sp. (5.6%, 12.8%, 3.2% and 2.4%) and other *Enterobacteriaceae* mix infections (5.6%) were higher in the control group compared to *sireh* treatment group (0.8%, 4%, 1.6%, 0.8% and 2.4% respectively). Although the bacterial prevalence in the treatment group was predominate by *A. hydrophila* (16%) compared to control (7.2%), the typical clinical symptoms of MAS was not observed. *Sireh* leaf extract in feed improve fish appetite thus effective as growth promoter in *patin* culture.



PRELIMINARY STUDY ON PRODUCTION OF NEOFEMALE BROOD STOCK OF GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii* IN HATCHERY

Badrulnizam Basri*, Balton Martin and Azhar Hamzah

Fisheries Research Institute, Kg. Pulau Sayak, 08500 Kota Kuala Muda, Kedah, Malaysia

*Corresponding Author's Email: bard4547@hotmail.com

Productivity of giant freshwater prawn is generally associated with the brood stock management and it can be improved by two methods: selective breeding and all male culture. Few studies have indicated economically advantages of all male culture. However, conventional methods by manual sexing and selecting the male prawn during grow-out is time and labour consuming. Another method to produce male fry is to use 'neo female' parent. This study was conducted to produce the 'neo female' brood stocks and evaluate the empirical percentages of success rate in the hatchery. A batch of 20,000 post larvae were nursed in a 40 tonnes rounded concrete tank. At day 45 of nursing, first segregation to identify male PLs was carried out by checking the presence of a pair of 'genophore complexes' located at the edge of fifth walking leg. Out of 2,000 PLs, only 600 were males (representing 30% of the population). These males were subsequently undergoing 'androgenic gland ablation' by removing the a pair of glands in a minor surgery. After a week of the removal procedure, $87.7 \pm 3.0\%$ of the males survived. After a month, the survived males were examined to check the presence of 'appendix masculine' (male organ structure). It was found that $84.7 \pm 3.5\%$ of the candidates were successfully reverse to female as their 'genophore complexes' and 'appendix masculine' were absence. They were raised in tanks for six to eight months until maturity. Mating of these 'neo female' brood stocks with normal males at a ratio of 4 to 1 have produced $50.3 \pm 3.5\%$ berried stocks. Relative fecundity of the 'neo female' is comparable to the normal brood stock ranging from 408 – 1080 larvae/g brood stock. Result also showed that the success rate of producing the neo female was $22.3 \pm 2.6\%$.

SPECIAL SESSION ON EURASTIP: REINFORCING INTERNATIONAL COOPERATION ON SUSTAINABLE AQUACULTURE PRODUCTION BETWEEN THE EUROPEAN UNION AND COUNTRIES FROM SOUTH EAST ASIA

David Bassett, EURASTIP Project Coordinator

European Aquaculture Technology and Innovation Platform (EATiP), Liège, Belgium
david@eatip.eu

The European Asian Aquaculture Technology and Innovation Platform (EURASTiP) is a three year support action project, funded through the EU Horizon 2020 programme, and coordinated by the European Aquaculture Technology and Innovation Platform (EATiP).

EURASTiP will provide a structured basis for multi-stakeholder dialogue in the aquaculture community between South East Asia and the EU.

The EU is the hub of the largest global market in seafood, of which circa 65% is imported, the majority being from Asia. With capture fishery yields proving static and increasingly subject to further regulation, global seafood markets must look to aquaculture to meet the forecast increase in demand from consumers. The FAO believes that global population growth will require an increase in aquaculture production of 1.5 million tons to satisfy demand. Modern commercial aquaculture practices exist using many processes and technologies that were developed originally in Japan and later on in other regions in the world including Europe, where growth and development has stagnated in recent years. Asia is by far the largest aquaculture production region in the world (circa 85% of all production). The driving question is “how to provide consumers with high quality, healthy seafood that is produced sustainably?”. A core objective of EURASTIP is to provide answers that enable long-term sustainable growth of aquaculture, promoting a win-win situation for all stakeholders.

Priorities for the EURASTiP project include: food safety; sustainability; environmental and social impacts; consumer issues (including the scope of production standards, product quality and conformity); aquatic animal health and welfare. These components are integral to developing the potential of commercial opportunities between European and SE Asian professional interests.

Further consideration will be given to: sharing of best practices and experience in SME development and financing; capacity building and technology transfer in aquaculture and food products; and the promotion of mobility of students/academics and strengthening of collaborations between educational organisations.

Consideration will also be given to developing and strengthening networks facilitating employment opportunities, work placements, etc.

The EU experience proves that achievement of such multiple development objectives is best facilitated through a multi-disciplinary and multi-stakeholder approach. The European Union has many successful examples, notably with the European Technology Platforms, where professionals, researchers, administrators and NGOs work together, providing a bottom-up approach to issue identification, needs and research prioritisation and problem-solving. A prerequisite for achieving successful cooperation between Europe and South East Asia is therefore to provide the necessary structural support to allow for constructive and pro-active dialogue between the industry and relevant stakeholders at both the national and international levels.

This session aims to introduce this new EU project to Asian stakeholders, review the different work packages and explore opportunities for concrete interaction between SE Asian and European stakeholders.

MASS PRODUCTION OF ROTIFERS (*Branchionus calyciflorus*) IN OUTDOOR CULTURE SYSTEM FOR AQUARICULTURE

Mangesh M. Bhosale, S. Felix, Mahadevi and Cheryl Antony

Fisheries College & Research Institute, TNFU, Ponneri (TN) 601 204

In the present study, mass production of freshwater rotifer, *Branchionus calyciflorus*, using *Chlorella vulgaris* food type was investigated. *C. vulgaris* stock was inoculated using agar plating method while egg bearing rotifer stock was prepared using pipetting method from wild and allowed to grow in 1L capacity plastic bottles fed with Baker's yeast for 5 days. Outdoor cement cistern tanks (1000L capacity) were fertilized using ground nut oil cake (250g), urea (10g) and single super phosphate (5g) and *C. vulgaris* was inoculated on the same day. Cultured *C. vulgaris* was exclusively used to feed rotifers at the intervals of 2x and 3x per day in continuous culture method. The highest population growth was attained with 3x feeding interval of *Chlorella* sp. (80-100 individuals/ml), followed by 2x (60-80 individuals/ml). The least population growth of *B. calyciflorus* was recorded in control (15-20 individuals/ml) where no feeding was done. The highest cell density of *C. vulgaris* was observed on 5th day onwards which is continued upto 15th day of culture and then again the fertilizers were added for culture continuation. The peak population growth was attained on day of 5 for *Chlorella vulgaris* (50000 cells/ml) and on 8 for Rotifers (130 individuals/ml). This study indicated that the quantity and quality of food have significant role on mass production of rotifer in ornamental fish culture.

EFFECT OF BIOTRONIC TOP LIQUID ON GROWTH PERFORMANCE AND DISEASE RESISTANCE IN NILE TILAPIA (*Oreochromis niloticus*)

Ram C. Bhujel¹, Anusha Perera¹, Antonia Tacconi², and Rui A. Gonçalves²

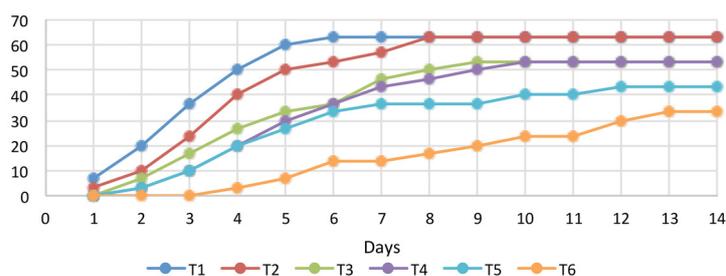
¹Aqua-Centre, FABS, SERD, Asian Institute of Technology (AIT), Thailand

²BIOMIN Holding GmbH, Erber Campus 13131 Getzersdorf, Austria

An 84-day feeding trial was conducted to determine the effects of Biotronic® Top liquid on growth performance and disease resistance on Nile Tilapia (*Oreochromis niloticus*). The trial was conducted using 18 tanks, of 100 L water volume with aeration. Each tank was initially stocked with 45 male Nile tilapia with the average weight of 16.17 ± 0.40 g (mean \pm SE). Biotronic liquid (BIOMIN Holding GmbH) is composed of three organic acids (formic acid, propionic acids and acetic acids) and the BIOMIN Permeabilizing Complex. Three replications were used for six treatments of commercial diets supplemented at varying level of Biotronic Top Liquid i.e. 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 L of Biotronic® Top liquid per ton of feed. At the end of the feeding trial, immunological, biochemical and biometrical parameters were assessed. From the 56th day of feeding and onwards, the fish were immersed with 1×10^8 CFU concentration of *Aeromonas hydrophila*. The initial feeding rate was 4% of the total body weight, which was fed dividing into three meals daily. Part I of the experiment was to investigate the growth performance and feed utilization efficiency of Nile tilapia and Part II, was to analyze disease resistance during the challenge test when exposed to 1×10^8 CFU *Aeromonas hydrophila*.

Cumulative survival of tilapia decreased with the increasing dose of Biotronic® Top liquid (Fig. 1). Similarly, highest dose i.e. 2.5 L/ton (T6) showed 38.4% of higher ($P < 0.05$) weight gain and significantly lower FCR value as compared to that of control (T1). Total bacteria counts also showed a significant decrease ($P < 0.05$) after immersion compared to control treatment. Blood sample analysis also showed significant difference ($P < 0.05$) with control. Therefore, present experiment suggests that the highest dose of Biotronic Top Liquid i.e. 2.5L/ton tested is the best in terms of growth rate, feed conversion rate and survival. However, more research can be done to evaluate the effects of higher dosages, bearing in mind the economics within a commercial aquaculture system.

Fig. 1 Accumulated mortality rate (%) of tilapia after challenged with *Aeromonas hydrophila*



AQUACULTURE AND FISHERIES EDUCATION, RESEARCH AND EXTENSION IN CHANGING CONTEXT

Ram C. Bhujel¹

Aqua-Centre, Asian Institute of Technology (AIT)
Klong Luang, Pathumthani 12120, THAILAND
Presenting author's email: Bhujel@ait.ac.th

Aquaculture as well as fisheries has attracted a lot of public attention due to remarkable role it has played in food and nutrition security, and overall livelihood of millions of people. At the same time, private sector is increasing investment in aquaculture because of its profit potential and technological advancement. While there is a need to double the annual aquaculture production by 2050, the industry is suffering by frequent occurrence of diseases with high mortalities (e.g. shrimp) often causing its collapses. Several other problems have emerged recently e.g. genetic deterioration in carps, decline in fishmeal supply and impact on environment. Aquaculture is often blamed for environmental damages. Changing climate and associated disasters are even threatening the industry. However, there is hope due to advancement in technologies such as biotechnology, genetic improvement, probiotics, and vaccines, which could make the industry sustainable and even take it to a higher level.

In order to address the issues, and leap forward education, research, and extension services need to be well functioning. Success may depend on how well they cooperate among themselves in sharing and transferring knowledge, research outcomes, and experiences. In traditional model, education was designed to provide knowledge to the graduates most of whom were expected to join research and extension institutions to provide services to the farmers or the end users.

Research institutions are supposed to dedicate to carry out innovative research based on the need and then provide the outcomes to the extension agents. However, in reality, instead of cooperating these organizations are competing each other and their activities are overlapping. On the other hand, ultimate-users i.e. farmers do not appear in the traditional model. They should be placed in the middle, and all of these institutions should try to reach them so that they are supported adequately (Fig. 1). It means education and curricula need to be farmer-oriented. Situation has changed and more graduates need to become farmers who should be able to improve farming. This would also help solve the problem of unemployment; which is becoming increasingly serious among new graduates. Similarly, research organizations need more practical field-oriented than lab-based high-tech type research. But long-term strategic research, and commercialization of technologies is lacking in Asia. Extension organizations often overlook research and pass research outcomes to farmers without testing in scientific way. They need to be regularly updated and develop packages of ready-to-use technologies.

In addition, private sector and non-government organizations (NGOs) are also duplicating these roles. In some cases doing effectively, but often misleading public with biased statements and some times with exaggerations. Careful coordination of all stakeholders is required to have a sound and sustainable sector to double its production by 2050.

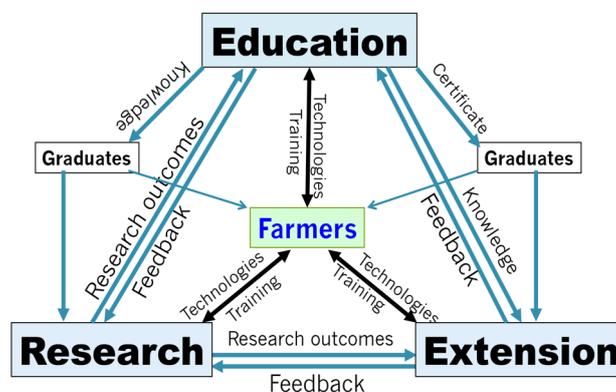


Fig. 1 Model showing relationship between Education, research and extension

PREVALENCE OF *Vibrio* sp. IN CULTURED WHITELEG SHRIMP (*Litopenaeus vannamei*) ASSOCIATED WITH CLINICAL SIGN OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

Iftikhar Ahmad A. R.*, Kua B. C., Kamisa A., Nur Ashikin A. & Nuruljannah H.

National Fish Health Research Division
Fisheries Research Institute Batu Maung
Department of Fisheries Malaysia
11960, Bayan Lepas, P. Pinang, Malaysia
iftikhar@dof.gov.my

Acute Hepatopancreatic Necrosis Disease (AHPND) is a bacterial disease caused by *Vibrio parahaemolyticus* and has caused high mortality in farmed Whiteleg shrimp. It appears within 30 days of stocking and causes symptoms such as lethargy, empty stomach and midgut, pale and atrophied hepatopancreas, darkened shells and mottling of the carapace. This study was conducted to relate the prevalence of *Vibrio* sp. in cultured Whiteleg shrimp associated with AHPND's clinical signs.

An epidemiological study of AHPND was conducted in one Whiteleg shrimp's (*Litopenaeus vannamei*) culture facility in Perak, Malaysia from April until June 2016. Sampling of shrimps were done on periodically basis on day of culture (DOC) 28, 62 and 85 involving gross observation, bacteriology and Polymerase Chain Reaction (PCR) analysis of each shrimp's hepatopancreas sample.

Results showed that 8.89% of 90 shrimps examined had clinical signs of AHPND. Analysis of PCR using AHPND AP4 kit showed all samples were negative. However, the presence of bacterium *V. parahaemolyticus* was high (90%) at DOC85, 60% and 37% at DOC62 and 28; respectively. Apart from *V. parahaemolyticus*, other bacteria found were *V. fluvialis*, *V. alginolyticus* and *V. vulnificus*. Prevalence of *V. fluvialis* at DOC28, 62 and 85 were 13, 27 and 60%; respectively while for *V. alginolyticus* ranging from 30 to 43%. *V. vulnificus* was found at DOC62 and 85 with prevalence of 33 and 10%; respectively.

This study showed that the presence of *V. parahaemolyticus* will not always coincide with the presence of bacterial toxin plasmid although the clinical signs of AHPND were visible in sampled shrimps.

EFFICIENT REPLACEMENT OF LECITHIN IN WHITE SHRIMP (*Litopenaeus vanammei*) DIETS WITH LYSOFORTE™ DRY (LYSOLECITHIN) SUPPLEMENTATION

Lakshmbai Vasanthakumari Bindhu* and C. Sugumar

Kemin Industries (Asia)Pte Ltd
12 Senoko Drive, Singapore
B.L.Vasanthakumari@kemin.com

Dietary phospholipids are essential for the normal growth and survival of shrimp. LYSOFORTE™ Dry is a biosurfactant enriched with lysophospholipids used in animal feeds to improve fat digestion and absorption. This paper presents the results of a study which evaluated the effect of LYSOFORTE on the growth performance and nutrient digestibility of white shrimp (*Litopenaeus vanammei*) fed diets with partial replacement of lecithin. Treatments consisted of (1) Positive control diet (2% lecithin), (2) Negative control diet (1% lecithin), (3) Positive control diet + LYSOFORTE (0.1%) and (4) Negative control diet + LYSOFORTE (0.1%). On top addition of LYSOFORTE resulted in 1.4% and 4% improvement in lipid and protein digestibility respectively. These improvements are also coupled with consistent improvement in shrimp production by 25g/aquarium, specific growth rate by 0.12% and body weight increase of 0.67 g/shrimp after 8 weeks. Negative control diet with LYSOFORTE showed enhanced protein and lipid digestibility of 12.6% and 7%, respectively, ($p < 0.05$). Interestingly, LYSOFORTE addition improved the hemolymph protein concentration, signifying its role in improving nutrient digestibility. When added to negative control diets, LYSOFORTE increased shrimp production by 51g, body weight by 1.35g/shrimp and specific growth rate by 0.25% after 8 weeks. Survival rate of shrimp improved by 4%, when LYSOFORTE was used to replace lecithin. LYSOFORTE addition to shrimp diets thus helps to enhance nutrient digestibility, resulting in improvements in growth performance of shrimp and supports economical shrimp production.

COMMUNITY BASED LAKE RESOURCES MANAGEMENT: A SUCCESSFUL MODEL IN LAKES OF POKHARA VALLEY, NEPAL

Jay Dev Bista*, Akbal Hussain and Ram Kumar Shrestha

Fisheries Research Centre
Pokhara, Nepal
jdbista@gmail.com

Nepal is rich in natural water resources and fishing is a long standing tradition in these waters. This paper mainly focuses on eutrophic lake Phewa (500 ha.) and highly eutrophic lake Rupa (100ha.) which are located in Pokhara valley, western mid hill of Nepal. Unplanned developmental activities, environmental changes, lack of proper management, these lakes are environmentally degraded threatening the aquatic biodiversity and livelihood of traditional dependents.

Community were encouraged and has realized about the importance of lake resources and form a legal community base organization (CBO) in Lake Phewa (300 members) and Lake Rupa 670 members, which act as a community base participatory co-management approach. Active participation of communities in cleaning the aquatic weeds, protection of spawning ground and habitat of local fishes, awareness campaign directed to prevent water pollution, stocking of fish in open water for capture fisheries. Besides these activities CBOs have launched eco-friendly plankton base cage aquaculture program in lake Phewa and producing 150 mt.(2014) fish from 650 (5m+5m+2m) floating net cages for their livelihood. The cooperative of lake Rupa after failure of cage aquaculture due to high infestation of aquatic plant over the lake, cleaning of aquatic weed and stocking of herbivorous fish control the weed and increase open water fish production from 6mt(2000) to 65mt(2008) and 90mt(2014). This income from fish is shared among cooperative member as well as to support in social welfare, education for disabled and conservation of Lake. These lakes are common property and are assessable for all; therefore, stakeholders prioritize their role and responsibilities and clear understanding among themselves. Community based participatory management not only supports livelihood of poor section of community living around the wetland but also contributes in maintaining aquatic ecosystem and conservation of biodiversity in sustainable way.

EFFECTS OF VDS FEED ADDITIVE MIX ON THE GROWTH PERFORMANCE OF *L. vannamei*

Koen Blanchaert*, Lodewijk Rosseel

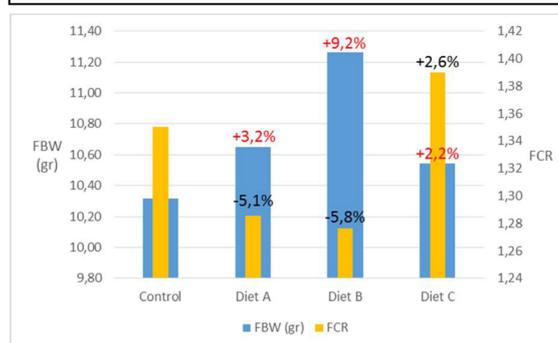
VDS nv
Paanderstraat 40
Deerlijk, 8540
koen@vds-afs.be

Improving the growth results of shrimp without a reduced survival rate is an important goal in shrimp farming. Certain feed additives can improve the gut health of the shrimp, creating beneficial conditions for optimal growth. Over the last 4 years VDS has been testing different combinations of additives used in aqua feed to find an optimal combination with effect to shrimp growth. In this paper we present our last trial with the additive mix resulting in the best growth performance. In this trial VDS selected 3 different mixes of several specific feed additives for a grow-out trial to compare their influence on standard growth parameters like FCR, Final Body Weight and survival.

A 55 days feeding trial was conducted in our in-house research facility in Belgium. 8 500l tanks in our recirculation system were stocked with *L. vannamei* PL₃₈ at a density of 140 shrimp/tank (180 shrimp/m²). The water temperature was maintained at 29°C ± 1 °C. The salinity was kept at 20 ppt throughout the whole trial. Other parameters like pH, ammonia and nitrite were monitored daily. 3 different additive mixes were tested against a negative control diet. Each diet had 2 replicates. The inclusion level of the additive mixes was 0.4%. The additive mixes were added on top of the negative control diet powder mix. Afterwards the mash was pelleted. The goal of this trial was to examine the effect of the additive mixes on the growth and survival of *L. vannamei* juveniles. The final body weight and survival were recorded at the end of the trial and the FCR and average weekly gain were calculated.

After 55 days diet B resulted in a significant better final body weight (FBW) and average weekly gain (AWG) than the negative control diet (Figure 1 and table 2). Overall survival was higher than 90%. There was no difference in survival between the treatments. The FCR was significantly higher in the control group compared to treatments B and A which is to be expected when a higher FBW is observed at the same survival rate. We can conclude from this trial that the treatment with additive mix A has the highest potential for improving the technical results of shrimp farming.

Figure 1. Final Body Weight (FBW) and FCR and the relative difference in FBW and FCR compared the control of *L. vannamei* fed 3 different additive mixes.



Tabel 1. Final body weight (FBW), average weekly gain (AWG), FCR and survival rate of *L. vannamei* fed 3 different additive mixes.

	Diet			
	Control	A	B	C
FBW (g)	10,32 ^a	10,65 ^{ab}	11,27 ^b	10,54 ^{ab}
AWG (g)	1,31	1,36	1,43	1,34
FCR	1,35	1,29	1,28	1,39
Survival (%)	91,1	93,9	92,5	92,1

EMULSIFIERS DO LITTLE TO ENHANCE THE PERFORMANCE OF BARRAMUNDI *Lates calcarifer*

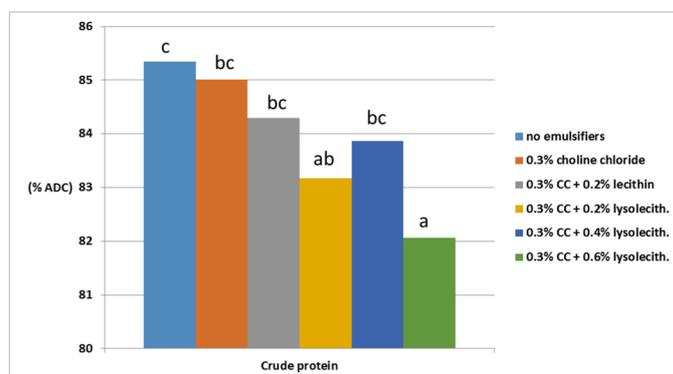
Mark Booth*, Simon Tabrett and Richard Smullen

NSW Department of Primary Industries
Taylors Beach, NSW, 2316, Australia
mark.booth@dpi.nsw.gov.au

This study was designed to test the efficacy of choline chloride (CC), lecithin powder or lysolecithin on the performance of juvenile Barramundi (aka Asian seabass; stock weight = 230g). Six diets were formulated with the same basic ingredient structure, with major protein sources being fishmeal (20%), poultry meal (18%) and blood meal (10%). Lipid inclusion was dominated by poultry oil (18%) and fish oil (5%). Carbohydrate sources included wheat (15%) and de-hulled lupins (7%). Monosodium phosphate was incorporated in all diets at 1.2%. Choline chloride, lecithin and lysolecithin supplementation varied according to the experimental design and ranged from 0.1 to 0.6%, however the design was not factorial (see figure).

The 13 week saltwater experiment was performed under controlled conditions (water temperature $\approx 27^{\circ}\text{C}$) in an indoor laboratory with 3 replicate tanks assigned to each diet. The first stage of the study focused on feeding (apparent satiation for 89 days) and growth response. The second stage was dedicated to collecting faecal material in order to determine the digestibility of each feed (31 days). Fish were sacrificed at the conclusion of the trial for compositional analysis and to determine other biometric indices including condition factor, hepatosomatic index (HSI), viscerosomatic index (VSI) and fillet yield.

The individual growth rate of Barramundi was acceptable and ranged between 4.8 – 5.3g day⁻¹. There were no significant differences among growth rates due to different diets (mean SGR = 1.33% d⁻¹) and we found no significant difference between feed intake or FCR of different diets (all FCR < 1:1 as fed basis). Likewise, there was no evidence of any differences in condition factor, HSI, VSI or fillet yield among dietary groups. The apparent digestibility coefficients of diets were high, but there was no difference among diets with respect to dry matter ($\approx 72\%$), lipid ($\approx 95\%$) or gross energy ($\approx 83\%$) digestibility. Interestingly, there was a significant difference in protein digestibility among diets, but it was difficult to relate this to the effect of single emulsifiers or the particular combination of emulsifiers examined in this study (see figure). Based on the results of the growth trial, it appears there is little advantage to adding choline chloride, lecithin or lysolecithin to feeds for Barramundi. Additional investigation of the data from this study is underway and will be discussed; particularly the digestibility of individual fatty acids and the retention of protein, lipid and energy in whole fish carcass. This data will shed further light on the efficacy of emulsifiers in aquafeeds for Barramundi, especially their role in enhancing lipid digestibility or as a source of phospholipids.



INCLUSION OF SOYBEAN MEAL AND SOY PROTEIN CONCENTRATE IN FEEDS FOR ASIAN SEABASS *Lates calcarifer*

Mark Booth*, Marc Campet, Jorge Alarcon, Lukas Manomaitis

NSW Department of Primary Industries
Taylors Beach, NSW, 2316, Australia
mark.booth@dpi.nsw.gov.au

Asian seabass *Lates calcarifer* (aka Barramundi) is an important aquaculture species in South East Asia (SEA) and Australia. They readily accept commercial aquafeeds containing a variety of animal and plant meals and are prime candidates for fish meal replacement. NSW DPI and the United Soybean Board of America (USB) have completed several research projects with Asian seabass. The research was aimed at determining the appropriate dietary inclusion of soybean meal (SBM) and soy protein concentrate (SPC) and assist SEA aquafeed companies develop sustainable, low-fishmeal aquafeeds for Asian seabass. To date this research has determined the apparent digestibility of SBM and SPC by Asian seabass as well as the digestibility of other ingredients including poultry meal, blood meal, corn gluten, wheat and gelatinised wheat starch. The digestibility of lower quality fishmeal has also been evaluated as this issue is problematic for feed manufacturers in SEA. The data on digestibility of ingredients was used to formulate a series of semi-commercial aquafeeds for Asian seabass which contained high dietary levels of SBM (up to 38%), or SPC (up to 60%), or feeds that contained a blend of both ingredients. These aquafeeds, along with appropriate controls, were tested on Asian seabass in tank trials in Australia. The successful outcomes of the tank based research resulted in the USB funding a sea-cage verification trial with Asian seabass in Vietnam. This research involved collaboration between NSW DPI, Marine Farms Vietnam (Van Phong Bay, Nha Trang, Vietnam) and the Ocialis Feed Company (Ho Chi Minh City, Vietnam). The aim of the field trial was to evaluate a range of aquafeeds containing high levels of SBM and SPC and benchmark these feeds against an industry standard. Our presentation will review the results of tank and field-based trials that tested the incorporation of SBM and SPC in aquafeeds for Asian seabass. It will also discuss the biological and economic outcomes of the field trial and the implications these have for the Asian seabass industry.

SUSTAINABILITY AND COMPLIANCE SOLUTIONS FOR ASIAN AQUACULTURE MARKET/VALUE CHAIN: INTRODUCING VERIFIK8

Emmanuelle Bourgois*

Fairagora Asia, 3rd Floor, Room 3/1, 94 Shinawatra tower Sukhumvit Soi 23, Klong Toei Nuea, Watthana, Bangkok 10110, Thailand

Sustainable aquaculture production is powered by the surge in consumer base as well as the drive for products that deliver quality and leave little impact on the environment as a result of its production. Regulations are being tightened hence the need to ensure that ingredient source as well as production process are truly sustainable. The need for a seamless flow between raw material sourcing, the production process and the marketing of products while still ensuring compliance with little effort is indeed the next level in terms of compliance monitoring. VerifiK8 helps seafood buyers to make safer and more responsible procurements through an affordable yet credible monitoring and verification software platform to de-risk operations in their supply chain.

VerifiK8 software uses mobile devices to collect and digitalize data directly from farms and cooperatives. It crosschecks information with other data streams to provide solid analytics and benchmarking of each farm or cooperative against the relevant sustainability standard. The system makes all aggregated data easily accessible on a single dashboard, from the farms to the buyers' desktop. Our unique technology allows a cost and time effective analytics and verification process to make certification more inclusive to medium and small-scale farms and fisheries. It builds trust and transparency between producers and buyers by de-risking procurements and improving farming practices and product quality. It secures aquaculture farms and fisheries' market outreach by proving their level of social and environmental compliance. It reduces labor and environmental risks, while increasing food safety and productivity.

In order to do these, VerifiK8 works through three channels: Data collection, Data analytics and on-Farm verification. The service suit also includes incentives for farmers.

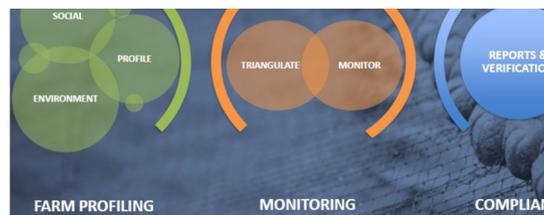


Figure 1: Verifik8 process flow



Figure 2: Incentives for Farmers/Fishers



Figure 3: Future outlook

ADDRESSING LABOUR ISSUES IN THE SOUTHEAST ASIAN SEAFOOD SUPPLY CHAIN

Emmanuelle Bourgois* (On behalf of the Digital Social Monitoring Expert Group)

Fairagora Asia, 3rd Floor, Room 3/1, 94 Shinawatra tower Sukhumvit Soi 23, Klong Toei Nuea, Watthana, Bangkok 10110, Thailand

Food safety, social responsibility, animal welfare, environmental sustainability and traceability are the core of responsible shrimp trade. Metrics and indicators exist for food safety, animal welfare, environmental impact and traceability. Social compliance in the seafood supply chain is rapidly emerging and new. 'Nowadays there is limited experience and research in social compliance in the seafood supply chain. To foster improvement in social, labor and human rights compliance within the seafood supply chain, social benchmarking should be developed to fit aquaculture farms and boats' needs. This means that relevant indicators must be identified and accordingly, new tools for social monitoring would be designed.

The digital social monitoring expert group aims at exploring digital social monitoring solutions to foster social and labor improvement in the seafood supply chain. The expert group is dedicated toward doing that by:

- Scoping for business implementation and civil society relevance
- Identify social performance indicators and matching metrics
- Deploying on-site digital monitoring systems as information source
- Continuous risk assessment to debug the digital monitoring system
- Co-creation process involving synergy and support

Currently there are 12 metric categories being developed

- Workers' representation
- Grievance Mechanism
- Child Labour
- Fair recruitment
- Fair remuneration
- Decent working hours
- Non-discrimination and Gender issues
- Health & Safety
- Fair Disciplinary procedures
- Community development
- Freedom of Movement
- Forced Labour

The metrics are expected to link environmental monitoring and social risk to compliance while also creating room for corporate social responsibility that engenders an in-depth evaluation of issues with surrounding communities concerning resource use.



A CRITICAL LOOK AT ESTIMATES OF APPARENT DIGESTIBILITY OF PROTEIN AND AMINO ACIDS

Dominique P. Bureau* and Guillaume Pfeuti

Fish Nutrition Research Laboratory
Dept. of Animal Biosciences, Ontario Agricultural College
University of Guelph
Guelph, ON, N1G 2W1, CANADA
dbureau@uoguelph.ca

Information of the apparent digestibility coefficient (ADC) of nutrients of different ingredients is increasing every year thanks to sustained research efforts. Estimates of ADC are regularly compiled in the reference literature and increasingly used by feed manufacturers who are now formulating their feeds on a digestible protein and amino acid basis. This progressive move from formulating on a 'total nutrient' basis to formulating on digestible nutrients is praiseworthy. However, increasing reliance by feed millers on published estimates of ADCs makes it critical to ensure that the information available is relevant and reliable.

ADCs of protein and amino acids are reported for a large number of ingredients fed to different aquaculture species. Significant variability is observable across studies and species. Some of the data suggest significant differences in ADCs of protein among species for the same ingredient. Differences in methodology and intrinsic variability in the composition of different batches of the same ingredient make it difficult to meaningfully compare ADCs across studies, let alone across animal species. A critical review of results from digestibility trials suggest that methodological shortcomings (e.g. marker analysis) and calculation errors (biased equations) result in published estimates of ADC that are not always highly reliable. There is frequently a need to examine the original data from trials to be able to identify sources of errors but this is rarely feasible for published studies.

The University of Guelph Fish Nutrition Research Laboratory (UG-FNRL) has been a pioneer in the assessment of the digestibility of fish feed ingredients. Cho and Slinger (1974) published reliable estimates of ADC of protein and energy to rainbow trout of practical ingredients. The sustained use of the same equipment and methodology by the UG-FNRL has highlighted significant improvements in ADC of protein of some ingredients over the past 40 years. These efforts highlighted significant variability in ADCs of protein and amino acids across different lots or batches of the same type of ingredient. These efforts also indicated that digestibility is a measure of "disappearance" of nutrients but not a direct measure of bio-availability of nutrients. Consequently, ADC of nutrients should ideally be corroborated by more direct assessment of the bioavailability of nutrients of ingredients. The processing and drying equipment and conditions used in the production of ingredients were observed to have a determinant effect on the digestibility of protein. An *in vitro* assay examining the susceptibility of protein to degradation by intestinal enzymes suggests the differences in ADC of protein observed for similar ingredients have a rational biochemical basis. However, the chemical determinants of digestibility are not well understood and characterized. Recent research efforts suggest that the level of protein cross-links present in feed ingredients may be strongly correlated with their digestibility of protein and bio-availability of amino acids. Protein crosslinks are either naturally occurring in proteins or formed as a result of heat or chemical treatment/damage. Protein crosslinks are mostly found as 1) disulfide crosslinks involving thiol-disulphide interchange reactions, and 2) amino acid cross-links derived from reactions between certain amino acids and chemical compounds. A simple processing technique targeting the disruption of disulphide cross-links was highly effective in improving the nutritional quality of relatively poorly digestible protein ingredients. Better understanding of the chemical determinants of protein digestibility should enable the development of rapid methods and tools to more reliably estimate the digestibility and nutritive value of protein ingredients.

POTENTIAL USE OF CINNAMON *Cinnamomum* sp. AS PHYTOBIOTICS FOR ORNAMENTAL FISH

Christopher M.A. Caipang*, Benjamin Quek, Clara M. Lay-yag, Sakinah Mulyana

School of Applied Science
Temasek Polytechnic, 529757 Singapore
ccaipang@tp.edu.sg

Phytobiotics are plant-derived substances that enhance the innate immune system of the animal. Immunostimulants are used as prophylactic measures and are possible alternatives to the use of chemotherapeutants such as antibiotics. Recently, phytobiotics are gaining popularity in aquaculture as a means of stimulating the immune responses of both fish and crustaceans. Previous studies demonstrated the beneficial effects of a commonly used spice, cinnamon, *Cinnamomum* sp. Cinnamon is known for its antimicrobial effects, and when incorporated in the diets of some food fish, this spice enhanced the growth performance and feed utilization. However the effects of cinnamon as an immunostimulant has not been widely studied in ornamental fish. Hence, these series of studies determined the effectiveness of cinnamon as an immunostimulant in ornamental fishes both *in vitro* and *in vivo*.

Commercially available cinnamon powder was obtained and a medicated ornamental fish feed using 1% of the fine powder was prepared by top dressing. Dietary administration of the medicated feed to two popular ornamental fish species, namely guppy, *Poecilia reticulata* and platy, *Xiphophorus maculatus* was done at 8% body weight for 15 days. Fish fed with the non-medicated feed were used as control for the experiment. Selected immune responses and anti-oxidant activity from tissue homogenates of the fish were assessed at 0, 3, 7 and 15 days during feeding. Experimental bath challenge with a bacterial pathogen was done after the duration of feeding with the medicated feeds and efficiency of the phytobiotics in protecting the fish was assessed by the relative percentage survival. Bacterial clearance and detection of the pathogen from the infected survivors was done by streaking tissues samples onto thiosulfate-citrate-bile salts (TCBS) agar.

Preliminary results showed modulation of the innate immune responses and anti-oxidant activities following dietary administration of cinnamon and the responses were species-dependent. Significant protection in the treated fish was observed following infection with a pathogenic bacterium in comparison with the control fish. Lower incidence of the bacterial pathogen was also obtained in infected survivors fed with the medicated feed compared with the infected control fish. Taken together, these results demonstrated the beneficial effects of cinnamon in ornamental fish by boosting their immune responses could further be developed as an immunostimulant for the ornamental fish industry.

DEVELOPMENT OF AN AUTOGENOUS BACTERIAL VACCINE AGAINST PATHOGENIC *Vibrio alginolyticus* AND AN ASSESSMENT OF ITS PROTECTIVE EFFICIENCY IN RED TILAPIA HYBRIDS *Oreochromis* sp.

Christopher M.A. Caipang*, Joel Tan, Clara M. Lay-yag, Sakinah Mulyana

School of Applied Science
Temasek Polytechnic, 529757 Singapore
ccaipang@tp.edu.sg

Several vaccines against bacterial diseases in fish have been developed and some of these have been routinely administered on a commercial scale. However, a single vaccine for a known bacterial pathogen may not confer high protective efficacy as there may exist various serotypes of a particular pathogen. Using red tilapia hybrids, *Oreochromis* sp as a model fish species, the study aimed to develop an autogenous bacterial vaccine against *Vibrio alginolyticus*, to implement a vaccination strategy for the delivery of this vaccine in fish and to assess the effects on the immune responses and protective efficiency of this vaccine in fish following experimental challenge with the pathogen.

Vibrio alginolyticus, isolated from local fish farm, was cultured in broth to a density of at least 10^8 colony forming units (CFU) ml^{-1} , harvested, heat-inactivated at 70°C for 24 h and diluted with phosphate buffered saline (PBS) to a final density of 10^9 CFU ml^{-1} . Intraperitoneal (IP) and intramuscular (IM) vaccine trials were conducted using 15-20 g red tilapia hybrid juveniles, each receiving 100 l of the heat-killed bacterial vaccine. Control tilapia were injected with the same volume of 1x PBS. Plasma samples were obtained from both the vaccinated and control fish at 0, 2, 15 and 30 days post-vaccination to assess the effects of vaccination on the immune responses and anti-oxidant activity. Protective efficiency of the vaccine in fish was determined following experimental challenge with live pathogen (100 l of 10^6 CFU ml^{-1} bacterial cells) by intraperitoneal injection and survival rate was monitored for a period of 10 days.

Immune responses of the fish were differentially modulated following vaccination and was affected by the route of vaccination. Anti-protease activity, total serum globulin levels, bacterial inhibition activity and scavenging activity were up-regulated during the early stages post-vaccination using the IM route, while the lysozyme, myeloperoxidase and alkaline phosphatase activities were up-regulated at a later time period post-vaccination. For fish vaccinated via the IP route, various components of the immune responses including amount of total serum globulin levels, myeloperoxidase, lysozyme, alkaline phosphatase, protease, bacterial inhibition and scavenging activities were up-regulated at different time periods post-vaccination. The autogenous vaccine resulted in high protective ability in fish following experimental challenge with the bacterial pathogen having a 75% and 87% relative percent survival (RPS) for the IP and IM vaccination, respectively. Taken together, the results showed the efficacy of developing an autogenous bacterial vaccine for fish. Further studies are required to better understand the effects of the vaccine on the adaptive immune responses of the fish and to assess cross-protective ability of this type of vaccine to various serotypes of the pathogen.

TEMPERATURE EFFECTS ON PERFORMANCE OF LARGE ATLANTIC SALMON (*Salmo salar*) HELD IN SEAWATER RECIRCULATION AQUACULTURE SYSTEMS

Chris G. Carter*, W.G. Nuez-Ortin, B.M. Codabaccus, M. Grünenwald, L. Adams, M. Adams, P. Hilder

Institute for Marine and Antarctic Studies (IMAS)
University of Tasmania
Hobart, Tasmania, Australia, 7001
Chris.Carter@utas.edu.au

Climate change and increasing environmental variability mean that Atlantic salmon are increasingly exposed to sub-optimum environmental conditions throughout the global range of their aquaculture. Recirculation aquaculture systems (RAS) is a technology with potential to remove both environmental extremes and variability for some, or all, of the production cycle. The development of commercial seawater RAS is underway but still requires research to improve the technology. In addition, RAS provides an important facility to understand the impact of sub-optimum environmental variables and their interaction with nutrition variables. The use of RAS as an experimental facility is applicable to tropical and temperature aquaculture research.

Experiments aimed to examine the effects of elevated temperature on aspects of feeding and nutrition of seawater pre-harvest Atlantic salmon of 1 to 4 kg in weight. For all experiments, Atlantic salmon grown on site for at least 6 months were distributed amongst 12 recirculation aquaculture systems each consisting of a 2500 L circular tank and associated recirculation and environmental control systems. A reference seawater temperature of 15°C was used. Feed intake was calculated each day and fish were measured for wet weight, fork length and external condition.

The effect of elevated temperature on the growth performance, protein metabolism, lipid utilization, fatty acid composition and pigmentation of large seawater Atlantic salmon held in RAS technology will be presented and discussed.

STUDY TO DETERMINE ATTRACTIVINESS, DIGESTIBILITY, GROWTH CONVERSION, HEALTH OF PL/JUVENILIES AND WATER QUALITY IN NURSERY SYSTEMS. COMPARING EPICOREBIONETWORK EPIBAL 500 & 700 VS PL FEED MILL FEED

*Fernando Castro, Roberto Moreira, Fabrizzio Vanoni, Fernando Garcia

Epicore BioNetworks Inc./Behn Meyer Chemicals Malaysia
4 Lina lane, Eastampton, New Jersey 08060, U.S.A.
www.epicorebionetworks.com
fcastro@epicorebionetworks.com

Shrimp farming in South East Asia has suffered significant losses in the past years due to low shrimp prices and outbreaks of WSSV, EMS viruses and EHP microsporidian a bigger problem spreading thru Asia causing slow growth, high FCRs in vannamei shrimp intensive ponds.

Farmers in Asia are implementing nursery/raceway systems or third phase additional system at the farm site. They develop nursery technology to improve the management of better care to PLs in the first stages of transition from hatcheries to farm ponds producing a stronger juvenile.

The need for high quality hatchery/nursery feeds during the first 10 days of culture in the nursery production cycle is required to produce a high quality juvenile for the ponds.

This paper discusses the benefits gained in feeding Epicore USA Epibal 500 & 700 as compared to conventional PL Feed Mill feed fines.

It has been eight years of technology development that led the industry to improve the design of these systems and their management. The use of better high quality feeds and probiotics has played a key role in increasing survivals by overcoming WSSV, EMS viruses and EHP microsporidian diseases, extending the residence time in the raceways/nurseries thereby improving nutrition and maintaining water quality in nursery production systems.

On September of 2016 a trail was done in Ecuador South America at Omarsa nursery farm systems to compare the benefit results of Epibal 500 and 700 versus Feed Mill feeds used for the first 10 DOC duration stage of PL stages to juveniles.

The direct benefit results achieved after 10 days of culture by using Epibal feeds are large PL growth sizes of 40 PLs/gram, more biomass of PLs harvested 34.87 kilos, lower FCR 1:1.029, consistent high-level 3 of lipids 97%, normal tubules no stress, and good digestibility or 74.3% fullness of guts/0% empty gut and higher survival results 92.98%.

Epibal feeds are high in lipids and protein reducing the need of live artemia or other high quality hatchery feeds during the first days of culture. The good digestibility and efficiency of Epibal feeds reduces the waste and maintains in good water quality conditions.

Epibal is an economic high quality dry diet for hatchery/nursery systems. Epibal has lower feed consumption and lower cost during the first 10 DOC as compared to Feed Mill feeds used with live artemia and other high quality hatchery feeds.

In Asia raceways or nursery system technology is growing fast and the need to improve nutrition during the third phase to produce a stronger more robust larve to challenge the WSSV, EMS and EHP occurring during days 50 to 60 DOC in ponds.

The technology in Asia for nursery technology was promoted in 2012 and over the last two years is growing fast the construction of nurseries in Vietnam, Thailand, Malaysia and other Asian countries.

ASIA SHRIMP CULTURE NEW TECHNOLOGICAL ADVANCEMENTS FOR VANNAMEI INTENSIVE CULTURE. HELPING TO IMPROVE PRODUCTION AND REDUCE COSTS

*Fernando Castro, Fernando Garcia, Fabrizzio Vanoni, Nurjeni Wahjudin, Huy Trang Quang and Bang Wang

EpicorebioNetworks Inc./Behn Meyer Chemicals Malaysia
4 Lina Lane, Eastampton, New Jersey 08060, U.S.A.
www.epicorebionetworks.com
fcastro@epicorebionetworks.com

Asia Pacific Aquaculture APA 2017
Kuala Lumpur, Malaysia July 24th - 27th 2017

During the last two years as Epicorebionetworks technical support I have visited hatcheries and shrimp farms in 10 Asia countries. Implementations of new technologies advancements at hatchery and grow out farm levels are improve pond survival, production yields and reducing production costs.

Shrimp farming in South East Asia has suffered significant losses in the past years due to low shrimp prices and continuous outbreaks of WSSV, EMS viruses and EHP microsporidian a bigger problem spreading thru Asia causing slow growth, high FCRs in vannamei intensive ponds.

This paper summarizes the new technological advancements being implemented in hatchery/shrimp farms in Asia to improve profits:

- At hatchery Alfa nauplii selection process.
- Probiotics use in intensive shrimp ponds.
- Nursery/Raceway Technology third phase systems.
- Automatic Feeders.
- Recirculation RAS farm system.

Hatchery- nauplii production unit collection of Alfa nauplii: From SPF brood stock nauplii selection protocol by phototropism. Lights are used in the nauplii holding tanks to select the strongest and best quality nauplii for PL production and better performance at farm level.

Light attraction harvest protocols are being used in hatcheries in Ecuador and now in Asia. Light separation and harvest of the strongest nauplii is done during the first 15 minutes to select the best quality nauplii. These nauplii are called Alfa nauplii that are stocking in PL production tanks. Post larvae survivals of 70% to 80% and better performance are noted.

Grow out results from Alfa post larvae in Indonesia northeast Java intensive farms. Uses automatic feeders, direct stocking of PL9 at 130-135 PLs/mt sq2 after 90 DOC days of culture harvest 45 shrimp/kilo and 70 DOC 66 shrimp/kilo, survival 85% to 90%. Feed conversion after 90 DOC is 1.1 to 1.2 FCR reducing production cost to \$3.0 USD/kilo and increasing production cycles to 4 per year. Increasing profits with low shrimp prices.

Results with non Alfa nauplii and no automatic feeders: Direct stocking of PLs, 130 PLs/mt2 after 110-115 DOC 3 cycles per year, harvest 30 shrimp/kilo or 35 tons/hectare with cost per kilo of \$3.75 USD.

Probiotics use in shrimp ponds: The practice of using probiotics in shrimp ponds has become a standard operating procedure for many years now. Consistent shrimp production results have been obtained with the use of high quality probiotics using proper application protocol.

In Ho Chi Ming City, Vietnam Epicorebionetworks farm product representative is OPS. The most consistent and successful farms are the once that use all of Epicore probiotics.

(Continued on next page)

Due to low shrimp prices many farmers are changing their probiotic protocols and purchasing cheaper price and quality probiotics brands leading to inconsistent production results. The investment of high quality probiotics and use in the 3 main applications: feed application to colonize the gut, water to colonize and control vibrio, and sludge or organic matter digestions sources of EMS and EHP. The good practice use of probiotics has lead to consistent and good harvest results in intensive farms in Vietnam.

Anh Khoa farms located in Lagi, Vietnam operates 4 farms with 70 ponds average size of 1.800 mt. sq., stock 200- 230 PL/mt², harvest 28 shrimp/kilo, 5 to 7 tons of shrimp in 105 DOC days of culture and FCR 1:1.3.

Epicore product protocol Epicin D in feed 4grs/kilo for gut health, 0-40 DOC days of culture Epicin use 70 grams/1800 mt sq pond every two days for vibrio control and 40 – 110 DOC days of culture use Epizym PST 227 grams/1800 mt sq pond every three days.

Probiotics are adjusted depending on water quaity and proper pond dry outs and live lime disinfection for EHP microsporidian are done between production runs.

Nursery/raceway technology: Third phase system at the farm site has been on going in the Americas for many years. Asia is now implementing nursery technology to improve the management of better care to PLs in the first stages of transition from hatcheries to farm ponds producing a stronger juvenile. It has been nine years of technology development that led the industry to improve the design of these systems and their management. The use of better high quality feeds and probiotics has played a key role in increasing survivals by over coming WSSV, EMS viruses and EHP microsporidian diseases, extending the residence time in the raceways/nurseries thereby improving nutrition and maintaining water quality in nursery production systems.

Improvements in shrimp production in Vietnam and Thailand has bee attributed to third phase systems. Nurseries are used to stock PLs and to harvest 0.6 to 1 gram size juveniles after 15 to 25 DOC. Compensatory growth of juveniles reduces the production cycle in the grow out phase to 60 DOC days of culture, harvesting 85% survivals and 40 to 50 shrimp/kilo.

Third phase nursery systems reduces production costs saving in feed 10% to 30% FCR feed conversion and reduces 45 DOC days of culture in grow out increasing turn over of ponds.

Production cost can be lowered from \$3.7 USD/kilo direct stocking of PLs to \$1.89 USD/kilo stocking 1 gram + juveniles. Juvenile stocking of ponds show fewer incidences of EMS virus and EHP microsporidian that occur after 50 to 60 DOC days of culture.

Automatic Feeders: The growing use and benefits of using Automatic feeders in intensive shrimp farms in Asia and extensive farms in Ecuador.

Automatic feeders are being manufactured in all Asian countries at around \$300 USD each. The benefits gained by using the feeders are reduction in manpower, lower feed conversion FCR and most of all the fresh application of feed on a continuous situation provides better quality feed to the shrimp.

In Indonesia intensive culture farm Mr. Sony, located in northeast Java uses automatic feeders and Alfa PLs, direct stocking of 130 PLs/sq. mt., pond size 3000-4000 sq. mts, partial harvest, production of 30 tons of 42 shrimp/kilo at 90 DOC days of culture with feed conversion 1:1.05 to 1: 1.1 FCR or 30% to 35% reduction in feed consumption.

Previous results broadcasting feed using normal PLs, direct stocking of 130 PL/mt. sq., partial harvest, production of 35 tons of 40 shrimp/kilo at 118 DOC days of culture with feed conversion 1:1.6 to 1:1.7.

Automatic feeders used in Asia are electric with 100 kilo feed capacity and are recharged once or twice a day with feed. Feeding frequency is 200 times per day at 1-second dispersemment of feed every 5 minutes, between 7 am to 12 mid nights. Best locations of the feeders are in the deep part of the pond at 7-meter distance from each other with platform access.

In Ecuador with extensive shrimp farms using automatic feeders in large ponds, stocking 7 to 8 Juveniles per meter square. Harvest 33 shrimp/kilo after 120 DOC days of culture with feed conversion of 1:1.2 FCR producing 1,5 tons/hectare of shrimp.

(Continued on next page)

They use solar powered automatic feeders; feeding frequency is 400 times per day at 1-second dispersement of feed every 5 minutes between 7 am to 12 mid night. Benefits reduces manpower and feed conversion ratio of 1:1.2 FCR or 25% saving in feed and better growth or less DOC days of culture.

Recirculation system RAS farm systems: The newest technology being implemented in few farms in Vietnam and Thailand with good consistent shrimp production results by recirculating the water and maintaining a more stable probiotics ecosystem and Aquamimicry balance of natural food and other marine species working together.

Recirculation RAS farm systems have been implemented in the past in Venezuela and Ecuador, South America in large farms with the use of probiotics to treat the effluent water producing more stable shrimp production results.

In Vietnam located in Can Gio area north of Ho Chi Minh City, Mr. Bang Wang farm is working for two years in developing the RAS Recirculation/Aquamimicry systems. The RAS farm consists of 4 production modules for shrimp production. Each module operates with one hectare grow out ponds and one hectare for recirculation ponds consisting of 3 small ponds for water treatment. first pond for sedimentation and organic matter/sludge removal, second pond for reducing ammonia and third pond for returning to production ponds.

The farm does direct stocking density of 70 PLs/mt. sq. production cycle, 90 DOC days of culture, and harvests 60 shrimp/kilo. No problems seen with WSSV, EMS viruses or EHP microsporidian in this difficult shrimp production zone.

A large shrimp farm company in Vietnam recently restructured 54 ponds for RAS systems and harvested successfully 52 out of 54 ponds stocked.

EVALUATION OF IMMUNOSTIMULATION PROPERTIES OF *Achyranthes aspera* SUPPLEMENTED DIETS ON *Labeo rohita* IN POND CULTURE SYSTEM

Rina Chakrabarti*, Amarjeet Singh, JaiGopal Sharma

*Aqua Research Lab, Department of Zoology, University of Delhi, Delhi 110 007, India

Diseases and various parasitic infections are responsible for major losses to aquaculture industry. Immunostimulation is one of the useful tools to prevent infectious diseases in the fishes. The use of herbal compounds as immunostimulants has been increasing rapidly in aquaculture to avoid the indiscriminate use of hazardous antibiotics.

Indian major carp rohu, *Labeo rohita* (153.81 ±2.88 g) were cultured in 3m² hapa (2.0 m × 1.5 m) under three different feeding regimes. Three replicates were used for each treatment. All hapas were kept in a pond of CIFE, Rohtak Centre, Haryana, India. Two test diets were formulated using seeds (0.5%) and leaves (0.5%) of *Achyranthes aspera*. The diet without plant ingredients served as control. Fish were immunized with c-RBC after 30 days of culture. Blood and tissue samples were collected after 7 days of immunization.

Final average weight was higher in rohu fed with plant supplemented diets compared to the control one. Myeloperoxidase and nitric oxide synthase levels were significantly ($P<0.05$) higher in seeds and leaves supplemented diets fed rohu compared to the control group. Thiobarbituric acid reactive substance and carbonyl protein levels were minimum in the experimental groups compared to the control one. Highest level of lysozyme was recorded in the seed supplemented diet fed rohu. The dietary supplementation of *Achyranthes aspera* increases the immunity in rohu under pond culture system.

EFFECT OF DIETARY ENCAPSULATED BUTYRIC ACID (BUTIPEARL™) ON THE GROWTH AND FEED UTILIZATION IN HYBRID CATFISH (*Clarias macrocephalus* x *Clarias gariepinus*)

Poh Soon Chan*, Alfred Chua, and Kah Heng Liong

Kemin Industries
12 Senoko Drive, Singapore 758200
pohsoon.chan@kemin.com

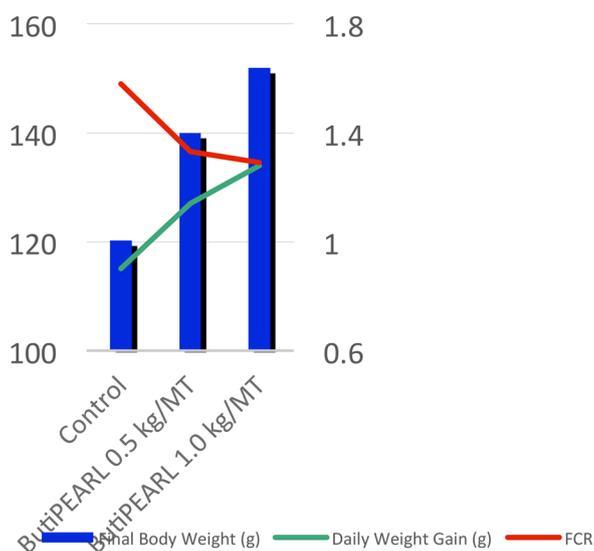
Butyric acid is an integral short-chain fatty acid acting as an energy source for epithelial cells. It improves gut health through development of the intestinal epithelium by increasing cell proliferation. ButiPEARL™ is an encapsulated form containing minimum 45% of calcium butyrate which is embedded in a matrix of vegetable oils and sugars. This encapsulation technology allows sustain release of calcium butyrate throughout the intestinal tract.

A 12-weeks feeding trial was conducted to evaluate the effects of encapsulated calcium butyrate (ButiPEARL™) on growth and feed utilization of hybrid catfish. In this experiment, the catfishes were randomly divided into 3 groups of 15 fish with 1 control group and 2 treatment groups, each group have 3 replicates. The experiment was conducted for 3 and a half months. The fish were kept for 1 week in the fish tank prior to the start of the experiment for them to adapt to the environment. Following that, the fish were fed with three experimental diets containing 0.5 kg/MT and 1.0 kg/MT of ButiPEARL™ respectively or none of these supplements (control diet) for 3 months.

The final results showed that 1.0kg/MT ButiPEARL™ feed fish group gave the heaviest final body weight (151.8 g/fish) compared to the control group (120.2 g/fish) and 0.5 kg/MT ButiPEARL™ feed fish group (140.0 g/fish). This suggests that butyric acid in the diet enhance feed utilization for growth performance. The body weight of all treatment groups were significantly different ($P < 0.05$) compared to from the control group, and fish fed with 1.0 kg/MT of ButiPEARL™ had the highest body weight gained with Feed Conversion Ratio (FCR) improvement of 29 points and 25 points respectively over the control.

In conclusion, ButiPEARL™ shows significant ($p < 0.05$) improvement with on performance (41.7% more in weight gain) and feed utilization (18.4% improvement in FCR) in catfish.

Graph 1: Animal performance improvement



MORPHOLOGICAL CHANGES OF GIANT GROUPER (*Epinephelus lanceolatus*) CRYOPRESERVED SPERMATOZOA WHICH TRANSPORTED USING DRY ICE

Che Zulkifli Che Ismail, Ivan Koh Chong Chu, Shahreza Md Sheriff, Sufian Mustafa,
Mhd Ikhwanuddin Abdullah

Institute of Tropical Aquaculture, Universiti Malaysia Terengganu
Email: zulkif009@gmail.com

Sperm Morphology is one of the parameters for quality measurement. Cooling and thawing activity on the spermatozoa causing a process of crystallisation and recrystallisation of the sperm cell. It may damage the morphology of the spermatozoa. The aims of the study was to determine the morphological changes of the external structure of spermatozoa after threated and exposed to the -79° Celsius from liquid nitrogen (-196° Celsius) for the purpose of shipping the cryopreserved semen. Giant grouper semen is very important for the production of hybrid grouper fingerlings to full fill the demand from marine culture industry. Cryopreserved semen was exposed to 24 hours, 48 hours and 72 hours in dry ice. Cryopreserved semen also were placed back to liquid nitrogen after 48 hours on dry ice. The spermatozoa sample from negative control (fresh semen) and positive control (thawing from liquid nitrogen) and four treatments were fixed, rinsed, washed and coated for scanning electron microscopy observation. The external morphology parameter was measured from the spermatozoa was head length, head width, mid piece length, anterior width of mid piece, posterior width of mid piece, flagella length and total length. Comparison of the external sperm morphology was made between the controls with four treatments. The treatment was T1: 24 hours in dry ice, T2: 48 hours in dry ice, T3: 48 hours in dry ice and immersed back to liquid nitrogen, T4: 72 hours in dry ice.

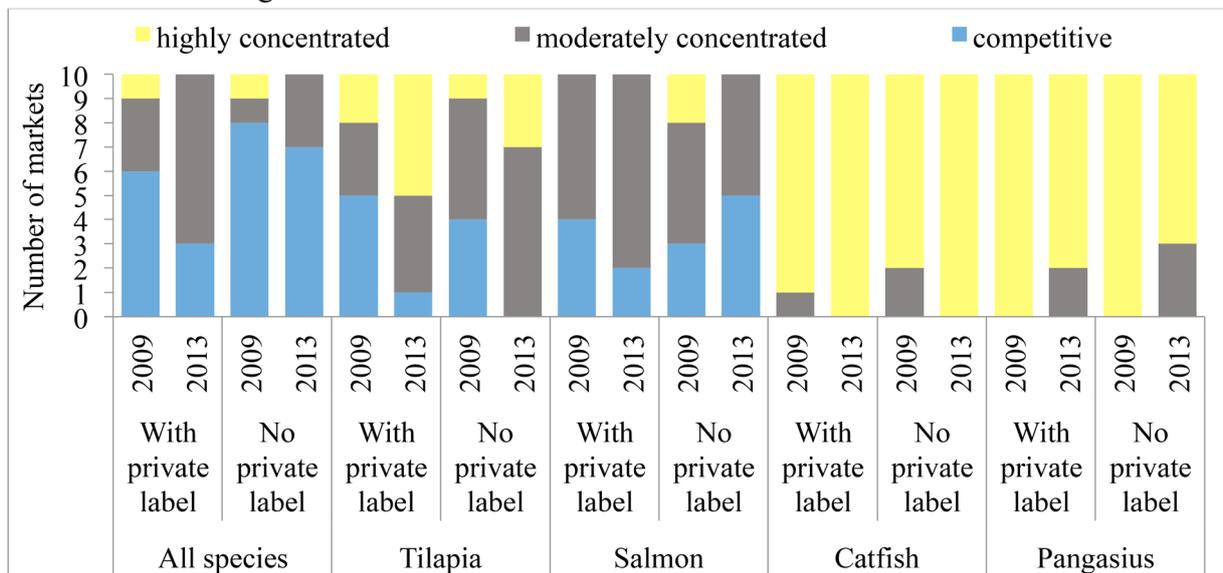
RETAIL MARKETS FOR CHILLED AND FROZEN FINFISH IN THE UNITED STATES: MARKET CONCENTRATION AND IMPLICIT PRICES OF PRODUCT ATTRIBUTES

Oai Li Chen* and Madan Mohan Dey

Nippon Foundation Nereus Program
 The University of British Columbia
 2202 Main Mall, Vancouver, B.C., Canada V6T 1Z4
 ochen1978@gmail.com or o.chen@oceans.ubc.ca

Today, seafood imports capture almost 94 percent of the U.S. domestic market share. This paper explores the current trends in the U.S. seafood retail markets based on available scanner data from the Nielsen Company that includes Wal-Mart data. It focuses on market concentration and implicit prices of seafood product attributes. The study reveals changes in the seafood market environment over the period from 2009 through 2013 across the ten metro markets, namely Atlanta, Boston, Chicago, Detroit, Los Angeles, New York, Philadelphia, Pittsburgh, San Francisco and Washington D.C. It highlights the likelihood of changing buying behavior towards more affordable seafood products. It further suggests that chilled and frozen seafood markets can be considered as a less differentiated industry as they are highly concentrated in terms of species, processed type and product form (Figure 1). Analysis of implicit price of seafood product attributes likewise confirms other literature findings that species and brand are the key attributes. Other potential price premium attributes are convenient product forms and smaller packaging sizes. The findings further highlight the likelihood of consumers' perceived indifference willingness to pay towards farmed white fish which include U.S. catfish, imported catfish, pangasius and tilapia in most of the markets. Results also show that private labels have managed to create strong brand positioning in all seafood markets. These results highlight the likelihood of tough competition facing the U.S. seafood industry in these coming years.

Figure 1: Market concentration in the U.S. seafood markets.



MODELING AND PROJECTING THE U.S. FISH SUPPLY, DEMAND AND TRADE TO 2030: A CLOSER LOOK AT THE U.S. FARM-RAISED CATFISH

Oai Li Chen* and Madan Mohan Dey

Nippon Foundation Nereus Program
The University of British Columbia
2202 Main Mall, Vancouver, B.C., Canada V6T 1Z4
o.chen1978@gmail.com mailto:or o.chen@oceans.ubc.ca

As fish is one of the most traded commodities and has become an important animal-protein element of the human dietary, it is not surprising that there is an increasing effort to incorporate fish in food sector analyses. This paper introduces a novel method by combining the consumer preference and market modeling literature to capture the complex dynamics of the U.S. aquaculture industry. It develops a U.S. fish supply, demand and trade simulation model (partial equilibrium model, referred as USFish) to generate projections of the U.S. catfish supply, demand and trade under alternative scenarios. Projections of the catfish supply and demand were simulated using the USFish model and the findings from the retail markets. The USFish model was used to run projections for baseline (assuming that the past trends continue) and seventeen alternative future scenarios. The model projects that the U.S. catfish output and consumption are projected to decline over time under the baseline scenario (Table 1). The alternative simulations further inform stakeholders the importance to consider measures in both the production and marketing segments to enhance price competitiveness, reduce substitution, and enhance market diversification (international). The USFish model is a rigorous analytical tool capable of analyzing alternative development scenarios. In addition to eighteen scenarios studied, the USFish model is flexible enough to be used to analyze other related technical and policy scenarios. The model can further be expanded to incorporate differential behavior of different consumer groups (i.e., income levels and demographic groups) and marketing agents (i.e., food service channels and retailers).

Table 1: Selected projected U.S. catfish output, average annual growth rate (%), 2012-30.

Scenario and deterministic assumption	Output
B Baseline (assuming that the past trends continue)	-1.13
1 Slower fish expenditure grows in the U.S.	-1.42
2 Faster growth in U.S. catfish exports price.	-1.06
3 Success in market positioning, yield lower own-price, cross-price and substitutability elasticities between U.S. catfish and other farmed white finfish.	-0.82
4 Improvement in policy and regulation that reduces imports of catfish and pangasius.	-0.16
5a Slower growth in feed price.	-0.05
5b Improvement in catfish farming productivity.	1.78
5c Improvement in catfish production environment that yield higher supply elasticity.	0.17
6 Opportunity in the domestic markets with faster fish expenditure trend.	-0.71
7a Combination of key drivers, including scenarios 2, 3, 4, 5a, 5b, 5c.	4.01
7b Combination of key drivers, including scenarios 2, 3, 4, 5a, 5b, 5c, 6.	4.56

Source: Authors' calculation based on the simulation exercises.

MANAGEMENT AND PRODUCTION OF GOOD QUALITY *Litopenus vannamei* SEEDS

Chen Ming Hsien

Hisenor (Vietnam), Aquatic Breeding Co. Ltd, Centralized Aquatic Breeding and Inspecting Zone,
An Hai Commune, Ninh Phuoc District, Ninh Thuan Province, Vietnam
shyan@pie.com.tw

This speech provides some technical guideline on how to improve and produce the health and good quality of white leg shrimp (*Litopenus vannamei*) postlarvae produced in hatcheries through improved facility and system maintenance, broodstock maturation, algae culture, larval rearing, feeding, water treatment and water quality management, biosecurity and health management of larviculture nursery. This speech also provides some information on how to standardized hatchery operating procedures and quality control interventions can be applied during hatchery production of *P. vannamei* postlarvae. Expected to facilitate the efforts of hatchery operators to produce good quality, disease-free, healthy *P. vannamei* postlarvae, thus improving overall production and the sustainability of white shrimp aquaculture.

CHARACTERIZATION OF *Mx* GENES RESPONSES FOR NODAVIRUS INFECTION IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

Yuan-Ning Wang, Young-Mao Chen, and Tzong-Yueh Chen*

Department of Biotechnology and Bioindustry Sciences, Institute of Biotechnology, Translational Center for Marine Biotechnology, Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan 70101, Taiwan
ibcty@mail.ncku.edu.tw

Groupers are the most important fish species in Asia aquaculture, but nervous necrosis virus (NNV) infection has been responsible for disastrous financial losses. During virus infection, virus infected grouper will induce many immune gene and antiviral protein expression, *Mx* protein is one of them. *Mx* proteins are main components of the antiviral innate immune response mediated by type I interferon (IFN). Therefore, we investigate the characteristics of three *Mx* genes in orange-spotted grouper response to NNV.

osgMx1, *osgMx2*, and *osgMx3* are cloned from orange-spotted grouper, and they are containing conserved GTPase binding domain and leucine zipper domain. Three *Mx* gene regulations in response to the NNV infection was analysis by real-time PCR and luciferase assay. Gene expression of *osgMx1* was about 132 times in the third day against to control group while *osgMx2* showed 1.4 times in 12 hours, and *osgMx3* showed 24.4 times in the third day. The luciferase assay results are similar to transcription activity. Both gene expression and promoter activity of *osgMx1* showed the highest fold change. It suggests a differential modulation of each *Mx* gene transcription over the immune response to NNV. Altogether, the results have provided new insight of viral disease prevention in grouper aquaculture industry.

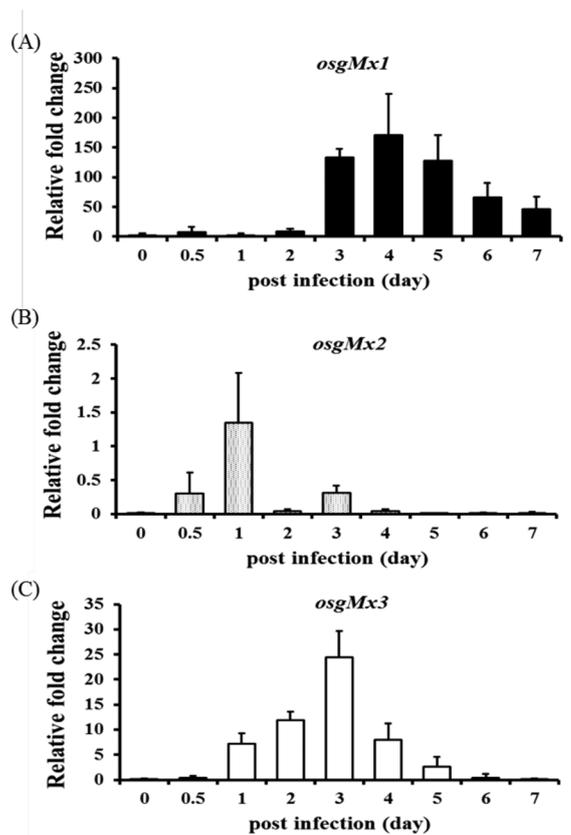


FIGURE 1. Transcription levels of (A)*osgMx1*, (B)*osgMx2* and (C)*osgMx3* in brain after infection with NNV. Gene expression is normalized against β -actin.

A GENETIC DIVERSITY STUDY OF MARBLE GOBY (*Oxyeleotris marmorata*) IN MALAYSIA AND TAIWAN USING MITOCHONDRIAL D-LOOP AS MARKER

Hao-Hsuan Hsu, Chaiw-Yee Teoh and Tzong-Yueh Chen*

Department of Biotechnology and Bioindustry Sciences, Institute of Biotechnology, Translational Center for Marine Biotechnology, and Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan 70101, Taiwan

Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900, Kampar, Perak, Malaysia
Email: zhuangtz91@gmail.com

In this study, genetic diversity of marble goby in Malaysia was analyzed using published mitochondrial D-loop region, wild type and farmed-breed marble goby from Malaysia was compared with farmed-breed ones from Taiwan to investigate the genotype differences between wild type and farmed-breed marble goby from Malaysia and to determine the genetic differences of marble goby species from Malaysia and Taiwan. The result shows that the haplotype of farmed-breed marble goby from Malaysia with the wild type contain significant differences, and the haplotype was found to be close to the haplotype of farmed-breed marble goby from Taiwan, indicating breed from this two region could inherit from same relative's species.

Malaysia wild type marble goby samples were collected from Tapah, Air Kuning (sample WT1, WT2) and Malim Nawar, Kinta River (WMM1, WMM2) while farmed-breed ones was collected from Johor Bahru, Kampung Teluk Jawa (FJB1, FJB2). While, Taiwan farmed-breed ones were collected from Guanmiao, Tainan, Taiwan (FTW1, FTW2). Total 8 samples were analyzed with D-loop region in mitochondria analysis, in the full length 817 bp nucleotides, 24 SNP sites were found in the samples and classified into different haplotypes. From genetic distance matrix analysis, we found there is significant difference between the farmed-breed and wild type haplotypes from Malaysia. However, farmed-breed haplotypes in Malaysia and Taiwan shows low genetic distance. Besides, the haplotypes of wild type contain certain genetic variation within their group. From the result of SNP sites analysis, there are 5 SNP sites found to be the specific diversify sites between wild type and farmed-breed, indicating the evolution of marble goby from different coastal region in same population.

Table 1. Analysis of SNP on D-loop sequence shows there are 24 polymorphic sites and different haplotypes were categorized.

single nucleotide polymorphic sites	5	10	17	24	33	84	85	92	95	155	158	185	186	195	198	231	558	562	566	635	734	745	758	766	
WMM1	C	T	T	G	C	C	C	A	A	T	T	A	A	C	T	T	C	A	A	A	A	A	A	G	-
WMM2	C	T	T	G	C	C	C	G	G	C	T	G	A	T	C	T	T	G	G	A	A	A	A	G	-
WT1	C	T	T	G	C	C	C	G	G	C	T	G	A	T	C	T	T	G	G	A	A	A	A	G	-
WT2	A	C	C	A	C	C	C	G	A	T	C	G	A	T	C	T	T	G	G	G	A	-	C	G	-
FTW1	C	T	T	G	T	T	T	A	A	C	T	A	G	T	T	C	C	A	A	A	A	A	A	G	-
FTW2	C	T	T	G	T	T	T	A	A	C	T	A	G	T	T	C	C	A	A	A	A	A	A	G	-
FJB1	C	T	T	G	T	T	T	A	A	C	T	A	G	T	T	C	C	A	A	A	A	A	A	G	-
FJB2	C	T	T	G	T	T	T	A	A	C	T	A	G	T	T	C	C	A	A	A	-	A	G	-	

Table 2. Genetic distance matrix analysis of marble goby samples. Comparison between haplotypes of the wild and farmed-breed from Malaysia shows high genetic distance.

	FJB1	FJB2	FTW1	FTW2	WMM1	WMM2	WT1	WT2
FJB1								
FJB2	0.0000000							
FTW1	0.0000000	0.0000000						
FTW2	0.0000000	0.0000000	0.0000000					
WMM1	0.0086625	0.0086625	0.0086625	0.0086625				
WMM2	0.0149374	0.0149374	0.0149374	0.0149374	0.0111662			
WT1	0.0149374	0.0149374	0.0149374	0.0149374	0.0111662	0.0000000		
WT2	0.0252097	0.0252097	0.0252097	0.0252097	0.0188032	0.0124641	0.0124641	

HEALTH MANAGEMENT AND FARM PRACTICES IN THAILAND

Prakan Chiarahkhongman*

International Technical Dissemination and Training Center
Aquaculture Promotion Company (APCO)
Charoen Pokphand Foods public company limited (CPF), Thailand
mailto:prakan@cpf.co.th

Thai shrimp and fish farmers are adapting new culture techniques in order to cope with recent disease challenges. A new concept of culture practices, referred to as the “5C” strategy, combines 5 basic hygiene concepts, i.e. Clean Seed, Clean Feed, Clean Water, Clean Pond & Bottom and Clean Environment. The combination of those 5 Clean concepts for aquaculture management in both shrimp and fish have proven to be effective in controlling the mortality and the production losses in Thailand.

Since years, Thai aquaculture suffers from a wide range of diseases such as WSSV, EMS, EHP, WFD in shrimp and Streptococcus, Flavobacterium spp., Aeromonas spp. and a diversity of parasitic infestations in Tilapia. The concept of 5 C has been introduced to the farmers to change their attitude including their management regimen to overcome the problems.

The Clean Seed concerns the stocking of diseases-free fry, either by passing through diseases screening processes or by using only SPF / SPR certified fry. Furthermore, juveniles are pre-grown during a “Nursery phase “ to pamper them for around 3-4 weeks until they are sufficiently strong to survive stocking in the grow out system.

The Clean Feed aims to recommend the farmers to use only a good quality feed which has a complete nutritional profile and includes health promoting feed additives to prevent infections and avoid using antibiotics. This includes a suitable feeding program and management, and good standard operation practices (SOP) of the nursery phase, such as oxygen and tanks management.

The Clean Water targets the most important part of aquaculture, i.e. controlling all optimum water quality parameters during their culture period, including water purification, disinfection, and the application of probiotics.

The Clean Pond & Bottom strategy aims at maintaining the conditions of the surrounding ponds and the bottom conditions throughout the crop including the waste management in the system both in the water column and in the bottom area to be as clean as it could be throughout the crop cycle. This requires the setup of centralized waste accumulation and sludge pumping machines since the waste proved to be a predisposing cause of the outbreak of diseases.

The Clean Environment considers the environmental impact caused by the farming operation. This includes the application of sludge treatment ponds and management procedures before discharging effluent water to the environment.

The 5C concept is a strategy allowing 100 % biosecurity and is being introduced to the farmers as a set of preventive measures to exclude and control a wide diversity of pathogens including bacteria , viral carrier and vector and parasitic and protozoa spore etc.

CHARACTERIZATION OF GONAD DEVELOPMENT IN GROUPEL WITH GONAD SPECIFIC MARKERS - PIWIL2 AND VASA

Tin-Han Chien*, Chih-Chan Wu, and Tzong-Yueh Chen

Department of Biotechnology and Bioindustry Sciences, Institute of Biotechnology, Translational Center for Marine Biotechnology, Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan 70101, Taiwan
E-mail: L68041060@mail.ncku.edu.tw

Groupers are famous for being a remunerative species of aquaculture in Asia. One of the bottlenecks in giant grouper breeding is their long-term sex maturation which takes as many as 7 to 10 years. The groupers of genus *Epinephelus* is a protogynous teleost that they only perform sex transformation until a specific body size is reached. To elucidate the gonad formation and sex development process, gonad specific expressed gene, Piwil-2 and Vasa, are cloned for monitoring the germ cells.

Piwil-2 and Vasa are cloned from *Epinephelus lanceolatus*. Piwil-2, containing conserved PAZ and PIWI domain, is involved in the piRNA pathway related to the germ cell survival and differentiation. Piwil-2 while Vasa is an RNA binding protein with an RNA-dependent helicase responsible for germ cell development. Both the genes are specifically expressed in gonad tissue while other somatic tissues only show low levels of Piwil-2 and Vasa. Anti-Piwil-2 and anti-Vasa antibody is produced in-house for protein level detection. Grouper embryos from 0 to 30 days post fertilization are collected for primordial germ cells detection with Piwil-2 and Vasa antibody. Primordial germ cells locate to the gonadal ridge around 3 dpf. It is shown that the molecular markers, Piwil-2 and Vasa, are potential tools for gonad development research.

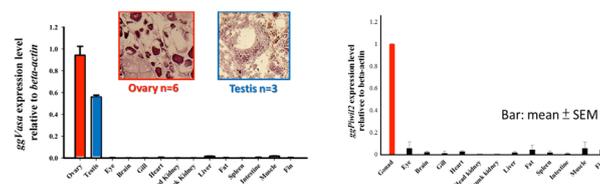


Figure 1. Tissue distribution shows Piwil-2 and Vasa specifically expressed in gonad.

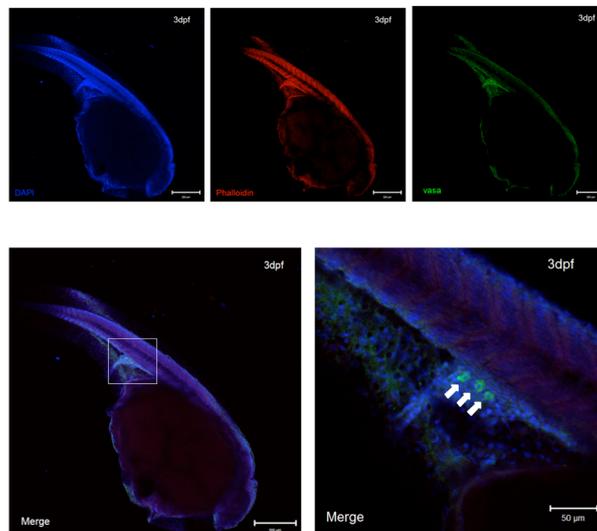


Figure 2. Detection of primordial germ cells (PGCs) with germ cell specific

ENVIRONMENTAL RISK ASSESSMENT & MANAGEMENT PLANNING: A CASE STUDY

Tony Chiffings*, Melissa Mary Mathews, Tania Golingi, Felix Ku Kok Hou, Marjorie Lim

DHI Water & Environment (M) Sdn Bhd, 11th floor, Wisma Perindustrian, Jalan Istiadat, Likas, 88400, Kota Kinabalu, Sabah, Malaysia
twc@dhigroup.com

Environmental risk assessment is increasingly becoming essential in considering major aquaculture developments. This is not just about minimising disease risks and ensuring a suitable environment for hatcheries and grow out, but is directly relevant to export quality guarantees associated with necessary accreditation for entry into high priced markets.

In Malaysia major aquaculture projects (greater than 50 hectares) may require a Detailed or Special EIA. This usually means well documented assessments of likely impacts on the environment by the project and how such effects can best be mitigated. By the same token, much of the work done for an EIA is directly relevant to assessing the environmental risk to the project and how these should be managed.

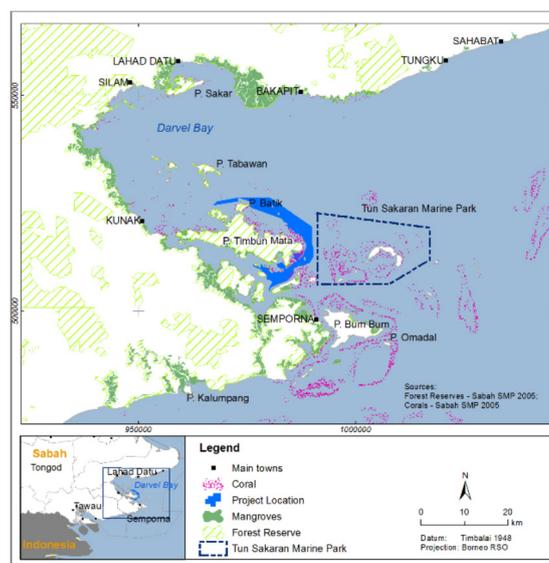
An environmental risk assessment for a proposed Lobster grow out facility near Semporna, South East Sabah, Malaysia is presented as a case study. The project is notable as it is intended to cover a sea space area of 9,300 ha and at full production produce 18 thousand metric tonnes of *Panulirus ornatus* lobster.

During the assessment a number of natural and anthropomorphic hazards were identified as risks to the project.

The geographic scale of this project means that ambient water quality may well be affected by land based, catchment scale processes over time. Water quality can also be impacted by marine based sources including oil spills and ballast discharges from shipping, and ecological hazards such as algal blooms. Tsunamis and pathogen introduction and transmission are also potential operational realities.

Key considerations within the environmental risks assessment and management process are:

- Identification and assessment of environmental risks, recommended mitigation and management measures.
- A comprehensive environmental monitoring programme.
- A strategy for communicating with the potential polluters and regulatory authorities.



WORTHLESS RECOVERY ATTEMPTS ON STUNTED GROWTH ASIAN SEABASS, *Lates calcarifer* LARVAE

Ching Fui Fui*, Anis Izzati Anwar, Nittaya A/P Ainik, Mohammed Noorfaiz bin Yusof, Shigeharu Senoo

Borneo Marine Research Institute,
Universiti Malaysia Sabah, Malaysia
cfuifui@ums.edu.my

Dwarf individuals are commonly observed within one single production of Asian seabass, *Lates calcarifer* and they are often cannibalised by larger individual and resulted in mass mortality related to cannibalism. This paper studied the recovery strategies for stunted growth *L. calcarifer*. Three recovery strategies were implemented separately through manipulation of stocking density (10, 20 and 30 per tank), feeding frequency (2, 4 and 6 times daily) and salinity (0, 15 and 30 ppt). Sorting was done to select and transfer only stunted growth larvae to experimental tanks. Initial average growth in total length and body weight were 1.0 ± 0.2 , 1.0 ± 0.2 and 2.40 ± 0.2 cm, and 0.05 ± 0.01 , 0.05 ± 0.02 and 0.3 ± 0.1 g respectively. Each experiment was conducted in 15 days. All findings revealed stunted growth larvae were unable to recover and remained smaller compare to non-stunted growth of same-age larvae. However, stunted growth larvae reared in 10 fish per aquaria (3.06 ± 0.39 cm; 0.53 ± 0.05 g), fed 4 times daily (1.49 ± 0.17 cm; 0.11 ± 0.01 g) in 0 ppt rearing water (3.94 ± 0.28 cm; 0.81 ± 0.19 g) were relatively larger but remained insignificant compared to other treatments. In addition, no significant difference was detected in term of survival among stunted growth larvae reared in all three experiments. Furthermore, histological analysis on larval nutritional condition revealed severe damage on larval digestive system in all three experiments coupled with weak feeding performance. Meanwhile, the overall production cost had increased with longer production period in all treatments. This study concluded recovery attempts for stunted growth *L. calcarifer* larvae are not practically worth and uneconomical.

PNA-PROBE BASED RT- PCR KIT FOR DETECTING THE NV GENE VARIATION OF VIRAL HEMORRHAGIC SEPTICAEMIA VIRUS ISOLATED IN SOUTH KOREA

Mi Young Cho¹, Hyun-Ja Han¹, Seong Don Hwang², Jee youn Hwang², Jaehun Cheong² and Sung-Hee Jung¹

¹Pathology Research Division, National Institute of Fisheries Science(NIFS)

²Aquatic life Disease Control Division, NIFS, 46083, ROK

³Pusan University, 46083, ROK

The viral hemorrhagic septicemia virus (VHSV) is a viral pathogen to cause severe losses in farming industry of olive flounder. Thirteen VHSV strains are isolated from olive flounders showing VHS symptoms and mortality in Korea, and then molecular variation of DNA and amino acid sequences of the NV genes were compared. Although common mutations were detected at four sites in DNA sequences of NV gene, there was no difference in the amino acid sequences among thirteen VHSV variants. Without common amino acid variation, we found six amino acid variations in mortality-derived VHSV NV proteins. Based on finding of NV-mediated decrease of intracellular ATP level, protein expression of six NV variants in flounder cells decreased more than control NV protein. We have developed a highly sensitive and simple methods using a peptide nucleic acid (PNA) mediated real-time PCR to detect the specific viral DNA of variation sites of NV gene. PNA probes (SeaSun Biomaterials, Korea) for each variation site were designed to maximize their hybridization efficiencies and to minimize any non-target hybridization. The use of real-time PCR and PNA probes have advantages in terms of flexibility because we can easily alter the targeted mutations by adding or removing PNA probes and primers. In this study, PNA based real-time PCR have been successfully applied for the highly sensitive detection of target viral DNA of VHSV mutations of the Korean isolates. The Kit developed in this study is expected to find increasing use in routine disease monitoring and diagnosis in aquaculture and field epidemiology.

HYPERPLASTIC AND HYPERTROPHIC GROWTH OF MUSCLE FIBER AS RELATED TO MUSCLE GROWTH IN OLIVE FLOUNDER *Paralichthys olivaceus*

Boin Lee^{1,*}, Yunkyung Lee¹, Bong Joo Lee², Kang Woong Kim², Kyung Duck Kim², Hyon Sob Han², and Young Min Choi¹

¹Kyungpook National University, Sangju 37224, Korea

²Aquafeed Research Center, National Institute of Fisheries Science, Pohang 37517, Korea
ymchoi1@knu.ac.kr

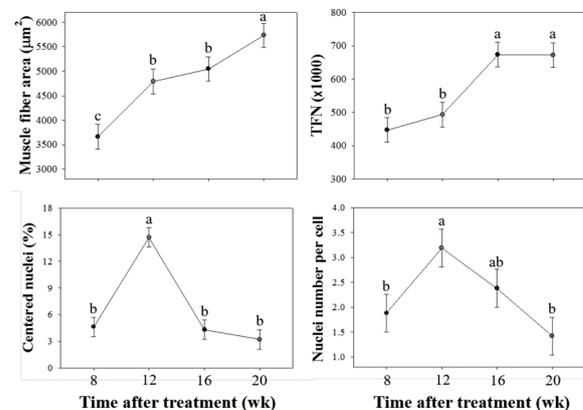
Understanding muscle growth is one of the most important goals in aquaculture. Ultimate muscle mass is largely determined by a combination of fiber hyperplasia (increase in number) and hypertrophy (increase in area), and the contribution of these factors depends on the fish species and ultimate body size. However, muscle fiber characteristics of olive flounder during growth periods and their influence on ultimate muscle mass have not been elucidated, even though olive flounder is one of the most important fish species in many Asian countries. Therefore, this study designed to extensively characterize the skeletal muscle of olive flounder to provide a growth model.

All fish for the treatment conditions were same, and were fed the same commercial diet. Start weight was 214 ± 10 g, and fish samples were randomly selected at 8, 12, 16, and 20 wk after treatment (11 samples per each period). Body weight and muscle cross-sectional area (CSA) of olive flounder continued to grow from 8 to 20 wk after treatment (Table 1). Like body weight, fiber area continued to increase during 8 to 20 wk (Figure 1). However, no significant difference was observed in fiber area between 12 and 16 wk due to a higher percentage of smaller muscle fiber (up to $2000 \mu\text{m}^2$) at 12 wk (data not shown). During 12 to 16 wk period, total fiber number (TFN) was increased (494.6 vs. 673.3×1000 , $P < 0.05$) unlike fiber area (4788 vs. $5046 \mu\text{m}^2$, $P > 0.05$). This increased fiber number is associated with greater percentage of centered nuclei (14.7% , $P < 0.05$) and nuclei number (18.4 , $P < 0.05$) at 12 wk. Therefore, there are separate periods of fiber hyperplasia, and fiber hyperplastic growth is more tightly associated with ultimate muscle mass in olive flounder.

TABLE 1. Body weight, total length, body depth, cross-sectional area (CSA), and fin muscle characteristics of olive flounder fed experimental diet during 20 week periods. Least square means with different superscripts in the same row significantly differ ($P < 0.05$).

	8 wk	12 wk	16 wk	20 wk
Body weight (g)	363.1 ^d	537.5 ^c	668.8 ^b	848.7 ^a
Total length (cm)	32.3 ^d	36.8 ^c	39.0 ^b	42.9 ^a
Body depth (cm)	12.1 ^d	13.6 ^c	15.3 ^b	16.8 ^a
Muscle CSA (cm ²)	17.1 ^d	21.9 ^c	31.9 ^b	39.3 ^a
Fin muscle area (cm ²)	3.36 ^d	4.48 ^c	5.48 ^b	6.93 ^a
Fin muscle (%)	16.5	17.1	14.7	15.0

FIGURE 1. Comparison of muscle fiber area, total fiber number (TFN), centered nuclei, and nuclei number per cell among the different time after treatment of olive flounder. Bars indicate standard errors of least square means. Different letters denote significant differences ($P < 0.05$).



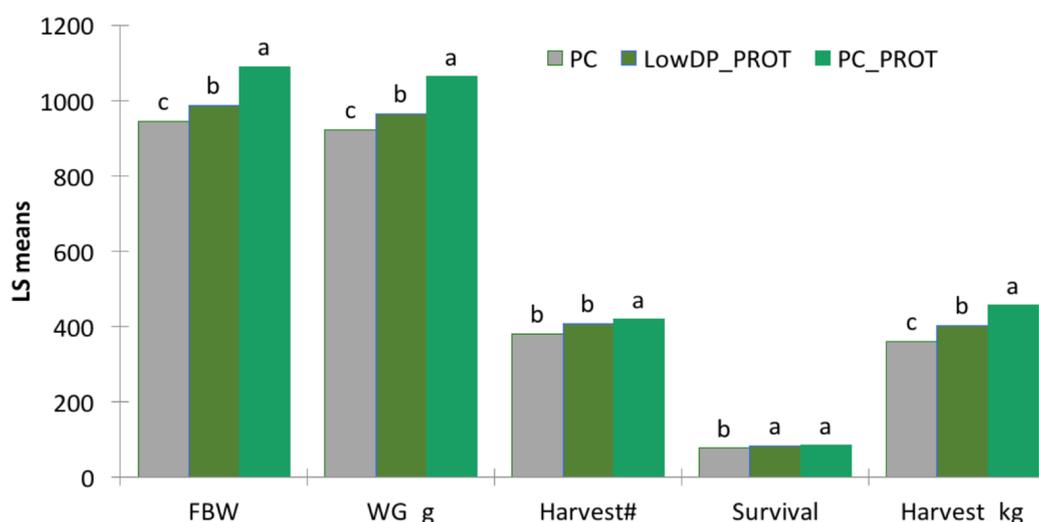
IMPROVED PERFORMANCE OF TILAPIA FED COMMERCIALY EXTRUDED DIETS SUPPLEMENTED WITH A PROTEASE COMPLEX IN BOTH FIELD AND LABORATORY CONDITIONS

M. A. Kabir Chowdhury*, Saharat Kuakij, Supornchai Sri-Nhonghang, Orapint Jintasataporn

Jefo Nutrition Inc.
5020 Jefo Avenue, Saint-Hyacinthe
Quebec, Canada J2S 7B6
kchowdhury@jefo.ca

Two growth trials with Nile tilapia (*Oreochromis niloticus*), Chitralda strain were conducted concurrently in both farm and laboratory conditions, where fish were fed three commercially manufactured extruded diets – (1) positive control (PC, regular diet), (2) low digestible protein diet + protease (LowDP_PROT), and (3) positive control + protease PC_PROT. Diets 2 and 3 were supplemented with a commercial protease complex (PROT) at 175 g/MT. All diets were isoproteic ($34.8 \pm 0.3\%$ CP) and isolipidic ($5.3 \pm 0.2\%$ CL). The duration of the field trial and the laboratory trial was 17 and 8 weeks, respectively. In both trials, the average initial body weight (IBW) of fish were similar (23.8 ± 1.3 g and 25.1 ± 0.3 g, for field and laboratory respectively). In the laboratory trial, there were no differences in performance among the treatments despite numerically better weight gain (61.5 ± 1.3 g), specific growth rate, SGR (2.19 ± 0.19) and feed conversion, FCR (1.24 ± 0.16) of fish fed the PC_PROT diets compared to those fed the PC (51.0 ± 1.4 g, 1.99 ± 0.08 and 1.43 ± 0.09 , respectively) and LowDP_PROT (49.2 ± 9.1 g, 1.94 ± 0.26 and 1.54 ± 0.20 , respectively). However, in the farm trial, weight gain, FCR, survival and protein efficiency ratio (PER) of fish fed both protease supplemented diets (LowDP_PROT and PC_PROT) were better than those fed the PC diet ($P < 0.05$) (Figure 1). This trial reconfirms the findings of the previously published laboratory scale tilapia studies conducted elsewhere on the efficacy of the specific protease in both laboratory and commercial field conditions.

Figure 1. Performance of tilapia at the commercial farm trial. Diet 1 – positive control diet; diet 2 – low digestible protein diet supplemented with a protease complex; and diet 3 – positive control diet supplemented with protease. FBW – final body weight in g, WG – weight gain in g. Different letters for a parameter denotes significant difference at $P < 0.05$.



SPAWNING AND CULTURE OF LICORICE GOURAMI, *Parosphromenus alfredi* (KOTTELAT & NG, 2005) UNDER LABORATORY CONDITION

Annie Christianus* and Muhamad Syafiq Zulkifle

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

Licorice gourami, *Parosphromenus alfredi* (Figure 1) is a rare native fish from Peninsular Malaysia, Borneo and Sumatra. It is sold at MYR30 per piece as ornamental fish. Thus the high market value has prompted this study to spawn this fish under laboratory condition. Adult fishes (2.2-2.5cm) were caught from the wild along roadside canals between Mawai to Desaru, Johor, Malaysia. Fishes were conditioned for 3 weeks prior to spawning trials. Pairs of 1 male : 1 female were placed in glass aquaria. Spawning ritual was between 1-2hr.

Newly spawn eggs were demersal, adhesive, whitish to yellow in color, slightly translucent and spherical in shape. Egg average diameter was approximately 1.33 ± 0.22 mm. Embryonic development was observed with hatching occurred between 48-49 hours after spawning, at water temperature of 27 ± 1 °C. A female *P. alfredi* was able to produce 22 larvae per spawning. Spawning occurs once in 10-20 days.

Figure 2 showed the total length increment of *P. alfredi* observed until 56 day after hatching (DAH). Newly hatched larvae were about 2.75mm in total length (TL) with mouth opened at 2 DAH. Yolk-sac absorption takes between 4-5 DAH. All fins were well developed by 28 DAH with small fin bud on anal and dorsal fin, with almost complete ray formation on caudal fin. Total length of the larvae at 56 DAH was 9.18mm. Live feed consisted of green water, *Panagrellus redivivus*, *Artemia* nauplii, Grindal worm (*Enchytraeus buchholzi*) and *Daphnia* neonates were given during the culture period. Water temperature, pH and dissolved oxygen measured in the culture tank were between 27-29°C, 4.8-6.3, and 6.9-7.6 mg/L, respectively.



Figure 1: *Parosphromenus alfredi*

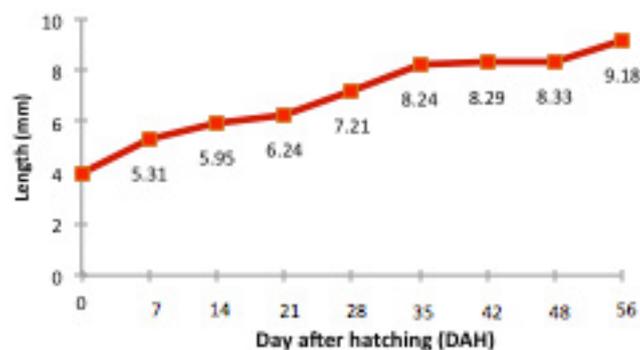


Figure 2: Total length (mm) increment of *P. alfredi* observed until 56 day after hatching

BEHAVIOURAL RESPONSE OF BIVALVE MOLLUSCS TO CALCIUM HYDROXIDE

Luc A. Comeau*, Rémi Sonier, Thomas Guyonnet, Thomas Landry, Aaron Ramsay and Jeffrey Davidson

*Atlantic Veterinary College, 550 University Avenue, Charlottetown, Prince Edward Island, Canada
E-mail: luc.comeau@bellaliant.net

Blue mussels (*Mytilus edulis*) cultivated in Prince Edward Island, Eastern Canada, are susceptible to heavy fouling on their shells as a result of the rapid proliferation of invasive solitary tunicates. The clubbed tunicate (*Styela clava*) is particularly difficult to manage. Its leather-like tunic cannot be perforated easily using high-pressure water. To control *S. clava* mussel growers periodically raise longlines in order to spray a highly alkaline (~12.7 pH units) calcium hydroxide solution onto the fouled mussels (Figure 1).

Laboratory experiments were conducted to assess the response of various bivalves to alkalinity levels they may encounter during calcium hydroxide treatments in the field. Behavioural responses were assessed by connecting bivalves to a system that closely monitored their valve movements (Figure 2).

Bivalves responded to increased alkalinity by completely or partially closing their valves. For *M. edulis* the avoidance behaviour intensified over a 3-day treatment period, suggesting that its sensitivity to alkalinity increases over time. Nevertheless, all behavioural responses were short-lived (0.2–4.7 h) and generally confined to the treatment period. In conclusion, it seems that spraying calcium hydroxide onto cultivated mussels has limited impact outside its intended purpose of eradicating fouling tunicates.



Figure 1. Mussel sleeves infested with *Styela clava* and manually sprayed with a saturated calcium hydroxide solution.



Figure 2. *Mytilus edulis*, *Mercenaria mercenaria*, *Argopecten irradians* and *Mya arenaria* wired with Hall magnetic sensor assemblies.

APPLICATION OF FERMENTED FEED ON SHRIMP CULTURE

Cui Luosheng

Guangdong Hisenor Group Co. Ltd, Guangzhou, Guangdong, China
cuils@haid.com.cn

As the improvement of shrimp culture history in main region and countries, the variation of the climate worldwide, the pollution of water resource and shrimp pond, the successful rate and production of shrimp culture falling down year by year, especially affected by shrimp diseases such as EMS, EHP, etc. The essence behind this phenomenon is the aging pond and instability of the water quality in the shrimp pond.

With this background, the fermented feed was studied by Sheng-Long Bio-Tech Company of Haid Group, which is considered to be the revolutionary product in shrimp culture in China. Totally, five strains of beneficial bacteria such as Bacillus, Photosynthetic bacteria, Lactic acid bacteria, *Streptococcus faecalis* and yeast added. To improve the synergistic effect, beneficial bacteria quantity level internal and external of the shrimp, all of the beneficial bacteria were compounded by orthogonal test, to guarantee the balance of beneficial bacteria in the shrimp gut and the balance between beneficial bacteria and beneficial algae in the pond water.

According to the farm test in Vietnam, the farmer who applied with fermented feed frequently can benefit from this product as follow: the survival rate of PLs increased by 15%, the FCR reduced by 0.15, the production of pond increased by 12%, the successful rate of shrimp culture improved by 16%. Above all, the vibrio in the gut and hepatopancreas can be controlled in safety level, which leads to improve the successful rate and production of shrimp culture.

HOW DOES PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS AND SOCIAL INTERACTION EFFECT GROWTH PERFORMANCE OF SPINY LOBSTER IN CAPTIVITY?

Audrey D. Tuzan*, Quinn P. Fitzgibbon, Chris G. Carter, and Stephen C. Battaglene

Institute for Marine and Antarctic Studies
University of Tasmania
Hobart, Tasmania 7001
Australia
Audrey.daningtuzan@utas.edu.au

Spiny lobsters can display large inter-individual differences in growth rate which have been reported as major drawbacks to the successful production in captivity. One explanation is that dominant lobsters use agonistic behaviours to control a disproportional share of food resources benefiting individual growth performance. The ability of an animal to compete for and control resources is often determined by the combination of that individual's morphological and physiological traits such as body size, sex, metabolic physiology and prior experience or condition. Thus, understanding how intraspecific diversity affects growth performance is an important consideration. Relationship between metabolic phenotype, social behaviour and growth performance has not been previously investigated in any spiny lobster species. In a laboratory experiments, we examined the influence of individual variation of metabolic phenotype on growth performance of juvenile spiny lobster, *Sagmariasus verreauxi* (5.99 ± 2.77 g) that were reared either individually ($n=17$) or as a group of 20 communally for 90 days. Furthermore, we investigated the influence of metabolic phenotype, body size, sex, feeding contest experience and rearing history on the social behaviour (dominance and aggressiveness) status of individual lobsters by examining their ability to compete for food in a series of randomly paired feeding contest experiments. Growth performance, survival and feed intake were significantly greater in communal rearing demonstrating that social interaction has an important influence on growth of lobsters. We also found a direct link between metabolic phenotype and growth of lobsters in the absence of social interaction but not in the communal setting. These results suggest that the effect of social interaction outweighs the influence of metabolic phenotype on lobster growth. Lobster social status was significantly linked with body size, metabolic phenotype and sex. Larger lobster are predicted to be dominant over smaller lobsters. Moreover, low metabolic rate lobsters displayed a greater ability to win over high metabolic rate lobsters. Female lobsters are also predicted to become more dominant than male lobsters irrespective of size and metabolic phenotype status. The combination of body size, metabolic phenotype and sex dependent responses of spiny lobsters dominance status and aggressiveness during feeding competition combine to determine the growth performance of individual lobsters. Hence, growth performance of spiny lobsters in captivity is determined by the link between individual lobster's morphological and physiological traits and social interaction.

GROWTH PERFORMANCE

Guillaume Daoulas

gda@ynsect.com

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

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

IMMUNO-STIMULANT PROPERTIES

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



Figure 1 : Evolution of the weight gain over time the pathogen

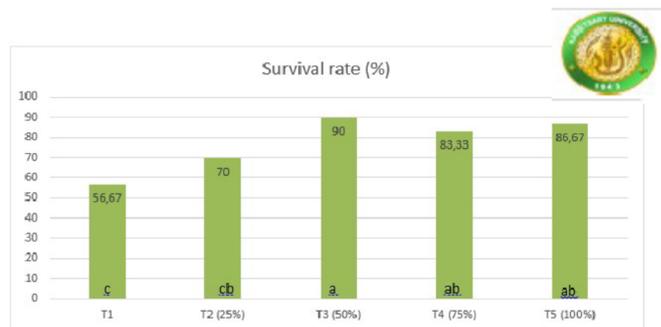


Figure 2 : Survival rate after 10 days after injection of

ORGANIC CARBON LOADING RATE AFFECTED PHYSIOLOGICAL STATUS AND DISEASE RESISTANCE OF AFRICAN CATFISH *Clarias gariepinus* JUVENILES REARED IN GLYCEROL BASED BIOFLOC SYSTEMS

Akeem Babatunde Dauda*, Nicholas Romano, Mahdi Ebrahimi, Murni Karim, Ikhsan Natrah, Mohd Salleh Kamarudin, Chou Min Chong, Abdullateef Ajadi, Teh Jun Chin

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
*tdabak@gmail.com

Biofloc technology (BFT) is a fish culture system that operates on stimulating heterotrophic bacteria by increasing the carbon/nitrogen (C/N) ratio in the system to convert toxic metabolites to consumable biomass. This can confer many advantages to the animal including improved immunity and disease resistance, but such response could depend on the C/N ratio. This study therefore examined the effect of different carbon/nitrogen ratios on the growth, muscle/plasma biochemical composition, and liver digestive enzymes of African catfish, *Clarias gariepinus*, and their subsequent resistance to *Aeromonas hydrophila* in glycerol based biofloc system.

There were three carbon/nitrogen ratios; 10, 15 and 20 and a control without carbon additions. Twenty-five groups of fish were triplicated in each treatment. After six weeks, all fish were measured for their sizes, while 15 were measured for various parameters. A total of 10 were left in each tank for another three weeks before being challenged with *A. hydrophila*.

Growth and survival were unaffected by BFT, but the muscle cholesterol, lipid peroxidation, and serum cholesterol/ triglycerides were significantly lower in BFT groups. The total granulocytes were higher in the BFT groups. Trypsin activity was not different but chymotrypsin was higher significantly in BFT than control. Fish challenged with *A. hydrophila* had increased resistance with increasing C/N ratio, with C/N 20 and 15 significantly higher than the control or C/N 10. Liver histopathology revealed substantially less damage in the C/N 20 treatment, followed by C/N 15, than the other treatments.

Although BFT had no effect on the growth of *C. gariepinus*, it improved their oxidative status and digestive enzyme activity. Such an improvement likely explains the enhanced resistance to pathogenic bacteria. Overall, a C/N ratio of 15 is recommended for the culture of *C. gariepinus* in a BFT-based system.

Table 1: Muscle cholesterol (MCHL) ($\mu\text{g ml}^{-1}$), lipid peroxidation (LPD) ($\mu\text{M g}^{-1}$), serum cholesterol (SCHL) (mmol/L), serum triglyceride (mmol/L), serum lysosyme (SLYS) (U ml^{-1}), and liver trypsin (TRYP) and chymotrypsin (CTRYP) (U/mg) activities of *C. gariepinus* reared in biofloc systems with different C/N ratios

	Control	C/N 10	C/N 15	C/N 20
MCHL	0.34 ^a	0.27 ^{ab}	0.22 ^b	0.22 ^b
LPD	0.15 ^a	0.03 ^c	0.07 ^b	0.06 ^{bc}
SCHL	3.43 ^a	2.84 ^b	3.05 ^b	3.11 ^b
STRIG	1.18 ^a	0.95 ^b	1.06 ^{ab}	0.93 ^b
SLYS	1.19 ^a	1.12 ^a	1.18 ^a	2.21 ^a
TRYP	0.18 ^a	0.22 ^a	0.32 ^a	0.15 ^a
CHTRYP	0.35 ^a	0.99 ^b	0.59 ^c	0.59 ^c

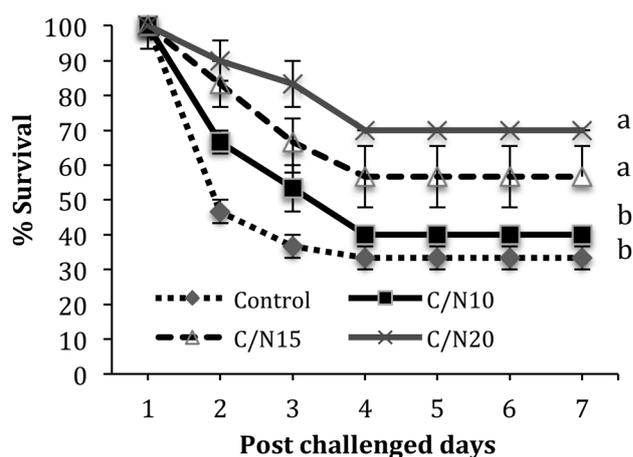


Figure 1: Survival of *C. gariepinus* challenged with *A. hydrophila* at different C/N ratios

BIOASSAY MODELS FOR PATHOGENICITY STUDY OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) CAUSED BY *Vibrio spp.*

Sridevi Devadas^{*1}, Sanjoy Banerjee¹, Fatimah Md. Yusoff¹, Subha Bhasu², and Mohamed Shariff Mohamed Din¹

¹Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

²Genetics and Molecular biology (ISB) and Centre for Biotechnology for Agriculture (CEBAR), 50300, University Malaya, Kuala Lumpur, Malaysia.
gs45950@student.upm.edu.my

Acute hepatopancreatic necrosis disease (AHPND) is caused by a unique strain of *Vibrio parahaemolyticus* (VP_{AHPND}), carrying one or more extrachromosomal ~70kbp plasmids (pVA1) that encode homologues of the *Photobacterium* insect-related (Pir) binary toxins (PirA^{vp} and PirB^{vp}). However, two other non-*V. parahaemolyticus* species, *Vibrio harveyi* and *Vibrio campbellii* have also been identified as carrying pVA1-like plasmids, indicating that these plasmids can be detected in other *Vibrio* species. It is crucial to perform bioassay challenges in order to determine the pathogenicity and to verify Koch's postulate of an isolated strain associated with the disease. Hence, this paper presents an overview of bioassay models for pathogenicity study of the AHPND causing-*Vibrio spp.* to facilitate future experimental work on AHPND and other similar shrimp diseases.

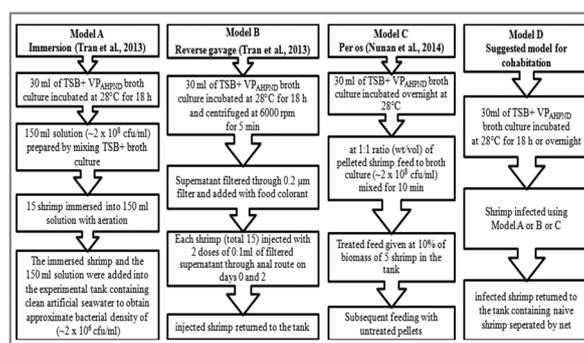
The VP_{AHPND} is determined as a causative agent of AHPND through an immersion (Model A) and reverse gavage (Model B) treatments. It has been shown that immersion and reverse gavage resulted in 100% mortality accompanied by typical AHPND pathology. Shrimps subjected to reverse gavage treatment survive longer than those that undergo immersion treatment. However, infected shrimps from both treatments recorded similar AHPND histological lesions.

Outbreak in Mexico was confirmed using the immersion model together with the *per os* (Model C) treatment. The *per os* method using contaminated pellet resulted in 100% mortality and showed the presence of AHPND histological lesions within 3 days of the treatment. Moreover, there have been indications that ingestion of the VP_{AHPND} strain could be the natural route of infection – in which case oral feeding may well produce more reliable results than the reverse gavage method.

No detailed model of the cohabitation is available for AHPND. However, the cohabitation model (Model D) is suggested based on existing experimental designs from other shrimp diseases. Prior to the cohabitation challenge, it is important to design appropriate routes of infection (Model A/B/C) to infect naive shrimp before using them in transmitting the disease.

Intramuscular Injection (IM) is not recommended for AHPND bioassay since the method fails to reproduce AHPND in the challenged shrimp.

Figure 1. Bioassay models for pathogenicity study of AHPND caused by *vibrio spp.*



TRIAL TO ASSESS THE EFFECTIVENESS OF CAPLUS ME ON GROWTH, SURVIVAL & FOOD CONVERSION OF RED NILE TILAPIA, *Oreochromis Niloticus*, DURING SEX REVERSAL

Alex Diana* and Tilman Wilke

Dr. Eckel Animal Nutrition GmbH & Co. KG

E-Mail: a.diana@dr-eckel.de

In order to obtain best outputs, most of the tilapia farmers choose to use only male tilapia in the grow-out stages. It is known that male tilapia is more profitable in terms of volume since they grow bigger and faster and they are energy efficient. Female tilapia tend to waste energy and time due to breeding. A common technique to produce only male tilapia is hormone sex reversal using methyltestosterone. The period of sex reversal is a critical phase in the production cycle and farmers are especially concerned about survival of fry.

The objectives of this studies was to test whether an organic acid product (CaPlus ME from Dr. Eckel animal Nutrition, Germany) added to the diet of red tilapia during sex reversal in hapas in PE-lined ponds is beneficial in terms of growth and survival of fry.

Ten 5m² hapas were set up in a two PE-lined ponds and stocked with 20,000 red tilapia swim-up fry. They were fed for 21 days on fish meal containing 60 ppm 17 α -methyltestosterone (MT) for a period of 21 days. The feed allocated per day was divided into five feeds and fed at 08:00, 9:30, 11:30, 13:30 and 16:30 by broadcasting the powdered feed over the water surface. The test diet was prepared by mixing the product into the fish meal before addition of the ethanol/MT solution and then mixed thoroughly in a blender-type mixer.

Aeration was provided in each pond via the use of 12 home-made diffusers made of PVC pipe and material. Hapas were changed with 10 m² hapas after 15 days to allow the young fish to have more space to grow.

Survival in the control was lower than usual, even for red tilapia, but the treatment showed much higher survival. The Caplus ME was also good with survival of 74% compared to the control of 52%.

Since survival was higher in the test treatments, individual fish size was less due to the effect of density.

Total harvest of fish per hapa was higher in the CaPlus ME treatment at 22283 individuals compared to 15599 in the control. Further studies will continue to see if the results can be repeated.



Figure 1 Hapas with red Nile tilapia during sex reversal.

TABLE 1. Performance results. Means in a row with different letters were significantly different ($F < 0.05$)

	Diet	
	Control	CaPlus ME
No. Harvested	15599a	22283b
Survival (%)	52a	74b
FCR	1.74	1.62
SGR (%bw/day)	11.3	11.6

FIRST CASE OF HEPATOPANCREATIC NECROSIS DISEASE (HPND) IN POND-REARED CHINESE MITTEN CRAB, *Eriocheir sinensis* ASSOCIATED WITH MICROSPORIDIAN

Zhengfeng Ding

Jiangsu Key Laboratory for Biofunctional Molecules, College of Life Science and Chemistry, Jiangsu Second Normal University, 77 West Beijing Road, Nanjing, China, 210013

An epidemic of hepatopancreatic necrosis disease (HPND) with a high mortality rate (40%–50%) recently occurred in the cultured Chinese mitten crab, *Eriocheir sinensis*, which is a very important economic crustacean species in China. Histology revealed infection by a microsporidian parasite within the cytoplasm of the epithelial cells of the hepatopancreas. Numerous discrete inclusions in the infected cells and presumably free parasite spores were also observed. By negative staining using electron microscopy, a typical morphology of spores was observed with a protuberant front of the anchoring disk. Infection was confined to the epithelial cells of the hepatopancreas, with no other organ implicated. By sequencing the PCR products using specific primers based on conserved regions of microsporidian small subunit (18S) ribosomal DNA, it was revealed that the parasite from HPND ponds had 99% sequence identity to that of *Hepatospora eriocheir*. Phylogenetic analysis also placed the microsporidian in the same lineage as *H. eriocheir*. This study reported the first case of widespread infections of *H. eriocheir* associated with HPND found in the pond-reared Chinese mitten crab, *E. sinensis*. The description of microsporidian in this important commercial host is fundamental for future consideration of factors affecting stock health and sustainability.

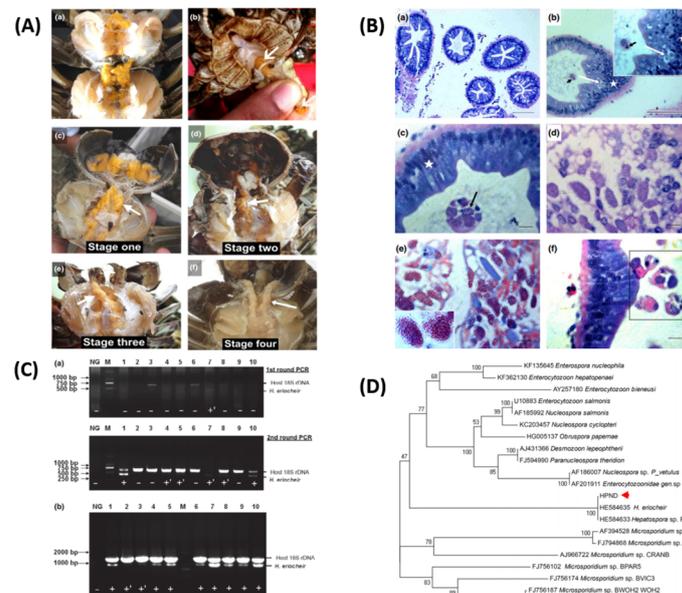


Fig. A, the hepatopancreas color turning from yellow-golden, light yellow to almost white; **B**, masses of apparently unicellular, basophilic and refractile parasite stages were observed throughout the hepatopancreas sections; **C**, two regions were obtained using the nested PCR and conventional PCR; **D**, phylogenetic analysis based on the 931 bp SSU rDNA placed the microsporidian in the same lineage as *Hepatospora eriocheir*.

SNAKEHEAD FISH FARMING PRACTICE AND FEED TECHNOLOGY IN MAJOR ASIAN COUNTRIES

Dong Qiufen*, Zhang Taoping, Zhang Song, Yang Yong

Guangzhou Nutriera Biotechnology Co., Ltd.
Unit1209, Building1, Zone 4, Helenbergh Creative
Industry Park, Panyu District, Guangzhou, Guangdong, 511400, China
qiufendong@gmail.com

Snakehead fish became a news topic since 2002 as it's reported as one of the notorious invasive species in most US states, but it's been a very important food source with high nutritive values in Asian countries since many years. More and more researches on the salutary values of snakeheads have been done, and scientists already have found out this fish has the properties of wound healing, antimicrobial, antinociception, osteoarthritic treatment, antioxidation, cardiological effects and neurological effects.

There are several main snakeheads are commercially farmed in Asian areas: *Channa maculata*, *C. striata*, *C. asiatica* and *C. argus*. China has the largest snakehead production volume with 300,000 MT yearly, the main farmed species is hybrid snakehead (*C. asiatica* ♀ x *C. argus* ♂). *C. striata* is dominant in Southeast and South Asian countries. Because of the restriction of fingerlings and feed, snakeheads are mostly farmed with big volumes in China, Vietnam and Thailand where have available artificial hatcheries and commercial feeds for this fish. With the technology promotion of Guangzhou Nutriera Biotechnology Co., Ltd., Myanmar and India have achieved successes on snakehead fry breeding, commercial farming and extruded feed development.

This presentation is a comparative analysis on snakeheads commercial farming and related feed used in the above Asian countries. The differences, problems and solutions of the farming practices and feeds will be listed, a practical farming procedure and a series of commercial feed formula are also given in the presentation.

TRANSFORMING LANGKAWI INTO A SUCCESSFUL AND SUSTAINABLE GROUPER INDUSTRY HUB IN MALAYSIA

Alexey Dorin

Chief Executive Officer
Fishance Berhad, Langkawi, Malaysia
No.31, 1st Floor, Baron Bandar Baru
Persiaran Putra, 07000, Kuah, Langkawi
Kedah Darul Aman

a.dorin@fishance.com

Grouper aquaculture is a promising industry with a huge potential and exciting opportunities. It proves to be economically feasible, innovative and sustainable in terms of investing financial resources into the projects. With demands from local and international markets, gradual increasing prices will be viable for the business. It also serves as the symbol of luxury and exclusivity which are the important link in tapping the market, thus guaranteeing huge potential for new companies to venture into grouper farming business.

Fishance Berhad is a newly-established aquaculture business specializing in indoor hatchery and cage system fish farming and is involved in the aquaculture industry since August 2016. Fishance started with 100 cages in Tanjung Rhu, Langkawi and now has up to 2000 cages located in Langgun Island. Fishance has organized the strategic plans not only to construct 10,000 cages by year 2019, but also will focus on the developmental of sustainable farming, strengthening operations and achieving business goals. Furthermore, we are committed to be accredited with the following certifications; MyGAP (Malaysia Good Aquaculture Practice), FQC (Fish Quality Certificate), BAP (Best Aquaculture Practice), ASC (Aquaculture Stewardship Council) by upgrading the infrastructure and services into world-class standard and establishing a Center of Excellence to train and develop experts in aquaculture-related fields as well as to be a leading company in the aquaculture business.

Fishance Berhad's main objectives are to engage in sustainable, clean and green aquaculture farming and also to be the largest producer of aquaculture marine fish in Malaysia. Our aim is to transform and highlight Langkawi as the first sustainable and successful International Grouper Hub in the region. Langkawi has the advantage of a duty-free zone and ideal conditions for production of sufficient best quality of groupers to be produced. This will attract business ventures with Live-Fish export traders from China, Hong Kong, Singapore and other important South East Asia countries.

EFFECTS OF REPLACING FISH MEAL BY A BLEND OF FERMENTED CANOLA MEAL AND SOY PROTEIN CONCENTRATE IN DIETS FOR JUVENILES RED SEA BREAM *Pagrus major*

Serge Dossou*, Shunsuke Koshio, Manabu Ishikawa, Saichiro Yokoyama, Philippe Lalèyè, Antoine Chikou, Mahmoud A.O. Dawood, Mohammed F. El Basuini, Olivier Adissin and Amr I. Zaineldin

Laboratory of Aquatic Animal Nutrition

Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima, Japan

sergedos@yahoo.fr

With the growing demand and high cost of protein sources in aquaculture, Rapeseed/Canola (CM) has become one of the most viable sources of plant protein because of the volume of production and its protein's nutritional and functional qualities. However, antinutritional factors in CM may restrict fish growth and affect protein utilization and health status of fish. Solid state fermentation has been shown to improve nutritional qualities of feedstuffs and leads of lowering food allergy, enhancing antioxidant activities and immune stimulator compounds in meals. Therefore, the present study was conducted to investigate the effects of using *Aspergillus oryzae* fermented CM (FCM) and Soy protein concentrate (SPC) as major dietary protein sources on red sea bream performances.

Five isonitrogenous and isocaloric diets were formulated replacing FM by a blend (2:1) of FCM and SPC to achieve an FM protein substitution level of 0% (FP0), 25% (FP25), 50% (FP50), 75% (FP75) and 100% (FP100) respectively. Triplicate groups of twenty fish were assigned to each dietary treatment for a 56-days feeding trial. FP25 fed group showed significantly higher ($P < 0.05$) growth performances than all other groups. However, there were non-significant differences ($P > 0.05$) in survival, feed/protein efficiency ratio in groups fed FP0, FP25 and FP50 diets. Blood total Cholesterol level followed the same trend. Triglyceride, blood urea nitrogen, glutamyl oxaloacetic transaminase, lysozyme and peroxide activities remained unchanged with the inclusion of the blend. Feed intake was unchanged in all dietary treatments but 75% and 100% substitution levels significantly reduced ($P < 0.05$) growth and feed/protein efficiency ratio. These effects could be related to reduced digestibility due to an increased dietary phytic acid.

These findings suggest that FM may be reduced to 24% (50% replacement) with FCM and SPC blend without detrimental effects on overall performances of red sea bream. Moreover, we recommend 25% replacement as this level has recorded the best growth, immune responses and anti-oxidative stress activities.

TABLE 1. Diet formulation for Red sea bream

	Diet				
	FP0	FP25	FP50	FP75	FP100
FM	48	36	24	12	
FCM		10.1	20.2	30.3	40.4
SPC		5	10	15	20
Other	52	48.9	45.8	42.7	39.6

TABLE 2. Final weight (FBW, g), specific growth rate (SGR, %/day), hematocrit (HT, %), Serum protein (T-Pr, g/dl), Catalase (CAT, KU/L) and antioxidant potential (BAP, $\mu\text{Mol/L}$). Means with the same letters are not significantly different ($P > 0.05$).

	Diet				
	FP0	FP25	FP50	FP75	FP100
FBW	18.1 ^c	19.4 ^d	17.8 ^c	15.7 ^b	13.6 ^a
SGR	2.1 ^c	2.2 ^d	2.0 ^c	1.8 ^b	1.6 ^a
HT	38 ^b	39.3 ^b	27.6 ^a	19.3 ^a	26 ^a
T-Pr	3.6 ^c	3.1 ^{bc}	2.6 ^{ab}	2.5 ^{ab}	2.15 ^a
CAT	175 ^{bc}	216.2 ^c	116 ^{ab}	89.4 ^a	68.1 ^a
BAP	3389 ^b	3895 ^c	2965 ^a	2698 ^a	2646 ^a

POTENTIAL GAPS IN MANAGING THE RISK OF TRANSFER OF AQUACULTURE PATHOGENS VIA SHIPPING – THE NEED FOR CROSS SECTORIAL COOPERATION

Drillet, Guillaume*; Wisz, Mary S.; Le Berre Lemaire-Lyons, Youna; Baumler, Raphael; Henn Ojaveer; Bondad-Reantaso, Melba G; Xu, Jiamin; Alday-Sanz, Victoria; Saunders, Justine; McOwen, Chris J.; Eikaas, Hans

DHI group
Cleantech One 03-05
CleanTech Loop
637141 Singapore
Guillaume Drillet: gdr@dhigroup.com

In November 2016, soon after Finland and the Republic of Panama ratified the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWMC), Nature published a correspondence in which we raised our concerns that the Convention may not be able to cover the potential risks generated by the transfer of aquaculture pathogens.

The Ballast Water Convention is a cornerstone of the regulatory regime in place to protect ecosystems from bio-invasions and it will substantially decrease the risk of alien invasions from shipping. The International Maritime Organization has worked since the end of the 1980's until today to develop a global and applicable regulation on Ballast Water. What has been a gargantuan adventure is finally becoming a reality. A presentation of the latest developments in the ballast water management sphere will be made. However, managing pathogens in ballast water might remain a challenge even after ships are fitted with Ballast Water and we are in the opinion that future work should be carried out to evaluate whether there are residual risks which could affect the aquaculture sector.

A NASCENT INDUSTRY TO ANSWER NEW MARKET CHALLENGES. HERMETIA MEAL, A SUSTAINABLE AND GLOBAL SOURCE OF PROTEIN

Franck Ducharne¹ and Emilie Devic¹

¹Entofood Sdn Bhd, Kuala Lumpur, Malaysia
franck.ducharne@entofood.com

For over a decade, increasing scarcity and market price of the fishmeal have led the animal feed industry to seek for sustainable and cost-efficient alternative to answer to the fast-growing demand of the sector. Aquafeed formulations evolved towards a reduction of the marine ingredients dietary inclusions and an increase use of plant-based products, including for carnivorous species. Nowadays, the challenge of the animal industry is to produce more with less pressure on natural resources and limited impacts on the environment. Different solutions are being explored to generate novel alternative protein sources such as single-cell, algae and insects.

Insects, in particular detritivores species such as *Hermetia illucens* (Black Soldier fly), offer various advantages. Extensive work and research have been dedicated to master breeding and zootechny of this species which can recycle and convert a wide range of organic side-streams. The year-round and global availability of large volume of organic by-products are key drivers to successful and sustainable development of a circular economy. Most challenges related to the scalability of the production of “*Hermetia* meal”, including breeding and automation, have been overcome and technical solutions are now available worldwide. In addition, thanks to the recent approval from the European Union Commission to use insect proteins in aquafeeds (1st July 2017), demand and use of insect meals are expected to grow. Although according to the literature, *Hermetia* meal is a high quality feedstuff that can be included in feed for various fish species, more work is still required to adapt commercial feed formulations to this alternative source of protein.

In Malaysia, *H. illucens* larvae fed plant-origin materials are further processed into a consistent high quality feedstuff with a nutritional profile comparable to fishmeal (Table 1). Moreover, functionalities related to the high levels of lauric acid of the product will be investigated. A vast R&D program is conducted in association with private and public organisations to test, validate and build a data base on the optimal inclusion of *Hermetia* meal in aquafeeds. So far, performance of the various species fed diets containing inclusions of *Hermetia* meal (*Lates calcarifer*, *Penaeus vannamei*, etc.) were not significantly different from those of the animals fed fishmeal-based control diets. This presentation will present more in details the outcomes of our recent studies.

Table 1: Nutritional composition of fishmeal (menhaden) and whole and defatted *Hermetia* meal

	Fishmeal	<i>Hermetia</i> meal	
	<i>Menhaden</i>	<i>Whole</i>	<i>Defatted</i>
Composition (% , as fed basis)			
Crude protein	64.5	47.9	55.5
Crude lipid	9.6	28.1	14.6
Ash	19	8.2	9.3
Crude fibre	0.7	5.7	6.7
Chitin	-	~ 3	~ 4
Essential Amino Acids (% , as fed basis)			
Arginine	3.66	2.45	3.08
Histidine	1.78	1.67	1.91
Isoleucine	2.57	2.03	2.48
Leucine	4.54	3.20	3.93
Lysine	4.81	2.61	3.75
Methionine	1.77	1.27	1.11
Cystine	0.57	0.32	0.54
Phenylalanine	2.51	2.23	2.68
Tyrosine	2.04	3.02	3.83
Threonine	2.64	1.74	2.35
Valine	3.03	2.84	3.44

HEMOLYMPH EXTRACTION UNDER THE LENSE: VISUALIZATION OF THE CARDIOVASCULAR SYSTEM OF THE BLUE MUSSEL (*Mytilus edulis*)

Mieke Eggermont*¹, Pieter Cornillie, Nancy Nevejan, Peter Bossier, Manuel Dierick, Dominique Adriaens, Patrick Sorgeloos, Tom Defoirdt, Annelies Maria Declercq

¹Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Rozier 44, 9000 Gent, Belgium

Bivalve hemolymph is commonly used in a broad range of research domains such as eco(toxico)logy and immunology. The minimally described hemolymph withdrawal protocols and reported locations (adductor muscles and heart) raise questions regarding the exact origin of the aspirated hemolymph and possible contamination with other body-fluids, which might have biased the conclusions that were drawn from these studies. A good description of the species-specific anatomy is lacking in many bivalves but essential for a correct hemolymph withdrawal. In this study we visualized and discussed the cardio-vascular anatomy of the blue mussel (*Mytilus edulis*) as model organism and generated three-dimensional reconstructions based on micro-CT and histological slides. Other organs, such as the gastro-intestinal system, the muscular system and body-cavities, were included as well because of their close relationship to the cardio-vascular system.

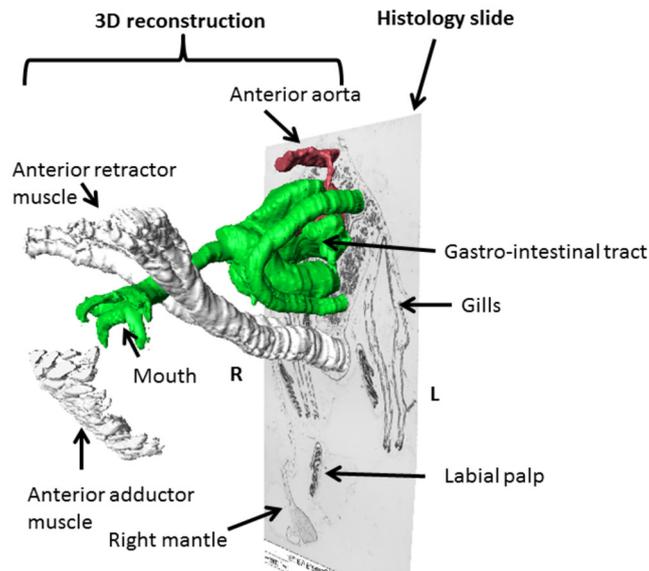


Fig. 1: 3D reconstruction of the blue mussel based on histology

ISOLATION OF VIBRIONACEAE FROM WILD BLUE MUSSEL (*Mytilus edulis*) ADULTS AND THEIR IMPACT ON BLUE MUSSEL LARVICULTURE

Mieke Eggermont^{*1}, Peter Bossier, Gde Sasmita Julyantoro Pande, Vyshal Delahaut, Ali Md. Rayhan, Nipa Gupta, Shikder Saiful Islam, Elsie Yumo, Nancy Nevejan, Patrick Sorgeloos, Bruno Gomez-Gil, Tom Defoirdt

¹Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Coupure Links 653, 9000 Gent, Belgium

The blue mussel (*Mytilus edulis*) is known as a robust mollusk species, although its larviculture appears to be highly susceptible to diseases. In this study, we isolated 17 strains from induced mortality events in healthy wild-caught blue mussel adults and demonstrated that they caused between 17 and 98% mortality in blue mussel larvae in a newly developed, highly controlled immersion challenge test model. Eight of the isolates belong to the *Splendidus* clade of vibrios, while the other isolates belong to the genus *Photobacterium*. The genomes of the most virulent *Vibrio* isolate and the most virulent *Photobacterium* isolate were sequenced and contained several genes encoding factors that have previously been linked to virulence towards mollusks. *In vitro* tests confirmed that all 17 isolates were positive for these virulence factors. The sequenced genomes also contained a remarkably high number of multidrug resistance genes. We therefore assessed the sensitivity of all isolates to a broad range of antibiotics and found that there were indeed many strong positive correlations between the sensitivities of the isolates to different antibiotics. Our data provide an ecological insight into mass mortality in blue mussels as they indicate that wild mussels contain a reservoir of pathogenic bacteria.

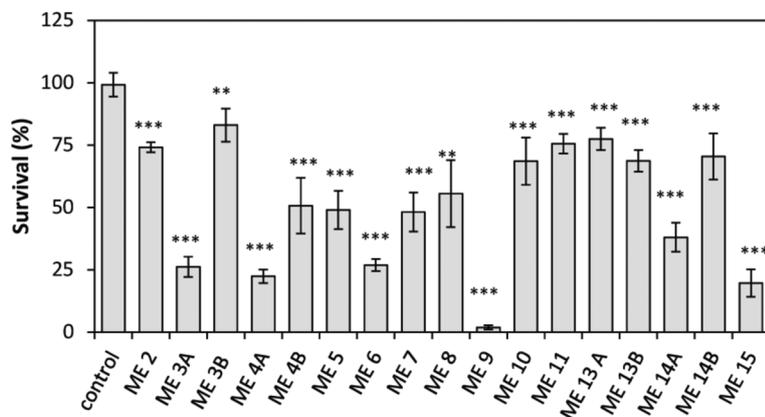


FIGURE 1: Survival of blue mussel larvae after 5 days of challenge with the isolates. Error bars represent the standard deviation of 4 replicate mussel cultures. Asterisks indicate a significant difference in survival when compared to the survival in the control treatment (independent samples t-test; **: P < 0.01; ***: P < 0.001). "Control" refers to non-challenged larvae that were otherwise treated in the same way as challenged larvae.

MULTIPLE-REGRESSION: A COMPREHENSIVE APPROACH FOR ANALYSIS OF WEIGHT-LENGTH RELATIONSHIPS IN *Macrobrachim rosenbergii*

MO Elsheikh^{1,3*} and S Bhassu^{1,2}

¹ Division of Genetics and Molecular Biology, University of Malaya, Malaysia

² Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Malaysia

³ Department of Genetics and Animal Breeding, University of Gezira, P.O Box 20, Wad Medani, Sudan

*Presenting author: Mohamed Omer Elsheikh

Email: mohamed1173536@yahoo.com

The Malaysian giant prawn, *Macrobrachium rosenbergii*, is an important crustacean aquaculture candidate cultured all year round in hot climate regions and seasonally in some temperate areas. According to the authors' knowledge, there is no reported multiple -approach attempt so far, at least for shrimps, for estimating weight using morphometric measurements. The authors hypothesized that the few morphometric traits, with proved simple significant relationships, are expected to affect body weight simultaneously. Hence, in this study they decided to use a multiple approach using these morphometric measurements as predictors for body weight. The approach was found to be more effective and accurate compared to simple relations, as it explained 73- 94% of the total variance and showed significant differences between populations of the species collected from different geographical locations. Accordingly, the authors confidently recommend this method to be used for such estimations in other crustaceans and another metazoan as well

SOCIO-ECONOMIC CHARACTERISTICS OF COASTAL FISHERMEN SOCIETY IN PULAU MOROTAI

Erna Ratnawati * and Ruzkiah Asaf

Balai Penelitian dan Pengembangan Budidaya Air Payau
(Research Institute for Coastal Aquaculture)
Jl. Makmur Dg. Sitakka 129 Maros 90512, Sulawesi Selatan
E-mail: ernaratnawati60@yahoo.co.id

Pulau Morotai in North Maluku is a border area which is a regional division with a view to the progress of development and improved welfare. This study examines the social and economic characteristics of fishing communities in the District of Morotai Island. The research aims to study the social and economic aspects of the fishing communities include fishing communities, education, etc. The study was conducted using a combination of qualitative and quantitative approach that emphasizes an explorative descriptive analysis. The combination of the two approaches are part of the triangulation theory both in terms of resources, data collection techniques, data sources and analysis. Data collected through in-depth interview with the respondents and through a process of observation. There are a few of those interviewed include the fishermen, gatherers, village governments and local governments are being sampled in this study.

Social conditions Population

of Agriculture as the principal livelihood for the population is predominantly of plantation crops such as cloves, nutmeg and coconut, then the results of the food crops such as rice, corn, cassava and others. While the results of the fishery in the dominance of the catch of fishermen with fishing gear are predominantly used hand line and amounted to 1,038 units (Morotai Island Regency in Figures 2015).

The structure of population by religion is dominated by the Moslem population and there are at most in the District of South Morotai, followed by Christianity and the highest in the District of West South Morotai, then Catholicism that most adherents in the District of Morotai Jaya.

Educational facilities which is a forum for learning and teaching is a concern that is not less important for the government of the District of Morotai Island, seen with full facilities available in each district starting from the level of kindergarten, elementary, middle, and high school / vocational school, so there is no excuse for residents to not denounce school. Not only to the high school level but for male and female students who will continue again education, no longer need to exit the area because in the district capital of existing universities, namely the University of the Pacific.

Analysis Process Flow Aquaculture

From the results of data collection and interviews with some of the respondents with respect to development of the cultivation in the District of Morotai, then the process flow analysis (marketing channels) to further discuss the relationship between sub-processes that influence the development of aquaculture in the District of Morotai as follows:



Description:

Product Flow:

Flow Information:

Financial Flows:

(Continued on next page)

Based on the results of research in the field can be seen that there are three streams that flow products, financial flow and information flow in the supply chain results aquaculture products. The third stream flows in the chain such as farmers / fishermen, retailers, entrepreneurs, traders and consumers. Efforts to optimize the three streams that exist in the supply chain results aquaculture activities can be done with the system approach involving multiple parties, such as farmers, retailers, entrepreneurs, traders, consumers and government as policy maker. Supplies products of aquaculture activities in Pulau Morotai been insufficient because the fishermen or farmers only dependent on the capital given to each farmer group and the constraint in cultivation that water quality conditions of cultivation and the disease were found in the continuous production of which affects the outcome of aquaculture production. Parties involved in the supply of product yield farming activities are fishermen or farmers because if they do not do cultivation optimally, the available cultivated will not be fulfilled until the level of the consumer, for the fulfillment of the government's cooperation in this regard is needed in terms of policy and the availability of technology and capital in doing cultivation.

GROWTH PERFORMANCE OF GIANT FRESHWATER PRAWN AND VEGETABLES (CHINESE CABBAGE AND LONG BEAN) IN AQUAPONICS WITH AND WITHOUT GRAVEL AS MEDIA FILTERS

Abentin Estim *, Mhd Jefri Lacing, Azizul Julirin and Saleem Mustafa

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah
Email: bentin@ums.edu.my

This paper presents the growth performance of giant freshwater prawn (*Macrobrachium rosenbergii*), chinese cabbage (*Brassica rapa*) and lettuce (*Vigna unguiculata*) in aquaponic systems with and without gravel as media filter. The study was conducted in 60 days using glass aquarium (150 litres). The aquaponic system was designed by following the 'one-pump rule' where the culture water was lifted by a 40 watt submersible pump to the highest portion of the system before its flow to the bio-filtration tank, allowing the flow of the water by gravity along the plant tanks (PVC pipe with diameter 100 mm and 80 cm long) and finally returning back to the prawn-holding tank (glass aquarium with size of 92 cm X 46 cm X 46 cm).. Results from the t-test analyses showed that there were significantly differences ($p < 0.05$) of *V. unguiculata* and nitrate concentration in the aquaponic system with and without gravel. The growth performance of *V. unguiculata* and $\text{NO}_3\text{-N}$ concentrations were recorded higher in the tank with gravel media. The other parameters, such as DO, pH, temperature, ammonia, nitrite, phosphate and growth performances of *M. rosenbergii* and *B. rapa* were no significantly differences ($P < 0.05$). These results showed that the gravel media did not affect water quality and the growth performance of prawn and cabbage. This paper suggested that the filter media of gravel is the best and feasible integrated aquaponic system for giant freshwater prawn, Chinese cabbage and lettuce.

IMPROVING NUTRITIONAL SECURITY THROUGH THE METHOD OF DRYING *Heteroclarias* AND *Oreochromis niloticus* FOR PRESERVATION

Fapohunda, Olawumi* and Esan, Ibiyemi

Department of Fisheries and Aquaculture Management
Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti
E-mail: olawumi.fapohunda@eksu.edu.ng

This study was conducted to compare the effect of Sun drying and Oven drying on the nutritional composition of two fish species (*Heteroclarias* and *Oreochromis niloticus*). Ten freshly harvested catfish (*Heteroclarias*) and Tilapia (*Oreochromis niloticus*) were shared into two parts; a part was sun - dried for 12 days (7 hours daily) between the hours of 9 a.m – 4 p.m at an average temperature range of 23 – 35.9°C, temperature range in Iworoko Ekiti between February – March (According to Norwegian Met Institute) and the other part was dried using laboratory electric oven at a temperature range of 80°C for 5 days (4 hours daily). Each part was in three (3) replicates. The proximate compositions of the dried samples (Mean moisture, protein, lipid, ash, fibre, carbohydrate) were determined. The changes in moisture, protein and carbohydrate contents including energy value were found to be significant ($P < 0.05$) for the two processing (drying) methods. Ash and lipid contents showed no significant differences ($P > 0.05$) for the two drying methods used in this study. The changes in the fibre contents was found to be significant ($P < 0.05$) in the *Heteroclarias* species for both processing methods while it was not significant ($P > 0.05$) in the *Oreochromis niloticus* species for both processing methods. This result indicates that drying methods have effects on the proximate compositions of fish species (*Heteroclarias* and *Oreochromis niloticus*). The organoleptic properties of Oven dried and Sun dried fish species are shown on Tables 1 and 2. The overall assessment showed that; for taste; the highest mean value recorded was 6.0 ± 0.0 and 5.3 ± 0.6 in Oven dried *Heteroclarias* and Oven dried *Oreochromis niloticus* respectively. The least mean value 3.0 ± 0.5 was recorded in the sun dried *Oreochromis niloticus* followed by 3.4 ± 0.6 in sun dried *Heteroclarias*. The result further indicated that there is significant difference between organoleptic qualities of oven dried and sun dried samples of the same fish species.

Table 1
Organoleptic Properties of the Oven – dried and Sun - dried *Heteroclarias*

Fish Species	Taste	Texture	Palatability	Flavor
Oven dried <i>Heteroclarias</i>	6.0 ± 0.0	5.3 ± 0.6	5.7 ± 0.4	5.6 ± 0.6
Sun dried <i>Heteroclarias</i>	3.4 ± 0.6	3.7 ± 0.8	2.9 ± 0.7	3.1 ± 0.5

Table 2 Organoleptic Properties of Oven – dried and Sun – dried *Oreochromis niloticus*

Fish Species	Taste	Texture	Palatability	Flavor
Oven dried <i>O. niloticus</i>	5.3 ± 0.6	3.8 ± 0.1	4.5 ± 0.0	4.5 ± 0.1
Sun dried <i>O. niloticus</i>	3.0 ± 0.5	2.5 ± 0.5	2.1 ± 0.5	3.1 ± 0.6

DEVELOPMENT OF INTEGRATED MOBILE KNOWLEDGE BASED AND REPORTING SYTEMS FOR AQUACULTURE MANAGEMENT OF TILAPIA INDUSTRY PLAYERS

Sharihan Fathi*, Aizul Nahar Harun, Shuib Rambat

Universiti Teknologi Malaysia Kuala Lumpur, Malaysia-Japan International Institute of Technology,
Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia
sharihanfathiz@gmail.com

Aquaculture industry generate a large number of untapped information to benefit all stakeholders in the aquaculture sector. The information including the current research in the industry, the players involved in the sector, problem solving towards the specific issues in breeding, farming or technology utilization. However, these information only benefit to a few group of stakeholders since the shared information unable to reach timely to the targeted group. Moreover, little is known about the interaction problem between aquaculture stakeholders that contribute to the failed in delivering and sharing the significant information among stakeholders. Hence, it leads to the need of effective information sharing among stakeholders that feasibly utilized current technology to strengthen the interaction among the stakeholders in term of sharing the knowledge, communication and relationship perspective. Therefore, this paper contributes to offer a novel idea on developing an integrated mobile knowledge based and reporting systems as a tool to assist the aquaculture management issue of Tilapia stakeholders. Specifically, this study emphasis on the effectiveness of the interaction among stakeholders mainly among Department of Fisheries (DOF) as the government entity and other stakeholders in Tilapia supply chain that consists of hatchery systems till grow out ponds. To achieve the objective of the study, this research will adapt to the Design Science Research (DSR) as a research philosophy with Generic Design Cycle as methodological approach. A literature review of aquaculture management, stakeholder management and trust among stakeholders will be explained during the study. These studies further will be analysed to form a systematic functional design of mobile application for aquaculture stakeholders. As a consequence, the aquaculture information with dependency of online mobile connectivity will be a functional platform to connect with all stakeholders in the aquaculture sector which resulted to the new paradigms for aquaculture management. Thus, aquaculture mobile application for knowledge based and reporting systems will be develop to benefit the stakeholders in the industry.

GONADAL DEVELOPMENT AND EARLY MATURITY OF GIANT GOURAMI IN INDONESIA

Rita Febrianti *, Sularto, and Nunuk Listyowati

Research Intitute for Fish Breeding.
Sukamandi - Subang, West Java
Post Code 41263 Ph. 0260-520500 Fax:0260-520662
E-mail : rfchemistipb@yahoo.com

Gonadal development is part of the reproduction of fish before spawning. The stages of gonad maturity necessary to determine the ratio of fish to spawn or not (Suwarso and Sadhotomo, 1995). According Suwarso and Sadhotomo (1995), information on the life cycle in the reproductive aspects including the development of gonadal maturation from spawning until the next spawning. In addition, it is used to find out about the size or age of the fish were first ripe gonads, spawning time, and the intensity of spawning for one year (Effendie, 1979).

Research on the biological aspects of reproduction of giant gourami (*O.gorami Lac*) has been done using stem technology development surrogate in particular to determine the morphology and composition of spermatogonia cells of giant gourami in various sizes (Mauluddin, 2009). Gonad maturation level (GML) on ornamental has also been carried out with the addition of leaf application ketapang (Kadarini T *et al*, 2010). Research on the early gonadal development as a mature sex determination in has not been done. Therefore this research. In addition, to determine the gonad maturity level (GML), giant gourami of male and female gonad maturity index (GMI), gonad index (GI), and morphology and histology ovaries and testes in the male and females giant gouramy.

The results showed that there were observations morphology and histology giant gourami (*O.gorami Lac*) can be divided into five stages, namely the development of GML I (resting phase), GML II (developing phase), GML III (Maturing phase), GML IV (partially spent), and GML V (full spent). The gonads of female gonad have been ripe at the age of 23 months with the average weight of 20.85 ± 6.29 gram. The weight of 23-month-old male gonads was 0.57 ± 0.08 gram. The number of eggs were 257 eggs per gram weight of the gonads. Gonad maturity level of male and female on the morphology and histology can be seen in the Table 1.

COST OF NUTRIENTS IN DIFFERENT METHODS OF FERTILIZATION FOR AEROBIC MICROBIAL FLOC PRODUCTION IN INDOOR TANKS

S.Felix, A.Gopalakkanan, M.Menaga and Yuvarajan

The aim of this study was to evaluate the effect of two methodologies of fertilization on the volume and characteristics of floc. Nine aquarium glass tanks (75 litres each) were divided into three treatments. The treatment 1 followed the fertilization regimes of including nitrogen fertilizers, treatment 2 was studied with the inclusion of both nitrogen and phosphorus fertilizers and treatment 3 was the control where the conventional method of fertilization was adopted. In treatment 1 & 2, the tanks received daily molasses fertilization throughout the entire rearing period. In control, molasses was used only initial fertilization was done. Distillery spentwash obtained as effluent from sugarcane industries were used as the carbon source. The tanks were operated with no water exchange and the total suspended solids concentration were kept between 300 and 400 mg L⁻¹. The sludge produced at harvest were quantified and their characteristics were determined. The production of TSS in the treatment 1 was higher than in the treatment 2 ($P < 0.05$). The analysis of the sludge revealed a high amount of volatile solids in both treatments, between 636 and 702 g kg⁻¹. The BOD: TSS ratio was also low in both treatments, but the treatment 2 showed lower values ($11.3 \pm 0.6\%$) than the treatment 1 ($15.9 \pm 0.0\%$) ($P < 0.05$). Both fertilization strategies were able to modify the characteristics of sludge produced during cultivation. Moreover, the nitrogen and phosphorus content in treatment 2 showed a increased floc volume (15.42 ± 0.2 ml) within 7 days and it was much easier to maintain the floc in both the treatments. The TPC was significantly different in treatment 2 ($P < 0.05$) indicates the growth of heterotrophic bacteria. The study concludes that cost of application of nitrogen and phosphorus fertilizers in treatment 2 was comparatively higher and increases the floc volume within seven days.

OYSTERS IN THE OUTBACK: OYSTER SPAT CULTURE IN POTASSIUM DEFICIENT INLAND SALINE GROUNDWATER

D. Stewart Fielder, Michael C. Dove, Laura M. Parker, Wayne A. O'Connor

Port Stephens Fisheries Institute
Locked Bag 1
Nelson Bay NSW 2315
AUSTRALIA
stewart.fielder@dpi.nsw.gov.au

The increasing prevalence of oyster diseases has had both direct and indirect impacts on oyster production world-wide. Beyond initial stock losses during disease outbreaks, secondary imposts can arise from biosecurity protocols enacted to protect unaffected populations. By moving hatcheries to locations where the disease is absent, where carriers are not present and where introduction of the disease is less likely, risks can be reduced. One such option in New South Wales, Australia, is to move inland where large supplies of saline water are available.

Considerable research effort has been invested into inland saline aquaculture in Australia, but to date the emphasis has been on the production of fish and crustaceans. One challenge has been natural deficiencies in potassium in the saline ground water. For fish this has been addressed through potassium supplementation, but it is not known if this would be necessary for molluscs. This trial was undertaken as a preliminary assessment of the use of saline ground water for hatchery production of oyster seed before more extensive investigation is undertaken.

Sydney rock oyster (*Saccostrea glomerata*) spat were taken to the Inland Saline Aquaculture Research Centre at Wakool in western New South Wales, where they were held at one of two salinities (17ppt and 33ppt) and one of 5 potassium concentrations (0, 25, 50, 75, and 100% of the concentration present in seawater at that salinity). Two additional treatments of 17ppt and 33ppt artificial seawater (Instant Ocean) were included as controls. Spat were fed a standard hatchery algal diet and growth (increase in shell height) and mortality were determined every 7 days for 3 weeks.

Spat survival was significantly reduced in potassium deficient saline ground water, with poorest survival in 0% potassium at 33 ppt. Growth was significantly reduced at 17 ppt in comparison to 33 ppt. Growth also differed significantly between potassium treatments with no growth in 0 and 25% potassium at both salinities. There was no significant interaction between salinity and potassium. Spat held at 50% potassium and greater showed significant increase in shell height, the equivalent of that observed in artificial seawater controls.

Investigations into effects of potassium deficient saline groundwater on the physiology of Sydney rock oysters are ongoing.

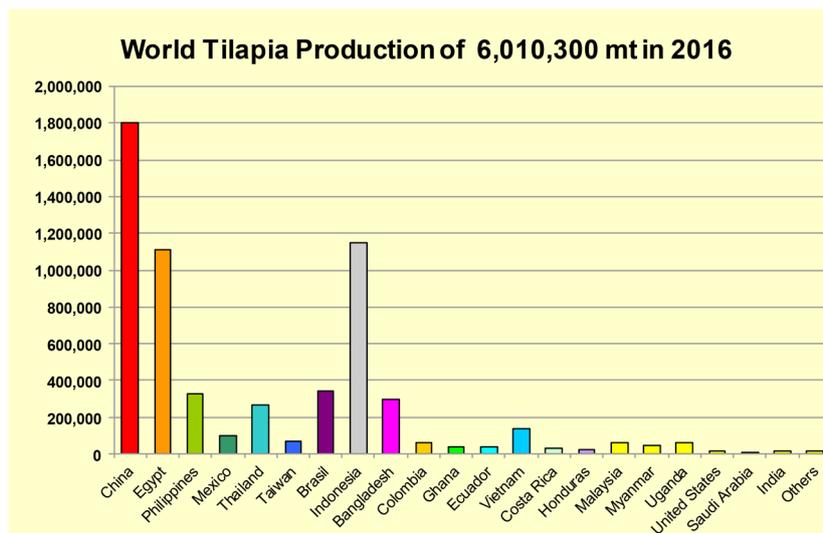
2017 - SUPPLY AND DEMAND IN TILAPIA MARKETS

Fitzsimmons, K.* and Kay LwinTun

University of Arizona, Forbes 306, Tucson, Arizona, 85719
kevfitz@ag.arizona.edu

Tilapia demand and prices in the US, Canada and parts of Western Europe have dropped in 2015, 2016, and early 2017. This drop seems to be completely attributed to several misleading reports posted on the internet. Google, Bing, and Yahoo searches for tilapia bring up several sites in their top results with claims that tilapia is worse than bacon for your health. Sites with more accurate and valid information are buried lower in the search results. It is the lead heading of worse than bacon that seems to engender the most attention, even though the claim has been discredited by nearly every nutrition expert, medical doctor and dietician. The comments were included in a technical paper comparing omega 3 to omega 6 fatty acid concentrations in farmed tilapia and other fishes. The first figure in the article shows the measured omega 3 levels in farmed tilapia to actually be much higher than many common warm water wild caught marine species. But a focus on the relatively high ratio of omega-6 to omega-3 led to the spurious comments. Strategies to correct the misinformation and raise the search results for more accurate information will be discussed. While the drop in demand seems to be greatest in English speaking countries, questions about quality have arisen from many countries. Despite the drop in demand in some markets, global production and consumption have continued to rise.

Production and consumption have increased in several countries that are particularly important on a global basis. First, Indonesia has reported rapid increases in production but only minimal increase in exports. The government reports for 2015 and 2016 are for over a 1,110,000 metric tons. Second, Egypt has also rapidly increased production, to more than 1,000,000 tons, mostly for domestic consumption with some exports to the Gulf Arab countries. Third, Bangladesh has increased its production at a tremendous rate in recent years. In 2002 the production was under 10,000 mt and by 2016, reliable estimates are for more than 300,000 mt essentially all for domestic markets. New production in India and Pakistan encourage us to believe that these two huge markets could become key leaders in further tilapia research and development and markets.



FEASIBILITY STUDY OF USING DUCKWEED AS TILAPIA-FEED INGREDIENT IN VARIOUS PROCESSING LEVELS

Laila M. Gallego*, Yew-Hu Chien

National Taiwan Ocean University
Keelung, Taiwan, R.O.C.
laila_gallego@yahoo.com

Duckweed has been considered a good source of dietary plant protein for fish due to its high nutritive value, up to 40% crude protein; fast growing, 8 days a generation; easy to harvest, overflow collection and other favorable production features. To assess the potential of duckweed's being used as a commercial feed ingredient, the digestibility and assimilation efficiency of the processed duckweed (also considered for storage) have to be studied. Therefore, this experiment was to find out the differences in growth performance of red tilapia fed duckweed processed in various levels.

Four experimental isoproteous diets containing duckweed processed in various levels were formulated and prepared as shown in Table 1. From simple to complicate processing of duckweed, those diets were noted as FF (fresh frozen), FD (fresh dried), DP (dried powder) and FP (fermented powder). A commercial tilapia feed was used as a reference but noted as C(control). Red tilapia ($4.7\pm 0.5\text{g}$) was fed at a daily ration of 8% body weight in a 9-week duration. Little stress was from water quality or stocking density.

The results show that FP diet had the best performance in growth by WG and SGR. FF diet had the poorest feed efficiency, or FCR. No difference in survival was found. Although the processed duckweed appears improve red tilapia's rearing performance, if the net benefit is parallel to its processing cost still require an economic analysis.

Table 1. Formulation of the experimental diets.

Ingredients	FF	FD	DP	FP
Fresh frozen	800		0	0
Fresh dried	0	800	0	0
Dried powder	0	0	800	0
Fermented powder	0	0	0	460
Squid meal	30	30	30	30
Corn meal	40	40	40	130
Carboxymethyl cellulose	50	50	50	300
Fish Oil	30	30	30	30
Vitamin premix	20	20	20	20
Mineral premix	20	20	20	20
Cr ₂ O ₃	10	10	10	10

Table 2. Final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and percentage survival (SR) of red tilapia fed with duckweed in various processing level for 9 weeks. Means in a column with different letters were significantly different ($P<0.05$)

	Diet				
	C	FF	FD	DP	FP
FW (g)	68.9 ^{ab}	63.3 ^{bc}	61.6 ^c	67.9 ^{bc}	75.2 ^a
WG (g)	40.5 ^b	35.0 ^{bc}	33.3 ^c	39.5 ^{bc}	47.6 ^a
SGR(%)	2.55 ^b	2.21 ^{bc}	2.10 ^c	2.49 ^{bc}	3.00 ^a
FCR	1.05 ^c	2.10 ^a	1.13 ^c	1.47 ^b	1.17 ^c
SR(%)	91.7 ^a	91.7 ^a	95.8 ^a	91.7 ^a	95.8 ^a

THE COMPARATIVE AEROBIC METABOLISM OF BARRAMUNDI *Lates calcarifer* AND BROWN-MARbled GROUper *Epinephelus fuscoguttatus*

Steven Gamble*, Igor Pirozzi

Centre for Sustainable Tropical Fisheries and Aquaculture & College of Science and Engineering
James Cook University
Townsville, Queensland
Australia

Barramundi, *Lates calcarifer*, and brown-marbled grouper, *Epinephelus fuscoguttatus*, are both important tropical aquaculture species. They are euryhaline and eurythermal with a high degree of plasticity to increasing temperature. Little has been documented on the aerobic metabolism of these species. Here, we determine the routine metabolic rate (RMR), maximum metabolic rate (MMR) and aerobic scope (AS). Aerobic metabolism of barramundi with a mean body weight of 262.5 ± 3.85 g and brown-marbled grouper with a mean body weight of 294.5 ± 2.61 g was measured across a 20°C temperature range (18, 28 and 38°C) and expressed as a function of temperature (T) per $\text{mg O}_2 \text{ kg}^{-0.8} \text{ h}^{-1}$ as: Barramundi $\text{RMR} = 3.464T - 27.605$, $\text{MMR} = 7.67T + 0.2067$ and $\text{AS} = 4.212T + 27.684$. The energy equivalent was calculated for RMR as 11.49, 22.32 and $34.08 \text{ kJ kg}^{-0.8} \text{ day}^{-1}$ for routine energetic demand and for MMR as 43.47, 73.35 and $93.51 \text{ kJ kg}^{-0.8} \text{ day}^{-1}$ for maximum energetic demand at 18, 28 and 38°C , respectively. Brown-marbled grouper $\text{RMR} = 0.0827T^2 - 0.8139T + 8.5516$, $\text{MMR} = -0.686T^2 + 44.686T - 461.38$ and $\text{AS} = -0.768T^2 + 45.468T - 469.59$. The energy equivalent was calculated for RMR as 6.75, 16.49 and $31.63 \text{ kJ kg}^{-0.8} \text{ day}^{-1}$ for routine energetic demand and for MMR as 39.37, 82.19 and $80.27 \text{ kJ kg}^{-0.8} \text{ day}^{-1}$ for maximum energetic demand at 18, 28 and 38°C .

The aerobic metabolism of juvenile barramundi continues to increase linearly with temperature to the upper extreme of the ecologically relevant temperature range (38°C). In contrast the response of brown-marbled grouper followed a bell-shaped curve with the vertex at 29.6°C implying a thermal optima for this species.. This provides practical information for suitable thermal range for aerobic scope i.e. at 28°C AS for barramundi is 22.3% less than brown-marbled grouper, as well as describing the thermo sensitivities of barramundi and brown-marbled grouper across an ecological relevant temperature range.

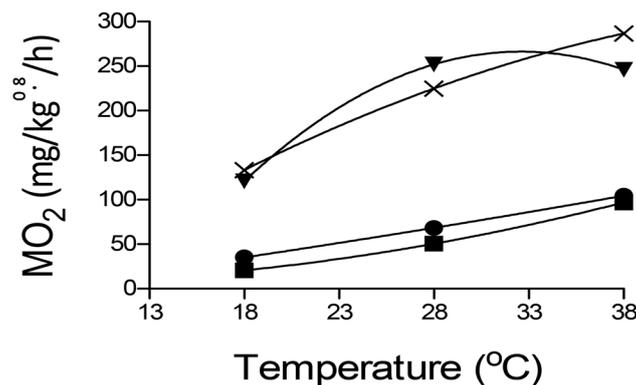


Figure 1: RMR (solid circle) and MMR (cross) of juvenile barramundi, mean body weight of 262.5 ± 3.85 grams and RMR (solid square) and MMR (solid triangle) of juvenile brown-marbled grouper, mean body weight of 294.5 ± 2.61 grams across a broad temperature range (18, 28 and 38°C).

STABLE ISOTOPE ANALYSES ON CARBONATE SHELLS OF CULTURED GEODUCK IN NEAH BAY WASHINGTON

Yongwen Gao

Makah Fisheries Management
P.O. Box 115, Neah Bay, WA 98357, USA
mailto:gaoy@olympen.com

The Pacific geoduck, *Panopea abrupta*, is an economically important species and mainly cultured in the southern Puget Sound of Washington State, USA. There are no examples of geoduck aquaculture along the Washington coast. Since 2007 the Makah Fisheries Management (MFM) has started a pilot project on intertidal geoduck aquaculture in the Port of Neah Bay, the northwest tip of the Olympic Peninsula of Washington. At the same time and location, MFM has established a monitoring program for the geoduck aquaculture beds and collected environmental parameters (e.g., temperature, salinity, pH, dissolved oxygen concentration, and pressure) and seawater samples on a weekly basis. The environmental parameters were measured with a YSI device, and the water samples were analyzed for $^{13}\text{C}/^{12}\text{C}$ ratios (or $\delta^{13}\text{C}$) from dissolved inorganic carbon (DIC) and $^{18}\text{O}/^{16}\text{O}$ ratios (or $\delta^{18}\text{O}$) of seawater. These monitoring data and measurements constitute the background information on the Neah Bay beach conditions and a valuable reference for stable isotope analyses on carbonate shells.

In this study I report stable isotope ratio analyses on Neah Bay cultured geoduck shells sampled in the summer of 2015. Two geoduck shell samples were selected, with clear growth increments and annuli on the outside of each shell and a length of about 130 mm from the umbo to the ventral margin. Because the shell is composed of calcium carbonate (CaCO_3) the principles of carbonate geochemistry can be applied to cultured geoduck samples. Preliminary results showed that $\delta^{13}\text{C}$ values of Neah Bay geoduck shells ranged from -0.62 to $+1.81\text{‰}$ VPDB (Vienna Peedee belemnite), whereas $\delta^{18}\text{O}$ values of the same samples ranged from -0.23 to $+1.84\text{‰}$ VPDB. Over the geoduck's full life-span (about 8 years) the $\delta^{13}\text{C}$ profile showed seasonal variations, with a distinct difference in $\delta^{13}\text{C}$ patterns after age-1. The $\delta^{18}\text{O}$ profile, in contrast, did not show seasonality but a steady decrease in $\delta^{18}\text{O}$ values from age-1. The MFM monitoring data indicated that seawater parameters (especially temperature, salinity, and pH) reflect a natural variation and have not changed significantly over the monitoring time. No significant changes were obtained from seawater analyses in $\delta^{13}\text{C}$ from DIC for about one-year collection, and the $\delta^{18}\text{O}$ values of seawater largely correlated with seasonal changes of temperature. Therefore, the isotopic composition of cultured geoduck shells in Neah Bay are markedly different from the previously reported isotopic data on subtidal geoduck shells from the Hood Canal of southern Puget Sound. In particular, the seasonal $\delta^{13}\text{C}$ values over the animal's full life-span did not show a steady decrease as the isotopic indicator of climate change and ocean acidification. More sampling and analyses will be needed to verify the isotopic variations and interpretation on these shells.

INFLUENCE OF COOLING OF MUD CRAB JUVENILES IN ICE BATH ON SURVIVAL AND PHYSIOLOGICAL CHANGES DURING STORAGE

Genodepa, Jerome*, Geromiano, Merlyn

University of the Philippines Visayas
Miag-ao Iloilo, Philippines
jerome_genodepa@yahoo.com

Considering that mud crab aquaculture is primarily dependent on the limited supply of wild seeds, mortalities associated with capture, handling and storage is serious concern for the industry. In lieu of the practice of cooling crabs in a chamber, crabs (*S. olivacea*, 27-44g) were directly immersed in seawater of different temperatures (4°C, 7°C, 15°C and 29°C/ ambient) prior to tying and storage. Crabs that were immersed at 15°C for 3 min obtained ~90% survival which was significantly higher than the rest of the treatments after 48h and 72h of storage ($P < 0.05$) (Fig.1).

Based on vigour index, crabs cooled at 15°C obtained the highest percentage of crabs that were classified as lively (~53%) after 48 h and 72 h storage (Table 1). Among the crabs that survived the 48 and 72 h storage, those cooled at 15°C also obtained the highest survival in the recovery tanks after 12 and 24 h.

The survival and vitality results may be explained by the lower glucose and lactic acid levels detected in the haemolymph of crabs that were cooled at 15°C and 7°C ($< 1 \text{ mmol L}^{-1}$) compared to 29°C/ ambient and 4°C (Table 2). Glucose and lactic acid levels below 1 mmol L^{-1} were reported to indicate that the crabs were not stressed.

This study therefore showed that cooling of crabs by immersion can be a technique for improving survival of harvested crabs.

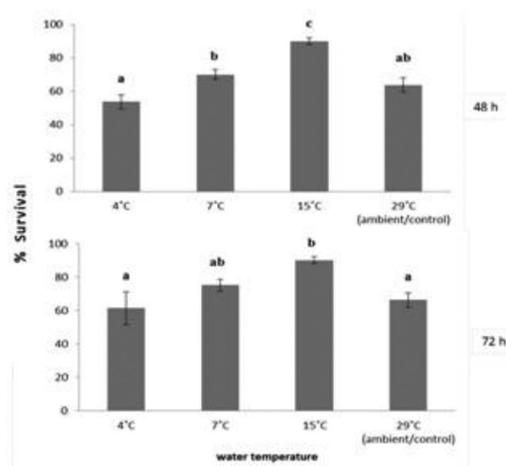


Fig. 1. Mean (\pm SE) survival of mud crab juveniles subjected to cooling at different temperatures prior to handling/tying and storage for 48 and 72 h. Columns under the same storage period with the same letters are not significantly different ($P > 0.05$).

Table 1. Mean (\pm SE) percent distribution of crabs according to vigour index (Poole et al., 2008). Columns with the same lowercase letters under the same storage period are not significantly different ($P > 0.05$). Rows with the same uppercase letters under the same period of storage are not significantly different ($P > 0.05$)

Immersion Temperature	Storage Period	Vigour Index			
		% Dead	% Weak/very slow	% Slow	% Lively
4°C	48h	46.3 \pm 0.8 ^{cd}	16.5 \pm 0.6 ^{ab}	25.8 \pm 0.6 ^{bc}	11.4 \pm 0.8 ^{AA}
	72h	38.8 \pm 9.9 ^{ab}	10.0 \pm 1.3 ^{AA}	35.0 \pm 12.3 ^{AB}	16.2 \pm 10.1 ^{AA}
7°C	48h	30.3 \pm 0.9 ^{bc}	9.0 \pm 0.07 ^{AA}	39.4 \pm 1.3 ^{cd}	21.3 \pm 4.3 ^{AB}
	72h	25.5 \pm 2.5 ^{ab}	6.0 \pm 1.3 ^{AA}	48.0 \pm 1.8 ^{cd}	20.5 \pm 2.0 ^{AB}
15°C	48h	10.0 \pm 2.0 ^{AA}	8.3 \pm 2.7 ^{AA}	27.9 \pm 4.8 ^{AB}	53.8 \pm 4.3 ^{bc}
	72h	10.0 \pm 2.0 ^{AA}	7.5 \pm 3.2 ^{AA}	28.8 \pm 6.9 ^{AB}	53.7 \pm 4.7 ^{bc}
29°C	48h	39.3 \pm 1.1 ^{bc}	8.6 \pm 1.3 ^{AA}	14.3 \pm 0.8 ^{AB}	37.8 \pm 0.6 ^{bc}
	72h	33.8 \pm 3.1 ^{cd}	10.0 \pm 4.1 ^{AA}	21.2 \pm 1.4 ^{AA}	35.0 \pm 1.7 ^{cd}

Table 2. Mean (\pm SE) haemolymph glucose and lactic acid levels (mmol L^{-1}) of crabs cooled at different temperatures after 48 h and 72 h storage. Rows with the same letter are not significantly different ($P > 0.05$)

		4°C	7°C	15°C	29°C
Glucose	48 h	1.10 \pm 0.14 ^{bc}	0.98 \pm 0.04 ^{ab}	0.76 \pm 0.02 ^a	1.26 \pm 0.09 ^d
	72 h	2.16 \pm 0.36 ^{bc}	1.10 \pm 0.09 ^{ab}	0.84 \pm 0.08 ^a	1.57 \pm 0.11 ^{bc}
Lactic Acid	48 h	1.14 \pm 0.05 ^c	0.64 \pm 0.09 ^b	0.36 \pm 0.05 ^a	1.4 \pm 0.04 ^d
	72 h	1.4 \pm 0.03 ^b	0.7 \pm 0.05 ^a	0.58 \pm 0.07 ^a	1.6 \pm 0.05 ^d

USING PORTABLE DNA SEQUENCERS FOR RAPID AND PRECISE CHARACTERIZATION OF PHYTOPLANKTON BLOOMS

Christaline George*, Caroline Chénard, Sek-Man Wong, Chee Yew Leong, Scott Rice, Diane McDougald, Patrick Martin, Guillaume Drillet, Aurore Trottet, Simone M. Low, Federico Lauro

Asian School of the Environment
Nanyang Technological University
50 Nanyang Avenue
Singapore 639798
christalyne_25@hotmail.com

The phenomenon of harmful phytoplankton blooms has been an alarming concern for the tropical waters of Singapore causing significant economic losses to the fishing and aquaculture industries. Many research studies have been performed with regards to such blooms around the world. However the exact combination of factors that contribute to the occurrences of such blooms remain blurred. Therefore, these events are still difficult to forecast and mitigate. A further complicating and limiting aspect of research and management in this field is the traditional identification of the causative organisms through microscopy and other conventional methods, which may be tedious, requiring trained personnel and might not be adequate to precisely characterize the bloom causing organisms. New rapid molecular methods for the detection and characterization of microorganisms are gaining popularity due to their relatively low costs and precise rapid results. We will present the first application of third generation sequencing technology using portable MinION™ sequencers for the rapid characterization of phytoplankton communities. This approach holds the promise for the early detection of harmful algal blooms even in the most remote locations where expensive laboratory facilities and professional expertise are not readily available. Such genetic characterization can further add insights into investigating the exact nature of the biotic interactions and dynamics which may either cause, perpetuate or lead to the decay of such harmful algal blooms. A thorough understanding of these interactions will help to develop methods to better forecast water quality and therefore support a better zone management of highly sensitive aquaculture production areas.

CHILLING TOLERANCE OF MUD CRAB *Scylla olivacea* JUVENILES

Geromiano, Merlyn*, Genodepa, Jerome

Institute of Aquaculture
University of the Philippines Visayas
Miag-ao Iloilo 5023, Philippines
memer852@gmail.com

Immersion of crabs in ice bath has been practiced to facilitate easy handling during harvest but no scientific evidence has been reported as to the extent of the low temperature that crabs can tolerate, as well as the period of immersion that will not compromise the crab's chances of survival. The tolerance of juvenile *S. olivacea* (27.2-44.2g) to 4°C, 7°C and 15°C were thus determined based on the period of cooling that would result to mortality. Since previous reports have used different indicators of end point of dying crabs, these indicators were first tested for accuracy prior to the temperature tolerance experiment. Crabs were immersed in 7°C seawater until they were presumed dead (based on cessation of movement of claws, eyestalk, appendages, or the mandibles) and were then allowed to recover in tanks with ambient/ 29°C temperature. Results revealed that the cessation of movement of mandibles was the most accurate indicator of dead crabs (Table 1).

The cessation of movement of the other body parts were not sure indicators of endpoints of dying crabs since some of the crabs that were identified as dead were able to recover when returned to holding tanks.

The tolerance test showed that crabs immersed at 15°C were able to tolerate this temperature and no mortality was recorded after 24h, while crabs immersed at 7°C and 4°C all died in less than 9 h. The tolerance of crabs immersed at 7°C was twice longer than that of crabs immersed at 4°C (Fig.1). The crabs immersed at 7°C started dying after 2h 19 min and reached 100% mortality after 8h 32 min while crabs immersed at 4°C started dying after 1h 39 min and had 100% mortality after 3h 6 min.

Crabs immersed in all the three temperatures showed slowing down of movement within 3 min and all the crabs also showed minimal resistance when they were caught and tied. These results suggest that crabs can be cooled in an ice bath to facilitate easier handling and 15°C is the safest temperature

Table 1. Mean (\pm SE, n=5) time (h: min) crabs were observed to die after immersion in 7°C seawater and the % accuracy of the various endpoints (cessation of movement of claws, appendages, eyestalk and mandibles) used as indicator of dead crabs. Means within the same rows with the same superscript are not significantly different ($P>0.05$)

	claws	appendages	eyestalk	mandibles
time(h:min)	3:33 \pm 0:21 ^{ab}	3:03 \pm 0:18 ^a	3:59 \pm 0:24 ^{ab}	4:44 \pm 0:33 ^b
% accuracy	98.60 \pm 0.24 ^b	97.80 \pm 0.37 ^a	98.80 \pm 0.20 ^b	100 \pm 0.00 ^c

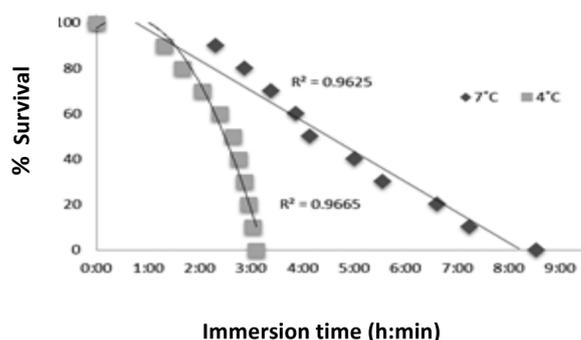


Fig 1. Correlation between the percentage of surviving crabs and immersion time under different temperatures (4°C and 7°C).

ALL-FEMALE CRUSTACEAN CULTURE – FINALLY A REALITY

Raveh Gill-More*, Assaf Shechter, Ohad Rosen, Tom Levy, Brit Eilam, Dudu Azulay, Idan Zohar, Eliahu D. Aflalo and Amir Sagi

*Enzootic HK Ltd., unit 1109, 11/F., Kowloon Centre, 33 Ashley Road, Tsimshatsui, Kowloon, Hong-Kong
Corresponding author email: raveh@enzootic.com

Monosex culture has been adopted by virtually every farmed animal sector as sex-specific trait(s) in sexually dimorphic species can be exploited to generate higher economic values. On this note, penaeid shrimp are no different, with size differences and FCR both favoring females. Moreover, all-female culture is expected to improve product uniformity, and help stabilize the genetic basis of the industry by maintaining the integrity of elite lines and avoiding influx of inferior genetics. This will allow the whole industry to move forward in issues such as disease management. As a result, all-female crustacean culture has been the goal of many groups over the past two decades but never reached commercial level implementation.

We have successfully developed a proprietary cost-effective and efficient all-female biotechnology based on parental cell transplantation (Figure 1), without using chemicals, hormones or genetic modification.

The technology which can be tailored to all decapods, was first applied to the giant river prawn *M. rosenbergii*, and was tested in a large-scale field study in earthen ponds. Our field study results show clear all-female superiority over mixed population in all the tested parameters including survival, yield, uniformity (Figure 2) and FCR.

Following these groundbreaking results in prawns, we are already implementing the technology in *L. vannamei* shrimp and expect to launch this product commercially in late 2017.

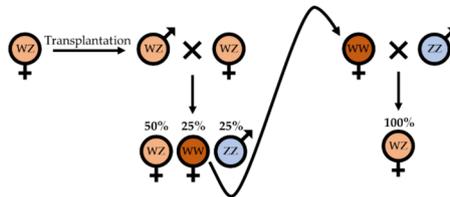


Figure 1. Reproductive scheme for producing all-female crustacean culture

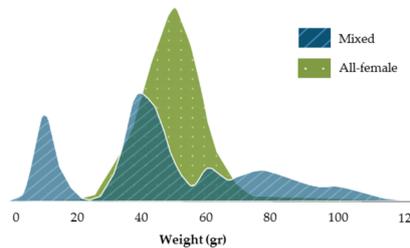


Figure 2. The unifying effect of culturing all-female compared to mixed populations, as demonstrated in prawns

GROWTH AND SURVIVAL OF SPINY LOBSTER *Panulirus homarus* FED DIFFERENT CHARACTERISTIC OF FORMULATED FEED DURING THE NURSERY PHASE

Nyoman Adiasmara Giri*, Bedjo Selamat, Sudewi, Haryanti, Wawan Andriyanto, Ibnu Rusdi, Gede S. Sumiarsa, Clive Jones and Simon Irvin

Institute for Mariculture Research and Development (IMRAD), Gondol-Bali, Indonesia

Email: s_u_dewi@yahoo.co.id

To date, spiny lobster farming in South East Asia is relying on trash fish as main source of nutrition. Therefore, it is required to develop formulated diet as a replacement for trash fish diet that could be used by farmers. The aim of this study was to assess survival and growth performances of spiny lobster *Panulirus homarus* fed different formulated diets.

The study was conducted in 25 net cages (60 x 60 x 60 cm) which were placed in a canvas tank (6 m in diameter). The canvas tank was equipped with flow-through water system and aeration. Fifteen juveniles of lobster at initial weight of 3 g was randomly distributed into each cage. The juveniles fed experimental feeds according to the designed treatments, twice daily. Four formulated feeds with 48% protein and 10,6% lipid were prepared in this experiment. Feed (A), the benchmark feed was produced with ingredients sourced from Australia. Three feeds (B to D) were formulated as feed A, but with ingredients sourced from Indonesia. Feed D was identical to feed C in formulation and composition; the exception was the feed dry matter content, 92% (dry) and 83% (moist) respectively. The fifth feed (E) consists of a mixed fishery product feed.

Results of the experiment showed that the best survival was achieved on lobster fed formulated diet with imported fish meal (C), and the lowest survival was obtained on lobster fed trash fish diet (E). It was difficult to distinguish mortality of lobster due to cannibalisms or natural mortality. In many cases no dead body was found in the rearing cages. There was no difference in growth between lobster fed moist and dry feeds. Lobster fed diet that prepared with local fish meal (B) showed higher growth than that of feed prepared with imported fish meal feed (C) (Figure 1). This results suggested that dry feed which was formulated by using local fish meal could be applied for rearing of spiny lobster during the nursery stage.

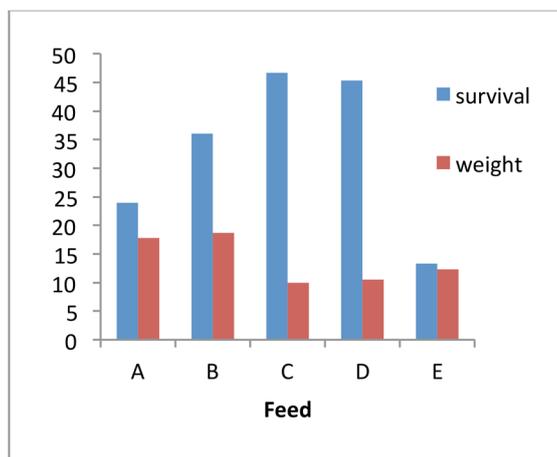


Figure1. Survival (%) and weight (g) of juvenile lobster fed experimental feeds for 12 weeks rearing period.

MODELLING THE APPLICATION OF THE IDEAL PROTEIN CONCEPT IN FISH AND SHRIMP

Brett D. Glencross*

Institute of Aquaculture, University of Stirling, FK9 4LA, Stirling, Scotland, United Kingdom
b.d.glencross@stir.ac.uk

The ideal protein concept (IPC), sometimes called the ideal amino acid concept, works on the premise that an animal requires the ten proteinogenic essential amino acids (EAA) in a ratio relative to lysine (or some other quantitatively determined amino acid) as defined by their own natural body amino acid composition (Table 1). Using a quantitative determination of a key amino acid this ratio is then applied to define the quantitative requirements of all the other EAA. Because of this relationship and the capacity to model protein and energy demand, using standard factorial bioenergetic modelling (FBM) methods, by extension it also becomes possible to model demand for each of the ten essential amino acids.

There are several steps to facilitating this modelling of the quantitative requirements for the ten EAA. The first step is to have a factorial bioenergetic model that predicts energy and protein demand of a healthy, growing animal. This requires a predictive growth model, a series of body composition equations, and determining key utilisation coefficients of energy and protein and having estimates of maintenance requirements for energy and protein. In addition to these standard parameters for development of a FBM, we also need a robust analysis of the amino acid composition of the whole animal with varying body size. This information then needs to be paired with a similarly robust assessment of the quantitative requirements for any of the EAA, usually as defined using a simple dose-response study. Each of these different parameters can then be integrated together to create an adaptation of the FBM that includes predicative outputs for the EAA.

In this paper, we will explore the application of the IPC in combination with the FBM to define the quantitative requirements for each of the ten EAA across five key aquaculture species. From these derived estimates, we will then compare and contrast across species and different animal sizes to assess the robustness of this modelling strategy for this application.

Table 1. Ratio of essential amino acids in five aquaculture species relative to Lysine*

Amino Acid	<i>S. salar</i>	<i>L. calcarifer</i>	<i>S. lalandii</i>	<i>O. niloticus</i>	<i>P. monodon</i>
Arginine	76%	89%	135%	82%	110%
Histidine	28%	27%	73%	34%	30%
Isoleucine	51%	56%	66%	61%	60%
Leucine	89%	99%	110%	66%	119%
Lysine	100%	100%	100%	100%	100%
Methionine	35%	44%	43%	52%	36%
Phenylalanine	50%	56%	65%	73%	62%
Threonine	52%	61%	77%	73%	57%
Tryptophan	13%	-	16%	20%	14%
Valine	60%	57%	74%	55%	69%

Salmo salar, Lates calcarifer, Seriola lalandii, Oreochromis niloticus, Penaeus monodon

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

G. I. Godahewa^{1,2*}, N. C. N. Perera^{1,2} and Jehee Lee^{1,2}
E-mail: imarshana@gmail.com

¹Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea

²Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea

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

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

G. I. Godahewa^{1,2*}, N. C. N. Perera^{1,2} and Jehee Lee^{1,2}
E-mail: imarshana@gmail.com

¹Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea

²Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea

Natural killer enhancing factor (NKEF) has been remarked as a cytosolic protein that enhances the natural killer cell cytotoxic activity. Apart from the cytotoxic activity of the NKEF, it has shown potential antioxidant function similar to that of peroxiredoxin members. Hence, natural killer enhancing factor A (NKEF-A) is also referred as peroxiredoxin 1. It protects the host cells from different oxidative damages such as hydrogen peroxide, alkyl hydroperoxide and heavy metals. In the present study, molecular features, functional properties and immune responses of *Hippocampus abdominalis* NKEF-A (HaNKEF-A) were assessed. Putative open reading frame encoded 594 amino acids with 29.9 kDa polypeptide and a *pI* of 6.43. Two conserved domains (Prx_typical_2Cys and Thioredoxin_like) and several active sites including, catalytic triad, dimer interface, decamer, peroxidatic and resolving cysteines were identified through bioinformatics tools. It shared the highest identity (93.4%) and similarity (98%) with the *Cyprinodon variegatus* Prx1. Multiple sequence alignment revealed the conservation of functionally active peroxidatic and resolving cysteines among the other NKEF-A/Prx1 counterparts suggesting the common peroxidase activity. Metal catalyzed oxidative (MCO) stress, cleaved the pUC19 DNA from supercoiled state into nicked state where, rHaNKEF-A protein could protect the pUC19 DNA cleavage by MCO system in a concentration dependent manner. The rHaNKEF-A catalyze the insulin reduction activity with the presence of 1,4-Dithiothreitol (DTT) in a time dependent manner. The results of the MTT assay revealed that the presence of the rHaNKEF-A increased the cell viability% against the H₂O₂ oxidative stress. Moreover, the activity was dose dependent and the highest cell viability percentage was gained with the 100 µg/mL of rHaNKEF-A. The same concentration of the rHaNKEF was given the highest reduction of the ROS level in the human LNCaP cells against the H₂O₂ oxidative stress. The HaNKEF-A transcripts were ubiquitously expressed in all examined tissues with highest expression in liver. Bacterial (*Edwardsiella tarda*, *Streptococcus iniae* and LPS) and viral (poly I:C) immune stimulated liver tissue showed significant HaNKEF-A mRNA expression after the post infection. Collectively, HaNKEF-A is belonging to the teleostean peroxiredoxin family member with its antioxidant function and potential immune responses upon bacterial and viral challenges. Also, it could be suggested that HaNKEF-A is an active member of seahorse antioxidant defense system.

SUPPRESSOR OF CYTOKINE SIGNALING 3 (SOCS3) MODULATES THE JAK/STAT GENES IN KOREAN BLACK ROCKFISH (*Sebastes schlegelii*)

G. I. Godahewa^{1,2*}, N. C. N. Perera^{1,2} and Jehee Lee^{1,2}
E-mail: imarshana@gmail.com

¹Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea

²Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province 690-756, Republic of Korea

Cytokines have crucial roles in the immune system by controlling the proliferation and differentiation of the immune cells. Correspondingly, Janus family tyrosine kinases (JAKs) are essential signaling molecules for cytokine signaling. However, members of suppressor of cytokine signaling (SOCS) protein family play an important role to regulation of cytokine signaling via JAK-STAT pathway. SOCS proteins inhibit cytokine signaling systems in a classical negative-feedback loop. SOCS molecules possess a central SH2 domain and a carboxyl terminal domain named the SOCS box. The target of each SOCS and CISH is determined by the SH2 domain which binds to phosphorylated tyrosine residues. Additionally, only SOCS1 and 3 possess a kinase inhibitory region (KIR), which is located at the N-terminus, immediately upstream of the central SH2 domain. Here in, we have characterized the molecular aspects of suppressor of cytokine signaling from black rockfish (RfSOCS3). Series of bioinformatics tools were used to discover the domain architecture of the RfSOCS3 protein. Transcriptional variation of *RfSOCS3* was determined under aseptic and septic conditions of rockfish. In order to analyze the STAT gene regulation effect of RfSOCS3, it was transfected into rockfish testis cells and evaluates the transcriptional variation of different STAT members of rockfish. According to the *in silico* analysis this protein has a molecular weight of 23 kDa and a theoretical pI value of 9.2. Domain architecture analysis revealed that this protein has a conserved SH2 superfamily domain and SOCS superfamily domain which are vital for the cell signaling transduction process. C-terminal SOCS box and a central SH2 domain are the characteristic features in this SH2 family members. SOCS3 is involved in the down-regulation of the JAK/STAT pathway and it inhibits JAK activity indirectly through recruitment to the cytokine receptors. There is no significant transcriptional modulation observed in RfSOCS3 transfected and pcDNA transfected cells in compared to control cells. However, there is significant up-regulation of RfSTAT genes in poly:IC treated cells compared to the un-treated cells. Later on the RfSTAT-1, -3 and -6 transcripts were down-regulated in RfSOCS3 transfected, poly:IC treated cells revealing the inhibition of RfJAK activity by RfSOCS3. Moreover, ubiquitous expression of RfSOCS3 transcripts was observed in healthy rock fish tissues with different magnitudes with highest expression in blood. Collectively, we can suggest that RfSOCS3 play an important role in the rockfish JAK/STAT pathway and down-regulate the STAT genes via inhibition of JAK activity.

SOME BIOLOGICAL PROPERTIES OF BOPYRID ISOPOD PARASITE *Epipenaeon ingens* Nobili (1906)

Mehmet Gökoğlu* Serkan Teker Jale Korun David Julian Aycan Ulutaş

Akdeniz University
Fisheries Faculty Antalya/Turkey
srkntkr@gmail.com

This study was carried out with a Bopyrid parasite *Epipenaeon ingens* found on *F. aztecus* in the Gulf of Antalya. Bopyrid isopod *Epipenaeon ingens* is a branchial parasite from the subfamily Orbioninae.

The parasites were carefully removed from the branchial cavity of the shrimp with the help of scissors and a pair of pens. Each parasite was separated, sexed and weighed in 0,001 g precision scale and measured in 0,01mm caliper. Parasites were separated according to the presence of eggs in the brood pouch of gravid female parasites or absence of eggs. The eggs were dis severed from the brood pouch of gravid female parasites and weighed. Eggs were counted by the method of gravimetric under stereomicroscope and egg diameter was measured and photographed.

The relationship of parasites total length (TL) and total weight (TW) was found $W=0,000020L^{3,5066}$ $r^2=0,8739$ for females and $W=0,0001L^{2,3349}$ $r^2 = 0,7945$ for males. The brood ranged between 16,000 and 279,000 eggs and average number of eggs was found $173,314 \pm 18,169$ (SE). Egg diameters were found $167\mu - 237\mu$. The smallest female parasite having eggs in sac was 14,36 mm long and 0,352g weighed. The smallest male parasite on the gravid female parasite was 3,28mm length and 0,001g weight.

EFFECT OF BROMELAIN ENZYME ON OCTOPUS (*Octopus vulgaris*) MUSCLE TEXTURE

Nalan Gokoglu^a

^aAkdeniz University Fisheries faculty, Antalya, Turkey
ngokoglu@akdeniz.edu.tr

The aim of this study was to investigate tenderization effect of bromelain enzyme on octopus (*Octopus vulgaris*) arm muscle.

Octopus samples were purchased just after landing and placed in cold storage bag with ice. Before treatments head, skin, viscera and tentacles of octopus were removed and arms were cut into cubes of 4 cm. Two solutions (0.001 and 0.004%) of bromelain were prepared using distilled water. Octopus pieces were dipped into these enzyme solutions. Then they were kept in a water bath at 60°C for 20 min to activate enzymes. Control samples were only immersed into distilled water they did not contain enzyme. After all treatments analyses were conducted. The Scientific and Technological Research Council of Turkey (TUBİTAK) supported this study (Project number 213O163).

After bromelain treatment, cooking loss, hardness and shear force values of octopus muscle decreased. Total soluble protein and water holding capacity increased. These increases probably occurred thanks to proteolysis by enzymes. Bromelain enzyme has been successful for tenderization of octopus muscle. These results will contribute to solve the problems regarding consumption in industry and consumer aspects. In addition, with increase of acceptability of octopus its value will increase.

OXIDATIVE ALTERATIONS OF LIPIDS IN FROZEN FISH AT DIFFERENT TIME AND SPEED DURING FROZEN STORAGE

Nalan Gokoglu* , Isin Aydın Unsal

Akdeniz University
Fisheries Faculty
Antalya, Turkey

The aim of this study was to determine the effects of temperature and time of freezing on lipid oxidation in anchovy during frozen storage.

Fresh anchovy (*Engraulis encrasicolus*) was used as material. The fish were frozen at three different temperatures (-20°C, -40°C and -80°C). During the freezing, temperature of thermal centre of fish was measured using a thermo couple and then time of freezing was calculated. Frozen fish were stored in a deep freezer at -18°C for 6 months. Analyses were conducted at monthly intervals. Freezing time and freezing velocity were calculated. Peroxide, thiobarbutiric acid (TBA), Para-anisidine (p-Av), free fatty acids (FFA) and conjugated diene (CD) values were determined during the storage.

The highest freezing speed and the shortest freezing time were observed for anchovies frozen at -80°C. Peroxide (PV), thiobarbutiric acid (TBA), para-anisidine (p-Av), conjugated diene (CD) and free fatty acid (FFA) values were significantly affected by freezing temperature. Low freezing temperature resulted in low oxidation level. Oxidation levels significantly increased during the storage. The lowest values were determined in the samples frozen at -80°C. The best freezing temperature in terms of delaying of lipid oxidation was found to be -80°C.

MYCOTOXINS IN SE ASIA AQUACULTURE PLANT-BASED MEALS

Rui A. Gonçalves*, Ursula Hofstetter, Dian Schatzmayr

*BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria

Among possible fish meal alternatives, e.g. animal by-products, fishery by-products, bacteria and algae concentrate, plant ingredients seem to be one of the most promising solutions. Numerous plant raw materials have been successfully tested (Gatlin et al., 2007). It is observed that an interrelationship between nutrition, immunology and disease resistance in fish and shrimp has been essential to evaluate the alternative plant ingredients. It is commonly agreed that a key negative aspect of plant meals are the presence of anti-nutrients that are detrimental to fish. Although there are processes to remove or inactivate many of these compounds, the same is not effective against mycotoxins that are relatively stable to processing conditions.

In order to evaluate the occurrence of mycotoxins in common plant nutrients used for aquaculture feeds production, samples were sourced over a period of two years (January 2015 - December 2016). In first year, 2176 samples of different plant sources were analyzed within the scope of BIOMIN Mycotoxin Survey program. The plant meals selected were: Soy Bean Meal (SBM), Wheat (WH), Wheat Bran (WB), Corn (C), Corn Gluten Meal (CGM), Cottonseed Meal (CSM), Rapeseed/Canola Meal (R/CM) and Rice Bran (RB). In the second year, 157 samples were analyzed, focusing on local plant meals. Common plant meals, alfalfa, broken corn, broken rice, cassava, corn bran, carrot cake, corn dust, full fat soy, ground corn, groundnut cake, peanuts cake, polish rice, rapeseed, sesame, soybean cake, sunflower cake, wheat bran and wheat flour were analyzed.

Mycotoxins were found in most commodities analyzed, showing that mycotoxins might represent a risk for the development of the sector. While in some cases the contamination levels are rather low, in other cases the contamination levels might represent a risk for aquaculture species. Generally, in 2015 samples it was observed that SBM, WH, WB, C, CGM, R/CM and RB were mostly contaminated with *Fusarium* mycotoxins (ZEN, DON and FUM)— the exception being CSM that was mainly contaminated by AF together with *Fusarium* toxins (ZEN and DON) in considerable amounts.

Regarding local plant meals, all samples were contaminated with mycotoxins. Corn dust, broken corn, ground corn, groundnut cake and soybean cake presented considerably high values which can, depending on inclusion levels, represent a risk for aquaculture species.

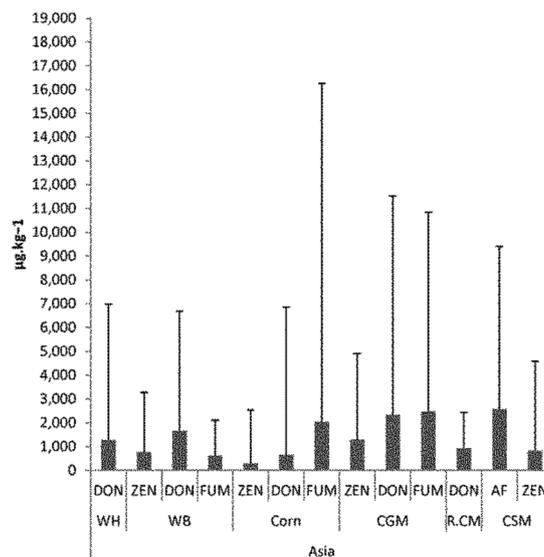


Figure 1. Average concentrations of aflatoxins (AF), ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FB) ($\mu\text{g kg}^{-1}$) in plant nutrient meals during 2015. Values displayed as mean \pm SE.

Acronyms for plant nutrients stand for: Wheat (WH), Wheat Bran (WB), Corn (C), Corn Gluten Meal (CGM), Rapeseed/Canola Meal (R/CM) and Cottonseed Meal (CSM).

MYCOTOXINS IN SE ASIA AQUACULTURE: A NEGLECTED THREAT?

Rui A. Gonçalves*, Ursula Hofstetter, Dian Schatzmayr

*BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria

Mycotoxins, and their effect in aquaculture species, have been a recognized threat since 1960, where aflatoxin-contaminated cottonseed meal caused an outbreak of aflatoxicosis in hatchery-reared rainbow trout (*Oncorhynchus mykiss*). Concern about mycotoxins in aquaculture has been growing, partly due to the gradual replacement of animal-derived proteins, such as fish meal, by plant proteins. According to Tacon et al., (2011), plant-based feedstuffs represent the major dietary protein source used within feeds for lower trophic level fish species like tilapia, carp or catfish, and the second major source of dietary protein and lipids after fishmeal and fish oil for shrimp and European high trophic level fish species.

Over a period of three years (January 2014–December 2016), 68 samples of finished aquaculture feed (FF) for both shrimp and fish, were analyzed within the scope of the BIOMIN Mycotoxin Survey program. All samples were sourced in SE Asia. In 2014 and 2015 the samples were tested for aflatoxins (AF, sum of AFB1, AFB2, AFG1 and AFG2), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FB, sum of FB1 and FB2) and ochratoxin A. In 2016, beside the previous mycotoxins there were also analyzed metabolites and masked mycotoxins, in total 18 mycotoxins were analyzed (e.g. nivalenol; 3-acetyldeoxynivalenol; 15-acetyldeoxynivalenol and fusarenon X-glucosid).

DON and AF were the most prevalent mycotoxins for 2014, with 68% of the samples testing positive, followed by ZEN and FB (58% positive). In 2015 we saw a shift, with FB1 as the most prevalent mycotoxin followed by ZEN and DON. Concerning the average contamination levels, a decrease was observed for the 2015 samples. However when looking to the mycotoxin co-occurrence, the percentage of samples containing more than 1 mycotoxin increased from 84% to 90%, which raised the risk of synergistic effects of mycotoxins. In 2016 with the increased mycotoxin screen and differentiated analysis between fish and shrimp feeds, it was observed that fish and shrimp feeds present a different mycotoxin contamination pattern. Fish samples were mainly contaminated by FB1 while shrimp feeds were contaminated mainly by DON. The present study shows an increase in mycotoxin contamination from 2014 to 2016. Registered contamination values, especially for 2016 are within the sensitivity level of several important Asian Aquaculture species.

Table 1: Overall results of the main mycotoxin and metabolites detected during 2016 survey

Main toxins detected	Samples (N)	Maximum occurrence ($\mu\text{g kg}^{-1}$)	Average occurrence ($\mu\text{g kg}^{-1}$)	Mycotoxins detected per sample (N)
FF Fish				
15 AcDON ¹	3	139	29.16	4
DON ²	3	396	48.07	4
FB1 ³	6	708	110.82	6
FF Shrimp				
15 AcDON	1	60	25.12	5
DON	2	2287	429.95	4
ZEN ⁴	1	53	21.44	5

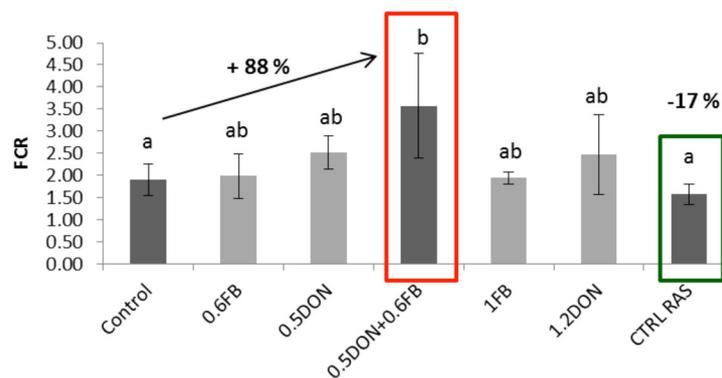
¹15-acetyldeoxynivalenol; ²Deoxynivalenol; ³Fumonisin B1; ⁴Zearalenone. FF= Finished Feed.

SENSITIVITY OF *Litopenaeus vannamei* TO *Fusarium* MYCOTOXINS: THE TOXICITY OF LOW DOSE OF FUMONISINS AND DEOXYNIVALENOL AND ITS ACCUMULATION IN CLOSED SYSTEMS

Rui A. Gonçalves*, Dian Schatzmayr, Michele Muccio, João Sendão

*BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria

The awareness of mycotoxin-related issues in aquaculture has grown as feed manufacturers and producers realize the importance of mycotoxins and their potential to impact production. Recent reports show that plant meals sourced in Asia, e.g., soybean meal, wheat, wheat bran, corn gluten meal, and rice bran, were mostly contaminated with *Fusarium* mycotoxins (zearalenone, deoxynivalenol and fumonisins)(Gonçalves *et al.*, 2016). This contamination is later reflected in the finished feeds for shrimp and fish on values that can be higher than 2 ppm (Gonçalves *et al.*, 2017). A 36-day feeding trial was conducted to determine the effects of low contamination levels of Fumonisins (FB) and Deoxynivalenol (DON), alone and combined, on growth performance and selected health indices of white leg shrimp. Seven treatments were established in triplicate. Treatments: Control (no mycotoxins); 0.6FB (0.6 ppm of FB); 0.5DON (0.5 ppm of DON); 0.6FB+0.5DON (0.6 ppm of FB combined with 0.5 ppm of DON); 1FB (1 ppm of FB) and 1DON (1 ppm of DON), were established in the same recirculating system. The treatment CTRL RAS (independent RAS; no mycotoxins) was designed to have all the same physical and chemical rearing conditions as previous treatments, however reared on an independent RAS. Mycotoxin levels in feed were confirmed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) before starting the experiment and water of RAS system was analyzed for DON and FB at the end of the trial. Low levels of FB (0.6 ppm) and DON (0.5 ppm), alone or combined showed a clear negative impact on growth performance of *Vannamei*. FCR was the most impacted parameter. Within the 6 treatments sharing the same RAS, the combination of DON and FB, significantly increased the FCR by 88% compared to control. Results confirm that *Litopenaeus vannamei* is sensitive to DON and FB on levels normally found on shrimp feeds (Fegan and Spring, 2007; Gonçalves *et al.*, 2016; Gonçalves *et al.*, 2017). The presence of FB in water brings an extra concern, especially for closed production systems.



SEAFOOD TRACEABILITY AND QUALITY AUTHENTICATION USING NUCLEAR TECHNIQUES

Karthik Gopi*, Debashish Mazumder, Jesmond Sammut, Neil Saintilan, Patricia Gadd, and Attila Stopic

School of Biological, Earth & Environmental Sciences
The University of New South Wales
Sydney NSW 2052
Australia
k.gopi@student.unsw.edu.au

Seafood provides an essential source of omega-3 polyunsaturated fatty acids, proteins and other micronutrients. Seafood is increasingly recognised as a healthier source of protein than red meat because of its role in cardiac health and mitigating against vascular diseases. Currently, the global seafood trade is valued at over US \$94.11 billion. The amount of seafood being consumed is increasing and placing pressure on capture fisheries to meet demand. Consequently, aquaculture has boomed and has become one of the fastest growing food production industries. Although aquaculture has been scrutinised over environmental impacts, largely based on its uncontrolled expansion decades ago, it can nonetheless, when well-managed, provide a sustainable source of food. In many countries, where access to land is highly competitive, wild and farmed seafood has become essential for food and income security.

Australia exports a large percentage of its seafood products because overseas markets are more lucrative. To meet local demand, seafood products (up to 72%) are imported, and are often packaged or filleted. Whilst this export/import market is beneficial for the Australian economy, imported products could be contaminated and processed seafood (eg fish fillets) might be fraudulently relabelled. Recently, imported seafood has come under scrutiny due to quality authentication issues. Consumers are mindful of health risks and overpaying for products that are possibly re-labelled and of questionable quality. More accurate and reliable methods to trace and authenticate seafood products are needed for regulatory authorities, Australian seafood traders and consumers.

The Australian Nuclear Science and Technology Organisation (ANSTO) is leading a collaborative research program in partnership with the University of New South Wales (UNSW), Macquarie University and multiple industries, to determine the effectiveness of isotopic and nuclear techniques in food traceability and authenticity research. We have analysed seafood obtained from aquaculture and the wild, and from across different geographical locations in Australia and Asia, and found that isotopic and elemental compositions of samples are distinct across geographical and environmental origins. The results provide insight into the effectiveness of nuclear techniques to trace the geographical origin of seafood, which is important for quality certification, to prevent 'food fraud' and ensure retailers and consumers are not impacted by contaminated or incorrectly labelled seafood products.

CRUDE PALM OIL AS A DIETARY LIPID SOURCE IN THE PRACTICAL DIETS OF HYBRID GROUPER, *Epinephelus fuscogutattus* X *Epinephelus lanceolatus*

Sandra Natalie Gudid*, Annita Yong Seok Kian, Gunzo Kawamura, Lim Leong Seng and Rossita Shapawi

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu Sabah, Malaysia
Email: naturelover1302@gmail.com

A long-term feeding trial (4 months) was conducted to evaluate the performance of crude palm (CPO) oil-based diets (fish meal and soybean meal were used as sources of protein) during the grow-out period of hybrid grouper, *Epinephelus fuscogutattus* X *Epinephelus lanceolatus* in sea net cages. The diet was formulated with 50% crude protein and 16% crude lipid. Fish oil (FO) was substituted with crude palm oil at 25% increment level and represented as 25CPO, 50CPO, 75CPO and 100CPO. Meanwhile, the control diet (100% fish oil) was labeled as FO. Hybrid grouper of about 200g average weight were randomly distributed into triplicate groups of 30 fish in square net cages (2.0 m depth and 1.5 m diameter) and were fed once a day. Growth monitoring was carried out on a monthly basis. At the end of feeding trial, the fish were also sampled for blood analysis and organoleptic test by using a 5-point hedonic scale of 44 untrained panels.

No significant difference ($p>0.05$) was detected in term of growth performance of fish fed with different experimental diets. At the end of feeding trial, the percentage of body weight gain (BWG) and specific growth rate (SGR) ranged from $170.42 \pm 4.17\%$ to $181.63 \pm 8.33\%$ and $0.82 \pm 0.04\%/d$ to $0.86 \pm 0.02\%/d$, respectively. Numerically, the fish fed with 50CPO diet had the highest BWG and SGR. Meanwhile, the fish fed with 75CPO diet has the lowest BWG and SGR. Survival rates of the fish were above 90%. The food conversion ratio (FCR) of experimental diets ranged from 1.82 ± 0.08 to 1.91 ± 0.02 , with no significant differences were observed ($p>0.05$). There were also no significant differences seen on the body indices, fillet yield as well as the condition factor of the fish. The results of the blood analysis were in the form of total cholesterol, total protein, red blood cell and hematocrit counts. Except for the total cholesterol, no significant differences ($p>0.05$) were detected in these parameters among the experimental diets. The total cholesterol for the fish fed with FO and 50CPO were significantly higher ($p<0.05$) than the fish fed with 75CPO and 100CPO. The findings from organoleptic test showed that all fillets were well accepted by the consumers without any significant differences detected in the tested attributes (odour, appearance, flavor, texture and overall acceptance). In conclusion, crude palm oil is an excellent source of lipid to replace fish oil in the grow-out diet for hybrid grouper, *Epinephelus fuscogutattus* X *Epinephelus lanceolatus* .

EFFECT OF DIETARY HYDROXY-SELENO-METHIONINE ON FEED PERFORMANCE, SERUM BIOCHEMICAL INDICES, AND ANTIOXIDANT ABILITY IN WHITE SHRIMP *Penaeus vannamei* FED LOW- OR HIGH-LEVEL FISH MEAL DIETS

Zhang Chunxiao(*), Chen Hong(**), Guerin M. (***)

(*) JingMei University, Xiamen Shi, Fujian Province, China, 361021

(**) Adisseo Life Science(Shanghai) Co., Ltd, Shanghai 201204, P.R. China

(***) martin.guerin.aqua@gmail.com

Selenium is a life-essential component of selenoproteins such as Glutathione peroxidase, a critical weapon in the biological antioxidant arsenal providing optimum immuno-protection and resistance to oxidative stress. *P. vannamei* is prone to devastating disease outbreaks aggravated by stress. This study looked at the effect of graded levels of hydroxyl-selenomethionine (OH-Se-Met), a safe, highly stable and bio-available form of organic Selenium, on feed performance parameters with 2 fishmeal inclusion levels, as well as Selenium levels in the muscle, and serum and hepatopancreas antioxidant status.

P. vannamei shrimp were allocated 10 diets: 2 fish meal inclusion levels, 15% and 30%, to which 5 levels of organic Selenium (0, 0.15, 0.3, 0.6, and 0.9 ppm) were added. They were divided at the rate of 50 shrimp per tank in a recirculating water system, and fed the test diets for 8 weeks.

Selenium and Fish meal levels had both a significant effect on shrimp growth. 0.3 ppm organic Selenium gave the best growth performance for both fish meal levels and best PER at 15% fish meal inclusion.

Level in Feed		Final weight (g)	Weight gain (% \square)	SGR \square (%/d \square)	FCR	PER \square (% \square)
Fish meal	Se ppm					
30% (High)	0	742.8 ^c	1619.4 ^c	4.74 ^c	0.79	303.2
	0.15	769.3 ^{bc}	1644.4 ^{bc}	4.77 ^{bc}	0.72	330.5
	0.3	819.4^a	1817.2^a	4.92^a	0.74	323.8
	0.6	780.2 ^{abc}	1797.8 ^a	4.91 ^{ab}	0.76	318
	0.9	808.2 ^{ab}	1752.1 ^{ab}	4.81 ^{abc}	0.77	317
15% (Low)	0	749.6 ^{AB}	1608.5 ^{AB}	4.73 ^{AB}	0.83 ^B	287.4 ^{AB}
	0.15	745.7 ^{AB}	1626.0 ^{AB}	4.75 ^{AB}	0.79 ^{BC}	298.5 ^{AB}
	0.3	788.7^A	1689.8^A	4.81^A	0.75^C	316.6^A
	0.6	732.9 ^B	1553.2 ^B	4.71 ^B	0.82 ^{BC}	287.9 ^{AB}
	0.9	732.0 ^B	1538.1 ^B	4.70 ^B	0.91 ^A	278.6 ^B

The content of Se in body and muscle increased linearly with the supplementation of OH-Se-Met in diets, and antioxidant capacity in serum and hepatopancreas of shrimp was also improved by OH-Se-Met.

EVALUATION OF GROWTH AND HETEROSIS OF JUVENILE OF NILE TILAPIA *Oreochromis niloticus*, BLUE TILAPIA *Oreochromis aureus* AND THEIR HYBRID REARED IN THE BRACKISH WATER POND

Bambang Gunadi*, Adam Robisalmi and Priadi Setyawan

Research Institute for Fish Breeding
Jalan Raya 2 Sukamandi Pantura
Patokbeusi, Subang 41263
West Java, Indonesia
bbgunadi@gmail.com

Growth is one factor that was an indicator of success in aquaculture. Growth performance, especially the character of weight, can be used as a determinant of the successfulness of the selective breeding program through hybridization method. The hybrid between Nile tilapia *O. niloticus* and blue tilapia *O. aureus* is expected to have better performances than their parents. This experiment aimed to observe the growth performance and the value of heterosis of the hybridization between Nile tilapia and blue tilapia. The research was carried out for 6 months at the Research Institute for Fish Breeding at Sukamandi, West Java and brackish water pond at Cirebon (Indonesia). There were two strains or species of tilapia involved in this experiment, i.e. Nirwana strain of Nile tilapia and blue tilapia. Inter and intra species of spawning were conducted communally in a freshwater pond sized of 25 m². Tilapia broodstock were stocked to spawning pond with male to female ratio of 10:30. After 14 days of stocking, larvae were collected then reared in the subsequent nursery hapas sized of 2x2 m for 60 days or after fingerlings reached the average individual length and weight of 4.82 cm and 2.20 g.

At the growing-up stage, fingerling were reared in the 3x5 m² net which was installed in the brackish water pond sized of 7000 m² at water salinity of 25-40 g/L for 120 days. The stocking density applied at this stage 10 fingerling/m² strain population revealed the highest growth performances with individual weight of 111.74 g, specific growth rate (SGR) of

The fish were fed with floating commercial fish feed (30% crude protein) at a rate of 5-10% of biomass which was given twice per day. The results showed that the crossing between female Nile tilapia strains and male blue tilapia showed the highest growth performances and value of heterosis on the length, weight and survival characters (Table 1). It meant that the hybrids to have traits superior to those of either parent.

Table 1. Final body weight, weight gain, specific growth rate (SGR), growth rate (DGR), feed conversion ratio, survival and value of heterosis

Parameters	Population			
	Blue tilapia ♀ x Nile tilapia ♂	Blue tilapia ♀ x Blue tilapia ♂	Nile tilapia ♀ x Blue tilapia ♂	Nile tilapia ♀ x Nile tilapia ♂
Initial weight (g)	1.71±0.59	2.08±0.66	2.33±0.80	2.66±0.95
Final weight (g)	181.92±33.72	164.72±14.98	198±30.12	143.6±13.08
Weight gain (g)	180.21±33.28	162.64±14.98	194.66±30.26	140.94±10.98
SGR (%/day)	3.89±0.28	3.64±0.24	3.7±0.28	3.32±0.23
DGR (g/day)	1.5±0.28	1.35±0.12	1.62±0.25	1.17±0.11
FCR	1.69±0.02	1.93±0.15	1.56±0.05	2.10±0.04
Survival (%)	76±1.76	75.5±1.08	78±2.12	71±1.06
Heterosis (%)				
Weight	18.01		27.79	
Length	6.48		7.02	
Survival	3.05		5.76	

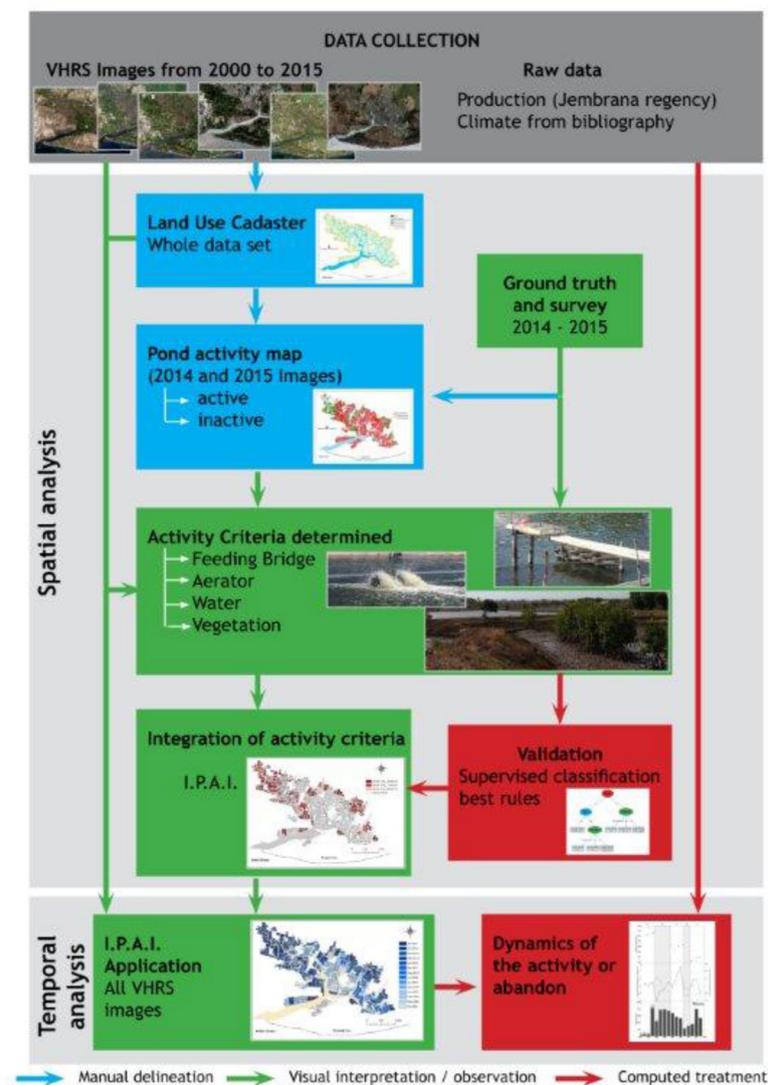
MONITORING POND ACTIVITY USING VHSR IMAGES: CASE STUDY PERANCAK ESTUARY, BALI, INDONESIA

Niken Gusmawati*, Benoît Soulard, Nazha Selmaoui-Folcher, Christophe Proisy, Akhmad Mustafa, Romain Le Gendre, Thierry Laugier, Hugues Lemonnier

Pôle Pluridisciplinaire et Matière de l'Environnement (PPME)
University of New Caledonia
Campus de Nouvile, BP R4, Nouméa CEDEX, New Caledonia 98851

From the 1980's, Indonesian shrimp production has continuously increased through a large expansion of cultured areas and an intensification of the production. As consequences of disease and environmental degradations linked to this development, there are currently 250,000 ha of abandoned shrimp ponds in Indonesia representing 37% of the total pond surface. To implement effective procedures to undertake appropriate aquaculture ecosystem assessment and monitoring, an indicator has been developed to discriminate between active and abandoned ponds. It is based on very high spatial optical satellite images.

To test this tool, shrimp aquaculture history of the Perancah estuary, Bali, Indonesia a production area in decline, has been analyzed between 2001 and 2015. This study highlights two risk factors that could contribute to explain dynamics of abandonment: (i) climate conditions and (ii) geographical positioning of ponds within the estuary, suggesting that a spatial approach should be integrated in our future thinking to operationalize pond rehabilitation.



STUDY ON GROWTH PERFORMANCE IN JUVENILE AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822) FED WITH PROBIOTICS SUPPLEMENTED DIETS

Nur Hidayahanum H.^{*}, Hassan M.D., Hasliza A.H., Md Sabri M.Y., Ruhil Hayati H.³, Siti Fairus M.Y., Nora Faten Afifah M. and Siti Nadia A.B.

Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 Serdang, Selangor
Malaysia
nur_hidayahanum86@yahoo.com

Probiotics has been widely known to have the ability to stimulate appetite, improve nutrients absorption and immune system of livestock and aquaculture production. This study were carried out to evaluate the effect of probiotics comprised of E1 and E2 as dietary supplement on growth performance of the juvenile African catfish, *Clarias gariepinus* (5.13 ± 1.03 g) under laboratory conditions for 50 days. The probiotics were previously isolated from vegetable wastes (Mung bean sprouts, *Vigna radiate*) and cucumber, *Cucumis sativus*) which have been fermented for 7 days. The experimental fish were divided into i) control, fed with commercial diet ii) E1, fed with practical diets containing diets supplemented with 10^8 CFU/ml of *Enterococcus faecium* isolated from fermented cucumber, iii) E2, fed with practical diets containing diets supplemented with 10^8 CFU/ml of *Enterococcus faecium* isolated from fermented mung bean sprouts. The experimental fish were measured for every 10 days of interval and the growth performance were assessed on weight gain (WG), length and specific growth rate (SGR) were calculated.

Probiotics supplementation showed improved growth performance especially in fish fed with E1 and E2 as compared to the control groups. The final weight and body weight gain of E1 and E2 increased significantly ($P < 0.05$) as compared to the control group (Table 1). In addition, SGR of the growth performances resulted with E1 and E2 showed significantly higher SGR ($P < 0.05$) compared to control group (Table 1). In conclusion, this study suggested that *E. faecium* were effective as probiotics for growth promoters in juvenile African catfish when supplemented as daily diet.

Parameters	Diets		
	Control	E1	E2
Initial weight (g)	5.26 ± 0.15^a	4.86 ± 0.12^a	5.27 ± 0.58^a
Final weight (g)	20.91 ± 0.81^a	27.22 ± 0.43^b	28.28 ± 0.54^b
Weight gain (g)	15.65 ± 0.66^a	22.36 ± 0.31^b	23.03 ± 1.12^b
Initial length (cm)	9.12 ± 0.16^a	9.20 ± 0.15^a	9.37 ± 0.20^a
Final length (cm)	14.35 ± 0.45^a	16.20 ± 0.20^b	16.68 ± 0.06^b
Specific Growth Rate (SGR) (%)	1.20 ± 0.01^a	1.50 ± 0.01^b	1.46 ± 0.11^b

Table 1: Mean growth performance of juvenile African catfish fed commercial diet supplemented with *E. faecium* for 50 days.

GENETIC IMPROVEMENT TO INCREASE HARVEST BODY WEIGHT IN RED TILAPIA (*Oreochromis spp*)

Azhar Hamzah^{1,*}, Ngo Phu Thoa² and Nguyen Hong Nguyen³

¹Fisheries Research Institute, Kg. Pulau Sayak, 08500 Kota Kuala Muda, Kedah, Malaysia

²Research Institute for Aquaculture No.2, Tu Son, Bac Ninh, Vietnam

³University of the Sunshine Coast, Maroochydore, QLD 4558, Australia

Email: azhar@dof.gov.my

Red tilapia (*Oreochromis spp*) has become popular in many countries because the fish are fetching a higher price relative to Nile tilapia due to their red colour that is similar to marine fish species. Our recent study conducted to evaluate performance of Red tilapia strains from three different sources (one from Taiwan, another one from Thailand, and a third population consisting of Malaysian stocks) showed that there were significant differences in production traits and survival among the three stocks. This information was then used in setting up the base population for this breeding programme by crossing them in a complete diallel design. Our analysis of the data collected from the three generations of selection from this breeding programme for increased harvest body weight showed that there was a significant genetic improvement for body weight in this species (12.3% per generation). Selection for high growth resulted in positive change in survival rate during growth. Avoidance of black spot parent mating also improved overall red colour of the selected population. The estimates of heritability for body weight (0.38 ± 0.09) indicated that the population will continue showing response to future generations. The large genetic variation in body colour, sexual maturity and survival also suggests that there is prospective for future improvement of these traits in this population. The genetic improvement program has successfully produced a genetic line of Red tilapia that possesses favourable characteristics for commercial production in Malaysia.

BREEDING AND EMBRYONIC DEVELOPMENT OF F₁ HYBRIDS, *Clarias gariepinus* (♀) (BUCHELL, 1822) AND *Pangasionodon hypophthalmus* (♂) (SAUVAGE, 1878)

Zarith Sofia Hamzah*, Yuzine, Esa, Annie Christianus, Muhammad Fadhil Syukri Ismail and Siti Fairus Mohamad Yusoff

Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
43400 Serdang
Selangor Darul Ehsan
zophia91@gmail.com

The present study conducted to breed and observe the embryonic development of F₁ hybrids, *Clarias gariepinus* (♀) and *Pangasionodon hypophthalmus* (♂) from fertilization until early hatched. The matured eggs and sperm were obtained by induced breeding using a commercial hormone, ovaprim. Dosages given for female and male were 0.5 ml and 0.25 ml ovaprim kg⁻¹, respectively. Fertilized eggs were adhesive, spherical, sticky and brownish green colour. Fecundity for female weighing 40 g and 60 g were 37,333 and 40,664 eggs/fish. Fertilization of eggs occurred 12 minutes after stripped the eggs and mixed with the sperm. Fertilization rates were 90% and 85% whereas hatching rates were 50% and 35%. The temperature during incubation was maintained to 27.7°C. The embryonic development ended when the eggs hatched after 16 hours and 15 minutes incubation (Figure 1).

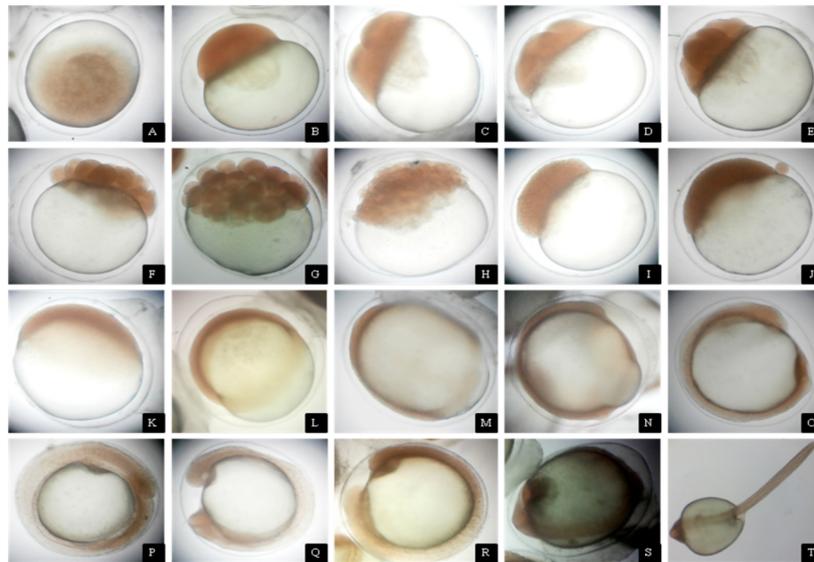


Figure 1: Embryonic development of hybrids, *Clarias gariepinus* (♀) × *Pangasianodon hypophthalmus* (♂). (A) Fertilized egg, (B) 1-cell stage, (C) 2-cell stage, (D) 4-cell stage (E) 8-cell stage, (F) 16-cell stage, (G) 32-cell stage, (H) 64-cell stage, (I) morula stage, (J) blastula stage, (K) early gastrula stage, (L) mid gastrula stage, (M) late gastrula stage, (N) blastopore stage, (O) embryo formation, (P) 5-myomere formed, (Q) optic vesicle formed, (R) tail separated and head formed, (S) embryo starts moving, (T) newly hatched larvae.

HAPLOTYPE DISTRIBUTION OF MITOCHONDRIAL DNA CYTOCHROME OXIDASE I GENE OF RED SPOTTED GROUPER *Epinephelus akaara* IN EAST ASIA

Sang-Hyun Han*, Yoo-Kyung Kim, Hong-Shik Oh, Chi-Hoon Lee, and Young-Don Lee

Educational Science Research Institute, Jeju National University, South Korea
102 Jejudaehak-ro, Jeju-si, Jeju Special Self-Governing Province, 63243, South Korea
hansh04@naver.com

The genetic structure and phylogenetic relationship were investigated in Korean red spotted grouper populations using the nucleotide sequence polymorphisms of mitochondrial DNA (mtDNA) *cytochrome c oxidase subunit I (COI)* gene. The *COI* gene was sequenced and compared with those previously reported. The obtained *COI* sequences showed 99.1-99.8% identities with the EF607565 previously reported. A total of twenty haplotypes were found, and the Korean population showed nineteen haplotypes. Among those, Hap_03 and Hap_08 showed Jeju-do and China-specific *COI* sequences, respectively. However, Hap_07 had twelve *COI* sequences from South Korea and the records from Hong Kong and Taiwan. The neighbor-joining (NJ) trees constructed from the phylogenetic analyses based on the polymorphisms of the *COI* haplotypes showed the monophyletic branching pattern within the genus *Epinephelus*, indicating that the red spotted grouper populations had evolved from common maternal ancestors. In addition, the Hap_08, which had the *COI* sequence recorded only from China Sea, has been found in the middle of the NJ tree nearby Hap_07 and showed a close relationship to that of Hap_07. Consequently, East Asian red spotted grouper populations are maternally related, as well as sharing the same evolutionary history, and still affected by the East Asian ocean current (Kuroshio).

In order to look for maternal relationship among the Red-spotted grouper populations in East Asia, haplotype of *COI* gene sequences of the mitochondrial DNA were determined and compared among the populations.

The results from the haplotype analysis for *COI* gene sequences showed twenty haplotypes, among those several haplotypes were predominant and shared together in most populations. Korean Red spotted grouper is also maternally related to other populations in East Asia. However, some of those showed location-specific patterns. The phylogenetic tree was also estimated the maternal relationship among the populations of Red-spotted grouper in East Asia.

This study helps to understand the genetic structure and phylogenetic relationship of red spotted grouper and also contributes to the significant role in research on artificial breeding and industrialization.

Table 1. *COI* haplotypes found in the Red Spotted grouper in South Korea

Haplotype	South Korea	China	Hong Kong	Taiwan
Hap_01	2			
Hap_02	39			
Hap_03	1			
Hap_04	2			
Hap_05	1			
Hap_06	1			
Hap_07	10		1	1
Hap_08		1		
Hap_09	1			
Hap_10	1			
Hap_11	3			
Hap_12	9			
Hap_13	1			
Hap_14	1			
Hap_15	1			
Hap_16	1			
Hap_17	1			
Hap_18	1			
Hap_19	1			
Hap_20	1			
Total	78	1	1	1

FIRST REPORT OF *Clavinema mariae* (LAYMAN, 1930) (NAMATODA: PHILOMETRIDAE) IN CULTURED ROCKFISH *Sebastes schlegeli* IN CHEONSU BAY, REPUBLIC OF KOREA

Hyun-Ja Han*, Jung-Soo Seo, Jeong- Su Park, Haeng- Lim Lee, Han-Gill Seo, Sung-Hee Jung and Se-Ryun Kwon

Pathology Division
National Institute of Fisheries and Science
Busan 46083, Korea
hjhan77@korea.kr

Rockfish (*Sebastes schlegeli*) commonly known as the rockfish, is an important species in aquaculture industry of the Republic of Korea. The first infection of nematode parasites in the epithelial tissue of rockfish observed in the Cheonsu Bay of the West Coast in July 2012. Nematode infection occurred throughout the year (May 2013 to April 2014), with the exception of October 2013.

Nematodes found in the epithelial tissues of cultured rockfish (*Sebastes schlegeli*) in the Cheonsu Bay of the West Sea in Korea were collected in April 2014 to June 2016 for identification. The fish were first examined macroscopically to detect infections female nematodes. 30 specimens collected in June 2016 were used for measurement. The body of the sub-gravid females was distinctly narrower in its posterior half. Body length ranged from 22.0-31.0mm, with a maximum width of 0.59-0.70 mm. The cephalic end was rounded, with an elevated esophageal bulb region. Small mouth papillae were observed, and mouth was slightly depressed. The anterior end of the esophagus formed a conspicuous, strongly muscular bulb that was well separated from the cylindrical portion of the esophagus. A nerve ring encircled the anterior end of the cylindrical portion of the esophagus. The intestine was wide throughout its length and attached by a short translucent ligament to the ventral. The parasite was identified as *Clavinema mariae* based on morphological studies using light and scanning electron microscopy. This is the first report confirming *C. mariae* infection in rockfish in Korea.

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

Hadzley Harith^{1,2}, Mohd Lokman Husain and Mohd Fadzil Mohd Akhir

Tel: 019-4651199, hadzley_harith@yahoo.com , gsk2105@pps.umt.edu.my, hadzley@dof.gov.my

¹Impact Assessment Research Division, Fisheries Research Institute, 11960 Batu Maung, Pulau Pinang

²Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu

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

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

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

THE EFFECT OF PROBIOTICS ON THE SURVIVAL, GROWTH AND IMMUNE RESPONSE OF SPINY LOBSTER *Panulirus homarus* CULTURE

Haryanti*, Zeni Widyastuti and Sari Budi Moria

Institute Mariculture for Research and Development
Br Gondol, Ds Penyabangan, Kec. Grogak-Buleleng
PO Box 140 Singaraja 81001, Bali
Indonesia
haryanti@indosat.net.id
haryanti0423@gmail.com

A number of problems including disease infections and significant mortality were occurred in lobster aquaculture. The aim of this research was to observe the performance of growth, survival and immune response on spiny lobster *P. homarus*. The method in this research was initiated by exploiting the potential probiotic candidates through the isolation, characterization-identification, and then tested the ability of enzymatic hydrolysis, vibriostatic activity, antagonistic, pathogenicity. The culture and application of probiotics was supplemented to the feed of spiny lobster. The spiny lobster was collected from Jembrana Waters – Bali- Indonesia. The Various supplemented moist diet were tested i.e. (A) the combination of probiotics and (B) Without probiotic (Control). In the present experiment spiny lobster were reared for three months in the concrete tanks with capacity of 15 m³. Initial weight of spiny lobster were 76.55 ± 12:03 g. Immunity responses were analyzed with quantitative value of immunity related gene expression (7 gene targets) by RT-qPCR after challenge test.

The results showed that, there were 4 strains of probiotics potential, i.e. *Photobacteria damsela* N-5, *Bacillus subtilis* C-1, *Bacillus oceanisediminis* H-3, and *Bacillus amyloliquefaciens* I-5. The growth rate of spiny lobster were obtained 189.21 g (combination of probiotics), while the control of 169.76 g. The survival rate was not significant different. Immunity gene expression analysis results indicate that supplemented combination probiotics for 3 months culture showed differences in increasing immunity responses. Expression immunity on ALFH_a-1, ALFH_a-2 and ALFH_a-4 after challenge test with *Vibrio harveyi* revealed by 2.0 -7.0 fold, 4.0 – 10.5 fold and 1.0 - 4.0 fold respectively compared with controls. At ProPO activating system (prophenoloxidase / ProPO). Expression immunity of spiny lobster as much as 1.5- 4.0 fold, while the clotting system (transglutaminase, clotting protein) expression immunity of 0.5 – 7.5 fold. Antioxidant defense mechanism (glutathione peroxidase / GPO) of 0.5 – 29.5 fold. Spiny lobster immunity gene expression was 1.5 -5.8 significantly different at the target gene SAA compared control (0.1-1.5 fold)

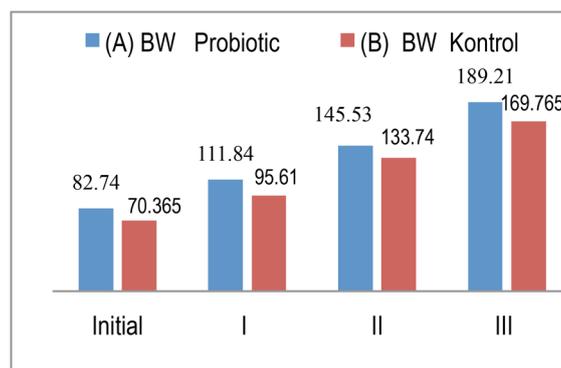


Figure 1 .Growth rate of spiny lobster *P.homarus* reared for three months with fed of combination of probiotics and without probiotic

SOCIO-ECONOMIC IMPACTS OF ADOPTION OF BRACKISHWATER TILAPIA (*Oreochromis niloticus*) CULTURE BY POND FARMERS IN ACEH PROVINCE, INDONESIA

Hasanuddin*, Muslim, Joanne Millar and Michael A. Rimmer

Brackishwater Aquaculture Development Centre Ujung Batee
PO Box 46 Banda Aceh
Aceh, Indonesia
hasanuddin_79@yahoo.co.id

Aceh province in Indonesia has an estimated 72,000 ha of brackishwater ponds which produce shrimp (mainly black tiger shrimp *Penaeus monodon*) and milkfish (*Chanos chanos*) using traditional extensive techniques. However, shrimp production has decreased due to the impacts of widespread white spot syndrome disease (WSSD) and milkfish culture is subject to unstable prices. A research and development project funded by the Australian Centre for International Agricultural Research (ACIAR) found that, while brackishwater tilapia production showed economic viability as an alternative to shrimp culture in coastal ponds, adoption by Acehnese farmers was constrained by limited availability of tilapia seedstock.

In response, farmer-managed tilapia seedstock production centres ('nurseries') were established at sites throughout Aceh. Production and sales of tilapia fingerlings were recorded by the nurseries, and the purchasers were interviewed by project staff to assess the farmers' responses to brackishwater tilapia culture. A structured questionnaire was used to interview a total of 55 farmers from 6 districts in 2 years (2013 and 2014).

The survey showed that the tilapia nurseries were successfully supplying local demand for tilapia fingerlings. In some cases, numbers of fingerlings were purchased by brokers for resale in adjacent districts. Most farmers reported a production cycle of 3–4 months, harvesting at a size of 6–7 fish per kg, and productivity of 200–500 kg per ha using extensive techniques. Many farmers (82%) used tilapia in polyculture with shrimp; the remaining 18% used a monoculture system. Farm gate prices ranged from IDR 13,000 to 25,000 per kg, with a mode of IDR 17,000 per kg. Cultured tilapia were also consumed by 92% of tilapia farmer households, contributing to improved nutrition in coastal farming communities.

Advantages to culturing tilapia in brackishwater ponds include: profitability (compared with other cropping options), ease of culture (using extensive culture methods), and rapid growth (faster than milkfish). The main constraints to tilapia production were identified as: predators, and high salinity during the dry season. It was noted that some farmers were alternately culturing shrimp during the dry season, and tilapia during the rainy season when shrimp disease outbreaks are more common.

GENÉTICA SPRING'S (GS) BREEDING PROGRAM AND ITS APPLICATION TO THE ASIAN SHRIMP FARMING INDUSTRY

Oscar Hennig*, Marcela Salazar, Morten Rye

Benchmark Breeding and Genetics
73-1353 Kaiminani Dr
Kailua-Kona Hawaii 96740 USA
oscar.hennig@bmkgenetics.com

During recent years Benchmark Breeding and Genetics has developed a solid presence in aquaculture genetics through acquisitions of Akvaforsk Genetics (AFGC), a leading provider of advanced breeding program design and technical services to global aquaculture, and the breeding companies SalmoBreed and Stofnfiskur (Atlantic salmon) and Spring Genetics (tilapia). With the recent incorporation of Genética Spring in 2016, Benchmark expanded its breeding business to include marine shrimp (*Litopenaeus vannamei*). Genética Spring continues and expands the pioneering shrimp breeding program initiated by CENIACUA in Colombia in 1997, a program designed and supervised by Akvaforsk Genetics. Today this is the worlds' most scientifically documented genetic improvement program for shrimp.

The cultivated shrimp industry is largely based on *L.vannamei*. Current strategies for disease management in Asia emphasize exclusion and eradication with strict quarantine and importation protocols. These strategies have not prevented a series of disease epidemics. Diseases have been introduced by illegal importations; fresh feeds and/or poor water management. Since commercial populations of shrimp are derived from imported SPF stocks that are susceptible to endemic diseases, amplification of the pathogen load occurs rapidly. The Asian *L.vannamei* sector will continue to be repeatedly exposed to new epidemics while depending on broodstock poorly adapted to local conditions. To break this vicious cycle there is the need of an alternative, integrated disease management strategy, based on: minimizing the risk of introduction of new diseases to the region; deployment of populations resistant to endemic diseases; and sanitary practices that minimize the likelihood of epidemics and delay infection.

Combining the SPF, SPR and APE approach Genética Spring has successfully bred *L.vannamei* populations with high levels of resistance to major diseases affecting the shrimp industry today such as: WSSV; EMS; NHP; TSV; Vibriosis.

This presentation will focus on the achievements of Genética Spring selective breeding program and its application for the Asia *L.vannamei* farming industry.

APPLICATION OF MUSTIKA CARP (*Cyprinus carpio*) ON PONDS AND FLOATING CAGE CULTURE: A NEW STRAIN OF KOI HERPES VIRUS RESISTANT

Yogi Himawan*, Khairul Syahputra, and Flandrianto Sih Palimirmo

Research Institute for Fish Breeding

Jl. Raya 2 Sukamandi, Pantura-Sukamandi, Subang, West Java, Indonesia

Email : yogihimawan@yahoo.com

Mustika is superior strains of carp with characteristics that resistance against KHV infection and has a high growth performance in the environment of cultivation. Mustika generated through program selection in carp strains Rajadanu assisted by markers of MHC-II and are now beginning to be developed in the community. Development of Mustika done through by dissemination and application programs directly to the farmer. The purpose of this study was to determine the performance of production carp Mustika on pond at nursery stage ground and enlargement in cage culture at Cirata Dam, West Java, Indonesia. The method are :

Materials used in the form are seeds of carp Mustika resulted from spawning of the broodstock sized male 0.8-3,8 kg and female 1- 1.7 kg. Container nursery is earth pond in the form of an area of 1000 m² calm water with a depth of 60-80 cm, as well as the size of the KJA 7x7x1,5 m in Cirata Dam for magnifying stage.

In the first spawning cycle, second, and third spawning obtained Mustika seed amount of 50,000 fish 2-3 cm in size, 380 800 ish 1-2 cm in size, and 80,000 fish in size of 2-3 cm resulted in nursery ground (Figure 1). Enlargement in 3 months at cage show that an average weight achieve 130.35 ± 38.12 g with a survival rate of 80%.

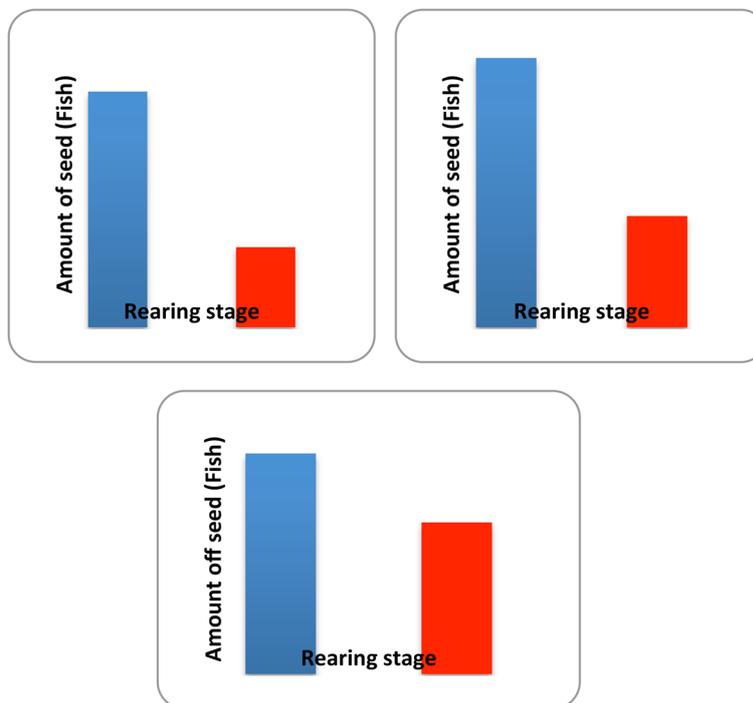


Figure 1. Amount seed of Mustika carp resulted in rearing at earthpond for three times spawning.

CHARACTERIZATION OF GLUCOCORTICOID RECEPTOR 1 AND 2 UNDER STRESS IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

Hsin-Yen Ho*, Chia-Jung Lee, Young-Mao Chen, and Tzong-Yueh Chen

Department of Biotechnology and Bioindustry Sciences, Institute of Biotechnology, Translational Center for Marine Biotechnology, and Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan 70101, Taiwan
ciny0214@gmail.com

Orange-spotted grouper, *Epinephelus coioides*, is one of the commercially important aquaculture fish in Asia. For successful aquaculture it is important to minimize stress. Due to inflammation is one of the important innate immunity in fish and tumor necrosis factor (TNF1) is first been expressed under stress in teleosts. The aim is to understand the characterization of the modulation of glucocorticoid pathway involved in modulating inflammation cytokine under stress condition.

GR1 and *GR2* are cloned from *Epinephelus coioides*. Cortisol produce their effect on responsive cells by acting through the glucocorticoid receptor, which regulates the transcription of target genes. Upon hormone binding, the GR translocates to the nucleus, where it acts as a transcription factor. The GR binds DNA at glucocorticoid response elements in the promoter regions of corticosteroid responsive genes, inducing transcription. Two *GR* gene regulations in response to cortisol treatment was analysis by real-time PCR. Immune stimulate of tumor necrosis factor 1, *GR1* and *GR2* in cortisol-treatment grouper compared with healthy grouper during 48 hours post-treatment. We used to FRET experiment conclude that cortisol exerts their influences by binding to the grouper GR and associating with HSP90AB, capable of regulating several genes. These findings have implications that biological effects of cortisol involve cross-talk with inflammatory and immune responses during nodavirus infection.

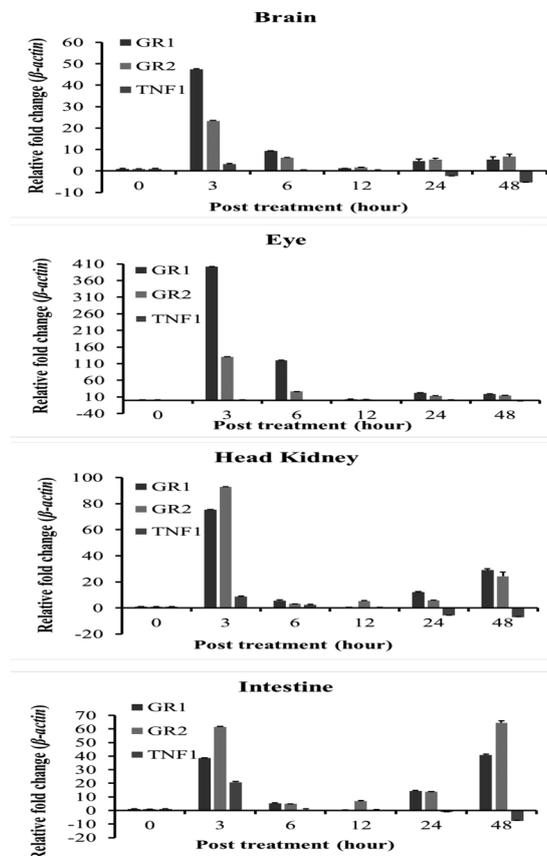


FIGURE 1. Immune stimulate of *osgGR1*, *osgGR2* and TNF1 in brain, Eye, Head kidney and Intestine after treatment with cortisol.

EFFECTS OF STOCKING DENSITY OF GRAY MULLET (*Mugil cephalus* Linnaeus, 1758) ON PRODUCTION AND NUTRIENT RECOVERY IN AN INTEGRATED FARMING OF WHITE SHRIMP (*Litopenaeus vannamei* Boone, 1931)

Manh Hoang Nghia^{1,2}, Phuoc Nguyen Ngoc², and Peter Bossier¹

(1) Laboratory of Aquaculture & Artemia Reference Center
Ghent University

Coupure Links 653, B-9000 Gent, Belgium

(2) Faculty of Fisheries, Hue University, Vietnam

Manh.HoangNghia@UGent.be

Shrimp farming has been facing a lot of problems consisting of environmental pollution, shrimp diseases outbreak, and overuse of chemical during operated culture activities. Polyculture is considered as an important alternative to utilize the uneaten feed and decrease organic pollution and prevent shrimp disease in shrimp farming. In Vietnam, Gray mullet (*Mugil cephalus* Linnaeus 1758) is being considered as a novel candidate for polyculture, especially with white shrimp because of its rapid growth rate, eating habit, and availability fingerling. The aim of this study was to assess the effects of different densities of gray mullet on production and nutrient recovery in the white shrimp integrated culture system.

An experiment (75 days) was conducted in twelve composite tanks (5 m³), with triplicates per each treatment (Table 1) and three tanks were stocked only white shrimp as control treatment. Shrimp (body weight of 0.50 g) were acclimated to laboratory conditions for 2 weeks prior to the experimentation. Gray mullet (*Mugil cephalus*) fingerling were reared in laboratory condition until they reached experimental size of 1.50 g by commercial diet (Growbest Co. Ltd. Vietnam). Water from the sea was filtered through a sand filter and pumped into a container tank (50 m³) where seawater was diluted with groundwater to produce experimental seawater which salinity of 15‰. Aeration was supplied in cultured tanks by air stones throughout the experimental period. Shrimp were fed four times a day using 35% protein commercial pellet (Growbest Co. Ltd. Vietnam) with feeding rate was 3 to 10% of biomass. No external feed was given to the fish during the experiment.

After 75 days, treatment II (10% gray mullet) shrimp and fish had significantly ($p < 0.05$) higher in the biomass, the weight gain, and the Nitrogen recovery compared to all other treatments (Table 2). These results showed that white shrimp can be cultured with gray mullet at the stocking density of 10% biomass of shrimp to improve overall productivity and nutrient utilization efficiency.

Table 1. Shrimp and gray mullet stocking density in the experiment

Treatment	Description
I (Control)	Only shrimp (60 shrimp/m ²)
II	Shrimp + gray mullet (10% biomass of shrimp)
III	Shrimp + gray mullet (20% biomass of shrimp)
V	Shrimp + gray mullet (30% biomass of shrimp)

Table 2. Production performance and nutrient recovery by white shrimp and gray mullet at the different experimental treatments

Indicator	Treatment			
	Control	10% Mullet	20% Mullet	30% Mullet
Total biomass (g/tank)	1879.62 ^a	2928.68 ^d	2423.03 ^b	2662.40 ^c
Total weight gain (g/tank)	1729.62 ^a	2763.65 ^d	2242.96 ^b	2467.50 ^c
Total Nitrogen recovered (%)	24.61 ^a	41.82 ^d	32.18 ^b	36.45 ^c
Total Phosphorous recovered (%)	9.40 ^a	27.53 ^b	35.76 ^c	34.52 ^c

Values were presented by Mean ± SE.

Mean values in the same row with different superscript letters (a, b, c, d) were significantly different ($p < 0.05$).

PERFORMANCE OF RED TILAPIA *Oreochromis sp.* FED DIET WITH FERMENTED BANANA *Musa acuminata* × *balbisiana* PEEL AT DIFFERENT STAGES OF RIPENESS CHALLENGED WITH *Aeromonas hydrophila*

Grace Honorio Mones* and Isagani P. Angeles Jr.

Provincial Institute of Fisheries
Isabela State University-Roxas Campus
Roxas, Isabela, Philippines 3320
monesgrace@yahoo.com / monesgraceh@gmail.com

This study evaluated the dietary effect of fermented banana (*Musa acuminata* × *balbisiana*) peel at different stages of ripeness on growth, antioxidant capacity, metabolic response and survival of Red tilapia (*Oreochromis sp.*) reared for nine weeks following *Aeromonas hydrophila* infection.

The fermented banana peel (FBP) with 100 ml DW was sprayed unto one kg of commercial tilapia feed; 200 ml immature FBP (iFBP), 200 ml ripe FBP (rFBP), 200 ml over ripe FBP (oFBP), while 300 ml DW for the control diet (C). Wf, SGR and PER of oFBP-fish were significantly higher and FCR was significantly lower than that of C-fish. WG of oFBP, rFBP, iFBP was increased by 80%, 43% and 29% as compared to the C, respectively. On the other hand, percentage survival of fish fed diet with FBP disregarding ripeness was significantly higher than the C-fish after nine weeks of rearing. SOD of oFBP, rFBP and iFBP- fish was 59, 43 and 35 % lower as compared to the C, respectively. GPx and GR activity of oFBP- fed fish were higher than that of the C-fed fish. oFBP and rFBP had 52 and 44 % lower Gluc level as compared to the C-fish, respectively. Furthermore, Lactate level of oFBP was lower than that of C- fed fish. Interestingly, all FBP-fed groups exhibited higher survival than the control.

Overall, these results indicated that FBP at different stage of ripeness, especially oFBP enhances growth performance, stabilizes both antioxidant capacity and metabolic response and improves resistance of Red tilapia against *A. hydrophila* infection. FBP could be therefore considered as potential alternative to synthetic growth promotant and antioxidant products used in aquaculture industry.

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

Tiago Hori*, André Dumas, Jason Stannard, Debbie Plouffe and John Buchanan

The Center for Aquaculture Technologies Canada
20 Hope Street
Souris (PE) C0A 2B0, Canada
thori@aquatechcenter.com

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

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

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

DEVELOPMENT OF A SNP MARKER PANEL FOR PARENTAGE, DIVERSITY AND RELATEDNESS ANALYSES IN BARRAMUNDI, *Lates calcarifer*

Melissa Allen, John Buchanan, Tiago Hori, America Fujimoto, and Jason Stannard

Center for Aquaculture Technologies
8395 Camino Santa Fe, Suite E, San Diego, CA 92121
jstannard@aquatechcenter.com

Barramundi, or Asian sea bass, are of significant economic importance, being both fished and farmed extensively throughout the Indo-Pacific and increasingly in the northern hemisphere. Global aquaculture production of this species has increased dramatically over the past two decades from roughly 20,000 metric tonnes in 2000 to over 100,000 metric tonnes in 2015. As demands rise along with its growing popularity around the globe, the need to establish reliable genetic tools to assess parentage, relatedness and diversity among cultured stocks is of increasing importance. We report here on the development and characterization of a single nucleotide polymorphism (SNP) panel for barramundi. A panel of ~ 178 SNP markers was established for use in genotyping wild or cultured populations of barramundi. The panel has been tested on varied geographic populations; and the majority are highly informative with an average genotyping success rate of ~98%. The ability of the SNP panel to be able to distinguish between all individuals in all populations (measured as the discrimination rate) was excellent, with a discrimination rate of 99.99%, even when 2 mismatches were allowed. These qualities will allow for increased accuracies concerning parentage assignment and various measures of population diversity. Some examples will be presented on the use of the SNP panel in providing information on population structure, inbreeding and relatedness within barramundi populations.

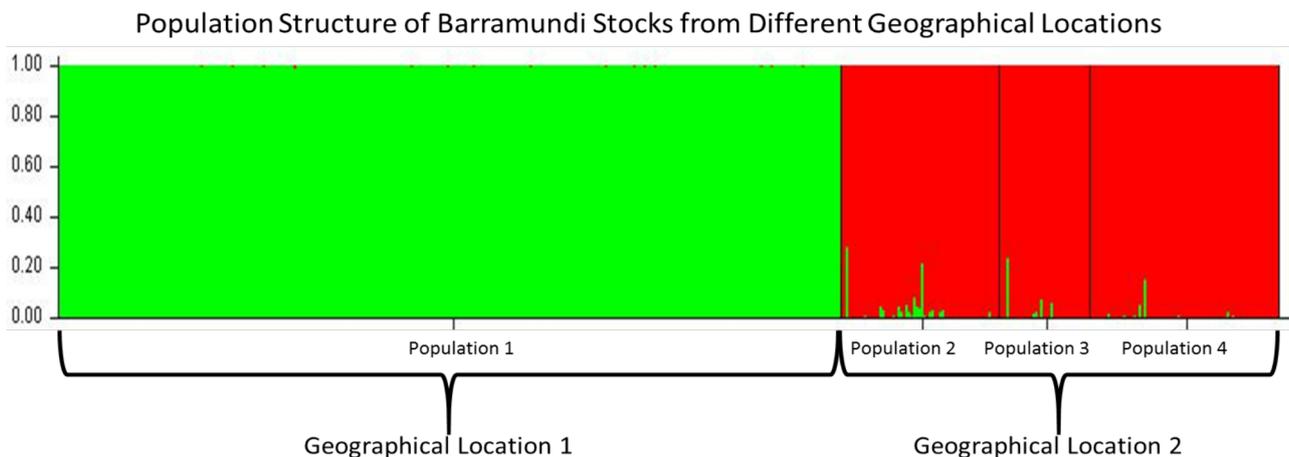


Figure 1. Showing a representation of the proportion of all individual's genomes belonging to 1 of 2 populations. The structure analysis indicates that individuals from population 1/ geographical location 1 are genetically distinct from individuals sampled from the 3 other populations within geographical location 2.

IMPROVEMENT OF NUTRITIONAL QUALITY OF CULTURED SEA BREAM (*Sparidentex hasta*) MUSCLE BY PREHARVEST FEEDING TECHNIQUE USING FINISHER FEEDS

M.A. Hossain*, K. Al-Abdul-Elah, and S. El-Dakour

Aquaculture Program
Environment and Life Sciences Research Center
Kuwait Institute for Scientific Research
PO Box # 1638, Salmiya 22017, Kuwait

The aim of this study was to improve the muscle and nutritional quality of the cultured sea bream (*Sparidentex hasta*) to be at par with that of the wild. This aim was achieved by feeding them with finisher feeds with high decosahexaenoic acid (DHA) content at the latter part of the grow-out stage so that they become equally acceptable as the wild to the consumers. The highest dietary levels of DHA in finisher feeds were chosen to match the level of DHA in wild sea bream muscle in the pre-spawning season when DHA is in its highest level. The level of DHA in the finisher feeds were maintained by incorporating high DHA tuna oil to a best performed grow-out diet for sea bream. A grow-out feed was used from the beginning until the end of the trial was considered as the control (Diet 1). Diet 2 and Diet 3 were formulated to contain 9.0 and 10.5% DHA, respectively, by incorporating high DHA tuna oil into a sea bream grow-out diet. Diet 4 was a commercial finisher feed from a feed company (Skretting, Italy). The experiment was carried out with grow-out sea bream (202.9 using a flow-through system consisting of 12 1-m³ circular fiber glass tank. The stocking density was 20 fish/m³, and the fish were hand fed twice daily at satiation level. All the fish were fed with the same grow-out feeds (Diet 1) from the start of the experiment for 4 months and then, the finisher feeds were fed for another two months. The results showed that all the finisher diets resulted in significantly ($P < 0.05$) better growth performance, feed utilization and higher muscle eicosapentaenoic acid (EPA) and DHA content in sea bream compared to those fed the control diet. The DHA and EPA in fish fed finisher diets were also higher than those of the whole year average DHA and EPA content of wild sea bream. An organoleptic comparison of muscle quality of cultured and wild sea bream showed no significant ($P > 0.05$) differences between sensory attributes. A study to determine the best time duration for feeding finisher diet showed that 6 weeks of feeding is optimum for assimilation of DHA in fish muscle.

TABLE 1. Growth performance and feed utilization by sea bream fed different finisher diets¹

Parameter	Diet			
	1	2	3	4
Mean initial weight (g)	203.36 ^a ±1.96	202.16 ^a ±2.70	202.57 ^a ±4.85	203.40 ^a ±2.36
Mean final weight (g)	563.93 ^b ±10.14	644.06 ^a ±14.33	642.47 ^a ±16.26	648.36 ^a ±20.27
Mean weight gain (g)	360.57 ^b ±8.19	441.90 ^a ±11.73	439.90 ^a ±15.83	444.96 ^a ±18.29
Specific growth rate (%/day)	0.57 ^b ±0.01	0.65 ^a ±0.01	0.64 ^a ±0.01	0.64 ^a ±0.01
Condition factor	2.68 ^a ±0.03	2.70 ^a ±0.02	2.71 ^a ±0.06	2.73 ^a ±0.03
Hepato-somatic index	1.53 ^a ±0.11	1.59 ^a ±0.09	1.59 ^a ±0.10	1.57 ^a ±0.13
Feed intake (g/fish/day)	3.75 ^b ±0.06	4.25 ^a ±0.07	4.32 ^a ±0.10	4.22 ^a ±0.08
Feed conversion ratio	1.87 ^a ±0.03	1.74 ^b ±0.02	1.76 ^b ±0.03	1.73 ^b ±0.03
Protein efficiency ratio	1.17 ^c ±0.02	1.30 ^b ±0.02	1.31 ^b ±0.03	1.38 ^a ±0.02
Apparent net protein utilization (%)	24.03 ^b ±0.39	27.35 ^a ±0.24	27.63 ^a ±0.83	27.76 ^a ±0.29
Survival (%)	95.00 ^a ±5.00	98.33 ^a ±2.89	98.33 ^a ±2.89	96.67 ^a ±2.89

Values (mean ± SD) in a column with different superscripts are significantly ($P < 0.05$) different determined by Tukey's test.

PROSPECTS AND PROBLEMS OF FISH PROCESSING PLANTS IN SYLHET REGION, BANGLADESH

Md. Motaher Hossain* and Md. Anisur Rahman

Dept. of Fisheries Technology and Quality Control, Sylhet Agricultural University, Sylhet-3100, Bangladesh
E-mail: motaher03@yahoo.com

The present study conducted to find out the prospects and problems of Fish Processing Plants in Sylhet region. The research period was from January -2015 to October -2015. The study was performed by questionnaire- based data collection and the findings of the individual interviews. The study focused on two EU approved Fish Processing Plants through which fish and fishery products (mainly white fishes) are being processed for export. The findings of this study reveals that there are lot of opportunities to establish Fish Processing Plants such as availability of raw materials, low cost land, transportation facilities, suitable environment and particularly buyer's demand of the fish species of this region. About 110 indigenous fish species were found in this region which almost half of the total number of fish species in Bangladesh. A large number of people of Sylhet region live in abroad. So, the main markets of processed fish are UK, USA and Middle East. Beside this potentiality and opportunity to enhance the export through these Fish Processing Plants the study also reveals that fish processors are facing lot of problems such as seasonality of fish species, lack of skilled personnel, high operational cost, weak infrastructure and storage facilities, international market competition, government policy, standardization, proper advertisement, low salary of workers, incentive problems with other exportable products, high interest of bank loan etc. These problems hamper the production of processed fishes of these Fish Processing Plants and also discourage the plant owners to export. To minimize the problems of this sector government should take necessary initiatives. So, it would create great opportunity to earn foreign currency by exporting the fish and fishery products and as well as contribute to the development of our country.

CHARACTERIZATION OF I κ B POST-MODIFICATION DURING NODAVIRUS INFECTION IN ORANGE-SPOTTED GROUPER *Epinephelus coioides*

Chi-Yu Hsieh*, Yu-Lin Tsai, Young-Mao Chen, and Tzong-Yueh Chen

Department of Biotechnology and Bioindustry Sciences, Institute of Biotechnology
Translational Center for Marine Biotechnology, and Agriculture Biotechnology Research
Center, National Cheng Kung University, Tainan 70101, Taiwan
cheetaholic1007@hotmail.com

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

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

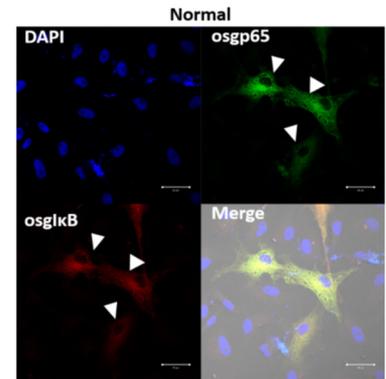


Figure 1. The distribution of *osgp65* and *osgI κ B α* in GF-1 cells.

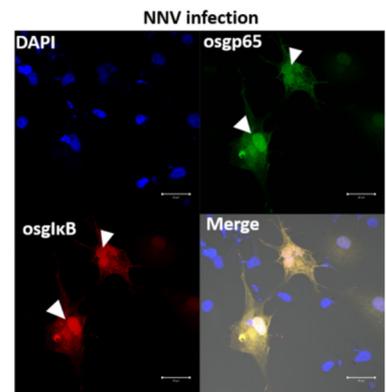


Figure 2. The distribution of *osgp65* and *osgI κ B α* in GF-1 cells under NNV infection.

EFFECTS OF DIFFERENT FORMS OF FEEDS ON FECUNDITY OF FRESHWATER PRAWN, *Macrobrachium rosenbergii*

Saadiah Binti Ibrahim^{ab}, Mohammed Suhaimi Abd. Manaf^b & Ng Wing Keong^a

^aSchool of Biological Sciences, Universiti Sains Malaysia
11800 Penang, Malaysia

^bFRI Pulau Sayak, Fisheries Department of Malaysia, 08500 Kota Kuala Muda, Kedah
sadiahibrahim7@gmail.com

Four different forms of maturation diet for freshwater prawn, *Macrobrachium rosenbergii* were tested to determine their effect on the fecundity and eggs quality. Two types of dry pellet form (Dry basic, DB & Dry mixed, DM), semi-moist feed (SM) and fresh feed (FD) form were used in this study. Diet DB, DM and SM were formulated to be isonitrogenous (45%) and isolipidic (9%). Fresh feed was used as a control diet. The study was conducted in 10 ton fiberglass tanks with two replicate tanks for each diet. A total of 30 female broodstocks in the range of 17 – 42 g were used in each tank. The feeding trial for females was conducted for 24 days prior to 10 pieces of male broodstock were introduced in each tank for mating. Results showed that the FD form gave the best performance compared to the other diets for the percentage of berried female, fecundity and quality of eggs. The percentage of berried female produced for broodstocks fed with FD, DM, DB and SM were 76.7%, 61.7%, 48.3% and 46.7%, respectively. Data of larval fecundity shows that broodstocks fed with FD gave the best result of 1219 ± 214 larvae/g female which was not significantly different compared to diet SM and DB with the value of 1155 ± 130 and 951 ± 140 larvae/ g female (Fig. 1). Eggs quality in terms size indicated that the highest length was obtained from broodstock fed the FD diet. However, the average volume of eggs from broodstock fed SM showed significantly larger size than other diets ($P < 0.05$) (Fig. 2). In conclusion, the fresh feed gave the best outcome for larvae fecundity and length, thus the investigation on its nutritional content will be conducted in order to increase the performance of pelleted feed.

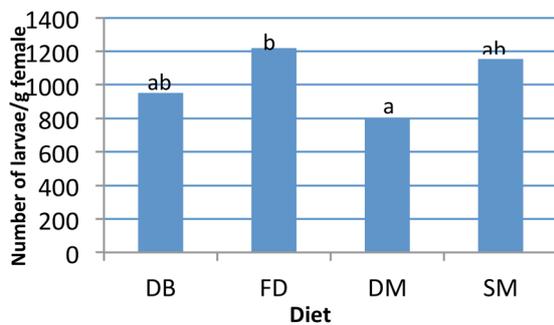


Figure 1: Larvae hatch fecundity

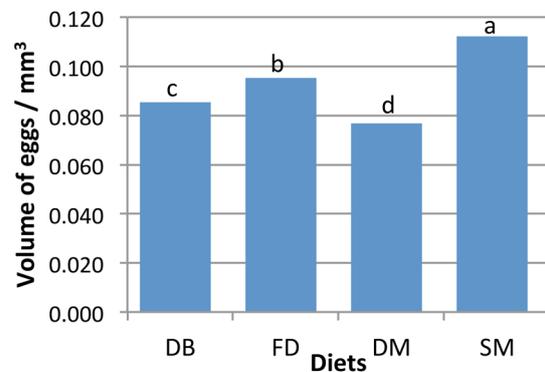


Figure 2: Volume of eggs with diets

SEA CUCUMBER *Holothuria scabra* JUVENILE GROWTH PERFORMANCE IN SALTWATER PONDS

Zaidnuddin Ilias*, Nik Nazley Effendy Ramli, RA Roki Mohamed and Zainoddin Jamari

Fisheries Research Institute Langkawi, Pejabat Perikanan Daerah Langkawi, 07000 Langkawi Kedah

* zaiali01@yahoo.com

Sea cucumber aquaculture in Malaysia is still in an early stage of development. Artisanal wild seed collection and grow out had been practised in small number in Sabah. Due to the seasonal and low number of seed available and also to prevent over exploitation of the natural resources the Department of Fisheries Malaysia has started a study on seed production of various species of sea cucumber which were *Stichopus horrens*, *Stichopus vastus* and *Holothuria scabra*. *Holothuria scabra* as the main species focused in the aquaculture was selected in this study. The issue faced was the slow growth and survival of juveniles in hatchery. A study was carried out to determine the potential of saltwater pond as the nursery system for *Holothuria scabra*. Survival and growth of sea cucumber juveniles cultured in were observed and recorded. The juveniles used were produced using matured sea cucumber held in sea pen at Langkawi island. Weight, length and survival number were collected monthly. The result from this study showed that juveniles cultured in ponds has higher growth rates where at week 5 reached the average weight of $15.9 \pm 5.9\text{g}$ whereas in hatchery was $4.1 \pm 1.9\text{g}$. The heaviest average was $21.7 \pm 6.2\text{g}$ (Figure 1).

Juvenile Growth in pond compared to Hatchery

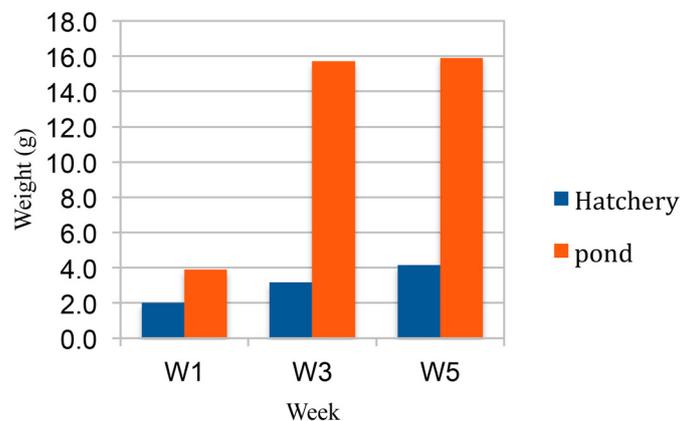


Figure 1: Sea cucumber juveniles, left 2 from pond

EFFECT OF VARYING LEVELS OF PROTEIN FROM DIFFERENT ANIMAL SOURCES ON GROWTH AND SURVIVAL OF CARP, *Cirrhinus mrigala*, REARED IN CEMENTED CISTERNS

Rehana Iqbal,^{1,*} Muhammad Ali,² Naeem Tariq Narejo³ and Kashif Umer

¹Institute of Pure and Applied Biology, Bahauddin Zakariya, University, Multan

²Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya, University, Multan

³Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro

rehanaiqbal82@gmail.com

A study was undertaken to determine the effect of protein from different animal sources on the growth and body composition of *Cirrhinus mrigala*. A total of 210 *Cirrhinus mrigala* fingerlings were distributed in completely randomized way into six treatments with three replicates each. Six experimental protein diets of varying protein level were formulated utilizing fish and chicken waste *viz.*, fish as protein source like fishmeal with 30% protein (FM30), fishmeal with 35% protein (FM35) and fishmeal with 40% protein (FM40). Similarly chicken waste as protein source like chicken meal with 30% protein (CW30), chicken meal with 35% protein (CW35) and chicken meal with 40% protein (CW40). Controlled group diet contained a balanced mixture of proteins from both animal sources. The daily feed ration was 8% body weight of fish. Highly significant ($P < 0.001$) results were observed with respect to growth and body composition parameters. Chicken waste feeds showed better BWG, SGR, FER and PER over fish meal. With respect to body composition, chicken waste group showed higher %water content than other feeding group. Fat content was higher in FM40. In the same way protein content was higher in control group which was not also significantly ($p > 0.05$) different from FM30, FM35 FM40 and CW40. In conclusion, CW30 showed better growth performance of *C. mrigala* reared in cemented cisterns.

Table: Mean values and standard deviation (Parenthesis) of growth parameters of *C. mrigala* for feeding groups from animal source.

Growth parameters	1				2		
	Fishmeal				Chicken waste		
	Control	FM30	FM35	FM40	CW30	CW35	CW40
Body weight gain	603.3 ^a	888.5 ^b	1045.3 ^c	877.8 ^a	1253.3 ^d	1244.8 ^d	1018.6 ^c
(BWG) ¹	(±114.7)	(±8.67)	(±10.6)	(±7.46)	(±11.1)	(±11.5)	(±12.3)
Specific growth rate	2.1 ^a	2.5 ^b	2.6 ^c	2.5 ^b	2.8 ^d	2.8 ^d	2.6 ^c
(SGR) ²	(±0.01)	(±0.00)	(±0.01)	(±0.00)	(±0.00)	(±0.00)	(±0.00)
Feed efficiency ratio	1.3 ^a	1.8 ^b	2.2 ^c	1.8 ^b	2.8 ^e	2.8 ^e	2.3 ^d
(FER) ³	(±0.01)	(±0.00)	(±0.01)	(±0.00)	(±0.00)	(±0.00)	(±0.02)
Protein efficiency ratio	0.07 ^a	0.10 ^c	0.11 ^d	0.07 ^a	0.15 ^f	0.13 ^e	0.09 ^b
(PER) ⁴	(±0.01)	(±0.00)	(±0.00)	(±0.00)	(±0.00)	(±0.00)	(±0.00)
Feed conversion ratio	45.3 ^b	55.9 ^c	45.4 ^b	54.5 ^c	36.4 ^a	36.2 ^a	47.2 ^b
(FCR) ⁵	(±0.2)	(±0.24)	(±0.22)	(±0.23)	(±0.20)	(±0.23)	(±0.54)
Condition factor (CF) ⁶	0.92 ^c	0.85 ^a	0.93 ^c	0.87 ^b	0.87 ^b	0.95 ^d	0.96 ^c
	(±0.02)	(±0.00)	(±0.00)	(±0.00)	(±0.00)	(±0.00)	(±0.00)

1-Body weight gain (%)

= (Final weight – initial weight) × 100 / initial weight

2-Specific growth rate (%day⁻¹)

= (ln final weight – ln initial weight) × 100 / days

3. Feed efficiency ratio

= (Final weight – initial weight) / Total dry feed intake × 100

4-Protein efficiency ratio (g)

= Final weight – Initial weight / protein intake

5. Feed conversion ratio

= Total feed intake / weight gain

6. Condition factor

= Final weight / Final length³ × 100

ALLOMETRY OF MANGROVE HORSESHOE CRAB, *Carcinoscorpius rotundicauda* FROM THE EAST COAST OF PENINSULAR MALAYSIA

Syuhaida, N.I*, Rozihan, M., Akbar John, B., Akmal, M.S, Joni, H.D and Esa, Y.B

Department of Aquaculture, Faculty of Agriculture
University Putra Malaysia
43400 Serdang Selangor, Malaysia
syuhaidaisa@yahoo.com

Carcinoscorpius rotundicauda is the species usually inhabit in mangrove ecosystem. The values information on morphometric variability of *C. rotundicauda* is still limited especially along eastern coast in Peninsular Malaysia. In this study, the allometric relationship between male and female crabs were compared through analysing morphological parameter of *C. rotundicauda* collected in two different location along east cost of Peninsular Malaysia [Setiu, Terengganu (Lat 05.40386 N, Long 102.43.032'E) and Pantai Balok, Pahang (Lat 3° 56, 25 7' N, Long 103° 22.568' E)]. Total length for male *C. rotundicauda* was 16.6-37.0cm and female was 26-39cm in Setiu, Terengganu while in Pantai Balok, Pahang, the averaged for total length in male was 22.5-31.5cm and female was 26.0-36.0cm. This study also showed values of all parameters in male and female populations of Setiu, Terengganu were recorded slightly similar as horseshoe crab collected from Pantai Balok, Pahang. The values for all morphometric parameter include total length, carapace length, prosoma width, interocular length and body weight were converted into logarithmic value as allometric growth were analysis. Both population showed positive allometry growth ($b > 1$) between body weight on total length-body and prosoma width relationships in male and female of *C. rotundicauda*. Positive allometry growth indicated that *C. rotundicauda* have increased in all size parameter. Therefore, all these relationship proved that wetland habitat in both populations is the most suitable for the growth and development of *C. rotundicauda*.

FUNCTIONAL FEED ADDITIVES TO PREVENT FISH PARASITES

M.M. Isern-Subich, Sam Ceulemans and Peter Coutteau

Nutriad International NV
Dendermonde
Belgium
*mm.isern@nutriad.com

Aquaculture is expected to continue to expand and fulfill the increasing global needs for fish and shellfish. But as the industry grows and intensifies its production systems, biological plagues are becoming more critical factors affecting profitability of aquaculture producers. All commercial aquaculture species such as salmon, shrimp, marine fish, tilapia and pangasius suffer from a variety of parasites which often cause important economic losses. Gill and skin flukes are causing increasing problems in fish farms, such as *Sparycotyle crisolphrii* in Mediterranean seabream *Sparus aurata*, or Gyrodactilids in tilapia worldwide. A wide diversity of protozoan ectoparasites infests freshwater fish, including white spot, *Trichodina spp.*,... Endo-parasites such as digenean worms, acanthocephalan, to coccidians, mixosporidians, and microsporidians (*Eimeria spp.*, *Enteromyxium spp.*) attack the digestive system and internal organs. The traditional approach to combat fish parasites, based on the use of chemicals and some therapeutics once the parasite outbreak is detected, is increasingly hampered by the development of resistance and the increasing restrictions on the use of chemicals. Health promoting feed additives are a crucial component of effective disease prevention strategies and have already become a standard ingredient in premium brands of salmon feeds designed to reduce the impact of sea lice. A wide range of additives with different mode of actions are currently offered including yeast extracts, phytobiotics, probiotics, prebiotics, organic acids and their derivatives. Functional feeds containing gut health promoters deliver with every meal an adequate concentration of natural compounds which can work through multiple mechanisms to reduce the success of the parasitic infestation. Natural compounds with anti-parasitic activity can work directly on gut parasites or reach the blood and/or mucus to affect ecto-parasites, whereas immune modulators can change the composition and thickness of the mucus.

This presentation will review the latest advances in the research and development of health promoting feed additives, both in laboratory and field conditions, showing their efficacy to boost fish health and reduce the economic impact of parasites on fish production. A number of case studies will be presented, including the prevention of gill flukes and endoparasites in Gilthead seabream *Sparus aurata* and European seabass *Dicentrarchus labrax*.

CAPTIVE BREEDING AND REARING OF THE BIG-BELLY SEAHORSE (*Hippocampus abdominalis*)

Mohamad Saupi Ismail* and Muhammad Fadzil Harun

Fisheries Research Institute, Batu Maung, 11960 Pulau Pinang, Malaysia
saupi@rocketmail.com

The big-belly seahorse *Hippocampus abdominalis*, is one of the largest seahorse species in the world and normally found in southeast Australia and New Zealand. This study shows result on *H. abdominalis* growth under captive conditions over a period of 100 days. Four healthy captive bred F1 *H. abdominalis* (2 males and 2 females) acquired from KL Aquaria, KLCC. The seahorses were conditioned, paired and bred at the Tunku Abdul Rahman Aquarium, Pulau Pinang. A few batches of juvenile seahorses were produced from a single pair of these species. Using mean water temperature of 19°C, salinity of 33 ppt and enriched adult *Artemia* along with a diet complement of live mysid shrimps, it was possible to obtain close to 70% young survival 60 days after hatching. At this age, juveniles had reached an average height of 9 cm and dry weight of nearly 2 g. They, then, reached the mature size of 13 cm in less than 100 days. To our knowledge, culture of the big-belly seahorse in Malaysia had not yet been reported. Thus, the result obtained from this study could help aquarists to culture *H. abdominalis* and be useful in developing seahorse aquaculture in Malaysia.



Big-belly seahorse broodstock

GROWTH PERFORMANCE OF JELAWAT (*Leptobarbus hoevenii*) FED ON VARIOUS LEVELS OF PROTEIN

Farahiyah Ilyana Jamaludin*, Zainal Abidin Abdul Rahman, Ahmad Aman and Wong H.K.

Animal Science Research Centre
Malaysian Agricultural Research & Development Institute (MARDI)
Serdang, Selangor, Malaysia
filyana@mardi.gov.my

Jelawat (*Leptobarbus hoevenii*) is one of the native freshwater fish species in Malaysia. It is found mainly in big rivers of the northern part of Peninsular Malaysia. Jelawat is one of the popular fish served for exclusive and expensive cuisine in some big restaurants in Malaysia. There is however, very limited research done on this particular species. Most studies conducted were from the 70's to 90's with a few reports on nutrition. Therefore specific requirement of nutrients for the growth of this species is mainly unknown. Protein requirement in fish is influenced by the habitat, feeding behaviour and the species of fish. The river fish is known to require high protein in their diets due to their physiology and habitat origin where protein is utilized for muscle build up. The present study was conducted to observe the growth performance and FCR of Jelawat fed on various levels of protein.

A 16 week feeding trial was conducted to investigate the effect of 4 different levels of protein on growth performance of Jelawat. A total of 600 fishes (initial weight of 17 ± 1 g; 3 inch in length) were randomly assigned to 4 treatments with 3 replications per treatment. The fish were fed with 4 different diets containing different levels of crude protein (CP): Treatment A (25% CP), Treatment B (32% CP), Treatment C (38% CP) and Treatment D (44% CP). Fish were fed at 5% of total body weight, twice daily at 8 am and 4 pm. The fish were reared in a 1000-litre circular polytank, and water exchange was done twice a week, depending on the quality of water. Water quality was measured every fortnight to ensure that water parameter is in the range of cultured freshwater fish requirement. The fish were then weighed every fortnight to measure the feed intake, weight gain and to adjust the portion of feed.

Treatment C (Table 1) showed the best growth performance with the lowest feed conversion ratio (FCR) of 1.58, highest specific growth rate (SGR) of 0.91 and highest weight gain (1473.33 g) but was not significantly different ($P > 0.05$) compared with treatment B and D. Treatment A showed the poorest growth with FCR exceeding 2. This may be due to insufficient dietary protein for the growth of Jelawat. The results also showed that the trend of the growth performances of Jelawat increased as the CP level increased. However, the growth performances and FCR were slightly reduced when the CP levels increased above 38%. In conclusion, a CP level of 38% is sufficient for the growth of Jelawat.

TABLE 1. Growth performance of Jelawat fed 4 different levels of protein.

Treatment	Weight gain (g)	Feed intake (g)	FCR	SGR
A	1053.33 \pm 110.15 ^b	2197.67 \pm 128.41 ^a	2.10 \pm 0.23 ^a	0.71 \pm 0.05 ^b
B	1286.67 \pm 133.17 ^{ab}	2304.33 \pm 98.23 ^a	1.81 \pm 0.25 ^{ab}	0.83 \pm 0.08 ^{ab}
C	1473.33 \pm 23.09 ^a	2334.00 \pm 26.91 ^a	1.58 \pm 0.04 ^b	0.91 \pm 0.04 ^a
D	1440.00 \pm 124.90 ^a	2413.00 \pm 63.17 ^a	1.69 \pm 0.17 ^{ab}	0.88 \pm 0.09 ^{ab}

^{ab}Means with different superscripts within the same column differ significantly ($p < 0.05$).

MOLECULAR, TRANSCRIPTIONAL AND FUNCTIONAL INSIGHTS INTO C1 INHIBITOR, MAIN REGULATOR OF THE COMPLEMENT SYSTEM, FROM *Sebastes schlegelii*

Jehanathan Nilojan^{1,2*}, Jehee Lee^{1,2}

¹Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Republic of Korea

²Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province, Republic of Korea
Email: niloan90@gmail.com

The complement system plays a crucial role in innate and adaptive immunity against microbial infection. Since uncontrolled activation of the complement pathway leads to serious pathological conditions, tight regulation of the activation of this system is vital in assuring homeostasis. This system is activated through classical, alternative and lectin pathways. Complement component 1 inhibitor inhibits the activation of classical and lectin pathways. C1 inhibitor ortholog from *S. schlegelii* was identified from previously constructed databases and designated as SsC1inh. Identified cDNA sequence was 2161 bp long with an ORF of 1797 bp, encoding 598 amino acids with a signal peptide of 20 amino acids. The amino acid sequence possessed two immunoglobulin-like domains and a serpin domain. Multiple sequence alignment revealed that serpin domain of C1inh was highly conserved among analyzed species where the two immunoglobulin-like domains showed divergence. Theoretical molecular weight and isoelectric point of SsC1inh were 64.8 kDa and 5.38, respectively. SsC1inh showed the highest identity (76.3%) and similarity (87.8%) with *Oplegnathus fasciatus*. Transcriptional analysis showed the highest expression level in liver, with 588699-fold compared to heart, in which the lowest expression was recorded, under normal physiological conditions. In RT-PCR analysis, after immune challenge experiments with lipopolysaccharide, *Streptococcus iniae*, and polyinosinic: polycytidylic acid, SsC1inh mRNA expression was significantly downregulated from 6 to 24 hours, post injection for all three stimuli. After 24 hours, the levels came back to normal but in LPS challenge, the level remained downregulated until 72 hours, post injection. To check the protease inhibitory potential of C1inh invitro the complete open reading frame (ORF) was cloned into pMAL-c5X, transformed into *Escherichia coli* BL21, recombinant SsC1inh (rSsC1inh) protein was over expressed by IPTG induction and purified by maltose affinity chromatography. Protease inhibition potential of rSsC1inh was assessed against C1 esterase and thrombin, two serine proteases, using colorimetric assays. In protease inhibition assays, approximately 30% and 57.6% of human C1 esterase and thrombin activity was inhibited by 50 μ g rSsC1inh. The results in this study prove the potential of SsC1inh to act as a regulator of the complement system to maintain homeostasis.

EFFECT OF AQUAVI® MET-MET SUPPLEMENTATION ON THE SUCCESS OF REDUCING FISHMEAL LEVELS IN DIETS FOR SHRIMP REARED IN GREEN WATER CONDITIONS

Orapint Jintasatoporn^{1*}, Cláudia Figueiredo-Silva², Dhanapong Sangsue³

¹Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

²Evonik Industries, Nutrition and Care, Animal Nutrition, Hanau, Germany

³Evonik (SEA) Nutrition and Care, Animal Nutrition, Singapore

*Email: ffigora@ku.ac.th

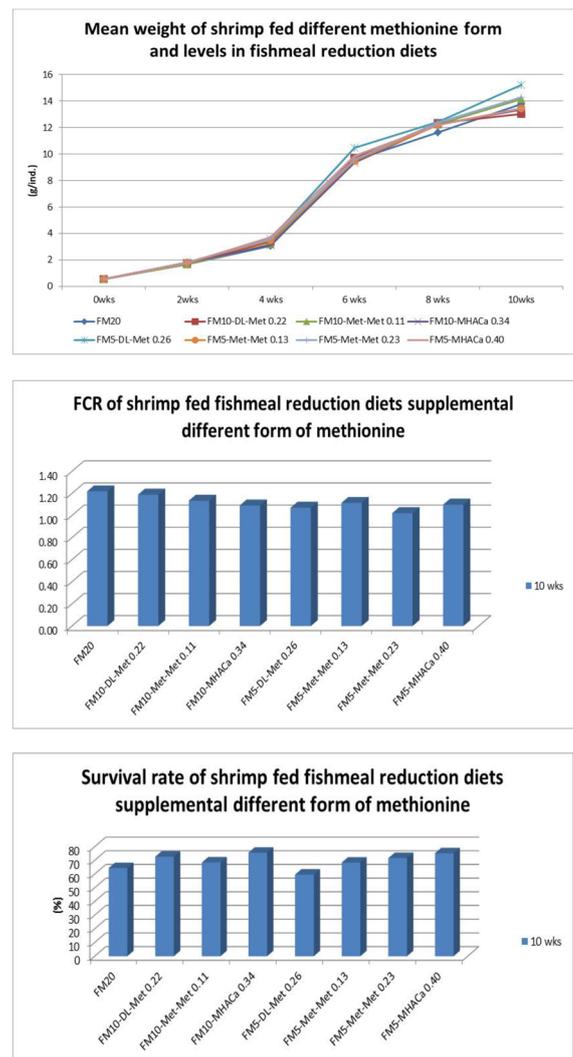
The objective of this study is to evaluate efficacy of 200% for AQUAVI®Met-Met, 100% for DL-Methionine and 65% for MHA-Ca in meeting the methionine requirement of white leg shrimp (*Litopenaeus vannamei*) fed with low fishmeal diets in green water conditions.

The study was assigned in a completely randomized block design, with 8 treatments and 6 replications in 2 ponds with each of 3 replications. The treatments were fishmeal 20% (T1 FM20), the other 3 treatments of 10% fishmeal with different forms and levels of methionine source: DL-Methionine 0.22% (T2 FM10-DL-Met 0.22), AQUAVI®Met-Met 0.11% (T3 FM10-Met-Met 0.11), MHA-Ca 0.34% (T4 FM10-MCa 0.34) and other 4 treatments of 5% fishmeal: DL-Methionine 0.26% (T5 FM5-DL-Met 0.26), AQUAVI®Met-Met 0.13% (T6 FM5-Met-Met 0.13), AQUAVI®Met-Met 0.23% (T7 FM5-Met-Met 0.23) and MHA-Ca 0.40% (T8 FM5-MCa 0.40). Shrimp were reared in 2*2.5*0.9 m net cage at stocking density of 100 pieces/m² in brackish water of 10 ppt. Shrimps of 0.57±0.02g were allocated to treatment cage in each pond. The growth parameters measured were production (g/cage) body weight (BW, g/ind.), body weight gain (BWG, g/ind.), average daily gain (ADG, g/ind./d), specific growth rate (SGR, %/d), feed intake (FI, g/ind.), daily feed intake (DFI, g/ind./d), feed conversion ratio (FCR), survival rate (SR, %), after 10 weeks of study.

The results after 10 week study showed that supplementation different levels of methionine in forms of DL-Methionine, AQUAVI®Met-Met, and MHA-Ca (T2-T8) showed the same ($p>0.05$) growth performance, feed utilization and survival rate as shrimp fed fishmeal 20% (T1).

The proximate composition of shrimp (whole body) showed significantly different ($p<0.05$) between forms and levels of methionine on moisture, protein and energy. The lipid content in shrimp body showed no significantly different ($p>0.05$). Over all of nutrient deposition in group of shrimp fed high levels of DL-Methionine, AQUAVI®Met-Met, and MHA-Ca in 5% fishmeal diet exhibit the high protein, lipid and energy accumulation.

Figure 1. Growth performance of shrimp



Therefore, supplemental DL-Methionine, AQUAVI® Met-Met, and MHA-Ca in fishmeal reduction diets (5-10%) for shrimp reared in green water conditions can improve shrimp production, feed utilization and survival rate closed to diet of 20% fishmeal.

EFFECTS OF YEAST CELL WALL ON RESISTANCE AGAINST *Vibrio parahemolyticus* IN WHITE SHRIMP (*Litopenaeus vannamei*)

Orapint Jintasataporn, Isabela Barros*, Melina A. Bonato, Glycon D. Santos

ICC Brazil

Av. Brigadeiro Faria Lima, 1768 4C,
São Paulo/SP, CEP 01451-909, Brazil
isabela@iccbrazil.com.br

The objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* cell wall and nucleotide source on survival rate and resistance against *Vibrio parahemolyticus* (Early Mortality Syndrome agent) in juvenile white shrimp (*Litopenaeus vannamei*).

The shrimps were distributed in a completely randomized design, with 5 treatments: 1- Control; 2- 0.5% of *Saccharomyces cerevisiae* as a source of nucleotides (YNU - Hilyses®, ICC Brazil); 3 - 1% of YNU; 4 - 0.5% of *Saccharomyces cerevisiae* cell wall (YCW - ImmunoWall®, ICC Brazil); and 1.0% of YCW, with 8 replicates each. These included 4 replicates for growth performance (28 days) and 4 replicates for immunity (bacteria challenge). Each replicate consisted of 100L fiber tank with stocking density of 360 pieces/m³ at 20 ppt salinity. Shrimps PL 8 were purchase from a commercial hatchery and were acclimated for 5 days, and then allocated to treatment tanks. The growth parameters measured were total feed intake (TFI, g/tank), feed intake (FI, g/ind.), daily feed intake (DFI, g/ind./d), body weight (BW, g/ind.), body weight gain (BWG, g/ind.), average daily gain (ADG, g/ind./d), length (cm), specific growth rate (SGR, %/d), feed conversion ratio (FCR), survival rate (SR, %) at 14 and 28 days. Also the shrimp size was evaluated (large, medium and small) at 28 days. Shrimp was challenged by *Vibrio parahaemolyticus* immersion or orally at of 1.0-2.9x10¹² CFU/mL under normal and salinity stress condition (35 ppt). The immunological parameters were evaluated weekly and daily for mortality rates. The data were analyzed using the GLM (SAS), and means were compared by Duncan's test (P=0.05).

Table 1. Growth performance of shrimp

Param.	Control	YNU 0.5%	YNU 1%	YCW 0.5%	YCW 1%	P-value
Weight (g/ind.)						
Initial	0.009	0.009	0.009	0.009	0.009
14 d	0.028	0.024	0.028	0.030	0.030	0.495
28 d	0.088	0.079	0.086	0.093	0.082	0.737
Specific growth rate (%/d)						
14 d	8.2	7.1	7.9	8.5	8.7	0.567
28 d	8.2	7.8	8.1	8.3	8.0	0.761
Feed intake (g/ind.)						
14 d	0.042 ^a	0.039 ^b	0.041 ^a	0.040 ^{ab}	0.039 ^b	0.008
28 d	0.086 ^a	0.082 ^b	0.086 ^a	0.083 ^{ab}	0.082 ^b	0.037
Feed conversion ratio						
14 d	2.5	2.8	3.4	2.3	2.0	0.633
28 d	1.5	1.6	1.6	1.4	1.4	0.841
Survival rate (%)						
28 d	73.0	81.5	80.6	78.1	85.0	0.465
Shrimp Size (%) – at 28 d						
Large	6.33 ^c	8.84 ^{bc}	10.72 ^{ab}	12.25 ^a	10.68 ^{ab}	0.006
Med.	38.07 ^c	46.84 ^b	51.79 ^{ab}	56.23 ^a	53.46 ^a	<0.001
Small	55.60 ^a	44.33 ^b	37.49 ^c	31.52 ^c	35.86 ^c	<0.001

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

Table 2. Survival rates (SR, %), *Vibrio ssp.* count (cfu/g and Log cfu/g) results for different challenges with *Vibrio parahaemolyticus*

Param.	Control	YNU 0.5%	YNU 1%	YCW 0.5%	YCW 1%	P-value
Vibrio challenge - oral administration						
SR (%)	96.3 ^a	98.8 ^a	98.8 ^a	98.8 ^a	97.5 ^a	0.736
V. (cfu/g)	3.4x10 ^{5a}	2.3x10 ^{5b}	1.9x10 ^{4d}	1.0x10 ^{5c}	5.3x10 ^{4cd}	<0.001
V. (Log)	5.53 ^a	5.37 ^b	4.28 ^c	5.00 ^c	4.73 ^d	<0.001
Vibrio challenge - immersion						
SR (%)	80.0 ^b	87.5 ^a	93.8 ^a	92.5 ^a	93.8 ^a	0.004
V. (cfu/g)	2.6x10 ^{4a}	1.2x10 ^{4b}	1.0x10 ^{3c}	3.2x10 ^{3c}	1.3x10 ^{3c}	<0.001
V. (Log)	4.41 ^a	4.09 ^b	3.01 ^d	3.50 ^c	3.12 ^d	<0.001
Vibrio challenge - immersion + 35 ppt salinity						
SR (%)	4.44 ^b	53.33 ^a	46.67 ^a	44.44 ^a	51.11 ^a	<0.001
V. (cfu/g)	9.6x10 ^{6a}	4.2x10 ^{6b}	8.6x10 ^{5cd}	2.5x10 ^{6bc}	3.2x10 ^{5d}	<0.001
V. (Log)	6.98 ^a	6.63 ^b	5.93 ^c	6.38 ^b	5.43 ^d	<0.001

*Different letters in the same row differences (P<0.05).

In general, the inclusions of 0.5 to 1% of YNU and YCW were able to improve the shrimp *Litopenaeus vannamei* disease resistance against virulent bacteria, *Vibrio parahemolyticus*, mainly under high salinity conditions.

(Continued on next page)

There is no significant difference ($P < 0.05$) among treatments for growth and performance results, except for FI parameters, where the supplementation of YNU and YCW decrease ($P < 0.05$) the FI compared to control group; and for shrimp size, where all feed additives increased compared to control (Table 1). The additives supplementation also increased (numerically) the survival rates compared to control (Table 1). For *V. parahemolyticus* challenge via oral, both YNU and YCW decrease the hepatopancreas *Vibrio spp.* count ($P < 0.05$). However, for immersion challenge and/or with salinity stress, YNU and YCW improved survival rate and decrease the *Vibrio spp.* count ($P < 0.05$) (Table 2).

In general, the inclusions of 0.5 to 1% of YNU and YCW were able to improve the shrimp *Litopenaeus vannamei* disease resistance against virulent bacteria, *Vibrio parahemolyticus*, mainly under high salinity conditions.

THE EFFECTS OF CHINA ROSE POWDER CONCENTRATIONS ON THE INCREASING NUMBER OF CHROMATOPHORES IN ORNAMENTAL FISH BLUE GOURAMI (*Trichogaster trichopterus*)

Maryam Jorjani*, Amin Mirhashemi Rostami, Mostafa Sharifrohani, Tayebah Enayat Golampour, Koroush Amini, Aileen Tan Shau Hwai

Inland waters Aquatics Stocks Research
Gorgan, Golestan Province, Iran
maryam_jorjani@yahoo.com

Currently, ornamental fish are gaining more importance not just because of their aesthetic value but also its commercial value in export trade around the globe (Joseph et al., 2011). Ornamental fish are characterized by their wide diversity of colours and patterns. Natural carotenoids from plants are considered to be a good and economical alternative in inducing growth and pigmentation in fish. Blue gourami (*Trichogaster trichopterus*) is an ornamental fish of great importance that contributed greatly in the international trade because of its colour and behavior. This research was to evaluate the effect of China Rose (*Hibiscus rosasinensis*), a natural carotenoid, on the distribution and number of chromatophores in the ornamental fish, blue gourami. China Rose powder was added to the artificial diet (Biomar Company) at 0.0.5.1.5 and 2.5%. A total of 120 fish were stocked in 12 aquaria (60×30×30cm) containing 10 fish for 70 days. Each treatment had three replicates. Fishes were feed twice a day. Water was changed daily. At the end of experiment, scales were removed and tested under digital microscope. Result indicated that two out of five chromatophores (punctate-stellate and stellate) were observed in blue gourami fish based on the results of scales chromatophores analysis). Results showed that the addition of China Rose could increase significantly the number of punctuate-stellate chromatophores in the scale of blue gourami ($P<0.05$). A total of 2.5 % of China Rose powder was the highest enhancement at the compare of control group. There were no significant differences between 1.5 and 2.5%. There were significant differences between 1.5% compared to control group. The results also indicated that there were significant differences between 0.5% compared to control for stellate chromatophores ($P<0.05$). These results show that China Rose can affect increasing number of chromatophores in ornamental fish blue gourami (*Trichogaster trichopterus*).

EFFECTS OF PRO YG TABLETS ON GROWTH PERFORMANCE, SURVIVAL RATE AND VIBRIO CONTROL OF LITOPENAEUS VANNAMEI CULTURE PONDS

K

In aquaculture practices, probiotics are considered a valid alternative to antibiotics and in particular to maintain optimum water quality and to control pathogen loads. The present work emphasizes the efficacy of the Pro YG Tablets (product from Royal Bio-Marine, Pernambut, India, Certified by Coastal Aquaculture Authority of India (CAA Reg. No. CAA/F16/FA/00817) role in water quality management, soil quality and survival rates in *Penaeus vanammei* culture. The study was carried out for 122 days to know the growth and survival rate of *P. vanammei* by applying Pro YG Tablets in Ganapavarm, West Godavari district. Andhra Pradesh, India. Four experimental ponds (0.8 hec pond) were selected of which two were PRO YG Tablets treated ponds and remaining two is control. Crab fencing and bird netting was done before pumping water to prevent the autoentrants. After pond preparation, PL10 (average weight of each 0.98 ± 0.04 mg (mean \pm SD) was stocked at the rate of 51 per m² following polymerase chain reaction (PCR) tested by Using IQ 2000 PCR kit from Aqua Lab. The commercially available branded feed was used during the entire study period. Transparency, salinity, pH, dissolved oxygen (DO), temperature, total Ammonia Nitrogen (TAN) were recorded by standard measurements. The average final body weight of the harvested shrimp is 22.5, 23 g in probiotics ponds and 18.2, 18 g in controlled ponds and the difference was significant ($P < 0.01$) between these two productions. The average survival rate was 91.5 % in probiotic pond and 76 % in controlled pond. The average Vibrio bacterial (yellow and green colonies) counts were found to be significantly ($P < 0.01$) lower in Pro YG Tablet treated (Pond 1, Pond 2) ponds compared to control ponds (Pond 3, Pond4). Especially dominant green colonies were lowered in both the experimental ponds and increased Vibrio levels are noticed in Pond 3 and Pond 4 in periodical weekly sampling in water and animal samples.

The result showed that Pro YG Tablets plays an important role in maintaining water quality parameters, soil sludge digestion, health management, reduced Vibrio bacterial count and improved total yield of shrimp survival rates.

POND PERFORMANCE DETAILS (Control and Experimental)

POND DETAILS	EXPERIMENT		CONTROL	
	POND 1	POND 2	POND 3	POND 4
Pond No.				
Pond area (Ha)	0.8	0.8	0.8	0.8
Initial stocking	410,000	410,000	410,000	410,000
Density (m ²)	50	50	51	50
Total culture days	120	121	120	119
Harvested size	22.5	23	18.2	18
Total quantity harvested	8300	8240	6400	6150
Survival rate	91	92	78	74
Total feed used (Kg)	9900	10050	9800	9000
FCR	1.19	1.21	1.5	1.46
Production (Kg/Ha)	10375	10300	8000	7687.5

INTERACTIVE EFFECTS OF DIETARY PROTEIN LEVELS AND PROTEIN QUALITY ON GROWTH PERFORMANCE OF MALAYSIAN PRAWN, *Macrobrachium rosenbergii*

Md. Abdul Kader*, Mahbuba Bulbul, Fauzani Mahmud

School of Fisheries and Aquaculture Sciences
Universiti Malaysia Terengganu, 21030 Kuala Terengganu
Malaysia
*abdulkader_fc@yahoo.com

A feeding trial was designed to determine the effects of dietary protein levels and dietary fishmeal (FM) replacement levels; and the interaction between them on growth performance and feed utilization of freshwater prawn. This experiment was conducted in 2 factorial design. *Macrobrachium rosenbergii* post larvae culture in tank 100L (fill with 80L) for 50 days. The experimental treatments were assigned in triplicates. Six test diets were formulated to contain two different protein levels (P35% and P40%) and three levels of FM replacement (FM0%, FM35%, FM70%). The dietary FM was replaced with a blend of palm kernel meal (PKM) and fish bone meal (FBM). The diets were designated as Diet 1 (P35 FM0), Diet 2 (P35 FM35), Diet 3 (P35 FM70), Diet 4 (P40 FM0), Diet 5 (P40 FM35) and Diet 6 (P40 FM70), respectively. Result of the present study indicated that both dietary protein levels and FM replacement levels had significant ($P < 0.05$) effects on growth performance and feed conversion ratio, while no interaction was found between the two factors on the analyzed parameters. Increasing dietary protein from 35% to 40% increased the growth performance of prawn. On the contrary, replacement of FM significantly decreased the growth performance of prawn. The freshwater prawn fed Diet 4 (P40 FM0) showed significantly highest growth performance, while the lowest was found in prawn fed Diet 3 (P35 FM70). On the other hand, survival rate was not significantly varied among the treatments. The highest level of PKM & FBM (70%) inclusion in diets gave significantly lower growth performance and nutrient utilization of *M. rosenbergii* in both dietary protein levels. However, differences were not found in the performance of prawn fed Diet 1 (P35 FM0) and Diet 6 (P40 FM70). Similar results were evident for feed intake and protein efficiency ratio. Therefore, the present study concluded that at 35% dietary protein level, a mixture of fish bone meal and palm kernel meal could replace up to 35% FM and increasing dietary protein to 40% could replace up to 70% fishmeal with the blend of PKM and FBM. Therefore, PKM and FBM are proven potential alternative protein sources which could replace a significant portion of fishmeal protein in aquafeeds. Fishmeal replacement levels could be further improved by increasing 5% dietary crude protein.

POTENTIAL OF MICROALGAE AS AN ALTERNATIVE FOR PREVENTION OF VIBRIOSIS

Ince Ayu K.Kadriah*, Sahabuddin and Nurbaya

Research Institute for Coastal Aquaculture
Jl. Makmur Dg. Sitakka No. 129.Maros 90511, South Sulawesi
E-mail: inceayu@gmail.com

Aquaculture is a rapidly growing food sector in the world. However, infectious disease caused by pathogens either viruses or bacteria dramatically reduces the productivity of aquaculture and has obstructed economic development in many countries. Pathogenicity of microorganisms that infect in aquaculture system is closely related to the release of factors regulated by *quorum sensing* (QS). *Vibrio* bacteria use an *Acyl Homoserine Lactone* (AHL) compound for *quorum sensing* signal. QS inhibition is a potential therapeutic target, where the inhibition of bacterial cell communication could be a promising strategy to suppress bacterial virulence.

This study was aimed to determine the potential of coastal and marine microalgae as an alternative material for the prevention of vibriosis through the method of inhibition of quorum sensing signal. This research was conducted in the Laboratory of Fish Health and Environment, Research Institute for Coastal Aquaculture (BPPBAP, Balai Penelitian dan Pengembangan Budidaya Air Payau). In this study, 10 types of microalgae obtained from P₂O LIPI, BBAP Situbondo and BBPPBL Gondol were used. Furthermore, we succeeded in performing isolation of 3 types of microalgae from Losari Coastal Area, Lae-Lae Island and Coastal Area of Barru Regency. Screening of microalgae producing metabolites of Anti Quorum Sensing (AQS) was conducted in several stages, started from the isolation of supernatant through centrifugation method which was followed by metabolites extraction using Ethyl Acetate solvent. Later, evaporation was performed to evaporate the solvent. The extract was dissolved again with sterile aquades. Once the microalgae extract was obtained, bioassay test with disc diffusion method using biosensor of *Chromobacterium violaceum* bacteria as a marker of anti QS on LB media was performed. QS inhibition was detected by the presence of a clear ring around disc paper by microalgae which produce AQS degrading AHL.

Based on the result of bioassay test, five types of microalgae which were positive to have the ability to inhibit Quorum sensing signal were found, namely: *Melosira*, *Porpyridium*, *Vulgaris*, *Phaeodactylum* and *Nannochloropsis*. **Keywords:** *Quorum Sensing*, *Microalgae*, *Vibriosis*, *Acyl Homoserine Lactone*

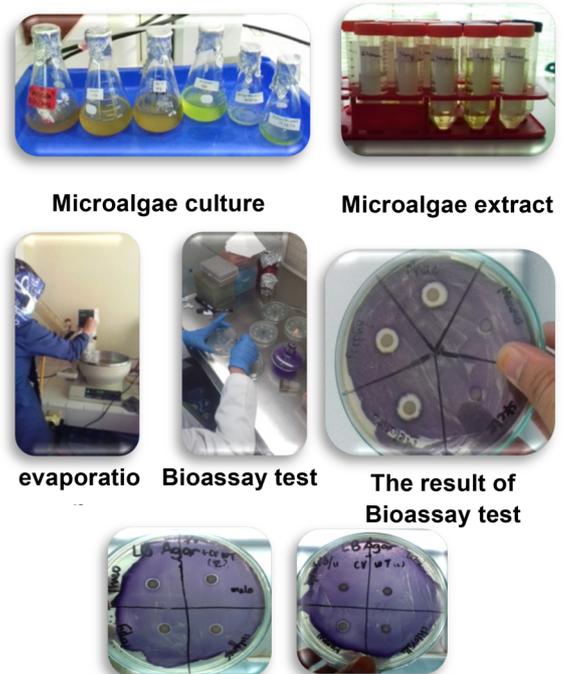


Table 1. The Result of Bioassay test

MICROALGAE	AQS	CLEAR ZONE
<i>Melosira</i>	++	1.1 cm
<i>Porpyridium</i>	++	1.2 cm
<i>Isochrisis</i>	-	-
<i>Vulgaris</i>	+	0.6 cm
<i>Phaeodactylum</i>	++	1.1 cm
<i>Chlorella</i>	-	-
<i>Nannochloropsis</i>	+++	1.5 cm
<i>Navicula</i>	-	-
<i>Tetraselmis</i>	-	-
<i>Spirulina</i>	-	-

COMBINATION OF PRE-GELATIZED AND ISO-MALTOSE ON THE PELLET CHARACTERISTICS AND SUBSEQUENT EFFECTS ON THE FEEDING EFFICIENCIES AND NUTRITIVE VALUE OF AFRICAN CATFISH, *Clarias gariepinus*

Naga Kanmani, Nicholas Romano*, S.M. Nurul Amin, Mahdi Ebrahimi, Mohd Salleh Kamarudin

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

* Email address: romano.nicholas5@gmail.com

The two main functions of starch in aquafeeds are to act as a binder as well as an inexpensive energy source to fish. Both these characteristics can potentially be improved through pre-gelatinization, which usually involves the application of heat and water to degrade the less digestible regions of starch. While this can improve both the pellet characteristics as well as fish growth, a potential downside can include less intestinal bacteria. This may be mitigated by the inclusion of a prebiotic, which is an indigestible carbohydrate that can act as a substrate for beneficial bacteria in the intestine. In this study, we compared different diets that consisted of native tapioca starch (TS), TS with isomaltose (TS+Iso), pre-gelatinized TS (PGTS) and PGTS with isomaltose (PGTS+Iso) on the growth, feeding efficiencies, whole-body proximate composition, and liver glycogen content to African catfish (*Clarias gariepinus*) after 7 weeks. Pellet characteristics were also measured that included bulk density (BD), pellet durability index, water solubility index (WSI), water absorption index (WAI), and water stability (WS).

All diets were isonitrogenous and isolipidic and in the isomaltose diets, this was added at 0.5%. Triplicate groups of 15 fish (initial mean weight = 7.51 g) were fed their respective diets to apparent satiation.

Results showed that the WSI and WAI were significantly higher by in the PG diets than the others, while the pellet durability index was slightly better. There are no significance differences between treatments in growth of catfish ($p > 0.05$), but feed intake was significantly less in the PGTS and PGTS+Iso treatments. Crude protein and lipids were significantly lower and higher in the PGTS treatments, but the crude protein content was significantly improved when Iso was present with PGTS (i.e. the PGTS+Iso diet).

Overall, the PGTS+Iso diet could be recommended since this significantly reduced feed intake, but without compromising growth. This result, along with better pellet characteristics, could substantially reduce feeding costs during the production of *C. gariepinus*.

Growth performance, feeding efficiencies and proximate analysis of catfish fed after 7 weeks

	Experimental diets			
	TS	TS+Iso	PGTS	PGTS+Iso
SGRW	3.77±0.2 ^a	3.71±0.17 ^a	3.27±0.22 ^a	3.61±0.26 ^a
FCR	0.93±0.2 ^a	0.85±0.06 ^a	1.07±0.08 ^a	0.86±0.05 ^a
Feed intake	1.07±0.15 ^a	1.18±0.08 ^a	1.0±0.09 ^a	1.17±0.07 ^a
Crude protein (%)	57.74±0.92 ^a	59.54±1.2 ^a	49.75±0.90 ^c	52.35±0.22 ^b
Crude lipid (%)	28.00±2.30 ^a	28.96±2.05 ^a	34.77±0.69 ^b	36.2±0.78 ^b

GROWTH OF *Kappaphycus alvarezii* AND *Euचेuma denticulatum* CULTIVATED IN FLOATING CAGES

Kasim M.¹⁾, Muzuni²⁾, Mustafa A.³⁾, Indrayani.⁴⁾, Jalil W.⁵⁾

¹Faculty of Fishery and Marine Sciences, Halu Oleo University, Indonesia
email : marufkasim@hotmail.com

²Mathematic and Natural Science Faculty, Halu Oleo University, Indonesia
email : muzuni71@yahoo.co.id

³Faculty of Fishery and marine Sciences, Halu Oleo University, Indonesia
email : astafa_611@yahoo.com

⁴Faculty of Fishery and marine Sciences, Halu Oleo University, Indonesia
email : indrayani_tajudin@yahoo.com.au

⁶Faculty of Fisheries, Dayanu Iksanuddin University
email : wardha.jalil@yahoo.co.id

Seaweed (Euचेumatoid) cultivation continues to expand with a variety of methods to increase production. *Kappaphycus alvarezii* and *Euचेuma denticulatum* are two species were belonging to genus Euचेuma. Both species are very popular simultaneously cultivated in all cultivation regions in Indonesia. The purpose of this study to observe at both species of growth by using new cultivation methods in Indonesia and named floating cage. This research was conducted during March-November 2015 in Southeast Sulawesi Province, Indonesia. The results showed that the growth rates of *E. denticulatum* in floating cage were faster and had better thallus morphology than *K. alvarezii*. The total weight of *E. denticulatum* and *K. alvarezii* increased from 5 kg in day 0 to about 31 and 25.6 kg, respectively in day 50. Thallus growth of *E. denticulatum* and *K. alvarezii* was different in September and October. The growth of *E. denticulatum* and *K. alvarezii* increased from 50 g in day 0 to about 253 g and 168.5 g, respectively in day 45. The average Specific Growth Rate (SGR) of *E. denticulatum* cultivated in Floating cage reached 3,6 % day⁻¹ whereas *K. alvarezii* achieved its highest SGR in 3,1% day⁻¹ in July and August.

FISH BRAND CREATIONS: PROGRESSIVE AQUACULTURAL APPROACHES THAT CONTRIBUTE TO REGIONAL DEVELOPMENT

Yukichika Kawata*

Faculty of Economics
Kindai University
4-1 Kowakae 3-chome, Higashiosaka, Osaka 577-8502, Japan
ykawata@kindai.ac.jp

Worldwide, the importance of aquaculture has increased, especially recently. In 2013, the total global fish consumption exceeded the total global beef consumption. The state of the global marine fish stocks has deteriorated; 30% of marine fish stock is overfished. However, the share of cultivated fish has been increasing, satisfying growing demands. In 2014, the global fishery capture and aquaculture production were 93.4 and 73.8 million tons, respectively. Because quality control is easier in cultivated fish than in wild fish, it is more feasible to establish fish brands with cultivated fish.

The state of the Japanese marine fish stocks is similar to the global situation. According to the FY2015 assessment by the Japanese ministry, 50% of the marine fish stocks are low and the importance of aquaculture has increased. However, the long economic depression and low prices on imported fish have driven domestic fish prices down, negatively affecting the Japanese aquaculture industry. Increasing prices through aquaculture fish branding and reducing production costs through proactive ingenuity are two promising solutions for this problem.

A promising initiative to increase fish price is fruit fish cultivation, in which fish is cultivated using food containing fruit flesh, juice, and skin, improving the flavor of the fish and preventing lipid degradation. Aquafarmers may utilize fruit residuals produced by other industries, such as fruit remnants after squeezing juice. In such cases, there may be cost reductions in both fruit and aquaculture industries.

A promising fish branding initiative to reduce production cost is inland puffer cultivation. Ocellate puffer, virtually the only cultivated puffer, is a marine fish; however, it is suitable for inland cultivation. Some aquafarmers use a pre-existing vacant pool, such as that of an abandoned school building, rather than building a new pool, for puffer cultivation, thereby reducing costs. Additionally, by using water with components that differ from those of seawater, aquafarmers can cultivate puffers with special flavor. In this way, aquafarmers can create novel fish brands, which may cause a rise in fish prices.

In this study, we examined influence of the abovementioned initiatives (i.e., fruit fish and inland puffer cultivation) on regional development, particularly cost reduction through using industrial wastes and unused facilities, and price rise through creating cultivated fish brands.

DIFFERENT *Bacillus* spp. ISOLATES VARY IN THEIR BIOREMEDIATION CAPACITY

Jutta C. Kesselring*, Barbara Weber

Biomin Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria
jutta.kesselring@biomin.net

Poor water quality negatively impacts animal health in aquaculture. After prolonged exposure, challenging water quality is detrimental to animal performance and will cause significant economic losses. Probiotics can be applied to remove toxic compounds and to clean up ponds. Although a range of bacterial species are capable of nitrification and / or denitrification, not all species are applicable for bioremediation products.

Nitrification and Denitrification was investigated for several *Bacillus* spp. and *P. pantotrophus* strains under aerobic and anaerobic conditions.

After 24 hours and 48 hours cultivation with active aeration, an increase, steady levels and an efficient reduction of ammonium ions were measured for different strains, reflecting their strain-specific capabilities. In general, the performance of *P. pantotrophus* PaP#768 and of *B. amyloliquefaciens* BA#895 was superior in comparison to the other tested strains.

Under anaerobic conditions, all strains reduced nitrite at 24h and 48h, except for *B. amyloliquefaciens* BA#855. This strain requires 48 hours to decrease the nitrite levels in the culture medium. Under aerobic conditions, most strains reduce nitrite, but often not as efficient as under anaerobic conditions.

Under anaerobic conditions, almost a complete removal of nitrate was detected for *P. pantotrophus* PaP#768, all *B. amyloliquefaciens* strains, all *P. polymyxa* strains, *B. licheniformis* strains BL#746, BL#857, BL#879 and BL#896, and *B. subtilis* strains Bio9, BS#588, and BS#652. Compared to these strains, only a low reduction of nitrate was observed for *B. licheniformis* BL#858 and *B. pumilus* BP#856. Under aerobic conditions, only four strains, namely *P. pantotrophus* PaP#768, *B. licheniformis* BL#857 and BL#858, and less pronounced also *P. polymyxa* PP#866 reduced nitrate.

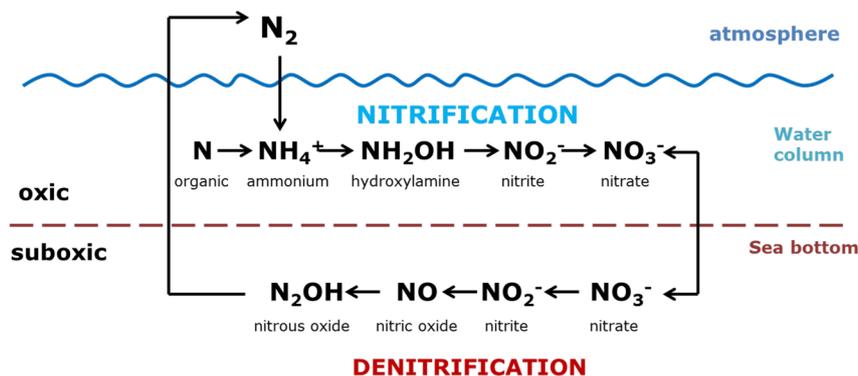


Figure 1: Bacterial nitrification and denitrification in aquatic systems. Bacterial nitrification is the oxidation of ammonium / ammonia (NH_4^+ , NH_3^+) to nitrate (NO_3^-) via hydroxyl amine and nitrite (NO_2^-). Denitrification describes the reduction of nitrate to nitrous oxide and finally to nitrogen gas, which returns into the atmosphere.

FIRST RECORD OF THE GENUS *XIPHOPENAEUS* (SMITH 1869), THE ATLANTIC AND PACIFIC SEABOB SHRIMP *Xiphopenaeus kroyeri* (HELLER 1862), AND *Xiphopenaeus riveti* BOUVIER, 1907 (DECAPODA, PENAEIDAE) SOUTHEASTERN MEDITERRANEAN COAST OF EGYPT

Amal Ragae Khafage*; Somaya Mahfouz Taha

National Institute of Oceanography and Fisheries, Quiet Bey, Alexandria, Egypt
Email address: amal_khafage@live.com

One hundred and one specimens of the seabob shrimp, genus *Xiphopenaeus* (Smith 1869), the Atlantic *Xiphopenaeus kroyeri* (Heller, 1862) and the Pacific *Xiphopenaeus riveti* (Bouvier, 1907), were caught by local fishermen within the period of July-November 2016, from Ma'deya shores, Abu Qir Bay, Alexandria, Egypt. The genus probably has been introduced to the Southeastern Mediterranean as a result for several factors, among which are the increase of maritime traffics and global warming. This is the first record of the closed thelycum penaeid genus *Xiphopenaeus*, the two species *X. kroyeri* and *X. riveti* in Egypt.

Introduction. The Mediterranean Sea has been affected by several human activities, such as the construction of the Suez Canal, the Aswan High Dam, in addition to global warming, so many invertebrates were driven away from the warm waters of the Red Sea into the Mediterranean (Cheung *et al.*, 2013)

The finding of Western Atlantic species is a more of an unexpected event. Herein we report the capture of the Western Atlantic *X. kroyeri* (Heller 1862), and the Pacific *X. riveti* (Bouvier, 1907) penaeid species, a genus, *Xiphopenaeus*, never before recorded in the Mediterranean Sea.

Materials and Methods. During the routine monthly sampling of shrimp in the invertebrate lab, NIOF, Alexandria, Southeastern Mediterranean, Egypt, unfamiliar specimens were clearly observed among the indigenous penaeid shrimps. These species were caught from areas of Ma'deya shores off Alexandria coast. The collection went through taxonomic diagnosis and morphometric study.

Results and Discussion. The edible indigenous species of the shrimp commercial catch in Alexandria are *Melicertus kerathurus* (Forsskål, 1775) and *Parapenaeus longirostris* (Lucas, 1846) *Penaeus semisulcatus* (De Haan, 1844), *Marsupenaeus japonicus* (Bate, 1888), *Metapenaeus monoceros* (Fabricius, 1798), *Metapenaeus stebbingi* (Nobili, 1904), *Trachysalambaria curvirostris* (Stimpson, 1860) (Fischer *et al.*, 1987) and recently *Meliceratus hathor* (Burkenroad, 1959). The collected specimens by this study were identified as genus *Xiphopenaeus*, *Xiphopenaeus kroyeri* (Heller, 1862) and *Xiphopenaeus riveti* (Bouvier, 1907). The genus *Xiphopenaeus* is characterized by long rostrum, with

very elongate, styliform anterior part varyingly elevated and armed with only dorsal teeth (usually five) basally. Carapace with epigastric tooth situated posterior to the first rostral tooth. Longitudinal suture long, reaching about midlength of carapace. Last two pairs of pereopods are long, flageliform, with elongate multiarticular dactyls. Petasma produced distolaterally in pair of relatively broad horns. Thelycum with plate of sternite XIV broad, protuberance of sternite XII quite short, and slit between them almost horizontal. The genus contains only two closely similar species (Burkenroad 1934b); the Atlantic and Pacific seabob *X. kroyeri*, and *X. riveti*. The former has a maximum length, males, 115 mm; females, 140 mm, and has a wide geographical range throughout the western Atlantic Ocean from North Carolina south through the Gulf of Mexico and the Caribbean Sea to Santa Catarina, Brazil. The latter has a maximum length, females, 170 mm, and has a range from Gulf of California, Mexico, to Paita, Peru (Pérez Farfante, 1988).

References:

- Burkenroad 1934b, The Penaeidea of Louisiana with a discussion of their world relationships. Bull. Am. Mus. Nat. Hist. 68: 61-143.
- Fischer, W., Schneider M. & Bauchot, M. L. 1987. Fiches FAO, identification des espèces pour les besoins de la pêche. Méditerranée et Mer Noire zone de pêche 37. Revision I. Vol. I. végétaux et invertébrés.
- Pérez Farfante, I, 1988. Illustrated key to penaeoid shrimps of commerce in the Americas. NOAA Technical Report NMFS 64: 1-32.
- Cheung W, Watson R and Pauly D (2013) Signature of ocean warming in global fisheries catch. Nature 497: 365- 368.



Fig 1. *X. kroyeri* (Heller, 1862) from Ma'deya shores, Alexandria, Egypt.

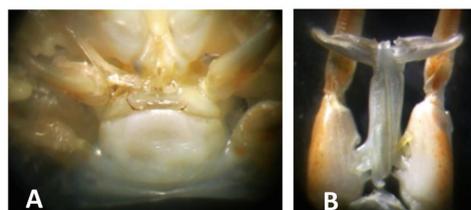


Fig 2. *X. riveti*, A. Female thelycum; B. Male petasma

EVALUATION OF AMMONIA EFFECT ON BLOOD PARAMETERS, STRESS, AND GILL HISTOLOGY OF THE BANDED CICHILID (*Heros severus*)

Ahmad Termeh Yousefi¹, Hossein Khara^{2*}, Mohaddeseh Ahmadnezhad³

1- Department of Fisheries, Science and Research of Guilan, Islamic Azad University, Rasht, Iran

2- Department of Fisheries, Lahijan Branch, Islamic Azad University, Lahijan, Iran

3- Iranian Fisheries Science Research Institute, Inland Water Aquaculture Research Center, Agricultural Research Education and Extension Organization (AREEO), Bandar Anzali, Iran
h.khara1974@yahoo.com

As a chemical stressor, increased ammonia concentration in water is a major problem in aquaculture which can cause non-specific physiological responses in the fish. If the appropriate concentration of ammonia is not taken into account in aquaculture, it will be certainly followed by the production cuts. The present research aimed to determine changes in blood parameters and study the pathological effects of ammonia on gill tissue of the banded cichlid, *Heros severus*. For this purpose, 5 treatments of ammonia (0.1, 0.2, 0.4, 0.8, and 1.2 mg/l) with a control and three replicates were established. A total of 180 banded cichlid specimens were exposed to different concentrations of ammonia in 50-liter tanks for 12 hours. At the end of the trial, blood samples were taken from fish specimens for measurement of blood parameters and stress indices (glucose and cortisol). In addition, their gill tissues were sampled and studied. The results showed that white blood cells count reaches its peak in exposure to different levels of ammonia in Treatment 3 ($P < 0.05$). The lowest red blood cells count was observed in Treatment 5 ($P < 0.05$). Similar to red blood cells, hemoglobin and hematocrit showed a slight downward trend with the increase in ammonia concentration, which was not significantly different ($P > 0.05$). Increased concentration of ammonia caused no significant difference between the five experimental treatments in MCV, MCH, and MCHC ($P > 0.05$). Neutrophils and lymphocytes, like white blood cells, showed an upward trend at first and then started to reduce with increasing doses of ammonia ($P < 0.05$). Monocytes and eosinophils also followed a variable and irregular trend, which was not significantly different ($P > 0.05$). The results obtained from the measurement of stress indices indicated that cortisol increased in the first two treatments and then decreased in the next three treatments. In addition, glucose reached its minimum level in the first treatment and then showed a significant increase in other treatments ($P < 0.05$). The results of histological studies revealed that tissue damages exacerbated with the increase in ammonia concentration from the control to Treatment 5, with the highest volume of damages in Treatment 5 (1.2 mg/l ammonia). The most common tissue damages observed in studied fish specimens included hypertrophy of lamella epithelial cells, adhesion of lamellae, lamella detachment and bulging, filament epithelium necrosis, severe necrosis in the lamella and filament epithelial, and necrosis and demolition of pillar cells. Finally, concentrations of ammonia tested on fish specimens in this study caused stress for the banded cichlid. Therefore, 0.1 mg/l dose of ammonia is recommended for the culture of the banded cichlid.

WASTE BIOFLOC AS A POTENTIAL FEED SUPPLEMENT FOR SHRIMP POSTLARVAE

Helena Khatoon*, Sanjoy Banerjee and Abol Munafi Ambok Bolong

Institute of Tropical Aquaculture
Universiti Malaysia Terengganu
21030 Kuala Terengganu
Terengganu
Malaysia
hlnkhatoon@gmail.com

Biofloc is a protein rich aggregate composed of bacteria, protozoa, and algae. Recently, it has drawn interest in shrimp farming for its potential nutritional benefits and to maintain water quality. Excess biofloc which is discarded as waste was collected from shrimp farm and used as feed supplement. In this study, commercial shrimp feed was supplemented with 0, 25, 50, 75 and 100% dried biofloc and fed to shrimp post larvae (PL) to evaluate the growth performance, survival rate and nutritional composition. Results showed that PLs fed with 50% biofloc-supplemented feed (BSF) had significantly higher ($p < 0.05$) specific growth rate compared to the other treatments. In addition, PLs fed with 50% and 75% BSF had significantly higher survival ($p < 0.05$) rate compared to those fed with commercial feed only. However, protein content of PLs fed with 50% and 75% BSF was comparable to those of 100% commercial feed. Total ammonium nitrogen, nitrite nitrogen and soluble reactive phosphorus were maintained in culture tanks with minimal water exchange throughout the experiment. This study illustrates that waste biofloc could be utilized as a potential and cost effective feed supplement for rearing shrimp PL. Consequently, there is conservation of environment due to efficient waste management.

DISTINCT BACTERIAL COMMUNITY ASSOCIATED WITH *Microcystis aeruginosa* (Kützing) kützing 1846 CULTURED IN DIFFERENT CULTURE MEDIA AFFECTS THE CYANOBACTERIUM COLONY AND MORPHOLOGICAL PROPERTIES

Yam Sim Khaw^{1*}, Nicholas M.H. Khong¹ and Fatimah Md. Yusoff^{1,2}

¹ Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

² Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

Email of presenting author: yskhaw@gmail.com

Cyanobacterial blooms with predominance of *Microcystis aeruginosa* is often considered a threat to human and environmental health, due to their bloom-forming and possible toxin-producing potentials; or a nuisance to recreation. The role of bacteria in influencing the growing environment of microalgae, and vice-versa has not been well described. The objective of this study was to study the bacteria community that grew with *M. aeruginosa* and the colony morphological properties of this cyanobacterium when cultured under different culture media. A tropical *M. aeruginosa* (strain UPMC-A0051) was isolated from Putrajaya Lake, Malaysia, and identified using microscopic and molecular techniques. Microcystin production profile was further examined using molecular method and the cyanobacterium was identified to be a non-microcystin producer. Next, this cyanobacterium was cultured in two different media, Blue-Green medium and Bold's Basal medium. The associated heterotrophic bacteria were isolated and identified from the two culture media. Distinct bacteria communities associated with *M. aeruginosa* in the two different culture media were belonged to *Actinobacteria*, α -Proteobacteria and β -Proteobacteria. Moreover, it was found that *M. aeruginosa* cultivated in different media with different bacteria diversity demonstrated distinct colony morphology and biomass. Distinct bacteria diversity particularly the different number of bacteria species in the two culture media possibly led to the dissimilar colony morphology of *M. aeruginosa*. The present study revealed insights to *M. aeruginosa* in adapting different nutrient conditions, which bacteria might aid in their survival ability.

WORLD FISH'S NILE TILAPIA (*Oreochromis niloticus*) BREEDING PROGRAMS

Hooi Ling Khaw*, and John Benzie

WorldFish

Jalan Batu Maung, Bayan Lepas, 11960 Penang, Malaysia.

h.khaw@cgiar.org

Aquaculture is one of the fast growing industries in the world. According to FAO statistic, during the past decade, aquaculture has increased its global food fish production from 47.3 million tonnes in 2006 to 76.6 million tonnes in 2015. In 2015, out of the 76.6 million tonnes of aquaculture food fish production, 5.7 million tonnes is accounted by tilapia production. From the total global tilapia production, 68% is from Nile tilapia (*Oreochromis niloticus*).

WorldFish is an international, nonprofit research organization that harnesses the potential of fisheries and aquaculture for reduce poverty and hunger. One of the approaches that WorldFish took to ensure food security is through enhancing production traits of aquatic animal species by genetic means. Currently, WorldFish has two Nile tilapia breeding programs, one located in Malaysia and another one in Egypt. The one in Malaysia is the well-known GIFT (Genetically Improved Farmed Tilapia) strain, which is hardy and faster-growing improved strain developed in 1980s. GIFT has a very strong and diverse base, where the founder were from four different wild stocks in Africa and four different domesticated stocks in Asia. Since GIFT is transferred to WorldFish Malaysia in 2000 to 2001, WorldFish continue the selection on growth for harvest weight and the population has currently went through 16 generations of selection. The genetic gain is about 5-6% per generation. Since the completion of GIFT project in Philippines, it has been disseminated to more than 10 different countries in Asia and South America. The demand for the strain and the satisfaction expressed by those that receive it from WorldFish attest to its genetic merit.

The Egypt Nile tilapia breeding program (named as Abbassa strain) managed by WorldFish is setup based on GIFT technology. The founder population of this breeding program were formed by 4x4 diallel cross of three different wild populations and one hatchery population in Egypt. The program started in 2002 and currently has went through 13 generations of selection. The average genetic gain is about 2% per generation. The improved strain, so far, has only disseminated to local aquaculture industry since 2013.

A successful breeding program is a program that can reach their targeted end-users with the improved fish seed and show impact on the industry or society. Thus, dissemination and adoption of improved fish seed is very important and essential for the benefit from the breeding program to the end-users.

DIFFERENT COLLAGEN PROCESSING AND EXTRACTION TREATMENT IMPACT THE STRUCTURAL PROPERTIES OF THE COLLAGEN PRODUCED

Nicholas M.H. Khong*, Fatimah Md. Yusoff, B. Jamilah, Mahiran Basri, Maznah Ismail, Che Azurahaman Che Abdullah & Kim Wei Chan

*Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

Collagen is a functional ingredient, valuable for the construction of medical devices as well as adding value to nutraceuticals, cosmeceuticals and nutricosmetics. Sources of collagen are naturally exclusive to animals and collagen from land-based animals differs from their aquatic-based counterparts. The study aimed to examine the effects of various collagen extraction processes to the structural properties of the collagens produced, especially ultrastructure and secondary structures. Collagen from the jellyfish, *Acromitus hardenbergi* is extracted using acid-assisted extraction (AAE), enzyme-assisted extraction (EAE) and a new physical-aided acid-assisted extraction (PAAE). The collagens produced were then subjected to Fourier Transform Infrared and electron microscope analyses. It was found that EAE caused the highest alterations to the collagens extracted followed by AAE; and PAAE exhibited minimal alterations to the structural properties of the collagen produced. Extraction procedures involving treatment which inflicts biochemical changes to the biomaterial affected the collagen produced in secondary structures significantly ($p < 0.05$). Understanding the impacts of various processing treatments onto the collagen produced would be most practical for effective development and delivery of collagen to the final product, as different industries would need collagen of different grade and quality.

GREEN HOUSE HDPE LINER POND MESOCOSM FOR CONSISTENT HATCHERY PRODUCTION OF MARINE FINFISH IN MALAYSIA

Eng Wah Khoo ,Jia Zin Khoo,Kar Fook Yu

GMS Aquaculture Solution Sdn Bhd.
Malaysia

Marine Finfish production of fish fries in Malaysia normally is carried out in Indoor Tank Hatchery or Out Door Pond Mesocosm Hatchery.

Fertilized eggs are released inside the tank or in the outdoor pond in which live feeds are supplied for about 30 days, the fish fries are then harvested. However the annual rainfall in Malaysia) is ranged from 1500 mm to 2500 pm with an average of 120-200 mm per month and that resulted poor result. Rainwater is normally acidic in nature with zero salinity and low alkalinity.

From the production data of the many hatchery operators in Malaysia, good results are related to the good climatic condition. During raining seasons, pond water contamination with rainwater of low salinity, low pH, low alkalinity as well as low temperature , poor live feed culture, and with soluble iron seepage from acid sulfate soil ,low performance is the consequence.

Green House HDPE Liner Pond Mesocosm is the solution of the teething problems of low survival in the hatchery production of marine finfish seed in Malaysia condition. Green House HDPE Liner Pond Mesocosm enhances good salinity, alkalinity, pH, water temperature, ample live feed supply with no soluble iron contamination.

GREEN HOUSE HDPE LINER POND-ENHANCED HATCHERY SEED PRODUCTION IN MALAYSIA CONDITION

Eng Wah Khoo, Jia Zin, Khoo, Kar Fook Yu

engwah.khoo@stac.com.my

Introduction

Malaysia is in the tropical region with annual rainfall ranged from 1500-2500 mm. The monthly average rainfall is from 120-200 mm, during raining season, the water of the aquaculture ecosystem will be affected.

The nature of rainwater normally with pH 5.5-6.5, salinity is 0 ppt, alkalinity is 0-5 ppm. During raining dissolved oxygen will be lower than normal with lower temperature as well. Rain water seepage during raining day results low pH low alkalinity and high soluble iron in coastal aquaculture earth ponds excavated on acid sulfate soil. Low and poor survival of the seed production in tropical countries are teething problems for the hatchery operators that result financial lost.

With many years of hatchery operation in open mesocosm system in earthen pond that experienced , STAC provided a consultancy commercial project in 2006 in Labuan and 2010 in Tanjong Karang using Green House HDPE Liner Pond for hatchery production of marine fish seed and marine crab seed production, the results proved the system is commercial viable and sustainable.

A third project is carried out at Bagan Segaris of Pantai Remis of Perak in a 6.5 acres coastal land with acid sulfate in nature where 3 x 1000 m² Green House and HDPE Liner Ponds, 3 x 250 m² Green House HDPE Liner Ponds Brood Stocks and 3 x 3 000 m² Liner Pond for nursery and reservoirs are in operation.



Green House HDPE Liner Pond-Enhanced Hatchery Seed Production In Malaysia Condition

(Continued on next page)

House and HDPE Liner Ponds, 3 x 250 m² Green House HDPE Liner Ponds Brood Stocks and 3 x 3 000 m² Liner Pond for nursery and reservoirs are in operation.



(Continued on next page)



(Continued on next page)



(Continued on next page)



METHIONINE REPLACEMENT WITH CYSTINE IN DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Soohwan Kim*, Chorong Lee and Kyeong-Jun Lee

Department of Marine Life Sciences, Jeju National University, Jeju 63243, South Korea

Corresponding author: kjlee@jejunu.ac.kr

This study was conducted to verify the use of cysteine for methionine and cystine (free L-Met, free L-Cys) on, cystine availability, replacement effect of methionine in diets of Pacific white shrimp .

Seven experimental diets were formulated to contain L-methionine and L-cystine in different proportions (100:0, 90:10, 80:20, 70:30, 60:40, 50:50 and 0:100). The shrimp averaging at 1.10g were randomly distributed into 28 acrylic tanks of 96 L capacity at a density of 15 shrimps per tank. After an 8 week feeding trial, no significant differences were observed in growth performance, feed utilization and survival. In non-specific immunity, no significant differences were found in phagocytic (NBT), penoloxidase (PO) activities and immunoglobulin. Whole-body composition was not significantly different among all the dietary treatments.

In conclusion, dietary cysteine supplementation is possible to replace the methionine up to 100% for Pacific white shrimp.

Table 1. Growth performance and feed utilization of Pacific white shrimp (IBW: 1.10±0.01) fed the seven experimental diets for 8 weeks.

Treatments	FBW (g)	FCR	Survival (%)
M10C0	6.23±0.56	2.18±0.22	96.7±6.67
M9C1	6.40±0.63	2.09±0.22	85.0±6.38
M8C2	6.09±0.42	2.22±0.30	80.0±14.4
M7C3	6.45±0.89	2.10±0.31	93.3±5.44
M6C4	6.15±0.90	2.25±0.47	98.3±3.33
M5C5	6.31±0.16	2.15±0.12	95.0±3.33
M0C10	6.08±0.80	2.27±0.27	81.7±11.4

Table 2. Non-specific immune responses of Pacific white shrimp fed the seven experimental diets for 8 weeks.

Treatments	NBT	PO	Ig
M10C0	1.22±0.26	0.140±0.010	28.3±3.63
M9C1	1.33±0.18	0.148±0.020	29.4±1.35
M8C2	1.41±0.19	0.150±0.014	29.6±11.9
M7C3	1.33±0.15	0.143±0.020	27.6±9.38
M6C4	1.40±0.23	0.146±0.019	28.3±5.07
M5C5	1.50±0.13	0.143±0.014	22.9±6.75
M0C10	1.38±0.24	0.155±0.013	28.0±2.02

EVALUATION OF A HYDROLIZED PIG BRISTLE MEAL AS A FISH MEAL REPLACER IN DIETS FOR OLIVE FLOUNDER *Paralichthys olivaceus* AND COMMON CARP *Cyprinus carpio*

Youjeong Kim*, Insun Yang, Soyeon Lee, Hwangwon Kwon and Kyeong-Jun Lee¹

Department of Marine Life Science
Jeju National University, Jeju 63243, South Korea

¹ Corresponding author: kjlee@jejunu.ac.kr

Pig bristle meal (PBM) is made from pig hair by hydrolysis process and contains about 80% crude protein. This study was conducted to evaluate PBM as a FM replacer in diets for juvenile olive flounder (Exp-1) and common carp (Exp-2).

In Exp-1, six diets (PBM0, PBM3, PBM6, PBM9, PBM12 and PBM15) were prepared to contain 0, 3, 6, 9, 12 and 15% PBM replacing FM. Triplicate groups of olive flounder (Initial BW, 8.69 g) were fed one of the diets to apparent satiation for 8 weeks. At the end of the feeding trial, growth performance was significantly affected by the dietary treatments. All groups showed significantly lower growth compared to the control group except for PBM3. Hemological parameters were not significantly different. PBM6 group showed the highest lysozyme activity while PBM9 group exhibited the highest immunoglobulin level. Increasing levels of PBM in diets resulted in decreased apparent dry matter and crude protein digestibilities. In Exp-2, six diets (PBM0, PBM4, PBM8, PBM12, PBM16 and PBM20) were prepared to contain 0, 4, 8, 12, 16 and 20% PBM replacing FM. Triplicate groups of common carp (Initial BW, 3.7 g) were fed one of the diets to apparent satiation for 8 weeks. At the end of the feeding trial, growth performance and feed utilization were significantly affected by the dietary treatments. Increasing levels of PBM in diets resulted in decreased growth performance. PBM4 and PBM8 groups showed significantly higher protein digestibility compared to the control group.

Those results indicate that up to 7.5% of FM protein can be replaced by PBM with supplementation of limiting amino acids in diets for olive flounder. In diet for common carp, up to 14% FM protein can be replaced by the PBM without supplementation of limiting amino acids.

GROWTH PERFORMANCE OF BLACK SEA BREAM *Acanthopagrus schlegelii* FED THE FREE OR DIPEPTIDE FORM OF LEUCINE

J.D. Kim^{1*}, S.S. Kim², S.J. Shin², H.S. Han³ and K.J. Lee⁴

¹College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

²Feed and Livestock Institute, CJ CheilJedang

³Aquafeed Research Center, National Institute of Fisheries Science

⁴Department of Marine Life Science, Jeju National University

menzang@gmail.com

Dietary leucine is essential for the optimal growth and health of fish and is necessary for protein synthesis in muscle tissues. Leucine deficiency can cause severe biochemical malfunctions that result in growth retardation. However, few studies have evaluated the effects of dietary leucine supplementation on aquatic animals. In the present study, therefore, we investigated whether juvenile black seabream could utilize the dipeptide form of leucine as a protein source and compared the efficacy of the dipeptide and free forms in terms of growth performance.

All experimental diets contained 0.4% leucine from fish meal. Six additional diets were prepared by adding incremental levels (0.3%) of two different forms of Leu to the control diet to be 0.7, 1.0, and 1.3% leucine level in diet. Leucyl-Glycine (Leu-Gly) was used as the Leu source in a dipeptide form (D) and crystalline L-Leu was used as a free form (F). The conditioned experimental fish averaging at 3.23 ± 0.003 g were then randomly distributed into twenty one 20 L tanks (15 fishes/tank) in a flow-through system with sand filtered seawater. Fish fed the leucine in dipeptide form (Leu-Gly) had significantly higher weight gain at all the three dietary leucine levels (0.7, 1.0, and 1.3%) than those fed free form, L-leucine. Weight gain and specific growth rate were significantly increased in fish fed the diets supplemented with free or dipeptide leucine compared to those in fish fed the control diet containing only the basal level of 0.4% leucine. The lowest feed conversion ratio (FCR) was found in fish groups fed the dipeptide diet. The highest FCR was found in fish groups fed the F-0.7% diets. The present study shows that the availability of AA could be better in fish when AA is provided as dipeptide forms than free forms, and that dipeptides can be used as AA source for AA requirement study in the fish.

Table 1. Growth performance of juvenile black sea bream (*Acanthopagrus schlegelii*) fed the experimental diets with different levels of leucine supplement and molecular forms for 4 weeks¹

	Control	Leu-Gly			L-Leu		
	C-0.4	D-0.7	D-1.0	D-1.3	F-0.7	F-1.0	F-1.3
IMW ² (g)	3.18	3.19	3.25	3.22	3.25	3.25	3.25
FMW ³ (g)	4.40	5.25	5.79	5.71	4.84	5.00	5.24
Weight gain ⁴	38.1 ^a	64.5 ^{bc}	78.3 ^c	77.4 ^c	49.0 ^{ab}	54.1 ^{ab}	61.2 ^{bc}
FI ⁵	2.84	2.59	3.02	3.68	4.06	3.52	4.05
SGR ⁶ (%)	1.24 ^a	1.91 ^{bc}	2.20 ^c	2.20 ^c	1.53 ^{ab}	1.66 ^{ab}	1.83 ^{bc}
FCR ⁷	2.35 ^c	1.26 ^a	1.17 ^a	1.49 ^{ab}	2.54 ^d	2.05 ^{bc}	2.12 ^{ab}
PER ⁸	0.91 ^a	1.73 ^{bc}	1.84 ^c	1.43 ^{abc}	0.96 ^a	1.09 ^{ab}	1.17 ^{abc}
Survival (%)	55.6	64.4	62.2	51.1	60.0	66.7	53.3

¹Means of triplicate groups; values are presented as mean \pm SD. ²IMW = Initial mean body weight. ³FMW = Final mean body weight. ⁴Weight gain (%) = $100 \times (\text{final mean body weight} - \text{initial mean body weight}) / \text{initial mean body weight}$. ⁵Feed intake (g/g body weight) = dry feed fed (g) / body weight (g). ⁶Specific growth rate (%) = $[(\log_e \text{final body weight} - \log_e \text{initial body weight}) / \text{days}] \times 100$. ⁷Feed conversion ratio = dry feed fed / wet weight gain. ⁸Protein efficiency ratio = wet weight gain / total protein fed.

PHYLOGENETIC POSITION OF THE ENDANGERED RED-SPOTTED GROUPEP *Epinephelus akaara* BASED ON COMPLETE MITOCHONDRIAL DNA SEQUENCES

Yoo-Kyung Kim*, Sang-Hyun Han, Chi-Hoon Lee, and Young-Don Lee

Marine Science Research Institute, Jeju National University, South Korea
102 Jejudaehak-ro, Jeju-si, Jeju Special Self-Governing Province, 63243, South Korea
moolmaru@hanmail.net

This study revealed phylogenetic position of the endangered Red-spotted grouper, *Epinephelus akaara* (Perciformes, Serranidae) based on the nucleotide sequences of complete mitochondrial DNA. Complete nucleotide sequences were determined from the mitochondrial genome of two individuals of the Red-spotted grouper caught in South Korea. The mitochondrial genome had 16 795 base pairs (bp) and 13 protein-coding genes, 2 ribosomal RNAs, 22 transfer RNAs, and a noncoding control region. The two mt genomes were highly homologous (99.71% similarity). The two mt genomes of *E. akaara* analyzed in this study were found in Clade I with those of *E. awoara*, *E. fasciatus*, *E. sexfasciatus*, *E. diacanthus*, *E. stictus*, and *E. morio*. Here, we reported the complete mt genome sequence of *E. akaara*, suggesting that this may be helpful to understand phylogenetic position of *Epinephelus* species including Red spotted grouper.

The Red-spotted grouper, *Epinephelus akaara*, is a western Pacific fish species and designated as endangered species. This species inhabits in Korea, southern Japan, Taiwan, and southern China, and it generally inhabits shallow coral and rocky reefs.

Genomic DNAs were extracted from two individuals (specimen IDs. BukbariG01 and BukbariG05) of *E. akaara*, one collected from the South Sea and the other, from a shallow coral area of Jeju Island, South Korea. The complete mitochondrial (mt) genome was sequenced (accession nos. KJ700439 and KJ700440). Both mt genome sequences of *E. akaara* had 16 795 base pairs and consisted of 37 genes. The two mt genomes were highly homologous (99.71% similarity).

The phylogenetic relationships of *Epinephelus* spp. were examined using mt 13 protein-coding genes of 21 species previously reported in *Epinephelus*. The phylogenetic tree showed three distinct clades (Clade I-III, Figure 1). The present mt genomes of *E. akaara* were found in Clade I with those of *E. awoara*, *E. fasciatus*, *E. sexfasciatus*, *E. diacanthus*, *E. stictus*, and *E. morio*. Clade II and III contained all other species used in the phylogenetic tree. Molecular studies including *E. akaara* have described by some researchers (Ding et al., 2005; Zhuang et al., 2013; Han et al., 2014). Here, we reported the complete mt genome sequence of *E. akaara*. This specific phylogenetic position may explain in the family Epinephelidae.

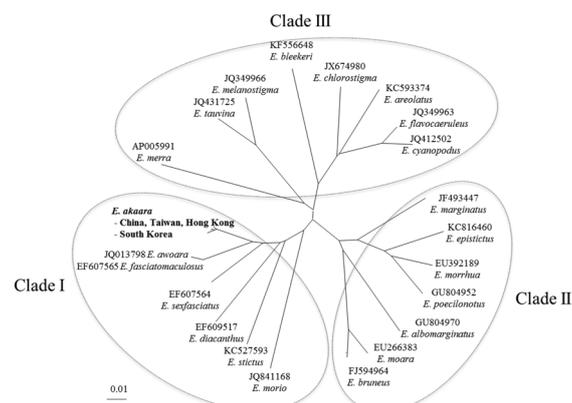


Figure 1. Molecular phylogenetic analysis of mt 13 protein-coding genes in 22 species of *Epinephelus*. The phylogenetic tree was constructed using neighbor-joining method based on the genetic distances. The rate variation model allowed for some sites to be evolutionarily invariable. The number above bar indicates genetic distance.

REPLACEMENT OF FISH MEAL BY DEHULLED SOYBEAN MEAL WITH METHIONINE AND LYSINE SUPPLEMENTATION TO DIET FOR JUVENILE STERLET STURGEON, *Acipenser ruthenus*

Jeong-Dae Kim^{1*}, Dong-Hoon Lee² and Seong-Ryul Lim²

¹College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

²Gyeonggi Province Maritime and Fisheries Research Institute, Korea

menzang@gmail.com

Soybean meal is considered to be one of the most suitable and stable supplies of an alternative ingredient for replacing fish meal to reduce the cost in commercial fish feed industries. This study was designed to investigate the effects of the replacement of fish meal by dehulled soybean meal (DSBM) with dietary methionine and lysine supplementation on meat quality for sterlet sturgeon. The DSBM of 25, 50, 75 and 100% was added to the basal diet (DSBM0) containing 42% protein and 11% lipid designated to DSBM25, DSBM50, DSBM75 and DSBM100.

Five groups (three replicates/ group) of 300 fish (60 fish per group) of a mean body weight of 125.4 g selected from 1,000 fish were randomly allotted to each of 15 tanks (2 × 1 × 0.4 m). Water temperature and dissolved oxygen levels were kept at 20±1°C and over 6 mg O₂ L⁻¹, respectively. Fish were fed the diets by hand at the rate of 3 to 4% of fish body weight per day at 08:00 h, 13:00 h and 18:00 h, for 10 wk.

Total amino acid contents of whole body (DSBM0, 14.8%; DSBM25, 14.1%; DSBM50, 14.7%; DSBM75, 15.0% DSBM100, 13.4%; % as-is basis) of fish groups were not significantly difference (p>0.05). Also, total free amino acid contents of whole body (DSBM0, 0.155, mg/100mg; DSBM25, 0.171 mg/100mg; DSBM50, 0.146 mg/100mg; DSBM75, 0.160 mg/100mg and DSBM100, 0.143 mg/100mg; as-is basis) of fish groups were not significantly difference (p>0.05). Aspartic acid and L-proline were, however, greatly different (p<0.05) among fish groups fed five experimental diets. In whole body fatty acid composition, saturated fatty acids [SFA, (DSBM0, 34.60%; DSBM25, 32.53%; DSBM50, 34.00%; DSBM75, 34.34%; DSBM100, 34.09; % of total fatty acids)], monounsaturated fatty acids [MUFA, (DSBM0, 41.65%; DSBM25, 39.65%; DSBM50, 42.00%; DSBM75, 40.72%; DSBM100, 39.45; % of total fatty acids)] and polyunsaturated fatty acids [PUFA, (DSBM0, 23.71%; DSBM25, 27.83% DSBM50, 24.01%; DSBM75, 25.93%; DSBM100, 26.46; % of total fatty acids)] were not significantly different in fish group (p>0.05).

From the present study, it was concluded that replacement of fish meal by DSBM with dietary methionine and lysine supplementation may not affect amino acid, free amino acid and fatty acid profiles in whole body of sterlet sturgeon. However, no comparisons with other studies could be made because investigations on the use of soybean meal in relation to meat quality of sterlet sturgeon have not yet been reported.

YIELD IMPROVEMENTS IN SHRIMP & PRAWN GROW OUT UTILIZING A NEW MINERAL-BASED TECHNOLOGY

Author John Knights*
jknights@calix.com.au

Co Author Dr. Mark Sceats
Calix Limited
9 Bridge St. Pymble 2073
NSW Australia

Grow out trials of *Litopenaeus vannamei* and *Penaeus monodon* in both lined and earthen ponds have yielded remarkable results. The product tested is AQUA-CAL+, produced from magnesite and dolomite. The following results have been consistently observed in a variety of pond conditions:-

1. Yield at harvest increased by at least 20%
2. FCR reduced by at least 20%
3. Reduction in Ammonia and Nitrite
4. Stabilization of pH and Alkalinity
5. Clean pond bottom
6. Improved phytoplankton growth
7. Evidence of control of Vibrosis
8. Improved water clarity
9. Reduced mortality
10. Suppression of toxic metals

AQUA-Cal+ is made by flash calcining a mixture of magnesite and dolomite to produce a unique, very high surface area powder, and then hydrating the powder to produce a slurry for ease of application to ponds and formulated to impact on both the aqueous and benthic ecosystems in the pond. Data will be presented that shows the results are a consequence of the formation of ROS (Reactive Oxygen Species) in the unique flash calcination process. The results of the trials will be presented and the mechanisms that give rise to these observations will be discussed.

EFFECTS OF FISH TRANSPORT ON CORTISOL AND GLUCOSE LEVELS OF RAINBOW TROUT (*Oncorhynchus mykiss*, W.)

J. Korun*

*Akdeniz University, Faculty of Fisheries, Campus, Antalya-Turkey, 07058

E-mail:jalekorun@akdeniz.edu.tr

Stress has two different effects on fish and these are grouped into direct and indirect effects. Cortisol and glucose changes and haematological values are included into the direct stress effects (Tapia *et al.*, 2012). Fish transport is an important process in fish farming and it causes acute stress on fish (Abreu *et al.*, 2008). The aim of this study is to inform comparison of cortisol and glucose levels of fish which were transported from commercial fish farm to research laboratory conditions and control group. Rainbow trout were transported from the commercial farm to the research laboratory. After transportation, the fish were examined as clinical findings and stress response parameters blood glucose and plasma cortisol were examined for each fish sample. The fish were firstly anesthetized with clove oil (75 mg/L for 15 min.) and then the body weights of the fish were measured. For control group, the study was carried out at the field conditions and these fish were not transported. For plasma cortisol analysis, 1 ml of blood was collected from the caudal vein of the fish samples. The blood clotting from the fish was taking within 30 sec. because of cortisol level rising related stress. 20 μ l of blood sample was used for glucose level determination. The glucose levels were measured by using glucose meter during the sampling. For cortisol analysis, the blood was taken into the eppendorf tubes containing EDTA and then the samples were allowed to clot for 2-3 h on the ice. After clotting, the upper part of the clotting blood was removed by using a micropipette and stored until analysis. The cortisol levels were determined by using ELIZA method which was done by a private laboratory. The results of both cortisol and glucose levels were evaluated according to Iwama (2004). The mean fish weights were 160 g. Plasma cortisol analyses showed that the levels changed from 5.0 μ g/dL to 9.9 μ g/dL and blood glucose levels were 3.16 mmol/L. Plasma cortisol levels of the control group was 0.5 μ g/dl and blood glucose levels of the group were 3.12 mmol/L. Iwama (2004) reported that plasma cortisol levels and blood glucose levels of trouts were >4-200 μ g/L and >5 mmol/L under stress conditions, respectively. Plasma cortisol levels of the fish which were transported were from 5.0 5.0 μ g/dL to 9.9 μ g/dL. and the results of the control fish were 0.5 μ g/dL. In conclusions, plasma cortisol levels of the transported fish were higher than the control fish group. These results may be indicator that the transported fish were under the stress; however, blood glucose levels of both control and transported fish were not indicated stress conditions.

Abreu J. S. de, A. I. Sanabria-Ochoa, F. O. Gonçalves, and E. C. Urbinati. 2008. Stress responses of juvenile matrinxã (*Brycon amazonicus*) after transport in a closed system under different loading densities. *Ciência Rural*, Santa Maria, 38: 1413-1417.

Iwama K. G., 2004. Stress in fish. *Fish Biol. Fisheries*. 8: 35-36.

Tapia P.J., M. C. Puebla, and A. Muñoz, E. Rojas, C. M. Marchant, M. A. Cornejo, and M. Futagawa. 2012. Evaluation of the cortisol stress response in a marine perciform fish, the San pedro *Oplegnathus insignis*. *North American Journal of Aquaculture*, 74: 438-442.

LARVAL FECUNDITY ON BASE POPULATION OF FAST GROWTH AND LATE MATURITY OF FEMALE GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*)

Hary Krettiawan* and Fajar Anggraeni

Research Institute for Fish Breeding
 Jalan Raya 2 Sukamandi Pantura
 Patokbeusi, Subang 41263
 West Java, Indonesia
 gkrett@gmail.com

Larval fecundity which expresses the number of larvae produced per gram of female, is a variable that directly describes the quantity of larvae produced per unit weight of the broodstock. The objective of this study was to evaluate the number of larvae produced per unit weight of the broodstock (fecundity larvae) at base population (F0) of fast growth and late sexual maturity.

Both of selected broodstock and controlled population were maintained in the spawning pond size of 200 m². The ratio of male: female was 1: 1. Commercial feed with a crude protein content of 26% has been used with the feeding rate as much as 3% of the prawn biomass. The mean of larvae produced on a selected population was 21.092 larvae and it was significantly different from the controlled population that was 14 972 larvae. Larval fecundity in both populations showed significantly difference at the 95% confidence level. This indicates that the broodstock of selected populations has a good reproductive performance. The broodstock in a selected population showed that the reproductive performance was not impaired, so it can be used to generate the next generation.

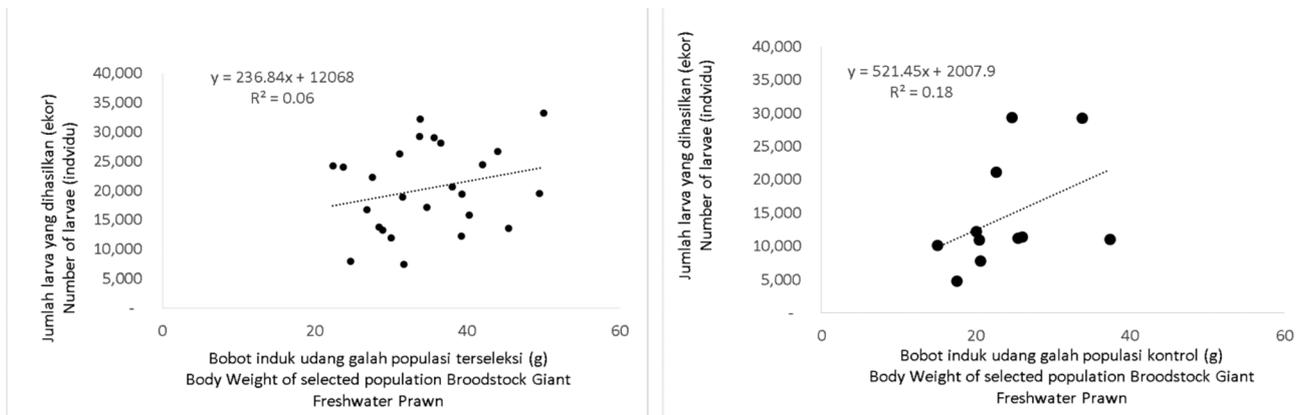


Figure 1. Linear regression between body weight and number of larvae on (a) selected population and (b) control population

OCCURRENCE OF PARASITIC ISOPOD *Cymothoa epimeria* IN CULTURED HYBRID GROUPEL (*E. fuscoguttatus* × *E. lanceolatus*) AT BT. TAMBUN, PENANG, MALAYSIA

Kua Beng Chu*, Nuruljannah H & Nur Shikin A

National Fish Health Research Division (NaFisH)
Fisheries Research Institute
Department of Fisheries Malaysia
11960 Batu Maung, Penang
kuaben01@dof.gov.my

The parasitic isopod genus *Cymothoa* Fabricius, 1793 is known as tongue-eating isopod or a production pest of marine grouper aquaculture. The isopod attaches itself to the fish's tongue and cause disruption of food intake. Due to their feeding activities and site attachment on the host, the effect caused by them should not be neglected. The current information on biology, pathogenic mechanisms, epidemiology and control methods of this isopod is limited. The aim of the study was to determine the status infestation of parasite isopod in hybrid grouper during early culture period at floating cages.

A total of 739 hybrid groupers with body weight ranging from 20g to 1kg cultured within period of 2 weeks to 9 months in floating cages at Bt. Tambun, Penang were examined for the mouth inhabiting parasitic isopod infestation. The results showed only one species of cymothoid isopod with prevalence ranging from 5 to 65 and mean intensity of one during the period of March 2016 until November 2016. The isopod was identified as *Cymothoa epimeria* and both adult or manca stages were observed at the mouth of hybrid grouper. The adult *C. epimeria* (average length 2.21 ± 0.24 cm, n=40) was first detected on 10th week after the hybrid grouper was placed in floating cages. It also indicated that within 10 weeks, manca stage went through several moulting before reaching adult stage.

The fecundity study showed each mature isopod carried 80 - 500 manca stage, which is highly productive. The adult *C. epimeria* releases the manca in mouth cavity and the manca will swims to mouth of new host. Once the fish was infested, the manca will stay in mouth until adult stage. Farmers need to remove the adult or manca isopod from fish mouth manually using hand else the infected grouper will show slow growth with big head and small body. The information obtained from this study showed the effect of isopod on hybrid grouper was severe. Apart from that, it can also help farmers to schedule the suitable period to remove the *C. epimeria* from the fish.

COMPARISON OF PRODUCTION PERFORMANCE AND ECONOMICS BETWEEN MONO-SEX AND MIXED-SEX TILAPIA *Oreochromis niloticus*

Mrityunjoy Kunda*, Aruna R. Deb and Md. J. Islam

Department of Aquatic Resource Management
Faculty of Fisheries, Sylhet Agricultural University
Sylhet-3100, Bangladesh
kunda.sau@gmail.com

Tilapia is one of the top ranked cultured species in the world. In Bangladesh tilapia culture is also increasing rapidly. Still a large number of consumers did not like hormone treated mono-sex tilapia, because there is a perception in the general people that there may have some negative effect on human health. Moreover, there is a good market demand of smaller size about 100-200g tilapia in the rural market due to lower price and which can be grown by 2-3 months. Considering these factors the study was conducted to assess the comparison of production performance and profitability between mono-sex and mixed-sex tilapia, *Oreochromis niloticus*. The experiment was carried out with two treatments each having six replications for a period of 99 days from 7 May to 12 August 2014, in 12 cages which were set up in a pond at Sylhet Agricultural University, Sylhet, Bangladesh. In the first treatment (T_1), mixed-sex tilapia was stocked with a mean initial weight of 1.743 ± 0.436 g. In the second treatment (T_2), mono-sex male tilapia was stocked with a mean initial weight of 1.756 ± 0.482 g. Fish were fed at the rate of 30% body weight of fish initially and gradually decreased up to 4% of body weight of fish at the last month of study. Feed was adjusted every 7 days interval depending on the body weight of tilapia. Water parameters, viz. water temperature, transparency, DO, pH, CO_2 , NH_3 , TDS, hardness, nitrite nitrogen were measured fortnightly.

During the study period water quality parameters found within the suitable range of fish farming. At 99 days of culture period, mono-sex male tilapia attained a significantly ($P < 0.05$) higher mean final weight, 167.15 ± 13.297 g in comparison to mixed-sex tilapia. However, there was no significant ($P > 0.05$) difference of food conversion ratio and survival (%) between the treatments at the end of the study. The benefit-cost ratio was calculated as 1.47:1 and 1.59:1 for mixed-sex and mono-sex male tilapia, respectively. But it was observed that there were no significant differences of individual weight and gross yield of tilapia, at 72 days among T_1 and T_2 (Table 1). The comparative study suggested that within two and half month culture period mixed-sex tilapia can be cultured successfully instead of mono-sex male tilapia which is very safe for human health as because of mixed sex tilapia is not hormone treated.

Table 1. Comparison of mean (\pm SD) production performance between two treatments

Parameters	Treatment-1	Treatment-2
Individual weight(g) at 58 days	67.019 \pm 2.255 ^a	75.652 \pm 10.255 ^a
Individual weight(g) at 72 days	103.82 \pm 5.135 ^a	110.76 \pm 4.701 ^a
Individual weight (g) at 86 days	144.88 \pm 5.860 ^a	155.95 \pm 5.257 ^b
Individual weight(g) at 99 days	167.15 \pm 13.297 ^a	189.67 \pm 19.142 ^b
Gross yield at 58 days (kg/m ³)	4.476 \pm 0.194 ^a	5.082 \pm 0.749 ^a
Gross yield at 72 days (kg/m ³)	6.936 \pm 0.428 ^a	7.431 \pm 0.285 ^a
Gross yield at 86 days (kg/m ³)	9.677 \pm 0.488 ^a	10.467 \pm 0.434 ^b
Gross yield at 99 days (kg/m ³)	11.157 \pm 0.835 ^a	12.724 \pm 1.272 ^b

ALL-MALE PRAWN TECHNOLOGY FOR DEFINITE ECONOMIC GAIN IN MALAYSIA

G. Kuppusamy*

Lot.2-239, Kompleks Metro Pudu, 1, Jalan Metro Pudu 2, Fraser Business Park, 55200 Kuala Lumpur
giva@gk-aqua.biz

Malaysia was the first country to discover a culture method for *Macrobrachium rosenbergii* but it has been unable to meet market demand due to inadequate production volume. Between 2006 and 2011, Malaysia produced 2,300 (FAO) metric tons (i.e. average of 400 mt per year), which is low compared to Bangladesh at 39,868 (FAO) metric tons annually. A poor-quality supply of post-larvae (from poor genetic management and low-quality broodstocks and poor farming practices have contributed to low yields.

The typical broodstock available in Malaysia results in substantial female populations (smaller in size, lower price), and potentially weaker hatching leading to low supply. These inconsistencies create a need for continuous and frequent partial harvesting over a nine-month period, which is operationally inefficient. Consequently, around a decade ago, traditional *Macrobrachium rosenbergii* farmers switched to growing the smaller, white marine shrimp (*Litopenaeus vannamei*), which initially delivered good economic return.

Successes in all male prawn technology using RNA silencing of the androgenic gland gene, GK AQUA was formed to commercialize and implement its cutting-edge technology to improve freshwater prawn farming efficiency. Through the RNA silencing technique GK AQUA will have “Neo Female” which can be naturally mated. Due to the chromosome pairings when mated, these neo-female prawns produce 100% male, virus and pathogen resistant broodstock offspring. This technique does not result in a genetically modified organism, for it uses traditional cross breeding technology producing normal prawns. All male harvests 3x larger in size are, capture a 100% higher market price, grow faster (4 vs. 9 months), and require harvesting in a single day. An average farmer using Giant Prawn’s broodstock will benefit from 134% greater yield compared to typical Freshwater prawn farming per year.

With the initial 20 contract farmers, GK AQUA’s operational target is to establish at 200 tons in the first year. By the fifth year, GK AQUA will expand to 100 contract farmers and reach production of 1000 metric tons/year and pave the way for freshwater prawn production in Malaysia. This production forecast based on minimum production of 800kg/acre and in real per acre size pond with all male culture can give rise to yield of 1200kg/acre.

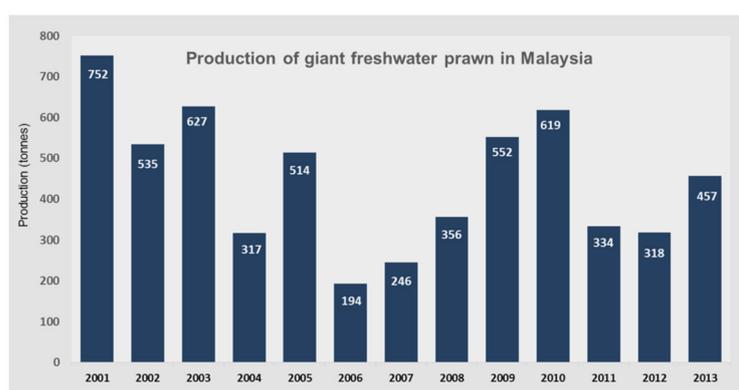


Figure 1 Source: Journal of Aquaculture, Research & Development, Vol.7, Issue 4, 2016

MALACHITE GREEN AND LEUCOMALACHITE GREEN RESIDUES IN LOCAL AND IMPORTED STRIPED CATFISH, *Pangasius hypophthalmus*

Penz Penz Kwan*, Nur Ain Syahira Ishak, Mohammed Shariff, Sanjoy Banerjee

Institute of Bioscience
Universiti Putra Malaysia
43400 Serdang, Selangor
pearlypearl.pk@gmail.com

Malachite green (MG) is commonly used in aquaculture as an antifungal and antiparasitic agent. However, due to its toxicological effects and potential carcinogenesis, the maximum permitted level (MPL) under European Union (EU) regulations of the total MG and its primary metabolite leucomalachite green (LMG) in fish is $2 \mu\text{g}/\text{kg}$. Hence, a sensitive method for the determination of MG and LMG in local and imported river catfish (*Pangasius hypophthalmus*) muscle was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS). Fish samples were purchased from seven local markets. The fish muscles were extracted with acetonitrile using a simple method without the need of solid phase extraction. Results showed that out of 14 samples, 7 samples showed residues ranging from 1.187 to $4.095 \mu\text{g}/\text{kg}$, and three of the samples contain sum of MG and LMG above $2 \mu\text{g}/\text{kg}$ which exceeds the MPL set by the EU. This indicates that there are residues of MG and LMG in striped catfish available from the market in Malaysia.

Table 1: Malachite green and leucomalachite green residues in muscles of *Pangasius hypophthalmus*

Market		MG ($\mu\text{g}/\text{kg}$)	LMG ($\mu\text{g}/\text{kg}$)	MG + LMG ($\mu\text{g}/\text{kg}$)
1	Import	$1.64^{\text{d}} \pm 0.00$	$0.59^{\text{e}} \pm 0.02$	2.23*
	Local	$3.51^{\text{g}} \pm 0.00$	$0.59^{\text{f}} \pm 0.00$	4.1*
2	Import	n.d.	n.d.	-
	Local	n.d.	n.d.	-
3	Import	n.d.	n.d.	-
	Local	n.d.	n.d.	-
4	Import	n.d.	n.d.	-
	Local	n.d.	n.d.	-
5	Import	$1.52^{\text{c}} \pm 0.01$	$0.21^{\text{b}} \pm 0.00$	1.72
	Local	$2.16^{\text{f}} \pm 0.03$	$0.35^{\text{c}} \pm 0.00$	2.51*
6	Import	$0.84^{\text{b}} \pm 0.02$	$0.88^{\text{g}} \pm 0.00$	1.72
	Local	$0.76^{\text{a,b}} \pm 0.03$	$0.43^{\text{d}} \pm 0.01$	1.19
7	Import	n.d.	n.d.	-
	Local	$1.77^{\text{e}} \pm 0.03$	$0.05^{\text{a}} \pm 0.00$	1.82

Values (mean \pm standard error) with different superscript letters in the same column are significantly different ($P < 0.05$); n.d.: not detected; * value exceeds the maximum permitted level set by the European Union

INJECTION OF OOCYTE DEVELOPER ENHANCED GONADAL MATURATION OF WILD MALE TIGER SHRIMP, *Penaeus monodon* AGAINST EYE STALK ABLATION

Dony Prasetyo, Asda Laining* and Oman Agus Sudrajat

*Presenting and Correspondence Author
 Research Institute for Coastal Aquaculture (RICA)
 Ministry of Marine Affairs and Fisheries
 Jl. Makmur Dg. Sitakka No.129, Maros 90512
 South Sulawesi, INDONESIA
 asdalaining@yahoo.com

Hormonal manipulation in crustaceans has been reported as an alternative technique to improve their reproductive performance. This experiment was conducted to evaluate the oocyte developer to induce the gonadal maturation of wild male tiger shrimp, *Penaeus monodon* against eye stalk ablation.

Oocyte developer (OD) used in the experiment is a hormone premix containing pregnant mare serum gonadotropin and antidopamin. Two dosages of OD were evaluated at 0.5 (OD0.5) and 1.0 (OD1) mL/kg and control was eye stalk ablation (AB). The OD was administered via injection two times with one week interval. The 75 wild male broodstocks were randomly distributed into three of 10 tonnages concrete tanks with density of 25 shrimps per tank. A week after the last injection, males were electrically shocked to release their spermatophores from ampulla terminalis. Variables observed were male reproductive performance and profiles of fatty acid of muscle of the male stocks.

Males injected with OD0.5 produced the highest number of spermatophores which was 80.8% followed by AB (68%) and OD1 (64%). Injection of OD shortened the time for maturation where all males injected with OD0.5 released their spermatophores within 7-d while AB matured until 21-d. Characteristic of spermatophore including weight of spermatophore and number of sperm cell did not differ ($P > 0.05$) among treatments. There was a positive correlation between weight of male and spermatophore weight where R^2 value was high at OD0.5 (0.60) and when all data was pooled (0.61).

Total fatty acid contents (%) in muscle of male induced with OD0.5 and OD1 were 2.64 and 2.65, respectively higher than those of ablated male. 2.26.

Based on number of matured males and several other parameters, the optimum dose of OD to enhance the reproductive performances of male tiger shrimp was 0.5 mL/kg.

TABLE 1. Number of matured male males releasing spermatophores at first maturation and rematuration

Treatment	Number of matured male (shrimp)		
	Maturation	1st Rematuration	2sd Rematuration
AB	17 (68%)	9 (36%)	1 (4%)
OD0.5	21 (84%)	3 (12%)	0
OD 1	16 (64%)	3 (12%)	0

TABLE 2. Number of matured male at different times of maturation

Treatment	Number of matured male based on maturation time (shrimp)		
	7 hari	14 hari	21 hari
AB	8 (47%)	5 (29%)	4 (24%)
OD 0,5	21 (100%)	0	0
OD 1	15 (94%)	0	1 (6%)

CULTURE OF TROPICAL ZOOPLANKTON SPECIES, *Moina micrura* WITH DIFFERENT TYPES OF DIETS

Nur Laishatulaini Latib^{1*}, Fatimah Md.Yusoff^{1,2}, Norio Nagao², and Nizar Hamadon¹

¹Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia

²Laboratory of Marine Biotechnology
Institute of Bioscience
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia

laishatulaini@gmail.com

In aquaculture industry, zooplankton is widely used as live feeds as it is rich in nutrient contents to promote better growth, and high survival for fish larvae and other aquatic animals. A study was conducted to investigate the effects of different feed types on the growth of a tropical cladoceran, *Moina micrura* consisting of two non-algal diets: Baker's yeast and *Spirulina* powder, and two mono-algal diets: *Chlorella sorokiniana* and *Chlorella vulgaris*. *Moina Micrura* samples were collected from a small pond in the Universiti Putra Malaysia (UPM). Live *Moina Micrura* samples were kept and brought to the laboratory for species identification, isolation, and mass culture. Experiments were performed with three replicates for each diet by providing *ad libitum* feeding to *Moina micrura* (about 1.0×10^6 cells ml⁻¹ density for algae diets and 0.01mg ml⁻¹ for non-algae diets). The experiments were terminated when the populations stabilized or began to decline. The *Moina micrura* population density was estimated every three days by removing 25 ml of the well-mixed culture and counted under dissecting microscope. The highest population density (4143.71 ± 694.90 individual l⁻¹) was produced in the culture fed with *Chlorella vulgaris* while Baker's yeast produced the lowest population density (1126.95 ± 200.26 individual l⁻¹) compared to other treatment ($p < 0.05$). Correspondingly, the highest specific growth rate ($p < 0.05$; $0.25 \pm 0.27\mu$) was achieved also in *Moina micrura* cultured with *Chlorella vulgaris*. *Spirulina* powder gave the lowest specific growth rate ($0.078 \pm 0.07\mu$). The body length of *Moina micrura* culture with *Chlorella vulgaris* produced the best results ($783.97 \pm 22.59\mu\text{m}$). The results of the present study illustrated that *Chlorella vulgaris* was the most suitable food for the growth of *Moina micrura*.

AN INTEGRATED APPROACH WITH FOCUS ON LARVAL FEED DEVELOPMENT, FEEDING STRATEGIES AND ROBUSTNESS FOR THE PRODUCTION OF HIGH-QUALITY SHRIMP POSTLARVAE: STRETCHING THE BALANCE BETWEEN EXPERIMENTAL FEEDS AND *Artemia*

Patrick Lavens*, Roeland Wouters, Dirk Grymonpre, Celine De Maesschalck, Virginia Calabuig, Pieter Joos, Anamaria Reckecki, Phuthongphan Rattayaporn, Geert Rombaut, Eddy Naessens

INVE Aquaculture
Hoogveld 93, 9200 Dendermonde, Belgium
r.wouters@inveaquaculture.com

This presentation reviews our continued research efforts towards improving feeds and feeding protocols for white shrimp (*Penaeus vannamei*) hatcheries, based on knowledge acquired during in-house and joint research projects on larval feed development, feeding strategies and improving resilience. And with a focus on maintaining a high standard with respect to postlarval quality as this is affecting later performance in the ongrowing unit. Larval nutrition entails work on nutrient requirements, in-depth ingredient quality assessment, *in vitro* digestibility assays, least-cost formulation, feed production technology and physical properties of the feed particles. Developing new feeding strategies entails testing different combinations in terms of feed delivery, feeding frequency and timing, feeding rates, and *Artemia*/formulated feed ratios in controlled culture environments.

One of the objectives was to further stretch the Best Balance concept and verify the effect on larviculture performance and postlarvae quality. Best Balance is a concept on which INVE has been working for several years and which seeks the right balance between formulated feeds and *Artemia* nauplii in larval feeding regimes, specific to the regional conditions or even tailored to a specific hatchery situation, even under low *Artemia* consumption protocols. In addition, new concepts for increased resilience and microbial management have been included.

The effects of varying combinations of experimental feeds, feeding strategies and health concepts have been tested in several experimental larval shrimp trials at INVE's tropical research center in Thailand (ITARC), in which larval development, survival, growth and postlarval (PL) quality were always monitored. To ensure the production of high-quality shrimp, a series of parameters were measured to evaluate the robustness of the PLs, PL size variation and performance during the post-hatchery phase. Results of these hatchery trials and PL quality evaluation tests will be presented and discussed.

CONFLICT BETWEEN SOMATIC AND GONADAL GROWTH IN SEA URCHINS: IMPLICATIONS FOR AQUACULTURE

John M. Lawrence*

Department of Integrative Biology
University of South Florida
Tampa, FL 33620, USA
lawr@usf.edu

Studies on aquaculture of sea urchins have focused on production of large, high quality gonads by large individuals. Sea urchins must reach a threshold of body size before they are marketable. Decreasing the length of time required to reach this threshold is desirable for aquaculture of sea urchins. Somatic growth in sea urchins is great initially and then decreases with size as gonadal production occurs. Somatic growth in *Strongylocentrotus intermedius* is initially fast, then decreases after initial HD = ca. 15 mm with increasing size to an asymptote (Fig.1). Gonads of *S. intermedius* begin to increase greatly in size after a HD = ca. 15 mm (Fig. 2). The marketable size of *S. intermedius* is ca. 50 mm.

Gonadal production exceeds somatic production seasonally in species with annual reproductive cycles, suggesting conflict. Conflict between somatic and gonadal production requires limited common nutrient requirements. Independent processes would suggest differences in nutrient requirements or preferential allocation. Scheibling and Hatcher (2013) concluded there is apparent conflict between somatic and gonadal growth in strongylocentrotids but that the hypothesis that they are independent could not be rejected. Field and laboratory studies have shown both somatic and gonadal production are related to food availability and quality. Species with a low allocation to somatic production have faster growth and greater gonadal production, indicating a genetic basis for production. Intrinsic control (hormonal) of allocation to gonadal production but not to somatic growth has been reported. Rapid growth to a marketable size is important in aquaculture. The studies suggest feeding a high quality feed would increase the rate of somatic growth and decrease time to marketable size but the increase in gonadal growth would decrease cost effectiveness.

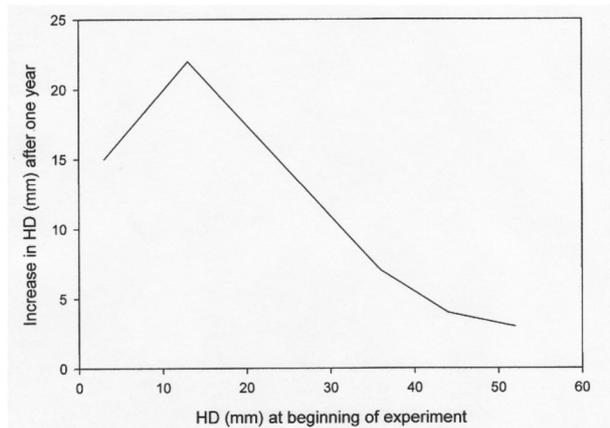


Fig. 1. Somatic growth curve of *Strongylocentrotus intermedius*. From data in Fuji, 1967.

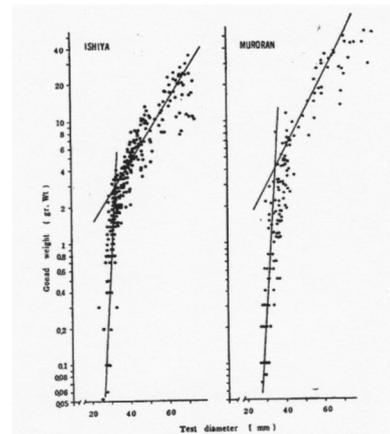


Fig. 2. Gonadal production and body size in *S. intermedius*. From Fuji, 1967.

UTILIZATION OF FREE OR DIPEPTIDE FORMS OF PHENYLALANINE IN DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Chorong Lee*, Soohwan Kim, Kyeong-Jun Lee

Department of Marine Life Science,
Jeju National University, Jeju 63243, South Korea
Corresponding author: kjlee@jejunu.ac.kr

Amino acids (AA) is not only the building blocks for protein synthesis but also have regulatory roles in key metabolic functions which are critical for maintenance, growth, immunity, larval metamorphosis, reproduction and resistance to pathogens in shrimp. Phenylalanine (Phe) is an aromatic AA and its requirement is influenced by dietary tyrosine level. Dietary Phe as an essential AA has great impacts on feed intake, growth performance, immunity and survival of fishes. However, few studies have been conducted to evaluate Phe requirement or availability in free or di-peptide forms for shrimp. A 4-week feeding trial was carried out to compare the efficacy of free Phe or dipeptide Phe-Ala in diets for white shrimp. Three diets were prepared with 1.4% supplementation of each free or dipeptide form of Phe in a basal diet (designated as F-1.4, D-1.4 and B-0.1, respectively). Two replicate groups of shrimp (initial body weight, 0.38 g) were fed one of the diets for 28 days. At the end of the feeding trial, growth performance was not significantly affected by the dietary treatments (Table 1). The highest survival rate was obtained in D-1.4 group which was significantly different from other dietary groups. The optimum dietary Phe level for juvenile *L. vannamei* was estimated to be less than 0.3% of dry diet based on growth performance results. The on-going experiment will be further addressed.

Table 1. Growth performance and feed utilization of *L. vannamei* (Initial BW: 0.38±0.02g) fed three experimental diets for 4 weeks.

Treatment	FBW ¹	WG ²	SGR ³	FCR ⁴	PER ⁵	Survival ⁶
B-0.1	1.01±0.15	164±41.4	3.45±0.56	2.06±0.48	1.37±0.32	80.0±7.07 ^b
F-1.4	1.11±0.39	202±93.9	3.86±1.13	1.87±0.98	1.72±0.90	72.5±10.6 ^b
D-1.4	1.08±0.06	176±17.6	3.62±0.23	1.84±0.04	1.52±0.03	100±0.00 ^a

Values are mean of triplicate groups and presented as mean ± SD. Values in the same column having different letters are significantly different (Tukey; P < 0.05). ¹Final body weight (g); ²Weight gain (%); ³Specific growth rate (%); ⁴Feed conversion ratio; ⁵Protein efficiency ratio; ⁶Survival (%).

SUPPLEMENTAL EFFECTS OF BIOFLOC POWDER ON GROWTH PERFORMANCE, INNATE IMMUNITY AND DISEASE RESISTANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Chorong Lee*, Se-Jin Lim, Kyu-Tae Lee, Kyeong-Jun Lee¹

Department of Marine Life Science
Jeju National University, Jeju 63243, South Korea
¹Corresponding author: kjlee@jejunu.ac.kr

Biofloc technology can minimize water exchange and water usage in shrimp aquaculture systems through maintaining adequate water quality with extra carbon sources within culture units. A 56-day indoor feeding trial was conducted to evaluate the effect of dietary supplementation of biofloc powder on growth performance, feed utilization, innate immunity and disease resistance of Pacific white shrimp. A fish meal based diet (Con) and four other diets were prepared with dietary inclusion of either biofloc powder at 2.0 and 4.0% (BF2.0 and BF4.0) or immunostimulant (IP2.0 and IP4.0). Triplicate groups of shrimp (initial body weight, 0.92 g) were fed one of the diets at 5~10% of wet body weight per day for 8 weeks. Growth performance was not significantly different among all the dietary treatment. At the end of a challenge test, significantly higher survival rate was found in BF2 group compared to the Con groups (Fig. 1). However, no significant difference was found among other biofloc and immunostimulant supplemented groups. The findings in this study indicates that the use of biofloc in powder-form can be used in feeds for Pacific white shrimp as an immune-stimulant when it is collected and prepared in a proper way. More detailed results will be addressed.

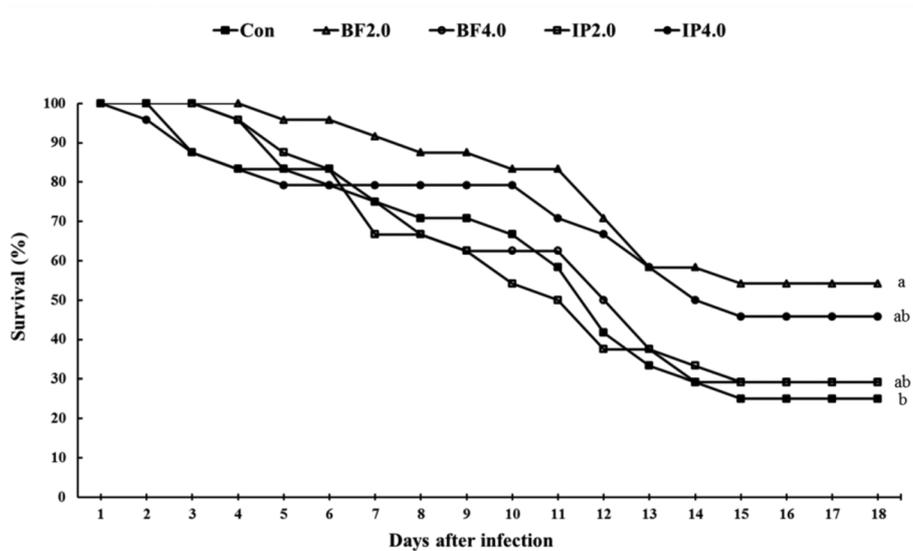


Figure 1. Survival rate of *L. vannamei* during a challenge against *V. harveyi*. The shrimp were injected intramuscularly with *V. harveyi* suspension containing 2×10^7 CFU mL⁻¹. The shrimp was not fed during the challenge period.

IDENTIFICATION, CHARACTERIZATION AND EXPRESSION PROFILE OF SERUM AMYLOID P-COMPONENT FROM THE BIG-BELLY SEAHORSE (*Hippocampus abdominalis*)

Seongdo Lee^{1*}, Thanthrige Thiunuwan Priyathilaka¹, Hyunsik Yun and Jehee Lee¹

¹ Department of Marine Life Science, Jeju National University, Jeju special Self-Governing province 63243, Republic of Korea

Pentraxins (PTXs) are humeral lectins which are evolutionarily conserved pattern-recognition molecules in tissue homeostasis and innate immunity. PTXs are divided into the two groups: short pentraxins and long pentraxins. Serum amyloid P component (SAP) is a member of short pentraxin family that play crucial roles in innate immune system which is specified to recognize and eliminate the invading pathogens. Herein, Serum amyloid P-component(SAP) was identified and molecularly characterized from the big-belly seahorse (*Hippocampus abdominalis*) (designated as *ShSAP*). The full cDNA sequence of *ShSAP* was identified from Seahorse cDNA database. *In silico* analysis was performed to determine the conserved domains and evolutionary position between other orthologs. Quantitative Real-Time PCR (qPCR) was performed to determine the tissue specific distribution and expression profile of *ShSAP* in response to the pathogenic stress. The full-length *ShSAP* was 829 bp consisting of 666 bp open reading frame which encodes the polypeptide with 222 amino acids. The deduced ShSAP protein contained pentraxin domain and signal peptide. The phylogenetic result showed that the *ShSAP* shared significant homology with other *SAP* orthologs. The *ShSAP* mRNA was abundantly expressed in liver tissue among the tissues tested, whereas the lowest expression level was observed in heart tissue. After immune challenge experiment with *Streptococcus iniae*, *Edwardsiella tarda* and polyinosinic:polycytidylic (poly I:C), the *ShSAP* mRNA expression levels were significantly up-regulated in liver. Collectively, our investigations suggest that the *ShSAP* plays an essential role in post immune responses upon the invasion of pathogens.

MANAGING PATHOGENS OF DISEASES IN MARINE FISH CULTURE IN FLOATING CAGES AND DISEASE MANAGEMENT PROTOCOLS

Dr. Leong Tak Seng

3 Cangkat Minden, Lorong 13
11700, Gelugor, Pulau Pinang
Malaysia
Email: leongtakseng@gmail.com

The study of diseases in marine fish cultured in floating cages in Peninsular Malaysia was undertaken by fish disease research group at Universiti Sains Malaysia, Penang between 1985 to 1999. During these period of studies, many parasites and bacteria pathogens were identified, diseases symptoms recorded and seasonal disease outbreaks observed. The author was a consultant to the newly established Intervet Norbio Singapore Pte Ltd from 2003-2005. During these period, Iridovirus, Nodavirus and *Streptococcus iniae* and *S. agalactiae* were confirmed as present in marine fish cultured in Malaysia. Many parasites and bacteria pathogens infecting marine fish culture in Malaysia were also found in same fish species cultured in other Southeast Asia countries. These studies on parasites and diseases were documented and published in 2014 as 'PARASITES AND DISEASES OF WARM WATER MARINE FINFISH IN FLOATING CAGE CULTURE'.

Scratches and hemorrhages on body surface, tail and fin rot with loose scales were frequently observed in diseased fish, as well as many dead fish recovered at the bottom of the net, when on net changing. Accumulated loses can be as high as 80% in any one batch of fish. Freshwater treatment of fish diseases was routinely undertaken by fish farmers since the eighties. They used this method of treatment to remove the "white worm" from the fish. They had some success in reducing mortality, with reasoning that they had to reduce these "white worms". Some time, this method of treatment did not solve their problem, resulting with continuing mass mortality of the fish in the cage.

The most problematic pathogens in marine fish culture are the populations of capsalid monogeneans and leech that are found throughout the external surface of the fish. The current study is to determine what happen to the populations of the capsalid monogeneans and leech when they are treated with freshwater. This is then following up with a determination of how rapid they re-infect the fish. The results of these studies are used to determine how frequent one has to change the net to manage a reduce populations of the pathogens so as to reduce mortality in the cage. Preliminary results of these studies will be presented.

After 30 years of research on diseases of marine fish culture in floating cages, a brief outline of protocols for diseases management is suggested. Each fish farm would have to examine the suggestion to determine how to adapt it to its farm. Fish farmers should voice out their problems in such meeting to seek assistance for solutions to their problems in the farms.

TRAF6 FROM MUD CRAB *Scylla paramamosain* PARTICIPATES IN ANTI-LIPOPOLYSACCHARIDE FACTORS (ALFs) GENE EXPRESSION

Shengkang Li*, Wanwei Sun, Xinxu Zhang, Xiaobo Wen

Marine Biology Institute, Shantou University
Daxue Road 243#, Shantou, P.R.China, 515063
lisk@stu.edu.cn

Tumor necrosis factor receptor-associated factor 6 (TRAF6) in mud crab *S. paramamosain* is a cytoplasm key signal adapter protein that mediates signals activated by tumor necrosis factor receptor (TNFR) superfamily and the Interleukin-1 receptor/Toll-like receptor (IL-1/TLR) superfamily. The full-length 2492 bp TRAF6 (*Sp*-TRAF6) contains a 1800 bp of open reading frame (ORF) encoding 598 amino acids, including an N-terminal RING-type zinc finger, two TRAF-type zinc fingers and a conserved C-terminal meprin and TRAF homology (MATH) domain. *Sp*-TRAF6 transcripts were predominantly expressed in the hepatopancreas and stomach, whereas it was barely detected in the heart and hemocytes in our study. Further, *Sp*-TRAF6 transcripts were significantly up-regulated after immune challenge with LPS. The *in vitro* binding and antimicrobial activity assays indicated that the recombinant *Sp*ALF5 and *Sp*ALF6 protein showed a varying degree of binding activity towards bacteria and fungus, and exhibited a broad spectrum of antimicrobial activities against Gram-positive, Gram-negative bacterium and fungi. Therefore, six ALF isoforms from mud crab had been reported up to now. To investigate *Sp*-TRAF6 activating *Sp*ALFs gene expression, RNA interference assay was carried out to examine the mRNA level of six *Sp*ALFs after silencing *Sp*-TRAF6 gene. The results shown that silencing *Sp*-TRAF6 gene could inhibit *Sp*ALF1, *Sp*ALF2, *Sp*ALF5 and *Sp*ALF6 expression in hemocytes, while *Sp*ALF1, *Sp*ALF3, *Sp*ALF4, *Sp*ALF5 and *Sp*ALF6 in hepatopancreas.

After knockdown of *Sp*-TRAF6 expression, we found the bacterial clearance ability of crab's hemolymph was significantly reduced. Moreover, the dual luciferase reporter assays were performed to detect the luciferase activity of six ALFs' promoters in *Drosophila* S2 cell in which *Sp*-TRAF6 protein was over-expressed. The results indicated that these ALFs' promoters could significantly enhance the luciferase activity, and confirmed that *Sp*-TRAF6 regulated the *Sp*ALFs transcription via the TLR/NF- κ B routes. Using Pull-down assay, we found that *Sp*-TRAF6 could interact with the upstream protein *Sp*Pelle and downstream *Sp*Ecsit protein, and the binding areas were located in the PAT1 domain of *Sp*Pelle with Ring/Zf-TRAFs domain of *Sp*-TRAF6, while MATH domain of *Sp*-TRAF6 with ECSIT domain of *Sp*Ecsit protein. Furthermore, the Far-Western blotting was performed to confirm the interaction relationship between *Sp*-TRAF6 and *Sp*Pelle or *Sp*Ecsit *in vitro*. Meanwhile, we verified the interaction function between *Sp*-TRAF6 and *Sp*Pelle or *Sp*Ecsit in *Drosophila* S2 cell. All those results suggested that *Sp*-TRAF6 participated in the immune response of *S. paramamosain*, and regulated the expression of *Sp*ALFs.

Taken together, the acute-phase response to immune challenges and the inhibition of *Sp*ALFs gene expression indicate that *Sp*-TRAF6 plays an important role in host defense against pathogen invasion via regulation of ALF gene expression in *S. paramamosain*.

TOTAL REPLACEMENT OF DIETARY FISH OIL WITH A BLEND OF VEGETABLE OILS IN THE MARINE HERBIVOROUS TELEOST *Siganus canaliculatus*

Yuanyou Li*, Shuqi Wang, Xuebing Liu, Cuihong You, Óscar Monroig, Douglas R. Tocher

School of Marine Sciences
South China Agricultural University
Guangzhou, China
yyli16@scau.edu.cn

The rabbitfish *Siganus canaliculatus* is a marine herbivorous teleost and feeds on algae and seagrass in nature. *S. canaliculatus* is a commercially valuable marine teleost fish widespread along the Indo-West Pacific coast and has become one of the most harvested species in southeastern Asia, including along the coast of southeast China. Therefore, the development of a suitable formulated diet is necessary for the culture industry. In our recent studies, we reported that *S. canaliculatus* may have the ability to convert 18:2n-6 and 18:3n-3 into long-chain polyunsaturated fatty acid (LC-PUFA) in both brackish water (10 ppt) and seawater (32 ppt), which suggested that fish oil (FO) may be partially or completely replaced by vegetable oils (VO). Moreover, our preliminary research results revealed that soybean oil (SO) can replace up to 67% or 45% of total dietary FO for *S. canaliculatus* without negatively compromising the growth performance or nutritional quality of fish.

To investigate the feasibility of total replacement of dietary FO with VO, and the optimal ratio of lipid sources and PUFA level in *S. canaliculatus*, six isonitrogenous (32% crude protein) and isolipidic (8% lipid) diets were formulated using different lipid sources. FO was used as the sole lipid source in the control diet (FOc), whereas varying blends of palm oil, SO, rapeseed oil, and linseed oil were used in diets VO1 - VO5, in which the dietary PUFA level was 41.95%, 38.18%, 33.83%, 29.94% and 27.12%, respectively. After *S. canaliculatus* juveniles were fed with the diets for 9 weeks, the growth performance exhibited no significant differences among all the dietary groups. The tissue fatty acids profile in liver and fillet generally reflected the dietary fatty acid composition, and showed no significant difference among the VO dietary groups. The results suggested that dietary FO can be totally replaced by VO in rabbitfish concerning the growth performance. Considering the resources and price of the experimental VO, diet VO2 with a blend of palm oil, SO, rapeseed oil, and linseed oil in a ratio of 2:2:1:1, and the dietary PUFA level was 38.18% of total fatty acids, was recommended.

This work was financially supported by the Major International Joint Research Project from National Natural Science Foundation of China (NSFC) (31110103913) and NSFC General Projects (No. 41276179).

IMMUNOSTIMULATORY INFLUENCE AND DEVELOPMENT OF NON-SPECIFIC IMMUNITY OF NILE TILAPIA *Oreochromis niloticus* AGAINST *Aeromonas hydrophila* USING GARLIC

Mary Joy H. Libatique, Heidi A. Quiboyen

Provincial Institute of Fisheries
Isabela State University-Roxas Campus
Roxas, Isabela, Philippines 3309
libatiquemaryjoy@yahoo.com

The study evaluated the efficacy of the different garlic constituents as immunostimulant to Nile Tilapia *Oreochromis niloticus*. Specifically, the study aimed to determine the immune response of Nile Tilapia fed diets containing different parts of garlic (whole garlic, garlic peel and garlic bulb) and its resistance against *Aeromonas hydrophila* infection. Treated fish were fed with diets containing 5% garlic within four weeks. Vitamin C serves as the positive control treatment while the negative control was treated with distilled water. The different haematological parameters as white blood cell, red blood cell, haemoglobin haematocrit and platelet counts were determined during 0, 14, 20, and 28 days pre and post immunization. After the feeding experiment, fish were challenged with *A. hydrophila* and survival was monitored.

Result showed that WBC, RBC, HGB, HCT and PLT in fish fed garlic diets at dietary inclusion level of 5% showed significantly higher values among treatments. The same results were observed on the survival of tilapia against the infection. Conversely, high levels of supplementation of garlic as immunostimulant did not significantly affect the non specific immunity of the tilapia. These results further suggests that use of lower than 5% garlic via oral administration as immunostimulant to nile tilapia will be considered for future feed formulations.

GENOMIC RESOURCES FOR GENETIC IMPROVEMENT OF MOZAMBIQUE TILAPIA (*Oreochromis mossambicus*)

Woei Chang Liew*, Xueyan Shen, Natascha M. Thevasagayam, Sai R. S. Prakki, Jolly M. Saju, Si Yan Ngoh and Laszlo Orban

Reproductive Genomics Group
Temasek Life Sciences Laboratory
1 Research Link
Singapore, 117604
Republic of Singapore

Tilapias are the second most farmed group of fishes in the world with global production predicted to reach around 5 million tonnes in 2015. More than half of that comes from Asia-Pacific, making tilapias one of the most important economic sources in this region. In 2011, we started a Mozambique tilapia (*Oreochromis mossambicus*) selection project at Temasek Life Sciences Laboratory (Singapore) in collaboration with the Agri-Food and Veterinary Authority of Singapore. The project aims to use genomic tools to select for fast growth fish in high salinity conditions. I will present a brief overview of the selection and improvement we have achieved so far.

I will also focus on our effort to develop molecular tools for Mozambique tilapia. We have assembled a draft genome that contains 910.5 Mb sequences distributed among 41,739 scaffolds with N50 of 304.2 Kb. Using the genome as a guide, we also put together a transcriptome. These genomic resources will be useful for genetic improvement of the Mozambique tilapia.

AMINO ACIDS AS FEEDING STIMULANT IN THE DEVELOPMENT OF SOYBEAN-BASED DIET FOR JUVENILE GROUPER *Epinephelus fuscoguttatus*

Leong-Seng Lim*, Rossita Shapawi, and Gunzo Kawamura

Borneo Marine Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400, Kota Kinabalu Sabah

Groupers are popular species for the mariculture industry especially in the Southeast Asian region. As groupers require diets with high protein level for optimum growth, farmers have to rely on the expensive fish meal (FM) based-compounded feeds to farm groupers. Therefore, many researches have been carried out to find the suitable alternative protein sources to substitute FM in the practical diets for groupers. Many studies have proven that soybean meal (SBM) protein indeed is the best candidate among the plant-based proteins, to partially replace the FM protein in the diets for juvenile groupers. However, high dietary inclusion levels of SBM protein generally will reduce the diets palatability and causes poor intake and fish growth. Supplementation of suitable dietary feeding stimulants (FS) can be practiced to solve this problem but there is still limited information on the suitable FS for groupers. Amino acids are the commonly known FS for fish. The taste preference in fish for amino acids is species-specific. Therefore in the present study, behavioral assays were conducted through video recording to identify the amino acids which are preferable by the juvenile grouper *Epinephelus fuscoguttatus*, and an 8-week feeding trial was conducted to evaluate the potential of the selected amino acids as FS for the fish.

In the behavioral assays, juvenile *E. fuscoguttatus* with the average body weight (BW) at approximately 22.9 g was used. Agar gel pellet was used as the medium to deliver 19 amino acids to the fish. The pure agar gel (PAG) and feed extract (FE) pellets were used as the negative- and positive-control, respectively. From the recorded videos, two parameters were observed: (I) the pellet was consumed or rejected [A] – if consumed, recorded 1; if rejected, recorded 0, and (II) frequency of the pellet been captured before it was consumed or rejected and ignored [B], and the preference index was calculated through [A] / [B]. The PAG pellet was totally rejected by the fish (index's value = 0). Among the 18 amino acids tested, only 6 amino acids were found acceptable to fish (index's value ranged from 0.07 to 1.00). These selected amino acids were then supplemented (1.0%) into the soybean meal (SBM)-based diet (40% fish meal protein replaced with SBM protein) to evaluate its potential to improve the feed intake of the SBM diet in the juvenile *E. fuscoguttatus* (average BW 15.6 g).

Through the feeding trial, it was determined that the dietary supplementation of the selected amino acids has significantly improved ($P < 0.05$) the feed intake of SBM diet by the fish (Fig. 1). In addition, fish fed SBM diet with the AAM supplementation (AAM10) attained higher weight gain and specific growth rate than those without the supplementation but no significant difference was found. Although the feed intake, weight gain and specific growth rate of fish fed AAM10 were not comparable to those fed with the control (FM-based) diet, the positive effective of the AAM as feeding stimulant for grouper was confirmed.

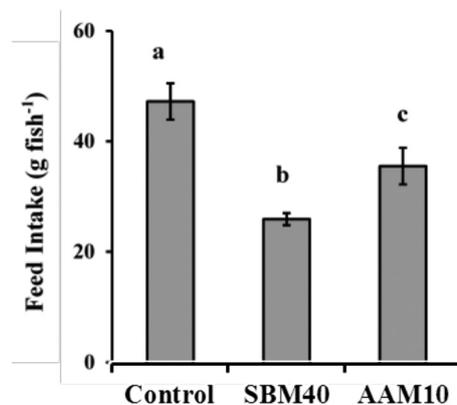


Fig. 1. Feed intake of the experimental diets in *E. fuscoguttatus*.

ACCUMULATION OF ASTAXANTHIN IN CHLOROPHYTES UNDER CULTURE MEDIUM-STARVED CONDITION

Keng C. Lim*, Fatimah M. Yusoff, Mohamed Shariff and Mohd S. Kamarudin

Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM Serdang
Selangor, Malaysia
kengchin.lim@gmail.com

The vegetative cells of chlorophycean microalgae were exposed to culture medium-starved condition. Growth medium deficiency effectively induced astaxanthin formation in the investigated chlorophytes. *Haematococcus lacustris* biosynthesized the highest cellular astaxanthin and total carotenoid contents ($26.773 \pm 2.235 \text{ mg g}^{-1}$ and $28.613 \pm 2.314 \text{ mg g}^{-1}$, respectively) compared to *Chlorella vulgaris* ($0.182 \pm 0.015 \text{ mg g}^{-1}$ and $3.176 \pm 0.062 \text{ mg g}^{-1}$, respectively), *Schizochlamydeella capsulata* ($0.228 \pm 0.015 \text{ mg g}^{-1}$ and $3.515 \pm 0.077 \text{ mg g}^{-1}$, respectively) and *Tetraselmis striata* ($0.253 \pm 0.012 \text{ mg g}^{-1}$ and $4.155 \pm 0.076 \text{ mg g}^{-1}$, respectively). The feasibility of astaxanthin accumulation corresponds to a multifunctional defence response of microalgae against adverse culture conditions.

GROWTH PERFORMANCE OF *Haematococcus lacustris* IN DIFFERENT CULTURE MEDIA

Keng C. Lim*, Fatimah M. Yusoff, Mohamed Shariff and Mohd S. Kamarudin

Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM Serdang
Selangor, Malaysia
kengchin.lim@gmail.com

In this research, we investigated the growth and biomass production of *Haematococcus lacustris* in three different culture media (MBBM, MBG-11 and MCHU-10). Cultivation in MBBM medium resulted in a significantly better growth, greater biomass production and higher specific growth rate ($P < 0.05$) when compared to MBG-11 and MCHU-10 media. Results suggested that varying compositions and concentrations of mineral nutrients between growth media exerted profound influence on the growth performance.

EVALUATION OF BILE SALT AS CHOLESTEROL REPLACER IN DIET FOR WHITE SHRIMP

Yu-Hung Lin¹

Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan
yuhunglin@mail.npust.edu.tw

For shrimp, fish meal is the most ideal protein source. It is important to find adequate alternative ingredients for fish meal due to the limited global supply and increasing price of fish meal. Soybean meal is the most widely used plant ingredient for shrimp aquaculture. Cholesterol functions in cell membrane and molting function in shrimp. However, due to absence of cholesterol in soybean meal and lack of *de novo* cholesterol-synthesis ability for shrimp, it is necessary to supplement cholesterol in diet for shrimp when the feed containing high levels of soybean meal. Our previous study found that dietary fish meal protein replaced by soybean meal caused poor growth, low tissue cholesterol concentration and depressed molting relative gene expression, including ecdysteroid receptor b, retinoic acid X receptor, hemocyanin, chitin synthase, chitinase isoenzyme and beta-actin gene. While cholesterol was supplemented in soybean meal-based diet, tissue cholesterol concentration and molting relative gene expression were improved for the shrimp. Because cholesterol is the most expensive ingredient in shrimp diet, it is worthy to find an alternate to cholesterol. In our recent work, a growth trial was to evaluate the potentiality of dietary bile salt as cholesterol replacer on cholesterol status and molting relative gene expression of white shrimp. A basal diet (soybean replaced 70% fish meal protein) was supplemented with 0.1 (SBMCA0.1) and 0.2% (SBMCA0.2) bile salt. The diet with all fish meal (FM) was also included for comparison. Hepatopancreas and hemolymph cholesterol concentration were higher in FM group than those in other dietary treatments. ECRb gene expression was the highest in FM and SBMCA0.2 groups, followed by the SBMCA0.1 group, and the lowest in SBM group. HCyn gene expression was higher in FM group than that in the SBM group. The results indicated that bile salt could partially replace cholesterol in diet for shrimp and maintain the normal cholesterol status and molting relative gene expression.

EFFECTS OF DIFFERENT DIETARY SOYBEAN MEAL LEVELS ON TISSUE CHOLESTEROL STATUS AND MOLTING RELATIVE GENE EXPRESSION IN WHITE SHRIMP, *Litopenaeus vannamei*

Yu-Hung Lin* and Jia-Jinn Mui

Department of Aquaculture
National Pingtung University of Science and Technology
Pingtung 912, Taiwan
yuhunglin@mail.npust.edu.tw

The study was to evaluate the effects of different dietary soybean meal (SBM) levels substituting to fish meal protein on growth, cholesterol status and molting relative gene expression in white shrimp, *Litopenaeus vannamei*. Diets supplemented with SBM replacing fish meal protein at 20 (SBM20), 40 (SBM40), 60 (SBM60), 80 (SBM80) and 100% (SBM100) were used as experimental diet. All fish meal diet was included as control. Total of 6 experimental diets were each fed to fed to triplicate groups of juvenile white shrimp (initial weight: 2.17 ± 0.05 g) in a recirculation rearing system for 8 weeks. Weight gain of shrimp fed with control and SBM20 diets were higher ($P < 0.05$) than that of shrimp fed with SBM80 and SBM100 diets. Cholesterol concentrations in whole body, hepatopancreas and hemolymph generally decreased by increasing dietary SBM levels. Hepatopancreatic ecdysteroid receptor gene expression was the highest in shrimp fed the control diet, followed by shrimp fed the SBM20 diet, and the lowest in shrimp fed SBM40, SBM 60, SBM80 and SBM100 diets. Shrimp fed control and SBM20 diets had the highest hepatopancreatic retinoic acid X receptor gene expression, followed by shrimp fed SBM40 and SBM60 diets, and the lowest in shrimp fed SBM80 and SBM100 diets. Chitin synthase gene expression was higher in shrimp fed control, SBM20 and SBM40 diets than other dietary treatments. Results indicated that dietary soybean meal supplementation reduced tissue cholesterol concentrations and molting relative gene expression for white shrimp.

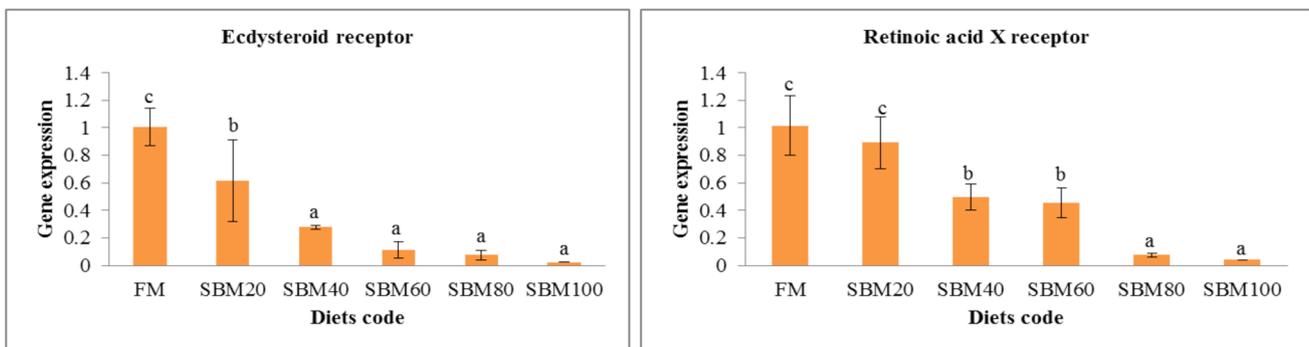


Fig. 1. Gene expression of hepatopancreatic ecdysteroid receptor and retinoic acid X receptor of Pacific white shrimp, *Litopenaeus vannamei* fed different diets for 8 weeks. The statistical analysis was based on comparisons of the relative expression ratios of ecdysteroid receptor and retinoic acid X receptor to the elongation factor 1 α by calculating $2^{-\Delta\Delta C_t}$ values. Data are presented as means \pm SD from three groups of shrimp fed on a same experimental diet

KEY POINTS OF COMMERCIAL FEED FORMULATION FOR *Litopenaeus vannamei* IN CHINA

Liu Ming*, Dong Qiufen, Zhang Song, Yang Yong

Guangzhou Nutriera Biotechnology Co., Ltd.
Unit1209, Building1, Zone4, Helenbergh Creative
Industry Park, Panyu District, Guangzhou, Guangdong, 511400, China
liuming.1212@163.com

China have the biggest *Litopenaeus vannamei* culturing amount in the world (>100 million MT/Year) and fierce competition between feed mills lasting more than 20 years. Global governments, universities and feed mills together promoted the development of research on *L. vannamei* in aspect of increasing growth speed, improve the survival rate of the larval, decreasing the cost of formulation and so on.

Fish meal is one of the primary proteins in shrimp feeds because of its known nutritional and palatability characteristics. But fishmeal's high cost and concern about the reliability of future supplies from plant and animal by-product sources have prompted efforts to identify and develop novel and relatively cheaper ingredients to function as fish meal substitutes: Spray-dried blood meal, ultra-low gossypol cottonseed meal, peanut meal, insect meal, corn gluten meal. For example: *Bacillus subtilis* E20-fermented mixture containing soybean meal and *Eisenia fetida* earthworm meal can replace fish meal completely with same feeding efficiency. A maximum level of 140g kg⁻¹ of peanut meal can be used in a commercial diet for the shrimp.

Lipid is of the highest energy density and many fatty acids from lipid metabolism are essential for normal growth and development of shrimp. EPA&DHA are essential fatty acid required by *L. vannamei* especially at early stage. Phospholipids and cholesterol are major constituents of membrane and are vital to the normal function related to ion permeability, can exert a modulating effect upon the activity of certain membrane enzyme systems and are essential.

About minerals, *L. vannamei* would have higher dietary magnesium (Mg) or potassium (K) requirement at low salinity, although marine species reared in seawater do not require dietary sources Mg and K. Because very low-salinity water will pose various negative effects.

Yeast is one of the probiotics, which is commonly used in aquaculture either alive to feed live food organisms, or after processing, as a feed ingredient. Yeast cells contain β -glucans, nucleic acid, oligosaccharides and polyamines, which may help to improve the immune response and growth performance as well as metabolism in *L. vannamei*.

This paper will give the detail evaluation of alternative protein ingredients with same performance but can decrease the cost of commercial formulation. Then essential elements requirement will be recommended like phospholipid, cholesterol and minerals when culture at low salinity. At last, this paper will describe the effect of yeast on the health of *L. vannamei*.

DEVELOPMENT OF AN INFECTION MODEL FOR THE WHITE FECES DISEASE ON WHITELEG SHRIMP *Penaeus vananmei*

Loc Tran^{1,2*}, Grace Chu-Fang Lo², Vy Van Nguyen¹, Phuc Hoang¹, Trang Nguyen¹

(1) ShrimpVet Laboratory, Ho Chi Minh City, Vietnam

(2) Department of Aquaculture Pathology, College of Fisheries, Nong Lam University, Vietnam

(3) College of Bioscience and Biotechnology National Cheng Kung University Tainan, Taiwan

(*) Corresponding Author: thuuloc@email.arizona.edu

White Feces Syndrome has been an idiopathic disease causing significant economic losses for shrimp farmers in Asia. The syndrome is characterized by transformation and sloughing of microvilli of hepatop ancreatic tubule epithelial leading to accumulation of aggregated, transformed microvilli (ATM) in the tubule lumens (Sriurairatana et al., 2014), white fecal materials in the gut, and the floating feces on pond water surface. This study aimed at determining the transmission nature of the potential pathogen(s), isolation, characterization, and development of the infection model for the White Feces Syndrome. Based on the natures of this syndrome: occurs mostly on animals of 45-60 days of culture, thick algal bloom, bad feed management, pollution, etc., we could focus on the bacterial etiology.

Several attempts of doing the transmission via intramuscular injection and feeding of white feces syndrome affected shrimp muscle tissues failed to produce the ATM. When the freshly prepared minced gastrointestinal (GI) tract on affected shrimp was mixed with shrimp feed and fed to the experimental shrimp, the formation of ATM and gross signs of white fecal matter happened within 2 days of challenged. A mixed culture of bacterial from affected shrimp was mixed with shrimp feed and fed to the experimental shrimp. The same pathology of white feces syndrome also occurred within 2 days of challenge. Several single isolates were obtained from white feces syndrome collected from the field could produce the same pathology in a laboratory challenge model using feeding method. Up to 15 different *Vibrio* isolates obtained and the Koch's postulate was completed.

In short, this study could prove the infection nature of white feces syndrome and propose an infection model for this disease. Further molecular biological analyses of the isolates are under investigation.

EFFECTS OF DIETARY POTASSIUM DIFORMATE ON JUVENILE TILAPIA IN COLOMBIA – A CASE STUDY

Christian Lückstädt*, Nicolas Greiffenstein and Ying Kang Chang

ADDCON, 53227 Bonn, Germany
christian.lueckstaedt@addcon.com

Global production of farmed tilapia in more than 140 countries exceeded 5.5 million t in 2015, an increase of more than 19 percent in only two years. This tremendous growth in production requires high-quality fish feeds. In particular, new, antibiotic-free and sustainable feed additives which support this production growth have caught the attention of the aquaculture industry. Dietary organic acids, and especially potassium diformate – the most widely tested organic acid salt in aquaculture, are among the various alternatives spearheading environmentally friendly and nutritionally-sustainable aquaculture approaches.

Dietary potassium diformate (KDF) has been used in tilapia aquaculture since 2005, to improve the performance and survival of the fish. Trials had mainly been carried out in Asia, Africa and Europe. However, data from Latin America had not yet been evaluated. This study analysed the impact of the additive on juvenile Red Tilapia in pond culture in Colombia.

A trial was carried out in a commercial pond farm for Red Tilapia in Huila, Colombia. Six ponds were used for the study. Three ponds served as a negative control, whereas fish in the other ponds were fed a commercial diet which included 0.2% potassium diformate. Almost 181,000 fish were used for the study. The duration of the trial was at least 164 days. Results for weight gain, FCR, mortality and the Fish Productivity Index are expressed as mean \pm standard deviation. Data were subjected to statistical analysis and a significance level of 0.05 was used in all tests.

Tilapia fed with 0.2% KDF had a numerically increased weight gain (309 g vs. 325 g for control and treatment respectively, +5.2%, $P=0.11$) within a culture period, which was 9 days shorter than the control (164 vs. 173 days). Furthermore, the feed conversion ratio of fish fed KDF was also improved (-5.0%). Due to the low number of replicates and the high variation between ponds, this result was non-significant. Finally, the fish productivity index, which is calculated on the basis of weight gain, survival rate and feed conversion - thus combining the three most important parameters in any fish production, improved impressively, by almost 13%. Based on the data listed above, the cost of weight gain in fish mass was reduced by more than 3 USD cents per kg.

Under commercial conditions, results show improved growth and FCR in tilapia fed with dietary potassium diformate, leading to an increased overall productivity. The use of KDF in tilapia feed under farm conditions in Colombia is therefore supported as a promising and cost-effective tool for the modern aqua-feed industry in order to contribute to ecologically sustainable tilapia production.\

\

EFFECTS OF DIETARY POTASSIUM DIFORMATE ON JUVENILE TILAPIA IN LATIN AMERICA – A PERFORMANCE ANALYSIS

Christian Lückstädt* and Nicolas Greiffenstein

ADDCON, 53227 Bonn, Germany
christian.lueckstaedt@addcon.com

Production of farmed tilapia worldwide is growing rapidly. In 2015, production exceeded 5.5 million t. This is only possible due to the use of high-quality fish feeds. Under such intensive aquaculture production conditions, bacterial diseases are a major cause of economic loss to producers, so antibiotic-medicated feeds became common practice. However, the regulatory authorities in most exporting countries now focus on the misuse of antibiotic growth promoters (AGP) in aquaculture, while public attention has shifted towards sustainable production methods. Alternative additives to replace AGP's have therefore had to be tested. Among the various alternatives available, dietary organic acids, and especially potassium diformate, which is the most widely tested organic acid salt in aquaculture, are enabling the transition to environmentally friendly and nutritionally-sustainable aquaculture.

The use of dietary potassium diformate (KDF) in tilapia feeds has been tested for over a decade, producing numerous publications and conference contributions from Europe, America and Asia. However, data from Latin America had so far not been evaluated. This study analysed the average impact of the additive from all studies in Latin America on its effect on performance parameters such as weight gain, feed efficiency and mortality as well as overall productivity.

The final data-set contained the results of 7 trials, carried out under scientific as well as commercial conditions with KDF-inclusion, which ranged from 0.2% to 1.0% and covered more than 293,000 fish. Data were subjected to statistical analysis and a significance level of 0.05 was used in all tests. Results are expressed as percentage difference from the negatively controlled fish.

The average level of dietary potassium diformate from the data-set in all treated fish was 0.44%. Performance of tilapia, based on weight gain, was significantly increased by 4.1% ($P < 0.001$). Furthermore, the feed conversion ratio of fish fed KDF was also significantly improved ($P = 0.01$); here the improvement was 6.1%. Data on mortality were inconclusive, since some of the trials were carried out under clean laboratory conditions, while others suffered from bacterially-related mortalities in cage culture. Under those conditions a numerical improvement of survival rates were noted. Finally, the fish productivity index, which is determined on the basis of the three most important parameters in fish production: weight gain, survival rate and feed conversion, improved significantly by more than 13% ($P < 0.05$).

In general, results show significantly better growth and FCR in tilapia fed with dietary potassium diformate, leading to an improved yield. The use of KDF in tilapia feed in Latin America is a promising alternative in the aquafeed industry to contribute to modern tilapia production.

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

Jianjian Lv ^{a,b,*} Baoquan Gao ^{a,b} Ping Liu ^{a,b} Jian Li ^{a,b}

^a Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture, P.R.China, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 266071 Qingdao, China

^b Laboratory for Marine Fisheries and Aquaculture, Qingdao National Laboratory for Marine Science and Technology, No. 1 Wenhai Road, Aoshanwei Town, Jimo, Qingdao, China

E-mail: lijian@ysfri.ac.cn

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

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

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

IDENTIFICATION OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN BLACK TIGER SHRIMP (*Penaeus monodon*) PACIFIC WHITE SHRIMP (*Penaeus vannamei*), AND FRESH WATER SHRIMP (*Macrobrachium rosenbergii*)

Kay Lwin Tun^{1,2*}, Siddhartha Kanrar, Kevin M. Fitzsimmons, Moe T. Oo, Hlain H. T. Kyi, May T. Oo, and Arun K. Dhar

¹Laboratory of Aquatic Bioscience, Department of Zoology, University of Yangon, University Avenue, Yangon, Myanmar

²Department of Zoology, Mandalay University of Distance Education, Mandalay, Myanmar
kaylwintun@gmail.com

The acute hepatopancreatic necrosis disease (AHPND) is an emerging disease that has caused a major loss in shrimp aquaculture in Asia since the original report of the disease in 2009 in China. The disease has now spread to many neighboring countries including China, Vietnam, Malaysia and Thailand in Asia and Mexico, Belize, Nicaragua and Honduras in the Latin Americas. AHPND now poses a serious threat to shrimp farming globally. The causative agent of AHPND was identified as *Vibrio parahaemolyticus*, *V. campbellii* and other *Vibrio* spp. that produces a toxin encoded by two genes *pir A* and *pir B* that are located in the plasmid DNA.

We have screened shrimp samples (N=36) from Myanmar that include *Penaeus vannamei*, *P. monodon* and *Macrobrachium rosenbergii*. These samples were collected from Rakhine, Yangon and Ayeyarwaddy regions of Myanmar. DNA PCR targeting *pir A* and *pir B* gene following an OIE Protocol detected both *pir A* and *pir B* genes in 5 samples, *pir A* only in 13 number of samples and *pir B* only in 3 samples. Our data clearly suggest that AHPND is present in Myanmar.

We are currently performing two tasks to further characterize the bacteria: 1) amplify and sequence the entire Open Reading Frames of *pir A* and *pir B* genes (ORF 23 and 24) to understand the genetic architecture of the toxin gene loci and, 2) amplify and sequence 16S rDNA using Next Generation Sequencing (NGS) representing different species of shrimp screened, as well as samples that originated from different culture systems to understand the population dynamics of bacterial species in the hepatopancreas of samples that are positive for *pir A*, *pir B* and *pir A& B* genes.

This is the first report of AHPND from Myanmar. Our study clearly shows the need to implement biosecurity protocol to manage any potential outbreak of AHPND in the near future.

PRODUCTION PERFORMANCE OF GIFT STRAIN *Oreochromis niloticus* WITH MAGUR *Clarias batrachus* AND GULSHA *Mystus cavasius* IN SEMI-INTENSIVE CULTURE MANAGEMENT

Yahia Mahmud*, A.H.M. Kohinoor and M. M. Rahman

Bangladesh Fisheries Research Institute
Mymensingh-2201

Monosex GIFT strain is a popular aquaculture species in Bangladesh. Most of the farmers in our country growing tilapia in mono culture management. They claimed that, they are not getting enough profit from Tilapia culture. This is mainly due to high price of commercial fish feed and comparatively less price of Tilapia in the market. Under these circumstances, a research program has been undertaken to grow GIFT with other high valued fish species to minimize the cost along with extra benefit. The present paper highlights the results of polyculture of GIFT (*Oreochromis niloticus*) with Magur (*Clarias batrachus*) and gulsha (*Mystus cavasius*) with different densities and also evaluates the economic benefit.

Production performance of monosex GIFT strain (*Oreochromis niloticus*) with magur (*Clarias batrachus*) and gulsha (*Mystus cavasius*) were carried out for five months during March to June 2014 in six farmer's pond at Dohakhola, under Gouripur upazila, Mymensingh. Three stocking densities of magur and gulsha were tested keeping the monosex GIFT Tilapia stocking density similar. Each stocking density of magur and gulsha was considered as treatment and replicated thrice. Fingerlings of magur and gulsha were stocked at the rate of 5000 & 50000; 10000 & 45000 and 15000 & 40000/ha in T-1, T-2 and T-3, respectively. In all the treatments monosex GIFT strain were stocked at the rate of 50,000/ha. The same regime of pelleted feed (28% crude protein) was applied in all the treatments. After five months rearing, the production obtained were 11808±631, 13525±389 and 13225±339 kg/ha from T-1, T-2 and T-3, respectively (Table 1). The highest production was obtained from T-2, where monosex were stocked with magur and gulsha at the stocking density 10,000 & 45,000/ha. The production level of treatment-1 showed significant difference ($P>0.05$) with T-2 and T-3. From the results, it was observed that high valued indigenous fish, magur and gulsha can be cultured with monosex GIFT strain in semi-intensive culture management for getting high production as well as net profit.

TABLE 1. Harvesting weight (g), survival, SGR and production of fish under different treatments

Treat	Fish sp.	Initial Wt. (g)	Harvesting Wt. (g)	Survival (%)	SGR (%)	Sp. wise Prod./dec.	Total Production (Kg/ha)
T-1	GIFT	6.25±0.64	232.40±10.06 ^a	93	3.02	42.22	11808±631 ^a
	Magur	5.50±0.88	160.20 ±7.90 ^a	70	2.81	2.24	
	Gulsha	2.30±0.41	20.09 ±3.66 ^a	69	1.80	2.77	
T-2	GIFT	5.89±0.69	246.15 ±9.85 ^a	95	3.11	46.77	13525±389 ^b
	Magur	5.84±0.87	151.10 ±7.80 ^b	67	2.71	4.02	
	Gulsha	2.60±0.50	23.90 ±3.87 ^a	77	1.85	3.31	
T-3	GIFT	6.12±0.51	240.77 ±8.85 ^a	91	3.06	43.82	13225±339 ^b
	Magur	5.60±0.95	145.60±8.6 ^c	65	2.72	5.68	
	Gulsha	2.47±0.62	25.27 ±3.29 ^a	82	1.94	3.40	

* Dissimilar superscript indicates significant difference at 5% level of probability

NUTRITIONAL EVALUATION OF DISTILLER'S DRIED GRAIN WITH SOLUBLE (DDGS) AS REPLACEMENT TO SOYBEAN MEAL IN DIETS OF MILKFISH, *Chanos chanos* AND ITS EFFECT ON FISH PERFORMANCE AND INTESTINAL MORPHOLOGY

Roger Edward P. Mamauag^a, Trisha J. Nacionales^a and Janice A. Ragaza^b

^aSoutheast Asian Fisheries Development Center, Aquaculture Department, Tigbauan, Iloilo, Philippines, 5021

^bDepartment of Biology, Ateneo de Manila University, Katipunan Ave., Loyola Hts., Quezon City, NCR, Philippines, 1108

A 90-day feeding trial was conducted on milkfish, *Chanos chanos* with an initial mean body weight of 3.07 ± 0.17 g. Six treatment diets were formulated to contain 0 (Diet 1), 15 (Diet 2), 25 (Diet 3), 30 (Diet 4), 35 (Diet 5) and 45% (Diet 6) DDGS. All the dietary treatments were isonitrogenous (38% crude protein) and isolipidic (6% crude fat). Result of the feeding trial indicated that growth rates, feed intake and feed efficiency was not significantly ($P > 0.05$) affected by inclusion levels of DDGS by up to 45% in the feed. Proximate body composition (crude protein, crude fat, ash, fiber) in fish fed the dietary treatments were not significantly ($P > 0.05$) affected as well. The DDGS when used as a milkfish ingredient has a protein digestibility of 91%, fat digestibility of 85%, carbohydrate digestibility of 75% and a dry matter digestibility of 52%. Results from the intestinal morphology displayed no apparent pathological changes in the digestive tract of fish fed all dietary treatments. These results indicate that DDGS can be efficiently utilized by milkfish by up to 45% without negatively affecting performance parameters and intestinal morphology.

COMPARISON OF A ZERO FISH MEAL FORMULATED FEED, A U.S. SOY-OPTIMIZED FORMULATED FEED WITH THREE PERCENT FISH MEAL AND COMMERCIAL FEED USING THE U.S. SOYBEAN EXPORT COUNCIL'S MARKETING PROGRAM'S LOW VOLUME, HIGH DENSITY CAGE AQUACULTURE TECHNOLOGIES WITH TILAPIA *Oreochromis niloticus* IN A COMMERCIAL PHILIPPINE TILAPIA CAGE FARM ON TAAL LAKE

Levy Loreto L. Manalac*, Mark W. Newman and Lukas Manomaitis

United States Soybean Export Council (USSEC)
Singapore Representative Office
541 Orchard Road
#11-03 Liat Towers
Singapore 238881
www.soyaqua.org
lmanalac@ct.ussec.org

The U.S. Soybean Export Council's (USSEC) Soy-In-Aquaculture (SIA) Program conducted a comparative feed demonstration project using U.S. soy products with tilapia (*Oreochromis niloticus*) on a commercial farm in Taal Lake, Batangas, Philippines using the USSEC Low Volume High Density (LVHD) cage culture production methodology.

The objective of this feeding demonstration was to evaluate the growth performance and economic return of tilapia cultured from fingerling to market stages in cages using three diets; a zero fishmeal diet formulated by USSEC, a U.S. soy optimized diet formulated by USSEC with 3% fishmeal and a commercial diet produced by Southeast Feeds Corp. All production cages were managed using the USSEC LVHD cage culture methodology.

The demonstration project used 15 units of 3x3x3 m (27m³) steel floating cages that targeted a final biomass of 30kg/m³ (810kg/cage) of tilapia. Five cages used the zero fishmeal tilapia formulated diet, five used a U.S. soy optimized formulated diet with 3% fishmeal and five used commercially available feeds. The tilapia using the USSEC formulated feeds targeted a 32% crude protein, 6% crude lipid (32/6) extruded, floating tilapia feed. The tilapia using the commercially available feeds targeted a 33% crude protein, 5% crude lipid (33/5) extruded, floating tilapia feed.

Tilapia were stocked at an average size of 42g and a stocking rate of 3,240 fish per cage. After 88 days of culture the fish fed with the USSEC zero fishmeal formulated diet had a biomass of 643kg or 23.8kg/m³ with average body weight (ABW) of 319g, FCR of 1.53 and survival rate of 62.4%, while fish fed with the USSEC U.S. soy optimized formulated diet with 3% fishmeal had a biomass of 664kg or 24.6kg/m³ with ABW of 325g, FCR of 1.47 and survival rate of 63.4% and the fish fed with the commercial feed had a biomass of 682kg or 25.6kg/m³ with ABW of 339g, FCR of 1.46 and survival rate of 62.0%.

Based on cost per unit gain (cost of feed to produce a kilo of fish) it showed that the tilapia fed with the USSEC zero fishmeal formulated diet has a cost of US\$ 0.995/kg, while the tilapia fed with the USSEC U.S. soy optimized formulated diet with 3% fishmeal has a cost of US\$ 0.993/kg and the tilapia fed with the commercial feed has a cost of US\$ 1.050/kg.

GROWTH PERFORMANCE OF AFRICAN CATFISH *Clarias gariepinus* LARVAE AT VARIOUS FEEDING REGIMES IN CONTINUOUS SALINE AND DARK CONDITIONS

Arlyn Mandas*, Analyn Baldove, Jhumar Jamis, Joseph Morada, Valeriano Corre Jr, Jerome Genodepa and Konrad Dabrowski

*Institute of Aquaculture, College of Fisheries and Ocean Sciences
University of the Philippines - Visayas
Miagao, Iloilo, Philippines
arlynmandas@yahoo.com

African catfish production in the Philippines cannot meet market demand due to several challenges including poor growth and survival during larval rearing. This 12-day study was conducted to determine effects of various feeding regimes on growth performance of *C. gariepinus* larvae under combined saline and dark conditions. Larvae at 48 h after hatching (mean weight = 2 mg; total length = 7mm) were distributed randomly into 12 black tanks (6-liter volume) at the rate of 10 larvae per liter. Except for the control which salinity was 0 ppt, the rest of the tanks had a salinity of 3.5 ppt. All tanks were provided with a black plastic cover to create a dark condition. The tanks were aerated and placed in a water bath to maintain an average temperature of 28°C. Two groups of larvae in 0 ppt (SA 0) (control) and 3.5 ppt (SA 3.5) were fed continuously with Artemia the entire duration of the experiment.

Two other groups of larvae were also reared in 3.5 ppt and fed Artemia for 3 (3DA 3.5) and 6 (6DA) days.

The Artemia nauplii were fresh-hatched and given *ad libitum*, whereas the formulated diet was fed 50% and 40% of the body weight of the catfish larvae for the first 3 days after the Artemia feeding days in 3DA 3.5 and 2nd six days of the culture in 6DA 3.5, respectively. Feeding of Artemia and formulated diet was done every 4 hours in 24 hours, 7 days a week.

Larvae exhibited significant differences in growth ($P < 0.01$). Mean weight and specific growth rate (SGR) were the highest in SA 3.5 and 6 DA 3.5. Growth of the larvae fed 3 days Artemia in 3.5 ppt (3DA 3.5) did not vary significantly from larvae fed continuously Artemia the entire culture duration in 0 ppt. Survival of catfish larvae was the highest in the control (SA 0) ($P < 0.05$) compared to the rest of the treatments. No significant differences in survival were seen in larvae reared in 3.5 ppt at various feeding regimes (SA 3.5, 3DA 3.5 and 6DA 3.5). No significant differences were also found in condition factor (K), performance index (PI) and feed conversion ratio (FCR) between treatments. Under the condition of the experiment, it is concluded that larval rearing of African catfish larvae at reduced Artemia feeding days does not sacrifice growth and the general condition and performance of the larvae are the same when cultured in 0 and 3.5ppt.

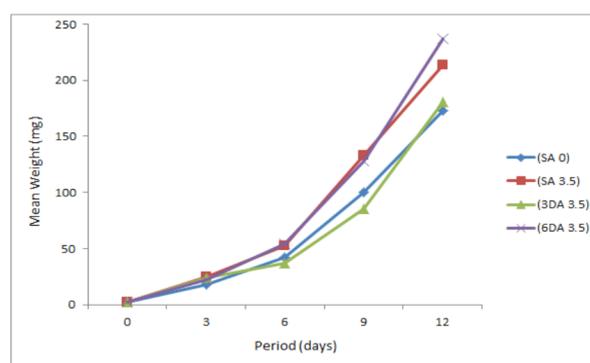


Figure 1. Growth pattern of African catfish larvae at various feeding regimes during the 12-day rearing culture

Table 1. Growth and survival parameters of African catfish larvae using live and formulated diets after

Feeding regimes	Mean weight (mg)	SGR (%)	Survival (%)	Condition factor	Performance index	FCR
Solely Artemia 0 ppt (SA 0)	173.2±4.1 ^b	37.2±0.2 ^b	64±2.0 ^a	0.55±0.03 ^a	0.92±0.04 ^a	-
Solely Artemia 3.5 ppt (SA 3.5)	213.4±5.9 ^a	38.9±0.2 ^a	43±5.6 ^b	0.65±0.02 ^a	0.76±0.09 ^a	0.69±0.06
3-day Artemia 3.5 ppt (3DA 3.5)	180.5±17.1 ^b	37.5±0.8 ^b	44±8.3 ^b	0.56±0.05 ^a	0.65±0.08 ^a	0.63±0.07

THE INTESTINAL IMMUNE RESPONSE (INFLAMMATION, TIGHT JUNCTION PROTEIN AND HUMORAL IMMUNE GENES EXPRESSION) OF GILTHEAD SEABREAM *Sparus aurata* FED MORINGA LEAVES SUPPLEMENTED DIETS

Abdallah T. Mansour^{*ac}, Liang Miao^b, Cristobal Espinosa^c, Jose-Maria G. Beltran^c, Diana Ceballos^c, and Maria A. Esteban^c

^aFish and Animal Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt
E-mail: a_taaq@yahoo.com

^bKey Laboratory of Applied Marine Biotechnology, Ministry of Education, Ningbo University, Ningbo 315211, China

^cFish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence “Campus Mare Nostrum”, University of Murcia, 30100 Murcia, Spain

The effect of dietary incorporation of *Moringa olifera* leaves (MOL) meal (0, 5, 10, 15%) in gilthead seabream *Sparus aurata* (138.75±4.65 g) on intestinal immune response was studied after two or four weeks. To do this, relative gene expression of intermediate immune response and immune-related genes in the intestine, such as anti-inflammatory (*il-10* and *tgf-β*), pro-inflammatory cytokines (*il-8* and *tnf-α*), tight junction protein (*occludin* and *zo-1*) and humoral immunity (*lyso* and *c3*). The results showed that relative expression of the mRNA levels of *tgf-β* significantly up-regulated with all MOL treatments all over the experimental period. Meanwhile, *il-10* was not affected with MOL inclusion treatments except down-regulation with 10% MOL after 2 weeks. The mRNA levels of pro-inflammatory cytokines showed a down-regulation trend of *il-8* and *tnf-α* after two or four weeks of feeding with the experimental diets. The tight junction proteins *occludin* and *zo-1* as a physical barrier in intestinal wall tended to increase after four weeks of treatment with all MOL levels especially 5% MOL. The intestinal mucosal immune components as humoral substances (*lyso* and *c3*) showed an increase of *lyso* mRNA levels with 15% MOL inclusion after two or four weeks, without any significant difference among other treatments and the control. Moreover, the mRNA level of *c3* significantly up-regulated with 15% and insignificantly with 5% MOL substitution than the control group. Therefore, it is recommended to incorporate MOL in *S. aurata* diets for intestinal immune stimulation.

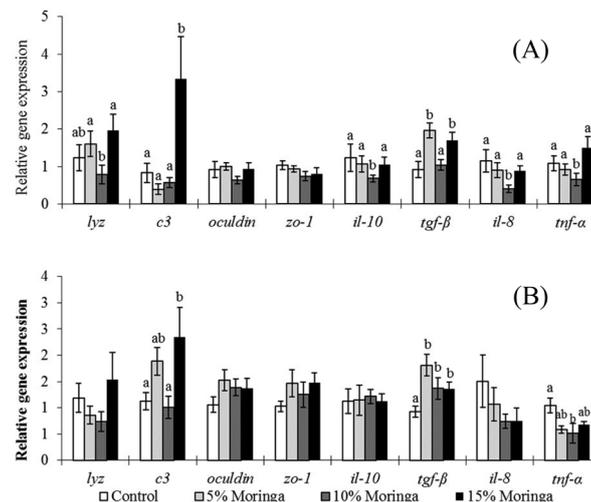


FIGURE 1. Relative expression of genes in intestine of gilthead seabream specimens fed with different experimental diets for 2 (A) or 4 (B) weeks. Bars represent the mean±SE (n=6). Different letters denote significant differences between treatment groups ($P \leq 0.05$).

IDENTIFICATION OF MUTIARA STRAIN OF AFRICAN CATFISH RESISTANT TO *Aeromonas hydrophilla* INFECTION USING MHC-I GENE AS THE MARKER

Huria Marnis, Bambang Iswanto, Imron & Selny Febrida

Research Institute for Fish Breeding
marnis.huria@gmail.com

In the previous study, MHC-I gene has been identified as a candidate marker for resistance to *Aeromonas hydrophilla* infection in Sangkuriang strain of African catfish. The results showed that the marker has high similarity to allele 9 and 17 of MHC-I gene. However, this marker has high polymorphism and was not effective when used for selective breeding program. The aim of this study was to evaluate MHC-I marker related to resistance against *A. hydrophilla* infection in Mutiara strain of African catfish strains resistant and susceptible to bacteria *Aeromonas hydrophilla* with single and specific band of DNA, so this marker has been effective when applied in the aquaculture.

In this study, we used Bacteria pathogen, *A. Hydrophilla* (Installation of Research and Development of Fish Diseases, Depok, Indonesia). The nucleotide coding sequences of MHC I (GenBank Accession numbers EU714302–EU714322) was aligned using a Pick primer NCBI to design 15 specific primer set for 22 allele. MHC I gene detection performed in live/survived/resistant and dead juvenile of African catfish post challenge test using PCR method. The PCR products were sequenced in 1 st Base Sequencing INT-Singapore. The data of sequencing were aligned by Bioedit software version 7.1.9. Furthermore, it was analyzed by BLAST (<https://www.ncbi.nlm.nih.gov/>). The MHC-I gene expression used PCR and β -actin as internal control. We detected candidate MHC-I marker from 454 broodstock (201 male fish and 253 female fish) and mated 60 female and 60 male fish, both having (P) and 3 female and 3 male fish no MHC-I (N) to progeny test.

The result of this study showed LD₅₀ of *A. hydrophilla* bacteria was 10⁸ CFU mL⁻¹. Among the 15 primer sets tested, a set primer is African Catfish grouped in two categories. First category is resistant/survival fish and it has been specific band about 1000 bp. Second, non-resistant (dead), it has not been PCR product (Figure 1). A set of primers and allele is MHC I (Clga-UAA) mRNA Clga-UAA 07 allele.

The results alignment of PCR product nucleotide at position 1000 bp sequence with GenBank: EU714308.1 showed similarities 99%, Identity 181/183(99%) and Gaps 1/183 (0%) (Figure 2).

Nucleotide sequence PCR product of about 1000 bp identical size as much as 99% with MHC I (Clga-UAA) mRNA Clga-UAA allele 07 of African catfish. Gene MHC I (Clga-UAA) mRNA Clga-UAA07 allele expressed in survived/resistant fish post challenge test with *A. hydrophilla*. The expected size of its gene was 285 bp (Figure3).

The percentage of male broodstock of African catfish carried MHC-I gene about 97.01% (195/201) and 97.52% (247/253) for female fish and about 97.36% total broodstock carrying MHC-I gene. The results of the progeny test, both having MHC-I gene were 90%-100% carrying MHC-I gene. Otherwise, the progeny test from crossed male and female broodstock of African catfish that did not carry the MHC-I gene was all offspring negative MHC-I gene.

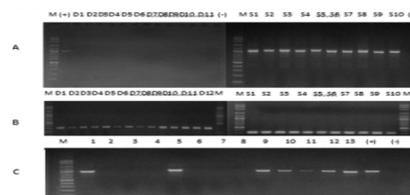


Figure 1. A. Detection of major histocompatibility complex I as molecular marker in African catfish (*Clarias sp.*) strain Mutiara (S1-S10= survived/resistant fish post challenge test); (D1-D12= dead fish) B. β -actin was used as internal control of genomic DNA and C. Fish without injection (number 1-13 = individual number). M= marker of DNA fragment size (100-3000 bp, Vivantis), (+) positive control and (-) negative control. The expected size of β gene-actin e was 300 bp.

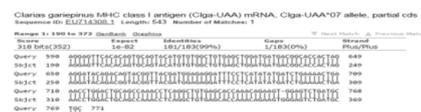


Figure 2. Nucleotide alignment of with PCR product 1000 GenBank: EU714308.1 bp DNA fragment of MHC I (Clga-UAA) mRNA Clga-UAA 07 allele in the survived/resistant African catfish Mutiara strain (*Clarias sp.*) post-challenged with *Aeromonas hydrophilla*

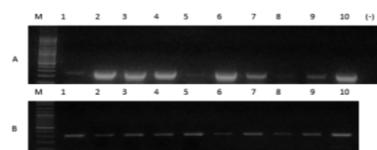


Figure 3. A. Expression gene MHC I (Clga-UAA) mRNA Clga-UAA07 allele survived/resistant African catfish Mutiara strain (*Clarias sp.*) post challenge test with *Aeromonas hydrophilla*. M= marker of DNA fragment size (100-3000 bp, Vivantis), (+) positive control and (-) negative control. The expected size of gene MHC I (Clga-UAA) mRNA Clga-UAA 07 allele was 285 bp. B. The expected size of β gene-actin e was 300 bp as internal control.

PERFORMANCE OF WHITE LEG SHRIMP (*Litopenaeus vannamei*) FED HIGH OR LOW FISH MEAL DIET SUPPLEMENTED WITH INCREASING LEVELS OF DL-METHIONYL-DL-METHIONINE

Karthik Masagounder*¹, Dhanapong Sangsue², Cláudia Figueiredo-Silva¹
and Muhammad Agus Suprayudi³

¹Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany

²Evonik (SEA) Pte. Ltd., Singapore

³Department of Aquaculture, Bogor Agricultural University, Bogor 16680 Indonesia

E-mail: karthik.masagounder@evonik.com; agus.suprayudi1965@gmail.com

Shrimp feed industry is constantly looking for opportunities to reduce inclusion of dietary fish meal. Replacement of fish meal with alternative protein sources requires balancing diets for dietary nutrients, while maintaining palatability. Methionine (Met) is typically the first limiting amino acid in soybean-based shrimp feed and thus, supplementation of methionine is essential in those diets. DL-Methionyl-DL-Methionine, 'Met-Met' in short (AQUAVI® Met-Met.), is a dipeptide of DL-Methionine and is shown to be an effective supplemental methionine source for shrimp in the recent studies. The objective of the current study was to evaluate and compare the performance of shrimp fed low fish meal diet (8%) with increasing levels of supplemental Met-Met, relative to those fed a high fish meal diet (16%) under green water system.

Shrimp (3.47±0.02 g, mean ± SD, initial weight) were randomly stocked into 20 cages (1×1×1.5 m). The experiment consisted of five dietary treatments (~38% crude protein) including: a positive control (PC) diet containing 16% fish meal with 0.10% DL-Met supplementation (D1), a negative control (NC) diet containing 8% fish meal without supplemental methionine (D2) and three other NCs with increasing levels of Met-Met supplementation at 0.03%, 0.06%, and 0.10%, respectively (D3-D5). Analyzed Lys, Met and Met+Cys levels (as-is basis) were 1.83%, 0.76% and 1.23%, respectively in the PC diet and 1.75-1.81%, 0.57-0.72% and 1.05-1.20%, in the NC diets with or without supplemental Met. Each dietary treatment was allotted randomly to four replicate cages and shrimp were fed four times daily to apparent satiation for 42 days. Data were analyzed with ANOVA and means were separated using Tukey test ($P < 0.05$).

Results of the study are shown in Table 1. Shrimp fed the NC diet showed significantly lower weight gain, feed intake and protein retention efficiency (PRE, %), marginally lower specific growth rate (SGR) ($P=0.06$) and similar feed conversion ratio (FCR) relative to those fed the PC diet. Growth rate, FCR and PRE significantly improved for the groups fed with increasing levels of supplemental Met-Met (D3-D5). Shrimp fed NC+0.10% Met-Met diet (0.72% Met and 1.20% Met+Cys) showed the best performance with significantly better body weight gain, and FCR relative to the group fed NC diet without supplemental Met. Overall, results showed that AQUAVI® Met-Met can be used as an effective supplemental methionine source in reducing fish meal level from 16% to 8% in shrimp feed without compromising growth performances.

Table 1. Summary of essential amino acid (EAA) requirements (% diet, total basis) of juvenile Nile tilapia from published studies

EAA	Met	Met+Cys	Lys	Thr	Trp	Arg	Iso	Leu	Val	His	Phe+Tyr
N ¹	7	7	5	5	2	3	2	2	2	3	2
Mean (% diet)	0.82	1.11	1.75	1.17	0.33	1.55	0.88	1.23	0.97	0.61	1.56
SD ²	0.21	0.26	0.35	0.24	0.06	0.39	0.01	0.39	0.26	0.18	0.01

¹ = number of studies used; ² = standard deviation

AMINOACID NUTRITION OF NILE TILAPIA: A REVIEW OF AVAILABLE DATA AND PROGRESS IN MODELLING APPROACH

Karthik Masagounder

Evonik Nutrition and Care GmbH
 Rodenbacher Chaussee 4
 63457, Hanau, Germany
 karthik.masagounder@evonik.com

Tilapia is currently the second largest produced farmed fish, just after carp. The industry is however facing major challenges with rising feed cost. Among nutrients, protein has a major share of diet cost and there has been continuous efforts in finding alternative cost-effective protein sources. Quality of the dietary protein is determined by its amino acid composition and digestibility in relation to the requirements of fish. Improving our understandings on the amino acid requirements of tilapia is therefore very important in order to be flexible with the diet formulations and maximize profitability. Knowledge on the amino acid requirements of tilapia is increasing over the years. Quantitative requirements for essential amino acids (EAA) of Nile tilapia was first reported back in 1980s. Since then several studies ($n > 15$) have been published on the amino acid requirements of Nile tilapia. In due course, efforts were also made to provide amino acid recommendations for feed industry which included table recommendations given by NRC, and also by Evonik for different growth stages of tilapia. The main objective of this project was to do a critical review of the available datasets and provide recommendations determined via factorial approach.

Studies in the past have used dose-response approach, diet dilution technique and deletion method for determining amino acid requirements of tilapia, with dose-response approach being the most commonly used method. Among the EAA, requirements for sulfur amino acids (methionine and cysteine), lysine and threonine were most commonly studied, understandably because these are often the first three limiting amino acids in practical fish feeds. Table 1 illustrates the summary of various published data on the amino acid requirements of Nile tilapia. Data show a wide range of variations in the requirement data published for several EAA. Variations among studies are partly because of differences in methods used and mathematical models adopted for the estimation of EAA requirements. Furthermore, Nile tilapia has been genetically improved over the years for better growth which suggests that dietary EAA requirements for modern tilapia can be quite different and likely higher than the previously reported values. Finally, effort was also made to provide EAA recommendations for different life stages and production scenarios of Nile tilapia based on factorial approach. Factorial approach treats the total requirement for an amino acid as the sum of its amounts needed to meet the physiological demands of fish for maintenance and growth. Available data on maintenance requirements, amino acid retention and utilization were considered in developing amino acid recommendations for Nile tilapia.

Table 1. Growth performance (mean \pm SD) of shrimp fed high or low fish meal diets supplemented with increasing levels of Met-Met over 42 days

Diets (Description)	Weight gain (g/shrimp)	SGR (%/d)	Feed Intake (g/shrimp)	FCR (g fed/g biomass gain)	Protein Retention Efficiency (%)	Survival (%)
D1 (PC)	8.60 \pm 0.26bc	2.95 \pm 0.06abc	12.51 \pm 0.18b	1.72 \pm 0.08ab	27.28 \pm 0.67bc	84.00 \pm 0.82
D2 (NC)	7.58 \pm 0.71a	2.75 \pm 0.15a	11.48 \pm 0.53a	2.05 \pm 0.13a	22.17 \pm 1.39a	77.25 \pm 2.50
D3 (NC+0.03%MM)	8.26 \pm 0.42ab	2.89 \pm 0.10ab	12.28 \pm 0.31b	1.75 \pm 0.07ab	25.23 \pm 0.49ab	84.00 \pm 1.83
D4 (NC+0.06%MM)	9.02 \pm 0.04bc	3.04 \pm 0.03bc	12.13 \pm 0.12ab	1.58 \pm 0.05ab	29.94 \pm 1.37cd	84.67 \pm 2.08
D5 (NC+0.10%MM)	9.36 \pm 0.32c	3.11 \pm 0.09c	12.10 \pm 0.19ab	1.49 \pm 0.07b	30.83 \pm 2.35d	85.75 \pm 1.71

PC, positive control; NC, negative control; MM, DL-Methionyl-DL-Methionine (Met-Met)

Means in each column sharing different alphabets are significantly different ($P < 0.05$, ANOVA)

AGE AND LIPOFUSCIN COMPOSITION RELATIONSHIP OF BLUE SWIMMING CRAB *Portunus pelagicus* IN CAPTIVITY

Hilmi, M. Ghani*, Ambak, M. Azmi, and Ikhwanuddin, M.

Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu

*Corresponding author: e-mail: hilmi.matghani87@gmail.com

The blue swimming crab, *Portunus pelagicus*, was one of the main species of crab at Setiu Wetlands and have been caught by fishermen for decades due to their high commercial value. Until now the status of the *P. pelagicus* in the Setiu wetlands have not been studied by any researcher. This study was carried out to estimate the age of the *P. pelagicus* using lipofuscin extraction for better understanding with their population. The samples were cultured in a hatchery and every month the eyestalks from available juvenile crabs were taken and LF extracted. The procedure was repeated until 1 year of data completed. As for the correlation analysis between males and females, there are no significant difference at $p = 0.05$. There are both positive linear relation of LI and age for males and females of blue swimming crab. The linear regression of males *P. pelagicus* was $LI = 3.3634(t) - 1.1509$ with $R^2 = 0.768$ and $LI = 3.8246(t) - 3.7268$ with $R^2 = 0.8478$ for females. Overall, the regression line for total samples combined was $LI = 3.5908(t) - 2.4058$ with $R^2 = 0.8071$, respectively. The LI of both male and female eyestalks were not significant at each other. This has showed that the metabolic rate in crab's cells is at equal rate for the different sexes. Since the traditional length based methods of age determination have proven inaccurate, population analysis using LF as an index of age should be used to develop more robust population dynamics models. This would help the fishery sectors to accurately understand the population biology and contribute to the knowledge regarding age and growth in crustaceans.

TOWARDS DEVELOPMENT OF MICROBOUND DIET AND ITS APPLICATION ON LARVAL AND POST LARVAL OF FRESHWATER PRAWN, *Macrobrachium rosenbergii*

Noordiyana Mat Noordin*, Nik Nur Ayu Hafizah N. Kamaruzaman, Mhd. Ikhwanuddin

*School of Fisheries and Aquaculture Science, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, MALAYSIA
diyananoordin@umt.edu.my

Effects and application of microbound diets (MBD) in larvae and post larvae (PL) of *Macrobrachium rosenbergii* were investigated in series of experiments. Microbound diets were formulated with different binders (agar, zein, alginate, carrageenan and carboxymethyl cellulose) and the MBD stability was analyzed by monitoring the dry matter retained and turbidity of water over progressive time up to 360 minutes. MBD with zein showed higher dry matter retained, followed by MBD with agar. After 20 minutes of immersion, the dry matter retained of MBD with zein (97.16%) was significant highest than that of MBD with CMC (88.76%) ($P < 0.05$) and after 40 and 90 minutes of immersion time, water stability of MBD with zein was significantly higher than MBD with carrageenan and CMC ($P < 0.05$). At the end of 180 minutes, the water stability of all diets was found to be almost the same. Absorbance value of MBD water sample was increased with the increasing disintegration of the MBD in the water over time. Throughout the experiment, MBD with zein consistently showed the lowest value of absorbance.

Survival, development and growth performance of larvae and PL of *M. rosenbergii* fed with MBD similar to the above were also evaluated for 14 days. At the end of the feeding trial, survival rate of larvae fed with live feed and MBD formulated with agar, zein, alginate, carrageenan and CMC were 77, 53, 48, 42, 47 and 43%, respectively. For developmental stages, larval fed rotifer had variety of stages (stage VII, VIII, IX, X and XI) compared to the all larval which fed with MBD (stage X and XI). Stage X is a dominant stage for all treatments receiving MBD meanwhile larval fed on rotifer had dominant stage IX ($p < 0.05$). For PL of *M. rosenbergii*, PL fed with MBD with agar consistently showed higher survival rates, similar to those achieved by larvae fed with rotifer. For growth performances, there were no significant difference ($P > 0.05$) was observed for all MBD treatments including when the larvae were fed on rotifer.

In third experiment, MBD formulated with agar were fed to PL of *M. rosenbergii* at various frequencies and timing (0800, 2000, 0800-2000, 0800-1400-2000 and 0800-1200-1600-2000) for two weeks. The effects of this feeding regime on PL of *M. rosenbergii* was investigated using survival, growth performances and fatty acids composition as parameters. Results showed that PL fed three times a day, 0800-1400-2000 had significantly higher survival rates (45%) compare to other treatments. No significant different was obtained from growth performances however, 0800-2000 feeding had the best growth performance in weight gain, specific growth rate and daily growth rate compared to other treatments. For body composition and fatty acid composition, no significant different was observed among all treatments ($P > 0.05$). Results from these experiments have high practical value and readily applied. Appropriate binder type and optimal feeding regime will boost hatchery culture of *M. rosenbergii* by optimizing their survival, growth, reduce management cost and manage good water quality.

ENHANCING AQUACULTURE ENVIRONMENT FOR SUSTAINABLE GROWTH OF INDUSTRY: ROLES OF ECOLOGICAL DIVERSITY, STABILITY AND RESILIENCE

Debashish Mazumder*, Fatimah Md.Yusoff

*Australian Nuclear Science and Technology Organisation (ANSTO)
Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
dma@ansto.gov.au

The world's population is steadily increasing with research suggesting that it will reach 9.7 billion in 2050 and 11.2 billion by 2100. The rapid increase in population will increase the competition for water resources globally. Water resources are an essential component of aquaculture development, which plays an important role in human nutrition and global food supply. Fish are currently the major source of animal protein for around 1.25 billion people globally, and are a source of livelihood for millions of people. The production of fish from capture fisheries have been declining globally, due to overexploitation, and detrimental effects of habitat degradation and pollution. Capture fisheries accounted for 88 million tonnes per year in the 1980s, this number declines to around 81.5 million tonnes per year in 2014. Despite the decreasing supply, global per capita fish consumption continues to rise from an average of 9.9 kg in the 1960s to 19.7 kg in 2013. Aquaculture therefore has tremendous potential to fill out the gaps and meet the increasing demands for fish in most regions of the world. In a global perspective, aquaculture is one of the fastest-growing food production sectors, with a growth rate of 5.2 to 7.2% between 1995-2014. Aquaculture in the Asia-Pacific regions accounts for about 89% of the global production. Hence, the contributions of aquaculture to food and nutrition security and livelihoods are hugely significant. This signifies the importance and urgency of sustainable approaches for enhancing aquaculture productivity without compromising the biodiversity of aquatic environments, which plays a substantial role in ecosystem stability and resilience.

Despite the huge potential, the major challenge for aquaculture expansion is particularly dependent on the contention of ecological footprint of aquaculture. The ecological footprint of aquaculture will be larger than anticipated if the following issues are not dealt appropriately. The main issues for the expansion of aquaculture include inefficient use of water, especially in land-based aquaculture, habitat modification, interferences to the food web, introducing non-indigenous species when cultured species escape from cages, nutrient pollution, and the use of antibiotics and chemicals. Adoption of innovative technologies and ecological based approaches to aquaculture helps mitigate environmental concerns as well as contributing to the stability and resilience of the aquatic ecosystem. Our collaborative research works in the Asia-pacific region to quantify the source of energy and nutrients of fish in the ecosystems, and how diversity and resilience of fish are affected by the changing nutrient dynamics. This research will contribute to the knowledge of the above issues and will help enhance aquaculture production sustainably.

ISOTOPES FOR AQUACULTURE

Debashish Mazumder*, Jesmond Sammut, Angela Liu, Michael Dove, Elizabeth Fabian and Karthik Gopi

*Australian Nuclear Science and Technology Organisation (ANSTO)
Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
dma@ansto.gov.au

Aquaculture is the fastest growing food-producing sector in Australia and around the world, where it accounts for one third of global fish production. Its rapid growth has been catalysed by the decline of wild fisheries and advances in aquaculture technologies. However, the rapid growth of aquaculture has presented new challenges. The main issues are the cost and quality of feed products, contamination of farms by runoff and soil chemical interactions, and waste generation that can impact the viability of local industries. There may also be cumulative impacts on nearby industries and public/environmental health in general. Because of these issues, production and profitability from aquaculture are often considered unsustainable. Fish 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. Application of new technologies and better management practices in aquaculture help address some of these challenges. Stable isotopic and other nuclear techniques can help resolve technological bottlenecks and contribute to sustainable solutions for aquaculture production problems.

Our work is testing the efficacy and practicability of nuclear technologies in shellfish and finfish research to determine the contribution of nutrients, from different natural and formulated feeds, to growth performance. These techniques can help to reduce aquaculture production costs, which will increase profitability for small-holders and large-scale producers. Importantly, nuclear technologies are now providing a useful tool in feed ingredient replacement studies which can lead to reduced pressure on wild fish stocks for fishmeal. Nuclear technologies are also a potential independent or complementary tool for aquaculture product certification and traceability.

CONSUMER ACCEPTANCE TESTING OF GENETICALLY IMPROVED FARMED TILAPIA (GIFT)

Wan Norhana Md. Noordin*, Mohd Nor Azman, A. and Masazurah A.R.

Fish Safety and Quality Section, Fisheries Research Institute
11960, Batu Maung, Penang, Malaysia
Corresponding author: *norhana@dof.gov.my

Genetically Improved Farmed Tilapia (GIFT) is one of the significant outputs from the collaboration between the Department of Fisheries, Malaysia and WorldFish. From this project, GIFT broodstock has been disseminated to many countries where they were well-received by the consumers. However the consumers in Malaysia are perceived to prefer red tilapia over GIFT tilapia. This statement is backed up by the recent findings from the GIFT tilapia value chain analysis study which indicated that local farmers from the three top producing tilapia states in Malaysia were not favouring GIFT because of the low demand from the consumers. Hence a survey was conducted out during the Malaysia Agriculture, Horticulture and Agro-Tourism Exposition (MAHA) in December 2016 to evaluate the consumers' acceptance of GIFT Tilapia. A sensory evaluation acceptance test was carried out on ninety nine (99) randomly selected individuals from different socio-economic backgrounds among MAHA patrons, using a 7-point hedonic scale (extremely dislike (0) to extremely like (7)) to determine the level of acceptance for 4 main attributes of GIFT (taste, odour, texture and overall appearance). In general, more than 80% of the consumers liked all of the GIFT attributes tested. The attributes of GIFT that were liked by the consumers were the texture (N=96, 97%) odour (N=89, 90%) followed by, appearance (N=87, 88%), and taste of GIFT (N=80, 81%) respectively. The texture of GIFT was described as tender yet firm, and pulled-off from the bones easily. Among the attributes, taste recorded the lowest liked percentages (81%) and highest dislike percentages (7%). All participated consumers (100%) were not bothered with the grey-green coloured skin of the GIFT tilapia. According to the consumers, they bought red tilapia because it was easily available in the market compared to GIFT. A small percentages (<5%) of the consumers did not consume GIFT believing that they were being cultured in sewage treatment ponds. A few of the Muslim consumers were doubtful of the feed used on the GIFT. The results from this study indicates that the main impediment to GIFT consumption is the non-familiarity and the lack of availability to the Malaysian consumers. Promotion on GIFT Tilapia to farmers and consumers in Malaysia could overcome the current lack of demand of GIFT. The production of GIFT from MyGAP certified farms should be labelled as such to increase the consumers' confidence towards the quality of GIFT tilapia.

FEEDING ECOLOGY AND FOOD PREFERENCES OF CHEROK PALOH, PAHANG COASTAL HORSESHOE CRAB, *Tachypleus gigas*

Mohd Razali, M. R. *, Zaleha, K., Asnor Azrin, S. & Ismail, A.

Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia.

*Email: razalirazak5379@gmail.com

Horseshoe crabs are known as omnivorous organisms. Intensive previous studies on their feeding ecology were mostly focus on *Limulus polyphemus*. Their food preference might be different depend on the availability and abundance of feeds in the particular environment. The aims of this experiment were to investigate the feeding ecology and food preferences of *Tachypleus gigas* by comparing their gut content between the open sea, pre-mating (beach) and post-mating (beach) migration phases at CheroK Paloh, Pahang coastal area. Ten samples of male and female horseshoe crabs, *Tachypleus gigas* were trapped in fishing net at the open sea area near to the opening of CheroK Paloh River during the incoming high tide and 20 samples of male and female were hand-harvested at the spawning beach; ten samples during pre-mating and post-mating respectively. Their gut content was collected from the oesophagus to anus. Results showed that horseshoe crab coming to spawn with full gut content and however, the content decreased as the horseshoe crab migrated inshore. The decreases were faster in females (20%) compared to males (4%). At the open sea, echinoderm served as a main food composition in the gut of males (48.43%) and females (51.28%). This food item remained in their gut until post-mating phase. The main food composition was substituted by macrophyte in males (59.51 - 65.15%) and females (38.32 – 58.10%) as they migrated inshore to spawn. Based on Electivity Index, male crabs showed positive preference toward polychaete at the spawning site (*EI*: 0.04) while, the females showed positive preference toward bivalve (*EI*: 0.46). Further study is needed to determine the exact food item of horseshoe crabs along the Malaysian coastal water in order to improve the reproductive success and growth of *Tachypleus gigas* in Malaysia.

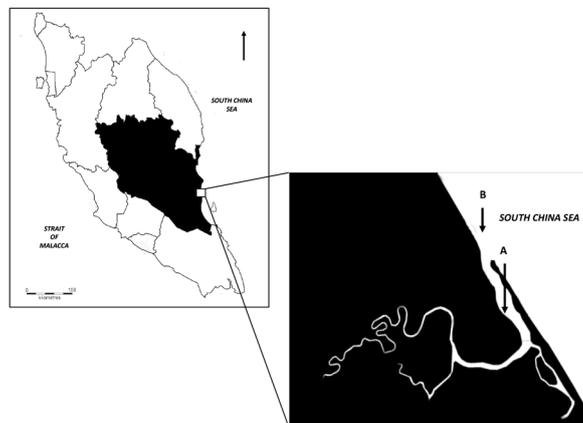


Figure 1: Locations of CheroK Paloh, Kuantan, Pahang.
 A: Beach, hand harvesting (03°36'27" N, 103°23'36" E).
 B: Open sea, netting (03°37'33"N 103°23'16"E)

DISTILLERY SPENTWASH AS ALTERNATIVE CARBON SOURCE IN AEROBIC MICROBIAL FLOC TECHNOLOGY

M. Menaga*, S. Felix, A. Gopalakannan and P. Ezhilmathi

Distillery effluents are a major resource in the country with about 150 distilleries producing 900 million litres of alcohol annually resulting in 10,000 million litres of spent wash. The efficacy of aquaculture as a tool for treatment of such wastes has been demonstrated at Advanced Research Farm Facility, Tamil Nadu Fisheries University, Madhavaram. The parameters analyzed include pH, EC, TDS, Total Hardness, Calcium, Magnesium Hardness, Total alkalinity, Chloride, Fluoride, nitrite, Nitrate, Phosphate, Sulphate, DO, COD, BOD and TOC are higher side on the permissible limits of aquaculture. However due to its increased level of TOC makes it as an efficient carbon source in zero or minimal water exchange systems. The dark colour of spent wash due to the presence of water-soluble recalcitrant colouring compounds called melanoidins. This may affect the transparency of water and hence a minimum concentration of distillery spent wash along with rice for carbon enrichment. It also helps in treating the aquaculture effluents through microbes. Many trials on using carbohydrate enriched distillery spent wash in shrimp and GIFT tilapia rearing under aerobic microbial floc technology has been attempted successfully. Our findings recommended that on the application of 13 ppm distillery spent wash aids in treating aquaculture effluents and the pollutants of spentwash can also be degraded.

GROWTH OF TILAPIA INDUSTRY IN INDIA

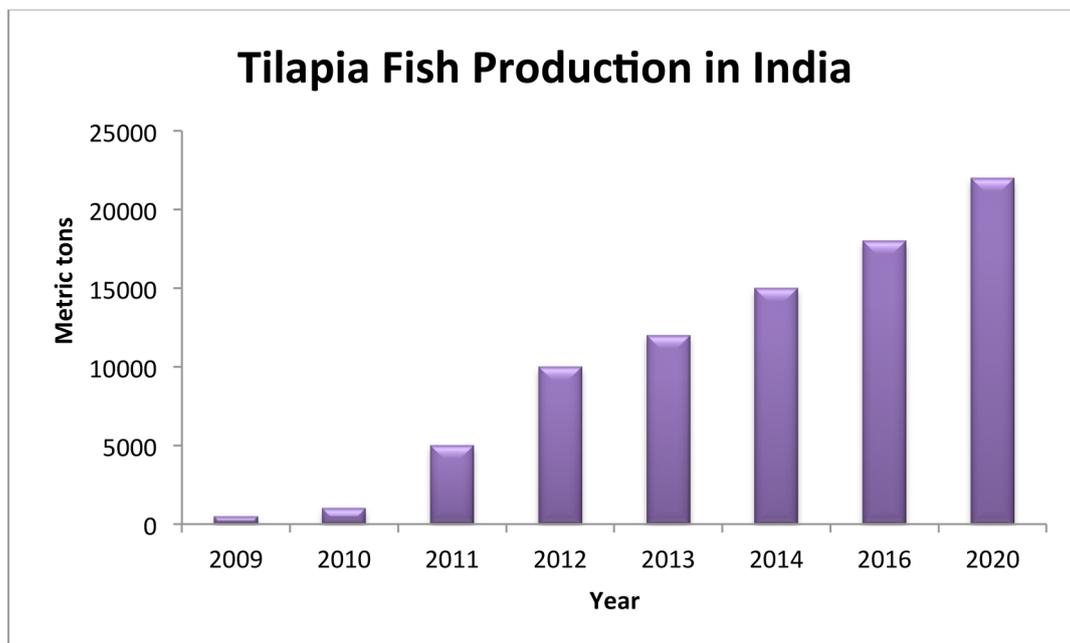
Menaga, M.* and Fitzsimmons, K.

Fisheries College and Research Institute, Ponneri
Tamil Nadu Fisheries University, INDIA
mena.fishcos@gmail.com

In 2009 the Indian government realized that undocumented shipments of tilapia are entering the country and that many farmers were beginning to work with the fish without any organization or planning. Marine Product Export Development Authority (MPEDA) decided to organize a conference to address this issue and invited an international panel of experts to discuss the situation and provide recommendations for how tilapia aquaculture should be organized. Subsequently the government developed guidelines for importation, biosecurity, quarantines and licensing. This guidance has allowed the tilapia industry to rapidly expand in an organized fashion. Production in 2016 has grown to 18,000 metric tons per year.

Tilapia farming is clustered in Andhra Pradesh and Kerala states. The most common production systems are in ponds, cages, raceways and tanks. Tilapia are also used in aquaponics systems contributing less than 1% of the national production. Tilapia have been incorporated into polyculture with shrimp, providing an additional cash crop for the farmer and reducing incidence and severity of viral and bacterial diseases in the shrimp. Polyculture with carps has also been reported in Andhra Pradesh, Gujarat and Tamil Nadu states. The shrimp and carp polyculture provide about 3 % and 5% of national production of tilapia.

Most of the tilapia being farmed are *Oreochromis niloticus* (Nile tilapia) although there are some red tilapia hybrids present, especially in areas using higher salinity water for polyculture. Virtually all of the tilapia produced in India is sold in domestic markets in a whole fish on ice mode.



THE IMPACT OF THYROXIN HORMONE ON THE SURVIVAL, GROWTH AND DEVELOPMENTS OF IRANIAN STURGEON *Acipenser persicus* EGGS AND LARVAE

Mirhashemi Rostami Seyed Amin*, Mansouri Behrooz

Inland Waters Aquatics Stocks Research Centre
Gorgan, Iran. P.O.Box,139
Rostamy_a@yahoo.com

In this study, the effect of thyroxin hormone bath on the survival, growth and development of Persian sturgeon (*Acipenser persicus*) eggs and larvae was examined. Field activities carried out at Shahid Marjani sturgeon hatchery and culture center located at the southeast region of the Caspian Sea coast in the Golestan province. Fertilized eggs (150 g per tub) from one mature wild female Persian sturgeon broodstocks prepared and then in following experiment, fish larvae after hatching, placed in plastic tubs (60-litres) for six hours bath with different concentrations of thyroxin (0 ppm as a control , 0.1, 0.5 and 1 ppm as treatments) in triplications. Variables such as the onset of eggs hatching, hatching rate, survival rate and also the mean weight and length of larvae till the start of first exogenous feeding were evaluated in a completely randomized design. For statistical analysis ($\alpha < 0.05$), the one-way ANOVA were used which followed by Duncan's test using the SPSS software. All the curves and tables were processed using Microsoft Excel software. The results indicated that there were no significant differences in mean hatching rate, survival percentage and also mean weight and length of fish larvae between treatments and control group.

PARAMETERS ASSOCIATED WITH THE STRIPED GREY MULLET *Mugil cephalus* L. FRIES MORTALITIES IN IRAN

Mirhashemi Rostami S.A.*, Amini Kourosh, Jorjani Maryam

Inland Waters Aquatics Stocks Research Centre
Gorgan, Iran. P.O.Box, 139.
Rostamy_a@yahoo.com

Nine years old cultured breeders of *Mugil cephalus* L. specimens were subjected to eight artificial propagation treatments from December till February in 2003. Some female breeders received two injections in an interval of 24 hours and some others received a gradual daily injection of 500 IU HCG per kilogram of body weight for five days and then subjected to two injection protocol. Male breeders were given 5-10 mg of 17- α MT in addition to HCG. The result of these artificial propagations was the production of 117 – 2000000 larvae in six trials for each breeder. The temperature and salinity of water during artificial propagation process were 20-23° C and 32 ppt respectively. There have been two trials for larvae culture. The final density of larvae was 20 individuals per liter. The water temperature and salinity in larvae culture tanks were 22-24°C and 32-33 ppt respectively.

The larvae were fed from the second day post-hatching with *Nannochloropsis oculata* with the density of 500000 cells/l, rotifers (*Brachionus plicatilis*) with density of 20 individuals/ ML. and also *Artemia* Nauplii with the density of 3-200 individuals/l. The particle size of dry feed used was 100, 300 and 500 micron. The daily water exchange in larval culture tanks at first, the second and third weeks of post-hatching were 10, 20-30 and 40-50 percent respectively.

At the first round of larval culture trials which carried out by means of ten circular tanks with water depth 80 cm in each; whole larvae died 12 days post-hatching. However at the second experiment, which carried out by three black color circular fiberglass tanks with 3000 liters volume and working water depth of 100 cm, the survival rate estimated 0.9 percent 50 days post-hatching.

There are two vertical migrations during first two weeks of the grey mullet larvae culture. The first sinking takes place after the second day post-hatching and probably related to the rapid absorption of the yolk sac and the resulting change in specific gravity. The second sinking happens 8th -11th days post-hatching and probably related to fully absorption of oil globule and the resulting increase in specific gravity. These migrations have been coincided with high larval mortalities (critical points).

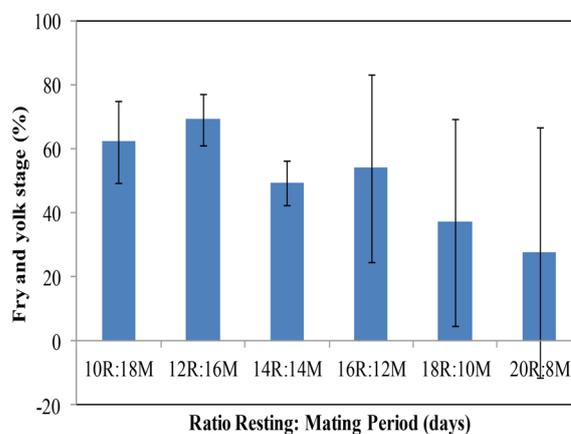
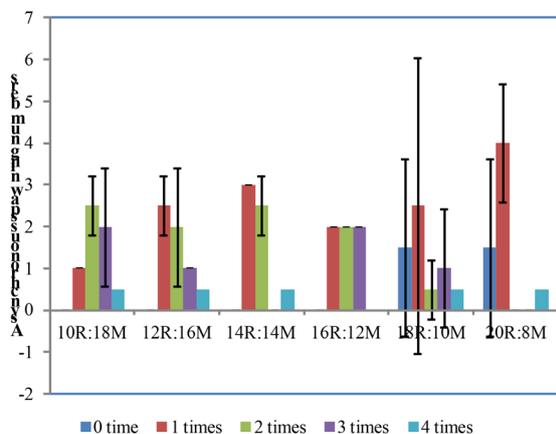
It seems that the characteristics of larval culture tank (such as shape, volume and depth), the nutritional circumstances of larvae and the aeration procedure are some important and effective factors to pass on these critical periods.

EFFECT OF DIFFERENT MATING PERIOD TO BREEDING PERFORMANCE OF RED TILAPIA HYBRID BROODSTOCK

Siti Norita M*, Mumtaziah A.H, Nurazaidah, M and Mohd Fariduddin O

Freshwater Fisheries Research Division, FRI Glami Lemi, Titi, 71650 Jelebu, Negeri Sembilan, Malaysia
 ctnorita@dof.gov.my; noritappat@gmail.com

The present study was conducted to evaluate the effect of mating period on the seed production of red tilapia hybrid broodstock, *Oreochromis* spp. under hatchery system. Females broodstock with mean body weights of 215.0 ± 50.0 were individually tagged and stocked in six different resting (R) and mating (M) period (10R:18M, 12R:16M, 14R:14M; 16R:12M, 18R:10M, 20R:8M) at a male : female ratio of 1:2 in $1.0 \times 3.0 \times 0.50$ m (W \times L \times H) fiberglass tanks. Fecundity (eggs/fry per spawn) and development stage removed from individual incubating broodfish at specified interharvest intervals (28 days) was monitored for 4 months. The results showed no significant different in total seed production and seed production/body weight. However higher spawning synchrony discovered in shorter resting period (10R:18M, 12R:16M and 14R:14M) than longer resting period of 20R:10M and 18R:10M (Fig 1). The mean percentage of seeds in yolk-sac and swim-up fry stages was highest at 12R:16M and 10R:18M (Fig. 2). The percent of hatchability decreased with eggs than yolk-sac developmental stages. However, the hatching rate was not affected by mating period. Numbers of eggs produced were higher compared to fry/yolk-sac per spawn implying that development stage of egg was influenced by mating period. Allowing female broodstock to remain undisturbed for a longer period in tanks may lead to inhibition of spawning activity which might be related to the absorption of eggs for growth. Egg and yolk-sac/fry yields for red tilapia averaged 6.86 ± 2.0 eggs/g female/spawn and 5.1 ± 1.7 yolks/g female/spawn; respectively. This data could be adopted in operating broodstock multiplication center (BMC) for better seed production and might have important implications for broodstock management in the commercial hatcheries.



- FIGURE 1: Asynchronous spawning numbers at different resting (R) and mating (M) period for 4 month.
-
- FIGURE 2: Mean percentage of seeds in yolk-sac and swim-up fry stages.

PRELIMINARY STUDY ON GROWTH OF HYBRID GROUPEL CULTURED IN CAGES AT LANGKAWI

R A Roki Mohamed*, Nik Nazli Effendy Ramli, Zaidnuddin Ilias, Idris Ahmad, Zainoddin Jamari and Halim Zainal

Fisheries Research Institute Langkawi
Department Of Fisheries
Bukit Malut
07000 Langkawi, Kedah
Email: roki@dof.gov.my

The study was conducted to determine the growth of hybrid groupers, female tiger grouper *Ephinephelus fuscoguttatus* x male giant grouper *E. lanceolatus* cultured in sea cages. Two batches of hybrid groupers with average sized (21.54 ± 1.87 g, Wt. ; 11.37 ± 1.59 cm, TL) were stocked in six HDPE cages measured 3m x 6m, stocked with 1,000 pieces of fries for each cages. The experiment was conducted in six months. The fishes were fed with formulated feed twice daily until satiation for the first three months and with trash fish onwards. Thirty pieces of fish were taken as sample every month in every cage to measure their body weight (BW) and total length (TL). After six months, mean total length for Batch 1 hybrid grouper was 29.28 ± 3.87 and 28.98 ± 2.19 cm for Batch 2. Meanwhile, the mean body weight was 615.7 ± 280.82 and 517.05 ± 107.49 g, respectively. There was no significant difference between Batch 1 and Batch 2 ($p > 0.05$). It's showed that hybrid grouper can grow faster in this study.

THE MORPHOMETRIC VARIATIONS OF HORSESHOE CRAB (*Carcinoscorpius rotundicauda*) COLLECTED FROM TWO DIFFERENT ECOLOGICAL HABITATS OF PENINSULAR MALAYSIA

Rozihan, M*, Nabilah, N.W, John B.A, Joni, H and Yuzine, E

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

The muddy horseshoe crab or the specific name is *Carcinoscorpius rotundicauda* can be found on most of the states in Malaysia. However, the information regarding morphometric variation and genetic variation of *C. rotundicauda* in Malaysia were limited. The objectives of the study were to measure the morphometric parameters of the horseshoe crab, *C. rotundicauda* in Pantai Klang, Selangor and Pantai Merlimau Melaka. The average of the total length for male horseshoe crab was 20.93 ± 2.13 cm, while for the female horseshoe crab was 23.34 ± 2.02 cm in Pantai Klang (Table 1). For Pantai Merlimau, the average total length for male was 20.46 ± 2.12 cm, while for the female horseshoe crab was 27.95 ± 2.73 cm. Mean of weight for male and female of *C. rotundicauda* in Pantai Klang were 44.44 g and 52.5 g respectively while in Pantai Merlimau were 85.2 g and 131.0 g (Table 1). The allometry relationship between different body parameters in Pantai Klang and Pantai Merlimau mostly had positive allometry relationships. Therefore, this study showed the morphometric variation information of *C. rotundicauda* in some regions of Peninsular Malaysia. These information can be useful for the study of the conservation and genetic population of *C. rotundicauda* in the future.

Table 1 Mean of the different body measurements of *C. rotundicauda* in Pantai Klang, Selangor and Pantai Merlimau, Melaka.

Parameters	Pantai Klang, Selangor		Pantai Merlimau, Melaka	
	Male $\bar{x} \pm SD$	Female $\bar{x} \pm SD$	Male $\bar{x} \pm SD$	Female $\bar{x} \pm SD$
Total length (cm)	20.93±2.13	23.34±2.02	20.46±2.12	27.95±2.73
Carapace length (cm)	10.27±0.75	11.56±1.12	10.34±0.92	13.39±1.19
Carapace width (cm)	10.49±0.77	11.28±0.99	9.45±1.72	13.98±1.47
Eye to eye (cm)	5.24±0.37	5.82±0.66	5.21±0.52	7.25±3.14
Opisthosoma length (cm)	6.16±0.52	6.88±0.75	6.01±0.49	9.18 ±1.57
Prosoma length (cm)	5.19±0.38	5.68±0.54	5.24±0.51	6.57±0.99
Telson length (cm)	12.19±1.23	12.26±0.90	11.25±1.49	15.8±2.28
Weight (g)	44.44±25.29	85.20± 32.55	52.50±13.94	131.0±30.92

MICROALGAE INCLUSION AFFECTS BACTERIAL COMMUNITY COMPOSITION IN RECIRCULATING AQUACULTURE SYSTEM

Norulhuda Mohamed Ramli*, C. Giatsis, F.M. Yusoff, J.A.J Verreth and M.C.J Verdegem

Wageningen University and Research
Current address;
Department of Biological and Agricultural Engineering
Faculty of Engineering, Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia
norul49huda@gmail.com

Literatures provide untested observations that fish in the green water are more vital, robust and less sensitive to diseases than those reared in algae-free systems. We hypothesized that the underlying mechanism could be related to the vitality of the microbiota in the system.

In this study, the inclusion effect of microalgae on bacteria community in recirculating aquaculture system (RAS) was investigated. The RAS with microalgae (RAS+A) used in this study included a fish tank (65 L), a sedimentation tank (42 L), a moving bed reactor (nitrification tank) (14 L), two units of microalgae tank (14 L each) and a sump (112 L). RAS without microalgae (RAS-A) had the same set up as RAS+A, except that the tank for microalgae was filled with water and contained no microalgae. To determine the water bacterial composition, water samples from the fish, nitrification and microalgae tanks were collected at the start of the experiment (d0) and on the final day of the experiment (d28). Bacteria community in the system was analysed using denaturing gradient gel electrophoresis DGGE (for day 0 and 28) and 16S metagenomic analysis using Illumina MiSeq technique (Illumina Inc, San Diago, USA) (for day 28).

PERMANOVA test using Bray-Curtis resemblance matrix from DGGE data showed a significant difference of bacterial community between treatments (RAS+A and RAS-A). 16 S metagenomic analysis revealed 5561 operational taxonomic units (OTU) at a similarity threshold of 97% which were grouped into the bacteria domains. No difference of bacterial community composition based on 16 S metagenomic was found between RAS+A and RAS-A (Pseudo-F = 3.9; P-value = 0.056; Unique permutations = 60). Nonetheless, similarity percentage analysis (SIMPER) showed that algal-associated bacteria were more abundant in the RAS+A than in the RAS-A.

This finding suggested that microalgae inclusion could influence bacterial community in the RAS. In the future, the role of microalgae to steer bacterial community to the desired composition should be explored.

COMPARISON ON EMBRYONIC AND LARVAL DEVELOPMENT OF *Pangasianodon hypophthalmus* (SAUVAGE, 1878) AND *Pangasius nasutus* (BLEEKER, 1863)

Siti Fairus Mohamed Yusoff*, Annie Christianus, Hassan Mohd Daud, Yuzine Esa and Muhammad Fadhil Syukri Ismail¹

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia
Email: bicarahati_fai1186@yahoo.com

River catfish, *Pangasianodon hypophthalmus* is a popular fish in the aquaculture industry due to several reasons, high growth rate, omnivorous feeding habits, strong resistance to disease and tolerance to low dissolved oxygen level. *Pangasianodon hypophthalmus* has been successfully produced through artificial fertilization and cultured in Malaysia and several South East Asian countries. Nevertheless, its close relative, *Pangasius nasutus* production is almost nonexistent due to major constraint in seed production. Low fertilization, hatching, fry survival and growth were observed for *P. nasutus* produced through induce breeding technique. Important information on the basic biological features of oocytes and eggs, and larval development of this species is still lacking and could limit aquaculture production. Thus, the present research was conducted to observe the embryonic stages and determine the morphological differences between *P. nasutus* and *P. hypophthalmus*, which may contribute to the success of it's seed production.

Ovulations were induced by two injections of commercial hormone, Ovaprim (Syndel Asia) 0.1 ml/body weight for first injection and 0.5 ml/body weight for second injection at a 14 h interval. Artificial fertilizations were carried out 8 h after second injection. The embryonic stages were observed under a microscope. Larval growths were measured weekly and survivals were counted on the fourth week for both species. Results showed percentage of fertilization for *P. hypophthalmus* and *P. nasutus* were 80.0 % and 40.0 %, respectively. Meanwhile, incubation period was relatively longer in *P. nasutus* (25 h) compared to *P. hypophthalmus* (21 h). This result showed that the embryonic development is faster in *P. hypophthalmus* than in *P. nasutus*. Larvae commence first feeding at 38 h after hatching (hAH) for *P. hypophthalmus*, which is earlier than *P. nasutus* (42 hAH), while yolk sac absorption was completed at 4 day after hatching (dAH) and 5 dAH, respectively.

Growth and larval survival were higher in *P. hypophthalmus* (total length, 2.01 ± 0.58 mm; survival, 70.0 %) than *P. nasutus* (total length, 1.97 ± 0.37 mm; survival, 15.0 %). Species-specific and temperature are two main factors influencing embryonic development for both species. Cannibalism was observed in *P. nasutus* which affects the larval survival. In this case, it is recommended that first feeding should be provided sufficiently at the right time to prevent cannibalism. Even though these species are under the same family, they showed species-specific biological features. Thus, this finding can be used as guidelines to improved and managed eggs incubation and larval rearing of *P. nasutus*.

EFFECTS OF MAGNETIC WATER ON THE GROWTH PERFORMANCE AND SURVIVAL OF HYBRID RED TILAPIA *Oreochromis niloticus*

Vean Salih Mohammad*, Yuzine Esa, Mohd Salleh Kamarudin, and Annie Christianus

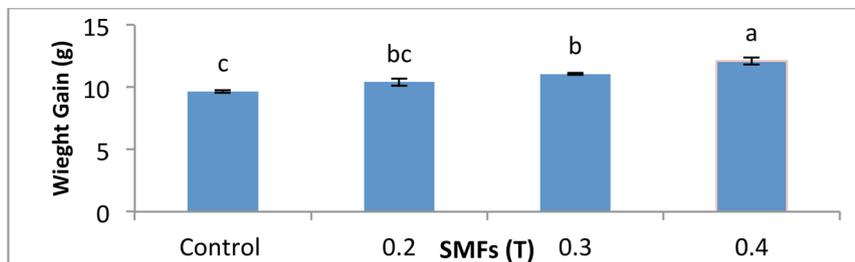
*Department of Aquaculture
Faculty of Agriculture
University Putra Malaysia UPM
43400 Serdang, Selangor, Malaysia
Vyan.mohammad@yahoo.com

Environmental factors affecting living animals, including fish, are divided into two types: changeable (biocoenotic, climatic etc.) and stable, or relatively stable (gravitation, earth magnetism). Water is needed to transport compounds via the blood, to maintain cellular structural integrity, regulate temperature, etc. (Reuter, 2004). Restructured water or magnetic water (MW) can be formed using permanent magnets. Magnetically treated water has its effect on the human body when taken internally and regularly for a considerable period of time (Lam, 2001). Therefore, this study was conducted to assess the effects of MW on growth performance and survival of red hybrid tilapia *Oreochromis* sp.

Experiment was carried out between September to December 2015 in Aquaculture Research Station, UPM, Puchong, Selangor, Malaysia. Red hybrid tilapia *Oreochromis niloticus* fingerlings were acquired from local supplier, then acclimated to the experimental conditions for 2 weeks. Treatments were control and three strength of static magnetic fields (SMFs) of (0.2, 0.3, and 0.4)T. Randomly, 240 tilapia were distributed in 12 aquaria (n=20) and divided into four different groups, with each treatment triplicated. Temperature was kept at 26 – 29 °C with dissolved oxygen greater than 5 mg/L. Experimental fish were fed twice daily (0900 and 1600) at 4% body weight per day with commercial fish pellet containing 34% crude protein.

Both the weight gain WG and specific growth rate SGR of the animals exposure to the different SMFs were significantly different ($P < 0.05$) compare to the control. The highest values, 12.11 g and 2.68 % respectively were recorded in treatment 0.4T. While the lowest survival 93 % observed in the control was significantly different from other treatments. These results indicated the effect of magnetism the living cell. Earlier researches showed that the equilibrium of living cell can be restored with the help of magnets. Water passed through the magnetic field acquires finer and more homogeneous structures. Based on the results obtained, the water treated by static magnetic fields could positively influence the growth, weight gain and survival of hybrid red tilapia fingerlings.

Fig 1: Weight gain (g) of red tilapia fingerlings reared in different magnetic water



BEST GROWTH CONDITION FOR *Gracilaria manilaensis* (RHODOPHYTA) UNDER DIFFERENT SALINITIES, pH AND LIGHT INTENSITIES AND IT'S TYPE OF CARRAGEENAN

Normawaty Mohammad Noor*, Nor Salamah Muhamad Hidayat, Deny Susanti, Yukinori Mukai and Shahbudin Saad

Department of Marine Science
Kulliyyah of Science
International Islamic University Malaysia
Jalan Sultan Ahmad Shah
25200 Bandar Indera Mahkota
Kuantan, Pahang

Seaweed from genus *Gracilaria* is well known for their characteristic as agarophyte and has the potential as source of carrageenan, a polysaccharides. For commercial production, an enormous amount of raw seaweed is required. Thus, mariculture can become the alternative way to meet the growing demand of seaweed as raw materials. Based on literatures, it is well documented that growth rate and carrageenan yield depend on environmental factors such as salinity, pH, temperature, light intensity and water movement. Therefore, in this study, growth rate and carrageenan yield of *Gracilaria manilaensis*, a red seaweed, were determined at different conditions. *G. manilaensis* was cultured under laboratory conditions in a 500 mL flask of seawater at different light intensities (100, 500 and 1000 lux), salinities (15, 20, 25 and 30 psu) and pH (7.6, 7.8, and 8.0) for 21 days. At the end of experiment, the growth rate was determined followed by carrageenan analysis. Then, the ground seaweed samples were used for Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) to identify the carrageenan type of each treatment. From the analyses, the highest growth rate was observed at high light intensity, low salinity and slightly alkali pH (1000 lux, 15 psu and pH 7.6). Meanwhile, the carrageenan yield was highest at low light intensity, high salinity and more towards alkaline pH (500 lux, 25 psu and pH 8.0). This indicates that the quality of *G. manilaensis* in term of carrageenan content can be optimized under certain growth conditions. Analysis of carrageenan types showed that different types of carrageenan i.e. kappa, iota and lambda were observed under different conditions. Findings from this study provide important information in developing culture of *Gracilaria* for high biomass and/or for carrageenan production. The identification of different types of carrageenan from *Gracilaria* at different growth conditions indicates that this species has high potential to be commercialized however further study is needed to quantify carrageenan's yield.

THE USE OF RECIRCULATION SYSTEM ON PRODUCTION OF ROTIFER (*Brachionus plicatilis*) IN FISHERIES RESEARCH INSTITUTE TG. DEMONG

Shaharah Mohd Idris¹, Aluwi Sulaiman^{1,1}, Nik Haiha Nik Yusoff¹, Zainuddin Jambari², and Siti Zahrah, Ahmad³

¹ Fisheries Research Institute, TanjungDemong, Besut, Terengganu

²Fisheries Research Institute, Batu Maung, Penang

³National Fish Health and Research Centre (Nafish), Fisheries Research Institute (FRI), Penang, Malaysia

Studies on high density production of rotifer using recirculating aquaculture system were conducted in FRI Tg. Demong with collaboration from Nafish for RMK 11. The continuous culture system consists of 500L of culture tank, 1ton of settlement tank, a protein skimmer and 500L of aeration tank. Preliminary trial run of the system indicated that there a few problems that need to overcome in order to run the system, and preliminary results have shown that the rotifer counts increased from 500 ind/mL to 3000 cells/mL but decrease due to clogging of filter. In addition water qualities were also improved by using probiotic in biological filter which reduces the concentration of ammonia from 1 mg/L to 0.02 mg/L. In general terms it can be stated that the use of a recirculation system has proved to reduce labour and maintenance cost while ensuring stable physic-chemical rearing condition resulting in more reliable and healthy rotifer.

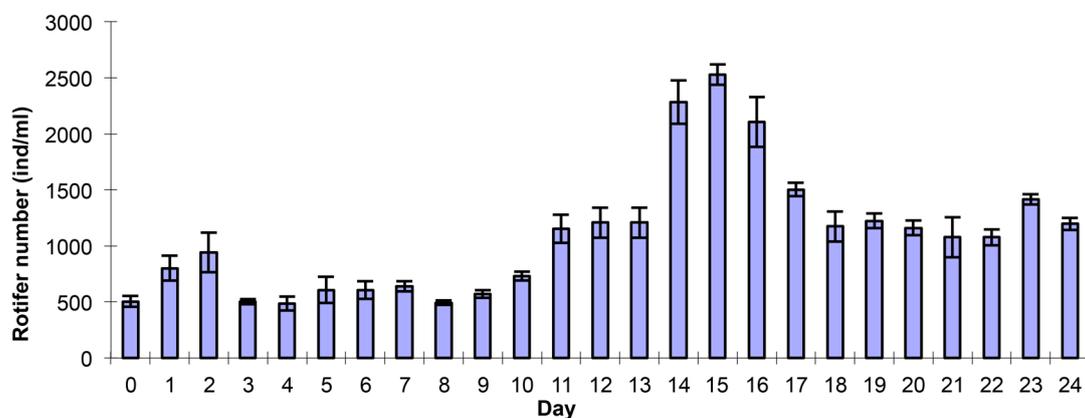
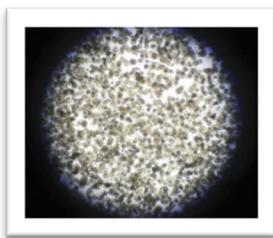


Figure 1: The number of rotifers produced per day

GENETIC VARIABILITY OF *Tachypleus gigas* (MULLER), HORSESHOE CRAB FROM JOHOR, INFERRED BY CYTOCHROME OXIDASE SUBUNIT 1 GENE

Mohd Lazim Mohd Saif*, Masazurah A. Rahim, Abu Bakar Tumin, Amatul Samahah Md Ali and Azlina Apani

Brackishwater Aquaculture Research Division, Fisheries Research Institute, 81550 Gelang Patah, Johor, Malaysia

*Corresponding Authors e-mail: lazimsaif@gmail.com

In Malaysia, horseshoe crab *Tachypleus gigas* (Muller) research on abundance and morphology have been done but little information on phylogeny and genetic. This study was conducted to investigate the variability and structure of population in Mersing and Sedili in East Coast while Johor Bahru, Pontian, Batu Pahat and Muar in West Coast. The results of sequencing analysis of 49 samples, there are only 7 different sequences. We can note, sequence or haplotype TG26 only exist in the east coast locations (Mersing and Tanjung Sedili), while TG4 haplotype shared by all locations. The constructed phylogram clearly indicated that the horseshoe crabs are closely related. Among the sampled collected, 7 haplotypes as well as with *T. tridentatus* sample as an out group clearly clustered *T. gigas* in separate branch indicating high phylogenetic cues in mtDNA COI gene. A total of 49 individuals of adult horseshoe crab, *T. gigas* were collected from 6 different shorelines in Johor, Peninsular Malaysia, namely Mersing, Tanjung Sedili, Johor Bahru, Pontian, Batu Pahat and Muar. Live samples for each population were collected from the fishermen and were brought back to the laboratory. A gills clip of the horseshoe crab *T. gigas* was extracted using DNeasy blood and tissue kit (Qiagen, Germany). The partial cyt b gene was amplified by a pair of primers, L14841 (5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3') and H15149 (5'-AAACTGCAGCCCCCAGCAATGATATTTGTCCTCA-3'); [9]. Thermocyclic condition for PCR included the initial denaturation at 94°C for 15 sec, annealing at 45°C for 15 sec, and extension at 72°C for 10 sec, with a final extension at 72°C for 10 min, followed by indefinite hold at 4°C. Final PCR products were sent to FirstBase Laboratories Sdn Bhd for sequencing. Sequences was align using Clustal application of MEGA 6.0 software. Genetic structure analysis is carried out using MEGA6.0, DNsp and Arlequin. Detail of the sequences used to generate the phylogram. The highest number of *T. gigas* found in Pontian(22) meanwhile 5 in Mersing, 4 Muar, 5 Batu Pahat, 2 Johor Bahru and 8 Tanjung Sedili, Kota Tinggi. Based to analysis, indicated there are 7 of haplotypes was found. Among these haplotypes 1 (TT26) is unique to locations on the east coast of peninsular Malaysia (Mersing & Tg Sedili). Neighbor-joining (NJ) phylogram (fig. 1) showing the relationship among haplotypes of *T. gigas*. The numbers at each node represents the bootstrap percentage values based on 1000 pseudo replications for NJ/MP analyses. *T. tridentatus* (TT) used as an out group was clearly clustered in separate branch proves the reliability of the constructed phylogram. The constructed phylogram clearly indicated that horseshoe crabs are closely related. Among the sampled collected, 7 haplotypes as well as with *T. tridentatus* sample as an out group clearly clustered *T. gigas* in separate branch indicating high phylogenetic cues in mtDNA COI gene. The partial cyt b sequences in the phylogram constructed clearly showed that *T. gigas* (Mersing and Sedili) location on the east coast of Peninsular Malaysia contain haplotypes 1 (TG26) which unique to this location that not exist in sequence represent by *T. gigas* in West coast of Peninsular Malaysia (Pontian, Batu Pahat, Muar and Johor Bahru). But further studies need to be conducted to prove this concept by analysing fully cyt b gene or other DNA markers.

DIETARY CRICKET MEAL: EFFECT ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF AFRICAN CATFISH

Norhidayah M. Taufek*, Syuaib Supani, Shahrul Hafiz M. Zaini, Noor Hidayati A. Bakar, Hasniyati Muin, Shaharudin A. Razak

AquaNutri Biotech Research Laboratory, Institute of Biological Science, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia

Glami Lemi Centre for Biotechnology Research, Glami lemi, Titi, 71650, Jelebu, Negeri Sembilan, Malaysia

hidayahtaufek@gmail.com

The high demand of fishmeal (FM) as aquafeed in aquaculture industry has become a limiting factor for African catfish farming in Malaysia. Cricket meal (CM) is one of many insect meals that have been studied as fishmeal replacement for African catfish. However, there have been no controlled studies, which compare differences in crude protein level of cricket meal in African catfish broodstock diet. It is well established that reproductive performance and egg quality of fish broodstock are influenced by dietary nutrient intake. Protein could affect rate of spawning, quantity and quality of eggs as well as gametogenesis in the female fish. This research examined the growth and reproductive performance of African catfish fed with varying levels of crude protein (CP) at 35% and 40% from cricket meal compared to 35% CP from fishmeal as control in duplicate treatments of 10 fish per replicate. The feeding trial was conducted over a period of 56 days before the fish being induced through hormonal treatment by using Ovaprim for reproductive performance.

We observed that cricket meal-fed fish showed higher body weight gain (BWG), specific growth rate (SGR) and food conversion ratio (FCR), but lower protein efficiency ratio (PER) than fishmeal-fed fish. In term of reproductive performance, our studies also reported higher gonadosomatic index (GSI), fecundity rate and fertilization rate in fish fed with cricket meal. However, lower hatching rate were observed in groups fed with cricket meal compared to the control group. No mortality was recorded during the experimental period. As no significant differences were found in between fish fed with 35% and 40% CP of cricket meal, we concluded that cricket meal with 35% crude protein level is sufficient to replace fishmeal with comparable performance on the growth and reproduction of the catfish.

Table 1. Growth and reproductive performance of fish fed the experimental diets

	Diets		
	CM (35% CP)	CM (40% CP)	FM (35% CP)
BWG (g/g)	48.07 ± 1.82 ^a	58.49 ± 6.47 ^a	40.59 ± 5.46 ^a
SGR (g/g)	0.99 ± 0.06 ^a	1.15 ± 0.11 ^a	0.87 ± 0.05 ^a
FCR	1.34 ± 0.01 ^b	1.36 ± 0.01 ^b	1.22 ± 0.01 ^a
PER	2.13 ± 0.01 ^b	1.84 ± 0.02 ^a	2.35 ± 0.01 ^c
GSI	10.14 ± 0.83 ^a	8.29 ± 0.17 ^a	6.93 ± 0.95 ^a
Fecundity rate (×10 ⁴)	5.09 ± 1.23 ^a	4.63 ± 0.25 ^a	4.15 ± 0.45 ^a
Fertilization rate (%)	59.93 ± 7.00 ^a	62.35 ± 15.30 ^a	58.44 ± 2.48 ^a
Hatching rate (%)	59.98 ± 3.07 ^a	61.68 ± 2.82 ^a	61.87 ± 0.54 ^a

EFFECT OF BEEF LIVER MEAL IN MATURATION DIET TO ENHANCE REPRODUCTIVE RESPONSE OF DOMESTICATED KELAH (*Tor tambroides*)

Hanan Mohd Yusof*, Muhamad Zudaidy Jaapar, Tazri Amil Shafie, and Mohd. Fariduddin Othman

Freshwater Fisheries Research Department
FRI Glami Lemi, 71450 Titi, Jelebu
Negeri Sembilan, Malaysia
hananppat@yahoo.com

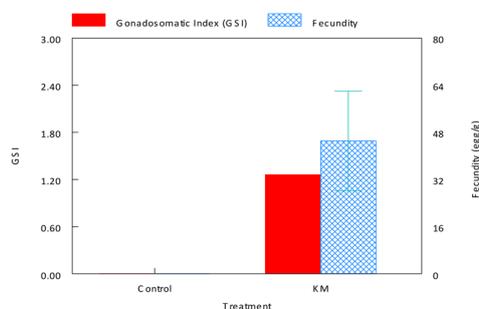
Kelah (*Tor tambroides*) is a riverine cyprinid, commonly known as the Malaysian Mahseer. As a fish with excellent taste however, the price is expensive that may fetch between USD 180 – USD 300 for a 1 kg live specimen. This has led to increase in fishing pressure on the natural resources. Artificial breeding of *Tor tambroides* for conservation and aquaculture is important and will ensure their survival and may reduce the negative impacts on natural stocks. However, to support this programme, it is need for specially formulated maturation diet to optimise and enhanced its maturity. Arachidonic acid (ARA) that can be found in beef liver was known to be a precursor for prostaglandins hormone (PGE2) to enhance maturation activity for fish broodstock. The objective of this study was to evaluate the effect of beef liver meal (BLM) to enhance reproductive response in the maturation diet of domesticated Kelah broodstock.

The study was conducted for 18 months using a fully indoor recirculating aquaculture system (RAS) tanks. 40 individual female Kelah broodstock of same parental breeding pair was individually tagged using PIT tag and randomly distributed in four 5MT RAS tanks. The experimental fish was fed with treatment diet, KM (BLM inclusion) and control diet (commercial diet) in duplicate. All diets were isonitrogenous with 34% CP and 6% CL. After six months of feeding protocol the sampling for breeding performance was conducted every three month intervals after undergoing artificial propogation for Kelah according to De Silva et al. (2004). The parameters for reproductive performance were breeding response, gonadosomatic index (GSI), relative fecundity, hatching rate, water hardened egg diameter and water hardened of individual egg weight were analysed and statistical tested using T-test.

After 18 months of experimental period, the result significantly ($P < 0.01$) showed an improvement of Kelah (final average size of 1.7 ± 0.2 kg) maturity with a 40% breeding response within the population compared non-response to Kelah fed with control diet. While, the comparison of some breeding performance of Kelah broodstock fed with different diets were shown in the presented table below.

The significant impact on breeding response and performance on Kelah broodstock fed with diet that had been formulated with BLM might be influencing the fish reproductive physiology. Further study are still ongoing to determine the effects of PGE2 hormonal level and reproductive performance of Kelah feed with broodstock diet.

Comparison of Breeding Performance (GSI and Fecundity) of Kelah Broodstock Fed with Treatment (KM) and Commercial Diet



INDUCED SPAWNING OF THE BLOOD COCKLE *Anadara granosa* IN HATCHERY

Mohd. Saleh, Mohd.Taha*, Hadzley, Harith,¹ Kua, Beng Chu.¹, Ku Kassim, Ku.Yaakob.¹, Masazurah, Abdul.Rahim.¹, Roziawati, Razali¹ and Md Fauzan K, Mohd.Yapandi.²

Crustacean Aquaculture Research Division
FRI Pulau Sayak
08500 Kota Kuala Muda, Kedah
salehtaha93@yahoo.com.my

¹ Fisheries Research Institute, Batu Maung, Pulau Pinang

² TNB Research Sdn Bhd, Bangi, Selangor

Induced spawning of the blood cockle, *Anadara granosa* in the hatchery using temperature differences of 5-6°C was successfully obtained in November 2016. The objective of this project is to investigate the technique of induced spawning on Malaysian blood cockle in hatchery and its potential application at spawning ground in Kapar, Selangor. A total of 3.0kg cockle broodstock from Sabak Bernam, Selangor (same broodstock source placed at Kapar) were procured and brought back to hatchery for spawning. Broodstock average size (n=31:range from 32mm–43mm) were 36.4±7.4mm in body length, 28.3±2.2mm in body width, 26.6±2.2mm in body height and 17.5±4.1g of body weight.

Prior to spawning, maturity study was done to examine the readiness of the broodstock for spawning. The result shows that sex ratio was 29% female and 61% male while 6% unknown due to the effect of total releasing gametes or spent stage. Gonad maturity examination showed that 90% of the gonad were at stage II (Ripe) and 4% at stage III (Release). All these biological assessments showed that the broodstocks were very ripe and ready for induced spawning. With these results, a breeding attempt was done on 22nd Nov 2016 at 2.00pm using 5-6°C differences of sea water temperature i.e. triggering with warm seawater of 34-35°C for a period of 2-3 hours in the spawning tank (normal seawater temperature in the broodstock tank was 28-29°C) and then the broodstock were put to rest by reverting to its normal temperature via feeding algae for up to 7 hours and all these processes were repeated. The matured broodstock responded to the stimulation process at 12.00 noon on 23rd Nov 2016 producing a total of 10 million eggs with the size of 53.3±4.0µm and metamorphosed the next day into straight hinged larvae sized 88.5±5.3µm in body length and 67.8±4.5µm in body width respectively with hatching rate of 50% producing up to 5 million straight hinged larvae. A control experiment was also done without a temperature shock and no spawning was observed.

This successful induced spawning in the hatchery suggested that it may be applied to the cockle spawning ground in Kapar, Selangor which experienced high temperature seawater discharge from electric power plant i.e. Kapar Energy Venture (KEV). The induced spawning shall occur during the Great Diurnal Tide (GT) and Lowest Astronomical Tide (LAT), where the temperature shocks on cockle happen during low tides. Through this studies all related information on broodstock conditioning, breeding methodology and life cycle of the cockle larvae were recorded to refine and understand of the biological needs during their life cycle stage and also to relate these information with their spawning ground at Kapar, Selangor.

INTRODUCING MACCAFERRI KIKKONET, AN EXCELLENCE MADE IN MALAYSIA AND USED IN CAGE AQUACULTURE WORLDWIDE

Marco Montagnoli – Product Development Manager
Email: marco.montagnoli@maccaferri-asia.com

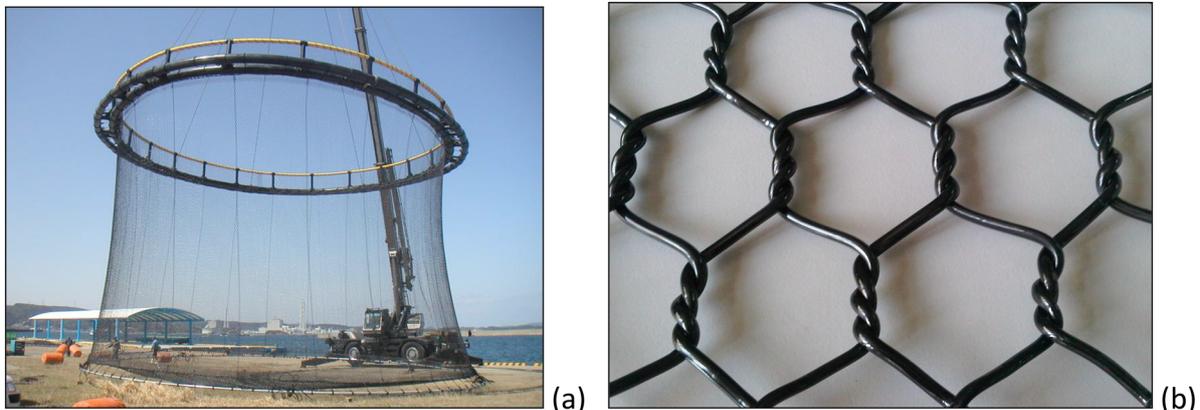
Maccaferri (Malaysia) Sdn Bhd
Unit 3A-11, Block G, Phileo Damansara 1
No. 9 Jalan 16/11, Off Jalan Damansara
46350 Petaling Jaya, Selangor Darul Ehsan, Malaysia
Ph/Fax: (60-3) 7957 8330

KikkoNet provides the best opportunity for less maintenance, better protection and safety for the fish in today's aquaculture. KikkoNet is a high performance net constructed from PET (polyethylene terephthalate) monofilaments that are double twisted in a hexagonal mesh.

The PET monofilament is a single, hard and smooth plastic wire; the surface area for parasites is limited compared to multifilament wire, where they could deposit the eggs between the fibres. Growth of biofouling is very limited and fouling detaches easily, without occluding meshes thus offering high dissolved oxygen in the water flowing in the cage.

Nets made of KikkoNet offer more protection against predators like seals, sharks and sea lions. The material has a higher abrasion and cut resistance compared to other types of netting. This results in better resistance, with the ultimate advantage in less stress and losses of fishes and less damages to the nets.

Since 2011 KikkoNet is being used in several marine net pens installed worldwide for farming operations of several fish species. Successful experiences are generating more demand as KikkoNet will create more benefits for farmers.



Pic. 1 – Fully preassembled KikkoNet connected to the floating collar and to the sinker tube (a); Hexagonal Double Twisted PET mesh detail (b).

COMPARATIVE HISTOMORPHOMETRICAL STUDY OF GILL IN MATURE AND IMMATURE SILVER CARP

Hassan Morovvati,* Sara Fallah, Mehrzad Mesbah, Zahra Minoosh Siavash Haghighi, Amineh Arefi

*Professor, Department of Histology, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran
hmorovvati@ut.ac.ir

This study was conducted on 10 immature silver carps with mean body length and weight about 10.95 ± 0.36 cm and 12.02 ± 1.08 gr and 10 mature silver carps with mean body length and weight about 41.4 ± 1.07 cm and 1.5 ± 66.6 gr, respectively to evaluate the morphology and morphometric measurements of gills in this species. Grossly gills consist of four gill arches within operculum which located on either side of the pharynx. Freshly this organ appears to be bright red in color. Samples of gills were harvested as 0.5cm in diameter and fixed in Formalin 10% solution, then the routine tissue processing steps were performed and samples were cut into 5 to 6 μ m in diameter and finally were stained with hematoxyline & eosin and acid Schiff solutions. Micrometric studies on the thickness of epithelial tissue covering the primary lamellae and gill rakers at both right and left sides in both mature and immature fishes, doesn't show significant differences. In mature fishes, the epithelial covering of gill rakers was measured thicker in apical area comparing to other parts. The results also revealed that the number of orifices of gill raker gap decreased with fish's weight but larger in diameters in both mature and immature fishes. The number of mucous cells in club-shape of primary lamellae was much greater in number than in the other parts which reflects more mucous secretion in these areas. The covering epithelium of lamella in this specific species, doesn't show any difference with other fishes in the family. The only difference was in the amount and length of the gill rakers.

REPLACEMENT OF SQUID AND KRILL MEAL BY SNAIL MEAL *Buccinum striatissimum* IN PRACTICAL DIETS FOR JUVENILE KURUMA SHRIMPS *Marsupenaeus japonicus*

Amina S. Moss*, Shunsuke Koshio, Manabu Ishikawa, Saichiro Yokoyama, Truong H. Nhu, Mahmoud A. O. Dawood, Weilong Wang

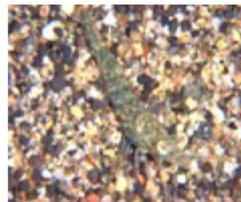
Laboratory of Aquatic Animal Nutrition, Faculty of Fisheries
Kagoshima University
Shimoarata 4-50-20 Kagoshima 890-0056, Japan
aminasmoss@gmail.com

Kuruma shrimps (*Marsupenaeus japonicus*) are considered quite valuable in Japan and they are some of the largest shrimp species in the penaeidae family with females reaching nearly 30 cm in body length. These carnivorous species require a higher amount of protein than other shrimp species at about 55%. This dietary requirement has made it difficult to create optimum diets for them. Fortunately, in Japan, squid and krill meals have been found to be most ideal protein sources for these shrimps. In an effort to promote the use of locally available ingredients to create potentially cheaper feeds, marine snails, *Buccinum striatissimum*, have been used in the present study to see their effectiveness in replacing squid and krill meal in the diets of kuruma shrimps.

A 60-day feeding trial was conducted in order to investigate the effects of substituting the marine snails for squid and krill meal into the diets of juvenile kuruma shrimps. Five experimental diets were formulated to contain varying levels of snail meal at 0%, 25%, 50%, 75%, and 100% (D1, D2, D3, D4 and D5, respectively) and fed to juvenile kuruma shrimp (initial mean body weight 0.27 ± 0.02 g). A natural light and dark regime was observed throughout the experiment and temperature, pH and salinity were recorded with mean values of $23 \pm 0.7^\circ\text{C}$, 7.7 ± 0.5 and 34 ± 0.06 g/L, respectively.

The results showed that growth, total body length, apparent feed intake and specific growth ratio were significantly improved ($P < 0.05$) with 75% and 100% substitution of snail meal. There was no significance ($P > 0.05$) detected in survival rate among diet groups. Lipid content in shrimps fed D1 and D3 were significantly higher while cholesterol content in shrimps fed D4 and D5 were significantly lower and polyunsaturated fatty acid contents were significantly higher in those groups. During the low-salinity stress test, diet groups fed 50-100% snail meal showed a significantly higher resistance ($P < 0.05$) to stress compared with the control group and shrimps fed 25% snail meal. These results suggest that supplementing snail meal for complete replacement of squid and krill meal at 100% can be done in order to improve juvenile kuruma shrimps' growth, stress resistance and to reduce their cholesterol levels.

	Experimental Diets				
	D1	D2	D3	D4	D5
Snail Meal	0	16	32	45	69
Krill Meal	18	13.5	10	6	0
Squid Meal	36	27	19	13	0
Fish Meal	5	5	5	5	5
Soybean Meal	5	5	5	5	5
Other	36	33.5	29	26	21



CHALLENGES ASSOCIATED WITH PhD. SUPERVISION IN SUB-SAHARAN AFRICA

By Austin Henderson Ndaona Mtethiwa and Daniel Sikawa

Lilongwe University of Agriculture and Natural Resources (LUANAR)
P.O. Box 219, Lilongwe, Malawi
Email: amtethiwa@bunda.luanar.mw

Research project supervision plays a paramount role in timely completion and outstanding quality of PhD. Thesis. Effective supervision is a conglomerate of various artifacts built around exceptional skills, capacity building as well as mutual understanding of supervisee/ supervisor.

A systematic literature review was conducted on the supervisee/supervisor relationship in sub-Saharan Africa. Literature was searched were using the keywords: “supervisor”, “Supervisee”, “PhD” and “Thesis” in combination with “Sub-Saharan Africa”. These searches were supplemented by reviews of lists for relevant publications in peer reviewed scientific journals or other media. The recovered information were included based on their regarding supervisor/supervise challenges and relationship.

Findings showed that there are a number of challenges that are faced in the supervisory process. Among them are negative attitude by the supervisor which are aggravated by student uncooperativeness, laziness, gender, origin as well as unclear rights and obligations. Besides students may become aggressive on to their supervisors due to untimely commenting on their submitted work due to either tight programs by the supervisors, negligence or insufficient knowledge in the subject area. This in many cases ends up in sour relationship that leads to untimely completion of the studies by the students.

The findings and suggested solutions to this study will from enhance an excellent relationships between supervisor and supervisee that will bring effective supervision leading to timely completion and excellent research output and theses.

AQUACULTURE SPECIES ARE EXTREMELY SENSITIVE TO MYCOTOXINS

Michele Muccio*¹, Rui A. Gonçalves¹

¹BIOMIN Holding GmbH, Austria

*Email: michele.muccio@biomin.net

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 as well, especially due to the increasing tendency to include plant-derived meals in aquatic diets.

Aflatoxins are potent carcinogens and are responsible for liver lesions and other hepatic alterations (reduction of viability of hepatic cells, lipid deposition, venous congestion, etc.) in several economically relevant aquatic species such as sea bream, tilapia, catfish, shrimps and pangasius (1).

Trichothecenes such as deoxynivalenol are inhibitors of protein synthesis. These mycotoxins are highly immunosuppressive and cause effects such as reduction of growth performance, feed efficiency and histopathological changes in aquatic species. Tilapia, carp and rainbow trout are among the most sensitive species to this class of mycotoxins and their effects have been reviewed in the literature (2, 4).

Zearalenone is an estrogenic mycotoxin and it is the major cause of hyperestrogenism in animals such as pigs and poultry. Shrimps in particular are quite sensitive to zearalenone and effects on growth and reproduction have been observed as well (5).

Fumonisin are potent neurotoxins and are common contaminants of corn and corn by-products. They affect the biosynthesis of complex sphingolipids and exhibit effects such as growth and immune suppression in several aquatic species such as the white leg shrimp (3). Fumonisin undergo synergistic interactions with aflatoxin B₁, increasing the severity of this mycotoxin.

References:

1. Gonçalves RA, Karin Naehrer K and Gonçalo A. Santos GA (2016). Occurrence of mycotoxins in commercial aquafeeds in Asia and Europe: a real risk to aquaculture? *Reviews in Aquaculture* 0, 1–18.
2. Hooft J.M., El Hakeem A., Ibraheem Elmor I., Encarnação P., Bureau D.P. (2011). Rainbow trout (*Oncorhynchus mykiss*) is extremely sensitive to the feed-borne Fusarium mycotoxin deoxynivalenol (DON). *Aquaculture* 311: 224–232.
3. Mexía-Salazar A.L., Hernández-López J., Burgos-Hernandez A., Cortez-Rocha M.O., Castro-Longoria R., Ezquerro-Brauer J.M. (2008). Role of fumonisin B₁ on the immune system, histopathology and muscle proteins of White shrimp (*Litopenaeus vannamei*). *Food Chemistry* 110: 471-479.
4. Pietsch C., Michel C., Kersten S., Valenta H., Dänicke S., Schulz C., Kloas W., Burkhardt-Holm P. (2014). *In vivo* effects of deoxynivalenol (DON) on innate immune responses of carp (*Cyprinus carpio* L.). *Food Chem Toxicol.* 68:44-52.
5. Supamattaya K, Bundit O, Boonyarapatlin M, Schatzmayr G, Chittivan V (2005) Effects of mycotoxin T-2 and zearalenone on histopathological changes in black tiger shrimp (*Penaeus monodon* Fabricius). *Songklanakarin Journal of Science and Technology* 27: 91–99.

AVAILABILITY OF MINERALS AND CARCASS COMPOSITION OF *Catla catla* FINGERLINGS FED PHYTASE SUPPLEMENTED MORINGA BY-PRODUCTS MEAL BASED TEST DIET

Muhammad Mudassar Shahzad* and Syed Makhdoom Hussain

Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan

*Corresponding Author: dr mudassarshahzad@gmail.com

Mobile #:+92-334-5934911

Present research work was carried out to estimate the influence of phytase supplementation on mineral digestibility of *Catla catla* fingerlings fed *Moringa oleifera* by-products meal based diet. Presence of anti-nutritional factors such as phytate in plant by-products reduces the bioavailability of minerals to fish and decreases carcass composition, resulting in poor fish performance. Moringa by-products such as *Moringa oleifera* leaf meal (MOLM) *Moringa oleifera* seed meal (MOSM) were used as test ingredients to formulate six test diets and were supplemented with phytase levels (0, 300, 600, 900, 1200 and 1500 FTU kg⁻¹). The fingerlings were fed at the rate of 4% of live wet weight twice a day and faeces were collected from each tank to estimate digestibility of minerals. Carcass composition (CP, EE, GE, Carbohydrates, Ash and Moisture contents) was analyzed using standard methods. On the basis of results it was noted that phytase supplementation showed significant ($p < 0.05$) improvement in bioavailability of minerals as well as whole body composition. Maximum minerals digestibility and higher carcass composition was noted at 900 FTU kg⁻¹ level of phytase supplemented MOLM+MOSM based test diet. It was concluded that phytase supplementation at 900 FTU kg⁻¹ level is helpful to develop an eco-friendly and cost effective fish feed by using MOLM+MOSM based diet.

GROWTH AND SURVIVAL OF THE SEA CUCUMBER *Holothuria scabra* LARVAE AT DIFFERENT FEEDING REGIMES

Sitti Raehanah Muhamad Shaleh*, Noor Adzlina Abidin, Ching Fui Fui, Mabel Manjaji-Matsumoto, Shigeharu Senoo & Saleem Mustafa

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS 88400 Kota Kinabalu, Sabah, Malaysia.
sittirae@ums.edu.my

Sandfish (*Holothuria scabra*) is one of the most widely cultured sea cucumber species in the Indo Pacific regions. Unfortunately, most of the sandfish farmer relies almost entirely on the wild captured sandfish. Therefore, hatchery seed production is now underway to meet the needs of aquaculture. Understanding the feeding regime is very crucial because it influenced the growth performance and affects the yield in the hatchery. In a factorial experiment, two microalgae species, *Nannochloropsis* sp. and *Chaetoceros calcitrans* at two different concentrations (2×10^4 and 4×10^4 cells ml^{-1}) were tested on sandfish larvae 2 days after hatching. The experiment was carried out for 2 weeks using plastic containers randomly arranged in a water bath (HDPE tank) with the temperature maintained at 29-30°C. The growth and survival of sandfish larvae were significantly affected by the feeding regime ($p=0.015$ and $p=0.000$), but not affected by the feed concentration ($p=0.160$ and $p=0.512$). The N2C feeding regime (2 days *Nannochloropsis* sp. + 12 days *Chaetoceros* sp.) at 4×10^4 cells ml^{-1} demonstrates the highest growth ($38.09 \pm 6.6\%$) while the highest survival was recorded in the larvae fed in a single species of *Nannochloropsis* sp. at 2×10^4 cells ml^{-1} ($1.37 \pm 0.13\%$). The results also show that larval metamorphosis was very slow as there is no doliolaria stage recorded until the end of the experiment. This study suggests that feeding regime affects the larval development rate and thus will determine the success in the seed production of sandfish.

ASSESSMENT OF PUERULUS CONDITION OF *Panulirus homarus* AND *P. ornatus* IN RELATION TO AQUACULTURE, IN INDONESIA

Muhammad Hidayat* and Clive Jones

Marine Aquaculture Development Centre, Lombok, Indonesia
blackelf85@gmail.com

Rock lobsters have a complex life cycle comprising a protracted larval (phyllosoma) phase, prior to metamorphosis to the adult-like puerulus. The phyllosoma rely on exogenous planktonic food to survive, building a store of energy to support the non-feeding puerulus, an active swimming stage with high energetic needs required to locate suitable habitat on which to settle. The energy reserves may vary in relation to the nutritional state at the time of metamorphosis and the time and energy consumed in locating suitable settlement habitat.

An experiment was performed to examine the energy reserves of newly settled pueruli of two species of tropical rock lobster, *Panulirus homarus* and *P. ornatus* in Indonesia. These species are abundant as settling pueruli, along the southern coastlines of Java, Lombok and Sumbawa, where they are fished commercially for supply to aquaculture farms. The study was conceived to assess if variability in condition may explain variable survival after stocking to aquaculture cages.

A total of 100 clear stage pueruli were randomly assigned to each of starved, fed (control) and point of no return (PONR) treatments for each species. All lobsters were maintained in individual experimental units (IEU) housed in indoor tanks supplied with recirculating sea water. Lobsters were maintained individually to enable close monitoring of development (moult interval), to eliminate cannibalism, and to preclude direct interactions between individuals. Daily observations were made throughout the experiment to record the date on which individual post-puterulus moulted, to allow calculation of the number of days between subsequent moults (moult frequency) and to immediately remove deceased animals. Dead pueruli were removed as soon as possible after death, their total length, weight, coloration (clear, white or full pigmented) and day of death (days since stocking) recorded and then frozen.

To test the effects of extended periods of starvation, post-puteruli were starved from first moult until either they died or were euthanised at specific time intervals for biochemical analysis (total lipid, lipid class, fatty acid, total protein and total glycogen). Individual survival and progress between consecutive moults was monitored daily, with a number of individuals randomly selected for euthanasia and biochemical analysis every 5 days.

To determine whether post-puteruli can subsequently feed and grow after starvation, 20 random specimens were fed after 10, 20 and 30 days of starvation from day 1. The influence of starvation on survival to the next moult was monitored. Post-puteruli were deemed to have recovered from starvation if further growth (moulting) occurred.

Data on mortality, moult interval, moult frequency and body composition are presented.

CULTIVATION OF INFUSORIA AS A STARTER LIVE FEED FOR MARINE FINFISH LARVAE

Yukinori Mukai^{1*}, Muhammad Mu'izzuddin Afandi² & Normawaty Mohammad Noor¹

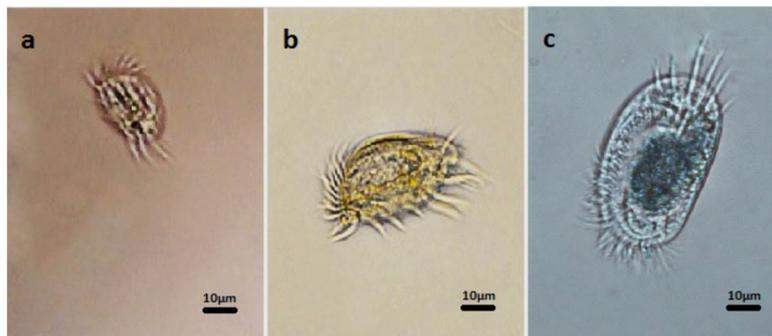
¹Department of Marine Science, Kulliyah of Science, International Islamic University Malaysia

²Department of Biotechnology, Kulliyah of Science, International Islamic University Malaysia

*mukai9166@iium.edu.my

Infusoria are the microscopic single celled animalcules belonging to Class, Ciliata and Phylum, Protozoa. Seed production of marine finfish is still facing problem particularly on suitable live feed for early stage larvae. Currently, S- or SS-type rotifers, which have sizes from 100 to 200 μm , are used as live feed, however we are still searching smaller size live feed for starter diets. Early stage larvae of many marine finfish select feeds less than 100 μm . For example, Asian seabass selected 40-60 μm microcapsules when offered a range of 15-150 μm . In limited studies, infusoria has been identified to be optimum live feed, because their body sizes are less than 100 μm . Infusoria have been successfully used as live feed for limited number of marine finfish larvae with small size mouths. In nature, fish larval stomachs are reported to be filled with infusoria.

In this study, we cultivated infusoria just using vegetable and fish meal. About 5000 cells /ml infusoria could be cultured. Larval rearing experiment was conducted using infusoria. Infusoria feeding larval group of Asian seabass showed better survival rate than rotifer feeding grouper.



Dominant species of Infusoria: *Euplotes sp*

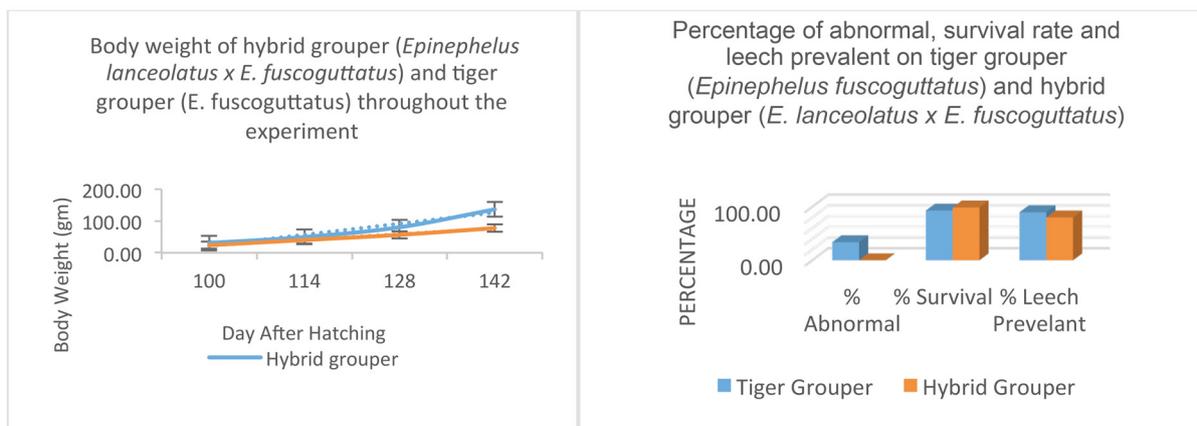
THE GROWTH PERFORMANCE OF HYBRID GROUPER (*Epinephelus lanceolatus* ♀ X *E. fuscoguttatus* ♂) AND TIGER GROUPER (*E. fuscoguttatus*) FINGERLING NURSE IN TANK SYSTEM

Sufian Mustafa and Nik-Haiha Nik Yusoff

Marine Fish Aquaculture Research Division
FRI Tg Demong, 22200 Besut, Terengganu, Malaysia
sufnor96@yahoo.com

Generally, this study aimed to investigate the difference performance of tiger grouper (*E. fuscoguttatus* ♀) and hybrid grouper (*E. lanceolatus* ♂ x *E. fuscoguttatus* ♀). Experiment was carried out for duration of 8 weeks. The fries of both tiger grouper and hybrid grouper were nursed until 100 DAH. Fast grower fish without abnormality were selected from both group for the experiment. About 100 fingerlings were placed in each of the six 3m³-fiberglass tank. Sampling were done every 2 weeks until fish attained average 80gm body weight.

Abnormality were observed highest in pure tiger grouper 33.74 % compared to hybrid grouper 0% (P<0.01). The survival rate between tiger grouper and hybrid grouper were 93% and 98.67% respectively (P>0.01). The Specific Growth Rate (SGR), Relative Weight Gain (RWG) and Weight Gain (WG) of the hybrid was higher compared to the tiger grouper. Abnormality in tiger grouper seed may be due to inbreeding. The hybrid grouper has an advantage because it reduces risk due to inbreeding in aquaculture. Fast growth hybrids in needed to shortened the culture period and reduce losses in production.



STUDY ON DETERMINATION OF CONCENTRATION CATEGORIES OF SOIL QUALITY VARIABLES IN DIFFERENT DEPTHS IN BRACKISHWATER PONDS OF JAVA ISLAND, INDONESIA

Akhmad Mustafa*, Kamariah, and Muhammad Chaidir Undu

Research Institute for Coastal Aquaculture
Maros, South Sulawesi
Indonesia, 90512
akhmadmustafa@yahoo.com

Soil quality plays pivotal role in increasing productivity and brackishwater pond successfulness, including brackishwater ponds of Java Island, Indonesia. However, the availability of data on concentrations categories for soil quality variables for brackishwater pond soils in Java Island, which is volcanic-dominated soils and the largest area for brackishwater ponds in Indonesia, is still scarce. This study was aimed to categorize concentration of soil quality variables as an initial point in interpreting pond soils quality of Java Island.

Soil samples were collected from two different soil depths of 923 sampling points representing 23 regencies/cities of West Java, Central Java, and East Java Provinces. Twenty soil quality variables were measured based on *in-situ* and *ex-situ* measurements. The t-Student and U-Mann Whitney tests were applied to analyse the differences in soils quality at each soil depth which were firstly analyzed using z-score to eliminate outlier data. The data were firstly rearranged from the smallest to the largest data prior to measuring data deciles; the deciles, as considered as the basic measurement, were used for categorizing each soil quality variable.

The results of this study indicated that the pH and nutrients are higher at the pond top soils, 0-0.2 m compared to that observed on the depth of 0.2-0.5 m. Concentrations of soil quality variables such as S_{KCl} , S_p , S_{POS} , TPA, TAA, TSA, dan pyrite relatively similar between the two depths. Data of each soil quality variable were categorized into very low, low, moderate, high and very high to facilitate a comparison to other data of pond soil qualities. The results of this study are not recommended for assessing the relationship between pond soil quality and pond productivity; yet are usefull in measuring which variable of pond soil quality is categorized as very low, low, moderate, high or very high; and also useful in making decision on brackishwater pond soil quality management in Java Island.

ANTIMICROBIAL RESISTANCE: AN INTRODUCTION TO FISH FARMERS

Gerald N Misol Jr¹, Wan Norhana Md Noordin², Noor Affizah Bujang Saili³

¹ Fisheries Biosecurity Division, Department of Fisheries Malaysia

² Fisheries Research Institute, Department of Fisheries Malaysia

³ Aquaculture Development Division, Department of Fisheries Malaysia

Email: gerald@dof.gov.my

Antimicrobial resistance (AMR) is a serious and growing global public health threat. AMR refers to microorganisms, such as bacteria, viruses, fungi and parasites, which have acquired resistance to antimicrobial medicines. AMR may occur naturally as organisms adapt to their environments. However misuse of antimicrobial agents in humans, animals and plants sectors has dramatically accelerated the emergence of AMR. In addition, the intensification of animal production methods to cater for the demand of increasing human population has made this problem worsen with an increase use of antimicrobials in agriculture including fisheries.

In view of the grave importance of AMR, the United Nations General Assembly has called for cooperation between the World Health Organization (WHO), the World Animal Health Organization (OIE) and the Food and Agriculture Organization (FAO) to provide support to the development and implementation of the National Action Plan on AMR at the local, national and international levels. In June 2015, at the 39th FAO Conference, delegates agreed to the Resolution 4/2015 AMR which is to identify the risk of threats to public health and the sustainability of food production. In the same year, during the 83rd OIE General Session, the 180 member countries including Malaysia officially committed to combat AMR and promote the prudent use of antimicrobials in animals. Global Action Plan has been established with four main objectives i.e: i) to improve awareness and understanding of AMR ; ii) to strengthen knowledge of AMR through surveillance and research iii) to support good governance and capacity building and iv) to encourage implementation of international standards. The National Action Plan on AMR in Malaysia has just been established with a cohesive and collaborative approach across federal departments including the Department of Fisheries (DOF) with mandates to address and mitigate AMR in respective sectors.

One of the fundamental ways to address AMR in aquaculture is to ensure that fish farm adhere to best practices for hygiene, biosecurity, and fish care and handling. This reduces the need for antimicrobials in the first place as does vaccinating fish to build their natural ability to withstand disease. In order to achieve this, the DOF has first to improve the awareness and understanding of AMR through effective communication, education and training. Thus the main goals of this paper are to create awareness and educate fish farmers in Malaysia about AMR. It is hoped that from this paper, farmers will understand what AMR is and the related risk and effects. It is also anticipated this paper will encourage the responsible and ethical use of antimicrobials in fish production in Malaysia.

CROSS BREEDING OF SUSTAINABLE HYBRID, *Hypophthalmichthys molitrix* (♀) X *Aristichthys nobilis* (♂), WITH INTRAMUSCULAR INJECTION OF OVAPRIM-C AT ISLAMABAD, PAKISTAN

Muhammad Naeem, Amina Zubari, Abdus Salam and Saima Yousaf

Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, 60800, Pakistan
(phone: +92-61-9210053; fax: +92-61-9210068
e-mail dr_naeembzu@yahoo.com

The experiment was planned to observe production of hybrid from sustainable hybrid fish, *Hypophthalmichthys molitrix* (♀) x *Aristichthys nobilis* (♂), by using single intramuscular injection of Ovaprim-C, number of eggs Kg⁻¹, fertilization rate and hatching percentage was studied during May - June, 1996 in fish hatchery Islamabad Fishes, Pakistan (Table-IV) were spawned and hybrid produce successfully following a single dose of Intramuscular injection of Ovaprim (LH-RH analogue) with 0.6 ml Kg⁻¹ for female (Table-II) and 0.1 ml Kg⁻¹ for male ova and milt were stripped simultaneous and mixture was stirred for 15 – 30 seconds during which fertilization occurred. Hatching occurred within 18 – 32 hours after fertilization. In the present study, ovulation, fertilization and hatching rate values were estimated to 100, 81.62 and 74.77%, respectively. (Table-I) Regression analysis was applied to assess body weight influence on absolute (Table-III)

TABLE I
EFFECT OF OVAPRIM-C ON SPAWNING OF HYBRID
HYPOPTHALMICHTHYS MOLITRIX (♀) X *ARISTRICHTHYS NOBILIS* (♂)

Parameter	Ovaprim treatment
No. of females treated	18
Total weight of females.	64.9 kg
Total no. of eggs.	4544166
Total no. of fertilized eggs.	3709165
Total no. of hatchling.	2773276
Overall fertilization percentage	81.62%
Overall hatching percentage	74.77%
Average no. of eggs/ Kg	70017
Average no. of fertilized eggs/Kg	57152
Average no. of hatching /Kg.	42731

TABLE III
DOSAGE OF OVAPRIM-C FOR CARPS AT DIFFERENT LOCATIONS

Fish Species	Dose of Ovaprim-C for (♀)	Reference
<i>Catla catla</i>	0.4 – 0.5	[30]
<i>Labeo rohita</i>	0.3 – 0.4	[30]
<i>Labeo rohita</i>	0.4	[31]
<i>Cirrhina mrigala</i>	0.25 – 0.3	[30]
<i>Cirrhina mrigala</i>	0.4	[31]
<i>Hypophthalmichthys molitrix</i>	0.4 – 0.7	[30]
<i>Ctenopharyngodon idella</i>	0.4 – 0.8	[30]
<i>Aristichthys nobilis</i> .	0.4 – 0.5	[30]
<i>Hypophthalmichthys molitrix</i> (♀) x <i>Aristichthys nobilis</i> (♂)	0.6	Present Study

(Continued on next page)

TABLE III
 STATISTICAL PARAMETERS OF BODY WEIGHT VERSUS TOTAL NUMBERS OF
 EGGS AND NUMBERS OF EGGS/KG OF HYBRID
 [*HYPOPHTHALMICTHYS MOLITRIX* (♀) X *ARISTRICHTHYS NOBILIS* (♂)]

Relationships	r	a	b	S. E. (b)
Wet body weight, (x) Total no. of eggs, (y)	0.896***	7100.94	68048.52	8438.34
Wet body weight, (x) No. of eggs / Kg, (y)	0.155 ^{n.s}	74685.35	-1224.83	1957.20
Log wet body weight, (x) Log total no. of eggs, (y)	0.882***	4.9107	0.8783	0.878
Log wet body weight, (x) Log total no. of eggs/Kg, (y)	0.149 ^{n.s}	4.8753	-0.0564	-0.094

*** $P < 0.001$, ^{n.s} = $P > 0.05$

TABLE II
 SPAWNING RESPONSE OF HYBRID [*HYPOPHTHALMICTHYS MOLITRIX* (♀) X *ARISTRICHTHYS NOBILIS* (♂)]

Month	Temperature (°C)	No of females	Total weight of females (kg)	Dose of Ovaprim (ml kg ⁻¹)	Total No of eggs	Total Fertilizatio n rate	Total No of Hatching
May, 1996	26.0	4	16.3	0.6	1186000	916667	680277
May, 1996	25.9	5	19.5	0.6	1409333	1257832	913666
June, 1996	26.5	4	14.0	0.6	973333	794166	620833
June, 1996	26.0	5	15.1	0.6	975500	740500	558500

OUTDOOR CONTINUOUS CULTURE SYSTEM BASED MASS PRODUCTION TECHNIQUE FOR CLADOCERANS (*Moina micrura*)

Mahadevi*, S. Felix, Mangesh M. Bhosle and Cheryl Antony

Department of Aquaculture
Fisheries College & Research Institute
Tamil Nadu Fisheries University, Ponneri (TN) 601 204

Appropriate live feed is necessary in larval rearing of commercially important species due to their small size, fragile nature, and nutritional quality as well as small mouth gap, partially developed receptors and digestive system of larvae. Considering the importance of zooplankton as live feed, present study reports mass production of freshwater Cladocerans (*Moina micrura*) using micro algae (*Chlorella vulgaris*) as food. Stock culture of *Moina micrura* was prepared by collecting samples from wild and segregated following the pipette-method which then allowed for growing in 1L capacity plastic bottles fed with Baker's yeast for 5 days. Mass production of *M. micrura* was carried out in outdoor cement cistern tanks (1000L capacity) which fertilized using ground nut oil cake (250g), urea (10g) and single super phosphate (5g) and inoculated with *C. vulgaris* on the same day. Cladocerans were continuously fed with Cultured *C. vulgaris* at the intervals of 2x and 3x per day. The high density of population was attained with 3x feeding interval of *Chlorella* sp. (35-40 individuals/ml), followed by 2x (20-30 individuals/ml). The low density of population was recorded in control (5-12 individuals/ml) in which feeding was not practiced. Out-door tanks were re-fertilized fortnightly to maintain the plankton production. The peak density population growth was found on 5th day for *Chlorella vulgaris* (50000 cells/ml) and on 8 for Cladocerans (40 individuals/ml). This study indicated that both the quantity and quality of food have significant role on mass production of Cladocerans in aquaculture.

mtDNA MUTATION OBSERVED IN MASU SALMON COLLECTED IN FUKUSHIMA

Muhammad F. B. Yusof, Gyo Kawada, Masato Watanabe, Shigehiko Izumi and Masamichi Nakajima*

Graduate School of Agricultural Science, Tohoku University
Aza-Aoba 468-1, Aramaki, Aoba, Sendai
Miyagi 980-0845, Japan

Fukushima Daiichi Nuclear Power Station accident occurred at March 11, 2011 led the deposition of the radioactive cesium (^{137}Cs) at freshwater fishery grounds. Although more than three years have passed, high level of ^{137}Cs has been detected from the muddy sediment and fishes in rivers of Fukushima. Since the situation of pollution by radioactive materials and its change is still unknown, the pollution will have physiological and genetic effects on freshwater fishes.

Mitochondrial DNA is clonally transferred from maternal parent to its offspring. From this characteristic of mitochondrial DNA, it is possible to survey the genetic change between maternal parent and its offspring and it is good material to consider the genetic effect of radioactive materials on fishes. We examined mitochondrial DNA in maternal parent and produced offspring collected from Mano river in Fukushima and compared with the masu salmon produced in Fukushima prefectural inland water fisheries experimental station (FP). Masu salmon *Oncorhynchus masou* is popular salmonid fish in this area and this species spend two or three years in the river until maturation.

From two females collected from Mano River, radioactive cesium was detected 632Bq/kg and 212Bq/kg, respectively. On the other hand, it did not detected in the females from FP. Two regions of mitochondrial DNA, Dloop and Cytb, were examined. Different sequences from maternal individual were observed in two sites of Dloop and four sites of Cytb, and calculated mutation rates were 1.76×10^{-5} in Dloop and 4.9×10^{-6} to 1.29×10^{-5} in Cytb. In these mutations, there are two amino acid substitutions was observed, however these change were already observed as polymorphism in masu salmon population. Other mutations were synonymous substitutions. There was no mutation in the individuals produced from PF. These results suggest the effect of radioactive materials on genome of freshwater fishes.

SINGEL NUCLEOTIDE POLYMPRPHISMS OF GROWTH HORMONE GENE IN THREE PANGASIID SPECIES: *Pangasionodon hypopthalamus*, *Pangaisus Jambal* AND *Pangasius nasutus*

Imron* and Khairul Syahputra

Research Institute for Fish Breeding
Jl. Raya 2 Sukamandi Pantura, Patokbeusi, Subang 41263
West Java, Indonesia
Corresponding author: imronnawawi@kkip.go.id

Growth performance in fish is known to be associated with of growth hormone gene. Preliminary study showed that *Pangasionodon hypopthalamus* and *Pangasius Jambal* show higher growth performance relative to *Pangasius nasutus*. It is interesting to know whether difference in growth performance among these species is also reflected in the structure of growth hormone gene. This study was aimed to explore the issue by looking at the profile of single nucleotide polymorphisms (SNP).

Genomic DNA of three pangasiid species were extracted and growth hormone gene were partially amplified in PCR system. DNA sequence of GH gene were obtained through running the sample in Miseq, an Illumina platform of next generation sequencing system. The resulting data were then aligned to both reference sequence, which is sequence of GH gene of *Pangasionodon hypopthalamus*, available on GeneBank by which SNPs were identified. Alignments were also carried out among species. Following this, deduced amino acid was carried out on exon region to see whether nucleotide substitutions resulted in amino acid changes

Out of 1200 nucleotide sequences obtained from the three species, significant number of SNPs were identified .Alignment between GH sequence *P. jambal* and *P. Hypopthalamus* found 61 SNPs, 57 occurred in intron and 4 occurred in exon., while alignment between of *P. Nasutus* and *P. Hypopthalamus* found 58 SNPs, being 55 occurred in intron and 3 occurred in exon. Alignment of GH sequence between *P. Jambal* and *P. nasutus* found only 25 SNPs, with 23 SNP were found in intron and 3 SNPs were found in exon. Further analyses showed that the nucleotide substitutions occurred in exon are synonymous that does not change the amino acid product. This study might indicate that differences in growth performance among the three pangasiid species may not be associated with differences in the structure of GH gene.

OCEAN MINERALS: EARTH'S ELEMENT SOUP - ITS PRODUCTION AND USE IN AQUACULTURE

Neoh Seong Lee*, Su Shiung Lam and Tse Seng Chuah

School of Food Science and Technology
Universiti Malaysia Terengganu (UMT)
21030 Kuala Terengganu,
Terengganu, Malaysia.
neohseonglee2004@yahoo.com

The ocean is a reservoir for almost all known elements. The synergistic beneficial effects of these elements on living organism has been speculated for a long time. However, six elements which make up 99% of the dissolved salt act as a gatekeeper, preventing the access of the other elements in seawater. This can be overcome by concentrating seawater. When seawater is concentrated, the elements precipitate out in order, starting with the major salts, such as sodium chloride. At one stage, the major salts are significantly reduced and other elements are concentrated, making it a natural diverse element solution. In order to tap into this abundant resource, a simple method to accurately reproduce seawater concentrate is needed. This study investigated the possibility of heat evaporating seawater up to 99.9%, using density of solution as the sole parameter for the consistent reproduction of various seawater concentrate. It was found that the representative elements in seawater concentrate, is strongly correlated with density, even in ultra concentrated form. This signify that various seawater concentrate, with consistent element profile can be reproduced to the targeted stage by using density as its sole indicator, making the production of seawater concentrate feasible for everyone. Various seawater concentrate produced were tested on an algae and it was found that seawater concentrate with solution density 1.348g/ml gave the best result, having 14.2% higher final yield and significantly lower nutrient use. It appeared that the diversity of elements in seawater, captured by concentrating seawater, is beneficial to algae. The research to investigate its effects on plants and other organism is ongoing at the time of this abstract.

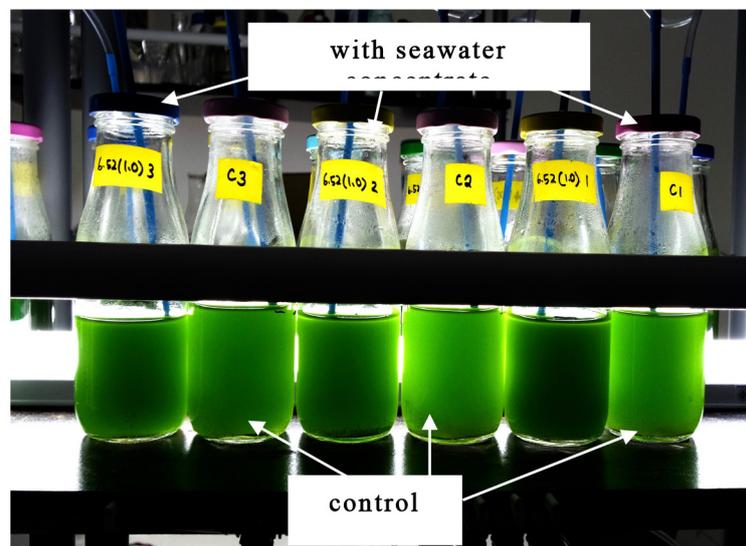


Figure: Algae treated with concentrated seawater versus non-treated control.

ESTABLISHING NATIONAL MULTI-STAKEHOLDER PLATFORMS IN ASIA

Richard Newton*, Chong Liu, David Little, Nhan The Dinh, Muhammad Rahman, Erik van Ballaer

Institute of Aquaculture
University of Stirling
FK9 4LA,
UK

*Corresponding author: rwn1@stir.ac.uk

The ultimate aim of establishing a technical and innovation exchange multi stakeholder platform (MSP) between Europe and Asia depends on the initial establishment of national pilot platforms (NPPs) within Asian countries. The pilot NPPs are being established in Bangladesh, Thailand and Vietnam. Development of a NPP requires several steps in understanding the dynamics of the industry on a national level 1) the formation of a stakeholder database 2) a period of stakeholder mapping which identifies the power and interest balance between different sectors within the aquaculture industry and outside actors 3) the setting up of a core working group in each country to direct the long-term establishment of the NPP. This paper presents the progress so far and the road map to the objective of establishing the three NPPs. Stakeholder databases were created in each country including a cross-section of aquaculture and its support industries. A series of stakeholder key informant interviews and surveys were conducted in each country to map the linkages between the different stakeholders, assess their relative influence and interest in the industry, and to identify common challenges within the industry that could be subjects for research. Power vs. interest grids, stakeholder issue inter-relationship diagrams and Venn diagrams were used as tools to assess and compare the relationships and common issues amongst stakeholders in each country and wider geographic area.

DETERMINING THE NUTRIENT AVAILABILITY OF COMMERCIAL TILAPIA FUNCTIONAL FEEDS UNDER FARM AND LABORATORY CONDITIONS USING ACID-INSOLUBLE ASH AS A NATURAL INERT MARKER

Wing-Keong Ng*, Chik-Boon Koh, Chaiw-Yee Teoh, and Siang-Bing Ng

Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
wkng@usm.my

The rapid expansion of tilapia production is due in large part to the intensification of culture systems made possible with the use of commercial pelleted feeds. Functional feeds are feeds with additional function, usually with health-promoting or disease preventing properties beyond their nutritional value. These feeds are now considered the new generation of aquafeeds. Our research into the use of organic acids as a functional feed additive in tilapia feeds as an alternative to the use of harmful antibiotics will be presented. Apart from their potential role in enhancing resistance to tilapia diseases, dietary organic acids are also known to improve nutrient digestibility of aquafeeds. The present study attempted to determine the impact of dietary organic acids on nutrient availability under commercial farm conditions and compared the results with that obtained under controlled laboratory conditions using dietary acid-insoluble ash as a natural inert marker.

Four 20-meter diameter plastic circular floating cages (2 replicates per treatment) in a tilapia farm were used in the study. All male Nile tilapia fingerlings (~25 g) were stocked at a density of 60,000 fish per cage, giving a stocking density of 16 fish/ m³. The tilapia feeds, containing no added organic acids or 2% of a prototype organic acids blend were produced and purchased from Cargill Sdn. Bhd., Malaysia. With the exception of the added organic acids, both diets were similarly formulated. Feeds were mechanically dispersed to the respective cages two times daily. Two to four weeks before the final harvest, 85 large fish were randomly sampled using a dip net from each cage. All fish were dissected and fecal samples were extracted from the terminal segment of the tilapia gut and were considered to contain almost fully digested feeds just before expulsion as fecal matter.

In the controlled laboratory trial, eight groups of 15 tilapia fingerlings (~ 16 g) were randomly allocated into each 95-L glass aquarium. Each commercial tilapia feed was fed to four replicates for seven weeks twice daily. After four weeks into the feeding trial, feces were collected once daily from each aquarium and pooled. Only intact fecal strands were carefully siphoned into a fine mesh net and collected.

The collected fecal samples were then analyzed for ash, protein, lipid, phosphorus and acid-insoluble ash (AIA) content to determine the apparent digestibility of nutrients.

Results indicated that AIA can be used to estimate nutrient digestibility of commercial feeds under commercial farm conditions. Dietary organic acids were shown to significantly enhance ($P < 0.05$) dry matter, ash, phosphorus and protein digestibility of feeds in the laboratory trial but significant differences between the two commercial feeds were not detected from the farm trial despite showing similar trends for dry matter and protein digestibility values. Challenges faced in determining nutrient digestibility of commercial feeds under commercial farm conditions will be discussed.

THE ROLE OF FISHER'S WIFE TO SECURE THE HOUSEHOLD ECONOMY IN MORODEMAK FISHING COMMUNITY - CENTRAL JAVA PROVINCE, INDONESIA: BREAKING THROUGH THE FRONTIER

Cholida A. Nia, Nur F. Lindrianti, Putra A.W. Prasetyo, Yermianto D. Puspito, and Indah Susilowati*

Faculty of Economics and Business, Diponegoro University
 Jl. Prof. Sudharto, UNDIP Tembalang Campus, Semarang 50275, Indonesia
 Phone: +62-24-76486851 Fax: +62-24-76486852.
 *Corresponding Author: indah.susilowati@undip.ac.id;
 indah-susilowati@rocketmail.com

Morodemak is one of fishing landing place in North coast of Central Java province. It lies about 60 km to the East of Semarang municipality (the capital city of Central Java). The fishing community are inhabited along the river of Morodemak. Fishers in Morodemak are considered as the small-scale with conventional pattern to operate their business. In majority they go for fishing for one day trip with the targeted fishing ground in the Java sea. It is often fishers went home with small in catch due to the existence of climate change. Thereafter, indeed need a help from wife to maintain the household's economy at least for survival purpose. From Javanese philosophy wife has an obligation on three roles: *kasur* (bed), *Sumur* (cleaning), and *dapur* (cooking). But under the global change such as climate change, the three conventional roles of wife is not sufficient, rather than stipulate beyond those roles to secure the household's economy. This study aims to explore what are the breaking through the frontier of wife's role in order to secure the household's economy under the existence of climate change. Mixed method was applied to analyse the data. About 98 wives had been interviewed in this study. The results indicated that wife is playing a significant role in fishing community to manage their family, particularly under the shocked situation such as climate change.

TABLE 1. The Profile of Wife Respondents

Description	Fisher's wives respondents (n=98)			
	Minimum	Maximum	Mean	Std. Deviation
Number of dependent (person)	0	6	2.59	1.259
Education (year)	0	12	6.67	2.708
Age (year)	21	67	38.80	9.570
Wife Occupation	0	1	0.42	0.496
Husband status	0	1	0.29	0.454
Household income (rupiah)	1	5	1.51	0.888

Notes :

Wife occupation: 0 = unpaid; 1 = paid

Husband status: 0 = boat crew; 1 = boat owner

Household income: 1 = <250,000; 2 = 250,000 – 500,000; 3 = 500,001 – 750,000; 4 = 750,001 – 1,000,000; 5 = 1,000,001 – 1,250,000

SEABASS (*Lates calcarifer*) BROODSTOCK NUTRITION AND EFFECTS ON THE QUALITY OF EGG

Nik Daud Nik Sin

Fisheries Research Institute
 TanjungDemong, 22200
 Besut, Terengganu, Malaysia
 nikdaud03@yahoo.com

The effects of broodstock nutrition on spawning performance of farmed seabass based on current nutritional practiced was monitored. Seabass broodstock was fed with fresh fish as first feeding trial for spawning performances in six months. They were spawned naturally every month and a total of 88.70 million eggs was collected with average 80% of fertilization rate. The quality of eggs produced is moderate; whereas the eggs diameter was decreased from 905.18 μm to average sizes 711.23 μm . The second feeding trial then was introduced by using fresh fish and broodstock diet (pelleted) and the spawning performances was monitored for the next six months. They were spawned naturally every month and a total of 83.33 million eggs was collected with average 80% of fertilization rate. The quality of eggs produced is no significant different compared to broodstock fed with fresh fish. Even though the eggs diameter was slightly increased to average sizes 733.58 μm but it's still consider smaller compared to most evaluating good egg size which is above 800 μm . In the year 2015, third feeding trials was introduced by using fresh fish and bio-omega (omega 3; DHA & EPA + vitamin E). The egg produced was show significant results with the fertilization rate increased and the egg diameter produced bigger in size. The used mixed diet of bio-omega containing omega-3; docosahexaenoic and eicosapentaenoic acid (DHA and EPA) and vitamin E enriched in sea bass feed was enhanced the egg diameter with the average size 830 μm (\uparrow 14.8%); and fertilization rate up to 90% (\uparrow 10%). The total of 121.49 million fertilised egg were produced until December 2015.

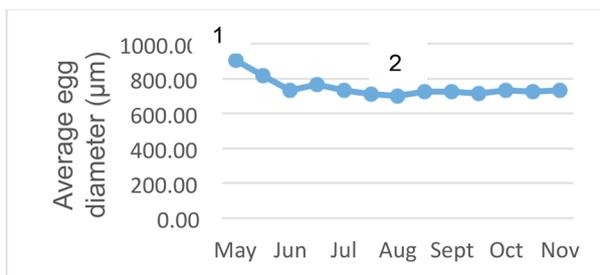


Fig.1 Average egg diameter with two different ration (1) Fresh food fish (2) Fresh food fish + broodstock diet (pellet)



1 2 3

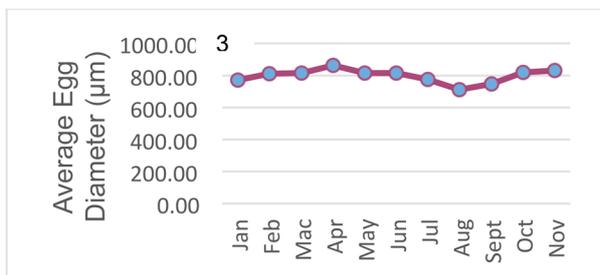
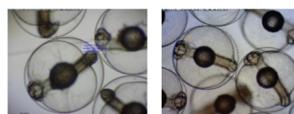


Fig.2 Average egg diameter with ration (3) Fresh food fish + bio-omega (EPA, DHA & Vit.E)



Fertilised sea bass

EFFECT OF SIREHMAX™ ON GROWTH PERFORMANCE AND SURVIVAL OF HYBRID GROUPER, (*Epinephelus lanceolatus* x *Epinephelus fuscoguttatus*)

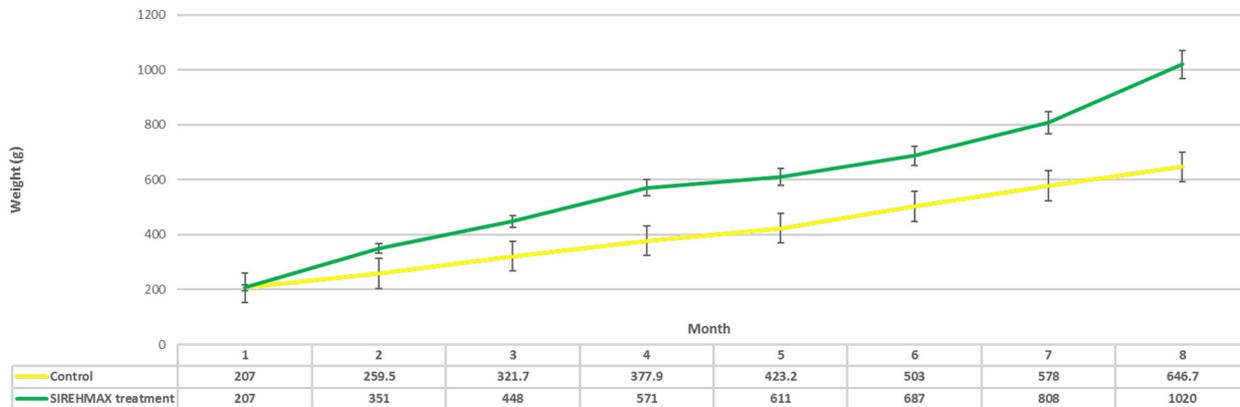
Nik-Haiha Nik Yusoff and Ahmad Baihaqi Othman

Marine Aquaculture Breeding Technology Centre
 FRI Tg Demong, 22200 Besut, Terengganu, Malaysia
 nikhaiha@dof.gov.my

SirehMAX™ is a herbal product containing betle leaves extract that has potential as an alternative to commercial antibiotic in aquaculture. It can be used in different species and at various stages of fish culture without any side effect. This study was conducted to evaluate the effect of SirehMAX™ as feed additives on growth performance and survival of hybrid grouper (*Epinephelus lanceolatus* x *Epinephelus fuscoguttatus*).

Hybrid grouper with an initial body weight of 12 g fish⁻¹ was reared in 4 different floating cages (3m x3m x3m) at a density of 14 fish per m for a 6-month period. Fish were fed twice a day at 5% body weight. Feed additive SirehMAX™ were added to the basal diet at 1ml kg⁻¹ feed and fed to fish once a week for the whole culture period. Fish fed on diet with SirehMAX™ showed significantly improved growth performance and survival rate (p < 0.05) compared to fish fed on control diet. From this observation, it can be concluded that 1ml kg⁻¹ feed of SirehMAX™ inclusion improves growth performance and survival of grouper. Thus it could be used in the fish feeding regime to shorten the culture period.

Growth performance of hybrid grouper in cage culture



ANTIBODY RESPONSE AND PROTECTIVE CAPACITY BY VIBRIO- VACCINE DELIVERED THROUGH LIVE FEED IN TIGER GROUPER LARVAE

Nik Haiha Nik Yusoff^a, Shuhada Aziz^b, Mohd.Zamri Saad^b, Mohd. Firdaus Nawi^b, Siti Zahrah Abdullah^c and Shaharah Mohd Idris^a

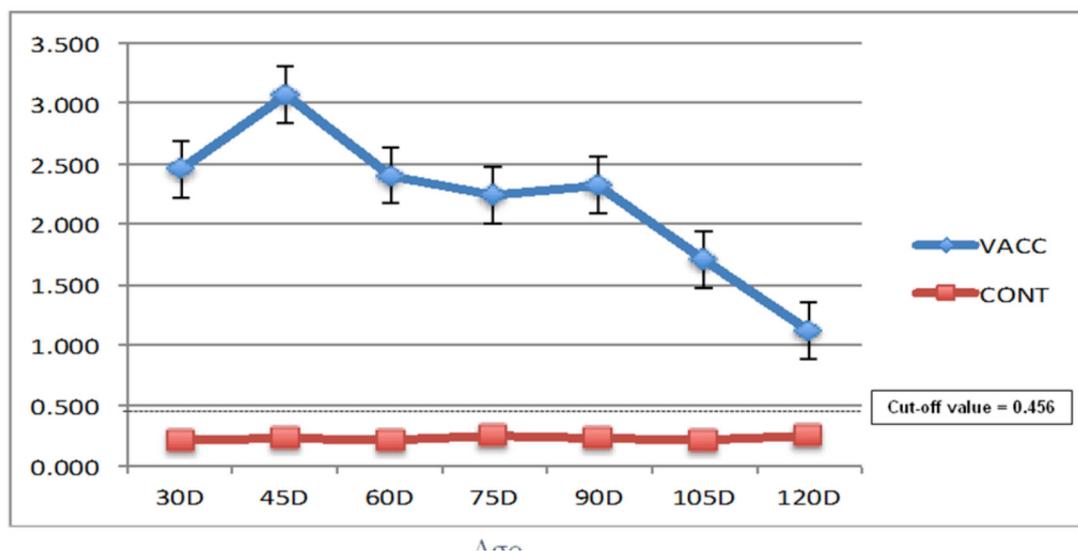
^aFisheries Research Institute, 22200 Besut, Terengganu, Penang, Malaysia

^bFaculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

^cNational Fish Health Centre, Fisheries Research Institute, 11960 Batu Maung, Penang, Malaysia

*Corresponding Author's Email: nikhaiha@dof.gov.my

Vibriosis by *Vibrio alginolyticus* is one of the major cause of mortality in cultured grouper especially at their early stage of life. The mortality due to this disease can reach up to 100% and caused extensive losses to the hatchery. Administration of vaccine against vibriosis at the larval stage was considered as one of the best solutions to developed strong and specific immunity in grouper as well as to increase the survival rate. However, delivery of vaccine by injection or immersion route are nearly impossible due to the nature of the grouper larvae that are very sensitive to handling. Hence, oral delivery by bioencapsulating the formalin-killed *V. Alginolyticus* (later termed as Vib0cine) into rotifer and artemia was selected as the best route to vaccinate the grouper larvae. The present study was conducted to determine the antibody immune response and protective capacity provided by oral administration of bioencapsulated Vib0cine in grouper larvae at 15-DAH, 30-DAH and last booster at 60-DAH. Then, fish were challenged by immersion with virulent live *V.alginolyticus* four weeks after last booster. Results demonstrated that the antibody level was significantly increased ($p<0.05$) fifteen days after first administration and went higher after administration of booster doses. Significantly ($P< 0.05$) higher survival rate also observed in vaccinated grouper that indicated the oral vaccination with bioencapsulated Vib0cine at early stage provided adequate protection against vibriosis in grouper.



COMPARATIVE STUDY ON THE EFFECT OF BLACK SOLDIER FLY *Hermetia illucens* LARVAE OIL ON GROWTH PERFORMANCE, DIGESTIBILITY, FATTY ACID COMPOSITION AND HAEMATOLOGICAL FOR NILE TILAPIA *Oreochromis niloticus*

Abu B. Noor-Hidayati*, Shaharudin A. Razak, Zazali Alias, Norhidayah M. Taufek, Hasniyati Muin, Ameenat A. Raji, and Firdaus Aspani

Institute of Biological Science
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia
noorhidayati_yat@yahoo.com

Tilapia has been cultured worldwide and is increasing popularity among farmers including Malaysia. In order to maximize growth performance and feed efficiency, tilapia is facing new challenges involving well-balanced diets with variety of alternative aquafeed source. Increasing cost of fishmeal and fish oil not only affect aquaculture industry in Malaysia but also worldwide. One of the major nutrient which is lipid, have very important roles in fish nutrition and metabolism. Insect or larvae have a great potential to be fish feed and was indicated to have rich in fats. Black soldier fly larvae oil is a potential lipid source for aquafeed with high content of lauric acid (35.2%). The aim of this study is to investigate the effect of fish oil (FO) replacement with black soldier fly larvae oil (BSO) on growth performance, digestibility, fatty acid composition and haematological for tilapia.

Five experimental diets were formulated with FO and with partial or complete (25%, 50%, 75%, or 100% BSO, respectively) replacement of FO with BSO. Chromic oxide was used as inert digestibility marker. The fish were fed at 5% of their body weight per day for 90 days. At the end of the experiment, tilapia from each experimental diet were individually weighted after fasting for 24 h. 3ml of blood was collected from caudal peduncle. Muscles were dissected for fatty acid and proximate analysis. Feces and diets were analysed to determine chromium content for apparent digestibility. All data were analyzed using SPSS, performed with one-way ANOVA.

The results showed that FO can be successfully totally replaced with BSO in tilapia diets without affecting fish growth performance. The highest growth (2.27%) and the lowest FCR (1.39) were obtained in fish fed the BSO25 diet. Similar to this study, current research suggested that saturated fatty acids could be effectively utilized by tilapia. There was no palatability problem in this study. The values obtained for haematological parameters were within the normal ranges for tilapia.

Table 1: Growth performance of Nile tilapia fed with different level of BSO.

Dietary treatments	FO	BSO25	BSO50	BSO75	BSO100
IBW (g)	7.39±0.00	7.18±0.12	7.19±0.04	7.17±0.08	7.00±0.11
FBW (g)	53.47±0.58 ^b	55.28±0.59 ^a	52.88±0.96 ^{bc}	51.23±0.51 ^{cd}	51.22±0.20 ^d
WG (g)	46.08±0.64 ^b	48.10±0.53 ^a	45.69±0.92 ^b	44.06±0.49 ^c	44.22±0.33 ^c
SGR (%)	2.20±0.31	2.27±0.46	2.22±0.32	2.22±0.55	2.21±0.42
FCR	1.41±0.04	1.39±0.12	1.56±0.10	1.50±0.05	1.45±0.07

MULTIPLE FEEDINGS IMPROVE GROWTH PERFORMANCE OF JUVENILE *Litopenaeus vannamei* REARED UNDER INTENSIVE CONDITIONS

Alberto J.P. Nunes*, Hassan Sabry-Neto, Adhemar R. Oliveira-Neto, Karthik Masagounder

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

Farm-raised shrimp are commonly fed by manual broadcasting or with feeding trays, from two to six times daily during grow-out period. Shrimp are slow benthic feeders, feeding continuously but at small quantities throughout the day. The present study investigated the effects of different feeding frequency on the growth performance of juvenile *L. vannamei* raised under an intensive green-water experimental rearing system. Shrimp of 1.06 ± 0.16 g were stocked at 100 animals/m² in 16 outdoor tanks of 1 m³ (1.02 m²). After 10 days of acclimation period, shrimp were fed an experimental pellet feed for 70 days. Experimental feed contained on a dry matter basis 32.06% crude protein, 1.93% Lys, 0.87% Met and, 1.33% Met+Cys was lab pelleted. Shrimp were fed manually twice (2x, 7:00 am and 1:00 pm) or four (4x, 7:00 am, 10:00 am, 1:00 pm and 4:00 pm) times daily, with equal amount of feed offered between meals. In the next two treatments, shrimp were fed multiple times a day using automatic feeders either diurnal (Day) from 07:30 am to 05:30 pm (10-h period), or both diurnal and nocturnal (D&N), 24-h period. Observed means for water temperature, salinity and pH were $30.2 \pm 1.01^\circ\text{C}$, 35 ± 2.1 g/L, and 8.5 ± 0.12 , respectively. At harvest, shrimp survival was $89.7 \pm 9.40\%$ and unaffected by feeding frequency. On the other hand, final shrimp body weight, daily growth, gained shrimp yield, and FCR improved with a higher number of feedings a day. Shrimp body weight improved significantly as feeding frequency increased from 2x, 4x, to multiple feedings a day (diurnal feeding), but settled at D&N. Daily growth, gained yield, and FCR (2.65 ± 0.35 to 1.62 ± 0.03) enhanced significantly as feedings exceeded 2x a day. In conclusion, juvenile shrimp need to be fed four or more times daily during grow-out period to maximize production performance.

Table 1. Performance (mean \pm SD) of *L. vannamei* fed 2 or 4 times in feeding trays and multiple times (day or day and night) with an automatic feeding dispenser. Common letters within the same line indicate statistically significant differences at 0.05 according to Tukey's HSD test.

Shrimp performance	Feeding Frequency			
	Manual/Feeding Trays		Automatic/Feeding Dispenser	
	2 times	4 times	Diurnal (multiple)	Day & Night (multiple)
Final survival (%)	80.4 \pm 7.7	89.5 \pm 13.6	92.4 \pm 4.6	97.1 \pm 15.1
Final body weight (g)	8.50 \pm 1.51a	10.55 \pm 1.82b	11.76 \pm 1.82c	11.22 \pm 1.78d
Daily weight gain (g)	0.09 \pm 0.01a	0.12 \pm 0.01b	0.13 \pm 0.005b	0.13 \pm 0.005b
Gained yield (g/m ²)	579 \pm 62a	844 \pm 64b	960 \pm 99b	989 \pm 48b
Feed delivered (g/shrimp)	15.2. \pm 0.71a	16.5 \pm 0.69b	16.1 \pm 0.61ab	15.9 \pm 0.54ab
FCR	2.28 \pm 0.35a	1.98 \pm 0.16b	1.74 \pm 0.10b	1.56 \pm 0.12b

DIETARY METHIONINE AND TOTAL SULFUR (MET+CYS) AMINO ACID REQUIREMENTS OF JUVENILES OF WHITELEG SHRIMP, *Litopenaeus vannamei*

Felipe N. Façanha*, Adhemar R. Oliveira-Neto, Karthik Masagounder, Alberto J. P. Nunes

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará, Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081, Brazil
felipenobre17@yahoo.com.br

Dietary methionine (Met) and methionine+cystine (Met+Cys) requirement have been estimated for two commercially relevant farm-reared penaeids, the Kuruma shrimp, *Marsupenaeus japonicus* (0.7 and 1.4% of the diet, respectively) and the black tiger shrimp, *Penaeus monodon* (0.9 and 1.3% of the diet, respectively). This study investigated the Met and Met+Cys requirements of juveniles of *Litopenaeus vannamei* fed a semi-purified diet supplemented with graded levels of DL-Methionyl-DL-Methionine (AQUAVI® Met-Met, Evonik Nutrition&Care GmbH, Germany). The work was conducted in a controlled system using clear-water tanks of 60 L (0.19 m² bottom area) for 56 days, after a 10-day acclimation. A total of 630 shrimp of 0.80 ± 0.05 g were stocked at 15 animals/tank in 42 tanks. Semi-purified diets were formulated with 23.0% (as fed) soybean meal, 20.0% dextrin, 15.0% wheat gluten meal, 12.0% salmon meal, and 3.0% squid meal, among other ingredients. A mix of essential amino acids (AA) were used to formulate on an ideal protein concept using lysine as the reference AA. L-Alanine and L-Glutamic acid were added to balance dietary CP to 37.9 ± 0.21%. From a control diet with 0.51% Met (0.87% Met+Cys; dry matter basis, DM), five other diets were supplemented with Met-Met at 0.14, 0.30, 0.46, 0.62, and 0.78% to achieve a total dietary Met (Met+Cys) of 0.68 (1.04), 0.82 (1.19), 0.99 (1.37), 1.20 (1.57), and 1.37% (1.75%). Shrimp survival (99.7 ± 0.01%) did not differ among treatments. Gained yield, final body weight, percent weight gain, weekly growth, and specific growth rate significantly increased as the dietary Met was raised from 0.51 to 0.99%. Results from the present study indicates that increased levels of dietary methionine enhanced the growth performance and feed utilization of juvenile *L. vannamei*. Based on a quadratic broken-line model (Fig. 1), the optimal dietary Met and Met+Cys requirement for maximum performance of juvenile Pacific white shrimp was estimated to be 0.97 and 1.34% of the diet (DM basis), respectively. This information will be useful in developing amino acid-balanced diets for intensive culture of juvenile whiteleg shrimp.

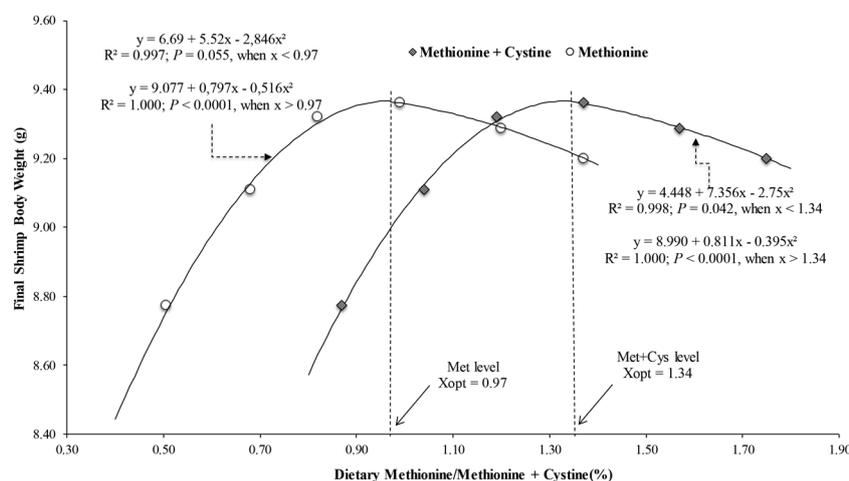


Figure 1. Maximum growth of *L. vannamei* in response to dietary Met and Met+Cys fed semi-purified diets.

KRILL MEAL IMPROVES FEED EFFICIENCY IN JUVENILE *Litopenaeus vannamei* AND REDUCES RELIANCE ON FISHMEAL AND OTHER COSTLY INGREDIENTS

Alberto J.P. Nunes*, Hassan Sabry-Neto, Esaú Aguiar Carvalho

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

Previous studies have shown that dietary inclusion of krill meal in diets for whiteleg shrimp, *L. vannamei*, increases the number of pellets eaten and prolong feed intake. The present study evaluated the amount of fishmeal that can be replaced by soybean meal, when low doses of krill meal are added in diets for juvenile whiteleg shrimp, *L. vannamei*. The control diet was formulated to contain 15.0% fishmeal, 36.4% soybean meal, 3.0% fish oil, 1.0% squid meal, 0.63% L-Lysine, 0.14% DL-Methionine, 0.26% L-Threonine, and 0.01% cholesterol. From this diet, three other diets were formulated by substituting half of fishmeal for soybean meal (SBM) and adding either 1.0, 3.0 or 5.0% krill meal at the cost of squid meal and cholesterol. Shrimp of 1.13 ± 0.19 g ($n = 3,210$) were stocked at 100 animals/m² in 30 outdoor tanks of 1.0 m³ (1.02 m²) and raised for 65 days with experimental diets after a 6-day acclimation period. Shrimp were fed using an automatic feeding device which operated daily between 07:30 am and 05:30 pm. To measure feed attractiveness, harvested shrimp were transferred to five indoor tanks of 0.5 m³ and stocked at 88 animals/m². Animals were fed twice a day for 7 days using four feeding trays per tank. Feed attractiveness was based on apparent feed-intake responses and consisted of offering all diets simultaneously in the same rearing tank. Each diet was placed individually in a feeding tray rested on the tank bottom opposite to each other. In the end of the grow-out period, shrimp survival was $96.2 \pm 3.0\%$ and unaffected by dietary treatment. Final shrimp body weight significantly dropped with fishmeal replacement, but stabilized at 5% krill meal compared to the control diet ($P > 0.05$). Daily weight gain (0.10 ± 0.01 g) and final shrimp yield (650 ± 41 g/m²) were not statistically different. FCR and the amount of feed delivered was significantly reduced when shrimp were fed 3 and 5% krill meal ($P < 0.05$). Results from the attractability assay indicated that diets containing krill meal showed a significantly higher apparent feed intake than the control diet. This study demonstrated that a 5% dietary inclusion of krill meal is sufficient to reduce the dependence on fishmeal and other costly ingredients, while maintaining shrimp performance through an improved stimulation of feed intake.

Table 1. Performance of *L. vannamei* fed graded dietary levels of krill meal in diets with reduced reliance on fishmeal and other costly ingredients. Superscript letters within the same line indicate statistically significant differences at 0.05 according to Tukey's HSD test. $29.3 \pm 0.7^\circ\text{C}$ temperature, 40 ± 1.8 g/L salinity, and 7.71 ± 0.31 pH.

Shrimp Performance	Experimental Diets			
	Control	1% Krill	3% Krill	5% Krill
Final survival (%)	95.1 ± 4.5	96.5 ± 2.4	96.2 ± 2.6	96.9 ± 2.2
Final body weight (g)	8.14 ± 1.07^a	7.81 ± 1.28^b	7.96 ± 1.15^{bc}	8.11 ± 1.18^{ac}
Daily weight gain (g)	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.001
Gained yield (g/m ²)	656 ± 33	631 ± 45	648 ± 52	667 ± 31
Feed delivered (g/shrimp)	9.57 ± 0.23^a	9.38 ± 0.30^a	8.66 ± 0.29^b	8.64 ± 0.26^b
Food Conversion Ratio	1.46 ± 0.08^a	1.49 ± 0.10^a	1.34 ± 0.09^b	1.30 ± 0.05^b

LARVAL DEVELOPMENT OF THREE INDONESIAN POPULATION OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*)

Dessy Nurul Astuti, Fajar Anggraeni and Hary Krettiawan

Research Institute for Fish Breeding
Jalan Raya 2 Sukamandi Pantura
Patokbeusi, Subang 41263
West Java, Indonesia
dessy.kertaditira@gmail.com

Macrobrachium rosenbergii has been introduced for aquaculture to many areas outside its natural range. It is farmed in China, India, Thailand, Vietnam, Bangladesh, Malaysia, Taiwan, and in Ecuador in South America. In the Pacific, it is farmed in Fiji Islands, Hawaii, and even New Zealand. The need to increase aquaculture production to meet market demand along with growing fishing pressure and deterioration of GFP natural habitats have attracted related stakeholders to study the species for the purpose of either aquaculture development or conservation of its natural resources. Indonesia is rich of giant fresh water prawn (GFP) germ plasms. Indonesia has 19th native species of *M. Rosenbergii* (Hadie and Hadie, 2002). GFP germ plasms are widely distributed from the western to the eastern part of Indonesia. Several natural populations such as populations Asahan from North Sumatra, Bone from South Sulawesi and Berau from East Kalimantan. The aim of this study was to evaluated the larvae development of third GFP germ plasms.

Prawn larvae reared in five fiberglass funnel with a capacity of 60 liters with a density of 50 larvae / liter using a system of clear water salinity 10-12 ppt. Observation of larval stage of freshwater prawns done every 3 days, by taking 20 larvae / funnel maintenance to be observed with the aid of binokular microscope. Feed used is naupli *Artemia* sp. and artificial feed (egg custard).

Parameters measured were the larval development of three population GFP germ plasms. The results showed at the first day larval stage starts from stage 1, larvae of Berau population has growth faster until 9th day of reraring . While larvae of Asahan has a faster development since 9-18 day of reraring. During 18th day of rearing larvae of Bone has slower development until 12th day than other germ plasms population (Figure 1).

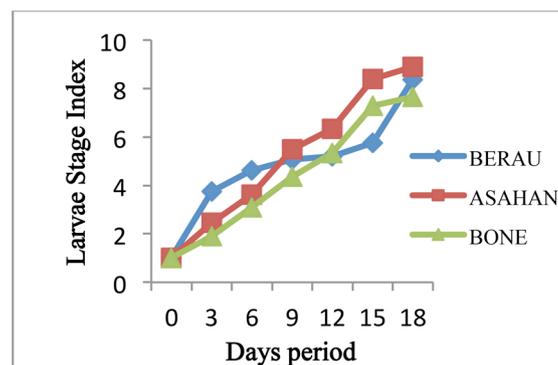


Figure 1. Larvae stage indeks (LSI) of three GFP germ Plasms : Berau, Asahan and Bone at larvae rearing.

MORPHOMETRIC AND MERISTIC VARIATION BETWEEN GIFT TILAPIA, UPM RED TILAPIA F2 HYBRID AND BACKCROSS (BC1F2)

Nwachi, O. F., Esa, Y.E., Christianus, A, Abdullah Rahim, A. and Kamarudin, M. S.

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang Selangor Malaysia

Email: fish2rod@gmail.com

The traits that could be used to explain morphometric and meristic traits of F2 Hybrid, Bc1F2, UPM red Tilapia and Gift Tilapia were examined using Principal Component Analysis in Unscramble@ X statistical tool. A total of 25 morphometric and 5 meristic observation were taking using the buss truss protocol. The score value, correlation loading and the bi-plot was used in explaining the discriminate value. The Score plot shows that the morphometric traits could be represented by the first 2 principal components (PC1 and PC2), with 85% of the original variance in the data set (PC1: 58% and PC2: 27%). Meristics observation used for the scores were the dorsal fin (DF), anal fin (AF), pelvic fin (PF) caudal fin and pectoral fin (PeF). They were represented in the first 2 principal components (PC1 and PC2) with original value of 82% (PC1: 44% and PC2: 38%). Most scores values from the morphometric traits were able to explain the differences in the strain of studies unlike the meristic traits.



Fig 1a UPM red Tilapia



Fig 1b GIFT Tilapia



Fig 1c Bc1F2



Fig 1d F1-Hybrid

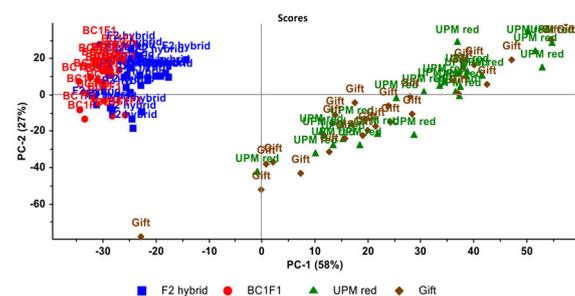


Figure 2: Score plots for morphometric variables

AN ASSESSMENT OF CONSTRAINTS TO AQUACULTURE PRODUCTION IN NORTH CENTRAL, NIGERIA

*Friday G. Ogbe; Unekwu Onuche and Ufedo M. Shaibu

Faculty of Agriculture
Kogi State University Anyigba, Nigeria
kanstarr@yahoo.com

High cost of animal protein has forced people to rely on fish. In sum, Nigeria's fish import has been growing owing to the decline of her artisanal fisheries output. It is hoped that aquaculture will alleviate this problem. However, while aquaculture contribution to total fish is high in some developing countries, its contribution to total fish supply in Nigeria is far less than 10 percent, although it is growing at about 20% per annum in recent time. Serious developmental effort at aquaculture is still nascent. Hence, aquaculture is bedeviled by problems. This study assessed constraints to fish production in North Central, Nigeria using Kogi State as a case study. A three staged random sampling technique was used to select 200 cat fish farmers in the state. Data obtained through structured questionnaire were analysed using mean score from a three point Likert type of scale. The findings indicated that the major constraint to cat fish production in the state is high cost of feed (M=2.80). This could be attributed to the fact that feed stuff and most inputs are sourced externally. Other problems include inadequate finance (M=2.61) and lack of encouragement from government (M=2.30). Amongst others, provision of inputs to cat fish farmers at subsidized rate was recommended.

MOLECULAR DELINEATION OF THREE CHEMOKINES FROM BIG BELLY SEAHORSE (*Hippocampus abdominalis*) AND THEIR PUTATIVE ROLE IN THE REGULATION INFLAMMATORY RESPONSES

Minyoung Oh*, Sukkyoung Lee, Jeongeun Kim and Jehee Lee

Department of Marine Life Science
Jeju National University
Jeju special Self-Governing province 690-756
Republic of Korea
E-mail: lucky5153@naver.com

Chemokines are a family of chemotactic cytokines that regulate leukocyte migration. They are classified into four groups namely, CXC, CC, C, and CX3C, based on the formation of disulfide-bridge. Among these, CXC chemokines are identified as the largest group of chemokines in human. In this study, we identified and functionally characterized a homolog of one CXC chemokine and two CC chemokines from *Hippocampus abdominalis* and designated them as HaCXCL, HaCCa and HaCCb, respectively. Complete coding sequences (CDS) of seahorse HaCXCL, HaCCa and HaCCb were comprised of 342, 276, and 333 base pairs (bp), respectively, which encode polypeptides of 113, 91, and 110 amino acids, respectively, with molecular masses of ~9 -13 kDa. The CXC and CC family specific small cytokine domain (SCY) were identified from the mature peptide region, which comprised of conserved cysteine residues. The recombinant proteins of each chemokine strongly induced the NO production on macrophage cells (RAW 264.7 cells), and showed the chemotactic effect on flounder peripheral blood leukocytes (Fig.1). Tissue specific profiling of HaCXCL, HaCCLa and HaCCCb showed a ubiquitous expression pattern in all examined tissues with the high abundance in spleen, gill and skin, respectively. Up-regulated pattern of mRNA expression was observed in blood and kidney tissues after immune stimulation by live bacteria (*Streptococcus iniae* and *Edwardsiella tarda*) and mitogens (LPS and poly I:C) suggesting their important roles in host immune defense against microbial infection.

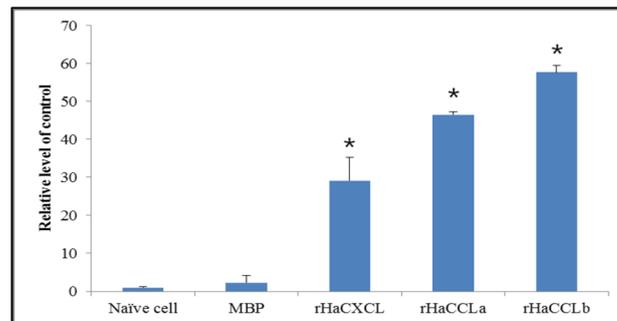


Fig.1. Chemotactic assay for rHaCXCL, rHaCCLa and rHaCCCb protein

TUBER YAM (*Dioscorea hispida*) COULD INFLUENCE REPRODUCTIVE HORMONE OF TIGER GROUPER (*Epinephelus fuscoguttatus*)

Ahmad Daud Om^{*a}, Kamarul Azlan Azizan^b, Syarul Nataqain Baharum^b

^aFisheries Research Institute, Tanjong Demong, 22200 Besut, Terengganu, Malaysia

^bInstitute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
1966adom@gmail.com.com

Tuber of different species of genus *Dioscorea* (*Dioscoreaceae* Family) are known as yam or Ubi Gadong in Malaysia. It more 600 species exist in Africa, Asia and Tropical America and about 19 noncultivated *Dioscorea* species in Malaysia. This important plant is lots of biological activities of have been reported for antioxidative, anti-fungal, anti-mutagenic hypoglycaemic, immunomodulatory effect and it is used as an important ingredient of dietary supplements. Some tubers have been evaluated for steroid hormone production. Therefore, an attempt has discover to analysis the role of tuber for enhancement reproductive hormone of tiger grouper.

The yields phytoestrogen as hormone in was analyzed with GC-MS, successfully with prostaglandin (PGE1) **0.378** mg/mL in 200mg sample (Fig.1). The fatty acid composition of tuber yam was dominating with Palmitic acid as saturated fatty acid (32%) and followed by linoleic acid the unsaturated fatty acid (30.0%). The effects brought by these substances were explored the hormones expressions in tubers Yam in the 50 days implant experiment (Fig.2). Tiger grouper test with tubers Yam (ethanol solvent) showed highest increased estradiol hormone after 40 days implanted.

It showed, tuber yam could be effects to enhance reproductive hormone of Tiger Grouper. It may stimulate the sex steroid production and in turns may potentially exert some positive actions on reproductive success of brood stock management

Fig. 1 GC-MS screening Tuber Yam

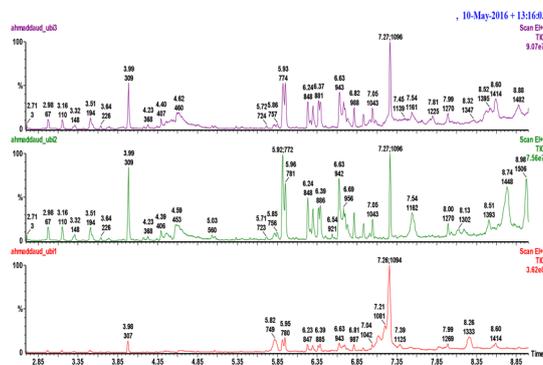
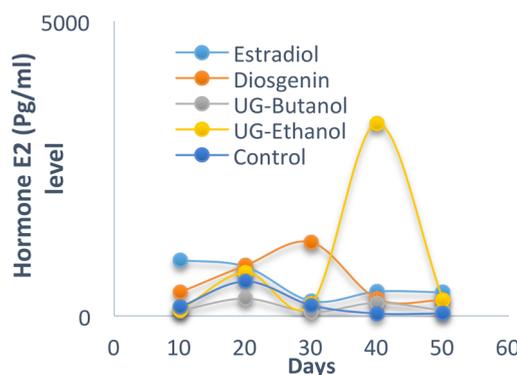


Fig.2 Effect Tuber Yam on reproductive hormone



EARLY ENHANCEMENT OF IMMUNO-RESPONSE IN SHRIMP INCREASES ITS ABILITY TO RESIST DISEASES

Si Mon Ong*, Sheau Yen Chin and Virna Fe Encio

GS Biotech Sdn Bhd, Malaysia
ongsimon79@yahoo.com

In the recent years, shrimp culture has been difficult due to climate change and emergence of new diseases. New products and technology that produce and sell in the market cannot cope with the fast-changing diseases causing the farmers to lose their crop and money. With an immunostimulant that can increase shrimp's immunity starting at the early stage of culture, it will help the animal to withstand the environmental problems and directly resist diseases. This will give more time for farmers to harvest at a certain profitable market size or even better to harvest at a desired day of culture just like in a normal crop. The immunostimulant Beta Defense tested in several farms in the Philippines and Malaysia have consistently attested its efficacy in enhancing the shrimp immunity against infectious diseases (WSSV, EMS and White Feces) and grow the shrimp to marketable size.

COST EFFICIENCIES OF CATFISH FIRMS IN KOGI STATE, CENTRAL NIGERIA

Unekwu Onuche* and Friday G. Ogbe

Faculty of Agriculture, Kogi State University
Anyigba, Nigeria

In this study, cost efficiency levels of catfish producing firms of Kogi State, north central Nigeria were estimated. Stochastic frontier cost function was applied to data obtained from 100 small scale catfish farmers. These catfish farmers were selected through a multistage sampling procedure. Results reveal that the venture is male-dominated. These fish farmers are aging, highly educated but have an average experience of 6 years. Costs of land and labour were significant determinants of cost inefficiency. Less than half (42 farmers or 42% of the Farmers) incur 5% more than the economically optimum cost. These constitute the most cost inefficient firms. Thus, allocative efficiency in catfish production in the area is fair. The average cost efficiency level in the area is 1.106, suggesting that farmers on the average incur about 11% more cost than necessary. The level of cost efficiency could improve with attempts at reducing inefficiency by this 11%. To achieve this, catfish farmers must pay some attention to pricing of land and labour, and seek to optimize the use of these factors of production. This can be achieved through training on cost and input relationship.

Table 1: Stochastic cost frontier estimates for catfish production in Kogi state

Variables	Parameter	Coefficient	t-ratio
Constant	β_0	76725.893	79193.568*
Output	β_1	0.924	3.334*
Feed Cost	β_2	0.084	0.298
Pond Cost	β_3	0.057	0.576
Land Cost	β_4	-25610.850	-46619.614*
Labour Cost	β_5	25611.025	31179.520*
Treatment Cost/drugs	β_6	0.042	0.450
Fingerlings Cost	β_7	0.630	1.058
Sigma square	δ^2	0.673	1.8**
Gamma	Γ	0.968	3.696*

*, ** = Significant at 1%, 5%

Table 2: Cost efficiency estimates of catfish firms in Kogi state

Efficiency Range	Frequency	Percentage	
1.00-1.05	58	58.00	
1.05-1.10	29	29.00	
1.11-1.15	4	4.00	
1.15 +	9	9.00	
Total	100	100.00	Mean: 1.106

TRANSFER OF MATERNAL ANTIBODY FROM BROODSTOCK TO EGG IN TIGER GROUPER (*Epinephelus fuscoguttatus*)

Rafidah, O.¹, Mohd Hafiz Nain Azman¹, Ching, F. F. and Mohd Zamri-Saad, M.²

¹Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu Sabah, Malaysia

²Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia

Corresponding author: rafidaho@ums.edu.my

This study evaluated the immune response of Tiger grouper broodstock following vaccination against vibriosis and passive transfer of maternal antibodies to eggs. Four tails of tiger grouper (*E. fuscoguttatus*) broodstock (Mean BW=8.66 ± 0.09 kg) were immunised intraperitoneally (IP) with 10⁶ CFU/ml of inactivated *V.harveyi* followed by a 1 ml/kg booster dose after 2 weeks and 4 tails were injected with PBS as Control unvaccinated group. Blood sampling was conducted within 5 months at 1- month intervals. The level of serum lysozyme of the vaccinated broodstocks showed a significant ($P<0.05$) increased from 4 ± 2.03 Unit mL⁻¹ to 17 ± 6.3 Unit mL⁻¹ after pre-vaccinated and there was no significant ($P>0.05$) at control group. Where as, serum antibody titer was increased significantly ($P<0.05$) from log₂ 3 to log₂ 129 at pre and 2 weeks post vaccination respectively. While there was no agglutination observed at control unvaccinated group. The eggs lysozyme level of the vaccinated group was 10 ± 1.5 Units mL⁻¹ is significantly ($P<0.05$) higher as compared to control 5 ± 1.8 Units mL⁻¹. The antibody titer in eggs from the broodstocks of the vaccinated group was log₂ 17 and no antibody was detected in eggs from the control group. This finding suggested that inactivated *V.harveyi* able to stimulate the immune response in broodstock and passively transferred to the eggs of the vaccinated grouper.

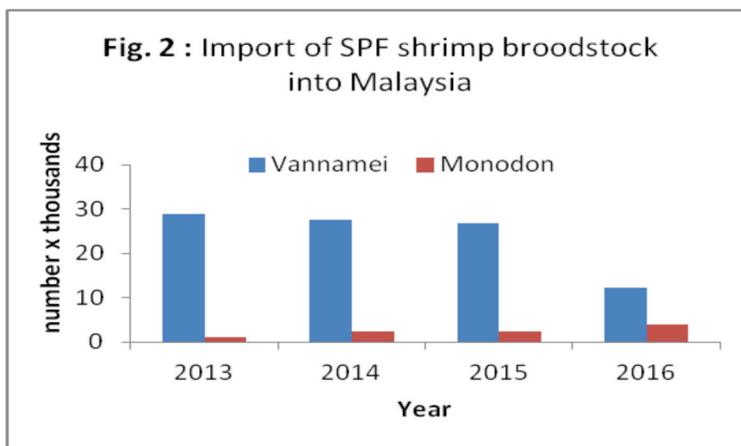
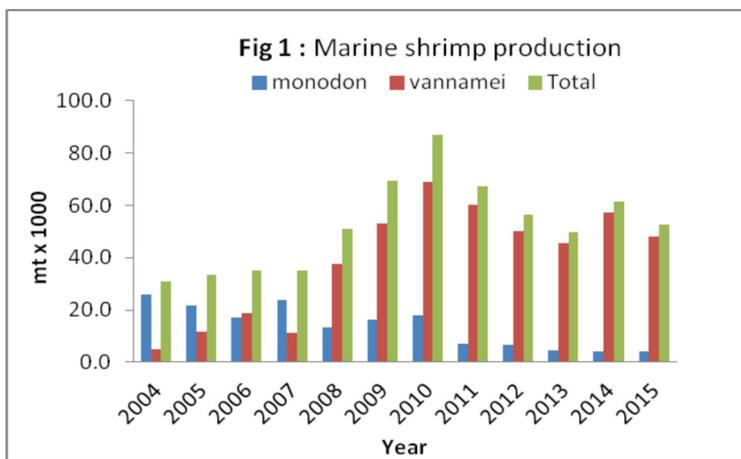
THE STATE OF BLACK TIGER SHRIMP *Penaeus monodon* AQUACULTURE IN MALAYSIA

Mohd.Fariduddin B. Othman*, Razak B. Buang, Afiq B. Razi and Mohd. Hafiz B. Hamdan

*Freshwater Research Division, Fisheries Research Institute (FRI)
 Gelami Lami, 71760 Titi, Jelebu Negeri Sembilan
 fariduddin @ dof.gov.my

Marine shrimp aquaculture in the country started in late 1970's farming local stock of *Penaeus monodon*. Consequently, following the WSSV disease problem, exotic white shrimp *Litopenaeus vannamei* were imported and gradually overtake. In total, a peak production of more than 87,000 mt was achieved in 2010 but take a down turn following emergence of EMS/ AHPND in *vannamei*. The impact was significant albeit the last 5 years output were 40% down or average 50,000mt annual harvest. Largely were due to lower density stocking and smaller size shrimp harvest from *vannamei* farming. *Monodon* harvest stay put with normal size of 40-45pcs/kg but gradually move to higher density stocking of 50-70 pieces per m² using post larvae from imported domesticated strains. Survival of more 80% and harvest of closed to 10 tons is kind of normal result after 120 DoC.

Currently, farming status are 90:10 *vannamei* to *monodon* and cultured within 7500 ha of pond areas. Despite government support and incentives to development, farm expansion seem pending subsequent from disease problem, rising cost of operation and difficulty with supply of SPF broodstock. During the 2013-2016 period about 95,000 *vannamei* and closed to 10,000 *monodon* SPF broodstock only were supplied despite a higher import quota requested and approved by DoF. Apparently, trend toward *monodon* farming is picking up and will be activated if sufficient SPF post larvae supply is guaranteed.



ELECTRIC SHOCK EFFECTIVENESS ON THE VIABILITY OF MUSTIKA CARP (*Cyprinus carpio*) SPERM CELLS

Flandrianto Sih Palimirmo*, Yogi Himawan, and Khairul Syahputra

Research Institute for Fish Breeding
Jalan Raya Pantura Sukamandi no 2 Patokbeusi
Subang Regency, West Java, Indonesia
fspalimirmo@gmail.com

Mustika is superior strain carp that formed by Research Institute for Fish Breeding is a superior breeding fish breeding by selective breeding program based on the molecular mark of MHC II with purposing to increase KHV disease resistant. Furthermore, increasing of Mustika carp genetic quality can be did by introduction of gene with electrophoresis method. Electrophoresis will be done by inserting gene transgene to gene target which later be expressed to increasing more genetic quality of Mustika carp. Gene inserted can be done by give electric shock though electrophorator machine to sperm cells so that making ease for transgene gene to inserting genetically structure of target gene. The aim of this research is to knowing the effectiveness of electric shock to viability of Mustika carp sperm cells.

This research used experimental method with three times repetitions on each treatment. 1 mL a total of Mustika carp sperm cells diluted to physiological solution (NaCl) with 10 times of dilutions, then electric shock given by Gene Pulser Xcell Bio-Rad electrophorator machine on 0, 25, 50, 75, and 100 volt with 0.5 mS long exposure and 0.1 S of resting time at three and five times. Viability of sperm cells observation used eosin 0.01g/mL coloration method, observed by under microscope with 400x magnification, and count based on the percentage of life sperm cells/total sperm cells.

Based on the observation, we obtaining the number of sperm viability at three times electric shocked on 0, 25, 50, 75, and 100 volt are 100, 90, 88, 80, and 70%; while at five times electric shocked on 0, 25, 50, 75, and 100 volt are 100, 88, 82, 75, and 57%. This show that power and number increasing of electric shock can be following by decreasing of sperm cells viabilities.

Table 1. Viability of carps's sperm after electric shock

Shock voltage	Number of shock	
	3	5
0 volt	100.00	100.00
25 volt	90.00	88.33
50 volt	88.33	81.67
75 volt	80.00	75.00
100 volt	70.00	56.67

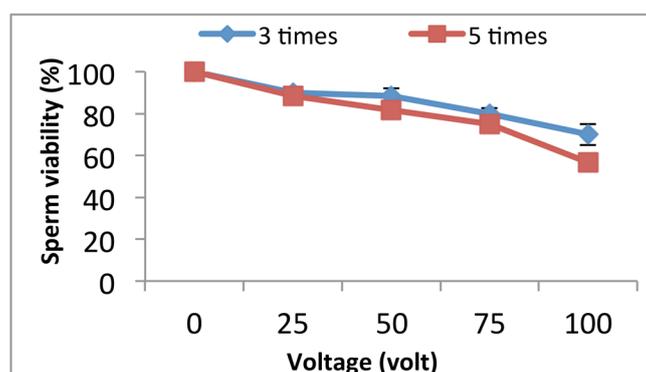


Figure 1. Viability of carps's sperm after electric shock

CHARACTERISTIC OF A VIRAL PROTEIN (VP-15) OF WHITE SPOT SYNDROME VIRUS ISOLATED FROM INFECTED TIGER SHRIMP *Penaeus monodon*

Andi Parenrengi*, Alimuddin, and Andi Tenriulo

Research Institute for Coastal Aquaculture, South Sulawesi, Indonesia, 90511
Agency for Marine and Fisheries Research and Development
andi_parenrengi@hotmail.com

Since 1990, tiger shrimp culture in Indonesia was faced the problems by the decrease environment quality and the viral/bacterial diseases. At least 20 viruses causing the shrimp diseases have been reported; where white spot syndrome virus (WSSV) is one of the major virulent viruses infected the shrimp in pond culture and hatchery. Structural proteins of WSSV, including VP-15, play very important roles in virus infection and morphogenesis process. VP-15 is located in nucleocapsid of virion virus, which involves in the life cycle of WSSV in host cells. The present study was conducted to isolate and characterize the VP-15 from infected tiger shrimp in providing a material base to construct the RNAi technology for controlling the shrimp diseases in aquaculture.

The tiger shrimp were collected from the pond culture at Takalar regency, South Sulawesi. The nine positively white spot diseases were selected for further study, such as 3 samples from outbreak in 2012, 1 sample in 2013, and 5 samples in 2014. DNA genome was extracted by CTAB method and diseases detection was conducted by using the commercial kit. PCR technique was performed to isolate the gene of VP-15 and target gene was then sequenced. Nucleotide sequence analysis was done by Genetyx 7.0 and BLAST-N/P program from NCBI.

A gene encoding VP-15 was successfully isolated in fragment size at 243 bp and had very high similarity (up to 99%) compared with VP-15 in GenBank. The VP-15 consisted of 80 amino acids, two start codons, one stop codon, and one Kozak context, and the VP-15 was rich amino acid of lysine (21.3%), arginine (22.9%) and serine (24.6%) (Figure 1). Three clusters were revealed in the present study corresponding to the time (year) of isolates collection (Figure 2). The characteristic of VP-15 suggested the great potentiality to be used as a material base to develop the RNAi technology.

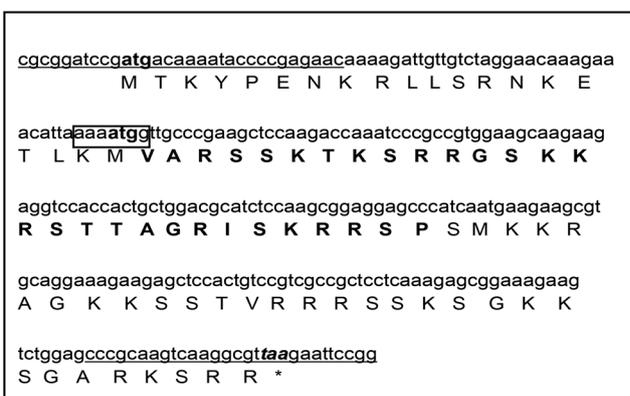


FIGURE 1. Nucleotide sequence and amino acid deduction of VP-15. Nucleotide symbol was small letter and amino acid was capital letter. Forward and reverse primer were underlined, the Kozak context was boxed, start codon was in bold nucleotide, and stop codon was in italic nucleotide, the N-terminal sequence was in bold amino acid.

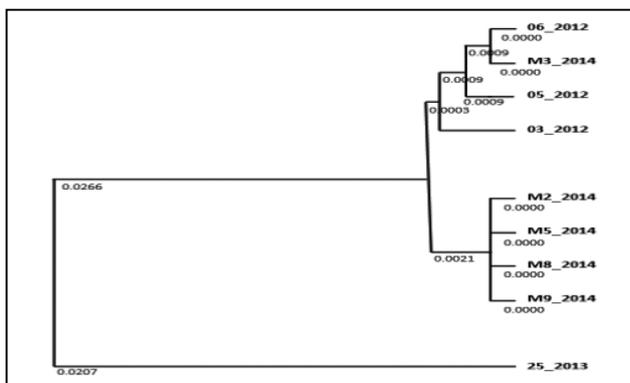


FIGURE 2. Phylogenetic tree of gene encoding VP-15 WSSV isolated from infected tiger shrimp in 2012 (03_2012, 05_2012, and 06_2012); 2013 (25_2013); and 2014 (M2_2014, M3_2014, M5_2014, M8_2014, and M9_2014).

EFFECTS OF TEMPERATURE ON THE EMBRYONIC AND EARLY LARVAL DEVELOPMENT OF HIGH-VALUED TROPICAL SEA URCHIN, *Tripneustes gratilla* (LINNAEUS, 1758)

Md. Shamim Parvez^{1*}, M. Aminur Rahman^{1**}, Fatimah Md. Yusoff^{1,2}, and Aziz Arshad^{1,2}

¹Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia

²Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia

*Presenting author's Email: parvezmdshamim@gmail.com

**Corresponding author's E-mail: aminur1963@gmail.com

Global warming has and will to be continued to warm the world's ocean, which may have unfavorable significances for oceanic bio-resources. Temperature is one of the most important factors affecting the normal functioning of marine invertebrates, especially for sea urchin, i.e. living in shallow tidal water. Studies weighing the impact of climate-change stressors on early life stages of marine invertebrates have focused on immediate fertilization success or larval development, but have so far not been considered gamete longevity. The study was evaluated the effects of different levels (16, 19, 22, 25, 28, 31, and 34°C) of temperature on the embryonic and early larval development of high-valued tropical sea urchin, *Tripneustes gratilla*. The growth performances of the larva were investigated in a wide range of water temperature between 16°C and 34°C under an organized laboratory condition. The critical lower and higher temperature for embryonic development was found at 16°C and 34°C, respectively. Embryos reared in both of these two temperatures exhibited 100% abnormality within 48 h post-insemination. The time required to reach these embryonic and larval stages was increased with temperature treatments from 28°C followed by 31°C, 25°C, 22°C, and 19°C each and individually. The developmental times of 2-cell stage until 4-arm pluteus larvae showed significant differences ($P < 0.05$) among the temperature levels evaluated. The larvae in the stage of prism and 2-arm pluteus were survived at temperature ranges from 19°C to 31°C, while 4-arm pluteus larvae survived at temperature from 22°C to 31°C. In regards of survivality, the survival rate of different stages of the larvae was observed in dissimilar at different temperatures (22°C to 43°C), and the highest values of survival at prism, 2-arm and 4-arm stages were always found at 25°C and 28°C temperatures. However, the development of larvae within the temperature range from 22°C to 31°C revealed that they were able to tolerate a wide range of temperature without getting any abnormality. The morphometric characteristics from prism to 4-arm pluteus larvae in all temperature treatments were differed significantly ($P > 0.05$). Among the temperatures evaluated, 28°C was found to be the best temperature in respect of highest growth and development of larvae in all stages. The findings appeared from our study will not only be helpful to understand the critical limits of temperature but also to find out the appropriate suitable temperature levels for the optimum growth and development of embryos and larvae as well as to facilitate us to develop captive breeding and mass seed production of economically important sea urchin (*T. gratilla*) and other important marine invertebrates for aquaculture in commercial scale.

DECIPHERING OF MOLECULAR CHARACTERS AND IMMUNE RELATED FUNCTIONS OF TWO AKIRIN2 HOMOLOGS FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

Amirthalingam Pavithiran*, S. D. N. K. Bathige, Roopasingam Kugapreethan, Jehanathan Nilojan, Jehhee Lee

Department of Marine Life Sciences, Jeju National University, Jeju, Republic of Korea

*apavithiran@gmail.com

Akirins are highly conserved nuclear localized proteins present throughout metazoan. Most of the vertebrate species have two homologs of akirin genes. In vertebrates, akirin2 is required downstream of NF-Kb in toll-like receptor (TLR), tumour necrosis factor (TNF) and interleukin (IL)-1 β signaling pathways for the production of inflammatory cytokines such as IL-6. In the present study, two homologs of seahorse akirin2 gene were identified from transcriptome database of seahorse and designated as Haakirin2(1) and Haakirin2(2). The molecular features of Haakirin2(1) and Haakirin2(2) were studied using bioinformatics tools. The spatial distribution, and transcriptional modulation during the immune challenges (LPS, Poly I:C and *Streptococcus iniae*) were determined in liver and kidney tissues by using qPCR.

The Open Reading Frame (ORF) of Haakirin2(1) cDNA was consisted of 543 bp which encoded for 180 amino acids with estimated molecular weight (MW) of 20.48 kDa. Meanwhile, 537 bp long ORF was identified from Haakirin2(2) which encoded for 178 amino acids with MW of 20.46 kDa. No putative conserved domain was detected from seahorse akirin2 homologs during the *in silico* analysis. Phylogenetic tree showed a closer relationship of Haakirin2(1) and Haakirin2(2) with respective orthologs from fish species belongs to Acanthopterygian taxa.

Tissues dependent relative mRNA expression of Haakirin2(1) and Haakirin2(2) showed that both homologs in seahorse were ubiquitous in all tissues with higher expression from ovary. During the temporal analysis, in kidney, HaAkirin2(1) showed a significant early up-regulation throughout all challenges. In contrast, the liver tissue showed a significant up-regulation of Haakirin2(1) in late phase of the challenges. Meanwhile, Haakirin2(2) homolog was showed an up-regulated expression after 12h post injection in liver. In kidney, Haakirin2(2) was significantly up-regulated in the mid phase of immune challenges. The results suggest that seahorse akirin2 homologs play a critical role in defense against bacterial and viral pathogens.

INTENSIVE WHITE SHRIMP *Litopenaeus vannamei* CULTURE IN ECO-BASED RECIRCULATION SYSTEM

Minh Anh Pham, Thanh Phong Chau

Neovia Aquaculture Research Center
Nha Be District, Ho Chi Minh City
Vietnam
anhpm@vn.neovia-group.com.vn

The white shrimp (*L. vannamei*) has become a common culture species in Asia and Latin America. Outbreak of several diseases including white feces and acute hepatopancreatic necrosis syndrome (AHPNS) has caused huge economic losses in many countries. A lot of efforts have been made on developing different farming systems to prevent the outbreak of those diseases. In this presentation, we will summarize the results of a trial on recirculation system which established for intensive aquaculture of white. This trial was conducted at NEOVIA AQUACULTURE RESEARCH CENTER, Nha Be district, Ho Chi Minh City, Vietnam. The system consisted of two circular tanks with a volume of 100 cubic meter each. Brackish water was recirculated from a sedimentation pond with tilapia at a stocking density of 1 fish/m². Shrimps at initial averaged live weight of 5 g from a nursery tank were stocked at a stocking density of 110 shrimps/m³. Shrimps were fed with commercial feeds for 70 days. Averaged live weight of shrimps at the harvest was 30.3 g per shrimps (33 shrimps/kg) and average daily weight gain was 0.36 g/shrimp/day. Survival rate was 93.9%. Total biomass was 3.1 kg/m³ (equal to 31 tons/ha/cycle). The present results indicate that it is possible to apply RAS for intensive white shrimp aquaculture with an estimated production of 31 tons/ha/cycle. In the next trial, higher stocking density will be evaluated.

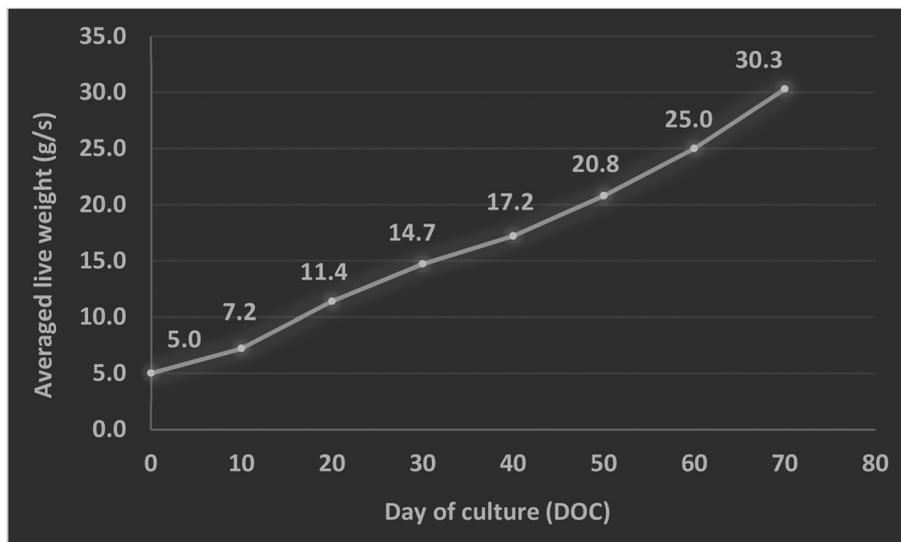


Figure 1. Growth rate during grow-out period

NUTRITIONAL MODELLING OF PROTEIN, AMINO ACID AND ENERGY REQUIREMENTS OF TIGER GROUPEP *Epinephelus fuscoguttatus*

Igor Pirozzi

Port Stephens Fisheries Institute, NSW Department of Primary Industries, Taylors Beach, NSW 2316, Australia

Centre for Sustainable Tropical Fisheries and Aquaculture & College of Science and Engineering,
James Cook University, Townsville, Queensland 4811, Australia

igor.pirozzi@jcu.edu.au

Tiger grouper are a high value marine fish species yet relatively little information is available describing their basic nutritional requirements for protein and energy. Nutritional modelling has been successfully applied to many aquaculture species to provide quantitative estimates of nutrient and energy requirements throughout growth production. While models have been developed for several species describing protein and energy requirements, few have considered the requirements for essential amino acids.

This study used a bioenergetic approach to quantify the digestible protein, amino acid and energy utilisation efficiencies, maintenance requirements, carcass composition and growth potential of tiger grouper. The daily digestible protein (DP) intake to achieve maximum predicted protein retention efficiency was $2.0 \text{ g DP kg}^{-0.7} \text{ day}^{-1}$. The utilization efficiency of dietary protein for tiger grouper was 0.58 with the corresponding cost of DP per unit of protein gain was 1.71 g g^{-1} . The daily digestible energy (DE) intake ($\text{kJ DE kg}^{-0.8} \text{ day}^{-1}$) to achieve a maximum predicted energy retention efficiency was $101 \text{ kJ DE kg}^{-0.8} \text{ day}^{-1}$. The utilization efficiency of energy for tiger grouper was 0.63 and equates to an energetic cost of production of $1.59 \text{ kJ DE kJ}^{-1}$ energy deposition. Utilisation efficiencies for digestible MET, LYS and HIS were 0.51, 0.54 and 0.30 respectively (Fig. 1). The reciprocal cost of production for these essential amino acids per unit gain was 2.0, 1.8 and 3.4 g g^{-1} respectively.

Detailed understanding of nutrient and energy requirements of tiger grouper throughout production will provide a platform to improve feed management and feed formulation.

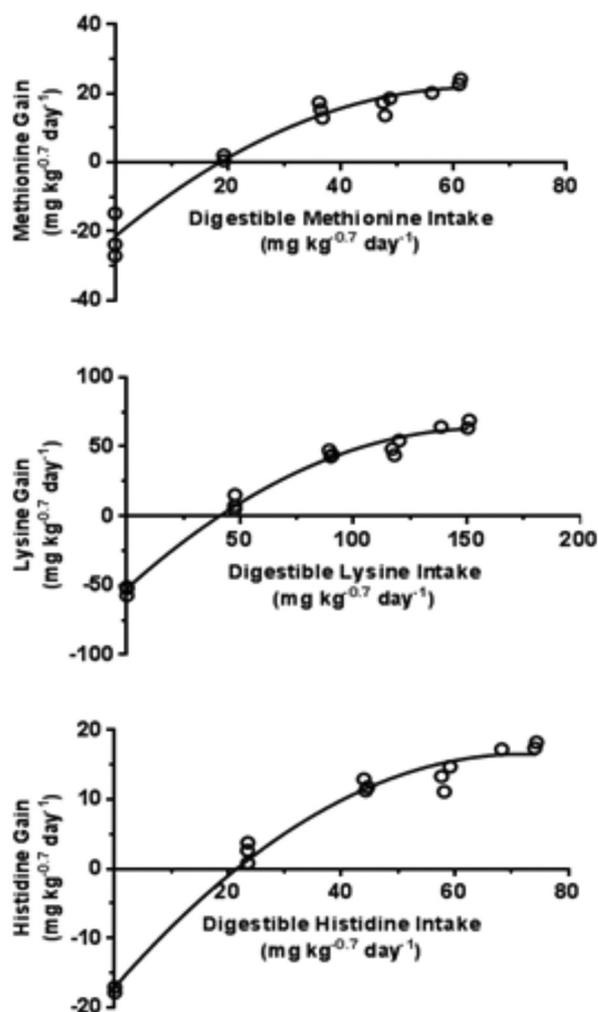


Fig. 1. Relationship between digestible amino acid intake ($\text{mg kg}^{-0.7} \text{ day}^{-1}$) and amino acid gain ($\text{mg kg}^{-0.7} \text{ day}^{-1}$) in tiger grouper.

THE STATUS OF SPINY LOBSTER AQUACULTURE IN INDONESIA

Bayu Priyambodo*, Clive M. Jones, Jesmond Sammut

School of Biological, Earth and Environmental Sciences
The University of New South Wales, Sydney, NSW, 2052, Australia
b.priyambodo86@gmail.com

Lobster aquaculture is a proven strategy to meet the demand for spiny lobster. For example, in Vietnam, annual production of farmed lobster can exceed 1,500 tonnes using a natural supply of puerulus. Surprisingly, farmed lobster production in Indonesia is only 300 tons despite a significantly larger supply of puerulus compared to that of Vietnam. Lobster aquaculture, and its development in Indonesia, has experienced dramatic changes over the last 4 years. The farmed lobster industry, based only in Lombok, consisted of around 2,000 sea-cages in 2012. The industry encountered several problems that impacted production, including: reduced availability of puerulus and post-puterulus due to redirection of seed to the lucrative export market (commenced in 2013), disease outbreaks (2011), and farmers' lack of skills and knowledge of best practice. A study tour of Vietnamese lobster aquaculture sites by Indonesian lobster farmers was undertaken to foster transfer of the Vietnamese technology to Indonesia. The overriding goal was to improve lobster production in Indonesia via technology adoption.

A ministerial regulation, "Decree No. 1/2015" in January 2015, banned the collection of lobsters less than 200g or 8cm in carapace length for aquaculture production. Consequently, this decree affected the seed collection and grow-out industries. The number of lobster farmers decreased to 5% of that of 2012. The ban led to a loss of jobs and income for more than 5,000 people; difficulties for fishers to pay children's school fees; collapse of seed collection and lobster grow-out operations; increase in crime; increase in the number of backyard loan sharks; and, middlemen losing funds invested in future crops that are now illegal. Although the ban has made puerulus collection and export illegal, a 'black market' has emerged which has become lucrative for those who participate in the illegal market chain. Ironically, the number of puerulus caught has increased dramatically from around 8 million of pueruli in 2014 (before the implementation of the regulation) to around 50-60 million pueruli in 2015, as new sources of puerulus have been discovered in Sumbawa, South East Sulawesi, Java, and Aceh.

Lobster farming has huge potential to create a sustainable livelihood alternative for coastal communities in Indonesia. There is an opportunity for the central government to support a sustainable puerulus export and lobster growout industry by fostering best practices based on technologies from Vietnam as well as through locally-based research. Additionally, a review of policies, and implementation of social and environmental impact studies, can help underpin decision-making processes that ensure the principles of Ecologically Sustainable Development are met. There is an opportunity to restore livelihoods and also to potentially improve wild lobster fisheries through stock enhancement using translocated puerulus.

EFFECT OF HERBAL BIOEXTRACT ON SURVIVAL RATE OF NILE TILAPIA FINGERLINGS INJECTED WITH *Aeromonas hydrophila*

Pattareeya Ponza*, Supachok Songsiri, Kankanit Pisamayarom, Touchpol Karakade and Supat Ponza

Program of Fisheries Science
Department of Agricultural Science,
Faculty of Agriculture, Natural Resources and Environment
Naresuan university, Phitsanulok province 65000, Thailand
email pattareeyap@nu.ac.th

Plant product have been identified and reported to accelerate growth and enhance immunity in fish to parasites and bacterial infection as their phytochemical richness such as alkaloids, terpenoids, saponin and flavonoids. Due to Thailand is a tropical countries, the availability of herbs and application has been well known. Kariyat (*Andrographis paniculata* Burm.f. Wall.ex Nees) has been used as local medicinal plant against cold in human for years. It was reported that the major compounds in this plant comprised of andrographolides. This plant has been extracted in water, methanol and ethanol prior to supplemented into fish feed. Another local used for general purposes is Noni (*Morinda citrifolia* L.) In this study, the plants were shredded into small pieces before fermenting with carbon source (brown sugar) and water to encourage bioextraction process for 30 days. After that the aqueous part was retrieved and mixed with the pelleted feed (30% protein) to feed tilapia fingerlings (3-5 g/fish) for 60 days. Growth performance, together with blood parameters, was monitored. Then the fish was challenged by intraperitoneally injecting with *Aeromonas hydrophila* at 3.8×10^6 CFU/fish. Mortality of the fish showing signs of diseases (hemorrhage at the fins and skin) was recorded for 14 days. Results of this study showed that the experimental diet did not affect growth of the fish and blood parameters observed ($P>0.05$). The survival rate of fish received *Andrographis* and *Noni* bioextract supplemented diets were lower than that of the control group (Figure 1). This study aimed to examine the effect of herbal bioextract in feed fed to tilapia fingerling on immunity and growth. It could be indicated that herbal bioextract in this experiment added to fish diet could possibly affect their immunity. Further experiment regarding including these herbs or its pure extraction in fish diet will be investigated.

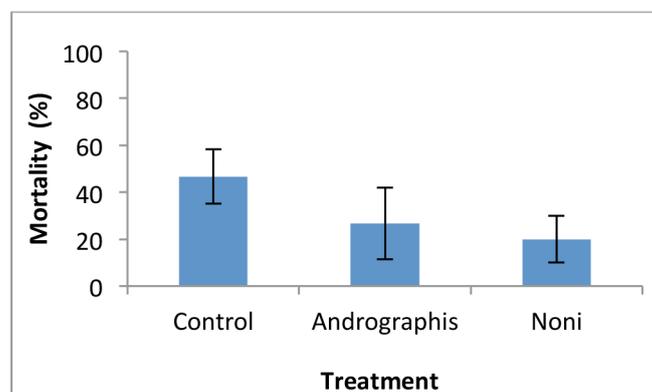


Figure 1: Mortality (%) of the fish in each treatment observed for 14 days post challenged with *Aeromonas hydrophila*.

EFFECTS OF PRO YG TABLETS ON GROWTH PERFORMANCE, SURVIVAL RATE AND VIBRIO CONTROL OF LITOPENAEUS VANNAMEI CULTURE PONDS

1.K.Habeeb Rahaman
Royal Bio-Marine
66/27. F.G.Street, Pernambut-635810
Tamil Nadu. India
info@royalbiomarine.com

2. B.Senthil Kumar
Professor and Head of the Department
Department of Zoology, Thiruvalluvar University, Vellore - 632 115

In aquaculture practices, probiotics are considered a valid alternative to antibiotics and in particular to maintain optimum water quality and to control pathogen loads. The present work emphasizes the efficacy of the Pro YG Tablets (product from Royal Bio-Marine, Pernambut, India, Certified by Coastal Aquaculture Authority of India (CAA Reg. No. CAA/F16/FA/00817) role in water quality management, soil quality and survival rates in *litopenaeus vannamei* ponds. The study was carried out for 122 days to know the growth and survival rate of *P. vannamei* by applying Pro YG Tablets in Ganapavarm, West Godavari district. Andhra Pradesh. India. Four experimental ponds (0.8 hec pond) were selected of which two were PRO YG Tablets treated ponds and remaining two are control. Crab fencing and bird netting was done before pumping water to prevent the autoentrants. After pond preparation, PL10 (average weight of each 0.98 ± 0.04 mg (mean \pm SD) was stocked at the rate of 51 per m² following polymerase chain reaction (PCR) tested by Using IQ 2000 PCR kit from Aqua Lab. The commercially available branded feed was used during the entire study period. Transparency, salinity, pH, dissolved oxygen (DO), temperature, total Ammonia Nitrogen (TAN) were recorded by standard measurements. The average final body weight of the harvested shrimp is 22.5, 23 g in experimental ponds and 18.2, 18 g in controlled ponds and the difference was significant ($P < 0.01$) between these two productions. The average survival rate was 91.5 % in experimental pond and 76 % in controlled pond. The average Vibrio bacterial (yellow and green colonies) counts were found to be significantly ($P < 0.01$) lower in Pro YG Tablet treated (Pond 1, Pond 2) ponds compared to control ponds (Pond 3, Pond4). Especially dominant green colonies were lowered in both of the experimental ponds and increased Vibrio levels are noticed in Pond 3 and Pond 4 in periodical weekly sampling in water and animal samples.

The result showed that Pro YG Tablets plays an important role in maintaining water quality parameters, soil sludge digestion, health management, reduced Vibrio bacterial count and improved total yield of shrimp survival rates.

EXPLORATION OF THE HIGH-VALUED MARINE BIORESOURCES IN THE INDO-PACIFIC AND THEIR EMERGING PROSPECTS, CULTURE POTENTIALS, CONSERVATION STRATEGIES AND BIOMEDICAL APPLICATIONS

M. Aminur Rahman*, Fatimah Md. Yusoff and Aziz Arshad

Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author's E-mail: aminur1963@gmail.com

Malaysian/Indonesian Archipelago belonging to the Indo-Pacific is the "Centre of Origin" of many diverged marine flora and fauna. The region has been considered as one of the richest marine biodiversity hotspot comprising unique species of echinoderms, mollusks, shrimps, crabs and fishes in the oceanic systems. These bioresources provide excellent opportunities for the studies of breeding biology, reproductive ecology, aquaculture, conservation, population genetics as well as species and speciation mechanisms (evolution) of many diverged taxa. However, this interesting area is quite new and yet to be explored in Malaysia. In the bottom-dwelling sessile invertebrates, echinoderms have been considered as the high-valued marine bioresource, having profound biological, ecological, aquacultural, conservational, nutritional and pharmaceutical significance. Among them, the sea urchins and sea cucumbers are both commercially fished and heavily overexploited. In sea urchins, the harvested product is the gonad of both sexes, commonly known as "Sea urchin Roe", which has been long traditions of consuming as a high delicacy food in Asian, Mediterranean and Western Hemisphere countries, and have long been using as luxury foods in Japan. The population of the Asian Pacific Region has been using it for long time as a remedy for improving general living tone and treatment for a number of diseases. In the sea cucumber, the principal product is the boiled and dried body-wall or 'bêche-de-mer' for which there is an increasing demand in many tropical and subtropical countries. In addition, sea cucumbers have also been popular as a traditional food tonic in China, Korea and Taiwan for thousands of years. Sea cucumbers are also believed to exert wound healing and reduce arthritis pain in humans, hence are widely used in Asian folk medicine. However, due to lack of proper management and conservation strategy in place, many sea urchin and sea cucumber fisheries are under threat. For this, cultivation of these species increasingly becomes a necessity, both for stock enhancement programs and as a means to meet the increasing market demand. Large-scale cultivation of commercial sea urchins through effective culture and reseedling methods of species has been practiced in Japan for many years. However, sea urchin cultivation, outside in Japan is still a recent practice, less than 15 years old, and mainly still at a research level, even though a number of commercial species are now being produced and cultured in captive rearing conditions. Culture of sea cucumber first initiated in Japan in the 1930s and is now well established there and in China. Procedures for mass production of the tropical *Holothuria scabra* are now well established in India, Australia, Indonesia, the Maldives and the Solomon Islands, and have recently been practicing in Philippine and Thailand. Alike many other marine organisms, echinoderms have been, and continue to be, examined as a source of biologically active compounds with biomedical applications. Most recently, a number of important bioactive compounds have been isolated from sea cucumbers, sea urchins and starfish, having distinctive biological and pharmacological activities including anti-angiogenic, anticancer, anticoagulant, anti-hypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor and wound healing activities. However, development patterns of echinoderm fisheries are largely expectable, often unsustainable and frequently too rapid for effective management. Proper steps should be undertaken on the potential ecosystem and human community consequences, appropriate aquaculture management strategies, and urge for better monitoring and reporting of catch and abundance, proper scientific research for stock enhancement and consideration of international biodiversity and trade regulations to ensure sustainable development and utilization of high-valued echinoderm fisheries in the Indo-Pacific to a greater extent.

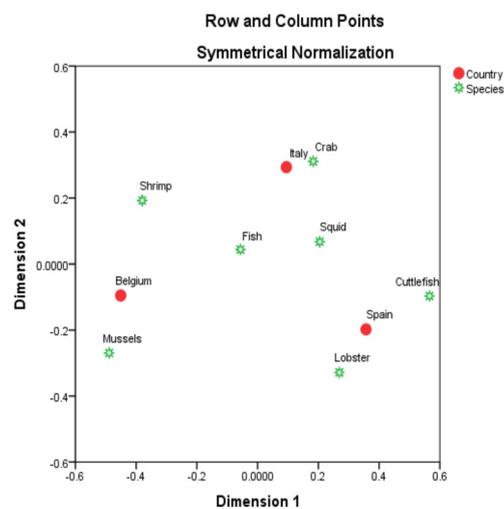
CONSUMER PREFERENCES OF SEAFOOD IN BELGIUM, SPAIN AND ITALY TO UNDERSTAND THE NEEDS OF THE MARKET

Nashila Rahmaniya * Mini N. Sekharan

School of Industrial Fisheries
Cochin University of Science and Technology
Cochin 682016
India
nashilar@gmail.com

Belgium, Spain and Italy are the major seafood export destination countries in Europe. Fish is an important element of the Mediterranean diet and also a part of the traditional European diet (Birch and Lawley, 2012). Studying the preferences of the consumers in these countries will help India to enhance export performance to these countries.

Data of 294 respondents from Belgium (148), Spain (87), and Italy (59) through a structured questionnaire by sequential snowball sampling technique. The questionnaire were filled from the natives of each country who attended the seafood expo at Brussels (2016) and also emailed to many respondents. Consumers were asked to rank their preference fish, shrimp, mussels, crab, lobster, squid and cuttle fish. Profile chart of the preference of three countries show that mussels are having closer relationship with Belgium while Spain is closely associated with lobster and cuttle fish and Italy with Crab. These results are significant for the marketers and producers of the Indian seafood Industry to help plan efficient marketing strategies and build new business opportunities.



APPLICATION OF ENZYMES FOR IMPROVING FEED DIGESTIBILITY AND PERFORMANCE IN CARP CULTURE

Rajalekshmi, M.* , Harikumar, S., and Vidya, A.

Kemin Industries South Asia Pvt. Ltd.
C-3, 1st Street, Ambattur Industrial Estate
Chennai 600058, India
rajalekshmi.m@kemin.com

Supplementation of enzymes to improve the digestibility is a well-established practice in production animals but not been exploited in aquatic animals to its full potential. Indian major carps (IMC) are freshwater fishes mainly reared in India; the species include rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*). Around 65% of IMC cultured in India are fed exclusively with mash feed, and 35% are fed with mix of mash and pellet feed. The mash feed prepared by farmers majorly consists of de-oiled rice bran (DORB) - 80%, groundnut cake - 10%, cotton seed cake/ sunflower cake - 10%. IMC also feed on zooplankton, phytoplankton and higher plants. The native enzymes in IMC include trypsin, lipase, amylase, glucosidase, maltase, sucrase, lactase, melibiase, and cellobiase. We hypothesized that supplementation of additional enzymes would improve the digestion of feed and phytoplankton.

Considering the type of feeding materials being used and amount of endogenous enzymes present in IMC, an enzyme formulation was developed specifically for carp culture. The formulation was further fine-tuned after taking into account the feeding pattern of carps, which includes phytoplankton, waterweeds, insects, mollusks etc. The formulation was tested for *in vitro* feed digestibility using DORB as a substrate, individually and in combination with IMC gut enzyme preparation. Crude enzyme extract from fish intestine was prepared by grinding the whole intestine of rohu (*Labeo rohita*) fingerling in chilled phosphate buffer saline, the protein content of the extract was determined in the supernatant of crude extract which was used as enzyme mix for the studies. An improvement in digestibility was measured based on *in vitro* sugar release.

The combination of enzymes in the formulation was observed to give improved sugar release as compared to the individual enzymes when used alone. A significant increase in sugar release was observed when the formulation was supplemented with or without the gut enzyme extract from fish. We also noted a synergistic effect for the formulation when used in combination with the gut enzyme extract from fish.

In carp culture farms in India, the feed is supplemented in submerged feed bags which posed a concern on the leaching of enzymes and possible requirement of higher dosages. A study was conducted with enzyme supplemented feed bags submerged in water for 3 h, and the leaching was measured based on release of cellulase in to water. It was observed that only less than 1 % of the total enzymes are leached in to water during this time period.

The formulation, Kemzyme® Aqua was tested at commercial carp culture systems. It was observed that supplementation of Kemzyme® Aqua in feed improves the body weight and enhances pigmentation in IMC. This would enable the farmers to reduce the culture period and fetch a better price for the produce as the pigmentation improves market price for IMC.

ISSUES RELATED TO BLOOD COCKLE *Anadara granosa* MORTALITY IN WEST COAST OF PENINSULAR MALAYSIA

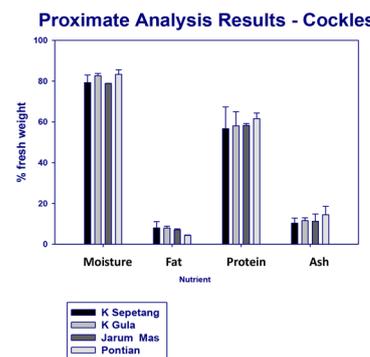
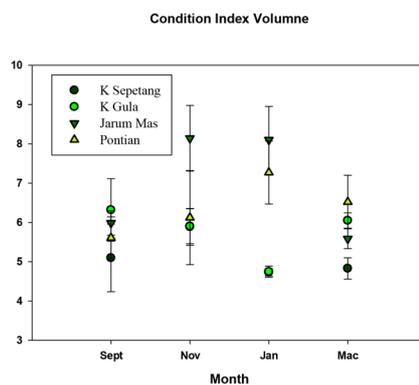
Shahunthala Devi Ramachandran*; Mohd Nawab.B Arshad; Pauzi B. Ahmad; Kamaruzaman B. Ismail

Fisheries Research Institute
11960, Batu Maung, Penang
Malaysia
shadev01@yahoo.com

The mudflats of the west coast of Peninsular Malaysia are very productive, producing 72,000 tonnes of marketable cockles and 11,000 tonnes of cockle seeds (Mohd. Mazlan, 2000). Based on the Fisheries Annual Statistic Report 2000, issued by the Department of Fisheries Malaysia, the total production of cockle landings in Selangor was 6,922 mt utilizing 654 ha farming area with a production rate of 11 mt/ha. Meanwhile, in the 2011 report, the total production of cockle landings had increased remarkably to 26,505 mt with further expansion of 5,593 ha of cockle farming area. It shows an increasing number of productions and area of cockle's farming. However, the production rates for 2011 had declined to 4.7 mt/ha. The declining of cockle's production rate was due to several factors. Poor water quality with special reference to ammonia, increased stocking density and invasion from the 'siput rantai' (clam) were some of the factors mentioned to explain the losses. This paper presents water quality data and sediment texture from several locations sampled over 2014- 2015 experiencing cockle mortality in an attempt to elucidate contributing factors to the aquaculture problem. Condition index values of the cockles over the growth period as well as proximate analysis data were determined as a basis of comparison between locations sampled.

Results indicate poor water quality especially dissolved oxygen and ammoniacal nitrogen during neap tide could be a contributing factor to cockle mortality. Condition index values reflect poor growth in cockles from areas experiencing poor water quality.

Laboratory bioassays carried out to determine LC 50 and EC 50 values for ammonia in solution exposures are ongoing. Preliminary findings show reduced feeding by cockles exposed to sub-lethal concentrations.



EFFECT OF DIETARY SUPPLEMENTATION OF *Cosmos caudatus* IN BAGRID CATFISH, *Mystus Nemurus* FINGERLINGS

Nor Maisarah Rameli ^{1,2*}, Md. Abdul Kader ^{1,3} and Ong Chee Hong ²

¹School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia

²Underwater World Langkawi Sdn. Bhd., Jalan Pantai Cenang, Mukim Kedawang, 07000, Langkawi, Kedah, Malaysia

³Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia

*Corresponding author: normaisarahrameli@yahoo.com

Aquaculture production globally had been increased thus raised up some related issues such as nutrition and food safety. As an attempt to overcome these issues, incorporation of herbs in feed is started. For this current study, *Cosmos caudatus* was used as food additive in bagrid catfish, *Mystus nemurus* fingerling. This herb is common and normally consumed by locals since it believed to help in improving human health. The crude of *C. caudatus* was incorporated in the fish diet at the level of 0, 0.5, 1.0, 1.5 and 2.0%. Six hundred fish with average of initial weight of 3.05 ± 0.39 g were distributed into 150L fiberglass tank in quadruplicate. The fish was fed twice a day until satiation level. From the data collected after 60 days of feeding, there was significant difference ($P < 0.05$) seen for the final weight of fish among treatments. This ongoing study is considered a preliminary study and the whole results will be presented later.

GROWTH RATE OF FIELD FARMING SEAWEED *Kappaphycus spp* IN LANGKAWI

Nik Nazli Effendy Ramli^{1*}, Zaidnuddin Ilias¹, R.A Roki Mohamed¹, Zainoddin Jamari¹

¹Fisheries Research Institut Langkawi, Kedah 07000 Malaysia

*Corresponding author email: nikhazlier@hotmail.com

The growth experiment of seaweed using floating basket system was conducted in Langkawi, Kedah. The experiment was conducted from Jun until August 2016. The present study of seaweed culture exhibit the results of species variation of daily growth rate with 3 different initial densities such as 50, 100 and 150g for *Kappaphycus spp*. The "hijau", the maximum daily growth rate (3.29 ± 1.12) was recorded with initial seedling density of 100g at 48 days. The minimum growth rate (1.73 ± 1.41) was observed in 50g of culture period.

THE EFFECT OF SALINITY ON SURVIVAL AND GROWTH PERFORMANCE OF GOLDEN TREVALLY *Gnathanodon speciosus* JUVENILES

Azmi R^{1*}, Fadzilah Y¹, Saber M¹, Abu B.A², Zainoddin J. and Farazi J¹

Brackish Water Aquaculture Research Division, FRI Gelang Patah
81550, Gelang Patah, Johor
Corresponding author: azmirani2005@gmail.com

The experiment was performed to determine the effect of salinity on survival and growth performance for Golden trevally *Gnathanodon speciosus* juveniles during nursery phase. Before that, *G. speciosus* juveniles were introduced to abrupt changes of salinity from 20ppt to 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45ppt. The salinities with mortality rate less than 50% within 72 hours were used in the study to find out the survival rate and growth performance of the juveniles during 60 days of nursery period. The salinities were 10, 15, 20, 25, 30, 35 and 40ppt. The Fingerlings exhibited highest survival rate at 20ppt (92.67%) followed by 35ppt (91.67%) and the lowest survival rate was at 10ppt (63.67%) whilst the Specific Growth Rate (SGR) showed highest at 15ppt (0.11g/day) followed by 20ppt, 25ppt, 30ppt, 40ppt, 10ppt and 35ppt. Somehow the differences among treatment were not significant ($P > 0.05$). The juveniles grew and survived satisfactorily at salinities range from 10–40ppt, implying that this species can be cultured successfully at wide range of salinity. *G. speciosus* can be considered as an ideal species to promote, in view of current and future climate variables as more and more coastal areas are going to be vulnerable to saline water inundation.

MUCOSAL MAPPING OF SALMON'S SKIN, GILLS AND ESOPHAGUS REACTION TO HYDROGEN PEROXIDE TREATMENT

Imelda Rantty¹ and Karin Pittman²

1. Fisheries Research Institute Sarawak, Jalan Perbadanan, Bintawa, 93744 Kuching, Sarawak, Malaysia
imeldarantty@gmail.com

2. Dept. of Biology, University of Bergen, 5020 Bergen, Norway
Karin.Pittman@uib.no

Sea lice infestation has caused major economic losses for the salmon industry imparting a negative review from the public because of its adverse effect on the welfare and environment (Boxaspen et al., 2006). Hydrogen peroxide (H_2O_2) is one of several treatments used to control sea lice infection in Atlantic salmon, *Salmo salar*, production although acts on the slimy barrier tissues of all fish. The barrier tissues of fish (skin, gills and guts) have mucosal epithelia (slime producing), which have been shown to play an important role in the defence against pathogens in Atlantic salmon (Svendsen and Bogwald, 1997). This study explores the effect of hydrogen peroxide treatment on the mucosal barriers of skin, gills and esophagus in commercial salmon farming using mucosal mapping and determines the recovery time from treatment. Mucosal mapping (Pittman et al., 2013) has been shown to allow direct comparison of volumetric size data and ratios of epithelia to mucous cell to indicate enhanced immunity dysregulation in the gut due to diet (Torrecillas et al., 2015), exposure to infectious agents in the gills and responses to parasites in the skin. Samples of commercially farmed Atlantic salmon (mean length 44 cm) in seacages were taken prior to large scale H_2O_2 treatment (Day -1), Day 4, Day 11 and Day 18.

Samples were taken from standardized body sites of dorsolateral skin and caudal ventral, second gill arch and esophagus, fixed in formalin, decalcified, embedded in Technovit, sectioned, stained with Periodic Acid Schiff and Alcian Blue for unequivocal marking of the mucous cells, and analyzed for mucous cell area and density, mucous cell area:density ratio and filament:lamella ratio for gills. The results show that different fish body regions exhibit different cellular architecture where mucous cell size and density varied significantly across the body sites with significant differences between groups. It takes at least two weeks for Atlantic salmon mucosal epithelia to recover after delousing. Mucosal Mapping may detect weaknesses, which are undetectable by traditional histopathology. It provides an unbiased reference to understand the effect of hydrogen peroxide on mucosal barriers of salmonids and the corresponding recovery time. Patterns in mucosal epithelia are immunologically important, objectively reproducible and comparable across time, species and treatment may detect weaknesses, which are undetectable using traditional histopathology.

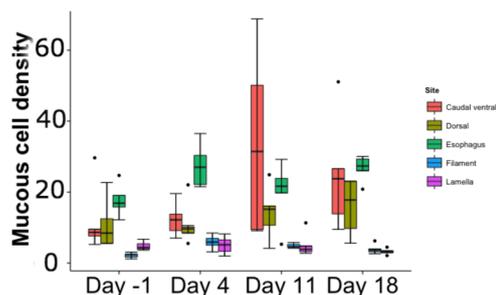


Figure 1: Mucous cell density (%) with time in skin (caudal ventral and dorsal), esophagus, and gill (filament and lamella) samples from Atlantic salmon undergoing a delousing treatment with hydrogen peroxide

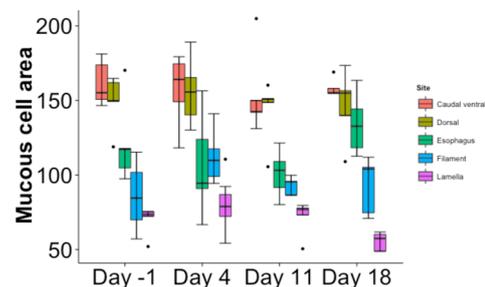


Figure 2: Mucous cell area with time in skin (caudal ventral and dorsal), esophagus, and gill (filament and lamella) samples from Atlantic salmon undergoing a delousing treatment with hydrogen peroxide

FARMER PERCEPTIONS OF FISH HEALTH MANAGEMENT AND BIOSECURITY ON GROUPER FARMS IN BALI, INDONESIA

Mardiana E. Fachry, Wahyudin P. Sasmita, Navneet K. Dhand and Michael A. Rimmer*

University of the Sunshine Coast,
Locked Bag 4, Maroochydore DC
Queensland, Australia.
Email: mrimmer@usc.edu.au

A socio-economic survey was undertaken for sea-cage farms in northern Bali, Indonesia, to better understand farmer attitudes with respect to fish health and biosecurity. The survey used a formal questionnaire that was administered to farm owners or managers. A total of 14 sea cage farms were randomly selected from a pool of 38 sea cage farms in northern Bali.

The main species farmed at the time of the survey were tiger grouper *Epinephelus fuscoguttatus*, the hybrid grouper ‘cantang’ ♀ *E. fuscoguttatus* × ♂ *Epinephelus lanceolatus*, and hybrid grouper ‘cantik’ ♀ *E. fuscoguttatus* × ♂ *Epinephelus polyphekadion*. These three species now dominate grouper production in Indonesia. Nine farms (64%) had cultured the high-value mouse grouper (*Cromileptes altivelis*) within the last year. Other species, including barramundi (*Lates calcarifer*) and pompano (*Trachinotus* spp.), were farmed in small numbers by only a few farms.

Common diseases reported by farmers are: viral nervous necrosis, iridovirus and parasite infestations. 59% of farms regarded fish losses due to disease as ‘medium’; 24% as ‘low’, and 18% as ‘high’. No farms regarded the frequency of disease occurrence as ‘low’; all regarded it as either ‘medium’ (57%) or ‘high’ (43%).

Farmer experience suggests that some grouper species are more prone to disease than others. The grouper hybrid ‘cantik’ was regarded by most farmers as having a generally high propensity for disease problems, while tiger grouper was rated moderate to high. In contrast, the hybrid grouper ‘cantang’ and mouse grouper were regarded as having a low propensity for disease.

Farmers ascribed disease-related losses to two main factors:

1. Poor availability and quality of ‘trash’ fish feed (the main feed source used in grouper farming); and
2. Water quality deterioration during the early part of the rainy season in November – February each year.

All 14 farms practised prophylactic treatment of the fish to reduce the incidence of disease outbreaks. Freshwater bathing was undertaken routinely by all the sea cage farms, at intervals of 3 days to 1 week. All the farms interviewed disinfected their equipment regularly. However, only the larger companies (21%) separated equipment that had been in contact with infected fish, and cleaned or replaced the nets after the fish were harvested.

All the farmers interviewed were confident that they could identify disease outbreaks visually. Swimming activity and loss of appetite were the main symptoms that farmers used to identify disease outbreaks, followed by changes in skin colour and signs of injury on the body. Formal diagnoses are rarely sought, and during disease outbreaks the farms primarily rely on the owner or technical staff to provide technical advice (93%). Only 7% of farms consulted with government technical institutions during disease outbreaks.

GROWTH PERFORMANCE OF THREE STRAINS TILAPIA IN BRACKISHWATER POND

Adam Robisalmi*, Priadi Setyawan, dan Bambang Gunadi

Research Institute for Fish Breeding
Jalan Raya 2 Sukamandi Pantura
Patokbeusi, Subang 41263
West Java, Indonesia
aa_salmi@yahoo.com

Tilapia is an euryhaline fish species which can be cultivated in either freshwater or brackishwater pond. Due to the fact that there is a lot of abandon shrimp ponds in Indonesia, increasing national tilapia production require a superior strain of tilapia which is able to grow well in high salinity ponds. This experiment aimed to evaluate the growth performance of three strains of Nile tilapia that is red tilapia *Oreochromis sp*, Srikandi hybrid tilapia *O. aureus x niloticus* and blue tilapia *O. aureus*.

Fish fingerlings were produced by natural spawning of each strains in the freshwater earthen pond. The mature broodstock were mated in the spawning ponds at male to female ratio of 10:30. After 14 days of broodstock mating, larvae were harvested and reared in hapa measuring 2x2x1 m with a stocking density of 250 fish/m². The fry were reared for 90 days to reach the fingerling size before they transferred the high salinity ponds for growing-out.

Rearing of fingerling was carried out in the coastal pond sized of 5000 m² in the 3x5 m² net at water salinity of 20-30g/L for 120 days. The fingerling were stocked at density of 10 fish/m². Feeding with floating commercial fish feed (30% crude protein) was applied at a rate of 5-10% of biomass twice a day. The observed parameters included of growth rate, survival rate and feed conversion ratio. The results showed that Srikandi strains tilapia revealed the highest growth (figure 1). Based on the growth rate, feed conversion ratio, and survival rate, Srikandi strain tilapia had the best performance among others (Table 1) and was recommended as the most suitable candidate for fish culture in the high salinity ponds in Indonesia.

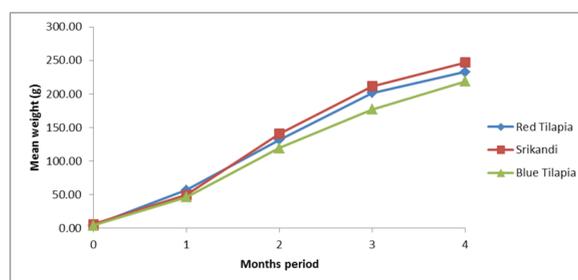


Figure 1. Growth pattern of three strains tilapia reared in brackishwater pond

Table 1. weight gain, specific growth rate, feed conversion ratio and survival rate of three strains tilapia

Strains / Parameter	Red Tilapia	Srikandi	Blue Tilapia
Weight gain (g)	228,59±2	240,93±2	214,54±26.98
SGR (%day ⁻¹)	3,33±0.02	3,13±0.26	3,28±0.16
FCR	1.67±0.06	1.75±0.11	1.89±0.23
Survival rate (%)	94.50±2.12	91.00±1.76	85.00±1.06

PREGELATINIZED STARCHES IMPROVED PELLET CHARACTERISTICS AND THE GROWTH AND PHYSIOLOGY OF TILAPIA

Nicholas Romano, Naga Kanmani, Mahdi Ebrahimi, S.M. Nurul Amin, Mohd Salleh Kamarudin, Vikas Kumar

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Starches are less well utilized in fish than terrestrial animals, but are included in aquafeeds as an inexpensive source of energy but also to act as a binder. Starch utilization as well as their binding properties may be improved through pregelatinization. This is when starches are heated in the presence of water and the crystalline regions break down and gels, which can allow more sites for digestive enzymes as well as binding of nutrients. The aim of the current study was to assess the potential differences of native or pregelatinized (PG) starches on the growth and various physiological parameters of tilapia after 9 weeks as well effects to various pellet characteristics.

Four isonitrogenous, isolipidic and isoenergetic diets were formulated to contain tapioca starch, PG tapioca starch, corn starch or PG corn starch, at 250 g kg⁻¹. The PG starches were made by mixing with water (1:1 ratio), autoclaved for 15 min., oven dried and then hammer milled. Pellets were extruded and after oven drying, the bulk density, expansion ratio, pellet durability index, water absorption index, water solubility index, water stability and protein solubility were measured and then the microstructure of surface and cross sections were examined. Pellets were then fed to triplicate groups of tilapia and the growth, muscle/plasma biochemistry, intestinal short chain fatty acids, and liver glycogen after 9 weeks.

The PG diets had significantly higher water stability, water solubility, bulk density, and protein solubility along with a smoother surface. Tilapia fed the TS diet had lower growth than all other treatments, but was significantly improved when pre-gelatinized. In the PG treatments, intestinal SCFA significantly decreased while plasma glucose, cholesterol and triglycerides as well as liver glycogen significantly increased. Fish fed the CS diet had significantly more long chain polyunsaturated fatty acids compared to the other treatments. Pre-gelatinization may improve fish productivity and offer greater flexibility during aquafeed production.

Growth performance, feeding efficiencies and survival of tilapia fed diets with different pre-gelatinized or native carbohydrate sources after 9 weeks.

	Experimental diets				2-way ANOVA		
	CS	PG-CS	TS	PG-TS	Type	Treatment	Interaction
WG (%)	184.18 ± 12.43 ^{ab}	192.47 ± 4.92 ^{ab}	145.29 ± 7.65 ^b	213.38 ± 22.38 ^a	NS	0.028	0.042
SGR	1.42 ± 0.03 ^a	1.45 ± 0.01 ^a	1.32 ± 0.02 ^b	1.49 ± 0.05 ^a	NS	0.021	0.035
FCR ^d	1.24 ± 0.08 ^a	1.21 ± 0.01 ^a	1.57 ± 0.09 ^b	1.08 ± 0.11 ^a	NS	0.011	0.019
PER ^c	2.47 ± 0.12 ^{ab}	2.50 ± 0.02 ^{ab}	2.11 ± 0.07 ^b	2.75 ± 0.21 ^a	NS	0.041	NS
Survival	88.34 ± 1.67	93.34 ± 1.67	86.67 ± 1.67	85.00 ± 2.89	NS	NS	NS

EVALUATION OF CRYOPROTECTANT (Methanol, Glycerol and Dimethylsulphoxide) FOR SPERM CRYOPRESERVATION OF TIGER SHRIMP *Penaeus monodon*

Rosmiati and Ike Trismawanti

Research Institute for Coastal Aquaculture
emirosmiati@yahoo.com.

The lack of wild male broodstock shrimp tiger as the spermatophore source, unconcurrent of gonad maturation period of female and male parent and unsynchronism between spermatophore (donor) supplying and female parent molt cycle as the receiver is required to do spermatophore supplying through cryopreservation technique development. One of the spermatophore cryopreservation successfully is determined by cryoprotectant used. The study aims to evaluate the best cryoprotectant used to sperm cryopreservation of tiger shrimp. Sperm cryopreservation was carried out by using extender of saline solution and cryoprotectant of methanol, glycerol and dimethylsulphoxide at the concentration of 5 % in sterile saline solution and keep for 30 days at the room temperature, -20°C and liquid Nitrogen (-196°C). The observation of sperm mortality was investigated at the initial and every 5 days of rearing time period. Findings showed that among the three of cryoprotectant used, glycerol was obtained to be the best cryoprotectant for sperm cryopreservation of tiger shrimp with the density of sperm and survival rate percentage of sperm were 0.8×10^6 cell/mL and 80.4 % at -196°C (liquid nitrogen) after 30 days of rearing period

Table 1. Total dan survival rate percentage of sperm cryopreserved in the different cryoprotectants at room temperature

Jenis pengawet (Kode)	Waktu penyimpanan (hari) x 10^6 sel/mL					
	5	%	10	%	30	%
Glycerol (GA)	0.4	37.52	0.4	36.3	0.3	24.5
Methanol (MA)	0.8	81.4	0.6	55.9	0.1	2.9
Dimethylsulphoxida (DA)	0.8	80.4	0.1	12.1	0.2	2.0
Saline Solution (SA)	0.4	38.2	lisis	0	lisis	0

Table 2. Total dan survival rate percentage of sperm cryopreserved in the different cryoprotectants at -20°C

Jenis pengawet (Kode)	Waktu penyimpanan (hari) x 10^6 sel/mL					
	5	%	10	%	30	%
Glycerol (GB)	0.9	85.3	0.8	80.4	0.7	67.6
Methanol (MB)	0,2	20.6	0.1	7.8	0.3	2.9
Dimethylsulphoxida (DB)	0,3	26.5	0.1	7.8	0.3	2.9
Saline Solution (SB)	0.2	2.0	0.3	1.3	0.1	1.0

Table 3. Total dan survival rate percentage of sperm cryopreserved in the different cryoprotectants at -196°C

Jenis pengawet (Kode)	Waktu penyimpanan (hari) x 10^6 sel/mL					
	5	%	10	%	30	%
Glycerol (GC)	0.9	91.2	1.0	89.2	0.8	80.4
Methanol (MC)	0.7	70.6	0.5	48.0	0.2	22.6
Dimethylsulphoxida (DC)	0.8	77.5	0.7	70.6	0.5	56.9
Saline Solution (SC)	0.4	3.9	0,2	2.0	0.1	1.3

EFFECT OF VDS FEED ADDITIVE MIX ON THE RESISTANCE OF *L. vannamei* TO WSSV

Lodewijk Rosseel*, Koen Blanchaert

VDS nv
Paanderstraat 40
Deerlijk, 8540
koen@vds-afs.be

The present trial aimed to evaluate if *L. vannamei* shrimp, which were subjected to a feeding trial with specific dietary treatments (see Paper “Effects of VDS feed additive mix on the growth performance of *L. vannamei*”), showed an improved resistance to WSSV infection. This was based on the assumption that the experimental diets contained a specific feed additive mix that can improve shrimp health in general and/or stimulate (antiviral) immunity specifically.

In order to evaluate the resistance to WSSV, 20 shrimp per treatment were orally inoculated (natural infection route) with 108.3SID50 (shrimp infectious doses 50% endpoint) WSSV solid inoculum under standard laboratory conditions. Three days before starting the WSSV challenge, shrimp were transferred from the feeding trial facility of VDS to the disease challenge facility of IMAQUA. The shrimp were housed individually in 10L glass tanks (infection units). Aside the negative control (CTRL) and the 3 different dietary treatments of VDS (A,B,C), an internal IMAQUA (commercial) control (ICTRL) was also included in the evaluation (Table 1). The clinical outcome of the WSSV challenge test was evaluated by the following parameters: Cumulative percentage of mortality by day 7; Cumulative percentage of diseased shrimp by day 7; Onset of mortality; Cessation of mortality.

Although we can see an improved survival in treatment B when compared to the CTRL (Table 1), this difference was not statistically significant. This was most probably originated by the low mortality outcome and a limited sample size (number of shrimp per dietary treatment). It could be expected that at a higher mortality level using younger animals and with a higher sample size (+35 animals) per treatment, this difference would turn out to be significant. The difference in mortality between B and ICTRL were statistically significant. This reinforces the assumption that treatment B can induce a higher resistance of shrimp to WSSV.

Table 1. Summary of the results obtained in the different parameters evaluated during the WSSV challenge. Values sharing the same label are not significantly different from each other ($p>0.05$). Values that have no label in common are significantly different from each other ($p<0.05$).

Dietary treatment	WSSV challenge			
	Final mortality (%)	Final diseased (%)	Onset of mortality (h)	Cessation of mortality (h)
Mock	0±0	0±0	-	-
ICTRL	40±0 ^a	80±0	60	90
CTRL	25±7 ^{ab}	55±7	54	90
A	25±21 ^{ab}	55±21	54	90
B	10±0 ^b	55±7	60	60
C	30±28 ^{ab}	50±14	54	84

PRODUCTION OF TIGER SHRIMP BROODSTOCK (*Penaeus monodon*) CANDIDATE IN POND WITH DIFFERENT DENSITY

Sahabuddin*, H. S. Suwoyo dan Andi Sahrijanna

¹Research Institute for Coastal Aquaculture

Jl.Makmur Dg.Sitakka No.129 Maros South Sulawesi Indonesia

*Email : s.abud_din@yahoo.co.id

The Tiger shrimp (*Penaeus monodon*) was the leading commodity in order to improve Indonesia's shrimp exports. One of the problems today's the shrimp farming is broodstock limitations because it only get up the natural stock, while the need broodstock increased for disease free . The limited number of broodstock from the sea can be overcome by maintaining broodstock in ponds because its potential is quite large in generating prospective broodstock candidate. Maintenance of broodstock in ponds is done to ensure the availability of brood as needed. Based on this reality, the supply of tiger shrimp broodstock in ponds be a right choice.

This research was conducted at 2 ponds of land with an area of each 2,500 m², which is then partitioned into two portions being replicates in the study. Then performed the pond preparation, includes drying, repair construction, processing of land, fertilizer and water filling, then dotted tiger shrimp juvenile was 22 days (average weight of 0.02 g/ind). The treatments that are : A) stocking 5 ind/m² and B) stocking 10 ind/ m².

Observation of biological variables such as body weight were measured every two weeks.

Observation of water quality variables such as temperature, salinity, pH, soluble oxygen and brightness was measured in situ, while ammonia, nitrite, BOT, alkalinity and plankton were observed every 2 weeks. Shrimp survival rate calculated at the end of the study.

The results showed that the production of tiger shrimp broodstock can be done in the pond with a density of 5-10 individuals / m². The density of 5 individuals / m² tiger shrimp broodstock candidate growth reaches a weight of 20.5 g / ind., within 60 days of maintenance, while the density of 10 ind. / m² growth of black tiger shrimp broodstock weight average is only 12.56 gr/ind. with the same of time maintenance.

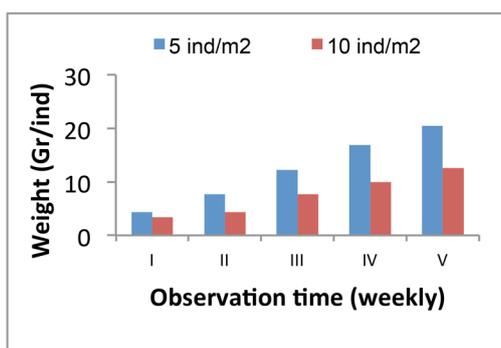


Figure 1. Growth of Tiger shrimp broodstock in pond at different density

GRADUAL REPLACEMENT OF FRESH FEED WITH FORMULATED DIET FOR SPINY LOBSTER *Panulirus spp.* WEANING

Ibnu Sahidhir*, Syafrizal, Clive M. Jones, Syahrul, Mike Rimmer

Brackishwater Aquaculture Development Center Ujung Batee
Jl. Laksamana Malahayati Km 16, Aceh Province
ibnusahidhir@yahoo.com

Effective artificial diets for lobster have been formulated with some level of success. However, feeding practice management has received little attention. The objective of this study was to evaluate growth performance and survival of lobster pueruli which were adapted gradually to formulated diet. The experiment was conducted in a recirculation system with round rearing tanks 56cm in diameter and 46cm high. The lobster pueruli (mean weight $0.31\text{g} \pm 0.01$) were reared for 30 days, fed with one of 5 different strategies i.e. formulated diet only (FD), 10% fresh diet replacement per day (D1), 10% fresh diet replacement every 2 days (D2), 10% fresh diet replacement every 3 days (D3), and fresh diet only (fish, crab and mollusc). Results showed that growth performance was significantly affected by the treatments but not survival. This study suggested that 10% gradual replacement of fresh diet every three days generates the best growth performance. High mortality and moulting were evident in the first week, in the transition phase between puerulus to post puerulus stage. At the completion of the experiment, the remaining lobster population was dominated by *Panulirus ornatus* and *P. homarus*.

Table 1. Growth and survival of lobster seed treated by different time of gradual replacement of fresh diet to formulated feed

Treatment	Initial weight (gr)	Final weight (gr)	Weight gain (%)	Specific growth rate (%)	Survival (%)
FD	0.31±0.01	0.76±0.02	141±3 ^a	2.94±0.04 ^a	26.6±1.9 ^a
D1	0.31±0.01	0.79±0.02	154±9 ^{ab}	3.09±0.12 ^{ab}	23.0±1.4 ^a
D2	0.31±0.01	0.79±0.04	157±2 ^{ab}	3.12±0.20 ^{ab}	28.7±0.8 ^a
D3	0.32±0.01	0.93±0.02	187±9 ^b	3.50±0.10 ^b	28.0±2.1 ^a
Fr	0.32±0.00	0.71±0.03	124±1 ^a	2.67±0.18 ^a	28.0±1.4 ^a

The result presented in means ± standard error. Data with different superscript in the same row are significantly different, $p < 0,05$, $n_0 = 60$



Panulirus ornatus
(57%)



Panulirus homarus
(31%)



Panulirus longipes
(6%)



Panulirus versicolor
(4%)



Panulirus sp. (1%)

Figure 1. Species of *Panulirus spp.* in the remaining lobster juvenile population.

SHOOTS MULTIPLICATION IN ENDEMIC AQUATIC PLANTS *Cryptocoryne elliptica* USING THIDIAZURON (TDZ)

Norhanizan Sahidin*, and Aziz Ahmad

Fisheries Research Institute
FRI Glami Lemi
71650 Titi, Jelevu
N. Sembilan, Malaysia
norhanizans@gmail.com

The effect of thidiazuron (TDZ) was investigated on *in vitro* proliferation from shoot tips explants of *Cryptocoryne elliptica* an endangered endemic aquatic plants. Murashige and Skoog (MS) medium and Linsmaier and Skoog (LS) medium containing TDZ (0.2 – 1.0 g/ml) were tested and effectively inducing shoot buds and inflorescences. The highest mean number (7.33 ± 3.077) of shoots per explant was achieved from shoot tips cultured on MS medium supplemented with 0.8 g/ml TDZ for 8 weeks. Inflorescences were shown on all explants cultured on medium supplemented with TDZ except phytohormone free medium.

Table 1: Effect of TDZ on multiple shoot regeneration from shoot tips of *Cryptocoryne elliptica* in MS/LS medium after 8 weeks of culture.

Concentration TDZ (g/ml)	Mean \pm SE of shoots regeneration	
	MS	LS
0	1.71 ± 0.91^b	3.44 ± 1.74^b
0.2	3.50 ± 2.43^a	6.10 ± 2.38^a
0.4	6.25 ± 1.28^a	6.90 ± 3.28^a
0.6	5.11 ± 2.42^a	6.75 ± 1.98^a
0.8	7.33 ± 3.08^a	5.88 ± 2.10^a
1.0	3.33 ± 0.58^a	5.30 ± 1.95^a

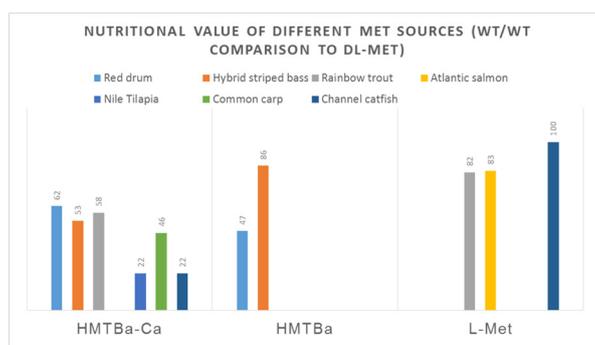
Values represent means \pm standard errors of two independent experiments of ten replicates. Means followed by the same superscript letter within columns are not significantly different by Tukey's test at 5% probability test.

SHORT REVIEW OF THE NUTRITIONAL VALUE OF DIFFERENT METHIONINE SOURCES IN FISH

Alexandros Samartzis* and Cláudia Figueiredo-Silva

Evonik (SEA) Pte. Ltd.
3 International Business Park #07-18
Nordic European Centre Singapore 609927
alexandros.samartzis@evonik.com

The 100% bioavailability of free amino acids (AA) has been demonstrated in several terrestrial and aquatic species and, as a result, the use of supplemental AA in animal feed has become common practice across different sectors of the animal feed industry, including aquaculture. The supplementation of low fishmeal or plant protein based diets with one or more limiting essential AA (EAA) to match animal requirements, was proven to be a cost-effective decision to reduce feed costs while maintaining or even increasing animal performance. Among the different EAA currently used in the fish feed, methionine (Met) is commonly the first-limiting EAA. Therefore, the interest of both industry and academia in assessing the nutritional value of the different Met sources available commercially, grew considerably in the last years. Different Met products are commercially available, including DL-Met, L-Met, 2-Hydroxy-4-(methylthio)butanoic acid (HMTBa) and HMTBa calcium salt (HMTBa-Ca). DL-Met and L-Met were shown to be both effectively utilized by terrestrial animals (Baker, 2006) and aquatic species, namely rainbow trout (Kim et al. 1992 & Powell et al., 2017) and Atlantic salmon (Figueiredo-Silva et al. 2014). Although not statistically significant, the slightly lower bioavailability of L-Met (82-83%) relative to DL-Met found in salmonids requires further investigation, but agrees with earlier data obtained in salmon (Sveier et al. 2001), rainbow trout (Kim et al. 1992) and in hybrid striped bass (Keembiyehetty and Gatlin III, 1995), showing that D- and/or DL-Met are at least as effective as L-Met. Further studies on the nutritional value of HMTBa products compared with DL-Met conducted in fish concluded that HMTBa and HMTBa-Ca are less available than DL-Met (reviewed by Lemme 2010; Lemme et al. 2012, Figueiredo-Silva et al. 2014; Powell et al. 2017). Applying regression analysis and comparing the slopes of the regression lines between sources based upon weight gain, nutritional value of HMTBa-Ca relative to DL-Met varied between 22% in Nile tilapia and channel catfish, and 62% in red drum (Figure 1). A critical review of the studies reporting on the nutritional value of different Met sources will be provided.



References

- Baker D.H. (2006):** Journal of Nutrition 136, 1670S–1675S.
- Figueiredo-Silva C., H. Folkers, C. Schulz and A. Lemme (2014):** Aquaculture Europe conference 2014, San Sebastian, Spain.
- Keembiyehetty C.N. and D.M. III Gatlin (1995):** Comp Biochem Physiol 112A: 155-159
- Kim K.I., T.B. Kayes and C.H. Amundson (1992):** Aquaculture 101: 95-103
- Lemme A. (2010):** Evonik AMINONews, Special Edition, March 2010.
- Lemme A., Wenhua, W. Gao and C. Kobler (2012):** ISFNF 2012, Molde, Norway.
- Powell C.D., M.A.K. Chowdhury and D.P Bureau (2017):** Aquaculture Research 48: 332-346.
- Sveier H., H. Nordås, G.E. Berge and E. Lied (2001):** Aquaculture Nutrition. 7:169-181

A CRITICAL LOOK AT ESTIMATES OF APPARENT DIGESTIBILITY OF PROTEIN AND AMINO ACIDS

Dominique P. Bureau* and Guillaume Pfeuti

Fish Nutrition Research Laboratory
Dept. of Animal Biosciences, Ontario Agricultural College
University of Guelph
Guelph, ON, N1G 2W1, CANADA
dbureau@uoguelph.ca

Information of the apparent digestibility coefficient (ADC) of nutrients of different ingredients is increasing every year thanks to sustained research efforts. Estimates of ADC are regularly compiled in the reference literature and increasingly used by feed manufacturers who are now formulating their feeds on a digestible protein and amino acid basis. This progressive move from formulating on a 'total nutrient' basis to formulating on digestible nutrients is praiseworthy. However, increasing reliance by feed millers on published estimates of ADCs makes it critical to ensure that the information available is relevant and reliable.

ADCs of protein and amino acids are reported for a large number of ingredients fed to different aquaculture species. Significant variability is observable across studies and species. Some of the data suggest significant differences in ADCs of protein among species for the same ingredient. Differences in methodology and intrinsic variability in the composition of different batches of the same ingredient make it difficult to meaningfully compare ADCs across studies, let alone across animal species. A critical review of results from digestibility trials suggest that methodological shortcomings (e.g. marker analysis) and calculation errors (biased equations) result in published estimates of ADC that are not always highly reliable. There is frequently a need to examine the original data from trials to be able to identify sources of errors but this is rarely feasible for published studies.

The University of Guelph Fish Nutrition Research Laboratory (UG-FNRL) has been a pioneer in the assessment of the digestibility of fish feed ingredients. Cho and Slinger (1974) published reliable estimates of ADC of protein and energy to rainbow trout of practical ingredients. The sustained use of the same equipment and methodology by the UG-FNRL has highlighted significant improvements in ADC of protein of some ingredients over the past 40 years. These efforts highlighted significant variability in ADCs of protein and amino acids across different lots or batches of the same type of ingredient. These efforts also indicated that digestibility is a measure of "disappearance" of nutrients but not a direct measure of bio-availability of nutrients. Consequently, ADC of nutrients should ideally be corroborated by more direct assessment of the bioavailability of nutrients of ingredients. The processing and drying equipment and conditions used in the production of ingredients were observed to have a determinant effect on the digestibility of protein. An *in vitro* assay examining the susceptibility of protein to degradation by intestinal enzymes suggests the differences in ADC of protein observed for similar ingredients have a rational biochemical basis. However, the chemical determinants of digestibility are not well understood and characterized. Recent research efforts suggest that the level of protein cross-links present in feed ingredients may be strongly correlated with their digestibility of protein and bio-availability of amino acids. Protein crosslinks are either naturally occurring in proteins or formed as a result of heat or chemical treatment/damage. Protein crosslinks are mostly found as 1) disulfide crosslinks involving thiol-disulphide interchange reactions, and 2) amino acid cross-links derived from reactions between certain amino acids and chemical compounds. A simple processing technique targeting the disruption of disulphide cross-links was highly effective in improving the nutritional quality of relatively poorly digestible protein ingredients. Better understanding of the chemical determinants of protein digestibility should enable the development of rapid methods and tools to more reliably estimate the digestibility and nutritive value of protein ingredients.

PROBIOTICS STIMULATE MUSCLE GROWTH OF NILE TILAPIA *Oreochromis niloticus*

Vander Bruno dos Santos*, Vinicius Vasconcelos Silva, Edson Assunção Mareco, Paulo E. Pardo, Maeli Dal Pai Silva, Rondinelle A. S. Salomão

Agência Paulista de Tecnologia dos Agronegócios
Instituto de Pesca/Polo Alta Sorocabana
Presidente Prudente, SP Brazil
vander@apta.sp.gov.br

The objective of this study is to evaluate the effect of probiotic additive in tilapia growth, assessing the morphology of the muscular. Tilapia fingerlings of approximately 1.5 g were cultivated in recirculation systems containing 15 water tanks of 0.25 m³ each at 28 °C, with biological filter and UV system at 80 fish/m³. The fish are fed three times a day, with the same feed, using the probiotic additive supplied by Biomart Animal Nutrition. They were assessed the inclusion levels of 0.1 and 0.2% of probiotic additive. The probiotic was homogenized in 2% soy oil and sprinkled over the feed. The control treatment consisted only of the addition of vegetable oil in the same proportion. The histology of muscle was evaluated at day 30th and 90th of cultivation to quantify fibers muscle diameter. Fiber muscle were separated in different diameter class and plotted in a histogram. It was proceeded ANOVA and Multiple Comparison Procedures (Student-Newman-Keuls Method).

The muscle fiber diameter was higher with treatment with 0.1 or 0.2% of probiotic in relation to control. This occurred at days 30th and 90th of cultivation (Table 1). Actually at day 90th of treatment with probiotics, the difference in relation to control was more intense than day 30th (9.1% compared to 6.2%).

It was observed higher fiber frequency in the class 40-60 μm and smaller fiber frequency in the class >80 μm in control treatment when compared with treatment with probiotics (Figure 1). It wasn't observed differences in hyperplasia (fiber < 20 μm). Probiotics added in diet stimulates muscle growth by white fiber hypertrophy.

Table 1. Mean and standard deviation of muscle fiber diameter of tilapia fed with ration with different level probiotics.

Time (days)	Treatment	Muscle Fiber Diameter (μm)
30	Control	44.64 (1.80) b
	0.1%	48.04 (2.78) a
	0.2%	46.80 (2.08) a
90	Control	63.75 (3.98) b
	0.1%	70.65 (6.05) a
	0.2%	68.65 (5.22) a

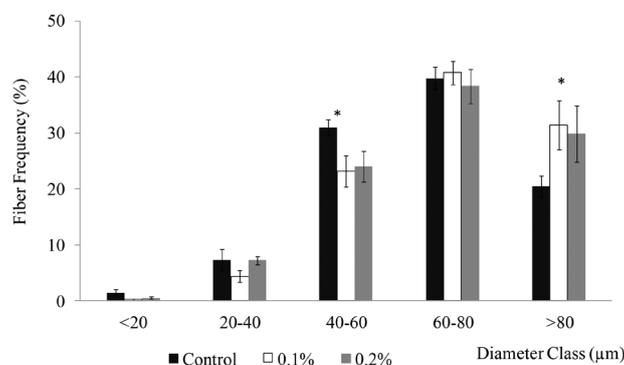


Figure 1. Frequency of white muscle fibers in different diameter class of Nile tilapia fed with ration content different probiotics level at day 90th. *Significant (P<0.05)

Acknowledgments: Financial support was given by Fapesp.

GROWTH OF NILE TILAPIA *Oreochromis niloticus* STRAINS IN BRAZIL

Vander Bruno dos Santos*, Edson Assunção Mareco, Rondinelle A. S. Salomão, Vinicius Vasconcelos Silva, Marcus V. Couto

Agência Paulista de Tecnologia dos Agronegócios
Instituto de Pesca/Polo Alta Sorocabana
Presidente Prudente, SP Brazil
vander@apta.sp.gov.br

The objective of this study is to evaluate the growth of Nile tilapia strains in Brazil, compared to genetically improved ones from Philippines, the Genomar Supreme. Tilapia fingerlings, male population, of approximately 8 g will be grown in water recirculation systems containing 0.5 m³ tanks, in initial density of 120 fish/m³, with four replicates (tanks). The fish will be fed three times a day with the same commercial feed for each particular growth phase according to biomass of each tank. Ten fish from each tank will be sampled at the 0, 60th, 120th, 180th and 240th days of cultivation. The study of the growth was conducted by fitting all data on fish weight for the Gompertz model given by $y = A \exp(-Be^{-Kx})$. The estimates were obtained by Weighted Least Squares. In addition it will be determined the absolute growth rates (AGR), the weight and age at inflection points (A/e and $(\ln B)/K$, respectively).

The initial weights were 8.72 (1.08), 8.60 (0.55), 8.70 (0.83) from Brazilian's strains and 8.14 (0.29) to Philippine's one and they were similar ($P > 0.05$). The final weights from Brazilian's ones were 875.45 g (52.89), 732.88 (31.44) and 713.60 (57.24), 60% smaller than Philippine's one (1302.19 g and 161.86, $P < 0.05$). The model fitted and AGR are presented in Figure 1.

Genomar Supreme strain presented higher weight (723.8 g), age (161.5 days) and absolute growth rate (7.67 g/day) at the inflexion point when compared Brazilian's ones (370-420 g, 135 – 150 days and 4.2 – 4.95g/day, respectively).

Data presented in this work don't represent the maximum growth of the tilapia strains. Genomar Supreme tilapia constitutes an important potential to improve tilapia farm in Brazil however, the use of this genetics needs to be concretized in this country.

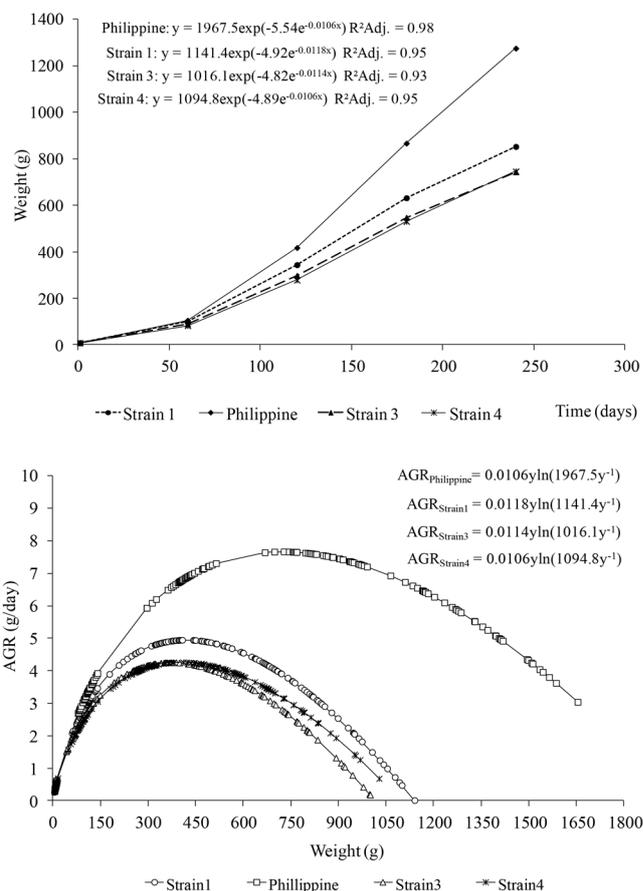


Fig 1. (A) Growth curves of Nile tilapia strains. (B) Absolute growth rate from different tilapia strain.

EFFECT OF CANNABIS ON GROWTH AND METABOLISM OF NILE TILAPIA *Oreochromis niloticus*

I. P. Saoud, Jessica Babikian, Nivin Nasser, & Samer Monzer

Department of Biology
American University of Beirut
Bliss Street, Beirut, Lebanon
is08@aub.edu.lb

Indian hemp, marijuana, pot, hashish, or *Cannabis sativa* is a hardy plant with a wide range of environmental tolerances including temperature variation and drought conditions. Although it is an excellent plant for semi-arid regions, it is forbidden in many nations because of its psychoactive effects. Lately, the beneficial properties of the plant extract are becoming better understood and perceptions are changing. As the aquaculture industry matures from a primitive extensive pond system to an industrialized intensive filtered system, fish stress and disease incidence are going to increase. In the present work we investigated whether cannabis oil extract would relieve stress, improve growth and feed conversion, and/or improve disease resistance. Three diets were made to contain either soy oil, industrial hemp oil or cannabis oil and offered to tilapia for eight weeks. At termination, survival, growth, feed conversion and blood parameters were assessed. Fish were returned to their tanks, offered the same feeds as during the experiment and respiration assessed. Results are in the table below. Cannabis extract was found to have a negative effect on fish growth and also increased metabolism. Cannabis had no effect on blood cell counts, total plasma protein, hematocrit or lysozyme activity.

	Control	Ind. Hemp	Cann. Oil
Survival (%)	100	100	100
IBW ± SE (g)	10.9	10.8	10.8
FBW (g)	49.96 ^a	43.19 ^b	40.26 ^b
TL (cm)	13.80 ^a	13.32 ^b	13.02 ^b
K	1.88 ^a	1.81 ^a	1.81 ^a
SGR (% / day)	2.58 ^a	2.37 ^b	2.29 ^b
FCR	1.97 ^a	2.34 ^b	2.48 ^b
R (mg O ₂ /g/h)	0.137 ^a	0.162 ^{ab}	0.185 ^b

IMPORTANCE OF LOTUS *Nelumbo nucifera* CULTIVATION WITH FISHES

AMITA SAXENA

Fisheries Resource Management, GBPUAT, PANTNAGAR 263145, INDIA

From ancient times the SCARED lotus has been a divine symbol in traditions . The growth of its pure beauty from the mud Lotus is an aquatic plant and grows in natural waters. Earlier it was grown in temples ponds with soun machlyao , now there are common practices to grow lotus plant with fishes specially ornamental fishes, . for sanctity religious views ornamental purposes and for food value .because various parts (rich in nutrients)of the plant are used .So cultivation if this plant in mineral rich water bodies has great value to farmers for enrichment. As this plant absorbed minerals and dirt and clear the water for fishes to grow This one way purify the water and on the other hand beautify the water body.

STUDY OF OSMOREGULATION TREND IN *Rutilus frisii kutum* EXPOSED TO CASPIAN SEA WATER

Iranian Fisheries Science Research Institute, Inland waters Aquaculture Research Center, Agricultural Research Education and Extension Organization (AREEO), Anzali, Iran

Mohammad Sayyad bourani, Hassan Magsoudieh Kohan, Mohadese Ahmadnejad, Sohrab Dejandian
mohammadborani@yahoo.com

The study was done in nutrition and live food in aquaculture research station was located in Bandar Anzali Ghaziyan. Juveniles weighted average 0.5, 1, 2.5 gr were randomly selected in three groups Caspian Sea with a salinity of 11 ppt, water 7 ppt and freshwater (with three replicates per group) were included.

To study the microstructure of gill for each treatment, tissue samples by classical histological methods and stained with hematoxylin - eosin slides were prepared. The frequency and location of the enzyme Na^+ , K^+ - ATPase and chloride cells with immunohistochemical localization was performed. Studies micrometric gill chloride cells by software Image tool (version 2.0) was performed. Data analyzed by one-way ANOVA (Oneway ANOVA) with Tukey's test was performed. Results of gills chloride cells number per square millimeter of the surface gills in *Rutilus frisii kutum* fingerlings showed that number of cells in 0.5, 1 and 2.5 gr juveniles were 9000, 9000 - 10000 and 10070- 12060 cells respectively. Overall, the results of measuring ions and osmotic pressure on the tenth day of treatment, the osmotic potential juveniles 2.5 gr in Caspian sea water and all groups except the juveniles 0.5 gr in water of 7 ppt confirmed.

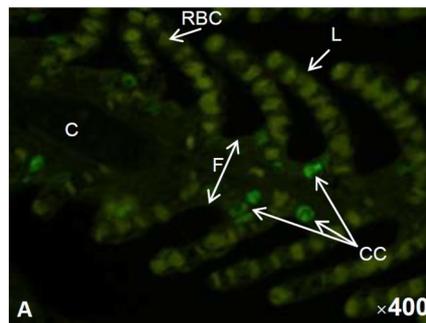


FIG.1. Immunohistochemical staining of the Na^+ , K^+ -ATPase in the gills of *Rutilus frisii kutum* (0.5 gr)

STUDIES OF CASPIAN TROUT GROWTH (*Salmo trutta caspius*) BY BACK-CALCULATED FISH LENGTHS METHODS

Mohammad sayyad bourani¹, Saltanat Najar Lashgari², Shahram Ghasemi³, Mohadese Ahmadnejad¹

1) Iranian Fisheries Science Research Institute, Inland Waters Aquaculture Research Center, Agricultural Research Education and Extension Organization (AREEO), Anzali, Iran
mohammadborani@yahoo.com

The aim of this project is to study the growth and age structure in the Caspian trout, comparison with other coldwater species and check the trend of these parameters in recent years. This study was conducted during 2013 till 2015. Biometric parameters such as length, weight and age of the fish were recorded. Base on Back calculation method in 1393, the average length of fish at ages 1, 2 and 3 years old were 18.98 ± 3.5 , 30.5 ± 7.24 and 41.7 ± 9.1 cm. So these age groups are under the adult age and don't approaching to near the beach and rivers for spawning behavior. Therefore, these length groups cannot be observed in catch composition.

Minimum age and maximum age of this species determined 4 years and 7 years (mean = 5.6) and the most frequency allocated to 5 age group and the frequency of 6 and 7 years has been remarkable.

The average length of salmon was 69.2 ± 6.2 cm (minimum 57 and maximum 81 cm) and the average weight was measured 3323 ± 677 g (2400 to 5600 g) in the catch composition. Growth parameters such as k , L_{∞} and ϕ' was measured 0.18, 104 cm and 3.289 respectively. L_{∞} and growth coefficient (K) on the Caspian trout were acceptable range, that it shows good growth the fish in the sea water.

Most of the fishes were caught from Cheshmehkileh River.

At present, Shilat uses just the broods of the Tonekaboon region for restocking of this species, we recommend using the broods of the western region separately for rehabilitation of the stocks of this region.

A COMPARATIVE STUDY OF THE GROWTH PERFORMANCE OF *Oreochromis Mossambicus* AND *Tilapia Rendalli*

Seabi JM

Department of Zoology, University of Zululand, P/Bag X1001, KwaDlangezwa 3886

The growth performance of *Tilapia Rendalli* and *Oreochromis mossambicus* was compared over a period of 12 weeks. A total of 10 *Oreochromis mossambicus* and 10 *Tilapia rendalli* fingerlings both with mean body weight of 1.9 g were separately stocked in 375 L glass aquaria feeding on macrophytes. In the second treatment, 10 *O. mossambicus* and 10 *T. rendalli* fingerlings with mean body weight of 1.3 g and 0.9 g respectively were stocked in another 375 L glass aquaria feeding on phytoplankton. A third experiment was set up and contained 10 *Oreochromis mossambicus* and 10 *T. rendalli* both with mean body weight of 0.9 g fed on pellets in fibreglass tanks. During the experiment water quality parameters were monitored on a weekly basis and the mass of the fish was measured at three week intervals. *Tilapia rendalli* feeding on macrophytes obtained a faster growth rate (3.5 g) as compared to *Oreochromis mossambicus* (2.4 g). However, *Oreochromis mossambicus* obtained a faster growth rate (4.2 g) as compared to *Tilapia rendalli* (3.7 g) when feeding on phytoplankton. *Oreochromis mossambicus* feeding on pellets obtained faster growth rate (6.7 g) than *Tilapia rendalli* (6.1 g) and had a better food conversion ratio (1.70) than *Tilapia rendalli* (1.78). This indicates that both species grew significantly faster when supplied with their natural food items. The implication of these results in the context of tilapia culture in South Africa is discussed.



PERFORMANCE TESTING OF 100-g NURSERY SIZE LOBSTER FED THE ACIAR BENCHMARK FEED PRODUCED WITH INGREDIENTS SOURCED LOCALLY IN INDONESIA

Bedjo Selamat*, Nyoman Adiasmara Giri, Sudewi, Haryanti, Wawan Andriyanto, Ibnu Rusdi, Gede. S. Sumiarsa, Clive Jones and Simon Irvin

Institute for Mariculture Research and Development (IMRAD), Gondol-Bali, Indonesia
bedjoselamet@yahoo.co.id

The production and use of a pellet is likely to start in farming of lobster at the village level and consist of a feed made from locally available ingredients. Previous studies have shown that feed for Nursery size lobsters should be semi moist and contain fishery product (>12%) to achieve optimal growth. The objectives of this study were first, successful production of a high performing locally produced feed will enable demonstration testing and further studies to refine the formulation. The second objective was to compare the growth performance of lobsters fed an identical feed which varies only in dry matter content.

The experiment was conducted to assess the growth and survival of lobsters offered different feeds. Twenty-five net cages (2m x 2m) were used in this experiment. The experiment was run as 5 treatments; each being replicated 5 times. Thirty lobsters in the calculated ideal weight range (100 ± 1g) were allocated to each of the 25 cages. The designed treatments were as follows:

- A. IMRAD feed (moist), with imported fishmeal
- B. IMRAD feed (moist), similar formulation to A with local fishmeal
- C. IMRAD feed (dry), the same formulation as B
- D. IMRAD feed (dry), with high protein, no fresh ingredient
- E. Trash fish

Survival for all treatments after 12 weeks rearing was around 80% (Figure 1). Lobster fed diet with local fishmeal (B) and high protein (D) showed high survival, whereas lobster fed trash fish (E) had low survival. The survival was dramatically decreased after 18 weeks rearing because of Milky Hemolymph Disease (MHD) outbreaks that were detected by Polymerase Chain Reaction (PCR) analysis.

Highest growth was achieved from local fishmeal feed and trash fish. Whereas lowest growth with imported fishmeal and high protein (Figure 2).

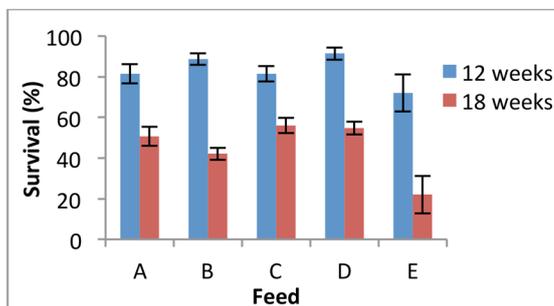


Figure1. Survival (%) of 100-g nursery size lobster after 12 and 18 weeks rearing with different feeds.

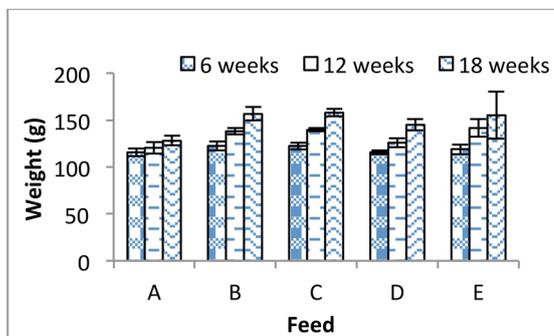


Figure2. Weight (g) of 100-g nursery size lobster after 12 weeks rearing fed different feeds.

ISOLATION, CHARACTERIZATION AND AUTOGENOUS BACTERIAL VACCINE DEVELOPMENT FOR A CHRONIC DISEASE AFFECTING BROWN-SPOTTED GROUPEL (*Epinephelus chlorostigma*)

Eng Khuan Seng * and De Xun Tan

Biotechnology Applied Research Group
School of Chemical & Life Sciences
Nanyang Polytechnic
180 Ang Mo Kio Ave 8, Singapore 569830
Seng_Eng_Khuan@nyp.edu.sg

In recent years high demand for seafood protein has led to intensive aquaculture farming. As a consequence, this has led to an increase incidence of disease outbreaks especially the the farms located in tropical region like South-East Asia (SEA). Often, at times, farmers lose their fish stocks without knowing the causal of the fish deaths. Such was the case in a fish farm in Singapore. They were experiencing chronic mortality in their brown spotted grouper which could lead up to 50-60% mortality rate. The farmers did not know the cause of death of their juvenile groupers.

Here, we attempted to investigate the causal of the chronic deaths. Judging from an quick autopsy of infected fish we speculated that the grouper could have died from vibrio infection. Hence, using two agar plates, Thiosulfate - citrate - bile sucrose (TCBS) and Anacker & Ordal (AOA) agar, 34 and 24 bacterial isolates were discovered respectively. After dereplication, 31 isolates were chosen for further purification. From here, 48 samples were isolated and tested using a Universal Vibro PCR primers (VibAll), 23 were found to be positive for Vibrio. Of the 23, 17 was later found to be *Vibrio harveyi* using specific primer set. From the 17 positive *V.harveyi* samples, 4 different isolates were selected for gram staining, biochemical & physical characterization and results is as shown in Table 1. Following that prototype vaccines for all 4 isolates characterized was done by inactivating the bacterial using formalin. It was found that formalin at a concentration of just 0.5% (v,v) is sufficient to kill the bacteria. Using this formalin killed bacteria, a prototype vaccine was formulated. All 4 prototype *V.harveyi* vaccines are currently in the midst of being tested in the diseased affected fish farm. If the prototype vaccine is found to be successful, this would be the 1st autogenous *Vibrio harveyi* fish vaccine fully developed in Singapore to protect fish from *Vibrio harveyi* infections in local farms.

Table 1. Biochemical and Physical Characterization of Bacteria Isolated from Diseased Grouper

Sample (Isolate)	7GA	7GC	7GD	22B
Organ	Kidney	Kidney	Kidney	Kidney
TCBS medium	Green	Green-Yellow	Green-Yellow	Yellow
AOA medium	Beige	Biege	Biege	Biege
Gram Stain	Neg	Neg	Neg	Neg
NaCl tolerance				
0%	-	-	-	-
3%	+	+	-	+
6%	+	+	+	+
8%	-	+	+	-
10%	-	+	+	-
Temperature				
27°C	+	+	+	+
36°C	+	+	+	+
40°C	-	+	+	-
Swarming	N	N	N	N
Legend Neg: negative ; - : No growth; + Growth observed; N- No swarming observed.				

SURVEILLANCE AND MONITORING OF DISEASES, INCLUDING NEMATODE INFECTIONS IN THE CULTURED ROCKFISH, *Sebastes schlegeli*, OF REPUBLIC OF KOREA FROM 2013 TO 2016

Han-Gill Seo *, Miyoung Cho, Sunghee Jung and Hyun-Ja Han

Pathology Division
National Institute of Fisheries and Science
Busan 46083, Korea
hjhan77@korea.kr

Nematode infection of cultured rockfish, *Sebastes schlegeli*, was first reported on the west coast of Korea, in 2012. Nematode infections of the epithelial tissues, including fins, operculum, nares, mouth, and head have caused serious economic losses in rockfish aquaculture in the western coast of Korea.

To survey the prevalence of pathogens in cultured rockfish, we performed diagnostic monitoring of fish in rockfish cages, from May 2013 to July 2016. A total of 1,945 samples were tested for bacteria, viruses, and parasites. Samples were collected from the western coast (Cheon su Bay and Hek san do), southern coast (Tong yeong and Nam hae), and eastern coast (Po hang) of Korea. Bacteria were detected in 373 of 1,364 fish. Bacteria isolated from fish included: *Photobacterium damsela* (8.9%), *P. piscicola* (2.3%), *P. sp.* (8.9%), *Aeromonas salmonicida* (1.8%), *A. sp.* (0.9%), *Vibrio scopthalmi* (1.5%), *V. sp.* (3.3%), *Streptococcus iniae* (1.2%), and other (8.0%).

In addition, 1,264 fish were infected with *Microcotyle sebastes*, and 334 fish were infected with the nematode *Clavinema mariae*. The prevalence rate of nematode infection in fish from Cheon su Bay, Hek san do, and Tong yeong was 35.3, 3.9, and 1.9 percent, respectively. Nematode infection was not detected in fish from Nam hae and Po hang. All of the fishes were not infected with viruses.

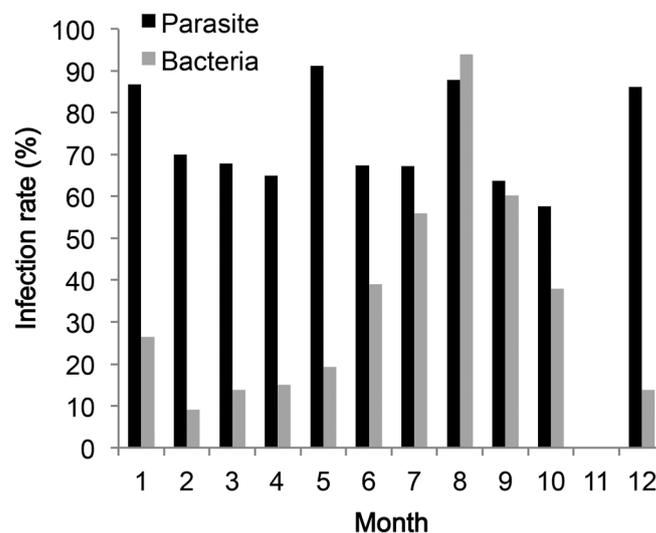


FIGURE 1. Monthly comparison of diseases prevalence of rockfish from 2013 to 2016 in Republic of Korea.

THE INFLUENCE OF VARIATION TIME WATER QUALITY OF INTEGRATED MULTI TROPIC SHRIMP CULTURE SYSTEM IN WEST SULAWESI

Andi Sahrijanna* and Early Septiningsih*

*Research Institute for Coastal Aquaculture

Jl. Makmur Dg. Sitakka No. 129, South Sulawesi 90 511

* Email: asarijanna@yahoo.com; earlyseptiningsih@gmail.com

This study aims to determine the time variation of water quality in shrimp culture ponds with integrated technology multitrophic Aquakulture (IMTA) is vaname shrimp, tilapia, kekeranga and seaweed. The research was conducted in West Sulawesi by using two ponds A. measuring 3,675 m² and 5,250 m² with treatment B. (A) vaname intensive shrimp, red tilapia, oyster and seaweed (B) faname semi-intensive shrimp, red tilapia, oyster and seaweed. Monitoring of water quality was monitored every 3 hours for 24 hours including the quality of physics observed in situ,

This research aims to study the time variation of water quality in shrimp culture ponds vaname with integrated technology multitrophic Aquakulture (IMTA). Material and Methos: The study was conducted in the village pond Sinyonyoi, Districtnamely oxygen, temperature, salinity and pH. Research results obtained average is oxygen (4.6 mg / L), temperature (32.76 ° C), salinity (23.7 ppt) and pH (8.7) and chemical quality in the form of ammonia, nitrite, phosphate, nitrate, organic matter and alkalinity in the analysis in the laboratory.

Kaluku, Mamuju, West Sulawesi province using 2 A. ponds measuring 3,675 m² and 5,250 m² with treatment B. (A) vaname intensive shrimp, red tilapia, oyster and seaweed and (B) vaname semi-intensive shrimp, red tilapia, oyster and seaweed. Stocking density vaname shrimp, tilapia and seaweed in each treatment is shown in Table 1, as follows:

Results and Discussion some physical parameters were observed in situ in the form of dissolved oxygen, pH, temperature and salinity can be seen in the following figure: Table 1. The range of parameter values kwaitas water in shrimp culture ponds with multitrophic technology integrated aquaculture (IMTA) during the study.

Description: Pond A (intensive), Pond B (semi-intensive)

By using advanced electronic IMTA into the pond prawn, tilapia, seaweed and oyster can improve water quality and environment-friendly, seaweed as a supplier of oxygen, through photosynthesis during the day, indigo eaters plakton and oyster can absorb excess nutisi and contamination toksit that toxic, From the observation of water quality for the maintenance of 3-5 ppm dissolved oxygen content, temperature 29-31oC, 15-20 ppt salinity, water transparency of 25-70 cm and a pH of 7.8 to 8.2. Water quality parameters during maintenance Table 1.The research pond A (intensive) and B (semi-intensive) in IMTA vaname shrimp is still within the limits eligible for shrimp farming.

stocking density	TREATMENT	
	A (intensive)	B (semi-intensive)
shrimp	600,000 ind/ha	300,000 ind/ha
vaname	2,000 ind/ ha	2,000 ind/ ha
Indigo	7500 ind/ ha	7500 ind/ ha
oyster	2 tons / ha	2 tons / ha
Seaweed		

Description: Pond A (intensive), Pond B (semi-intensive)

Parameter	Value Range (Range value)	
	Tambak A	Tambak B
Ammonia	0.0772 to 0.2826	0.0673 to 0.1821
nitrite	0.172 ± 0.06	0.1150 ± 0.04
phosphate	0.0070 to 0.0281	0.0027 to 0.0364
Nitrate	0.018 ± 0.0281	0.0217 ± 0.01
Total organic material	0.09333 to 0.3550	0.0988 to 0.7805
alkalinity	0.176 ± 0.09	0.4152 ± 0.28
	0.1233 to 0.3630	0.1182 to 0.3816
	0.238 ± 0.09	0.2222 ± 0.09
	58.81 to 66.94	52.55 to 66.32
	64.96 ± 2.97	61.62 ± 4.33
	87.20 to 239.80	87.20 to 117.73
	137.34 ± 60.14	103.91 ± 12.69

EFFECT HIGH CONCENTRATION OF NITRATE AND AMMONIUM ON GROWTH RATES, PRODUCTION, CHLOROPHYLL AND CARRAGEENAN *Kappaphycus Alvarezii*

Misbah Idam ^{*)}, Early Septiningsih ^{**)}, and Radjuddin Syamsuddin ^{***)}

^{*)} Students of Hasanuddin University Graduate Program

Jl. Jl. Perintis Kemerdekaan Km. Perintis Kemerdekaan: Km. 10, Tamalanrea, Makassar 90245 10, Tamalanrea, Makassar 90245, Indonesia

Email : Idhammisbah@yahoo.com Email: Idhammisbah@yahoo.com

²⁾ Balai Penelitian dan Pengembangan Budidaya Air Payau ^{**)} Research & Development of Brackish Water Aquaculture

Jl. Jl. Makmur Dg. Makmur Dg. Sitakka No.129, Maros Sitakka 129, Maros, South Sulawesi. Indonesia

E-mail: earlyseptiningsih@gmail.com E-mail: earlyseptiningsih@gmail.com

³⁾ Departemen Budidaya Perairan, FIKP-Universitas Hasanuddin ^{***)} Department of Aquaculture, FIKP-Universitas Hasanuddin

Jl. Jl. Perintis Kemerdekaan Km. Perintis Kemerdekaan Km. 10, Tamalanrea, Makassar 90245 10, Tamalanrea, Makassar 90245. Indonesia

This study aims to find the effect of NH_4^+ and NO_3^- alone or a combination of both that can generate growth, production and quality seaweed *Kappaphycus alvarezii* maximum. Penelitian ini dilaksanakan pada bulan April 2012 di Laboratorium Basah, Balai Budidaya Air Payau, Takalar, Sulawesi Selatan. Cultivation containers used were Styrofoam length, width and height of each 46x 32 x 28.5 cm amounts to 30 pieces. Rancangan percobaan yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan 10 perlakuan dengan masing-masing 3 ulangan yaitu: A (30 ppm NO_3^-), B (40 ppm NO_3^-), C (50 ppm NO_3^-), D (30 ppm NH_4^+), E (40 ppm NH_4^+), F (50 ppm NH_4^+), G (15 ppm NO_3^- dan 15 ppm NH_4^+), H (20 ppm NO_3^- dan 20 ppm NH_4^+), I (25 ppm NO_3^- dan 25 ppm NH_4^+) dan J (Kontrol). Data yang diperoleh dianalisis dengan menggunakan sidik ragam yang dilanjutkan dengan uji W-Tukey. Adapun parameter kualitas air dianalisis secara deskriptif berdasarkan kelayakan hidup untuk rumput laut *K. alvarezii*. Hasil penelitian menunjukkan bahwa laju pertumbuhan dan produksi rumput laut terbaik dihasilkan pada perlakuan kombinasi pupuk NO_3^- dan NH_4^+ dengan dosis masing-masing 20 ppm. Kandungan Karaginan yang terbaik dihasilkan pada perlakuan kombinasi pupuk NO_3^- dan NH_4^+ dengan dosis masing-masing 20 ppm. Kandungan klorofil a dan kandungan N-Total yang terbaik dihasilkan pada perlakuan kombinasi pupuk NO_3^- dan NH_4^+ dengan dosis masing-masing 25 ppm. The experimental design used was completely randomized design (CRD) with 10 treatment with each of three replications, namely: A (30 ppm NO_3^-), B (40 ppm NO_3^-), C (50 ppm NO_3^-), D (30 ppm NH_4^+), E (40 ppm NH_4^+), F (50 ppm NH_4^+), G (15 ppm NO_3^- and 15 ppm NH_4^+), H (20 ppm NO_3^- and 20 ppm NH_4^+), I (25 ppm NO_3^- and 25 ppm NH_4^+) and A (Control). Data were analyzed using analysis of variance followed by Turkey's W-test. The water quality parameters were analyzed descriptively based on the viability of seaweed *K. alvarezii*. the results showed that the rate of growth and production of the best seaweed produced in the combination treatment of fertilizer NO_3^- and NH_4^+ doses each of 20 ppm. The content of carrageenan is best produced on combination treatment of fertilizer NO_3^- and NH_4^+ doses each of 20 ppm. The content of chlorophyll a and the content of N-best total combination treatment of manure produced on NO_3^- and NH_4^+ with a dose of 25 ppm respectively.

KODE	TREATMENT	Daily Growth Rate (%)	Production	Kadar carrageenan	Chlorophyll-a	N rate
A.	NO_3^- 30 ppm	2,40 ± 0,03 ^a	76,17 ± 1,42 ^a	51,45 ± 2,32 ^{ad}	0,0127 ± 0,0040 ^{ab}	0,0993 ± 0,0087 ^a
B.	NO_3^- 40 ppm	2,45 ± 0,07 ^a	79,29 ± 3,83 ^a	45,91 ± 1,30 ^b	0,0130 ± 0,0026 ^{ab}	0,1150 ± 0,0056 ^a
C.	NO_3^- 50 ppm	2,38 ± 0,15 ^a	75,58 ± 8,05 ^a	46,30 ± 3,02 ^{ab}	0,0150 ± 0,0036 ^b	0,1180 ± 0,0056 ^a
D.	NH_4^+ 30 ppm	2,33 ± 0,33 ^a	73,75 ± 16,85 ^a	43,49 ± 1,15 ^{bc}	0,0140 ± 0,0026 ^b	0,1147 ± 0,0081 ^a
E.	NH_4^+ 40 ppm	2,11 ± 0,35 ^a	62,55 ± 15,64 ^{ab}	45,40 ± 1,03 ^{bc}	0,0160 ± 0,0030 ^b	0,1163 ± 0,0064 ^f
F.	NH_4^+ 50 ppm	2,07 ± 0,24 ^a	60,29 ± 11,30 ^{ab}	44,38 ± 1,08 ^{bc}	0,0170 ± 0,0036 ^b	0,1183 ± 0,0021 ^a
G.	NH_4^+ 15 ppm + NO_3^- 15 ppm	2,20 ± 0,27 ^a	66,36 ± 12,60 ^a	44,08 ± 1,68 ^{bc}	0,0130 ± 0,0020 ^{ab}	0,0907 ± 0,0190 ^a
H.	NH_4^+ 20 ppm + NO_3^- 20 ppm	2,67 ± 0,03 ^a	92,19 ± 2,03 ^a	53,65 ± 2,66 ^d	0,0163 ± 0,0032 ^b	0,1097 ± 0,0078 ^a
I.	NH_4^+ 25 ppm + NO_3^- 25 ppm	2,12 ± 0,28 ^a	62,80 ± 12,8 ^a	47,35 ± 0,90 ^{ab}	0,0177 ± 0,0031 ^b	0,1183 ± 0,0248 ^a
J.	Control	1,38 ± 0,05 ^b	32,85 ± 1,62 ^b	40,25 ± 1,41 ^c	0,0047 ± 0,0012 ^a	0,0297 ± 0,0078 ^b

SEAFOOD INSPECTION PROCESS BY THE UNITED STATES FOOD AND DRUG ADMINISTRATION

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

As the aquaculture industry continues to expand, concerns regarding the use of unapproved animal drugs and unsafe chemicals, and the misuse of animal drugs during aquaculture production has increased substantially. Aquaculture producers, particularly in developing countries, may use pesticides, general purpose chemicals or unapproved antibiotics that may cause food safety risks or harmful health risks to humans. Furthermore, use of antibiotics, such as nitrofurans and chloramphenicol, in animals can contribute to the emergence and spread of antimicrobial resistance in bacteria that may be transferred to and cause infections in humans, and reducing the effectiveness of these antimicrobial drugs vital for treatment of human disease.

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 import field examinations and sample collections of food products before the products may enter 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 FDA regulatory requirements. In addition to supplementing border surveillance and sampling, the program is intended to promote the development of cooperative relationships with foreign regulatory authorities and assist in building confidence in their seafood regulatory programs.

THE HATCHERY TECHNOLOGY OF GOLDEN TREVALLY FISH (*Gnathanodon speciosus* Forsskal) FOR THE SUSTAINABLE DEVELOPMENT OF THE AQUACULTURE IN THE INDONESIAN

Tony Setia Dharma, Gigih Setia Wibawa dan AA. Ketut Alit

Institute of Marine Research Aquaculture and Development
 Email: tonysetiadharna@gmail.com
 Po Box 140 Singaraja 81101

The golden trevally, *G. speciosus*, Forsskall is well-known as kue fish, belonging to the family of Carangidae, lived at the shallow sea water surface and coral reefs, swift swimmer, has oval, and flat body. It has high economical value with high price as exported commodity. This species can be used as a marine ornamental fish, with the commercial name in Indonesian of *pidana kuning, simba kuning or ikan kue*. The purpose of the present study was to know the management of technology spawn and gonad maturations and seed production of fish fry golden trevally in the control tank. The breeder rear on were conducted using two concrete tanks and fiber glass which have volumes of 30 m³ for larvae rearing of 6 m³. The size of breeder around 1.5-3.0 kg. The result of the experiment showed that fish in treatment A gave better best results of natural reproduction, and spawning on one year total number of eggs 11.450.000 pc and time spawning 156 times, and 48 times total number of eggs 3.240.000 pc for B (control). The hatching rate of eggs between 35,0-95,0 % with SAI around 2.25-4.80 for A treatment, and B treatment hatching rate of eggs between 20,0-92,0 % and SAI 2.00-4.20. The spawning season during one year, were on full moon and new moon. The larvae rearing survival rate have 5.8-8.20% during 28-30 days, and for nursery size to 5-8 cm have survival 60-70% during 20-30 day old.

The result of the experiment showed that fish in treatment A gave better best results of natural reproduction, and spawning on one year total number of eggs 11.450.000 pc and time spawning 156 times, and 48 times total number of eggs 3.240.000 pc for B (control). The hatching rate of eggs between 35,0-95,0 % with SAI around 2.25-4.80 for A treatment, and B treatment hatching rate of eggs between 20,0-92,0 % and SAI 2.00-4.20. The spawning season during one year, were on full moon and new moon. The larvae rearing survival rate have 5.8-8.20% during 28-30 days, and for nursery size to 5-8 cm have survival 60-70% during 20-30 day old.

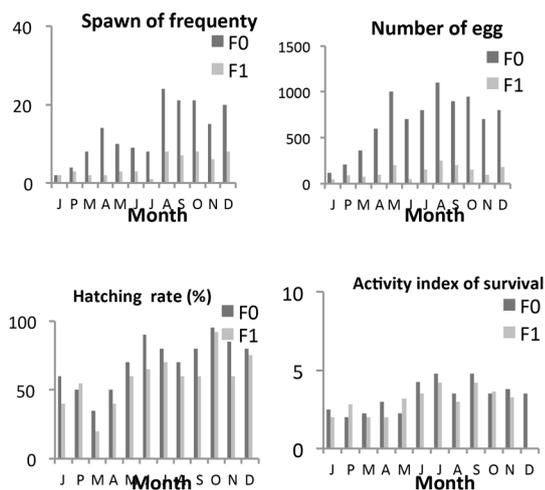


Figure 1. The performance of the spawn golden trevally fish breeder reared in the tank of 30 m³.



Figure 2. Develop of embryo, larvae and juvenile and result of nursery golden trevally fish

GROWTH AND FLESH QUALITY OF SRIKANDI STRAIN TILAPIA (*Oreochromis niloticus* x *aureus*) IN MARINE FLOATING CAGES

Priadi Setyawan*, Adam Robisalmi and Bambang Gunadi

Research Institute fo Fish Breeding,
Ministry of Marine Affairs of Fisheries, Indonesia
Raya 2 street, Sukamandi-Pantura,
Patokbeusi, Subang, West Barat 41263
setyawan_p@yahoo.com

Srikandi strain is a crossbreed between female Nile tilapia (*Oreochromis niloticus*) and male blue tilapia (*Oreochromis aureus*). High salinity tolerant on Srikandi strain is potential for marine culture development. However, high salinity medium can affect on growth and fish condition. This research was aimed to evaluate growth and flesh quality of Srikandi strain in marine culture. Srikandi seeds 3-5 cm was daily acclimated with 5 g/L/day up to 30 g/L. Marine floating cages (*poly-ethylene*) was used in Seribu Islands for six months rearing. Sampling was done at the end of experiment, flesh quality was analyzed in accredited laboratory. Result showed that Srikandi strain growth up to 457.2±97 g on females and 548.2±133.1 g on males with averages of total length are 27.4±2.4 cm on females and 29.6±3.0 cm on males. However, the survival rate is low in the range of 36%. Proximate test showed that protein contain of fish meat is 16.42±0.23%, water content 70.75±3.25 %, ash content 1.61±0.01%, carbohydrates 10.66±3.45% and total fat 0.57±0.04%. The content of omega-3 is 113.1±14.9 mg/100g, omega-6 167.8±62.5 mg/100g, omega-9 180.9±8.4 mg/100g, EPA 5.9±1.8 mg/100g and DHA 9.6±3.8 mg/100g. Growth out Srikandi strain in marine environment capable to produce fillet size tilapia with high nutrient content. Nevertheless, improving salinity tolerance and marine culture technology is needed to increase harvest productivity.

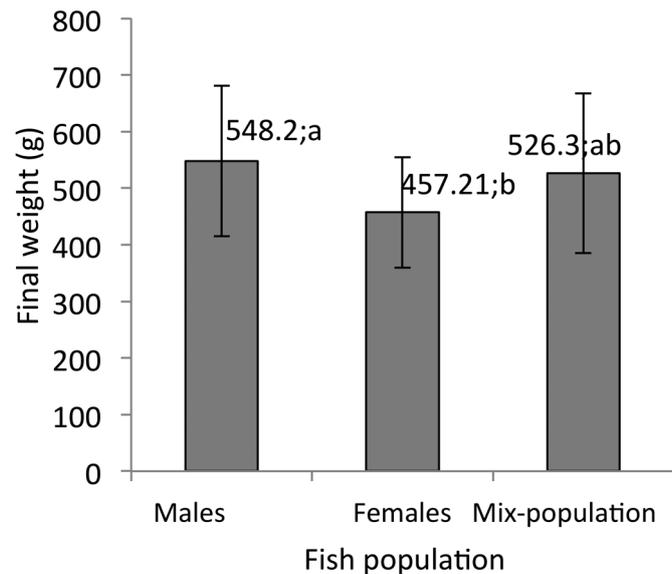


Figure 1. Body weight of Srikandi strain in marine floating cages

Table 1. Flesh quality of Srikandi strain on marine floating cages

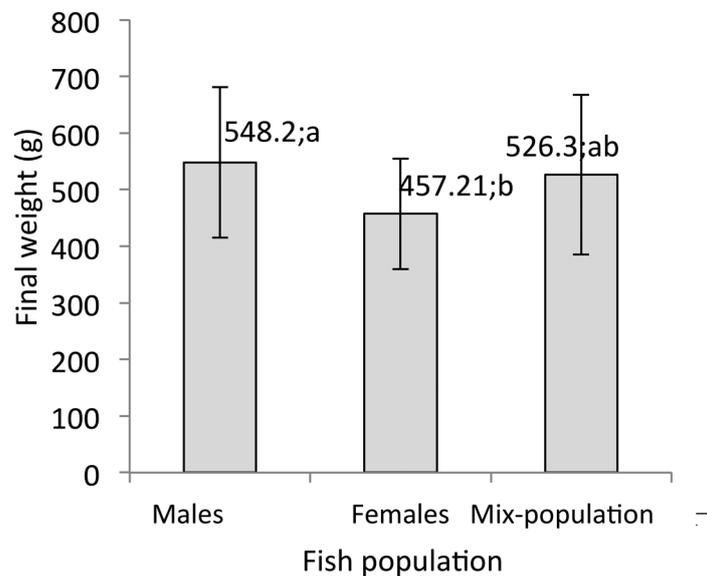
No	Parameters	Average	Units
1	Energy	113.8	kkal/100g
2	Water content	70.7	%
3	Ash content	1.6	%
4	Fat content	0.6	%
5	Protein	16.4	%
6	Total carbohydrate	10.7	%
7	Omega 9 fatty acid	180.9	mg/100g
8	Omega 3 fatty acid	113.1	mg/100g
9	Omega 6 fatty acid	167.8	mg/100g
10	EPA	5.9	mg/100g
11	DHA	9.6	mg/100g

GROWTH PERFORMANCE AND PROXIMATE ANALYSIS OF SRIKANDI STRAIN TILAPIA (*Oreochromis niloticus x aureus*) IN MARINE FLOATING CAGES

Priadi Setyawan*, Adam Robisalmi and Bambang Gunadi

Research Institute fo Fish Breeding
Ministry of Marine Affairs of Fisheries, Indonesia
Raya 2 street, Sukamandi-Pantura,
Patokbeusi, Subang, West Barat 41263
setyawan_p@yahoo.com

Srikandi strain is a crossbreed between female Nile tilapia (*Oreochromis niloticus*) and male blue tilapia (*Oreochromis aureus*). High salinity tolerant on Srikandi strain is potential for marine culture development. However, high salinity medium can affect on growth and fish condition. This research was aimed to evaluate growth and flesh quality of Srikandi strain in marine culture. Srikandi seeds 3-5 cm was daily acclimated with 5 g/L/day up to 30 g/L. Marine floating cages (*poly-ethylene*) was used in Seribu Islands for six months rearing. Sampling was done at the end of experiment, flesh quality was analyzed in accredited laboratory. Result showed that Srikandi strain growth up to 457.2±97 g on females and 548.2±133.1 g on males with averages of total length are 27.4±2.4 cm on females and 29.6±3.0 cm on males. However, the survival rate is low in the range of 36%. Proximate test showed that protein contain of fish meat is 16.42±0.23%, water content 70.75±3.25 %, ash content 1.61±0.01%, carbohydrates 10.66±3.45% and total fat 0.57±0.04%. The content of omega-3 is 113.1±14.9 mg/100g, omega-6 167.8±62.5 mg/100g, omega-9 180.9±8.4 mg/100g, EPA 5.9±1.8 mg/100g and DHA 9.6±3.8 mg/100g. Growth out Srikandi strain in marine environment capable to produce fillet size tilapia with high nutrient content. Nevertheless, improving salinity tolerance and marine culture technology is needed to increase harvest productivity.



1	Energy	113.8±12.73	ccal/100g
2	Water content	70.7±3.25	%
3	Ash content	1.6±0.01	%
4	Fat content	0.6±0.04	%
5	Protein	16.4±0.23	%
6	Total carbohydrate	10.7±3.45	%
7	Omega 9 fatty acid	180.9±8.45	mg/100g
8	Omega 3 fatty acid	113.1±14.9	mg/100g
9	Omega 6 fatty acid	167.8±62.5	mg/100g
10	EPA	5.9±1.84	mg/100g
11	DHA	9.6±3.82	mg/100g

EFFECTS OF CYANOBACTERIA, *Microcystis* spp. ON THE POPULATION GROWTH AND REPRODUCTIVE CAPACITY OF A CLADOCERAN, *Moina Micrura* Kurz 1984

Adibah Shakri^{1*}, Fatimah Md.Yusoff^{1,2}, & Intan Safinar Ismail³

¹Laboratory of Marine Biotechnology, Institute of Bioscience

²Department of Aquaculture, Faculty of Agriculture

³Laboratory of Natural Products

Universiti Putra Malaysia

Serdang 43400, Selangor, Malaysia

adibahshakri@gmail.com

To determine the effects of *Microcystis* spp. on a tropical cladoceran, *Moina micrura* was collected and isolated from a pond in the Universiti Putra Malaysia, Selangor, Malaysia, and mass cultured in the laboratory for use in population growth study and chronic bioassays (>10 days). *Moina micrura* was cultured and maintained in 20L aquarium using filtered-sterilized pond water (0.45 µm fiberglass filters), pH 7.8 ± 2 at room temperature 27.0 ± 2.0 °C under a photoperiod of 12 h light and 12 h dark (120 ± 2 µmoles photon m⁻² sec⁻¹ of light intensity) and fed *ad libitum* with *Chlorella vulgaris* (concentration of 4 × 10⁶ cells ml⁻¹). For the population growth study, experiment was conducted at a stocking rate of 200 female l⁻¹ under the same condition as above until the population began to decline. They were fed once in the morning every day with three different species of microalgae; *Microcystis aeruginosa*, *Microcystis viridis*, and *C. vulgaris* as a control. Algae cells were harvested at late exponential growth phase for feeding regimes. Our preliminary study showed that the food concentration of 1 × 10⁵ cells ml⁻¹ was the optimum concentration to initiate biological responses of *M. micrura* towards all three microalgae species. Each treatment was conducted in triplicates. 25 ml of the well-mixed culture was subsampled and examined in a petri dish under a dissecting microscope. For chronic bioassay, neonates (< 24h) were individually reared in 20 different glass vials containing culture medium and kept under 30 ± 4 µmoles photon m⁻² sec⁻¹ of light intensity. All the glass vials were checked daily (at 12h intervals) to determine age at first reproduction (day), fecundity (no of eggs female⁻¹), total offsprings (no. of offsprings female⁻¹) and longevity (no. of days). Both *Microcystis* spp. were toxic to *M. micrura*. The mortality of *M. micrura* subjected to *M. aeruginosa* and *M. viridis* was significantly higher (p ≤ 0.05) than the control treatment after 72h and 144h of exposure, respectively. After 96h of exposure to *M. aeruginosa*, *M. micrura* showed 100% mortality. For *M. viridis*, 100% mortality of *M. micrura* occurred at 168h of exposure. For the mean body size, *M. micrura* exposed to *M. aeruginosa* did not reach maturity as their mean body size only reached 627.80 SE ± 31.4 µm compared to *M. micrura* fed with *C. vulgaris* (814.94 SE ± 21.84 µm) and *M. viridis* (914.21 SE ± 12.64 µm). The population growth rate of *M. micrura* fed with *C. vulgaris* was 0.18 day⁻¹ while growth rates were negative when fed with *M. aeruginosa* (-0.046 day⁻¹) and *M. viridis* (-0.023 day⁻¹). In the chronic bioassay, the exposure of *M. micrura* to *M. aeruginosa* resulted in delayed production of *M. micrura*'s first offspring, which only occurred on day 6 compared to *M. micrura* fed with *C. vulgaris* which produced their first offspring earlier on day 3. This study showed that exposure of *M. micrura* to both *Microcystis* spp. reduced the fecundity, total offsprings production and longevity of *M. micrura* compared to those fed with *C. vulgaris*.

THE STUDY OF IMPACT OF PARTIAL REPLESHMENT OF FISHMEAL WITH PLANT BASED DIET ON THE DIGESTIVE PHYSIOLOGY OF CARP *Labeo rohita*

JaiGopal Sharma*, Ravi Kumar Goswami, Rina Chakrabarti

*Department of Biotechnology, Delhi Technological University, Bawana Road, Delhi 110042, India
e-mail: sharmajaigopal@yahoo.com

Nutrition plays central role in aquaculture. In intensive aquaculture, more than 60% of production cost is due to feed as fishmeal is used as ingredient. The whole aquaculture industry is looking towards the alternative source of fish meal. The present study aimed to evaluate the impact of plant ingredients on the digestive physiology of rohu *Labeo rohita*.

Indian major carp *Labeo rohita*, rohu (10.66±0.53 g) were randomly distributed in glass aquaria (10 fish/ 50 l aquarium) inside the wet laboratory facility. Four plant-based diets were formulated using almond oil-cake, *Terminalia catappa*, duck weed, *Lemna minor*, water fern *Salvania molesta* and combination of almond oil-cake, duck weed and water fern (1:1:1). In these diets, 50% fish meal was replaced. The diet containing fish meal served as control. Fish were cultured under five different feeding regimes for 90 days. Diet was given at the rate of 3% of body weight. The total amount was divided in two parts and delivered at 9.00 a.m. and 5.00 p.m. Crude protein, fat and moisture contents were analyzed following the standard methods. All fish were harvested after 90 days, survival rate and growth of individual fish was recorded.

There was no significant ($P > 0.05$) difference in the survival rate rohu cultured under five different feeding regimes. Significantly ($P < 0.05$) higher average weight was found in fish fed with *Lemna minor* supplemented diet. Amylase activity was significantly ($P < 0.05$) higher in fish fed with plant-based diets compared to the control one. Trypsin and chymotrypsin activities were significantly ($P < 0.05$) higher in fish fed with fish *Lemna minor* supplemented diet compared to other diets fed rohu. Lipase activity was significantly ($P > 0.05$) higher in rohu fed with almond oil cake supplemented diet. The impact of diets was also recorded in the water quality parameters of the culture systems. This study shows that there is scope for replacement of fish meal in the diets of herbivore fish. This will help in the production of cost-effective diets of fish in a sustainable way.

APPARENT DIGESTIBILITY COEFFICIENTS OF VARIOUS FEED INGREDIENTS FOR OLIVE FLOUNDER *Paralichthys olivaceus*

Jaehyeong Shin* and Kyeong-Jun Lee

Department of Marine Life Science, Jeju National University, Jeju 63243, South Korea
Corresponding author: kjlee@jejunu.ac.kr

Most digestibility studies in olive flounder have usually been conducted in the stage of juveniles. Digestibility information on growing or brood stages of the fish is not available up to date.

Olive flounder were fed a reference diet and test diets containing 5 fish meal (anchovy, sardine, pollack, mackerel and white), 4 animal protein sources (meat meal, meat and bone meal, feather meal and poultry meal) and 4 plant protein sources (corn gluten meal, soybean meal, soy protein concentrate, wheat gluten). Apparent digestibility was determined using a reference diet with 1% chromic oxide and test diets containing 70% reference diet and 30% test ingredients. Olive flounder averaging 650 g were stocked in 600L acrylic tanks at a density of 40 fishes per tank. Feces sample were collected from triplicate groups of fish using a fecal collection column attached to a modified Guelph system tanks.

Apparent digestibility of protein, lipid, energy and dry matter were provided in Table 1. These digestibility data could be used for the development of low cost and environment friendly diets for olive flounder in growing and brood stages.

Table 1. Apparent digestibility coefficients of protein, lipid, energy and dry matter of tested protein sources for olive flounder (%).

Ingredients	Crude protein	Crude lipid	Energy	Dry matter
Anchovy	92.3±0.14	70.6±0.35	67.7±0.35	57.0±0.22
Sardine	95.5±0.03	78.5±2.23	73.4±1.03	56.0±1.80
Pollack	95.0±0.06	82.7±1.94	66.2±0.71	51.2±0.76
Mackerel	95.0±0.02	85.3±0.70	75.3±0.89	64.7±0.18
White fish meal	94.2±0.03	67.9±2.09	68.2±1.07	56.4±0.38
Meat meal	71.6±0.25	64.8±0.15	71.4±0.35	59.1±0.02
Meat and bone meal	78.3±0.17	67.7±0.28	73.1±0.92	49.8±0.07
Feather meal	73.6±0.73	68.2±0.17	68.5±0.50	37.0±0.95
Poultry meal	81.7±0.25	67.0±0.66	80.0±0.90	68.5±0.01
Corn gluten meal	79.0±1.55	72.8±0.28	79.0±0.68	63.4±0.01
Soybean meal	80.1±0.76	65.4±0.71	73.0±0.70	50.1±0.06
SPC	74.5±1.39	65.5±0.67	72.8±2.41	46.1±0.02
Wheat gluten	88.6±0.03	79.5±0.14	80.7±0.88	59.5±0.13

GROWTH AND FEED UTILIZATION OF STARRY FLOUNDER *Platichthys stellatus* FED DIETS WITH GRADED LEVELS OF PROTEIN

S.J. Shin^{1*}, S.S. Kim², H.S. Han³, K.J. Lee⁴ and J.D. Kim¹

¹College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

²Feed and Livestock Institute, CJ CheilJedang

³Aquafeed Research Center, National

Institute of Fisheries Science

⁴Department of Marine Life Science, Jeju National University

mailto:ostrallo@naver.com

Starry flounder is being cultured as a new candidate species, because olive flounder and rockfish occupy more than three quarters of marine cultured fish production in Korea. Although growth rate is slow, starry flounder is disease-resistant and grows under wide range of temperature. Furthermore, its farm-gate price demands higher than those of two main species. Nevertheless, very few nutritional research has been conducted, thus fish meal-based diet is being commercially produced for the fish. Therefore, an attempt to establish the optimum level of protein in diet was carried out.

A total of 360 fish with an average weight of 76.7 g was randomly distributed into each (150 L) of 18 tanks (20 fish/tank). Fish were fed one of six diets containing 30, 35, 40, 45, 50 and 55% protein to apparent satiation twice a day. Graded protein was prepared using a combination of casein and α -starch. Feeding trial lasted for 4 months during which water temperature varied from 15.6 to 25.0 °C. Although daily feed intake (DFI, %) was not significantly different, fish group fed 55% protein showed a significantly higher ($p < 0.05$) weight gain (WG) and specific growth rate (SGR) than other groups. However, feed efficiency (FE) was highest in fish fed 45% protein, although it was not significantly different among groups fed more than 35% protein. Higher protein efficiency ratio (PER) was found in fish fed lower protein diets. From the present results, it was concluded that optimal protein level in diets for starry flounder would be in the range of 45 to 55%.

Table 1. Growth performance of starry flounder *Platichthys stellatus* fed the experimental diets¹

	Diets					
	30%	35%	40%	45%	50%	55%
IW ²	75.3±4.51	78.0±1.00	77.7±5.13	77.0±5.20	77.0±3.00	75.0±1.73
FW ³	96.9±4.74	104.2±1.89	104.3±4.02	107.6±11.64	106.5±3.91	115.5±3.50
WG ⁴	28.7±2.01 ^b	33.5±1.52 ^b	34.4±3.93 ^b	39.5±5.54 ^b	38.6±10.15 ^b	54.1±7.84 ^a
SGR ⁵	0.25±0.02 ^b	0.28±0.01 ^b	0.29±0.03 ^b	0.33±0.04 ^b	0.32±0.07 ^b	0.42±0.05 ^a
FE ⁶	102.0±11.98 ^b	119.5±15.50 ^{ab}	110.3±16.20 ^{ab}	135.0±25.12 ^a	132.0±11.30 ^{ab}	119.6±13.56 ^{ab}
PER ⁷	3.11±0.37 ^a	3.20±0.41 ^a	2.75±0.40 ^{ab}	3.07±0.57 ^a	2.68±0.23 ^{ab}	2.21±0.25 ^b
DFI ⁸	0.69±0.07 ^{ns}	0.62±0.09	0.68±0.09	0.64±0.04	0.59±0.07	0.71±0.07

¹Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

INFECTION KINETICS OF *Kudoa yasunagai* AND EFFECTIVE ULTRAVIOLET IRRADIATION DOSE FOR PREVENTION

Sang Phil Shin^{1*}, Chang Nam Jin¹, Je Hee Lee¹, Sho Shirakashi²

¹Department of Marine Life Science, Jeju National University, Jeju Self-Governing Province, 63243, Republic of Korea

²Fisheries Laboratory, Kinki University, Shirahama, Nishimuro, Wakayama 649-2211, Japan

Kudoa yasunagai is a myxozoan parasite that infects the brains of various marine fishes, including important aquaculture species. The fish-infective stage of *K. yasunagai* (and any Kudoid myxozoans) remains unknown until date and very little information exists regarding their infection process in the fish host. We monitored parasite abundance in different organs/tissues (gills, skin-muscle, intestine, blood, spinal cord, and brain) of juvenile *Seriola lalandi* for one month using *K. yasunagai*-specific quantitative PCR assays. In addition, we investigated the minimum ultraviolet (UV) irradiation dose to rearing water to prevent *K. yasunagai* infection.

K. yasunagai DNA was first detected in the intestine after a week, and attained a plateau phase after 3 weeks. Parasite DNA was detected in the brain after 3 weeks and increased to an estimated intensity equivalent to 10^5 myxospores mg^{-1} tissue by 5 weeks. In contrast, the estimated parasite intensity remained below 1 myxospore mg^{-1} in other organs throughout the experiment. These findings indicate that *K. yasunagai* infection in fish likely initiates from the intestine and the parasite then migrates to the target organs such as the brain and spinal cord for further development and multiplication. The infection prevalence in *Seriola lalandi* reared in untreated water reached 45 % while that in fish reared in UV-treated water at 5 mJ/cm^2 remained below 10%. Additionally, 5 mJ/cm^2 UV irradiation significantly reduced spore formation in the brain. No infection was detected when the water was treated with UV at the doses 15 and 30 mJ/cm^2 . These results indicated that *K. yasunagai* actinospores are relatively vulnerable to UV irradiation and the estimated minimum effective dose lies somewhere between 5 and 15 mJ/cm^2 .

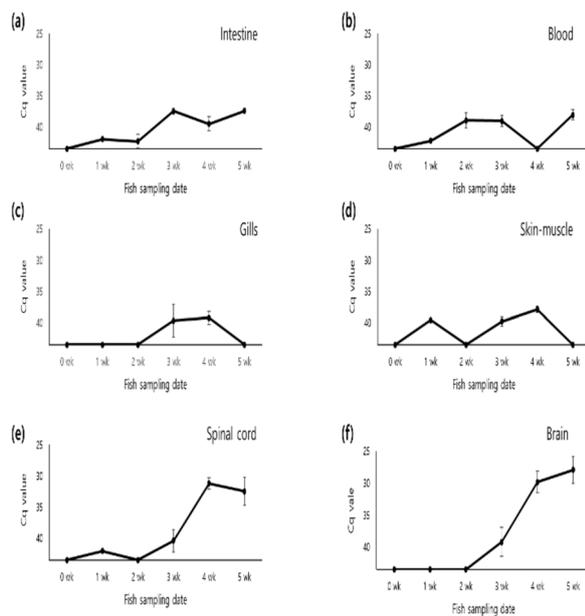


Table 1. Infection prevalence (n=20) of *Kudoa yasunagai* in *Seriola lalandi* reared in the water treated by different UV irradiation doses

Detection methods	UV Doses	Weeks				
		3	4	5	6	7
Microscopy	0 mJ/cm^2	10% ^a	0% ^c	35% ^a	40% ^a	40% ^a
	5 mJ/cm^2	10% ^a	0% ^c	5% ^b	5% ^b	0% ^c
	15 mJ/cm^2	0% ^c				
	30 mJ/cm^2	0% ^c				
qPCR	0 mJ/cm^2	20% ^a	15% ^a	35% ^a	45% ^a	40% ^a
	5 mJ/cm^2	0% ^c	5% ^b	5% ^b	5% ^b	0% ^c
	15 mJ/cm^2	0% ^c				
	30 mJ/cm^2	0% ^c				

MIXOTROPHIC SYSTEM WITH TWO DIFFERENT NEW APPROACH FOR SUPER-INTENSIVE SHRIMP

Dr Farshad Shishehchian

Blue Aqua International Pte Ltd
8 Temasek Boulevard, Suntec Tower 3
Penthouse Level, Singapore 038988

Farshad.shishehchian@blueaquaint.com

A good understanding of the science behind shrimp culture is essential to improve on the current way of farming shrimp and slowly overcome many challenges faced in this industry and work towards a sustainable aquaculture.

The pond is a naturally occurring ecosystem, where nutrients are constantly being broken down and recycled with the help of naturally-occurring micro-organisms.

Maintaining moderate amount of phytoplankton is desirable for a good culture. Healthy phytoplankton bloom, such as bloom of green algae and diatoms, increases the pond's primary productivity by providing food for the microscopic animals, like zooplankton, which in turn supports larger forms of life such as shrimp.

The nitrogen cycle plays a crucial part in the recycling of nitrogen. Nitrification is one important process that helps to convert ammonia, that is highly toxic to the aquatic organisms, to less toxic forms of nitrogenous compounds, such as nitrite and nitrate.

Oxidation-Reduction Potential (ORP) and pH are the major water parameters that should be monitored closely. Maintaining pH stability in pond is crucial in order to prevent fluctuations in pH that is fatal to the cultured species. ORP, measures of the cleanliness of the water and its ability to break down contaminants. A high value of ORP is related to oxidative, aerobic conditions, that favor nitrification, organic matter degradation and biological phosphorus removal. ORP should be regularly checked to ensure it is within optimal range, hence preventing the production of toxic products through anaerobic respiration.

The placement and management of aerator is another crucial part of the system that will affect how efficient the system runs. Aerators should be placed in positions that will facilitate the flow of water in such a way that will lead to the central drain.

Mixotrophic is a patented system to culture shrimps super-intensively. The three phases that mixotrophic manipulates are the phytoplankton phase, phytoplankton-probi- -otic phase and the probiotic phase. Maintaining good water quality and pond bottom management through promoting the proliferation of natural micro-organisms is the key to the right aquatic environment and healthy cultured species.

ORAL VACCINE AGAINST VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS) IN OLIVE FLOUNDER (*Paralichthys olivaceus*)

Jae-Hyun Sim*, Hyun-Boem choi, Myung-Hwa Jung, Sung-Ju Jung

Department of Aqualife Medicine, Chonnam National University, Yeosu, Republic of Korea

Viral Haemorrhagic Septicaemia (VHS) is one of the most serious viral diseases in farmed Olive flounder and several wild marine species in Korea, Japan and China. Administration of vaccines is a meaningful option to prevent and eradicate the disease outbreaks. Previously, we developed an injection-based immunization method using inactivated VHSV antigen (V-Ag) in olive flounder resulting into 60-90% RPS in contrast to control fish. But vaccination through parental routes like intramuscular/ intraperitoneal injection is stressful to fish and not applicable for small fish. The oral route offers the most attractive approach to vaccination of fish. However, the inactivated viral antigen when orally administered to olive flounder gives little or no protection (7% RPS) when challenged with VHSV (10^7 TCID₅₀/fish) 4 weeks post immunization. The lower level of protection might be due to the inadequate stimulation of host immune response or due to degradation of antigen in the hostile stomach environment before they reach the sites where immune induction occurs. In this context, antigen encapsulation can be a promising intervention in increasing the effectiveness of the viral antigen. The current study examines the potential efficacy of alginate and chitosan as a carrier vehicle for the viral antigen. The encapsulated NPs using alginate and chitosan was mixed with commercial feed pellets and fed to olive flounder (10 ± 1.6 g) at a calculated dose of 8.75mL V-Ag/g of body wt/3 days for 3 consecutive days with subsequent challenge with VHSV at an infective dose of 10^7 TCID₅₀/fish 4 weeks post vaccination. A moderate immune protective effect, relative percent survival (RPS) of 38.5~50% was recorded. This result suggested that viral antigen encapsulation can be an effective immunization strategy to enhance protective immune induction in the host as well as protect the viral antigen against digestive degradation. In retrospect, the findings also inferred that there is a scope for further improvement in the efficacy of encapsulated V-Ag with a more targeted delivery mechanism into the host.

EFFECT OF DIFFERENT LEVELS OF CARBOHYDRATE ON RELATED METABOLIC ENZYMES AND INSULIN LIKE GROWTH FACTOR-1 IN *Clarias batrachus* (LINNEAUS, 1758)

A. Singh*, K. K. Jain, M. D. Aklakur, S. Sahoo, N. P. Sahu, S. Kumar

ICAR- Central Institute of Fisheries Education(CIFE)

Panch Marg, Versova, Andheri West, Mumbai-400001

* Corresponding author- aprajitasingh68@gmail.com

Insulin like growth factor -1 was assessed in *Clarias batrachus* fingerlings fed with enriched carbohydrate diets. The animals were fed for 60 days with different levels of digestible carbohydrate diets (20%, 30% and 40%). Activities of amylase and G-6-PDH enzymes were significantly higher ($p < 0.05$) in T3 group, whereas both T2 and T3 groups showed higher AST and ALT enzyme activities. No significant changes was found in the activity of hexokinase enzyme ($p > 0.05$). Real time PCR was done to quantify IGF-1 expression at fifteen days interval of feeding trial. mRNA expression of IGF-1 was found to be highest ($p < 0.05$) in T2 group. There was no significant difference ($p > 0.05$) in expression on 15th and 30th day of feeding trial in all the groups but a gradual increase was observed on 45th and 60th day. IGF-1 serum quantification with ELISA resulted in higher protein concentration in T1 and T2 but significant ($p > 0.05$) lower in T3 during the feeding trial. In summary, it can be concluded that dietary inclusion of 40% carbohydrate can be used effectively in aquafeed formulation for *C. batrachus* upto 60 days of feeding, though there was no significant difference ($p > 0.05$) in weight gain between two groups T2 and T3. Feeding this level of digestible carbohydrate beyond 60 days needs to be studied since IGF-1 gene expression and protein concentration was significantly lower in T3 than T2, although amylase activity was more in T3 treatment group.

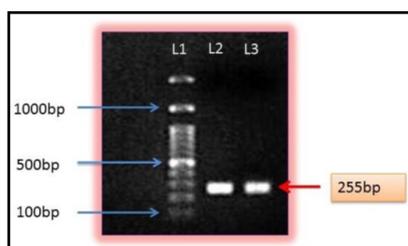


Fig 1. *C. batrachus* RT-PCR product amplified with IGF-1F and IGF-1R primers

```
>5'GACTCTGTGCGGGGCGGAGCTTGTAGACACGCTGCAGT
TTGTGTGTGGAGACAGGGGCTTTTATTTTCAGCAAACCGAC
AGTCTATAAGCCTAGTTCGAGACGGTCGCACAACCGCGGC
ATCGTGGACGAATGCTGCTTTCAGAGCTGTGAGCTAAGCC
CCCTCTACATGTACTGTGCACCCGTAAAGCCCGGCAAAAC
TCCCCGTTTCTACGAGCGGAACGGCACACAGACACCGCCC
AAAACACCAAAGAAACC>3'
```

Fig 2. 255 bp long sequence of Insulin like Growth Factor-1 (IGF-1) gene of *C. batrachus*

Keywords: Digestible carbohydrate, *Clarias batrachus*, growth parameter, Insulin like growth factor-1, metabolic and digestive enzymes, mRNA expression, serum quantification.

REARING OF SCALLOPED SPINY LOBSTER (*Panulirus homarus*) POST LARVAE IN FLOATING NET CAGE WITH DIFFERENT ARTIFICIAL DIET

Bejo Slamet*, Nyoman Adiasmara Giri* and Clive Jone

*Institute of Mariculture Research and Development
Gondol, PO Box 140 Singaraja 81101, Bali Indonesia

**Australian Centre for International Agricultural Research (ACIAR)
bedjoslamet@yahoo.co.id.

Scalloped spiny lobster (*Panulirus homarus*) is an export commodity and high economic value in Asian-Pacific markets, but the wild population was already over fishing. The purpose of this study was to determine the best artificial feed for scalloped spiny lobster nursery in floating net cage.

The nursery were conducted using 9 floating net cage 1x1x1 m in size with four treatment and 3 replicates. Four different artificial diet were used as treatments, i.e.:

Pellet comertial for grouper (A), pellet comertial for shrimp (B), and pellet formula Gondol Institute of Mariculture Research and Development (IMRAD) (C). The stocking density of post larvae (total length 2.1 cm and body weight 0.28 g) was reared at 100 ind./cage. A complete random design was used as an experiment design. Data was analysed using ANOVA and descriptive statistics. Feeding times were twice a day at 08.00 and 15.00 at local time; wit dose 5% of biomass. The sampling of fry on nursery were conducted every month to measure of survival rate(SR), total length (TL) and body weight (BW). On the end of the experiment also take a sample of blood for haemocyte counting. The result of experiment during 4 month rearing show that there was not significant different ($P>0.05$) among treatments for growth of total length and body weight. The best survival rate was on treatment C with survival rate (39.0%), followed by treatment A (29.7%: and treatment B (16.7%). The number of haemocyte on the nursery was almost the same between treatments with haemocyte number about 250-285 x 10³ per ml. From these results it can be concluded that artificial feed/pellet formula Gondol Institute of Mariculture Research and Development can be used for rearing of scallop spiny lobster post larvae.

Table 1. Survival rate, total length gain, body weight gain of scalloped spiny lobster (*Panulirus homarus*) during 4 month nursery in floating net cage with different artificial diet

Parameter	Artificial diet		
	Comertial pellet for grouper (A)	Comertial pellet for shrimp (B)	Pellet formula IMRAD (C)
<i>Survival Rate (%)</i>	29.7 ^b	16.5 ^a	39.0 ^c
<i>Initial total length (cm)</i>	2.1	2.1	2.1
<i>Final total length (cm)</i>	4.9 ± 0.95 ^a	4.7±0.93 ^a	5,1±0,98 ^a
<i>Total length gain(mm)</i>	2.8	2.6	3.0
<i>Daily total length growth (mm/day)</i>	0.233	0.217	0.250
<i>Initial body weight (g)</i>	0.28	0.28	0.28
<i>Final body weight (g)</i>	4,29 ± 2,13 ^a	3,84 ± 1,95 ^a	4,44 ± 2,27 ^a
<i>Body weight gain (g)</i>	4,01	3,56	4,16
<i>Daily weight growth (g/day)</i>	0,033	0,030	0,035

A CAUTIONARY TALE FOR FORMULATORS - HOW ENVIRONMENTAL OR FARMING CONDITIONS MIGHT NEGATIVELY IMPACT DIET PERFORMANCE EVEN WHEN THEY UTILISE PREMIUM QUALITY RAW MATERIALS: THE EFFECT OF RESTRICTED FEEDING ON PERFORMANCE OF ASIAN SEABASS *Lates calcarifer*

Richard Smullen*, Michael Salini, Simon Tabrett, Mark Booth, Simon Irvin, David Blyth, Barney Hines, Sue Cheers, Nicholas Bourne, Cedric Simon

Ridley Aqua-Feed
Unit 4 31 Robart Court, Narangba, QLD 4504, Australia
richard.smullen@ridley.com.au
Tel: +61 428765717

When one traditionally thinks about dietary requirements, one tend to focus on the amount of nutrients required in the feed to give optimal performance. However, when conditions are sub-optimal and intake is reduced, a previously adequate feed can become limiting, particularly with some of the amino acids and micronutrients. Furthermore, during periods of high temperature, it has been shown in Asian seabass (*Lates calcarifer*), that the maintenance protein and energy requirement increases dramatically while the utilisation decreases¹. In addition, Asian seabass above 33°C show a significant reduction of feed intake and commensurate growth rate².

Much of the data published therefore is from studies where conditions are optimal and feed intake is not restricted and the focus is on the level of a nutrient in the feed rather than the nutrient intake in grams per fish. In order to understand the impact of a single raw material under a restricted ration, a pair fed experiment was devised using juvenile Asian seabass where a high quality poultry meal (HQP) sequentially replaced a lower quality poultry meal (LQP). Five diets were formulated to be isoenergetic and providing all nutrients in excess of requirements for Asian seabass. A slight restriction of the satiated response of the fish fed the highest inclusion of LQP was used to determine the feeding level of all treatments. Apparent dietary digestibility of protein, energy, lipid, dry matter and methionine all increased with increasing HQP addition, however, surprisingly, growth decreased and FCR increased. In conclusion, the restricted feeding ration had a negative impact on performance and micronutrients rather than macro-ingredients were investigated further. This work highlights the dearth of information in the area of nutrient requirements in Asian seabass.

1. Glencross, B.D., Bermudes, M., (2011a). Effect of high water temperatures on the utilisation efficiencies of energy and protein by juvenile barramundi, *Lates calcarifer*. Fisheries and Aquaculture Journal
2. Bermudes, M., Glencross, B.D., Austen, K., Hawkins, W., 2010. Effect of high water temperatures on nutrient and energy retention in barramundi (*Lates calcarifer*). Aquaculture 306, 160-166.

LINDE AQUACULTURE SOLUTIONS FOR HEALTHY FISH –EQUIPMENT PORTFOLIO

Benben Song*

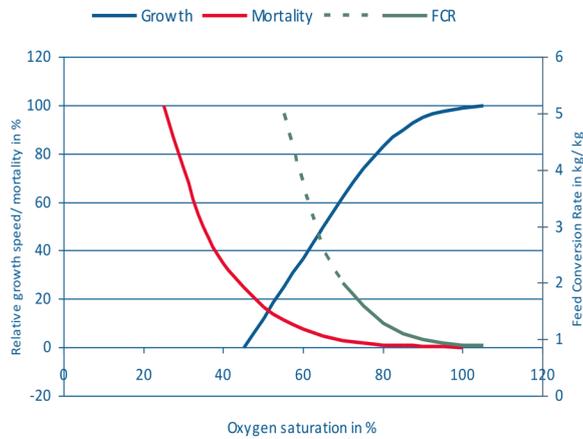
Linde Technology Center, BOC(China) Holdings Co.,Ltd-A member of the Linde Group
3rd Floor, Build D, 255 GuiqiaoRoad, Pudong,Shanghai,PRC
Email: benben.song@linde.com

The most important factor for achieving healthy fish is to have healthy water. Thus, controlling the concentration of oxygen dissolved in water is crucial in aquaculture.

Generally, the closer the oxygen concentration is to air saturation, the better the growth and health conditions are. Maintaining the right level of oxygen in the water will improve utilization of feed (FCR from 1.2 to 1), reduce the growth period (From 5% to 2%), reduced production cycle, reduce fish mortality and reduce the need for vaccination and antibiotics, improve growth rate and stock rate, meets animal welfare standard.

With the SOLVOX® product line, Linde has the right solution to meet these specific needs. SOLVOX® comprises a variety of oxygen dissolution and distribution systems. Linde offers the full range of oxygen supply modes: from cylinder and bulk storage to on-site generation, Linde has the experience to deliver the total engineered solution. Our portfolio includes: cost-effective substitution of air by oxygen; oxygenation equipment to improve and increase production in fish and seafood farms; liquid and compressed gases and related supply and control systems; tailor-made solutions for individual customers; a thorough understanding of fish farming through our international network of fish farming experts.

Get the latest aquaculture technology from Linde-your experienced and reliable partner for fish farming.
WWW.linde-gas.com/aquaculture



UTILIZATION OF *Durio zibethinus* RIND CRUDE EXTRACTION FOR IMMUNOSTIMULATION AGAINST BACTERIAL CHALLENGE IN NILE TILAPIA *Oreochromis niloticus*

Supachok Songsiri*, Pattareeya Ponza and Supat Ponza

Program of Fisheries Science
 Department of Agricultural Science
 Faculty of Agriculture, Natural Resources and Environment
 Naresuan University
 Phitsanulok province 65000, Thailand
 Corresponding author email supatp@nu.ac.th

Durian rind is considered agricultural waste which is still being usable for tilapia feed since there are nutrients and polysaccharide gel which is important as immunostimulant. This study therefore aimed to observe the effect of including crude extract of durian rind in fish feed on growth and welfare of the fish. Crude extract of durian rind was prepared by boiling the white part of the rind in water and was included into the fish diet at 2%, 4% and 8% (w/w), along with control group (0%) as shown in Table 1. Tilapia fingerlings of an average total length 5-6 cm/fish were fed with the experimental diets for 12 weeks. After that, fish was challenged with *Aeromonas hydrophila* at a level of 3.2×10^5 CFU/fish by intraperitoneal injection. It was shown that fish received 4% of durian crude extract showed non-significantly higher blood count and hematocrit value than the other groups ($P > 0.05$). However, result from bacterial challenge in Table 2 showed that fish fed with 4% durian crude extract exhibited a significantly highest survival rate (67%) among all treatments ($P < 0.05$). This could indicate that durian rind had possibility to be immunostimulant and feed contains 4%PG might be at least in part of enhancing immune responses against *A. hydrophila* in tilapia. Further investigation of identification gut microbes will be done for more understanding on immune responses activation which will developing the application of plants polysaccharide as immunostimulant in aquaculture.

Table 1: Nutrition value of experimental diets; Control (0%PG), 2%PG, 4%PG and 8%PG.

Diets	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)
0%PG	40.04±0.26	5.47±0.37	1.34±0.13	25.73±0.01	3.79±0.13
2%PG	39.83±0.60	5.20±0.63	1.68±0.28	24.48±0.01	3.93±0.03
4%PG	41.12±0.75	4.26±0.76	1.38±0.08	24.06±0.01	4.07±0.10
8%PG	40.87±0.36	5.45±1.18	1.41±0.18	24.73±0.01	3.34±0.21

Table 2: Result of bacterial challenge.

	0%PG	2%PG	4%PG	8%PG
Survival rate (%)	26.67±4.62 ^{bc}	0.00±0.00 ^c	66.67±5.77 ^a	13.34±2.31 ^{bc}

Data was shown as Mean±SD with different alphabets as significantly difference between groups ($P < 0.05$).

STATUS OF RESOURCE MANAGEMENT AND AQUACULTURE IN MALAYSIA

Veena Soni

veenasony8@gmail.com

Malaysia is a maritime nation and its fishing industry is a source of income for 134,000 fishermen. In 2012, the fisheries sector produced 1.7 million tons of fish valued at RM10.8 billion and generated trade worth RM6 billion. The landings from capture fisheries are expected to increase from 1.32 million tons in 2010 to 1.76 million tons in 2020 at an annual growth rate of 2.9%. In 2012, 65% of total catch was contributed by the coastal fisheries as compared to 35% from deep sea fishing. Landing from deep sea fishing is expected to rise from 381,000 tons in 2012 to 620,000 tons in 2020. Deep sea fishing has been identified for its potential to contribute to the increase in the country's fish production. With a growing population and an increasing preference for fish as a healthy source of animal protein, the National Agro-food Policy (2011-2020) estimated that the annual demand for fish will increase to 1.93 million tons by the year 2020. The Department of Fisheries (DOF) has developed Capture Fisheries Strategic Management Plan (2011-2020) based on three main documents i.e.; National Agro-food Policy (NAP, 2011-2020), Department of Fisheries Strategic Management Plan (2011-2020), and Malaysia National Plan of Action on Sustainable Fisheries for Food Security towards 2020. Aquaculture is now being promoted in Malaysia as an important engine of growth and eventually to become the mainstay of the nation's economy. Situated in a region with abundant supply of land and water, two determinant factors for aquaculture activities, Malaysia has always strived to ensure that this sector is not sidelined in their development efforts. With a growing population and an increasing preference for fish as a healthy source of animal protein, it has been estimated that the annual demand for fish will increase to 1.7 million tons in 2011 and further to 1.93 million tons by 2020. From the present annual aquaculture production of about 525,000 tons, this output would need to be raised to 790,000 tons to meet the projected demand by 2020. In a move to develop the aquaculture industry, the DOF, has initiated the Aquaculture Industrial Zone (AIZ) Program involving the development of 49 zones, located across Malaysia, which will be used for culture of various types of high value aquatic species. The DOF has identified several strategic areas that would be developed for downstream activities such as fish seed production, feed mills, fish processing plants, and other supporting industries. Aquaculture is also currently listed amongst the 16 Agro-food's Entry Point Projects (EPP) of the National Key Economic Area (NKEA). The government aims to double the Agro-food sector's contribution to Gross National Income (GNI) from Malaysian Ringgit (RM) 20.2 billion in the year 2010 to RM49.1 billion by 2020, or an increase of RM28.9 billion.

CAN AQUACULTURE BECOME THE NEW BLUE BIOTECHNOLOGY OF THE FUTURE?

Patrick Sorgeloos

Ghent University, Belgium

Aquaculture is the fastest growing food-production industry. It all started in Asia, especially in China, but it was only in the last 3 to 4 decades that spectacular growth took place with a progressive increase in the contribution of aquaculture to global seafood needs from less than 10 percent in the 1970s to about 50 percent of what we consume now. This trend will continue as demands increase and fisheries stocks are exploited near or greater than maximum sustainable yields. Calculations based on present per capita consumption and estimated population size 10 years from now reveal that aquaculture will have to provide more than 50 percent more on an annual basis within the next decade. When considering the available global resources for food extraction or production, it is clear that land for crops and pasture will come under serious pressure. It should be clear that aquaculture is expected to expand very significantly. However, can this all be achieved following our current expertise and experience? Can it be accomplished without causing environmental risks or human health problems? The answer is yes but only when we adopt new approaches when addressing sustainability issues. In future aquaculture developments we must embrace ecological principles and reconsider the monoculture approaches that we have increasingly introduced with the modern forms of business aquaculture. A detailed example will be given how management of the water microbiota in larviculture systems according to ecological selection principles can decrease opportunistic pathogen pressure and result in an improved performance of the cultured animals.

We will also need more international cooperation and multi-stakeholder interactions.

IMPROVING THE HEALTH STATUS OF FISH AND CRUSTACEAN LARVAE BY COMBINING MICROBIAL & STRESS MANAGEMENT

Patrick Sorgeloos, Peter De Schrijver, Peter Bossier and Geert Rombaut

Laboratory of Aquaculture & Artemia Reference Center, Ghent University (Belgium) and INVE Technologies, Dendermonde (Belgium)

To establish a more sustainable fish and crustacean production, there is a need for new microbial management strategies that focus on 'join them' and not the traditional 'beat them' approaches. To efficiently manage the microbiota in the system to minimize disease risk, a lot can be learned from research on intensive larviculture of several fish species where detrimental host-pathogen interactions are a normal phenomenon. It is argued that ecological theory could serve as a foundation for developing sustainable microbial management methods that prevent pathogenic disease in larviculture (De Schryver and Vadstein, 2014).

Management of the water microbiota in larviculture systems according to ecological selection principles has been shown to decrease opportunistic pathogen pressure and to result in an improved performance of the cultured animals. We hypothesize that such an approach will prove its value for the shrimp culture business in the context of AHPND as well.

Improving robustness of organisms is a more holistic approach to tackle the many infectious and non-infectious disease problems encountered in aquaculture. It encompasses improving the energetic and nutritional reserves, homeostatic capacities (osmotic regulation etc.), defense and immune systems, as well as the microbial flora in and on the animals. All of these levels have an additional/synergistic effect on how well the cultured organisms can fight off pathogens and deal with environmental stress. One of the central concepts in this kind of stress management is (xeno)hormesis (Calabrese and Baldwin, 2002). It is characterized by a low dose stressor inducing a response in an organism of which the net outcome is favorable for the organism. The search for products which allow animals to better deal with stress is one of the most obvious avenues to obtain improved production in a short term.

References

Calabrese, E.J. and Baldwin, L.A. (2002). Defining hormesis. *Hum Exp Toxicol.*, 21(2):91-7.

De Schryver, P. and O. Vadstein (2014). Ecological theory as a foundation to control pathogenic invasion in aquaculture. *The ISME Journal* (2014), 1-9

THE DIAGNOSTIC AND TREATING STRATEGIES OF EHP - AN EXAMPLE FROM A CHINESE SUPER-INTENSIVE FARMING SYSTEM

Chen Su*, Yun-Chieh Hsieh, Wei Lu, Li-Juan Ma, Chuan-Fu Tsai, Yi-Miao Chen

GeneReach Biotechnology Corp.
ScienChain Biotechnology Corp.
suchen@scienchain.com

Super-intensive farming system, also known as “shrimp factory”, is getting popular in northern China. The average stocking density of these indoor facilities is 500 to 700 pieces per square meter. Ideally, the icy sea water at winter time plays the role as a natural bio-security system to clear any potential threat from acute hepatopancreatic necrosis disease (AHPND) and *Enterocytozoon hepatopenaei* (EHP) in the water. However, an EHP outbreak still occurred after an unexpected overflow of untreated sea water at one facility in Oct, 2016.

On-site PCR tests were applied after the slow growth was observed, followed by further real-time PCR quantitative analysis done on the biggest and the smallest shrimps. The data showed that the infectious levels of these two groups were similar. Both were above 1000 copies/ng hepatopancreatic DNA. The real-time PCR analysis checked two EHP genes: the small subunit ribosomal RNA (SSU rRNA) gene and the spore wall protein (SWP) gene regions. From the Ct number of the data, the SSU rRNA gene system was more sensitive than the other.

At the same time, the neighboring ponds were confirmed as EHP positive by PCR without slow growth symptoms. The real-time PCR data showed the infectious level was below 10000 copies/ng hepatopancreatic DNA. Several potential treatment procedures were applied after the diagnosis, and the effects were monitored by the farmers' observation.

To establish an EHP control protocol, a strategy which combined the diagnosis, symptom observation, and treatment has been designed at this super-intensive system. A periodic sampling of 50 animals was applied. The growth curves of the average, smallest, and the biggest animals were plotted to identify the slow growth symptom. The smallest pieces were screened by PCR. When the EHP positive result was found, the real-time PCR analysis was followed to check the infectious level. A total of 6 infectious scenarios were identified for different treatment procedures. Besides the diagnostic and treating protocols, an intensive disinfection protocol was also applied to clean the infected ponds.

SUSTAINABLE, NOVEL & COST EFFECTIVE PROTEIN FOR THE AQUACULTURE MARKET

Ezhil Subbian*, Sandeep Kumar, Purvesh Shingala, Naga Sairam, Chetan Kurthukoti, Laavanya S, Elakkiya Chandran & Vinod Kumar.

*String Bio Pvt Ltd
Bangalore Bioinnovation Center
Biotech Park
Electronics city Phase I
Bangalore -560100
subbiane@stringbio.com

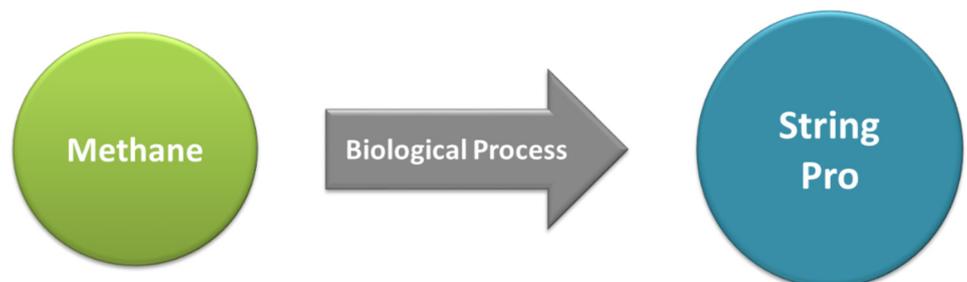
Imagine an ecologically sustainable world! What if everyday products were made of value chains that are circular? At String, we are using our experience and energy to enable this possibility. Our goal is a world where robust, cradle-to-cradle solutions provide us cleaner ways of living.

String Bio leverages methane as a source of carbon to manufacture green chemicals and feed ingredients. The products address multi-billion dollar markets. We are amongst a handful of companies worldwide and the only Asian company to successfully enable methane based value chain for proteins.

There is a growing gap in worldwide protein supply. To elaborate, on the demand side the world population is projected to reach 9.6 billion by 2050 - 70% of this growth is expected to be in urban areas with high meat consumption. On the supply side, the existing protein sources for the animal feed market, ie fishmeal and soyabean meal, are both constrained by climate variability and land/water availability. For instance, over the last decade the cost of fishmeal, the widely used protein source for aquaculture, has almost tripled. This presents a significant market opportunity for sustainable and cost effective protein. *String Pro*, our product, is a superior protein from methane that addresses this growing gap.

The advantages of *String pro* are 1) cost-effective, sustainable and novel protein 2) proprietary technology platform, 3) significantly lower cost of production, 4) reliable protein supply, 5) easy traceability, 6) environmental sustainability and, 6) cradle-to-cradle solution for a low carbon future.

With >65% protein and an amino acid profile similar to fishmeal, String Pro, is a very attractive replacement for fishmeal. String Pro is also cost-competitive with fishmeal. Currently, we are testing the use of *String Pro* for the aquaculture markets.



MILKYHEMOLYMPHDISEASEOFSPINYLOBSTER(MHD-SL)ANDITSEXPERIMENTAL INFECTION IN *Panulirus homarus*

Sudewi*, Zeny Widiastuti, Haryanti, Bedjo Selamat, and Ketut Mahardika

Institute for Mariculture Research and Development, Gondol-Bali, Indonesia
Email: dewigrim@gmail.com

Milky Hemolymph Disease of Spiny Lobster (MHD-SL) was reported as the most devastating disease in farming of spiny lobster. Outbreaks of the disease were recorded almost year round in Lombok from 2012 to 2016, and in Pegametan Bay, North Bali in 2016. It was stated that economic losses due to the outbreaks were considerably high. Therefore, this study was conducted first, to investigate milky disease occurrence in wild and farmed spiny lobster *Panulirus homarus* from different locations, second, to identify and to compare the causative agent of milky disease from other countries, and lastly, to perform experimental infection of milky disease in *P. homarus*.

Wild lobsters were captured from Banyuwangi, Lombok, and Jembrana coastal areas, while farmed lobsters were collected from net cages in Pegametan, Pangandaran, and Lombok. MHD-SL was detected by Polymerase Chain Reaction (PCR) analysis using 254F and 254R primers. Milky disease agent was identified by nucleotide sequence analysis, and a phylogenetic tree was built using MEGA6. Experimental infection study was done by several modes of infection: injection with undiluted and diluted infected hemolymph, water immersion, per os exposure, and two control groups as comparisons.

Three lobsters out of 35 samples were found to be infected with MHD-SL (Figure 1). Milky disease agent in this study was similar to milky disease agent from Vietnam. The phylogenetic tree showed strength relationship between our sample and other milky disease agent in some Crustacean from Vietnam, Tanzania, Madagascar, and Mozambique. All the experimentally affected lobsters exhibited disease symptom and pattern of mortality as naturally infected lobsters. The mortality pattern of the experimentally infected lobsters was found to be varies with the modes of infection.

In summary, milky disease was found only in the farmed lobster *P. homarus*, and no milky disease was detected in the wild lobsters. Milky disease agent in this study was classified as group of uncultured bacteria that cannot be cultured using various types of agar-based media. Experimental infection study indicated that milky disease could be transmitted horizontally from the diseased lobsters to the healthy lobsters by injection of undiluted and diluted affected hemolymph, by water immersion, as well as by per os exposure.

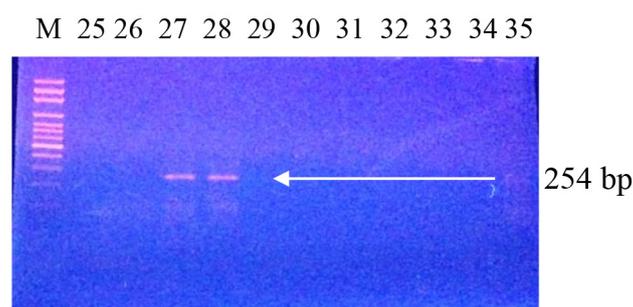


Figure1. Result of DNA amplification using 254F and 254R primers. M = Marker DNA Ladder 100 bp, 25-35 = PCR-ID. The positive results were indicated by band of 254bp.

STUDY ON MILKY DISEASES INFECTION IN WILD AND CULTURED LOBSTER, *Panulirus homarus* IN INDONESIA

*Ketut Sugama¹, N.L Anggra Lasmika², Isti Koesharyani¹ and Clive Jones³

¹. Center for Research and Development of Aquaculture, Jakarta Indonesia

² Lombok Quarantine office, West Nusa Tenggara, Indonesia

³ Centre for Sustainable Tropical Fisheries and Aquaculture, Collage of Marine Environmental Sciences

James Cook University Australia

ketut_sugama@yahoo.com

Milky Haemolymph Disease (MHD-SL) in spiny lobster (*Panulirus homarus*) is the most pathogenic diseases. Research on MHD-SL infection has not been undertaken in Indonesia. Therefore present study tries to determine pathogenic level and treatment to overcome of MDH-SL. The MHD-SL was confirmed by using DNA molecular technique. The study also tries to find an option for MHD-SL treatment. The spiny lobster samples were collected from Awang Bay, Telong-Elong Bay and Gerupuk Bay of Lombok Island and samples were analyzed in laboratories. MHD-SL was confirmed by PCR with specific primer of 254 bp and histology by staining HE and gram twort to finds tissue damaged and Rickettsia Like Bacteria (RLB). OTC test was determined by LC50.

The result showed that infected MHD-SL was found only in cultured spiny lobster on cage located at Gerupuk Bay. Determination of LC50 was found at diluted of MHD-SL $10^{-3.2}$. and infected MHD-SL could be treated by Oxytetracycline (OTC) at a dosage of 0.01 ml/100 g of weight, and after 3 weeks of treated by OTC shows that all 35 lobster treated were all survived and negative MHD-SL. Picture below shows the spiny lobster not infected and infected MHD-SL



Not infected MHD-SL

Infected MHD-DL

COST OF NUTRIENTS IN DIFFERENT METHODS OF FERTILIZATION FOR AEROBIC MICROBIAL FLOC PRODUCTION IN INDOOR TANKS

S.Felix, A.Gopalakkanan, M.Menaga and Yuvarajan

The aim of this study was to evaluate the effect of two methodologies of fertilization on the volume and characteristics of floc. Nine aquarium glass tanks (75 litres each) were divided into three treatments. The treatment 1 followed the fertilization regimes of including nitrogen fertilizers, treatment 2 was studied with the inclusion of both nitrogen and phosphorus fertilizers and treatment 3 was the control where the conventional method of fertilization was adopted. In treatment 1 & 2, the tanks received daily molasses fertilization throughout the entire rearing period. In control, molasses was used only initial fertilization was done. Distillery spentwash obtained as effluent from sugarcane industries were used as the carbon source. The tanks were operated with no water exchange and the total suspended solids concentration were kept between 300 and 400 mg L⁻¹. The sludge produced at harvest were quantified and their characteristics were determined. The production of TSS in the treatment 1 was higher than in the treatment 2 ($P < 0.05$). The analysis of the sludge revealed a high amount of volatile solids in both treatments, between 636 and 702 g kg⁻¹. The BOD: TSS ratio was also low in both treatments, but the treatment 2 showed lower values ($11.3 \pm 0.6\%$) than the treatment 1 ($15.9 \pm 0.0\%$) ($P < 0.05$). Both fertilization strategies were able to modify the characteristics of sludge produced during cultivation. Moreover, the nitrogen and phosphorus content in treatment 2 showed a increased floc volume (15.42 ± 0.2 ml) within 7 days and it was much easier to maintain the floc in both the treatments. The TPC was significantly different in treatment 2 ($P < 0.05$) indicates the growth of heterotrophic bacteria. The study concludes that cost of application of nitrogen and phosphorus fertilizers in treatment 2 was comparatively higher and increases the floc volume within seven days.

COMPARATIVE EVALUATION OF *INSITU* AND *EXSITU* FEEDING OF AEROBIC MICROBIAL FLOC IN THE NURSERY CULTURE OF TWO DIFFERENT STRAINS OF NILE TILAPIA, *Oreochromis niloticus*

S.Felix*, A.Gopalakannan and M. Menaga

This study evaluated the effect of aerobic microbial floc technology (AMF) application on immune response, water quality and production performance of two different strains of *Oreochromis niloticus* at *insitu* and *exsitu* raceways. The fish seeds of ± 0.1 gm at a density of 110 fries/m³ in Control (without external carbon input) and AMF treatments. The GIFT tilapia and Chitralada strains of *Oreochromis niloticus* with an initial average body weight ± 0.1 g was cultured in nursery raceways for 30 days. Distillery spentwash was added on AMF treatments as the organic carbon source at a C/N ratio of 15. Control treatments of each density tested showed more fluctuated water quality parameters throughout the experimental period. The highest TAN and nitrite-nitrogen were observed in two strains control treatment of 3.97 mg TAN/L, 4.01 mg TAN/L and 9.29 mg NO₂-N/L and 9.54 mg NO₂-N/L, respectively. The highest total yield was observed in GIFT strain of control treatment, whereas the highest survival and NBT activity was observed in AMF treatments of which *insitu* treatment of GIFT strain was relatively higher than chitralada strain. Total feed used in AMF was lower than that of control treatments ($P < 0.05$), however there was no significant difference between *insitu* and *exsitu* systems suggesting that AMF could be continuously harvested by the fish as other source of food.

COMPARATIVE EVALUATION OF *INSITU* AND *EXSITU* FEEDING OF AEROBIC MICROBIAL FLOC IN THE NURSERY CULTURE OF PACIFIC WHITE SHRIMP, *Penaeus vannamei*

Dr.S.Felix, Dr.A.Gopalakannan, Dr.Cheryl Antony and Ms. Menaga

This study evaluated the effect of aerobic microbial floc technology (AMF) application on immune response water quality and production performance of *Penaeus vannamei* at *insitu* and *exsitu* raceways. The shrimp seeds of ± 0.001 gm at a density of 1300 PL/m³ in Control (without external carbon input) and AMF treatments. Distillery spentwash was added on AMF treatments as the organic carbon source at a C/N ratio of 15:1. Control treatments of each density tested showed more fluctuated water quality parameters throughout the experimental period. The highest TAN and nitrite-nitrogen were observed in control treatment of 3.64 mg TAN/L, 3.84 mg TAN/L and 8.79 mg NO₂-N/L and 9.32 mg NO₂-N/L, respectively. The highest total yield was observed in control treatment, whereas the highest survival and NBT activity was observed in AMF treatments of which *insitu* treatment of vannamei was relatively higher. Total feed used in AMF was lower than that of control treatments ($P < 0.05$), however there was a significant difference between *insitu* and *exsitu* systems suggesting that AMF could be maintained and fed easily in *insitu* treatment than *exsitu*.

FORMATION OF SIAMESE CATFISH, *Pangasianodon hypophthalmus*, Sauvage, 1878 (PANGASIIDAE; SILURIFORMES) F2 BASED ON SELECTION OF CHARACTER GROWTH

Suharyanto, Jatmiko Darmawan dan Evi Tahapari

Research Institute for Fish Breeding, Sukamandi, West Java, Indonesia
E-mail: suhar.yanto83@ymail.com

Research on the formation of Siamese catfish F2 is one part of the assembly of superior strains of catfish. The research objective is to get a prospective parent of siamese catfish F2 selection results based on character growth. In 2015, the parent has done spawning populations F1 selection results the previous year and the parent population control. F2 seedling population resulting from the breeding between relatives maintained separately from the parent population selection and control. Siamese catfish seedling populations F2 consists of 10 family selection and eight families control in cohort 1 were then used as material selection in this year's study. Selections are made at the age of 11 months of age and the selection differential values obtained siamese catfish F2 cohort 1 are selected by 281.57 g which means that the average weight of the population of selection increased by 30.82%. In this year also made the establishment of seedling populations of catfish Siamese F2 consisting of 10 families and 3 families of selection on the control cohort 2 were then used as material selection in research in 2017. The population of siamese catfish F2 selection had a higher growth performance than the control. Values response character selection on body weight siamese catfish populations F2 cohort 1 aged 11 months in the amount of 21.34% ($\Delta G = 164.21$ g) and the siamese catfish populations F2 cohort 2 5 months of age in the amount of 10.50% ($\Delta G = 10.47$ g), so that the known value of the selection response average in the population of siamese catfish F2 of 15.92%. F2 siamese catfish population has the potential to be proposed as a candidate for release catfish is superior because it has the combined value of the selection response $\geq 30.00\%$, the combined value of the selection response between populations of siamese catfish F1 and F2 Siamese catfish population of 36.83%.

EFFECT OF STOCKING DENSITY ON TILAPIA PERFORMANCE AND WATER QUALITY DURING INTERMEDIATE GROWING PHASE UNDER BIOFLOC SYSTEM

Ashraf Suloma*, Rania S Mabroke, Azab M. Tahoun, Abd El-Naem F.A Zidan and Mohamed H.M. El-Shafiey

*Fish Nutrition Laboratory (FNL), Animal Production Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt

*Corresponding author: (Tel.): + 201006176195

Email: suloma2001@agr.cu.edu.eg.

The major constraint in the adoption of tilapia culture to marketable size is the longer duration of culture. Therefore, efforts are needed to reduce the culture duration by phasing out the culture period and it is important to develop intermediate growing phase for tilapia in order to use up the most space available, and improve management techniques. The objective of this study was conducted to determine the ideal stocking density for tilapia farming in biofloc system without water renewal under intermediate growing phase.

Nile tilapia with an average size of 35 g were reared for 84 days at densities of 20, 40 and 60 fish/m³ in a zero-exchange biofloc water system. Each group consisted of four tanks. Fish fed at a feeding rate of 2.5% body weight/day with 25% protein commercial pellets. During the culture period, starch was added into the BFT tanks to maintain carbon/nitrogen ration in water 10:1. Fish were fed six days a week. Data were analyzed as a one-way ANOVA using SPSS program. Means were separated using Duncan's multiple range test.

Total ammonia, nitrite, nitrate, and pH were within acceptable limits. The results indicated that the individual fish performance and feed utilization efficiency were inversely related to increasing stocking density. The net yield increased with increasing density levels. No significant differences in whole body chemical composition (moisture, crude protein, crude fat, total carbohydrates, macro minerals and micro minerals) were recorded. The results obtained indicate that from economically point of view, stocking 60 fish/m³ is recommended for cultivating tilapia under intermediate growing phase. This work was supported by Science & Technology Development Fund (STDF), Egypt under grant no 5671.

Table 3: Effect of stocking density on Nile tilapia performance in a zero exchange biofloc water system

Treatment	FBW (g/fish)	GAIN (g/fish)	FI (g/fish)	FCR	Net yield (kg m ⁻³)
20 fish/m ³	123.95 ^a ±3.05	89.95 ^a ±3.49	108.62±3.07	1.21 ^a ±0.03	2.48 ^a ±0.06
40 fish/m ³	110.22 ^b ±2.05	76.35 ^b ±2.16	106.61 ±2.01	1.40 ^b ±0.03	4.41 ^b ±0.08
60 fish/m ³	100.84 ^c ±2.12	66.59 ^c ±2.07	108.20 ±1.74	1.63 ^c ±0.03	6.05 ^c ±0.13

GROWTH PERFORMANCE OF SPINY LOBSTER *Panulirus ornatus* SEA CUCUMBER *Holothuria scabra* AND SEAWEED *Kappaphycus alvarezii* IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM

Milennie Vialantine Sumbing*, Sujjat Al-Azad, Abentin Estim, Saleem Mustafa

Borneo Marine Research Institute
Universiti Malaysia Sabah
88400 Kota Kinabalu Sabah
milennie.bibie@yahoo.com.my

Interest in development of lobster culture has grown significantly to meet the market demand. As with other species, this expansion of lobster culture has caused deterioration in water quality mainly because of the waste produce by the lobster and uneaten feed. Integrated Multi-Trophic Aquaculture (IMTA) offers a combination of different aquaculture species wherein waste, from one species turn into nourishment or fertilizer for another species. This study was conducted to determine the suitable diet for spiny lobster as well as the growth performance of sea cucumber and seaweed integrated in an IMTA system.

Spiny lobster (*Panulirus ornatus*), sea cucumber (*Holothuria scabra*) and seaweed (*Kappaphycus alvarezii*) were selected. The IMTA system consisted of (1 m × 0.5 m × 1 m) 500L tank for spiny lobster and two (1.3 m × 0.8 m × 0.4 m) 500L tanks, one for sea cucumber and other one for seaweed cultivation. The stocking rate was 5 ind/tank both for spiny lobster (mean BW 325.61 ± 14.32 g) and sea cucumber (mean BW 56.43 ± 2.33 g) while initial biomass of seaweed was 504.84 ± 0.59 g/tank. Spiny lobster was fed with three different diets, trash fish (Diet 1), mangrove clam (Diet 2) and commercial shrimp pellet (Diet 3) for 12 weeks. The feeding frequency was two time in a day (8:00h and 16:00h). *In situ* water parameters were recorded daily and sediment samples were collected at two weeks interval.

The specific growth rate of 0.15 ± 0.06 %/day in spiny lobster was significantly higher (P<0.05), when fed with Diet 1 compared to specific growth rate (0.12 ± 0.04 %/day) obtained with Diet 2 and Diet 3. The survival rate of 73.33 % was observed in spiny lobster fed with Diet 1 but it was lower (66.67 %) when lobster was fed with diets 2 and 3. Sea cucumber performance was poor in this trial. There was no significance difference (P>0.05) in specific growth rate of the seaweed among the three dietary treatments. The results indicated that waste generated from spiny lobster tank may not be sufficient for supporting growth of *Holothuria scabra* or may not be efficient to extract organic matter but *Kappaphycus alvarezii* indicated the assimilation of inorganic nutrients in this system. The results are interesting not only in terms of production efficiency but also environment-friendly method of farming that the IMTA system designed for this study has offered.

TABLE 1. Percentage weight gain, specific growth rate (SGR) and percentage survival for spiny lobster, sea cucumber and seaweed in IMTA system.

		Diet 1	Diet 2	Diet 3
Spiny lobster	Weight gain (%)	14.03 ± 6.07	11.39 ± 3.67	11.10 ± 4.30
	SGR (% day)	0.15 ± 0.06 ^a	0.12 ± 0.04 ^b	0.12 ± 0.04 ^b
	Survival (%)	73.33	66.67	66.67
Sea cucumber	Weight gain (%)	-5.34 ± 2.90	-6.66 ± 0.68	-3.61 ± 1.44
	SGR (% day)	-0.06 ± 0.09	-0.08 ± 0.07	-0.04 ± 0.14
	Survival (%)	86.67 ^{ab}	93.33 ^a	73.33 ^b
Seaweed	Weight gain (%)	167.81 ± 9.34	171.71 ± 5.23	173.87 ± 25.12
	SGR (% day)	1.09 ± 0.04	1.11 ± 0.02	1.12 ± 0.10

TILAPIA LAKE VIRUS: A NOVEL PATHOGEN THAT THREATEN WORLDWIDE TILAPIA CULTURE *HOW TO HANDLE A SITUATION?*

Win Surachetpong

Department of Veterinary Microbiology and Immunology
Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, 10900
fvetsp@ku.ac.th

Since 2015, the massive mortality of Nile tilapia and red tilapia have been observed in farm-raised tilapia in Thailand. The disease has been recognized by farmers as “tilapia one month mortality syndrome” “TOMMS” because the disease regularly occur during the one month period after the juvenile tilapias had been transferred from hatcheries to the grow-out cages. The mortality rate in affected farms ranges from 20 to 90%. We investigated 32 outbreaks of TOMMS during October 2015 to June 2016. The complete analysis of moribund fish revealed that the novel orthomyxo-like virus, named Tilapia Lake Virus (TiLV) [1] was associated with 22 outbreaks [2]. The virus has been detected and isolated from clinically diseased fish. Whole genome analysis of TiLV isolated in Thailand and previously reported TiLV in Israel revealed 95-100 percent sequence identity [2, 3]. Based on our field observations, TiLV is contagious as susceptible fish may acquire infection after cohabitation with infected fish for 5-7 days. It is possible that TiLV spreads through contaminated water, carrier fish or other potential vectors. Additionally, the movement of live fish or contaminated byproduct including infected carcass could spread the virus within the region. None of these hypotheses has been investigated or confirmed. Therefore, strict biosecurity such as removing moribund or dead fish is important to reduce the impact of TiLV outbreak. Moreover, disinfectants of equipment or elimination of the infected fish population will facilitate disease containment. Development of rapid diagnostic assays and vaccine will reduce the impact of this emergent virus. Given to the possible worldwide distribution of TiLV, active surveillance and control of fish movements at the farm, country, regional or global levels may limit the spread of this emergent viral disease.

FIGURE 1. Tilapia farm with TiLV outbreak. Massive mortality of red tilapia.



FIGURE 2. The clinical signs of TiLV infection. Red tilapia showed distinct skin redness (white arrow) and pale skin. Nile tilapia with severe skin erosion (black arrow).



TILAPIA LAKE VIRUS: AN EMERGING PATHOGEN IN THAILAND

Win Surachetpong^{1,2*}, Puntanat Tattiyapong^{1,2}, Taveesak Janetanakit³,
Nutthawan Nonthabenjanwan³, Alongkorn Amonsin³

¹ Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, 10900. *fvetwsp@ku.ac.th

² Center for Advanced Studies for Agriculture and Food, Kasetsart University Institute for Advanced Studies, Kasetsart University

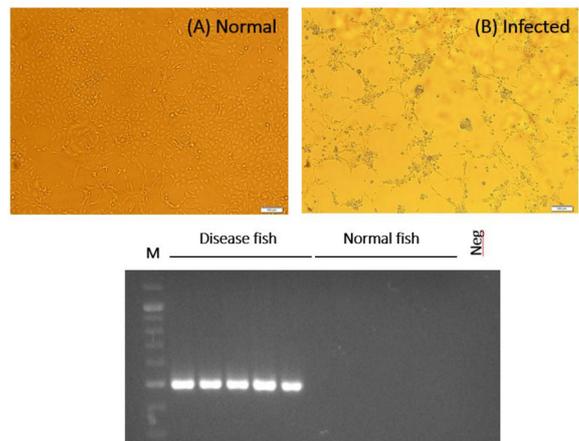
³ Faculty of Veterinary Science and Center of Excellence for Emerging and Re-Emerging Diseases in Animals, Chulalongkorn University, Bangkok, Thailand

Tilapia is the most important freshwater fish in Thailand with annual production of 200,000 metric ton. During 2015-2016, we investigated the massive die-off of red and Nile tilapia due to an unknown cause in Thailand. The disease has been called tilapia one month mortality syndrome (TOMMS). The cumulative mortality rate is 20-90 percent with peak of mortality occurred during 2 weeks since the moribund fish have been observed. The clinical signs of disease fish include lethargy, skin hemorrhage and congestion, skin and fin erosion, pale discoloration, exophthalmos and abdominal swelling. Here, we used reverse transcriptase polymerase chain reaction (RT-PCR) and virus isolation to confirm the presence of recently discovered tilapia lake virus (TiLV) in diseased tilapia in Thailand. The virus infected permissive cell line with cytopathic effect (CPE) development in 3 to 5 days post inoculation. Transmissible electron micrographs of infected cell and fish tissues showed round enveloped virions of 60 to 80 nm with characteristics very similar to those of *Orthomyxoviridae*. Additionally, genome analysis indicated that Thai TiLV is closely related to Israel TiLV. This is the first report of TiLV in Thailand and in South East Asia. At present, TiLV has been reported in Israel, Ecuador, Colombia, Egypt and Thailand. The spread of this emerging virus highlights that it is a potential threat to tilapia aquaculture in Thailand, Asia, and worldwide.

FIGURE 1. Clinical signs of infected red tilapia include skin and fin erosion.



FIGURE 2. Virus inoculation in (A) permissive cell line showing (B) CPE development within 3-5 days post inoculation. RT-PCR analysis indicated a positive band (491 bp) in all disease fish while normal fish have negative result. M = 100 bp, Neg = no template



ACCLIMATIZATION AND PRESERVATION OF SEAWEED *Kappaphycus alvarezii* AS PRODUCT OF *PaCS* (*Cytrat cintahse*) TRANSFORMATION FOR ENHANCED RESISTANCE TO ENVIRONMENTAL STRESS IN FLOATING NET CAGE IN PANGKEP WATER

Emma Suryati , Rosmiati and Rohama Daud

Research Institute for Coastal Aquaculture
emmasuryati@yahoo.com

PaCS (*cytrat synthase*) gene transformation in seaweed *K.alvarezii* has been successfully performed *in vitro* with the aim to improve resistance on environmental stress especially extreme environmental changes causing death and damage to the tissue cultivated seaweed. Nevertheless, the death often occurs during the regeneration of the flask cultures and acclimatization in the bath recirculation. Therefore, it is required an effort to improve the survival rate of gene *PaCS* (*Cytrat synthase*) inserted seaweed through the regeneration using the flask cultures continued with acclimatization and rearing of gene *PaCS* inserted seaweed on floating net cage (KJA), which is done in stages, namely: (1) the transformation of genes *PaCS* (*cytrat synthase*) into seaweed *K.alvarezii* followed by regeneration on the flask cultures in the laboratory; (2) acclimatization in cage on a net green (mess size of 100 um) with the size of 50x50x50 cm with the density of 200 explants and reared for 2 weeks, the explants were then after transferred to the net blue with the size of 50x50x50 cm (mess size of 200 um) with the density of 200 explants. After 4 weeks of the rearing period, regeneration of seaweed resulted of transformation was carried out by using long line method at the floating net cage, being tied up with a double ris rope with the distance of 15 cm. The measurement of weight, shoot length, and water quality was conducted at intervals of 2 weeks of rearing perid

Analysis of gene *PaCS* integration was done by PCR using primers *PaCS-F* and *PaCS-R*. Findings demonstrated that the daily growth rate (DGR) on regeneration in the culture flask was ranged from 0.11 to 0.18%, while the acclimatization using a net green exhibited the daily growth rate of 0, 62 to 1.3%, and the raring using net blue areas showed DGR increasing to 2.18% per day. Further enlargement of transgenic seaweed using a long line method showed DGR 1.42-4,3%. The integration of gene *PaCS* (*cytrat cynthase*) into seaweed showed the tape at the position of 1300 bp as the promoter fragment of *PaCS-F* and *PaCS-R* at *CaMV 35S* vector. Based on these results, it proved that the *PaCS* gene has been successfully transformed to the seaweed *K.alvarezii*

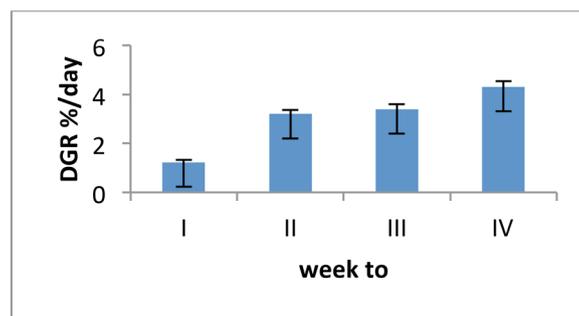


Figure 2. The daily growth rate (DGR) of seaweed shoot using a long line method

INHIBITORY TEST OF *Saccharomyces cerevisiae* YEAST TOWARDS *Vibrio parahaemolyticus* BACTERIA

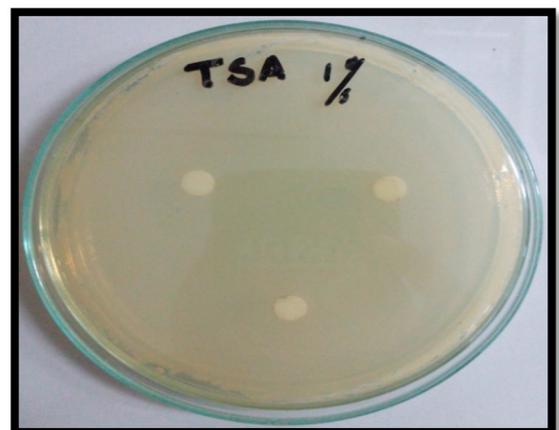
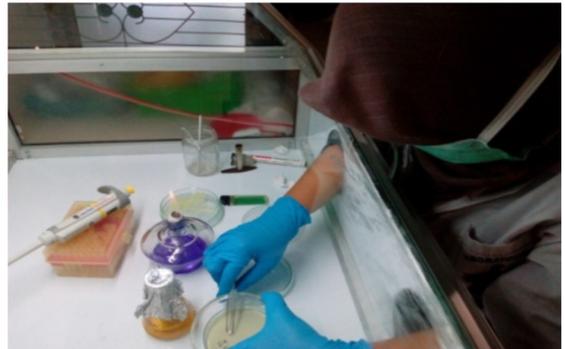
Endang Susianingsih, Nurhidayah, Ratna Sari

Research Institute for Coastal Aquaculture
 Jl. Makmur Dg. Sitakka No. 129, Maros South Sulawesi
 E-mail endang@kcp.go.id

Saccharomyces cerevisiae is yeast classified as eukaryotic which morphologically only forms cylindrical, oval or ovate blastospore influenced by its strain, reproduces by splitting itself through “budding cell”. *S. cerevisiae* can be utilized as probiotic, prebiotic and immunostimulant on shrimp and fish. This study was aimed to determine the inhibitory potency of *S. cerevisiae* yeast against *Vibrio parahaemolyticus* bacteria that are opportunistic pathogens.

Inhibition test performed using TSA media (Tryptic Soy Agar) with the addition of 1% NaCl (Tryptic Soy Agar) which is placed on a petri dish as much as 20µL / petri. Bacteria that have been rejuvenated (with a density of 109 CFU / mL) was inoculated into 1 ml TSA media and then flattened by using grinding and allowed to stand for 20 minutes. Fungus *S. cerevisiae* will be used rejuvenated into NB medium and taken as many as 200 mL and placed on the surface of the dish paper. Paper the dish and then placed on TSA media that already contains bacteria cultures were then incubated for 72 hours with positions reversed

The results showed that *S. cerevisiae*, by using disc paper, was known to have the ability to inhibit the growth of *V. parahaemolyticus* bacteria cultured together for 48 hours, which was characterized by the absence of clear zone formation resulted from the mixed culture. It concluded that the main function of *S. cerevisiae* yeast was not on the inhibition of bacteria. However, other functions of this yeast should be studied further.



WIFE'S ADAPTATION STRATEGY IN SECURING HOUSEHOLD ECONOMY OF FISHERS IN SUNGAI WAY TULANG BAWANG, TULANG BAWANG REGENCY, LAMPUNG – INDONESIA

Indah Susilowati*, Rizky Wilfrida Valentine, Mudjahirin Thohir

Diponegoro University
 Jl. Prof. Sudarto, UNDIP Tembalang Campus. Semarang
 indah.susilowati@undip.ac.id
 indah-susilowati@rocketmail.com

The main objective of this study is to formulate the adaptation strategy of wives to secure their household's economy. Mixed methods of quantitative and qualitative was involved to analyze the data. The results revealed that a woman who plays role other than reproduction is considered to be dealt with taboo subject. Assuming, the wives in the study area are disregarding with the taboo habit or belief in their daily practice, then the monetary values which may able to earn from their multiple roles activities (production, reproduction, and managing community) is incredible. The strategy proposed by this study among others are advocating for gender equity and guidance on changing the mindset of fisher's family against the suppositions without losing the heritage of ancestors, entrepreneurship training, campaign program for the important value of children's existence, loans access with low interest for fisher's wives under the supervision of financial management, and training of managing natural resources near Way Tulang Bawang River.

TABLE 1 Definition of The Operational Variables

Variables	Definition	Data Collection	Indicators
The Role of Production	Role which is directly related to activities or jobs that make money	<i>Indepth interview</i>	a. Job status b. Type of work Working hours (in a week)
The Role of Reroduction	Role which is related to maintain and endure the family life	<i>Indepth interview</i>	The implementation of reproductive activities, include: a. The childcare b. Housekeeping c. Deciding of a child's education d. Purchasing jewelry and electronics e. Managing Financial
The Social Role	Role which is related to civic and volunterrig activities	<i>Indepth interview</i>	a. regular social gathering b. recitation c. Mutual cooperation d. cultural activities

GROWTH PERFORMANCE OF TRANSGENIC TIGER SHRIMP BROODSTOCK (*Penaeus monodon*) AT DIFFERENT GENERATION

Hidayat Suryanto Suwoyo*, and Sahabuddin

Research Institute For Coastal Aquaculture
 Jl. Makmur Dg. Sitakka, No.129, Maros-90512, South Sulawesi
 E_mail: yayhat_95@yahoo.com

The provision of tiger shrimp (*Penaeus monodon*) broodstocks is part of the overall sustainability of tiger shrimp under hatchery production. Diseases resistant genes assemblage for tiger shrimp has been initiated by The Research Institute for Coastal Aquaculture (RICA), Maros, through transgenesis approach under anti-virus genes transfection. Gene antiviral was successfully transferred to the embryo from wild type broodstock to producing tiger shrimp founder (F_0). The breeding of founder (F_0) transgenic tiger shrimp has been conducted to produce F_1 generation. The present study aimed to evaluate the growth performance of broodstock candidates of tiger shrimp at different generation (F_0 and F_1).

This research was conducted at 4 ponds with size of 2000 m² in the experimental shrimp pond installation in Takalar Regency, South Sulawesi. The broodstocks of differences generations as the treatment, which were: broodstocks originated from F_0 generation, (A) and F_1 (B). The 22.63 to 28.57 g of broodstock candidates were stocked 0.5 ind.m⁻² and then reared for 128 days. During rearing period, these shrimp were fed using commercial pelleted feed with content 36-38% of protein in dosage of 10-4%/ body weight. Feeding frequency was applied for two times per day in the morning and in the evening. Measured variables were growth in weight, daily growth rate, size distribution, survival rate of tiger shrimp broodstock and Water quality, *i.e* temperature, salinity, dissolved oxygen, pH, alkalinity, nitrate, nitrite phosphate and TOM were monitored every week.

The results of this study indicated that the performances of these shrimps, growth rates, size distributions as well as survival rates between these F_0 and F_1 were not significantly different ($p>0.05$). Trend the growth pattern of tiger shrimp during the 128 days of rearing period on the each treatment relatively equal (Figure 1). Water quality measured during the experiment was still within the range of acceptable values for the growth of tiger shrimp.

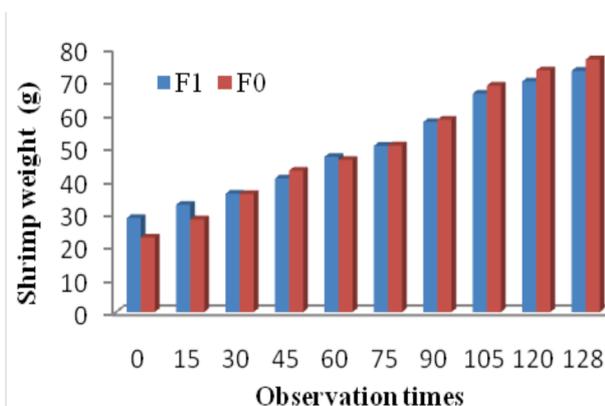


Figure 1. Growth of transgenic tiger shrimp broodstock in different generation

Table 1. Daily growth rate and survival rate of transgenic tiger shrimp broodstock during 128 days rearing period

Variables	Transgenic Tiger Shrimp in Different Generation	
	F_0	F_1
Initial weight (g)	22.63±2.89	28.57±3.37
Final weight (g)	76.74±4.53 ^a	73.27±3.22 ^a
Absolute weight (g)	54.11±4.53 ^a	44.69±3.22 ^a
DGR (g/day)	0.42±0.04 ^a	0.35±0.36 ^a
Survival rate (%)	73.35±2.05 ^a	51.7±31.68 ^a

EFFECT OF FEEDING FREQUENCY ON SURVIVAL AND GROWTH OF JUVENILE BAMBOO SPINY LOBSTER *Panulirus versicolor* IN INDONESIA

Syafrizal¹, Idat Galih Permana¹, Nur Bambang PU², Clive Jones³

¹Animal Nutrition and Feed Science, Bogor Agricultural University, Bogor, Indonesia
r27199@gmail.com

²Bogor Agricultural University, Bogor, Indonesia

³James Cook University, Cairns, Australia

In 2015 the Indonesian Ministry of Marine Affairs and Fisheries introduced a new regulation No. 1 / PERMEN-KP / 2015 specifying a minimum legal size for spiny lobster of 8cm carapace length. This effectively banned the export of lobsters smaller than 8cm carapace length, but provided opportunity for growout of seed lobsters to exceed the minimum legal size and provide an economic benefit to Indonesia. In Aceh, the tropical Bamboo spiny lobster *Panulirus versicolor*, is a common species, and potentially a suitable candidate for aquaculture. Vietnam provides a good example of the success of spiny lobster farming, and methods applied there are being assessed in Indonesia. The success of Vietnam lobster farming is based to a large extent on knowledge of the nutrient requirements of the lobsters which can be applied to formulation of manufactured diets. To date however, there has been little research on feed management and feeding frequency for lobster farming. An experiment was performed to assess the effect of feeding frequency on juvenile lobsters in Indonesia.

The experiment used a completely randomized design. 352 bamboo spiny lobsters (*Panulirus versicolor*) with mean weight 0.48 ± 0.51 g were reared for 4 weeks in 16 circular 80L tanks within a closed recirculation system. Four feeding frequencies were applied to 4 replicate tanks. The treatments were: FR1 fed once per day, FR2 two times per day, FR3 three times per day and FR4 four times per day. Initial feed ration was 100% of biomass per day, progressively reduced to 50%, 30% and 25% of biomass by week 4 of the experiment. The diet consisted of a manufactured pellet, formulated through an associated research project (ACIAR FIS/2014/059 Expanding spiny lobster aquaculture in Indonesia) and manufactured by the Institute of Mariculture Research and Development of Ministry of Marine Affairs and Fisheries (MMA-BRKP Gondol). Analysis of survival and growth was performed using SPSS version 23.0.

After 4 weeks of culture using 4 different feeding frequencies, there was no significant difference ($p > 0.05$) for weight, carapace length, moult frequency, specific growth rate (SGR), survival or food conversion ratio (FCR) (Table 1). Further research over a longer time period may be necessary to determine optimal feeding frequency for nursery culture.

Treatment	Final weight	Final CL	Moult	Survival %	FCR
FR1	0.87±0.17	11.30±0.71	0.77±0.77	28.50±4.36	5.81±0.66
FR2	0.97±0.20	11.96±0.61	0.72±0.85	30.50±4.36	5.14±0.62
FR3	0.91±0.23	11.52±0.89	0.70±0.64	36.25±3.69	5.36±0.47
FR4	0.97±0.26	11.82±0.96	0.74±0.61	31.75±6.13	5.04±0.50

THE PERFORMANCE OF WASTE WATER TREATMENT OF SUPER INTENSIVE *Litopenaeus vannamei* SHRIMP AQUACULTURE

Rachman Syah*, Mat Fahrur, Makmur and Muhammad C. Undu

Research Institute for Coastal Aquaculture (RICA)
Ministry of Marine Affairs and Fisheries, Indonesia
Jl. Makmur Dg. Sitakka No. 129. Maros, South Sulawesi, Indonesia
mailto:rachman222000@yahoo.com

High stocking density applied in super intensive *Litopenaeus vannamei* shrimp aquaculture has an inevitable impact to surrounding areas. Stocking density of 500 to 1250 ind/m² released TN and TP, originally from shrimp feed, as much as 47.9±5.4 kg N/ton shrimp and 15.5±1.2 kg P/tonnes shrimp, respectively; and 2.0±0.3 kg dry weight sediment/kg harvested shrimp. Thus, application of waste water treatments plant in a super intensive shrimp pond aquaculture should be an obligation. This study was aimed to evaluate the performance of waste water treatments plant in reducing nutrient loads released by super intensive shrimp ponds.

The plant was designed based on characteristics of effluent, the amount of operated ponds, the estimated volume discharged effluent every day and three days residence time. The waste water treatments plant consists a series of sedimentation ponds, two aeration ponds and equalization pond (Figure 1). The aeration ponds are facilitated with 5 aeration which are two paddle wheels and water jet; whereas a single paddle wheel is applied in equalization pond. Long-line cultivation of *Glacillaria* sp. and *Tilapia* sp. culture were taken place in the equalization pond.

The plant reduced 53.1 to 99.4% of effluent (Table 1). However, there is a need in increasing the ability of the plant in reducing concentrations of TOM which only 53.1%, considered as moderate, whereas nitrate is effectively reduced by the plant. The highly efficient was found in reducing concentrations of TSS, TAN, nitrite, TN and phosphate, which are 99.4, 92.7, 91.6, 96.8 and 95.2%, respectively.

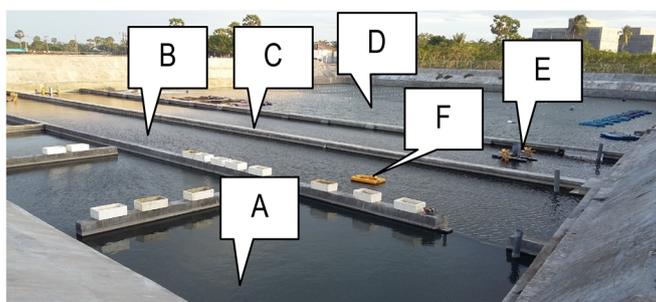


Figure 1. Waste water treatment plant of super intensive *Litopenaeus vannamei* shrimp aquaculture. A) sedimentation pond, B) aeration pond 1, C) Aeration pond 2, D) Equalization pond, E) Paddle wheels, F) water jet

Table 1. The average of suspended particles and dissolved nutrients concentrations in the plant.

Variable	Plant Inlet	Sedimentation ponds outlet	Aeration pond outlet	Plant outlet	Plant effectivity (%)	Standard effluent
TSS (ppm)	1715.41	437.11	402.78	10.14	99.4	< 70
TOM (ppm)	94.96	65.34	63.12	44.54	53.1	< 30
TAN (ppm)	6.91	2.42	2.10	0.50	92.7	
NO ₂ (ppm)	2.87	2.28	1.35	0.24	91.6	
NO ₃ (ppm)	2.35	2.13	1.91	0.69	70.6	
TN (ppm)	7.07	2.93	2.85	0.22	96.8	< 4
PO ₄ (ppm)	9.19	3.69	2.09	0.44	95.2	< 0.4
BOD ₅ (ppm)	19.80	12.83	10.74	7.02	64.1	< 20

DNA AUTHENTICATION OF STRIPED CATFISH *Pangasius hypophthalmus* AND *Pangasius macronema* FROM SOUTH SUMATRA INDONESIA

Mochamad Syaifudin*, Muslim, Dade Jubaedah, M. Rifqi Nanda Pratama

Program Study of Aquaculture, Faculty of Agriculture, Sriwijaya University
Jl. Palembang-Prabumulih Km 32, Indralaya, Ogan Ilir
South Sumatra, Indonesia

Corresponding author: Tel +62 (0711) 7371174; Fax +62 (0711) 580276; Email msyaifudin@fp.unsri.ac.id

Pangasiidae is highly economic important catfish in South Sumatra. Investigating fish diversity of catfish is of importance for species conservation. Many species or subspecies extincted due to environment decreasing caused by habitat loss, pollution and over fishing. Cytochrome C Oxidase subunit I is one of mitochondrial DNA markers used for species barcoding in freshwater, brackishwater and marine fish. This research aims to explore the use of COI gene for species barcoding, construct phylogenetic tree of Pangasiidae, and know physical and chemistry characteristic of habitat at Penukal Abab River, PALI Regency, South Sumatra. The methods used in the research consisted of DNA extraction, PCR (Polymerase Chain Reaction) amplification and sequencing mtDNA COI gene.

A 607 and 590 base pairs of partial coding sequences from *P. hypophthalmus* and *P. macronema* were obtained representing cultured and wild species. Nucleotide BLAST analyses showed COI gene of *P. hypophthalmus* had high identity (100%) to the same species from South Africa (KU568951.1) and Indonesia (KU692727.1). Meanwhile, *P. djambal* indicated 95% identity with *P. macronema* (KT289892.1) from Vietnam. Phylogenetic analyses indicated that obviously, *P. hypophthalmus* and *P. macronema* were at the same cluster from Pangasiidae family, and different cluster from others catfish (Bagriidae and *Clarias* sp) and *Oreochomis niloticus*.

Water quality criteria (brightness, temperature, ammonia, pH and alkalinity) at Penukal River were still in tolerance for survival rate. Further study using more species of Pangasiidae and habitat are needed to investigate the diversity of DNA from South Sumatra water resources.

REPRODUCTIVE BIOLOGY OF A COMMERCIALY IMPORTANT SEA CUCUMBER *Stichopus horrens* IN MALAYSIA: IMPORTANT INSIGHTS FOR AQUACULTURE DEVELOPMENT

Syed Zulfaqar*, M. Aminur Rahman**, Fatimah Md. Yusoff and A. Arshad

Laboratory of Marine Biotechnology
Institute of Bioscience, Universiti Putra Malaysia
43400 UPM Serdang, Selangor, Malaysia
*Presenting author's E-mail: syedzulfaqarsmk@gmail.com
**Corresponding author's E-mail: aminur1963@gmail.com

Sea cucumber fisheries have seen a period of relative increase in catch in the past decades. The rise in catch has brought together the unintended consequences of overexploitation and resource exhaustion. These circumstances have caused many closures of sea cucumber fishing sites in many parts of the world. Malaysia had also experienced such closure when the sea cucumber fisheries in one of the most famous landing site in Langkawi Island collapsed around mid-1980s. The tragedy has since sparked interest in a national aquaculture program for possible production of sea cucumbers without reliance upon capture fisheries. Despite these positive initiatives, Malaysia is still heavily relied on capture fisheries for sea cucumbers. As such, greater attention for management of these resources is to be demanded. There are however very limited accounts of the biology of sea cucumbers species available in Malaysia. Such knowledge, especially on the reproductive biology of sea cucumber species will not only be good for management of the capture fisheries but might also enhance the prospects of good aquaculture program. In a view to understand the modes of gonadal development and reproductive patterns, we investigated the commercially important sea cucumber, *Stichopus horrens* for a period of one year. Monthly samples of 20–40 sea cucumbers were collected from Pangkor Island, Perak, Peninsular Malaysia. The samples were dissected and their gonads were analyzed in the laboratory through macroscopic and histological examinations. The gonad weight and tubule diameter were significantly different between male and female, being heavier and longer in the latter. The gonad index (GI) was peaked at 0.76% in September, 2015 and gradually declined to the lowest at 0.03% in May, 2016. The proportion of matured species was also the highest in September, 2015. The spawning started in September, 2015 and prolonged up to April, 2016 with the majority spawned in November, 2015. The results also demonstrated that matured species can be found almost throughout the year. The prolonged release of gametes and availability of matured adults throughout the year might facilitate sustainable aquaculture program of this species. The period of enhanced spawning observed will also contribute to better management of broodstocks in such program to a greater extent.

COMPARATIVE STUDY OF MORPHOLOGY BETWEEN MALAYSIAN MASHEER HYBRID (*Tor tambroides* x *Puntius gonionotus*) AND JAVANESE CARP (*Puntius gonionotus*)

Fadhil Syukri*, Siti N. Ain, Nuruljannah Puaad and Azfar Ismail.

Department of Aquaculture
Faculty of Agriculture
University Putra Malaysia
43400 UPM Serdang
Malaysia
fadhil@upm.edu.my

Malaysian Mahseer, *Tor tambroides* locally known as kelah, is one of the highly sought after freshwater fish in Malaysia. However, mahseer have undergone decline in distribution and abundance due to significant degradation of their natural habits from deforestation, overfishing and high anthropogenic activities. Mass production of this species has still not been achieved. The sperm production of Mahseer is ever available. However, the eggs production cycle remains unclear. To overcome this problem, hybrid was introduced between male Mahseer and female Javanese Carp. The hybrid fish could be an alternative method to reduce the overexploitation of Malaysia Mahseer and also as a potential new aquaculture fish.

The experiment was conducted in the Wet Laboratory, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia. The broodstocks were injected with Ovotide hormone at 0.4ml/kg dosage and kept for 6 hours before the Javanese Carp eggs were ready to be stripped. Fertilization was done by stripping Mahseer sperm and mixed it gently with Javanese Carp eggs. Male Javanese Carp were also used in this experiments and fertilized with the eggs for comparison. The hybrid was raised and maintained for 3 months in aquaria with recirculating aquaculture system to maintain the water quality. Growth, fertilization rate, hatching rate, survival rate were observed in the experiment. The Truss Morphometric Network (TMN) analysis was used to detect differences among groups and to compare between species of confusable shape. Twenty-one landmarks were selected and measured using vernier caliper. The results were transformed and analyzed using SPSS (ver. 21) for comparison analysis. The fertilization rates were similar between the hybrid Mahseer and the pure Javanese Carp culture. Hatching percentage were significantly higher in Javanese Carp at 85.2±2.1% compared to the hybrid at 33.1±3.6%. However the growth rates were significantly faster in hybrid and can be observed after 14 days post hatching. Using the TMN, sixteen landmarks out of twenty-one landmarks showed significant differences between the hybrid and Javanese Carp. These results indicated that the hybrid fish have better performance compared to the pure Javanese Carp culture. The hybrid Mahseer could be a potential fresh-water fish aquaculture. However, further study is needed to improve the hatching rate and the fertility of the hybrid Mahseer need to be determined.

ANTIBACTERIAL POTENTIAL OF LOCALLY AVAILABLE AQUATIC AND TRADITIONAL MEDICINAL PLANTS AGAINST AQUACULTURE BACTERIAL PATHOGENS

Tan Chin Yueh*, Julian Ransangan, Jualang Azlan Gansau

Marine Borneo Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400 Kota Kinabalu
Sabah Malaysia
tan_chinyueh@hotmail.com

Aquaculture is the one of the fastest growing food-producing sectors in the world. However, occurrence of outbreaks of bacterial diseases obstructs its further development. Antibiotics and probiotics are the common methods used to treat and control bacterial diseases. Nevertheless both methods give rise to concern such as development of antibiotic resistance bacteria and accumulation of antibiotic residues in the fish and environment. Thus, search of new antibacterial substances that are not persistent in the environment for a long period of time is very much needed. The objective of the present study was to test antibacterial activity from extracts of four traditional medicinal plants against aquaculture bacterial pathogens including *Aeromonas hydrophilla*, *Aeromonas salmonicida*, *Aeromonas caviae*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio anguillarum*, using well diffusion method. Water, ethanol, chloroform and hexane were used as solvents to extract the plants samples. Inhibition zone of bacterial growth by the plant extracts at concentration of 10 milligram per millilitre was measured after 24 hour at 28°C. Of the four plants, *Artemisa* sp (mugwort) and *Cassia alata* (candle bush) displayed good antimicrobial activities based on the size of inhibition zone and number of bacteria inhibited. *Artemis* sp was able to inhibit all *Vibrio* species tested and *Cassia alata* was able to inhibit all the test bacteria. From the phytochemical analysis, it suggests that all the four plants were found to contain alkaloids, tannins, saponins, flavonoids and phenols. These may explain the antimicrobial activities exhibited by the plants. Although the plants may contain substances with antibacterial activities, their usefulness to aquaculture is limited until their non-toxicity to fish is proven.

EFFECTS OF DIFFERENT LIGHT SPECTRUMS ON GROWTH AND PROTEIN CONTENT OF *Chaetoceros calcitrans*

Gregory Tan Guan Yuan*, Sanjoy Banerjee, Helena Khatoon, Mohamed Shariff Mohamed Din, Fatimah Md. Yusoff

Institute of Bioscience
Universiti Putra Malaysia
Selangor, Malaysia 43400
gregtangy@gmail.com

Microalgae have recently received much attention as a new biomass source for protein, lipid, fatty acids and natural pigments. This makes microalgae a potentially rich source of chemical products with applications in the feed, food, nutritional and pharmaceutical industries. The growth and biochemical composition of microalgae are known to be greatly influenced by light. Limited availability of natural light (e.g. sunlight) due to diurnal cycles and seasonal variations impedes the viability of their production. Artificial lights such as fluorescent lamps are commonly used as a substitute to overcome light limitations. However, some of these light sources have high energy consumption. Therefore, energy-saving alternatives such as light emitting diodes (LEDs), with specific spectrums are encouraged to be integrated into culture systems to achieve faster growths and high quality biomass at a reduced operational cost. *Chaetoceros calcitrans* is widely used as a live feed for shrimp larvae. The growth and protein content of this commercially important species cultivated under 3 different light spectrums – blue, red LED, and compact fluorescent lamp (CFL; control) were evaluated in this study.

The highest specific growth rate (SGR) for *C. calcitrans* was blue and red LED treatments, which were significantly higher from the rest of the treatments ($p < 0.05$). The highest protein content was obtained in blue LED treatment ($p < 0.05$) (Table 1). These results indicate that blue and red LED promotes the growth of *C. calcitrans*. However, the protein content of the species was only higher when cultivated under blue LED.

TABLE 1. The growth and protein content of *C. calcitrans* cultivated under 3 different light sources.

Light source	SGR (/day)	Protein (% dry weight)
Blue LED	0.32 ^{a,b}	30.60 ^a
CFL	0.28 ^b	20.71 ^c
Red LED	0.35 ^a	26.35 ^b

NUTRITIONAL COMPOSITIONS AND GROWTH PERFORMANCE OF MEALWORMS *Tenebrio molitor* CULTIVATED WITH DIFFERENT AGRICULTURAL WASTES

S.W. Tan*, J.Y. Loh

Faculty of Applied Sciences, UCSI University Kuala Lumpur Campus
No. 1, Jalan Menara Gading, UCSI Heights (Taman Connaught), Cheras 56000 Kuala Lumpur, Malaysia
lohjy@ucsiuniversity.edu.my

Over-exploitation of natural fish stock and highly fluctuation of international price in fishmeal making aquaculture feed production using fishmeal is no longer a sustainable and environmental friendly approach. Hence, alternative protein source such as insect meal for feedstuff manufacturing is highly sought after to substitute fishmeal as the major ingredient. Larvae of *Tenebrio molitor* (yellow mealworm) is proposed as an alternative source of protein in this study, because of its ability to convert agricultural wastes into flesh mass and utilize them as energy sources. In present study, agricultural wastes e.g. watermelon rinds, broilers' eggshells, banana peels and the combination of these wastes were used in mealworms *T. molitor* cultivation. The nutritional effects and growth performance of mealworms *T. molitor* were tested after 28 days of feeding trial. The data of the proximate analysis and growth performance were analysed using ANOVA (Tukey's test).

Our results showed that watermelon rinds consist the most crude protein (11.57%), moisture (9.57%) and crude fibre (32.44%); broilers' eggshells has the most ash (89.54%) while banana peels contains the most crude fat (17.18%) and nitrogen-free extract (NFE) (43.18%) (Table 1). In terms of growth performance, watermelon rinds as a single diet showed the highest specific growth rate (SGR) (2.50%) and feed conversion efficiency (FCE) (0.10%) among all treatments, while banana peels improved the survival of mealworms *T. molitor* by 97.48%. In general, watermelon rinds and banana peels showed promising results in mealworms *T. molitor* mass cultivation. As a conclusion, our study provides an important dataset of the application of insect meal in aquaculture feed production.

Table 1 The proximate components of mealworms *Tenebrio molitor* after cultivation period.

Mealworms <i>T. molitor</i> fed on different diets	Proximate compositions (%)					
	Crude Protein	Moisture	Ash	Crude Fat	Crude Fibre	NFE
Control ^A	40.39 ^a	8.80 ^{ac}	1.31 ^a	39.54 ^a	7.36 ^{abc}	2.61 ^a
Commercial diet ^B	41.51 ^a	8.06 ^a	1.87 ^a	40.13 ^a	6.75 ^{ab}	1.69 ^a
Watermelon rinds (D1)	43.38 ^a	9.74 ^b	4.40 ^b	32.84 ^a	6.48 ^{ab}	3.17 ^a
Broiler's eggshells (D2)	42.49 ^a	9.55 ^{bc}	3.44 ^{bc}	33.57 ^a	8.73 ^c	2.23 ^a
Banana peels (D3)	38.53 ^a	8.13 ^a	2.48 ^{ac}	40.13 ^a	6.27 ^a	4.46 ^a
Mixture of D1, 2 and 3	42.78 ^a	8.48 ^a	3.62 ^{bc}	33.92 ^a	8.18 ^{bc}	3.02 ^a

NFE: Nitrogen-free Extract. Values with different letters are significantly different ($p < 0.05$), values with same letter are not significantly different ($p > 0.05$) among the nutrients. ^A Mealworms *Tenebrio molitor* without feeding. ^B Mealworms *Tenebrio molitor* fed on bread flakes.

CHARACTERIZATION OF TYPE 1 INTERFERON RECEPTOR (IFNR1) IN ORANGE-SPOTTED GROUPER (*Epinephelus coioides*)

Tang Zhi Zhuang*, Ting-Yu Wang, and Tzong-Yueh Chen

Department of Biotechnology and Bioindustry Sciences, Institute of Biotechnology
Translational Center for Marine Biotechnology, and Agriculture Biotechnology Research
Center, National Cheng Kung University, Tainan 70101, Taiwan
Email: zhuangtz91@gmail.com

Grouper aquaculture was among known high economic profits in aquaculture industry, raising interests in fish farmers. However, easily outbreak of virus diseases caused huge losses to fish farmers. Mechanism of first line of defense against virus diseases in teleost fish is activated by production of antiviral protein through signaling of secreted cytokine (type I IFN) to neighboring cells. Previous research proved up-regulation of gene expression level of grouper IFN and downstream Mx protein in response to virus infection.

To investigate interaction between type 1 IFN system with nervous necrosis virus infection, full length orange-spotted grouper IFNR1 was firstly cloned and identified as 1444 nucleotides sequence including untranslated region with ORF encoding 433 amino acids. Phylogenetic analysis indicated *osgIFNR1* shared high homology similarities with other teleosts. Relative expression of *osgIFNR1* was measured according to LPS and Poly I: C treatments which mimic microbial and virus infection respectively in immune organs. Gene expression of *osgIFNR1* was up-regulated during 6 hours of poly I: C treatment while LPS treatment showed highest expression level during 12 hours post-stimulation. The present study provides insight of cloning and identification of full length *osgIFNR1* follow by real-time PCR gene expression analysis showed effect of IFNR1 through up-regulation of gene expression level during 6 hours of poly I: C treatment. This study summarizes first analysis of orange-spotted grouper IFNR role in type 1 interferon system against nodavirus infection.

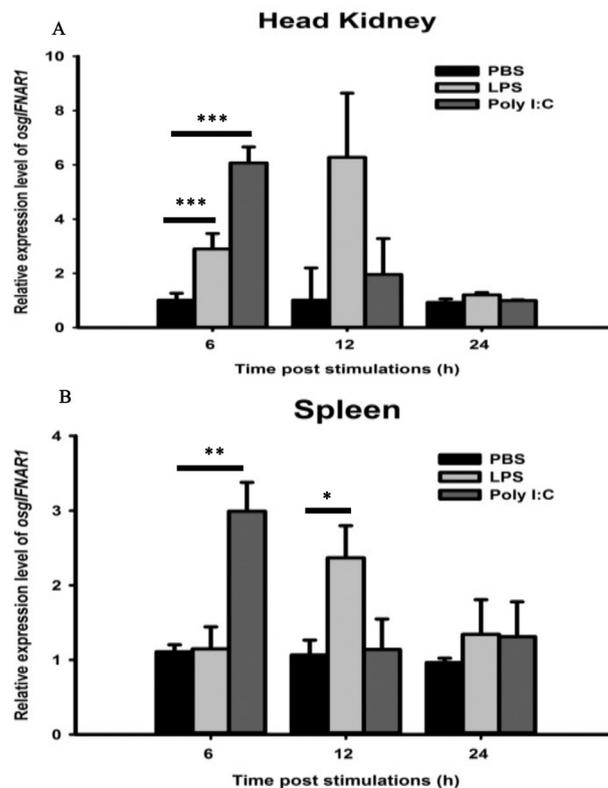


Figure 1 A and B: Gene expression analysis of IFNR1 in immune organ of orange-spotted grouper. Using beta-actin as internal control, IFNR1 expression in PBS, LPS and Poly I: C treatments on orange-spotted grouper in (A) head

EXPLORING BIOPHYSICAL AND MANAGEMENT CHALLENGES OF SMALL-SCALE SHRIMP FARM CLUSTERS IN INDONESIA USING FINE-SCALE GEOSPATIAL INFORMATION AND ANALYSIS

Tarunamulia*, Akhmad Mustafa and Hasnawi

Research Institute for Coastal Aquaculture (RICA)
 Jl. Makmur Dg. Sitakka, No 129, Maros, 90512
 South Sulawesi Indonesia
 litkanta@indosat.net.id

This study presents model applications of fine-scale geospatial information and analysis to explore bio-physical and management challenges of small-holders shrimp farm clusters in Suppa Sub-Districts, Pinrang District, South Sulawesi Province, Indonesia.

WorldView-2 multispectral satellite imagery with a spatial resolution of 2 m was used to create pond units layout (Figure 1A). To demonstrate the application of geospatial information and analysis, selected soil and water quality variables were obtained from Research Institute for Coastal Aquaculture (RICA) of which GIS-based spatial analysis such as image-vector conversion, interpolation and geospatial statistical models were applied.

The results generally indicated that geospatial information and analysis is an effective and efficient way to discover detailed technical constraints relating to biophysical characteristics and to assess the effective implementation and dissemination of aquaculture better management practices (BMPs). This study discovered aquaculture management constraints due to ineffective water exchange in some pond units as directly known from ineffective canal networks and from their correlations to spatio-temporal characteristics of salinity and dissolved oxygen values (DO). These pond units were also characterized by soil C/N ratio values > 10 which is an indication of slow in decomposition process of organic matter. The presence of Acid Sulfate Soils (ASSs) in most pond unit areas was also identified as indicated by $\text{pH}_F - \text{pH}_{\text{FOX}} > 3$ and mean S_{POS} value of 0.88%. The ratio of nitrogen and phosphorous (N/P ratio) of pond bottom soils < 4 indicated that N would be a limiting factor of pond productivity (Figure 1B). One of the more significant findings to emerge from this study is that the fine-scale geospatial analysis can be applied to monitor and evaluate spatial distribution of BMPs implementations and be compared with biophysical characteristics of the respective pond units. It is somewhat surprising that most BMPs implementations in this study area have not or just partially taken into account the spatial variability of the biophysical characteristics.

The empirical findings in this study provide a new understanding of the effective applications of fine-scale geospatial information and analysis on farm-level management. Its applications can help sustain the operation and productivity of small-scale shrimp aquaculture in the study area and other locations in Indonesia.

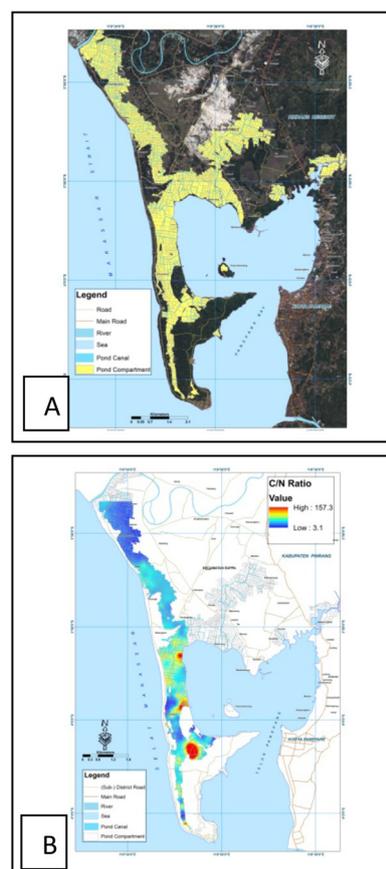


FIGURE 1. Original RGB WorldView-2 image overlay with vectors of pond units in 2016 (A) and spatial distribution of pond soil C/N ratio values (B) in Suppa Sub-Districts, Pinrang District, South Sulawesi Province, Indonesia

UNDERSTANDING BENTHIC CHANGE BENEATH LARGE OFFSHORE MUSSEL FARMS IN NEW ZEALAND

David I. Taylor*, Deanna Elvines, Emma Newcombe, Robyn Dunmore, Grant Hopkins, Nigel B. Keeley

Cawthron Institute
98 Halifax Street East
Nelson, New Zealand
david.taylor@cawthron.org.nz

New Zealand has approximately 19,000 hectares of water space allocated to aquaculture. Of this, over 10,000 hectares has been assigned for mussel farming in offshore areas. As part of ongoing environmental monitoring we have undertaken surveys to determine the benthic effects of large offshore mussel farms in the Tasman (749 ha) and Golden Bay (328 ha) areas since 2008 and 2009, respectively. During this time the benthic change beneath the farms has been considerable. The abundances and richness of infauna and epifauna species have increased significantly, as has sediment grain size. We used an enrichment stage index (ES - Keeley et al. 2013) to compare the effect of farm derived nutrients on benthic communities at sampling reference and farm sampling stations. Overall, ES did not increase significantly from reference stations at the Tasman Bay site, but in Golden Bay the ES score at stations beneath the mussel lines was significantly greater than reference stations. While enrichment may be having a minor impact on the seabed, in general the benthic environment appears to be having a net positive effect from having mussel farms above, which also provide some protection from bottom trawling and dredging.

IMMUNITY RESPONSE OF TRANSGENIC TIGER SHRIMP F1 AGAINST WHITE SPOT SYNDROM VIRUS (WSSV)

Andi Tenriulo, Andi Parenrengi, and Bunga Rante Tampangallo

Research Institute for Coastal Aquaculture
South Sulawesi, Indonesia, 90511
Agency for Marine and Fisheries Research and Development

Transgenic technology of tiger shrimp *Penaeus monodon* has been applied in order to produce a superior strain of tiger shrimp resistant to white spot syndrome virus (WSSV). Gene antivirus isolated from resistant tiger shrimp (survivor) was successfully transferred to the embryo in producing tiger shrimp founder (F0). In 2015, the breeding of founder (F0) transgenic tiger shrimp has been conducted to produce F1 population resistant WSSV. Gene antivirus insertion has been confirmed to the larvae by PCR technique. This present study aimed to evaluate the immunity response of transgenic tiger shrimp F1 against WSSV by challenge test.

A challenge test was performed to the transgenic shrimp F1 and non-transgenic shrimp as a control treatment. The virus was collected from infected white spot diseases tiger shrimp, prior to be injected to the shrimp by intra-muscular method in dosage of 100 μL (based on the LC-50). The transgenic and non-transgenic shrimp with size, weight of 7.93 ± 1.49 g and a total length of 9.15 ± 0.58 cm was stocked at 5 shrimp/16 L aquarium, completed with aeration. Each treatment consisted of three replications. Survival rate (SR) was observed at 1, 2, 3, 4, and 5 day after challenge, and immune responses such as total haemocyte count (THC), haemocyte differentiation (HD), pro-phenoloxidase (pro-PO) activity, and RNA concentration were observed at 1, 3, and 5 day after challenge from the shrimp haemolymph. Data of SR was analyzed by t-student test, and immune responses were descriptively explained.

After 5 days of challenged with WSSV, the transgenic shrimp exhibited higher ($P < 0.05$) resistance to the WSSV infection (52.0% survived) as compared to the control shrimp (8.0% survived) or equivalent with RPS (relative percentage survival) of 47.82%. The mortality of non-transgenic shrimp was sharply occurred at day-2 and continued until the end of study (Figure 1). The immune responses (THC, granular cell, pro-PO and RNA concentration) of transgenic shrimp showed higher value than the non-transgenic shrimp (Table 1).

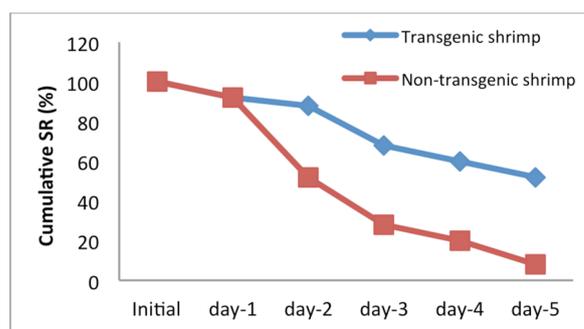


FIGURE 1. Cumulative percentage of survival rate of transgenic and non-transgenic shrimp after challenge test.

TABLE 1. The immune responses of THC, HD, pro-PO and RNA concentration of transgenic shrimp and non-transgenic shrimp.

Immune Response	Shrimp*	Initial	Day-1	Day-3	Day-5
THC (10^4 cell/mL)	T	2,666.7	1,255.6	2,622.2	2,222.2
	NT	916.7	1,300.0	1,266.7	1,066.7
HD** (%)	T	HC=14.3	HC=10.1	HC=18.4	HC=8.7
		GC=81.0	GC=71.9	GC=74.6	GC=81.1
	NT	SGC=4.47	SGC=18.0	SGC=7.1	SGC=10.2
		HC=16.1	HC=12.5	HC=13.2	HC=14.2
Pro-PO	T	0.0625	0.0545	0.0553	0.0743
	NT	0.0075	0.0147	0.0377	0.0040
RNA concentration ($\mu\text{g/mL}$)	T	66.6	38.2	101.0	33.8
	NT	56.4	15.0	44.2	20.4

*) T=transgenic and NT=non-transgenic

**) HC=hyaline cell, GC=granular cell, and SGC=semi-granular cell).

LIGHT EMITTING DIODES AS POSSIBLE LIGHT SOURCE FOR MICROALGAE *Nannochloropsis* sp.

Pik Neng Teoh, Zainoddin, Jamari, Nur Awatif Othman

teoh@dof.gov.my

This study is aim at assessing light emitting diodes (LEDs) as potential light source for culturing of *Nannochloropsis* sp. The microalgae were culture under three different source of lighting, fluorescent T5 tubes, warm white LEDs and warm white LEDs supplemented with red and blue LEDs. Initial result shows that *Nannochloropsis* sp. responded well to warm white LEDs with red and blue lights, cell density reached 4.961×10^7 cell/ ml followed by microalgae under warm white LEDs at 4.598×10^7 cell/ ml and 3.675×10^7 cell/ ml for flourescent warm white. Introduction of carbon dioxide and additional nutrient further enhance growth, with cell density reaching 1.17×10^8 cell/ ml, 9.49×10^7 cell/ ml and 5.42×10^7 cell/ ml for culture under warm white LED, warm white LED supplemented with red and blue light and fluorescent tubes.

TWO VARIANTS OF DISK ABALONE (*Haliotis discus discus*) MYD88 INVOLVED IN INFLAMMATORY RESPONSES VIA NF-KB ACTIVATION

Thanthrige Thiunuwan Priyathilaka*, S.D.N.K. Bathige, Yucheol Kim, Seongdo Lee and Jehee Lee

Department of Marine Life Sciences and Fish Vaccine Research Center
Jeju National University
Jeju Special Self-Governing Province, 63243
Republic of Korea
thiunuwan@gmail.com

Disk abalone (*Haliotis discus discus*) has become one of the commercially important marine gastropod species in Korean aquaculture industry. However, abalone production has been severely affected by pathogenic infections, such as bacteria, virus and parasites. Therefore, understanding about novel innate immune components, mechanisms and their responses against pathogens is vital for develop appropriate disease prevention strategies in abalone aquafarm industry. In present study, we identified two myeloid differentiation factor 88 (MyD88) molecules from disk abalone and their post innate-immune and inflammatory responses were characterized. The MyD88 is a critical adaptor in the TLR/IL-1R signaling pathway, which play an important role in immune responses via activating the NF-KB.

The abalone MyD88 1 and 2 (AbMyD88-1, 2) resembled typical domain structural features including characteristic N-terminal death domain and a C-terminal Toll/IL-1 receptor (TIR) domain known to be important for the functions of MyD88 in mammals. The phylogenetic analysis revealed that the AbMyD88-1 and 2 closely related to their in-vertebrate counterparts. Abalone MyD88-1 and 2 mRNA was ubiquitously expressed in all the tissues analyzed with the highest expression at muscles and hemocytes respectively. Moreover we examined the expression profiles of AbMyD88 genes at deferent developmental stages of disk abalone. The AbMyD88-1 and 2 mRNA transcripts were differentially modulated upon *Vibrio parahaemolyticus*, *Listeria monocytogenes*, viral hemorrhagic septicemia virus (VHSV), LPS and poly I:C in abalone hemocytes and gills. Overexpression of AbMyD88-1 and 2 in HEK293T cells exhibited significant activation of NF-KB. Thereafter, we determined their inflammatory responses by overexpressing the AbMyD88-1 and 2 in RAW264.7 murine macrophage cells. Significantly induced nitric oxide production and up-regulated expression of inflammatory marker genes, such as *iNOS* and *cox-2* were detected in AbMyD88-1 and 2 transfected RAW264.7 cells upon LPS stimulation. Furthermore, pro-inflammatory cytokines, including *IL-1 β* , *IL-6* and *TNF- α* were up-regulated in AbMyD88-1 and 2 transfected RAW264.7 cells after treatment with LPS. Collectively these observations suggest that the AbMyD88-1 and 2 involved in post-innate immune responses and inflammatory responses in abalone.

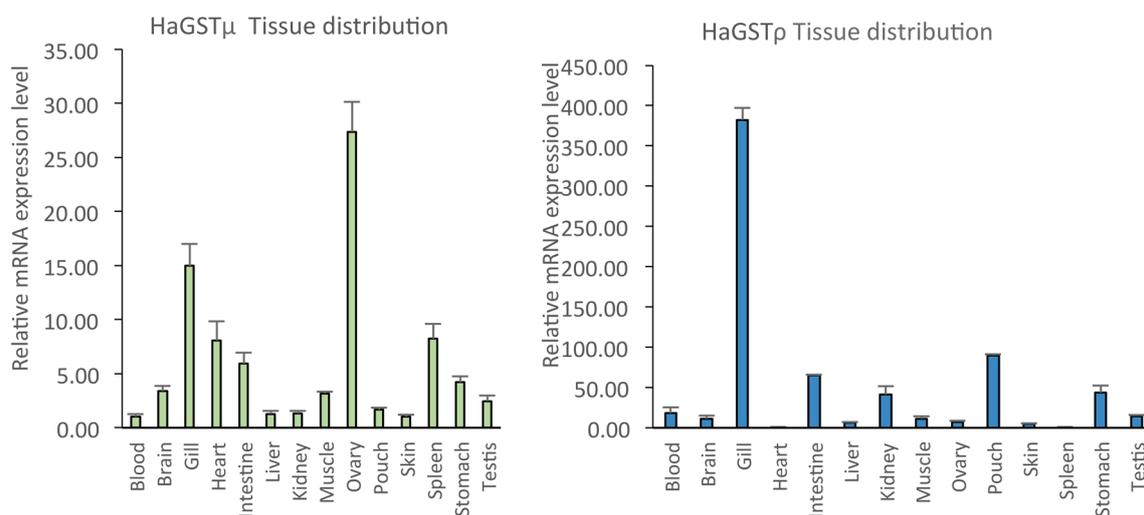
MOLECULAR CLONING AND CHARACTERIZATION OF TWO GLUTATHIONE S-TRANSFERASE FAMILY PROTEINS FROM BIG BELLY SEAHORSE *Hippocampus abdominalis*

M.D.Neranjana Tharuka*, S.D.N.K. Bathige, Gabin Kim, Jehee Lee

Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University
Jeju Self-Governing Province, Republic of Korea
neranjan10@gmail.com

Glutathione transferases (GSTs, EC 2.5.1.18,) are important phase II detoxifying enzymes which mainly catalyze hydrophobic electrophilic substrates with the conjugation of reduced glutathione (GSH). GST α and GST μ are cytosolic GSTs which have been studied in a variety of organisms in which GST α is fish specific. However, limited studies have been focused on teleosts. Those paralogs of big belly Seahorse (*Hippocampus abdominalis*; *HaGST α* , *HaGST μ*) were biochemically, molecularly and functionally characterized to determine their antioxidant range and protective capacities upon different pathogenic stresses. *HaGST α* and *HaGST μ* comprise coding sequences of 678bp and 654bp respectively, which encode respective proteins 226 and 217 amino acids in length. In silico analysis shown that all HaGSTs composed of characteristic N-terminal domains and C-terminal domains bearing GSH binding sites and substrate-binding sites respectively. The both recombinant proteins catalyzed the model GST substrate 1-Chloro-2, 4-dinitrobenzene (CDNB). Enzyme kinetic analysis revealed different K_m and V_{max} values for each rHaGST, presenting their dissimilar conjugation rates. The optimum conditions (pH and temperature) and inhibitory assays of each protein demonstrated different optimal ranges displaying their broad range of activity as a cluster.

GST α and GST μ were ubiquitously expressed in different amounts, however GST α was highly expressed in gills, whereas GST μ was highly expressed in ovary and gills. The mRNA expression of *HaGST α* in liver upon the LPS and Poly (I: C) challenges, shown a significant up regulated expression as well as up regulation upon the LPS, Poly (I: C), *E.tarda* and *S.iniae* challenges in blood. *HaGST μ* expression have been significantly increased in blood upon Poly (I: C), *E.tarda* stimulates respectively.



SELECTIVE BREEDING OF TILAPIA: STATUS AND PROSPECTS

Jørn Thodesen*, Morten Rye, Carlos Lozano, Sergio Vela Avitúa, Harry Johansen, Hideyoshi Segovia, and Jose Ospina

Akvaforsk Genetics
N-6600 Sunndalsøra, Norway
post@akvaforskgenetics.com

Geneticists at Akvaforsk Genetics, now a Benchmark company, have more than 30 years of experience working with selective breeding of tilapias and other species of fish and shrimp. Based on the highly successful GIFT-project for Nile tilapia in the Philippines, Akvaforsk Genetics has supervised the establishment of family-based selective breeding programs for Nile tilapia, Blue tilapia and red tilapia in Asia and the Americas. Based on the extensive experience from developing these programs, an in-house selective breeding program for Nile tilapia was established in the US in 2010. This state-of-the-art breeding program is operated by our Benchmark sister-company Spring Genetics.

The presentation will focus on the status of selective breeding of tilapias, highlighting achievements in three leading programs focused on improving the production efficiency (growth, survival), robustness (growth/survival in different environments, survival in environmental and specific disease challenge-tests) and product quality (external color). Pioneering work related to selection for improved resistance to *Streptococcus spp.* and application of genomic tools will be discussed.

CURRENT STATUS AND SUSTAINABILITY ISSUES OF MARINE RESOURCES AND BIODIVERSITY IN SUDANESE NATIONAL RED SEA WATER

Mubarak Eisa A. T.*¹ and Abdalla Nassir E. A.

¹ Department of Fisheries Science, College of Natural Resources & Environmental Studies, University of Bahri, Khartoum, Sudan zip code 11111
Email: mubaraktbn@gmail.com.

The Sudanese Red Sea is famous for its attractive and mostly pristine habitats, particularly its coral reefs. Three distinct depth zones are recognized: shallow reef-studded shelves less than 50 m deep, deep shelves 500 to 1,000 m deep, and a central trench more than 1,000 m deep, reaching a maximum of 3,000 meter off the city of Port Sudan. The current total annual fin fish production in Sudan is estimated to be 8000 tons from marine water (the Red sea). These marine resources divided into artisanal fishery (about 3000 tones), trawling (about 2000 tones), per sine fishery (about 2300 tones), shrimp from trawling (about 60 tones), shrimp from culture (about 6 tones), trochus (about 724 tones), mother of pearl shell (about 12 tones) and sea cucumber (about 60 tones). The Sudanese marine and coastal environment is in relatively good condition overall, with isolated badly degraded areas. The region, however, is subject to a mounting list of environmental impacts linked to urban and industrial development, and to overgrazing. coastal habitat destruction by development; oil industry spill risks; passing ship pollution; pollution from land-based sources; risk of importing invasive species in ballast water; fisheries management; mangrove cutting and overgrazing; and marine protected areas and tourism.

THE RESPONSE OF FISH TO STRESSORS IS GLOBAL: FROM HORMONES TO GENES AND FROM BLOOD TO MUCUS AND SCALES

Lluís Tort*, Felipe Reyes-Lopez, Ali R. Khansari, Mariana Teles, Camino Fierro-Castro, Eva Vallejos-Vidal and David Parra

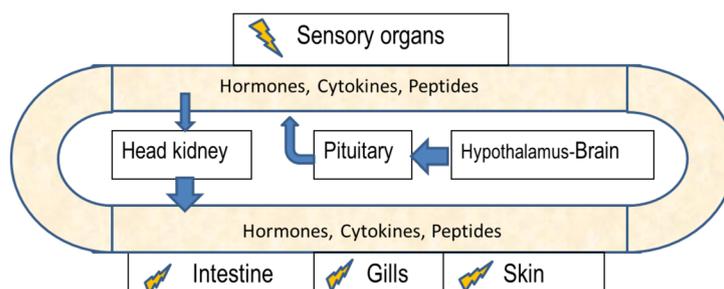
Dpt. Cell Biology, Physiology and Immunology. Univ. Autònoma de Barcelona. Spain

The stress response is an alarm mechanism that is common to all animals. As this response has been largely studied in vertebrates and particularly mammals and humans, emphasis has been made on the endocrine response, and more specifically in the activation of the hormonal axes leading to the secretion of adrenaline and cortisol. However, the nature of the stress response is necessarily global, and therefore an increasing number of biological structures and processes are recognized as having a role in the stress detection or in the stress response.

In fish, as in other vertebrates, the stress responses are well known in terms of the hormonal, metabolic and hematological changes produced by the stressors. Over the last two decades, the molecular and gene reactions have also been assessed and at the same time, responses at organismal level have been studied, as for instance, the behavioral response. This has allowed researchers to deep into the variation of the response through the investigation of high and low stress responders or proactive and reactive subsets of fish populations. By assessing these subgroups, a better knowledge of the respective reactions to stressors can be obtained and therefore this helps to manage stress episodes of fish batches under aquaculture conditions.

Recent work also shows that the stress perception may occur at the periphery of the fish and subsequently an initial response is initiated at these peripheral sites, in particular in the external surfaces of the fish that work as both barriers and portals in the interaction with water components, chemicals or pollutants and microorganisms (parasites, bacteria or virus).

New techniques to determine stressed status have been also studied over the last years with the objective of obtaining more accurate and complete record of the overall physiological changes but also, and particularly, with the objective of assessing the fish status using techniques as much non-invasive as possible. Thus, hormonal (cortisol) changes after stress can be determined not only in plasma, but also in fish mucus, in scales and also in the surrounding water, as part of the cortisol is secreted out of the fish. The measurement of cortisol in the scales gives also the advantage of assessing whether fish has been stressed before the sampling episode, since previous stress experiences give a printed record in the scale.



GENOTYPE BY ENVIRONMENT INTERACTION FOR GROWTH IN GIANT GOURAMI (*Osphronemus goramy*)

Sularto and Rita Febrianti

In the process of selection activities to obtain superior fish farming activities carried out through stages according to POS of rearing of such fish. The selection process is done by grading of the fish population that is taking groups of fish with the best growth performance to a certain extent. Selection activities carried out in certain sizes and different for each species of fish, in addition to any consideration or breeders have different reasons in determining the size or age of the fish that will be selected. In general, the selection of breeders agree should be done on the consumption size. After getting selected fish with the desired character, then the labeling or “tagging” to facilitate the next process in the “breeding program”.

Giant gourami (*Osphronemus goramy*) has a growth pattern in which males have more rapid growth than females (Sularto *et al.*, 2016). Therefore, we need a specific strategy in the framework of the selection process. Another thing to consider in the selection of giant gourami are environmental factors of cultivation. As reported by (Bangera *et al.*, 2015) that in aquaculture, environmental variables such as photoperiod, temperature and production system may significantly influence growth performance of fish across production environments. Differences in the environmental variables may induce a phenomenon called genotype by environment interaction ($G \times E$). Therefore, to reduce bias in the selection of giant gourami poses due to environmental factors, it is necessary to be carefully taken into account both factors. According to Bangera *et al.* (2015) that the genotype \times environment can be separated into two forms: re-ranking and heterogeneity of variances. Re-ranking means that the rank order of genotypic performance change across different environments, *i.e.*, the best genotypes in one production environment may not be the best in other production environments.

Table 1. Anova of Growth performance of 4 strains of giant gourami at the age of 18 months

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	334929.204 ^a	19	17627.853	59.243	.000
Intercept	842701.675	1	842701.675	2.832E3	.000
STRAIN	62633.009	3	20877.670	70.164	.000
BLOK	119057.590	4	29764.398	100.030	.000
STRAIN * BLOK	124330.884	12	10360.907	34.820	.000
Error	84802.693	285	297.553		
Total	1219004.880	305			
Corrected Total	419731.898	304			

a. R Squared = .798 (Adjusted R Squared = .784)

Table 1. Anova of Growth performance of 4 strains of giant gourami at the age of 18 months

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.511E6 ^a	19	132139.366	2.913	.000
Intercept	3492953.479	1	3492953.479	77.004	.000
STRAIN	314079.901	3	104693.300	2.308	.077
BLOK	336821.062	4	84205.266	1.856	.118
STRAIN * BLOK	1775985.206	12	147998.767	3.263	.000
Error	1.293E7	285	45360.665		
Total	1.943E7	305			
Corrected Total	1.544E7	304			

(Continued on next page)

This study used four carp populations, namely: Jambi, Kalimantan, Majalengka and Tasikmalaya. Fourth populations were initially kept in the same container cultivation, then at the age of 11 months do rankings based on body weight. Having obtained several groups in each population then maintained in a separate pool, after the age of 15 months are tagged with a micro-chip and then kept in the same pool until the age of 25 months. Sampling fish growth was done once a month, while the water quality observations carried out every two months.

The results showed that at the age of 11 months were the best growth in the population Majalengka (384.28 ± 75.95), followed by Kalimantan population (370.01 ± 71.09), Tasikmalaya (341.54 ± 65.34), Jambi (315.29 ± 50.46). There were indications that the environment affects the growth of giant gourami and there are indications that it was not always a strain or an individual has always excelled in all environments Table 2). At 18 and 25 months there were no differences between strains. This was presumably because it has entered the phase of gonadal maturation.

PROPHYLACTIC APPROACHES IN REDUCING THE IMPACT OF ENTERIC BACTERIAL DISEASE ON PENAEUS VANNAMEI

Loc Tran^{(1)(2)*}, Vy Van Nguyen⁽¹⁾, Phuc Hoang⁽¹⁾⁽²⁾

(1) ShrimpVet Laboratory, Ho Chi Minh City, Vietnam

(2) Department of Aquaculture Pathology, College of Fisheries, Nong Lam University, Vietnam

(*) Corresponding Author: thuuloc@email.arizona.edu

Early Mortality Syndrome or Acute Hepatopancreatic Necrosis Disease (EMS/AHPND) has been a pandemic disease to the shrimp farming since 2010. This disease alone has caused billions of dollar losses. White Feces Syndrome has been a mysterious disease that also caused significant losses to the shrimp farming industry in Asia. The ShrimpVet lab has been working on identifying the causation of White Feces Syndrome since 2016. Several single isolates were obtained from white feces syndrome collected from the field could produce the same pathology in a laboratory challenge model using feeding method. Up to 15 different *Vibrio* isolates obtained and the Koch's postulate was completed. In short, this study could prove the infection nature of white feces syndrome and propose an infection model for this disease. Further molecular biological analyses of the isolates are under investigation.

Antibiotics top-dressed on shrimp feed pellet is a very common practice in controlling bacterial diseases in shrimp farming in Asia. As more concerns of antibiotics resistance and antibiotics residue in seafood arises, there is an urgent need for the replacement of this practice. Several products have been tested at the ShrimpVet with the infection model to evaluate the efficacies of products against those bacterial diseases. Probiotics, bacteriophages, essential oils, immunostimulants, quorum sensing inhibitors, herbal extracts, yeast extracts, microbial metabolites, etc. are among of products/approaches tested using the challenge models. Many results indicate that several products can be mixed in the feed and delivered to shrimp orally as a prophylactic against enteric bacterial diseases. The outcome of those researches can lead to a formulation of a "functional feed" that can help reduce diseases without using antibiotics in feed.

INVESTIGATION ON THE DISEASE SITUATION OF KOI CARP (*Cyprinus carpio*) IN SEVERAL KOI FARMS IN THE MEKONG DELTA

Tran Xuan Minh^{1*}, Tu Thanh Dung²

¹Advanced aquaculture course 38

²Department of Pathology

College of Aquaculture and Fisheries, Can Tho University, Viet Nam

*Corresponding author: minh1209592@student.ctu.edu.vn

An investigation about pathogen infection on Koi carp (*Cyprinus carpio*) was conducted in the period from January to November 2016 in several koi farms the Mekong Delta provinces, Vietnam. The study purpose to determine the infection status on Koi carp and provide additional information for the study subsequent research. A total of 128 diseased Koi samples at the stage from 1 to 6 months of cultured were collected. Fish specimens were observed for clinical signs, examined for parasites and bacteria isolated. The clinical signs including reduced feeding, lethargy swimming, red spots on the body, red-abdominal fluid and visceral hemorrhage. Results on parasitic examination, indicated 5 genera of parasites, namely *Myxobolus* sp., *Trichodina* sp., *Dactylogyru* sp. and *Metacercariae* and *Argulus* sp.; found mostly in the skin and gills of the infected fish. The prevalence ranged from 14-72.3%, in which *Trichodina* sp. showed the highest intensity (3-28/40X) and *Argulus* sp., the lowest (2/10X). Remarkably, results on bacterial identification also isolated 32 strains of *Aeromonas* bacteria from internal organs of the affected fish. Conventional and rapid identification systems, PCR were used to identify the causative agents of the disease. Meanwhile, histological section of gills infected *Myxobolus* sp. with severed lamellar hyperplasia and chondrodyslasma of cartilage were also observed. In generally, hemorrhagic disease and *Myxobolus* sp. infection were the most two common diseases that caused the highest rate of infection and high mortality on the Koi carp in this investigation.

GONADOTROPIN RELEASING HORMONE ANALOGUE STIMULATED SPERMATOPHORES MATURATION OF HATCHERY-DISCARDED MALE TIGER SHRIMP, *Penaeus monodon* WITHOUT EYE STALK ABLATION

Ike Trismawanti, Agus Nawang and Asda Laining

Research Institute for Coastal Aquaculture (RICA)
Ministry of Marine Affairs and Fisheries
Jl. Makmur Dg. Sitakka No. 129, Maros 90512
South Sulawesi, INDONESIA
ic_ast@yahoo.co.id

The use of broodstock in hatchery for nauplii production is basically only for short periode. When the production target is achieved, normally both female and male stocks are discarded. A series of trial was conducted on male tiger shrimp which were discarded from hachtery in order to evaluate the effect of injection of salmon gonadotrophin releasing hormone analogue (sGRH-a) on spermatophores maturation against eye stalk ablation.

This study consisted of two trials; the first one was arranged with three treatments, injection of sGRH-a at two dosages of 0.1 (OV1) and 0.2 (OV2) mL/kg shrimp and eyed ablation (AB) as the control. Following trial comprised of two treatments in which the dosage tested was the optimal dose obtained in the first trial (0.2 mL/kg shrimp) and control ablation (AB). Discarded male stocks were randomly distributed with density of 14 males for the first trial and 12 males for the second trial. Injetion was carried-out three times with interval one week for the both trials. A week after the last injection, males were electrically shocked to release their spermatophores.

At the first trial, number of male releasing spermatophores at maturation and first rematuration was higher in shrimp induced with OV2 followed by OV1 and AB (Table 1). Number of male releasing spermatophore in the second trial was also higher in shrimp induced with 0V (58.3%) compared to AB which was 50% (Table 2). However, number of male releasing spermatophore at rematuration 1 and 2 was higher at AB than OV. Weight of spermatophore seemed not affected by the treatments but more related to the weight of the male used which in trial 1 were bigger than in trial 2. There was no clear trend of the cell number of sperm in spermatophore related to the treatments, however, ablated shrimp produced higher sperm cell (101.2×10^6 cell/shrimp) than OV (89.5×10^6 cell/shrimp) only at the first maturation. In general, the sperm cell number showed a decrease by increase the spawning frequency for both treatments.

Table 1. Number of male stock releasing spermatophore through electrical shock at first maturation, rematuration 1 and rematuration 2 in the first trial

Treat ment	Σ Male releasing spermatophore at first maturation (shrimp)	Σ Male releasing spermatophore at rematuratio n 1 (shrimp)	Σ Male releasing spermatophore at rematuration 2 (shrimp)
AB	4 (28.6%)	2 (14.3%)	1 (7.1%)
OV1	5 (35.7%)	2 (14.3%)	1 (7.1%)
OV2	8 (57.1%)	5 (35.7%)	3 (21.4%)

Table 2. Number of male stock releasing spermatophore at first maturation, rematuration 1 and rematuration 2 in the second trial

Treat ment	Σ Male releasing spermatophore at first maturation (shrimp)	Σ Male releasing spermatophore at rematuration 1 (shrimp)	Σ Male releasing spermatophore at rematuratio n 2 (shrimp)
AB	6 (50%)	4 (33.3%)	6(50%)
OV	7 (58.3%)	3 (25%)	1 (8.3%)

CARRYING CAPACITY ASSESSMENT OF A COASTAL ECOSYSTEM FOR MUSSEL AQUACULTURE

Aurore Trottet*

DHI Water & Environment (S) Pte Ltd
1 Cleantech Loop
#03-05 CleanTech One
Singapore 637141

Blue mussel (*Mytilus edulis*) culture industry in Canada started in the early 1970s and has steadily grown since. Over the last 35 years, a significant proportion of Eastern Canadian mussel production has been from the Magdalen Islands, Gulf of St Lawrence, Canada and has increased by more than a factor of ten. Contrary to shrimp or fish that need to be feed, mussels can grow by extracting plankton directly in the water column (extractive aquaculture). The sustainability of the industry depends on the ability to forecast the impact of mussel farms on the surrounding ecosystem based on knowledge of local conditions and ecosystem vulnerability. The aim of this project was to determine the carrying capacity of the GEL to determine the total bivalve biomass supported by this ecosystem without significant disturbance of the ecosystem.

In this presentation I will present the work published by myself and different members of the team from the Institute of Marine Science (ISMER) of the University of Quebec from Rimouski involved in the carrying capacity project of GEL. Monitoring was set up in summer 2003-2004 to assess the water column characteristics (physico-chemico-biological), and the impact of mussel filtration on plankton. The GEL is characterized in summer by: (1) low nutrient concentrations; (2) fairly high primary productivity; (3) dominance by small phytoplankton cells ($<10\ \mu\text{m}$); and (4) high biomass of heterotrophic microplankton. This indicates that the dominant trophic pathway in GEL is the microbial food web. Path analysis (Fig. 1) suggests a negative interaction of mussels on ciliates and heterotrophic nanoflagellates in GEL. Based on clearance rates of mussels in the area, we found that the mussel farm removed 16% of the biomass of ciliates, 8% for phytoplankton and 4% for phytoplankton daily production rates.

A calibrated fine resolution physical-biogeochemical model coupled with a dynamic energy budget (DEB) was used to investigate the local and system scale interactions between the mussel farm and the receiving coastal ecosystem. Using a set of published parameters for the DEB, the coupled model reproduces quite accurately both the local mussel growth and its spatial distribution over the farm area. Mussel related process rates are also well reproduced, allowing the study of mussel/environment interactions. The coupled model results show that the mussel stock could be greatly increased before reaching the maximum production capacity of Grande-Entrée lagoon. However, when the ecological aspect is accounted for, using model results along with objective criteria such as the depletion footprint curve, the overall carrying capacity of Grande-Entrée lagoon must be significantly reduced. The coupled fine scale numerical model developed for this study gives the opportunity to assess the ecological carrying capacity of a coastal region for shellfish culture accounting for both local and system scale processes.

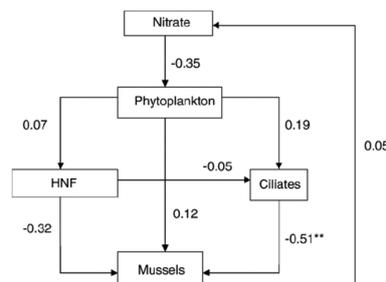


Fig.1: Path diagram of GEL mussel farm food web showing the trophic relationships between the taxa used for the path analysis. Arrows indicate direction of causality assumed in the model. Magnitudes of path coefficients are adjacent to arrows designating that path (** $p < 0.01$) (Trottet et al, 2008)

A STUDY ON *Gracilaria* Sp. GROWTH RATE CULTURED IN BRACKISHWATER ENVIRONMENT

Abu Bakar Tumin^{a*}, Mohd Lazim Mohd Saif^a, Rosmaria Abu Darim^a, Amatul Samahah Md Ali^a and Saberi Mawi^a

^aBrackishwater Aquaculture Research Division, Fisheries Research Institute, 81550 Gelang Patah, Johore, Malaysia

*Corresponding Author's Email: abubakar@yahoo.com, abubakar@dof.gov.my

A study was carried out to identify the growth rate of seaweed, *Gracilaria* sp. cultured in brackishwater environment. The information regarding the seaweed's growth is essential before further study related to *Gracilaria* sp. is to be conducted in such environment which records the salinity down to 10ppt especially during rainy season. The seaweeds were obtained from nearby brackishwater canal. The culture were done in ten floating baskets, in which cuttings of *Gracilaria* sp. with an initial weight of 49.9±2.8g were placed in each basket. The samples were submerged 40cm from water surface. Sampling for wet weight was done weekly and the experiment was terminated on week sixteen. Throughout the experiment, the lowest growth rate of *Gracilaria* was observed at 4.2±1.2g/day (week 8) and the highest growth rate was observed at 5.9±1.3g/day (week 6). Meanwhile, the final weight recorded was 612.6±141.3g.

ILOILO FISH PORT COMPLEX: ANALYSIS OF CAPACITY AND UTILIZATION, THE FISH AUCTION SYSTEM AND FUTURE PROSPECTS

Jesper Marie Turao*, Nelcie Jean Esmero, Jan Vincent Ybut, Rodelio Subade Rachel Aguilo, Fatima Dimaala, and Camille Deloria

University of the Philippines- Visayas
General Luna cor. Infante St., Iloilo City
jespermarie.turao31@gmail.com

Across the years various fish post have been constructed in major fish landing sites which are located in major cities across the Philippines. The fishports have provided the needed infrastructure and support facilities like freezing plants necessary for sustained delivery of fish to the downstream market channels up to the consumer level.

This paper reviews the marketing channels for fish in the context of the second largest fishport in the country, the Iloilo Commercial Fishport. Subsequently, it describes and analyzes the fish auction in Iloilo Commercial fishport, where the Dutch auction model locally known as "bulungan system" is employed.

Key informant interviews, focus group discussions and review of previous studies provides insights on the present level of the fishport's utilization and prospects for future management towards sustained fish supply particularly for Iloilo City and province.

TOWARDS AN ORAL DELIVERY MITIGATION FOR BOTH WHITE SPOT SYNDROME VIRUS AND EARLY MORTALITY SYNDROME SIMULTANEOUSLY

Eng Huan Ung*, Hweh Fen Goh

Biovalence Sdn Bhd, Unit 12-03, Blok C, 3 Two Square, Jalan 19/1, Seksyen 19, 46300 Petaling Jaya, Malaysia
Email: huanung@yahoo.com

White spot syndrome virus (WSSV) and early mortality syndrome (EMS), also known as AHPND are possibly the two most serious shrimp diseases that each cause over USD1 billion worth of loss annually. The fact that one is a viral disease and the other a bacterial disease makes it highly unlikely for the development of a single oral delivery treatment. We have developed an orally delivered probiotic bacteria named B11 that has proven efficacious for treating EMS/AHPND via feed delivery under laboratory conditions. We have also developed a broad spectrum oral delivery protein RetroMAD1 that has shown effectiveness against 17 DNA and RNA viruses including human viruses such as Ebola, Zika and Dengue as well as shrimp viruses such as WSSV, HPV and MBV. The WSSV virus has invested a lot of DNA into having 5 anti-apoptotic genes which we believe is a response against defensive apoptosis as a mechanism for viral accommodation. Cleavage of proteins such as WSSV 449 by caspase 3 can therefore potentially allow for more defensive apoptosis while the antiviral RetroMAD1 does its job in parallel killing viruses. This study is a first preliminary investigation into combining three orally deliverable platforms together to combat both EMS/AHPND and WSSV simultaneously. Although this work is still at a relatively early stage, its development may eventually allow for feed that is able to greatly diminish the catastrophic effects of WSSV and EMS/AHPND. This will allow farmers to eventually have a greater confidence to farm shrimp in areas or during seasons which either EMS/AHPND and/or WSSV is prevalent.

THE ROLE OF *Chromolaena odorata* BIOACTIVE COMPOUND IN ARTIFICIAL FEED FOR INCREASING OF *Penaeus monodon* SURVIVAL RATE AND GROWTH

Harlina¹⁾, Rosmiati²⁾, Syahrul Jafar¹⁾, Sukmawati³⁾, Nurhidayah²⁾

¹⁾Aquaculture Department, Faculty of Marine and Fisheries Science, Universitas Muslim Indonesia Makassar South Sulawesi

²⁾Research Institute for Coastal aquaculture, Maros, South Sulawesi

³⁾Faculty of Economic, Universitas Muslim Indonesia, Makassar South Sulawesi
linausman1965@yahoo.com

The finding of *Chromolaena odorata* Leave bioactive compound as antibacterial has been one of alternatives in vibriosis controlling, the causative agent of tiger shrimp mass mortality from seeding in the hatchery until growth out in the pond. The study aims to determine survival rate and growth of tiger shrimp *Penaeus monodon* through tiger shrimp immune response increasing. Bioactive compound of *Chromolaena odorata* Leave was tested by mixture in artificial feed at the concentrations of 1250 mg/kg feed, 1500 mg/kg feed and 1750 mg/kg feed. Feed containing the bioactive compound at those concentrations were fed to the tiger shrimp infected by *Vibrio harveyi*. Parameters measured were total haemocyte count (THC), differential haemocyte count (DHC). The results showed that the application of *Chromolaena odorata* Leave bioactive compound was able to increase immune response of tiger shrimp *P. monodon* infected *Vibrio harveyi*. The increasing of immune response of tiger shrimp *P. monodon* caused resilience of shrimp against *Vibrio harveyi* increased. As the result, the survival rate of shrimp infected with *Vibrio harveyi* was higher than the control (without bioactive compound).

Table 1 showed that treatment A (0 ppm, without bioactive compound) was obtained to give the lowest survival rate (3.33 %). In turn, post larvae treated with *Chromolaena odorata* leaves bioactive compound at the concentrations of 1000, 1250, 1500, and 1750 ppm resulted the survival rate of > 50 %. Terlihat pula bahwa terjadi peningkatan persentase sintasan sejalan dengan peningkatan konsentrasi bahan alami daun kopasnda

Tabel 1. Survival rate of post larvae infected with *V. harveyi* and treated with *Chromolaena odorata* leaves bioactive compound

Treatment (ppm)	Concentration (ppm)	Survival rate (%)
A	0	3.33
B	1000	77.78
C	1250	78.89
D	1500	84.44
E	1750	85.56

THE EFFECT OF IRON NANOPARTICLES ON GROWTH AND SURVIVAL OF RAINBOW TROUT (*Oncorhynchus mykiss*)

Alireza Valipour¹, Hamideh Kordi²; Alireza Shenavar Masouleh³; Mahmoud Hafezieh⁴

Inland Waters Aquaculture Research Center, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Bandar Anzali, Iran

²Ph. D. Student, Inland Waters Aquaculture Research Center, Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Bandar Anzali, Iran,

³International Sturgeon Research Institute, Agricultural Research Education and Extension Organization (AREEO), Rasht, Iran

⁴Iranian Fisheries Science Research Institute, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

This study was aimed at determining the effect of iron nanoparticles on the growth and survival of rainbow trout (*Oncorhynchus mykiss*) compared with FeSO_4 as iron resource in the diet. For this propose, fish (weighing 12.92 ± 0.47 g) were fed with four different dietary iron treatments containing 30 (T1), 60 (T2), 90 (T3) mg kg^{-1} iron nanoparticles or 60 mg kg^{-1} $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (T_c) for 8 weeks. Each treatment was performed in triplicate (three tanks/ treatment, twelve tanks in all) with 30 fish/ tank (90 fish/ treatment). The fish were fed twice a day, using a measured food ration of 5% body mass per day for the first month and 3% for the second month. Fish biomass per tank was measured fortnightly. Results showed there were significant differences between treatments in growth performance. The best WG, PWG, FCR and SGR was observed in T_2 and the worst in T_1 ($p < 0.05$). There was no significant difference between T_c and T_3 . Also, survival rates were not affected by different amounts of dietary iron (nano Fe_3O_4 and FeSO_4) during the entire experimental period ($p > 0.05$).

SHRIMP (*L. vannamei*) CHALLENGED WITH EMS/AHPND CAUSING BACTERIA AND THE EFFICACY OF CLAY, ALGAE AND COPPER PREMIX ON THE HEALTH PERFORMANCE

Maarten Jay van Schoonhoven*, Assoc. Prof. Dr Dang Thi Hoang Oanh

Olmix, Arnhemsestraatweg 8, 6880 AG, Velp, The Netherlands
mjvanschoonhoven@olmix.com

Over the years, the shrimp industry has had to deal with its share of diseases, which is a natural development in the evolution of the production of live animals. One of the more recent disease outbreaks which has had a very strong impact in the shrimp industry is AHPND, also known as EMS. Its slow but steady progress started in Asia where it spread into various countries, such as Vietnam, Malaysia and Thailand. EMS eventually made its way into the Latin American markets where it has also had a strong impact in the industries there.

Diseases are a natural part of animal production and the industry normally finds a way to deal with them. Generally past experiences in dealing with disease outbreaks are the first approach. Often small adjustments are developed to manage the disease. These tend to lead to preventive measures to be taken such as biosecurity and adapted culture techniques.

Preventive measures still allow for exposure to microbial environments, but also reduce risks of disease outbreaks. Improving shrimp gut health is a very effective method of preventing pathogens affecting the digestive tract. Use of clays, algae and copper in feed can influence the intestinal mucosa integrity and the microflora balance in the gut.

The present study consisted in evaluating the effect of a commercial premix based on clay (Bentonite), algae (*Ulva* sp) and copper on the health performance of shrimp (*L. vannamei*) after a challenge test with EMS causing bacteria (*V. parahaemolyticus*). PL5 shrimp, testing negative to common shrimp diseases (including *V. parahaemolyticus*) by PCR method, were reared following 4 treatments. T1 was the negative control, T2 the positive control, T3 had a continuous inclusion of 0,2% MFeed premix in the diet and T4 had a continuous inclusion of 0,4% MFeed premix. At PL25 shrimp were challenged via an immersion bath with *V. parahaemolyticus* causing EMS. Survival rates were monitored daily over a 14 day period until conditions were stable. First mortalities were observed 9 hours post challenge in the positive control, compared to 24 and 36 hours in the T3 and T4 respectively. Also preventive use of MFeed premix showed a 55% and 73% increase in overall survival for T3 and T4 respectively, when compared to the positive control group T2.

In conclusion, feed supplemented with the clay, algae and copper premix used in this trial can significantly increase survival rates of shrimp challenged with EMS causing bacteria when applied in a preventive manner.

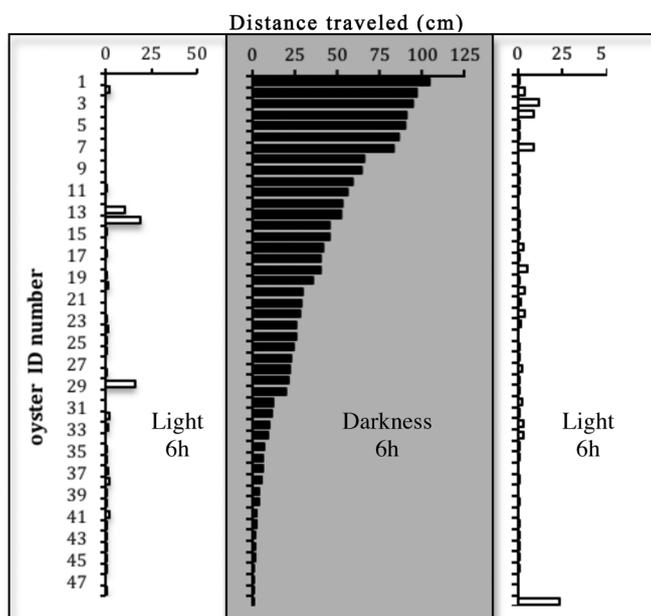


Fig. 1 Distances traveled by all oysters in all replicates under light and darkness throughout a 18 h experiment. Each bar represents one oyster. n=48

OVERCOMING THE ARTEMIA BOTTLENECK: CAN ARTEMIA NAUPLII BE REPLACED IN LARVAL SHRIMP DIETS?

Peter Van Wyk, Chris Stock, Ramir Lee, Satya Murthy, Diego Flores and Craig L. Browdy

Zeigler Bros., Inc.
400 Garners Station Rd.
Gardners, PA 17324

Hatchery production of both shrimp and marine finfish is highly dependent upon *Artemia* nauplii as a primary feed. Demand for *Artemia* cysts remains high in response to steady annual growth in the production of both shrimp and marine finfish. However, *Artemia* has several short-comings that can hinder industry advancement, including the fact that they are a limited resource. Annual production fluctuates with the weather, and annual harvests are approaching the maximum sustainable yield. The growing demand for a limited resource has resulted in escalating prices for artemia cysts, particularly when harvest from the Great Salt Lake are poor. The variable cost, supply and availability of *Artemia* cysts creates uncertainty and can be a potential bottleneck for the future growth of shrimp and marine finfish aquaculture. To continue to grow, these industries will need to find alternatives to *Artemia* in larval diets. Fortunately, shrimp larvae have evolved a requirement for the nutrients contained in *Artemia* nauplii, rather than a requirement for *Artemia* nauplii themselves. Data from commercial production trials show that an *Artemia* replacement diet that closely conforms to the nutritional composition of artemia nauplii can successfully replace 100% of the *Artemia* in larval shrimp diets with similar survival and PL quality. *Artemia* replacement diets have a number of advantages over artemia nauplii, including consistency in quality, and availability, lower product cost, lower labor cost, and reduced biosecurity risks.

LOCOMOTION IN JUVENILES OF THE WINGED PEARL OYSTER *Pteria penguin*: EFFECT OF LIGHT AND OYSTER SIZE

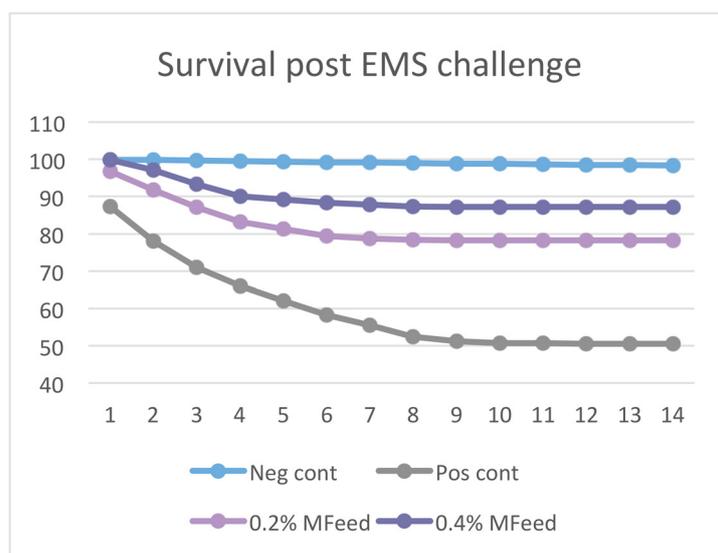
Hebert Ely Vasquez*, Xing Zheng, Aimin Wang

State Key Laboratory of Marine Resource Utilization in South China Sea, Marine Science College, Hainan University, 58 Renmin Ave., Haikou, Hainan 570228, China
hebertely@yahoo.com

The winged pearl oyster, *Pteria penguin*, is a tropical bivalve found from the Indian Ocean to Japan and throughout the Pacific islands. It inhabits a vast geographic range from Madagascar to Australia. It is also the most widespread cultured winged oyster for the production of large and valuable mabé pearls. Despite their economic and biological significance, surprisingly little is known about their behavior and ecology. The present study examines locomotion and attachment activity of *P. penguin* juveniles as a function of oyster size and illumination intensity.

In order to assess oyster movements, both shell height (SH) and hinge length (HL) of sixteen detached oysters (7.2~35.4 mm, SH) were measured. These oysters were then placed alternately large, then small, according to size, with 5cm spacing, in a 30L plastic tank containing 10L FSW at 25°C, 30 PSU. A lamp equipped with a timer was set approximately 50cm above the tank providing illumination measuring approximately 3500 lux. The experiment started with 6h of light, followed by 6h in complete darkness, repeated by another period of 6h of light to complete 18h of observation. The experiment was repeated three times under identical conditions. Juveniles were filmed using a surveillance camera equipped with an infrared illuminator. A total of 1080 still images were extracted from the raw video and merged using Adobe Premier CC 2014 software. Oyster movement was analyzed using the software application Tracker 4.95 (Open Source Physics Project, <http://www.opensourcephysics.org/>) to estimate the distance traveled by a moving object. Byssal attachment at the end of the experiment was confirmed by gently touching the juveniles with a pair of tweezers.

All oysters exhibited locomotion or rotation movements facilitated by foot. Locomotion was significantly higher in darkness (Tukey HSD test, $p < 0.01$) (Fig. 1). The average distance traveled was 3.3 ± 5.1 cm under lighted conditions, and 32.8 ± 31.2 cm in darkness respectively. The maximum distance traveled by a single individual was 104.6 cm in darkness. Moreover, movements were not observed to be significantly related to the oysters SH and HL ($P > 0.1$). Byssal attachment percentage was $62.5 \pm 6.5\%$ in all replicates. Results suggest that *P. penguin* juveniles undertake locomotion mostly in darkness, possibly an adaptation to reduce the risk of visual predation. Moreover, increased locomotive behavior in darkness may enable juveniles to search more successfully for environments that would augment survival rates, such as those providing more stable substrates for attachment.



FACTORS INFLUENCING THE GROWTH OF INLAND AQUACULTURE IN PAPUA NEW GUINEA

Havini Vira* and Jesmond Sammut

The National Fisheries Authority, Deloitte Tower, 11th Floor, Douglas Street
Port Moresby, NCD, Papua New Guinea
havini.vira@gmail.com

Freshwater fish farming was introduced to Papua New Guinea (PNG) in the 1950s to increase access to animal-based protein. Many people in PNG live on less than USD 1.50 and protein deficiency is a significant health issue. Rugged landscapes are not suitable for grazing large herds of animals. Although pigs are common in PNG, they are usually expensive and eaten at customary ceremonies and feasts. Hunting is not always a viable option for many PNG communities, and many people cannot afford to purchase fresh, frozen or canned animal protein. Freshwater fish farming is part of the Government of PNG's strategic development plans and now widely recognised as an important contributor to food security. Fish can be farmed alongside vegetable gardens and provide access to a source of protein without the need for refrigeration; fish can be harvested as needed.

By 2007 there were 10,000 farms in PNG and in under 10 years the number has grown to 60,000. We undertook a survey of the aquaculture sector in PNG and identified factors that have supported this growth, as well as bottlenecks to production.

Growth is attributed to improved farming practices and fingerling production, fish farming training programs, government funding of fish farming 'start up' projects, and increased collaborative research between the National Fisheries Authority (NFA), the Japan International Cooperation Agency (JICA) and the Australian Centre for International Agricultural Research (ACIAR). International collaborative research projects have partnered with non-government agencies to promulgate research findings and provide training to farmers. Lead farmers have also played a major role in disseminating findings. The industry is still constrained by poor access to affordable fish feed and feed ingredients. Husbandry practices are also poorly developed when compared to those used by fish farmers in nearby South East Asian countries.

The study also showed further potential for industry growth due to increasing knowledge transfer and growing realisation of the positive social and economic benefits of fish farming. NFA, in partnership with ACIAR, has developed a draft 10-year strategy to develop the industry sustainably. Research into reducing the cost of fish feed is the highest priority, followed by locally-practical fish husbandry practices.

PRELIMINARY STUDY OF POPULATION GENETIC STRUCTURE AND DEMOGRAPHIC ANALYSIS OF *Eleutheronema tetradactylum* (SHAW 1804) ALONG STRAIT OF MALACCA BASED ON MITOCHONDRIAL CO1 GENE SEQUENCES

Nurul Atikah Wahid*, Yuzine Esa, Rozihan Mohamed, and Mohammad Fadhil Syukri Ismail

Department of Aquaculture
University Putra Malaysia
43400 Serdang
Selangor Darul Ehsan
nurulatikahbtwahid@gmail.com

The four finger threadfin, *E. tetradactylum* (Shaw, 1804) one of the species of the Polynemidae family that can be found in Malaysian waters and widely distributed along Malaysia coastal water. *E. tetradactylum* is one of very high commercial and important fisheries species in Malaysia. This species is subject to intense exploitation in Malaysia. Knowledge on the population structure and demographic of *E. tetradactylum* in Malaysia coastal waters, which is important for proper management and to maintain the sustainability of the fishery resources, is lacking. In the present study, mitochondrial cytochrome c oxidase CO1 gene sequences was used to further investigate the population genetic structure and demographic analysis of *E. tetradactylum* along Strait of Malacca. Totally 58 sequences of CO1 gene were collected from 4 fish landing areas along Strait of Malacca. Among 58 individuals, 8 distinct haplotypes were defined. High level of haplotype diversity ($h=0.6$) in Selangor and low haplotype diversity in Perak. Nucleotide diversity (π) in Selangor higher than other locations and the lowest nucleotide diversity was in Perak ($\pi=0.03778$). Significant genealogical branches were recognized among the haplotype, this also proven in pairwise distance among the haplotypes. Haplotypes were divided into two groups (HET3, HET4) and (HET7, HET8, HET6, HET1, HET2, HET5). These results suggest that these two groups were from different species with same genus.

OPTIMUM SUPPLEMENTATION OF ASTAXANTHIN FOR KURUMA SHRIMP *Marsupenaeus japonicus*

Weilong Wang*, Manabu Ishikawa, Shunsuke Koshio, Saichiro Yokoyama

The United Graduate School of Agricultural Sciences, Kagoshima University
Faculty of Fisheries, Kagoshima University 762847770@qq.com

Astaxanthin (Ax) as an important pigment source and antioxidant is considered that it is necessary to be provided in the diet for cultured shrimp. But the optimum supplemental amount to diets for larval, post-larval and juvenile kuruma shrimp are still unrevealed.

Three individual trials were conducted on to evaluate the effect of chemically synthesized Ax (Carophyll Pink[□]) on growth performance, survival, stress resistance, colorimetric reading, Ax contents determination and immune response, respectively.

Six dietary levels of Ax (0, 50, 100, 200, 400 and 800ppm per kg diet) were added to the micro-bound diet (MBD) and fed to triplicate groups of kuruma shrimp. Firstly, 8-day feeding trial was conducted to investigate the effectiveness of dietary supplementation of Ax on survival (SR), growth, developmental stage (DS), metamorphosis to post-larval (PL) and formalin stress resistance (LT_{50}) on larval kuruma shrimp. The obtained results showed that shrimps fed diets supplemented with Ax exhibited higher SR, DS PL, TL and LT_{50} values significantly in dose dependent manner with the highest being in case of 200 ppm supplemented group ($P<0.05$).

Then, the second study was conducted to evaluate the effect of dietary Ax on the performances of post-larval kuruma shrimp for 30 days. Shrimps fed diets containing Ax showed higher final body weight (FBW), weight gain (WG) and specific growth rate (SGR) compared to the control group with the highest being in case of 100 and 200 ppm supplemented groups ($P<0.05$), however, no significant differences were detected in the other groups. After formalin stress test (LT_{50}), the data showed that LT_{50} was significantly higher in case of 100 and 200 ppm supplemented groups than the control group ($P<0.05$). Furthermore, the data of cumulative mortality index (CMI) for osmotic stress showed significantly lower values than the control group ($P<0.05$) with the lowest value in case of 200 ppm group.

Thirdly, another six dietary levels of Ax (0, 200, 400, 800, 1200 and 1600 ppm per kg diet) were added to the pellet diet and fed to triplicate groups of juvenile kuruma shrimp for 56 days. Shrimp fed diets containing Ax showed higher final body weight (FBW), weight gain (WG) and specific growth rate (SGR) compared to the control group with the highest being in case of 400 and 800ppm supplemented groups ($P<0.05$). After formalin stress test (LT_{50}), the 200, 400 and 800ppm supplemented groups showed higher data than other groups. After cooking, shrimp fed the diets containing the Ax exhibited a strong red color compared to the light pink color of control group. Colorimetric reading in cooked shrimp and Ax content for whole shrimp body demonstrated that pigmentation was increased in kuruma shrimp fed diets with increasing Ax levels in current study.

We concluded from the current studies that Ax was a necessary ingredient for kuruma shrimp. Considering the effect of Ax on comprehensive performance, the level of Ax supplemented in the diet for larval and post-larval kuruma shrimp should be between 100 and 200 ppm, and for juvenile kuruma shrimp should range from 400-800 ppm.

ESTABLISHING THE BIOSECURITY SYSTEM FOR SHRIMP AQUACULTURE INDUSTRY

Han-Ching Wang*, Chu-Fang Lo

Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Taiwan

(*) Corresponding Author: wanghc@mail.ncku.edu.tw

Although global shrimp aquaculture has considerable economic potential, several infectious diseases threaten of the viability of this industry, including WSD (white spot disease) and AHPND (acute hepatopancreatic necrosis disease). The latter disease, previously termed EMS (early mortality syndrome), has caused huge mortality and losses. Consequently, the use of genetic trait selection (for example, to promote disease and stress resistance and rapid growth) in breeding selection is critically important to ensure a sustainable aquaculture industry. In addition, effective bio-security, disease prevention and monitoring can reduce the probability of losses due to pathogens. In this talk, I will introduce several strategies/platforms developed by our team for implementing disease prevention and bio-security management systems, according to three major codes of practice in aquaculture bio-security management: 1) reduce the risk of introducing pathogens into facility; 2) reduce the risk of spread, in the case of pathogen introduction; and 3) reduce shrimp susceptibility to infection and disease. We believe it is to mitigate threats of infectious disease and to develop sustainable and profitable shrimp aquaculture.

MACROGARD: A POTENT IMMUNOMODULATOR IN MODERN SHRIMP PRODUCTION

Zurong Wang PhD

Marketing and Technical Director (Asia-Pacific Region)
 Biorigin, Singapore
 zurong.wang@biorigin.net
 www.biorigin.net

Modern shrimp production is facing a lot of challenges, such as pathogenic bacteria, virus, external parasite, ammonia, and other stressors. As shrimp are not able to respond to vaccination, enhancement of disease resistance has been attempted through stimulation of the immunological system.

Started in 1980s, MacroGard was a pioneer in researches of β -glucan in aquaculture. There are more than 100 published SCI articles on MacroGard. MacroGard, a purified branched β -1,3-1,6-glucans, has been shown a remarkable ability to enhance the non-specific disease in Salmon and Shrimp. Raa (1990) showed much more significant reduction of mortality to vibrio salmonicida compared to other β -glucan. Huang and Song (1999) showed that improved immunity to white spot syndrome associated virus (WSSV) due to MacroGard utilization could be maternal transmitted. Neo et al. (2015) reported that MacroGard improved survival rate from 25% to 45% of Litopenaeus vannamei challenging with the infectious myonecrosis virus (IMNV). Four commercial aquafarm trials (Basavaraja, 2013) showed that 1kg/ton of MacroGard in Shrimp feed significantly improved shrimp yield.

Overall, MacroGard is a very potent immunomodulatory to shrimp production with more than 40 years of experiments and field application.

Biography

Zurong Wang, has been marketing and technical director for Biorigin in Asia-Pacific region for years. Biorigin is a company focusing on yeast production and its further processed product. The super star product, MacroGard, is widely used in Salmon, shrimp, and other fish production, to fight against diseases from pathogenic bacteria, virus, parasites, and other stressors.

In 2005, Zurong Wang finished his bachelor and master in China Agricultural University. There his interest was astaxanthin production and processing technology. In 2008, he received his PhD in University of Arkansas, USA.

After that, Zurong Wang has worked for CP group for 4 years as different technical positions such as technical director in several countries --- China, Indonesia, and Singapore. Followed by that, he had joined LinkAsia Partners for 2 years, working as technical director in Asia-Pacific region for several European and American companies at the same time.

APPLICATION OF ICE SLURRY IN THE AQUACULTURE INDUSTRY

Ming-Jian Wang

Sunwell Technologies Inc.
180 Caster Avenue, Woodbridge, Canada L4L 5Y7
Tel: +1-905-856-0400
E-Mail: wang@sunwell.com

The rapid increase of world population in last decades puts added pressure on the aquaculture industry to improve fish production, quality and shelf life. One of the focuses is to implement advanced fish preservation means for all after harvest operations. Aquaculture species such as shrimp, yellowtail, cobia and salmon are high value and perishable products. They spoil fast at higher surrounding temperatures. Once quality is lost, no amount of manipulation can restore it. Proper cooling and preservation of these aquaculture products is therefore of great importance in maintaining its premium quality, especially during the harvesting and transportation process.

This presentation examines the physical characteristics, meltage rates, and cooling performance of various types of ice being used for the cold chain management. Special attention has been given to an advanced cooling and preservation technology – ice slurry, which has gained popularity in recent years in the aquaculture industry. Key features and benefits of ice slurry in both harvesting and processing operations are discussed in details. Installation examples given in the shrimp industry and in the salmon industry demonstrate that ice slurry is a rewarding technology. It provides three times faster cooling rate as compared to other ice technologies and maintains aqua products in low temperature levels, therefore improving quality and food safety. It improves yield and extends shelf life. It also saves operating costs in labor, energy and ice.

OPTIMUM SUPPLEMENTATION OF ASTAXANTHIN FOR LARVAL AND POSTLARVAL KURUMA SHRIMP *Marsupenaeus japonicus*

Weilong Wang*, Manabu Ishikawa, Shunsuke Koshio, Saichiro Yokoyama

The United Graduate School of Agricultural Sciences, Kagoshima University
Faculty of Fisheries, Kagoshima University
762847770@qq.com

Astaxanthin (Ax) as an important pigment source and antioxidant is considered that it is necessary to be provided in the diet for cultured shrimp. But the optimum supplemental amount to diets for larval, post-larval and juvenile kuruma shrimp are still unrevealed.

Three individual trials were conducted on to evaluate the effect of chemically synthesized Ax (Carophyll Pink[□]) on growth performance, survival, stress resistance, colorimetric reading, Ax contents determination and immune response, respectively.

Six dietary levels of Ax (0, 50, 100, 200, 400 and 800ppm per kg diet) were added to the micro-bound diet (MBD) and fed to triplicate groups of kuruma shrimp. Firstly, 8-day feeding trial was conducted to investigate the effectiveness of dietary supplementation of Ax on survival (SR), growth, developmental stage (DS), metamorphosis to post-larval (PL) and formalin stress resistance (LT₅₀) on larval kuruma shrimp. The obtained results showed that shrimps fed diets supplemented with Ax exhibited higher SR, DS PL, TL and LT₅₀ values significantly in dose dependent manner with the highest being in case of 200 ppm supplemented group ($P<0.05$).

Then, the second study was conducted to evaluate the effect of dietary Ax on the performances of post-larval kuruma shrimp for 30 days. Shrimps fed diets containing Ax showed higher final body weight (FBW), weight gain (WG) and specific growth rate (SGR) compared to the control group with the highest being in case of 100 and 200 ppm supplemented groups ($P<0.05$), however, no significant differences were detected in the other groups. After formalin stress test (LT₅₀), the data showed that LT₅₀ was significantly higher in case of 100 and 200 ppm supplemented groups than the control group ($P<0.05$). Furthermore, the data of cumulative mortality index (CMI) for osmotic stress showed significantly lower values than the control group ($P<0.05$) with the lowest value in case of 200 ppm group.

Thirdly, another six dietary levels of Ax (0, 200, 400, 800, 1200 and 1600 ppm per kg diet) were added to the pellet diet and fed to triplicate groups of juvenile kuruma shrimp for 56 days. Shrimp fed diets containing Ax showed higher final body weight (FBW), weight gain (WG) and specific growth rate (SGR) compared to the control group with the highest being in case of 400 and 800ppm supplemented groups ($P<0.05$). After formalin stress test (LT₅₀), the 200, 400 and 800ppm supplemented groups showed higher data than other groups. After cooking, shrimp fed the diets containing the Ax exhibited a strong red color compared to the light pink color of control group. Colorimetric reading in cooked shrimp and Ax content for whole shrimp body demonstrated that pigmentation was increased in kuruma shrimp fed diets with increasing Ax levels up to 400 ppm in current trial.

We concluded from the current studies that Ax was a necessary ingredient for kuruma shrimp. Considering the effect of Ax on comprehensive performance, the level of Ax supplemented in the diet for larval and post-larval kuruma shrimp should be between 100 and 200 ppm, and for juvenile kuruma shrimp should be around 400 ppm.

GENETIC DIVERSITY ANALYSIS OF FARMING GROUPS OF *Scylla paramamosain* IN CHINA ESTIMATED BY MICROSATELLITE MARKERS

Guizhong Wang*, Xiaojun Xu, Chaoshu Zeng and Haihui Ye

College of Ocean & Earth Sciences
Xiamen University, Xiang An, Xiamen, Fujian, China, 361102
gzwang@xmu.edu.cn

Our recent research has identified that the mud crab, *Scylla paramamosain*, in China may be sub-divided into northern and southern population. There were some biological differences among different populations. The seedlings of mud crab farming in China mainly came from natural sea. Whether the farming location of different population seedling is in line with its natural distribution will have a significant impact on the farming effect. To this end, the present study analyzed the genetic diversity of different farming groups of *S. paramamosain* in China. *S. paramamosain* is distributed only along the coastal areas of southeast China in the south of the estuary of Yangtze River. In the present study, six wild groups were collected. The collect locations from north to south were as follow: Ninghai (NH) of Zhejiang province, Langqi (LQ) near Fuzhou of Fujian province, Longhai (LH) near Xiamen of Fujian province, Zhaoan (ZA) in the south of Fujian province, the estuary of Zhujiang River (ZJ) of Guangdong province, and Dongzhai (DZ) of Hainan province. At the same time, two farming groups of the northerly location were also collected from Wenzhou (WZ) of Zhejiang province and Ningde (ND) of Fujian province. Six polymorphic microsatellite loci were used to estimate the genetic diversity of the farming groups and construct the UPGMA tree with the wild groups. A total of 129 alleles were detected in 62 individuals of these two farming groups. Both two farming groups showed high level of genetic diversity. The mean allele number for WZ and ND group were 16.333 and 17.667, the mean *He* and *Ho* for WZ and ND group were 0.8672, 0.8900 and 0.8490, 0.8778, respectively. Among 12 population loci, 1 showed significant deviation from the Hardy-Weinberg equilibrium. The UPGMA results showed that WZ farming group was closely clustered with ZA wild group, and ND farming group was closely clustered with DZ wild group. This means that the seedlings of two farming groups of the northerly location were probably captured from the wild coastal sea of ZA and DZ of the southerly location. The result suggested that the seedlings of north and south population were mixed in the farming of *S. paramamosain* in China. It's worthy to pay attention to this situation since the mix of the seedlings of north and south population may have certain impact on the effect of farming of *S. paramamosain*.

Acknowledgements

This work was supported by a grant (31472294) from National Natural Science Foundation of China (NSFC).

EFFECTS OF DIETARY PREBIOTIC FRUCTOOLIGOSACCHARIDE SUPPLEMENTATION ON GROWTH PERFORMANCE, HEPATOPANCREAS HISTOLOGY AND INTESTINAL SHORT-CHAIN FATTY ACIDS IN GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*) POST-LARVAE

Wee Wen Chen*, Fatin M. I. Natrah, Nicholas Romano, and Mahdi Ebrahimi

Department of Aquaculture
Faculty of Agriculture
Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia
weewenchen@gmail.com

The giant freshwater prawn, *Macrobrachium rosenbergii* is a valuable aquaculture species in many countries due to its commercial value. Some of the constraints in the expansion of this cultured organism include poor growth rates and diseases. Rising feed ingredient prices also have an impact on the aquaculture production cost. It is possible to reduce the cost of production if the prepared diets not only provide essential nutrients but also increase growth and development of the aquatic animals in commercial aquaculture. These may potentially be mitigated by dietary prebiotics but this research area remains scarce in *M. rosenbergii*. Prebiotics are indigestible carbohydrate that are capable to stimulate the symbiosis between host and microbiota particularly in the growth of beneficial bacteria which indirectly improves the growth and health condition of the host. Good formulated feeds supplementation could yield healthy and high quality seedling, resulting in better quality prawn. The goal of this study was to study the optimum inclusion level of fructooligosaccharide (FOS) required by *M. rosenbergii* post-larvae (PL) and the effects of prebiotic fructooligosaccharide supplementation towards the growth and health condition of *M. rosenbergii*.

The effects of dietary prebiotic FOS additives at 0.1%, 0.4%, 1% and 2% on the growth performance, antioxidant status, intestinal short chain fatty acids (SCFAs) and hepatopancreas histology of the freshwater prawn *Macrobrachium rosenbergii* PL (initial weight \pm SE of 22.8 ± 0.2 mg) were evaluated after 56 days of feeding.

The results showed that the specific growth rate for weight was highest in the 0.4% dietary FOS treatment compared to all others (Table 1). Furthermore, 0.4% FOS also significantly ($P < 0.05$) stimulated the highest SCFAs production compared to the control treatment. Increasing dietary FOS significantly enhanced the lipid peroxidation and suppressed superoxide dismutase activity (%), indicating oxidative stress to the prawns. Meanwhile, the hepatopancreatic tubules of prawns fed with 0.4% FOS were more closely packed with significantly more R- and E-cells. Based on the FOS inclusion level, 0.4% FOS significantly improved the growth performance and SCFAs production in prawns although FOS trigger oxidative stress in the prawns.

TABLE 1. Final weight (g), weight gain (g) and specific growth rate (SGR) of freshwater prawn (*M. rosenbergii*) after 56 days of being fed diets with increasing levels of FOS supplementations. ^{abcd} Mean value with different superscript letters are significantly different ($p < 0.05$).

Parameters	Experimental diets				
	Contro l	0.1% FOS	0.4% FOS	1% FOS	2% FOS
Initial weight (g)	0.02	0.02	0.02	0.02	0.02
Final weight (g)	0.23	0.26	0.34	0.30	0.27
Weight gain (%)	943.8 ^{9b}	1038 ^{.22b}	1376 ^{48a}	1165 ^{49ab}	106 ^{5.61_b}
SGR weight (% day ⁻¹)	4.18 ^b	4.34 ^b	4.79 ^a	4.53 ^{ab}	4.38 ^b

COMPARATIVE EFFICACY OF MONOCALCIUM PHOSPHATE AND WINDMILL® AQUAPHOS ON THE GROWTH PERFORMANCE, PHOSPHORUS RETENTION AND DIGESTIBILITY IN NILE TILAPIA *Oreochromis niloticus*

Zwart Sjo, Aliphos Rotterdam BV
Dias Jorge, Sparos

Aliphos Rotterdam BV
Zevenmanshaven Oost 139 3133CA Vlaardingen, The Netherlands
sjo.zwart@aliphos.com

A study was undertaken to evaluate the effect of two supplemental phosphate sources, Windmill® Aquaphos (MAP) and monocalcium phosphate (MCP, Aliphos® Monocal) on the growth performance, whole-body phosphorus (P) and calcium (Ca) retention, P and Ca digestibility and bone mineral composition of Nile tilapia.

The trial comprised three dietary treatments: a negative control diet (NC) without inorganic P supplementation; two other diets based on the NC formulation were supplemented with either MCP at 1.22% or Windmill® Aquaphos at 1.07%. Dietary total P levels were 0.60, 0.86 and 0.88 %DM for the NC, MCP and MAP diets, respectively. Diets were isonitrogenous (crude protein, 35% DM), isolipidic (9% DM) and isoenergetic (gross energy, 19.5 MJ/kg DM). Quadruplicate groups of 40 tilapias, with a mean initial body weight of 19.0 ± 1.2 g were fed one of the three experimental diets during 61 days.

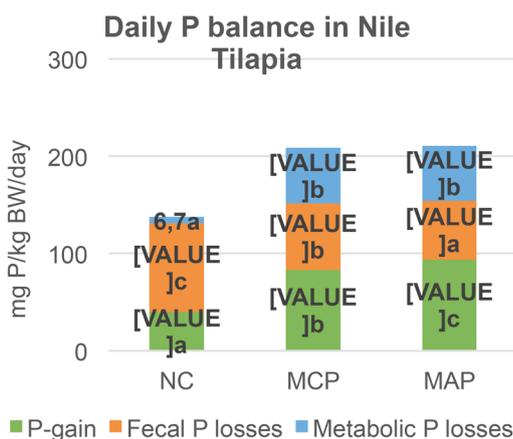
In this trial, fish fed the MAP diet showed a significantly higher FBW than those fed the MCP and control diet ($P < 0.05$). Fish fed the MCP and MAP diets showed a significantly lower FCR than those fed the NC ($P < 0.05$).

In comparison to the NC, both MCP and MAP diets resulted in significantly higher ash, phosphorus and calcium content in vertebral bone ($P < 0.05$). In comparison to the NC, both MCP and MAP diets resulted in a significantly higher retention of protein, phosphorus and calcium at the whole-body level ($P < 0.05$). Phosphorus digestibility of the MAP diet was significantly higher than that of the MCP and NC diet ($P < 0.05$). Daily P gain with the MAP diet was significantly higher than that achieved with the MCP and NC ($P < 0.05$) diets. Fish fed the MAP diet showed also significantly lower daily fecal P losses than fish fed the MCP and NC diets ($P < 0.05$).

Overall data confirms that both supplemental phosphate sources (MCP and MAP) are an effective strategy to enhance growth performance and P utilization in Nile tilapia. On a comparative basis against MCP, the MAP product (Windmill® Aquaphos) resulted on further gains in terms of FBW, P digestibility, body P gain and lower fecal P losses, making it a valuable nutritional tool to minimize the environmental impact of tilapia farming.

	Day 30			Day 61		
	NC	MCP	MAP	NC	MCP	MAP
FBW, g	55,01a	76,98b	80,99c	93,73a	140,73b	146,14c
FCR	1,10b	1,05a	1,02a	1,13b	1,04a	1,02a

Figure 1: Growth performances of Nile Tilapia fed different diets (statistical difference $P < 0,05$)



INORGANIC FEED PHOSPHATE IN AQUACULTURE: WINDMILL® AQUAPHOS, THE PHOSPHORUS SOURCE DESIGNED FOR FISHES

Zwart Sjo, Aliphos Rotterdam BV sjo.zwart@aliphos.com
Willems Emilie, Aliphos Rotterdam BV emilie.willems@aliphos.com

Aliphos Rotterdam BV
Zevenmanshaven Oost 139 3133CA Vlaardingen, The Netherlands

Phosphorus in aquaculture?

P is required for optimum growth, feed efficiency and bone development. Unlike other minerals, fish can't meet P requirements via uptake from the water. Thus a supplementation via the feed is essential. However diets for fish are changing. Less fish meal is used being replaced by vegetal protein sources. Vegetal protein sources have a low level of available P. Therefore inorganic feed phosphates must be added in the diets. They contain a high level of total and digestible P. Nevertheless wide differences remain among inorganic feed phosphates.

What is Windmill® Aquaphos?

Windmill® Aquaphos is a feed phosphate especially designed and produced by Aliphos for use in aquaculture nutrition. Windmill® Aquaphos is a highly soluble feed grade monoammonium phosphate. This high P solubility explains why Windmill® Aquaphos has the highest P-digestibility and P-retention amongst all feed phosphates used in aquafeeds, as proven in several trials with different aquaculture species.

Value of Windmill® Aquaphos in aquaculture

Over the past 13 years, Aliphos has carried out several trials to show the effectiveness of Windmill® Aquaphos on P-digestibility (dig.) and P-retention (ret.). Trials involved salmonids, sea bream and tilapia. The outcome of all these trials showed the same trend: Windmill® Aquaphos being a higher digestible or retainable phosphorus source than the normally used monocalcium and dicalcium phosphate.

On average Windmill® Aquaphos scores a P-bioavailability (based on P-digestibility and P-retention) of 90%. Contrary to MCP which reaches a value of only 67%. If used correctly, 6.4kg of Windmill® Aquaphos can replace 10kg of MCP. Creating space in formulations and saving but also lowering P-excretion in to the environment.

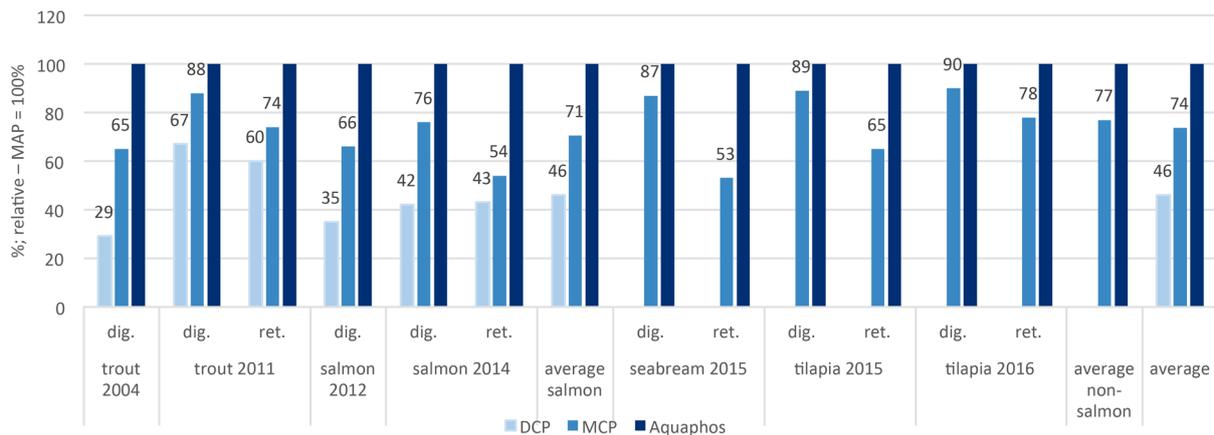


Figure: Values are expressed relative to Windmill® Aquaphos set on 100%

INHIBITION OF QUORUM SENSING BY BACTERIA FROM MARINE MICROALGAE

Yahya N. Ain*, Fatin M. I. Natrah

Laboratory of Marine Biotechnology
Institute of Bioscience
Universiti Putra Malaysia
43400 Serdang, Selangor
nurainyahya@gmail.com

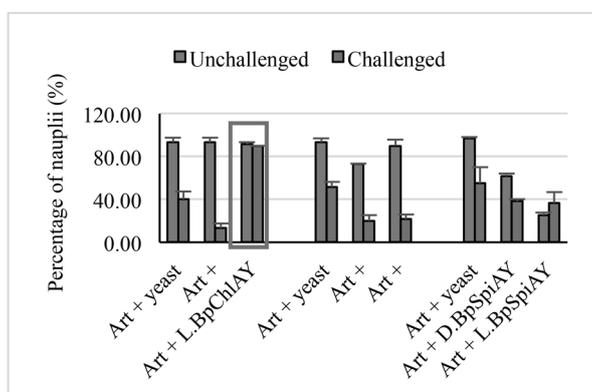
Quorum sensing (QS) is a bacterial cell-to-cell communication, resulted in behavioural regulations such as luminescence, biofilm, motility etc. Virulence factors productions by pathogens in aquaculture have also been to be regulated by QS. Thus, QS inhibition (QSI) is proposed as an alternative way to control diseases among aquatic organisms. We focus to evaluate the effects of live and dead (autoclaved cells) bacterial QS inhibitors to interfere QS system in pathogenic bacteria *Vibrio campbellii* BB120 using gnotobiotic *Artemia* as a model organism. Bacteria were isolated from microalgae and were screened for their anti-QS activity using *Chromobacterium violaceum* CV026. The selected QSIs were characterized and identified using 16S rRNA analysis. Their anti-QS activity were then quantified through acylhomoserine lactones (AHL) degradation assay, in comparison with positive and negative control strains, *Pseudomonas* sp. P3/pME6863 and *Pseudomonas* sp. P3/pME6000, respectively. The effects of QS inhibitors toward *Artemia* survival in challenge test were observed. Data were statistically analysed using students *t*-test ($p=0.05$), through SAS software version 9.4.

Three bacteria isolated from microalgae inhibited purple production of *C. violaceum* CV026. Analysis of 16S rRNA sequence revealed that those species belong to the genus *Bacillus*, and were deposited into GenBank database respectively under accession number KX356688 for BpChlAY, KX644098 (BpNofAY) and KX356689 (BpSpiAY). Degradation of AHL showed that BpNofAY was able to degrade the QS signal molecules within 6h, while BpChlAY and BpSpiAY within 9h. In *Artemia* challenged test, the highest *Artemia* survival was observed with live BpChlAY ($P<0.05$) (Figure 1). In contrast, dead BpChlAY, live and dead BpNofAY resulted to low *Artemia* survival ($P<0.05$) while there were no significant effect when fed with both live and dead BpSpiAY. In unchallenged group, dead BpNofAY, live and dead BpSpiAY showed low *Artemia* survival ($P<0.05$).

There were no significant effect when fed with both live and dead BpChlAY and live BpNofAY.

Live and dead bacterial QSIs showed different effects on *Artemia* survival to BB120. The probiotic effect of BpChlAY could serve as a biocontrol agent and has beneficial effect towards *Artemia* sp.

FIGURE 1 Effects of BpChlAY, BpNofAY and



BpSpiAY on the survival of *Artemia* upon challenged with *Vibrio campbellii* BB120

PARTIAL REPLACEMENT OF FISH MEAL BY A COMBINATION OF VARIOUS PROTEIN SOURCES IN PRACTICAL EXTRUDED PELLETS FOR STARRY FLOUNDER *Platichthys stellatus*

Il-Chang Yang^{1*}, Tae-Kyu Lee², Jung-Woo Choi¹, Tae-Hyun Yoon¹ and Jeong-Dae Kim¹

¹College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

²Cargill Agri Purina Inc., Seongnam 13630, Korea

mailto:yangsam14@gmail.com

Dietary fish meal is recognized to be the best ingredient with the high protein and good balanced amino acid profile for fish. Because the supply of fish meal is however static or even decreasing, a number of studies have been conducted to reduce its dietary incorporation level for farmed fish. Even though many ingredients have been tested as fish meal replacer for various species of fish, the research to find out the total substitute seems to be still on a long way to go. Differently from the other flatfish, starry flounder *Platichthys stellatus* grows well on diet composed of plant protein sources with low fish meal. Fish meal-based diet is, however, being commercially produced for the fish in Korea. Therefore, an attempt to investigate growth, hematological parameters and muscle fatty acid composition of the fish fed diets with different levels of fish meal was made under the field condition. A blend composed of lysine cell mass, corn protein concentrate and poultry by-product meal was employed to replace fish meal.

Dietary incorporation level of the alternative increased from 10 to 20, 30, 40 and 50%, while that of fish meal decreased correspondingly from 52 to 42, 32, 22 and 12%. The experimental diets were formulated to contain 52% protein and 10% lipid. A commercial diet containing the same levels of protein and lipid was used performed as positive control. The 5 experimental diets were extruded using a conventional twin extruder at the size of 3.5 mm.

Juvenile starry flounder with around 80~120 g of average body weight were randomly distributed in each (600~620 kg/tank) of 6 concrete tanks (8x8x0.5 m each) under a flow-through culture system. Water exchange rate was kept at 25 times a day and water temperature at 11.2 to 13.8°C during 6 week feeding trial. Fish were fed by hand twice a day. Weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and survival rate (SR) of fish fed the experimental diets were investigated. Hematological parameters and muscle fatty acid composition will be discussed.

COMPREHENSIVE EXPLOITATION AND UTILIZATION OF DEFATTED RICE BRAN IN AQUATIC FEED

Yongqing Ye, Xiaoxi Luo
Overseas Department General Manager, R&D Manager

Add: 6th, NO.8, Chengfeng Road, Gujian Village Committee, Daliang Street Office, Shunde District, Foshan City, Guangdong Province, China
Company: Guangdong Daynew Aquatic Sci-Tech Co., Ltd
E-mail: yyongqing@126.com

Rice is the staple food of almost all Asian countries and rice bran is the agricultural by-product of rice milling industry. Its by-product, defatted rice bran (DRB), which contains a number of valuable components – for example, proteins, carbohydrates and other phytochemicals – that exhibit health benefits. Direct use of the rice bran is problematic, because it rapidly becomes rancid due to the hydrolysis of neutral fat by highly active lipase enzymes right after the milling. Therefore DRB is considered a good source of aquatic feed. The aim of this study was to compare of rice bran and defatted rice bran. Subsequently, the contrast test results between DRB and wheat were described. A novel strategy of the replacement rice bran with DRB followed by experiment was proposed. Based on the results of this study, DRB can be a good source of aquatic feed that is suitable for applications in aquaculture industry and other new applications such as a potential material in the biomedical field.

MAGNESIUM HYDROGEN PHOSPHATE (MHP, MgHPO_4) RECOVERED FROM SWINE MANURE AS A NEW PHOSPHORUS ADDITIVE FOR FRESHWATER FISH

Tae-Hyun Yoon^{1*}, Dong-Hoon Lee², Seunggun Won³, Changsix Ra¹ and Jeong-Dae Kim¹

¹College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

²Department of Animal Resources, Daegu University, ³Gyeonggi Province Maritime and Fisheries Research Institute

taeplus@naver.com

Phosphorus (P) sources like monocalcium phosphate (MCP) and dicalcium phosphate (DCP) are being supplemented to the diet to meet the requirement of P for maximum growth. The present study summarizes the supplemental effect of various P sources and magnesium hydrogen phosphate (MgHPO_4 , MHP) as a new P source on growth, utilization of P and physiological responses in carp (*Cyprinus carpio*) and far eastern catfish (*Silurus asotus*). Either MCP, DCP, TCP (tricalcium phosphate) or MHP was added to the diets at the level of 2%, while control diet did not contain P source, which was replaced by cellulose. The major ingredients were constituted with fish meal (25%), soybean meal (40%), wheat flour (27%), fish oil (2%), and soy oil (2%) as a basal diet containing 42.5% protein and 6.5% lipid. The MHP was recovered from swine manure using a pilot scale reactor with the effective volume of 0.4 m³ and manufactured in Kangwon National University.

All experimental diets were manufactured to sinking pellets using a twin-extruder. Available P in diets was determined through digestibility trial. Following a 24 h fasting, respective 5 groups (three replicates/group) of 450 fish of a mean body weight of 6.5 g (carp) and 11.3 g (catfish) were randomly allotted to each of 15 tanks (0.4 × 0.6 × 0.36 cm, effective volume of 66 L). The feeding experiment lasted for 9 (carp) and 8 (catfish) weeks during which each diet was hand-fed to apparent satiety twice a day (08:30 and 16:30) during 6 days per week. A recirculation freshwater system where dissolved oxygen was maintained at 5.5~6.4 mg O₂ L⁻¹ was employed. Water temperature maintained under natural condition.

Carp fed the MHP showed the highest weight gain (WG) of 278%, which was not significantly different ($p > 0.05$) from that (270%) of fish fed the MCP, while fish fed the control and TCP showed lowest WG among the treatment groups ($p < 0.05$). Feed conversion ratio (FCR) ranged from 0.99 (MHP) to 1.29 (control). The highest protein efficiency ratio (PER) and specific growth rate (SGR) were found in fish fed the MCP and MHP, while fish fed the control and TCP showed the lowest. Relative weight gain (RWG) was significantly higher in DCP, MCP, and MHP among treatments. Catfish fed the MCP showed the highest WG (484%), which was not significantly different ($P > 0.05$) from those of fish fed the DCP and MHP. The same trends were also found in FCR, PER and SGR. P availability of MHP was 97.8 and 90.9% for carp and catfish, respectively. Inorganic P in plasma maintained equally at 6.7 mg/dL in carp fed MCP, DCP and MHP, while that was 15.4, 13.2 and 15.8 mg/dL in catfish fed the same diets, respectively.

From the studies, the potential use of MHP recovered from swine manure was proven sufficiently to replace MCP as an alternative P source with respect to WG and FCR as well as P availability.

ASSESSMENT OF DIGESTIBILITY OF SELECTED FEED INGREDIENTS FOR FINGERING SNAKEHEAD FISH (*Channa striata*) USING *IN VIVO* AND *IN VITRO* METHODS

Bundit Yuangsoi*, Narissara Suratipand Dhanapong Sangsue

Department of Fisheries, Faculty of Agriculture, Khon Kaen University
Khon Kaen, 40002, Thailand
bundyu@kku.ac.th

Snakehead fish (*Channa striata*) has become an economically important cultured freshwater fish in Thailand. The total production reported for this species to FAO for 1999 was 32,938 t. The countries with the largest produce were Thailand (27,500 t) and Philippines (5,438t) (FAO, 1999). Many farmers still use trash fish in preparing feed at the snakehead fish farm. From feeding trash fish, all nutrient leaching from trash fish and effect on water quality from uneatable feed. Knowledge of nutrient digestibility of the various feed ingredients used in formulating fish feeds is desirable so that effective substitution of one ingredient for another may be achieved. No information is yet available on the apparent digestibility of nutrients for snakehead fish in Thailand. The objective of this study was to determine apparent digestibility of nutrients in selected feed ingredients for snakehead fish.

Apparent digestibility of nutrients of three feed ingredients: fish meal (FM), soybean meal (SBM) and poultry meal (PM) were determined by incorporated of test ingredients to practical reference diet at a 7:3 ratio (70% of reference diet and 30% of test ingredient described by NRC, 2011). Chromic oxide (Cr_2O_3) is used as external digestibility marker at 0.5% in feed formulation.

The protein digestibility for fish meal was 84.51%, poultry meal was 77.17% while that for soybean meal was only 64.41%. The fat digestibility for fish meal was 81.26%, poultry meal was 66.84% while that for soybean meal was 54.95%.

The results shown that snakehead fish have the tendency to digest protein in feedstuffs of animal origin more efficiently than protein in feedstuffs of plant origin.

In vitro digestibility of different feed ingredients was investigated using an enzyme extract from whole digestive tract. The digested protein was determined as liberated reactive amino groups of the peptides using the trinitrobenzenesulphonic acid (TNBS) method. The concentration of the reactive amino groups was calculated using DL-alanine as a standard.

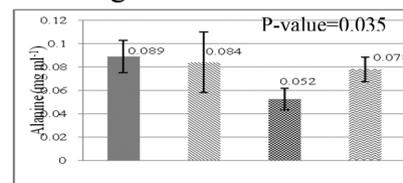
Studies of *in vitro* protein digestibility of different feed ingredients indicated that FM and PM are suitable ingredients for a formulated feed for fingerling stages.

In conclusion, data presented here suggest providing information on nutrient digestibility of feed ingredients used in complete feed for snakehead fish.

Table 1 Analyzed essential amino acid contents in faeces of snakehead fish.

Essential amino acid digestibility				
EAA	Reference	Fishmeal	Soybean meal	Poultry meal
Methionine	63.63	73.91	54.57	75.38
Cystine	57.29	65.79	50.72	67.43
MET+CYS	61.39	71.32	53.09	72.68
Lysine	74.21	84.81	72.57	82.20
Threonine	64.62	74.06	58.71	73.61
Tryptophan	60.37	70.71	57.89	69.81
Argenine	72.29	81.40	69.56	81.24
Isoleucine	63.63	74.08	57.10	73.26
Leucine	67.31	77.41	62.42	75.58
Valine	61.89	72.43	54.78	71.39
Histidine	71.73	81.66	69.30	81.14
Phenylalanine	67.93	76.25	61.65	76.48

Figure 1 *In vitro* protein digestibility of selected feed ingredients.



SitroPro AS AN ALTERNATIVE TREATMENT FOR SEABASS, *Lates calcarifer* INFESTED WITH MARINE LEECH, *Zeylanicobdella arugamensis*

Fadzilah Yusof, Azmi Rani, Saberi Mawi dan Zainoddin Jamari

Brackish Water Aquaculture Research Division
FRI Gelang Patah,
Johor Bahru Johor

Most global aquaculture practices nowadays turn their pathway to nature. The usage of chemicals especially to treat diseases of fishes is slowly reduced. Herbs extract was used everywhere as the alternative medicine in aquaculture. This so called phenomenon is due to food safety purposes as well as for the operators' wellbeing. This experiment was to ensure the ability of herbs extract developed by FRI GP namely SitroPro, to treat seabass (*Lates calcarifer*) infested with the marine leech (*Zeylanicobdella arugamensis*) and to get the optimum dosage of SitroPro to be used. Marine leech infected seabass were isolated into the treatment tanks and treated bath with 50, 75, 100, 150 & 200ppm SitroPro repeated for 3 consecutive days. The treatment then were continue with fish feed sprayed with SitroPro at 50, 75, 100, 150 & 200ppm (v / w) and fed in respective tanks for 7 days consecutively. Bath treatment with SitroPro 100ppm for 4 to 24 hours for marine leech infected seabass was effective enough to remove the marine leech from the fish body. The water had to be changed at least after 24 hours. The bath treatment then best to be followed with 100ppm SitroPro sprayed to fish feed and fed for at least 7 consecutive days.

COMPARATIVE STUDIES ON OSMOREGULATORY CAPACITIES OF TWO COMMERCIALY IMPORTANT PORTUNID CRABS: IMPLICATOINS FOR AQUACULTURE

Chaoshu Zeng*, Nicholas Romano

College of Science and Engineering
James Cook University
Townsville, Queensland 4811
Australia
chaoshu.zeng@jcu.edu.au

The mud crab, *Scylla serrata* and the blue swimmer crab, *Portunus pelagicus*, are two commercially important portunid crab species that have been targeted for aquaculture in the Indo-Pacific. Comparative studies on their osmoregulatory capacities showed that despite they are taxonomically closely related, their capacities on osmoregulation vary substantially. The juvenile mud crab *S. serrata* was shown as a strong osmoregulator, who was particularly adaptive to low salinities. The survival of juvenile *S. serrata* was found not significantly affected by a broad salinity range from 4 ‰ – 44 ‰ while the optimal salinity for their growth was identified as between 4 ‰ – 28 ‰. On the other hand, the juvenile blue swimmer crab *P. pelagicus* was identified as a weak osmoregulator, who was particularly sensitive to low salinities. It was shown that salinity ≤ 15 ‰ led to significant higher mortality of juvenile *P. pelagicus* while significantly lower growth was detected at salinity ≤ 10 ‰ and ≥ 45 ‰. The optimal salinity range for juvenile *P. pelagicus* was hence identified as with a comparatively narrow range of 20 ‰ – 35 ‰.

The above knowledge on osmoregulation ability difference of the two portunid crabs have significant implications on their aquaculture, including suitable farm site selection and adopting pre-emptive measures during monsoon season in the tropics for *P. pelagicus* culture. Since weak osmoregulation ability, particularly high sensitivity to low salinity, could represent an issue during monsoon season for *P. pelagicus* culture, experiments were conducted to evaluate whether the osmoregulatory capacity of the crab could be enhanced through dietary approach. The results showed that the resistance of juvenile *P. pelagicus* to osmotic shock substantially improved when they were fed diets with high phospholipid contents. Similarly, higher dietary highly unsaturated fatty acid (HUFA) contents significantly improved survival of juvenile *P. pelagicus* at low salinities while growth was significantly improved at both low and high salinity conditions, which was likely through improved haemolymph ion maintenance. These results demonstrated that dietary approach potentially can be adopted as a practical method to improve osmoregulatory capacity of cultured *P. pelagicus* under osmotically stressful conditions to improve their culture productivity.

PATHOGENICITY OF *Aeromonas hydrophila* AND PATHOGENESIS OF MOTILE AEROMONAS SEPTICEMIA IN CHANNEL CATFISH *Ictalurus punctatus*

Dunhua Zhang, De-Hai Xu, Craig A. Shoemaker, and Benjamin H. Beck

Aquatic Animal Health Research Unit
US Department of Agriculture - Agricultural Research Service
990 Wire Road, Auburn, AL 36832
USA
dunhua.zhang@ars.usda.gov

Outbreaks of motile *Aeromonas* septicemia (MAS) disease in warm-water fishes caused significant economic loss in aquaculture industries worldwide. Severe reoccurrence of the disease has been reported since 2009 on catfish farms of the Southeastern United States with approximate 2,000 tons of dead fish annually. To date, recommended management practices that have worked in the past seemed to be ineffective at limiting or preventing the MAS outbreak. The purpose of this study was to evaluate factors that predispose fish to *A. hydrophila* infection, virulence of *A. hydrophila* strains and the possible survival mechanism of *A. hydrophila* in catfish ponds.

Results of this study indicate that naïve and apparently-sound (healthy) fish were resistant to *A. hydrophila* infection while wounding on the fish body made fish highly susceptible to infection via waterborne route, suggesting that the (biological and/or physical) lesions occurred on fish could be a key factor predisposing catfish to MAS disease. Catfish weighing from 5 to 300 g were all susceptible and more than 90% mortality occurred within 48 h post immersion challenge in water containing with 2×10^7 colony forming units mL^{-1} of the pathogen. Cells of the pathogen were detected by quantitative real-time PCR as early as 1 h post challenge from most internal tissues of infected fish, implying that the pathogen was able to rapidly proliferate and spread through fish blood circulation system following infection. Different strains of *A. hydrophila* varied in virulence. A lineage of highly virulent strains of *A. hydrophila* (vAh) was responsible for the recurring outbreak of MAS in most US catfish farms. The genome of vAh encodes a suit of proteins for utilization of chitin, the most abundant organic matter in aquatic ecosystems. Assays *in vitro* showed that four chitinases, one chitobiase and one chitin binding protein were secreted extracellularly by vAh and participated in chitin degradation. The bacterium was able to effectively use not only N-acetylglucosamine and colloidal chitin but also chitin flakes as sole carbon sources for growth, enabling the bacterium to reach high densities in aquatic niches and constituting a potential threat to susceptible fish when abundant chitin is available.

Findings of this study improved our understanding of pathogenicity and pathogenesis of vAh in Catfish and will facilitate further studies of the disease prevention and control.

MICROBIOME DYNAMICS OF MUD CRAB *Scylla paramamosain* FOLLOWING MOLTING

Xinxu Zhang*, Shengkang Li

Marine Biology Institute, Shantou University
Daxue Road 243, Shantou, P.R.China, 515063
newsunzhang@stu.edu.cn

Symbiotic microorganisms living in the host or on its surface play a key role in the host's digestion and immunity, which is a research hotspot in recent years. The mud crab *Scylla paramamosain* is a commercially important aquaculture species in the southeast coast of China. Our previous results revealed the abundance and diversity of microbial communities in different mud crab tissues, including intestine, gill, hemolymph and subcuticular epidermis. Molting is an important process for the arthropods and some reptiles throughout its lifetime, which is related to its growth, reproduction and appendage regeneration. During a molting, the animal tries to escape from the confines of old exoskeletons and tissues (such as gill and intestine) when the connectives between the living tissues and the extracellular cuticles were loosen. However, little is known about the dynamic change of the microbiome in *Scylla paramamosain* following molting. In the present study, we aim to investigate whether molting affected the host microbiome, and to test the hypothesis that a recolonization event occurs in the host microbiome of the crab following molting. To this end, we have determined the microbial abundance and bacterial community compositions in different molting stages of the crabs, by using fluorescence microscopy and barcoded high-throughput sequencing of the V3-V4 region of the 16S rRNA genes. Distinct patterns of microbial communities were observed before and after molting, showing a dynamic change of the host microbiome following molting. Meanwhile, this study gives clues to develop microbial immunoenhancer, and to select appropriate administration routes for disease control.

IDENTIFICATION OF HEMOCYANIN GLYCOSYLATED SITES FROM SHRIMP *Litopenaeus vannamei* AND STUDY ON ITS CORRECTION WITH ANTIMICROBIAL FUNCTION

Yueling Zhang*, Zehui Zhang, Fan Wang

Department of Biology and Guangdong Provincial Key laboratory of Marine Biotechnology,
Shantou University
Daxue Road 243#, Shantou 515063, China
Zhangyl@stu.edu.cn

Hemocyanin (HMC) was documented to act as a important immune factors in invertebrates. In this study, we purified HMCs from *Litopenaeus vannamei* with gel-filtration chromatography and affinity chromatography (named as S-HMC and A-HMC). Only deglycosylated HMC by O-glycosidase led to decreased in agglutinative activities. Mass spectrometry analysis showed that *L. vannamei* HMC had O-glycan modifications, which has not been previously reported. 46 and 12 glycosylation sites were identified in S-HMC and A-HMC. Specifically, 23 and 20 glycosylation sites were identified in HMC subunit with higher and lower molecule weight, respectively.

Furthermore, together with our previous publications, the glycosylation sites of hemocyanin subunit C-terminal Thr537, Ser539 and Thr542 were replaced to Ala and expressed in yeast *Pichia pastoris* (named as *wt*-HMC and *mut*-HMC). Later results indicated that agglutination activities of *mut*-HMC were less than *wt*-HMC. We interestingly found that, compared with *wt*-HMC, the antimicrobial activities of *mut*-HMC were reduced significantly. Taken together, Thr537, Ser539 and Thr542 appeared to be closely associated with hemocyanin's antimicrobial activity, these results will be contributed to investigate hemocyanin function diversity mechanism of sugar molecules.

SIMPLIFIED METHOD TO MEASURE *Isochrysis galbana* CELL DENSITY IN THE HATCHERY

Xing Zheng*, Le Li, Hebert Ely Vasquez, Zhifeng Gu, Aimin Wang

State Key Laboratory of Marine Resource Utilization in South China Sea, Marine Sciences College of Hainan University.

58 Renmin Ave., Haikou, Hainan 570228, China

Zhengxing_edu@163.com

The flagellate, *Isochrysis galbana* Parke 3011, is a free living marine unicellular phytoflagellate of the order Chrysomonadales, which is rich in polyunsaturated fatty acids, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA), that are of nutritional value in the rearing of various bivalve larvae. Therefore *I. galbana* is widely used in the aquaculture industry. In some hatcheries in China, workers usually rely on the color intensity of the microalgae culture to determine the amount of food supplied to any given larvae husbandry. Surprisingly, information regarding the relationship between the microalgae density and color is scarce. Thus the aim of this research was to develop a simple and rapid method to indirectly calculate the microalgal concentration by describing the relationship between color parameters and cell density of *I. galbana*.

An 10-days culturing trial using 0.1 μm filtered seawater ($S=30$) in 5L flasks was conducted, at 26°C and constant illumination (5000 lux) measured at the surface of the flasks, as well as 30 L/min constant ventilation. Density and color parameters were measured everyday. Microalgae density in the samples was calculated by counting the cells using a blood count plate under the microscope. Sample color parameters were then measured in a Spectrophotometer CM-5 (KONICA MINOLTA, INC. Japan). Color parameters consists of $L^*a^*b^*$ units, and ΔE^* that was calculated automatically. The L^* parameter corresponds to the degree of luminance or lightness, ranging from 0 to 100, where $L^*=0$ yields black and $L^*=100$ indicates white. The parameters of a^* and b^* are the two chromatic components, in which $+a^*$ is red, $-a^*$ is green, $+b^*$ is yellow, $-b^*$ is blue, both a^* and b^* values ranging from -120 to 120. The color differences ΔE^* of the various concentrations were calculated using FSW as reference to zeroed the spectrophotometer. All correlations were performed using the software DPS (Ver. 16.05)

The relationships between culturing time and cell density are shown in Table.1, as well as color parameter. Results shown a strong relationship between the culturing time and the cell density, L^* , a^* , b^* , as well as ΔE^* . This simplified method can be used to other species of microalgae to indirectly calculate the microalgal concentration.

TABLE.1 Relationships of cell density, $L^*a^*b^*$ values, ΔE^* and culturing time

culture time and *	Formula	R ²
cell amounts	$y = 74.3035 * (0.5795^x) * x^{3.1981}$	0.9310
L^* values	$y = 102.084247 * (1 - e^{-((X_1 - 0.742842) / 12.519066)^{-0.645882}})$	0.9664
a^* values	$y = 7.944815 * (1 - e^{-((X_1 - 0.972369) / 5.490983)^{-3.756354}})$	0.9210
b^* values	$y = 60.488377 * (1 - e^{-((X_1 - 0.359247) / -4.679670)^{-1.678076}})$	0.9964
ΔE^*	$y = 68.267987 * (1 - e^{-((X_1 - 0.281180) / -5.062057)^{-1.786481}})$	0.9935

IMPROVEMENT OF THE NUTRITIONAL VALUE OF NILE TILAPIA USING FINISHER DIETS

Abd El-Naem, F.A Zidan*, Ashraf Suloma, Rania S Mabroke and Osama Elhouseiny

*Fish Nutrition Laboratory (FNL), Animal Production Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt

*Corresponding author: (Tel.): + 201115429602

Email: abd.elnaem@agr.cu.edu.eg

The wash out strategy using fish oil based diet before harvest affords the saving of feed costs and high fillet quality for human nutrition. The optimal duration for the inclusion of fish oil in tilapia diet should be determined to guarantee improvement of tilapia fillet by decreasing the content of ω 6, especially linoleic acid (LA) which is critical for economical and sustainable benefits.

This study was designed to examine the suitable duration for washing out ω -6 prior early harvest of tilapia fish when the average harvest weight ranged between 100-150 g. Two different lipid sources soy oil (SO) and fish oil (FO) were used to generate three different feeding regime; SO diet continuously for 6 weeks, FO diet continuously for 6 weeks, and SO diet for 3-wk followed by FO diet for 3-wk (SO/FO treatment). Growth performances of Nile tilapia fed on FO-finishing diet continuously did not have significant improvement in growth performance or feed utilization compared with tilapia fed SO diets and those subjected to SO/FO treatment. Tilapia fed SO diet continuously for 6 weeks had the highest significant LA (39.6) and ARA (2.03) in the fillet. While the lowest values were recorded for fish fed FO-finishing diet for 6 weeks. The decrease in fillet content of such fatty acid is consider beneficial for human consumer. High level of ω 6 is a precursor for inflammation and tumor diseases. Tilapia direct that much of fatty acids in viscera sector, therefore the tilapia by products included the Viscera could be considered as functional ingredient and could be used as lipid source in aqua-feed manufacturing.

Table 17. Effect of different finishing diet regimes on growth performance of tilapia (means \pm SE)¹

parameters	SO _{6wks}	SO _{3wks} + FO _{3wks}	FO _{6wks}
Mean initial weight (g/fish)	70.80 \pm 0.80	71.60 \pm 0.21	71.23 \pm 0.66
Mean final weight (g/fish)	122.69 \pm 8.96	119.62 \pm 1.09	134.98 \pm 4.22
Weight gain (g/fish) ²	51.88 \pm 8.16	48.02 \pm 1.30	63.74 \pm 4.89
Feed intake (g/fish)	80.62 ^{ab} \pm 3.94	77.39 ^b \pm 1.19	90.73 ^a \pm 1.63
FCR ³	1.58 \pm 0.17	1.61 \pm 0.06	1.42 \pm 0.08
SGR ⁴	1.30 \pm 0.14	1.22 \pm 0.02	1.52 \pm 0.09
PER ⁵	2.13 \pm 0.23	2.06 \pm 0.08	2.33 \pm 0.13

A PRELIMINARY STUDY ON THE PERFORMANCE OF “SEA VEGETABLE”, *Caulerpa macrodisca* (Decaisne) WEBER-VAN BOSSE IN CAPTIVITY

Wahidatul Husna Zuldin*, Rossita Shapawi and Sitti Raehanah Mohd Shaleh

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu,
Sabah, Malaysia
E-mail: wahidatul@ums.edu.my

Caulerpa macrodisca (Decaisne) Weber-van Bosse is a siphonous green macroalgae that has been reported to be widely distributed around South-west Asia (Sri Lanka), South China Sea, South-east Asia (Indonesia, Philippines, Singapore and Vietnam) and Pacific Islands (Samoan Archipelago). This species was known as “eaba-eaba” in Iloilo, Philippines which were collected seasonally from the wild by divers, sold in the local markets and consumed as fresh salad by the local people. However, the cultivation and consumption of *C. macrodisca* in Malaysia is very uncommon due to the limited research on this species occurrence. Recently, this species was found to be available around the West Coast of Sabah, Malaysia. This study reported the performance of *C. macrodisca* collected from Malaysian waters in captivity. The *C. macrodisca* was successfully grown in the tank for 40 days with the salinity ranged from 30.29 to 32.18 ppt, temperature of 28.03 to 31.42 °C, DO level of 4.75 to 5.21 mgL⁻¹, pH of 7.62 to 8.06 and light intensity of 50.77 to 87.55 μmol photons m⁻²s⁻¹. The specific growth rate of *C. macrodisca* in the tank was 5.13 g day⁻¹ and the weight gain was 281.85%. A proximate analysis was performed on the *C. macrodisca* with values of 21.52%, 23.85%, 1.09%, 13.08% and 97.01% for crude protein, crude fibre, crude lipid, ash and moisture content respectively. This study suggested that *C. macrodisca* would have a good growth performance in captivity and this species could be used as a kind of natural food and fibre for human. In conclusion, this study is significant to provide baseline data for the *C. macrodisca* culture in Malaysia.

ADDENDUM

FAIRAGORA ASIA, TECH SOLUTIONS FOR SUPPLY CHAIN TRANSPARENCY IN THE AQUACULTURE INDUSTRY

Emmanuelle Bourgois*, Pau Badia Grimalt, Flavie Denelle

FairAgora Asia, 3rd Floor, Room 3/1, 94 Shinawatra tower Sukhumvit Soi 23, Klong Toei Nuea, Watthana, Bangkok 10110, Thailand

Green consumerism is increasing, especially in the aquaculture sector. In Asia, the agrifood sector has been criticized many times due to the lack of sustainable standards, human trafficking and environmental concerns. This is why certification, pushed by consumer demand, is growing in Asia.

FairAgora Asia provides compliance services in Asia and has witness the fast changing food, agro and tech markets of Asia. Being involved in the co-design of various standards with the support of international donors or NGOs (Blue Brand – Oxfam, ASIC, USAID), FairAgora has developed an expertise into Ag Tech for seafood and aquaculture and even developed its own spin-off: an innovative verification application called Verifik8. VerifiK8 is an affordable yet credible monitoring and verification software platform to de-risk operations in their supply chain in crosschecking information with other data streams; it develops environmental and social metrics for both the seafood supply chain and other agricultural products: sugarcane, rice, etc.

Consequently, FairAgora Asia works with different partners such as ULULA (worker’s voice), Liberty Asia (anti-human trafficking in Asia), Blue number (unique ID number for farms and farmers), Asian Institute of Technology (AIT – developing and testing water sensors), Southeast Asian Shrimp Aquaculture Improvement Protocol (SEASAIP) or Mimosatek (pond sensors implementation in Vietnam), OXFAM (social issues), and the Social Expert Monitoring Group (experts creating social metrics for Verifik8). FairAgora will be presenting some of the existing Tech solutions for Aquaculture which are aiming at opening the industry to the Internet of ponds.

INTEGRATED SUSTAINABILITY & COMPLIANCE

		
ADVISORY SERVICES	MONITORING & TRAININGS	INNOVATION & RESEARCH
We identify the most relevant compliance solutions to support the development of your business, with a comprehensive sustainability-driven approach.	We develop tailor-made training, inspection and monitoring packages to support quality insurance and compliance needs in your business.	We develop innovative verification solutions to improve transparency in the agribusiness supply chain, with disruptive social and inclusive models.

CERTIFICATION SCHEMES FOR SHRIMP AQUACULTURE IN ASIA: TOWARDS EFFICIENCY, SOCIAL AND ENVIRONMENTAL INTEGRITY

Krishna R. Salin* and Gabriel Arome Atagube

Aquaculture and Aquatic Resources Management,
Asian Institute of Technology, Pathumthani 12120, Thailand. *Email: salinkr@ait.asia

Effective monitoring of aquaculture production of shrimps for export in relation to sustainable standards affecting the social and environmental aspects of the ecosystem has gained prominence in Asia considering the need to show evidence of sustainable production. Over the last two decades, Asia as a whole has been contributing nearly 90% of the world aquaculture production. Excluding China, the top crustacean producers in Asia are Indonesia, Vietnam, India and Thailand in that order. The whiteleg shrimp *Penaeus (Litopenaeus) vannamei* accounts for over 70% of Asia's shrimp aquaculture production effectively dwarfing *P. monodon* and *Macrobrachium rosenbergii*. There are several challenges to sustainable shrimp farming in the Asia Pacific region with the intensive scale of operation that is the norm.

A plethora of standards are in operation in Asia and the numbers are rising especially in the four biggest shrimp producing and exporting nations: India, Thailand, Vietnam and Indonesia. India has the highest number of certifications for BAP and ASC combined (247) closely followed by Thailand (209) (Fig. 1). The number is increasing yearly considering the numerous applications being processed for the two certificates. The focus has often been on the environment while social aspects are rarely mentioned. However, certification must move in the direction of labour and associated issues, and as enforcement is moved from state to private bodies, it should become less cumbersome to trace given innovative technologies are adopted. The use of technology will ensure a smooth interface of environmental compliance monitoring with social risk assessment and compliance. Recent research (2007 to 2017) has apparently centered on broader terms with specifics of social and labour issues being scant as evidenced from a focused literature search (Table 1). There is need to overcome this perceived disparity by adopting innovative ideologies to ensure social integrity of shrimp aquaculture.

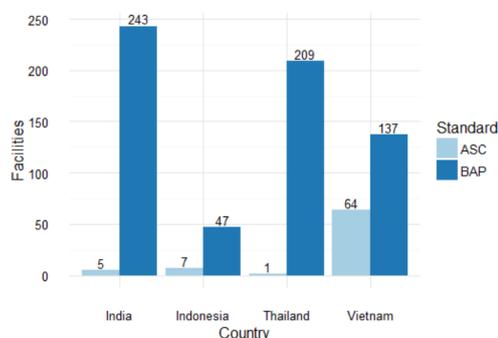


Figure 1: Number of certified aquaculture facilities by two standards in the four countries

Table 1: Number of articles under search terms from two publishing hubs (2007 – 2017)

Search Term	Sciencedirect	Taylor and Francis
Sustainable + shrimp	10764	2298
Aquaculture + labour	5125	1549
Aquaculture + social	9006	2959
Aquaculture + labour (Keyword)	5	0
Aquaculture + social (Keyword)	16	8
Aquaculture + shrimp (Keyword)	9	8

COMBINING RISK AND SUSTAINABILITY ASSESSMENT OF FUTURE AQUACULTURE

J. Schlundt

Professor, Director NTU Food Technology Centre (NAFTEC), Singapore

The most important animal food production system in SE Asia is aquaculture. The production of fish through fish farming is the fastest growing food production sector in the region (and globally). To achieve this type of growth, all aspects need to be optimized for maximum production efficiency and sustainability within the full production chain all the way to the consumer. Over the latest 25 years a significant number of food scandals have originated in a poor understanding of the full food production chain. This relates to zoonotic pathogens originating in animal farm settings, chemical contamination in the production chain, fraudulent practices related to primary production and antimicrobial resistance from use of antimicrobials in animal production. It is thus now globally recognized that the full production chain needs consideration in order to enable efficient and sustainable food production. Food technology research is thus no longer focused on the final production stage, but must consider the full food production chain.

Novel risk and sustainability assessment methodologies will in the future present an integrated framework for science based decision support. It is anticipated that improved efficiency and sustainability can be achieved by improving the health status of the fish and thereby reduce the antibiotic usage, improve profitability and ensure safer food. In addition, it is expected that novel feed compositions and vaccine formulations, early detection systems and risk assessment strategies can provide science-based solutions for fish production, enabling safer and more sustainable fish production.