# Green Culture Sustainable Production

# Book of Abstracts

## The 6th International Symposium on Cage Aquaculture in Asia 2018

**12th - 15th October 2018**

**Wangtai Hotel, Surat Thani, Thailand**

# AFS Message

Greetings!

I warmly welcome everyone to the 6th International Symposium on Cage Aquaculture in Asia (CAA6).

The International Symposium on Cage Aquaculture in Asia (CAA) is a specialized triennial symposium of the Asian Fisheries Society (AFS) focused on the status, growth, and development of cage aquaculture in the Asia-Pacific region. Since the first symposium in 1999 in Tunkang, Taiwan, CAA have been carried out in four countries including China in 2006, Malaysia in 2011, South Korea in 2013, and India in 2015.

The importance of aquaculture in Asian countries and its contribution to the world's aquaculture, and the continued expansion of cage aquaculture in the region in response to pressures from globalization and growing demand for aquatic products have made AFS decide to make the symposium a permanent symposium series of the Society. The main aim is to promote the sharing of latest information and technologies in cage aquaculture to meet the demand and interests of the Society's members and all key stakeholders involved in the industry.

For this year, CAA6 carries the theme "Green Culture, Sustainable Production," as it highlights notable discoveries on cage aquaculture towards reducing health and environmental impacts and also sustaining production with consideration of environmental, social and financial responsibilities for a longer term. This four-day symposium will include special lectures and keynote presentations together with a number of papers to be presented in the technical sessions on marine production systems, inland production systems, breeding and seed production, nutrition and feed, health and environment management, economics, livelihood and policies, and genetics, omics and biotechnology, and a field trip.

AFS is grateful to the Department of Marine Science, Faculty of Science, Chulalongkorn University for convening this important conference in collaboration with the National Science and Technology Development Agency, and the Prince Songkla University, Surat Thani Campus.

Congratulations to all CAA7 participants. We look forward to a successful symposium.

Alice Joan G. Ferrer, PhD
University of the Philippines Visayas
Vice-President, Asian Fisheries Society

# Welcome Message

On behalf of the Organizing Committee, I would like to extend our warm welcome to all participants of the 6th International Symposium on Cage Aquaculture in Asia 2018 (CAA6): Green Culture Sustainable Production. This symposium is organized by the Department of Marine Science, Faculty of Science, Chulalongkorn University in collaboration with National Science and Technology Development Agency (NSTDA) and Prince of Songkla University, Surat Thani Campus. I would also like to extend my sincere appreciation to Asian Fisheries Society for choosing Thailand to organize this event.

Asian countries have played a significant role in aquaculture industries, and have contributed over 90% of aquaculture production which provided livelihood directly and indirectly to millions of people in coastal and rural areas. Aquaculture is the fastest growing food production sector, and accounts for nearly half of the food fish supply globally.

Recently, cage culture has become more popular and has been a promising technique that can be an alternative livelihood option for communities. Thus, more research into cage culture techniques as well as socio-economic well-being of the Asian cage aquaculture industry are needed for sustainability. This symposium will provide a platform for scientists, government officers, non-government sectors, managers, students, and publics to update current status, discuss, and advance our understanding of the cage culture research in Asia.

I wish you a productive symposium, and hope you enjoy your time in Surat Thani.

Voranop Viyakarn, PhD
Chairperson of CAA6 Organizing Committee

# Welcome Message

Distinguished Delegates
Ladies and Gentlemen

On behalf of Prince of Songkla University, it is a great honour and pleasure to welcome you to the 6th International Symposium on Cage Aquaculture in Asia 2018 under the theme "Green Culture Sustainable Production" and I would like to express my sincere thanks for all participants who attend this symposium.

This symposium is organised by Department of Marine Science, Faculty of Science, Chulalongkorn University in collaboration with National Science and Technology Development Agency and Prince of Songkla University, Surat Thani Campus. As a co-host of this symposium, I would like to take this opportunity to initiate you that the purpose of symposium corresponds our university mission. Prince of Songkla University is a leading tertiary education centre of the Upper Southern Thailand. Our campuses which are Hatyai Campus, Pattani Campus, Phuket Campus, Surat Thani Campus and Trang Campus are located all over around the southern part of Thailand. PSU also comprises 39 faculties, colleges and institutes, four hospitals, and more than 40 excellence and research centres, all committed to academic excellence, strong social responsibility, and active engagement in community services. The central aims of the university are to raise general education standards and support regional industry and development. Moreover, the university aims to establish excellence in research and teaching, to provide academic services to communities, and to take an active role in the preservation of national heritage in arts and culture, especially for those from southern Thailand. Therefore, as a leading research university in Asia, PSU has contributed significantly to the development of the country and consistently turned out well-qualified graduates of high professional standing which the aquaculture is one of our expertise as well. Hence, the undergraduate and graduate courses are provided to serve the aquaculture field as well.

This symposium tries to provide the scholars, researchers and various educational institutions with the opportunity to present their current research study and exchange their knowledge and experiences among them, especially, for the advancement of technologies and technical expertise, socio-economic well-being of the Asian cage aquaculture industry leading to the mutual understanding and further research collaboration.

On behalf of Price of Songkla University, I would like to express my gratitude for your participation in this symposium and hope that we will have successful discussions to further mutual research scheme and collaboration.

Finally, it has been an honour to have all distinguished guests with us today. I am looking forward to the meaningful, fruitful and excellent outcomes of this symposium. Also, I hope we will have opportunity to welcome you in the next event again.

Thank you.

Chareon Nakason, PhD
Vice president of Prince of Songkla University for Surat Thani Campus
Co-Chairperson of CAA6 Organizing Committee

# Welcome Message

On behalf of the Scientific Committee, it is my great pleasure to welcome all participants to Surat Thani, Thailand and to the 6th International Symposium on Cage Aquaculture in Asia 2018 (CAA6) organized by Department of Marine Science, Faculty of Science, Chulalongkorn University in conjunction with Prince of Songkla University, Surat Thani Campus, and NSTDA. I would also like to extend my sincere appreciation to the Asian Fisheries Society (AFS) for choosing Thailand to organize this remarkable event.

With the increasing of world population, the duty ahead is finding ways to increase aquaculture production to meet the future global demands. The most sustainable alternative to capture fishery is green aquaculture. Asian Countries are main producers leading to the opportunity for contribution of the expansion of aquaculture production in practice.

This symposium will provide a platform for exchange of information and ideas, and disseminate updates on the development of both cage aquaculture and aquaculture biotechnology within the Asia-Pacific region. Over the next three days, we will deliberate on the research in cage aquaculture and related areas like nutrition and feed, health and environment management, economics and policies and the new session on genetics, omics and biotechnology. I would like to thank participants from National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA for their contribution of the new session.

On behalf of CAA6, I would like to thank our keynote speakers, and presenters who have come to share their valuable knowledge in this symposium. I would also like to thank our sponsors, particularly NSTDA for the financial support for conference reception and Thai Union Group (Thai Union Manufacturing Co., LtD. and Thai Union Hatchery Co., Ltd.) on excursion. Last but not least, I would like to congratulate members of the organizing and scientific committees and others who have helped to make this event a success.

Sirawut Klinbunga, PhD
AFS Councilor & Chairperson of CAA6 Scientific Committee

# MEMBERS OF ORGANISING COMMITTEE (OC)

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Dr. Narong Sirilertworakul, National Science and Technology Development Agency (NSTDA)

Assoc. Prof. Dr. Chareon Nakason, Prince of Songkla University, Surat Thani Campus

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## 6th International Symposium on Cage Aquaculture in Asia (CAA6)

### Chairperson

Dr. Sirawut Klinbunga, AFS and NSTDA, Thailand

### Members

Dr. Joykrushna Jena, AFS and Indian Council of Agricultural Research Fisheries Science Division, India

Prof. Alice Joan G. Ferrer, AFS and the University of the Philippines Visayas (UPV), Philippines

Prof. Dr. Shuolin Huang, AFS and Shanghai Ocean University, People Republic of China

Prof. Dr. Aziz Arshad, AFS and Universiti Putra Malaysia, Malaysia

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Prof. Dr. Atsushi Hagiwara, AFS and Nagasaki, University, Japan

Prof. Dr. Chen-Huei Huang, AFS and National Chiayi University, Taiwan

Prof. Dr. Han-Ching Wang, AFS and National Cheng Kung University, Taiwan

Prof. Dr. Nicholas Paul, AFA and University of the Sunshine Coast, Australia

Dr. Gopalakrishnan Achamveetil, AFS and Central Marine Fisheries Research Institute (CMFRI), India

Dr. Sorawit Powtongsook, NSTDA, Thailand

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## The 6th International Symposium on Cage Aquaculture in Asia 2018 (CAA6)
## Tentative Program

### Friday 12th October 2018

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| 14.00-17.00 | Registration |
| 15.00-18.00 | Poster mounting |

### Saturday 13th October 2018

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| 8.30-9.00 | Registration |
| 9.00-9.05 | Pay respects to His Late Majesty King Bhumibol Adulyadej |
| 9.05-9.15 | Culture Show |
| **Opening Ceremony** | |
| 9.15-9.25 | Opening message from<br>Prof. Dr. Alice Joan G. Ferrer (Vice-President of AFS) |
| 9.25-9.35 | Welcome message from<br>Provincial Governor of Surat Thani |
| 9.35-9.45 | Welcome message from<br>Dr. Sirawut Klinbunga (Co-Chair, Organizing Committee) |
| 9.45-9.55 | Welcome message from<br>Prof. Dr. Charoen Nakason<br>(Vice President of Prince of Songkla University, Surat Thani campus) |
| **Keynote lectures**<br>**Chair: Prof. Aziz Arshad** | |
| 9.55-10.35 | **Mariculture Park Program in the Philippines: Challenges and the Way Forward**<br>Keynote Speaker: Prof. Dr. Alice Joan G. Ferrer |
| 10.35-11.15 | **Genetics and biotechnologies for sustainable aquaculture of the black tiger shrimp *Penaeus monodon***<br>Keynote Speaker: Dr. Sirawut Klinbunga |
| 11.15-11.40 | *Coffee break* |
| **Session I: Marine Production Systems**<br>**Chair: Assist. Prof. Sanit Piyapattanakorn, Vice-chair: Dr. Sarayut Onsanit** | |
| 11.40-11.55 | **[S1O-06] R & D efforts in harnessing cage farming technology to increase fish production through mariculture in India**<br>Imelda Joseph and A. Gopalakrishnan<br>*ICAR-Central Marine Fisheries Research Institute, India* |
| 11.55-12.10 | **[S1O-05] Cage culture of orange spotted grouper, *Epinephelus coioides* off Visakhapatnam Coast: A novel initiative in India**<br>Ritesh Ranjan, Sekar Megarajan, Biji Xavier, Relangi Durga Suresh, Shubhadeep Ghosh, Imelda Joseph and Gopalakrishnan Achamveetil<br>*Visakhapatnam Regional Centre of ICAR-CMFRI, India* |
| 12.10-12.25 | [**S1O-09] Participatory farming demonstration of orange spotted grouper (*Epinephelus coioides*) at Kollam District of Kerala, India**<br>Santhosh B., Anil M.K., Muhammed Anzeer F., Aneesh K.S., Mijo V. Abraham, Unnikrishnan C., Jose Kingsley, Udayakumar A., Ritesh Ranjan, Philipose K.K., Boby Ignatius, Imelda Joseph, A. Gopalakrishnan3<br>*ICAR-Vizhinjam Research Centre of CMFRI, India* |
| 12.25-13.30 | *Lunch* |

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| **Session IV: Nutrition and Feed**<br>**Chair: Prof. Chen-Huei Huang, Vice-chair: Assist. Prof. Pattira Pongtippatee** | |
| 13.30-13.45 | **[S4O-04] Efficacy of Pueraria candollei extract to digestive system and growth performance in Marble Goby (*Oxyleotris marmorata*)**<br>Jiraporn Rojtinnakorn, Jaturong Matidtor, Ekawit Threenet, Sudaporn Tongsiri and Krisna R. Torrissen<br>*Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand* |
| 13.45-14.00 | **[S4O-03] Effect of herbal plants supplemented diet on the growth of a commercial carp, *Labeo rohita***<br>Zafar Igbal, Muhammad Hafeez ur Rehman and Bilal Ashraf<br>*Fish Disease and Health Management Laboratory, Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore, Pakistan.* |
| 14.00-14.15 | **[S4O-01] A combination of fresh feeds enhances sperm quality of domesticated male black tiger shrimp (*Penaeus monodon*)**<br>Kanchana Sittikankeaw, Rungnapa Leelatanawit, Sudtida Phuengwas, Jutatip Khudet, Suwanchai Phomklad, Somjai Wongtriphop and Nitsara Karoonuthaisiri<br>*BIOTEC, NSTDA, Thailand* |
| 14.15-14.30 | **[S4O-02] Study on nutrition compositions of protein hydrolysates and a possibility of using them as protein sources for shrimp feed, *Penaeus vannamei***<br>Chansawang Ngamphongsai and Seri Donnuea<br>*BIOTEC, NSTDA, Thailand* |
| **Session VI: Economics, Livelihood and Policies**<br>**Chair: Prof. Chen-Huei Huang, Vice-chair: Assist. Prof. Pattira Pongtippatee** | |
| 14.30-14.45 | **[S6O-01] Impacts of climate change on the tilapia value chain from cage culture in Luzon, Philippines**<br>Paul Joseph B. Ramirez, Edilyn V. Lansangan and Jairus Jesse M. Tubal<br>*University of the Philippines Los Baños, College, Laguna, Philippines* |
| 14.45-15.00 | **[S6O-04] Cage Culture of Monosex Tilapia for Food and Financial Security**<br>Ram C. Bhujel<br>*Aqua-Centre, SERD, Asian Institute of Technology (AIT), Thailand* |
| **Session III: Breeding and Seed Production**<br>**Chair: Prof. Chen-Huei Huang, Vice-chair: Assist. Prof. Pattira Pongtippatee** | |
| 15.00-15.15 | **[S3O-01] Identification of SNP in ribosomal protein S6 and ribosomal protein S6 kinase and their association with growth traits of the black tiger shrimp *Penaeus monodon***<br>Sirithorn Janpoom, Sirawut Klinbunga and Bavornlak Khamnamtong<br>*BIOTEC, NSTDA, Thailand* |
| 15.15-15.30 | **[S3O-02] Association between single nucleotide polymorphisms (SNPs) of X-box binding protein 1 and growth of the black tiger shrimp *Penaeus monodon***<br>Sirikan Prasertlux, Sirawut Klinbunga, Piamsak Menasveta and Bavornlak Khamnamtong<br>*BIOTEC, NSTDA, Thailand* |
| 15.30-15.45 | *Coffee break* |

| **Student session**<br>**Chair: Assist. Prof. Sanit Piyapattanakorn**<br>**Vice-chair: Dr. Jareeporn Ruangsri and Dr. Parichart Ninwichian** | |
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| 15.45-15.55 | **[S6O-02] Willingness to accept compensation for the establishment of mariculture operation in selected coastal communities in The Philippines**<br>Alice Joan G. Ferrer, Herminia A. Francisco, Canesio D. Predo, Benedict Mark M. Carmelita, and Jinky C. Hopanda<br>*University of the Philippines Visayas Foundation, Philippines* |
| 15.55-16.05 | **[S6O-03] Evaluation of Malaysia marine fishery sustainability in strengthening national food security**<br>Mohamad M. Fikri, Siti Rahyla Rahmat and Saidatulakmal Mohd<br>*School of Social Sciences, Universiti Sains Malaysia, Malaysia* |
| 16.05-16.15 | **[S5O-06] Parasites infection in seabass, *Lates calcarifer* (Bloch, 1790) in Earthen pond culture at Surat Thani province**<br>Supannee Sornkham, Kanda Kamchoo and Pongsak Laudee<br>*Department of Fishery and Coastal Resources, Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani Campus,Thailand* |
| 16.15-16.25 | **[S3O-03] Reproductive organs of triploid black tiger shrimp, *Penaeus monodon***<br>Muthita Sae-arlee, Wanita Semchuchot and Pattira Pongtippatee*<br>*Department of Fishery and Coastal Resources, Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani Campus, Thailand* |
| 16.25-16.35 | **[S7O-01] Effect of extenders and cryoprotectants on viability of spermatogonia and oogonia of striped catfish (*Pangasianodon hypophthalmus*)**<br>Pongsawan Khaosa-art[1] , Kensuke Ichida[1], Goro Yoshizaki[2] and Surintorn Boonanuntanasarn[1*]<br>*School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Thailand* |
| 16.35-16.45 | **[S7O-06] Development of microsatellite markers in the white scar oyster (*Crassostrea belcheri*) using next generation sequencing technology**<br>Ammarawadee Thepkum , Parichart Ninwichian, Jareeporn Ruangsri and Bavornlak Khamnamtong<br>*Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani Campus, Thailand.* |
| 16.45-16.55 | **[S7O-08] Differential expression of X-box binding protein 1 following ammonia stress in Pacific white shrimp *Litopenaeus vannamei***<br>Mookthida Kaewduang, Thaithaworn Lirdwitayaprasit, Sirawut Klinbunga and Bavornlak Khamnamtong<br>*Program in Biotechnology, Faculty of Science, Chulalongkorn University, Thailand.* |
| 16.55-17.05 | **[S7O-02] Recombinant Saccharomyces cerevisiae expressing delta 6 desaturase of Nile tilapia (*Oreochromis niloticus*)**<br>Araya Jangprai and Surintorn Boonanuntanasarn<br>*School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology,Thailand* |
| 17.05-17.15 | **[S5O-09] Evaluation of antibiotic resistance profiling in EMS-causing *Vibrio parahaemolyticus***<br>Nalumon Thadtapong, Pantaree Limvatanyu, Vanvimon Saksmerprome and Soraya Chaturongakul<br>*Department of Microbiology, Faculty of Science, Mahidol University, Thailand* |
| 17.15-18.30 | **Poster Session** |
| 18.30-20.00 | *Symposium Reception* |

**Sunday 14th October 2018**

| **Session I: Marine Production Systems (cont.)**<br>**Chair: Dr. Sarayut Onsanit, Vice-chair: Assist. Prof. Dr. Sirusa Kritsanapuntu** | |
|---|---|
| 9.00-9.15 | **[S1O-03] Culture of Indian pompano, *Trachinotus mookalee* in sea cage in India - A new potential candidate fish for coastal aquaculture and mariculture**<br>Sekar Megarajan, Ritesh Ranjan, Biji Xavier, Suresh Relangi Durga., Shubhadeep Ghosh, Boby Ignatius and Achamveetil Gopalakrishnan<br>*Visakhapatnam Regional Centre of ICAR-CMFRI, India* |
| 9.15-9.30 | **[S1O-04] Growth performance of hilsa (*Tenualosa ilisha*) at varying stocking densities in floating cages at Ukai reservoir, India: A maiden effort**<br>Shubhadeep Ghosh, Biswajit Dash, Ritesh Ranjan, Suresh Vettath Vadukootil Raghavan and Gopalakrishnan Achamveetil<br>*Central Marine Fisheries Research Institute, India* |
| 9.30-9.45 | **[S1O-08] Survival, growth and production of Asian Seabass, *Lates calcarifer* in high volume sea cage at Marine Farm at Karwar, Karnataka**<br>Jayasree Loka, Philipose K.K., Praveen Dube, Sanjeev Deshpande, Navanath Kumbhar, Imelda Joseph and Gopalakrishnan, A<br>*ICAR-Central Marine Fisheries Research Institute, India* |
| 9.45-10.00 | **[S1O-07] Growth performance of Asian seabass *Lates calcarifer* in open water cage farms in Kerala, South West coast of India**<br>Imelda Joseph, Shoji Joseph, Boby Ignatius, Reema George, Binoy Bhaskaran, and K.M. Venugopalan<br>*ICAR-Central Marine Fisheries Research Institute, India* |
| 10.00-10.15 | **[S1O-01] Flying fish resourses from southeast coast of India**<br>Muthukumarasay Srinivasan, Ramalingam Vinothkumar and Jaya praba<br>*Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Tamilnadu, Annamalai University, India* |
| 10.15-10.30 | **[S1O-10] Stock and mass culture of Bestiolina similis (Sewell, 1914) - a promising copepod live feed for marine finfish larviculture**<br>Santhosh B., Mijo V. Abraham, Muhammed Anzeer F., Aneesh K.S., Darsana S., Unnikrishnan C., Jose Kingsley H., Udayakumar A., Anil M.K., Imelda Joseph, Gopalakrishnan A.<br>*ICAR-Vizhinjam Research Centre of CMFRI, India* |
| 10.30-11.00 | *Coffee break* |
| 11.00-17.00 | *Excursion* |

**Monday 15th October 2018**

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| **Keynote lectures**<br>**Chair: Dr. Sirawut Klinbunga** | |
| 9.00-9.40 | **Aquatic vaccines in Japan and studies on fish DNA vaccines**<br>Keynote Speaker: Prof. Dr. Ikuo Hirono |
| 9.40-10.00 | **Session V: Health and Environment Management**<br>**[S5O-12] A comprehensive study of shrimp acute hepatopancreatic necrosis disease (AHPND)**<br>Prof. Dr. Han Ching Wang<br>*Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Taiwan* |
| 10.00-10.20 | **Session VII: Genetics, Omics and Biotechnology**<br>**[S7O-04] Molecular mechanisms of reproductive maturation in the black tiger shrimp (*Penaeus monodon*) through transcriptomic analysis**<br>Nitsara Karoonuthaisiri, Tanaporn Uengwetwanit, Rungnapa Leelatanawit, Umaporn Uawisetwathana, Juthatip Khudej and Somjai Wongtripop<br>*BIOTEC, NSTDA, Thailand* |
| 10.20-10.35 | *Coffee break* |
| **Session V: Health and Environment Management**<br>**Chair: Prof. Han-Ching Wang, Vice-chair: Dr. Jareeporn Ruangsri** | |
| 10.35-10.50 | **[S5O-08] Efficacy of dietary formalin-killed *Vibrio harveyi* to improve survival of the Pacific white shrimp through induction of granular-type hemocyte**<br>Rungkarn Suebsing and Kallaya Sritunyalucksana<br>*BIOTEC, NSTDA, Thailand* |
| 10.50-11.05 | **[S5O-01] Growth performance and microbiota of Pacific white shrimp *Litopenaeus vannamei* reared in Biofloc systems using different carbon sources**<br>Phimsucha Bunphimpapha, Panyisa Potibutr, Metavee Phromson, Siriporn Tala, Waraporn Jangsutthiworawat, and Sage Chaiyapechara<br>*BIOTEC, NSTDA, Thailand* |
| 11.05-11.20 | **[S5O-03] Attempt protection from Streptococcus iniae infection using incorporated feed and top-dressed feed vaccines in red hybrid tilapia (*Oreochromis* sp.) fingerlings**<br>Muhammad Adib Wafri Azaddin, Md Sabri Mohd Yusoff and Mohd Jamil Samad<br>*Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia* |
| 11.20-11.35 | **[S5O-02] Development of LAMP technique for detection of scale drop disease virus in Asian seabass**<br>Sirintip Dangtip and Wansika Kiatpathomchai<br>*BIOTEC, NSTDA, Thailand* |
| **Session VII: Genetics, Omics and Biotechnology**<br>**Chair: Assist. Prof. Dr. Kanda Kamchoo, Vice-chair: Dr. Jareeporn Ruangsri** | |
| 11.35-11.50 | **[S7O-05] Identification of G-protein coupled receptor from transcriptomics of premolt Y-organ in mud crab, *Scylla olivacea***<br>Sirinart Techa, Phimsucha Bunphimpapha, Manon Boonbangyang, Alisa Wilantho, Chumpol Ngamphiw, Sissades Tongsima, Boonyarath Pratoomchat and Sirawut Klinbunga<br>*BIOTEC, NSTDA, Thailand* |

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| 11.50-12.05 | **[S7O-07] Characterization of Insulin-degrading enzyme and association between its SNP and growth parameters of the Pacific white shrimp *Litopenaeus vannamei***<br>Wanwipa Ittarat, Sirikan Prasertlux, Bavornlak Khamnamtong and Sirawut Klinbunga<br>*BIOTEC, NSTDA, Thailand* |
| 12.05-13.15 | *Lunch* |
| **Session V: Health and Environment Management**<br>**Chair: Prof. Han-Ching Wang, Vice-chair: Dr. Jareeporn Ruangsri** | |
| 13.15-13.30 | **[S5O-04] Development of a multiplex recombinase polymerase amplification (RPA) assay for rapid and sensitive detection of $VP_{AHPND}$ and EHP in shrimp**<br>Narong Arunrut, Jantana Kampeera and Wansika Kiatpathomchai<br>*BIOTEC, NSTDA, Thailand* |
| 13.30-13.45 | **[S5O-07] Development of 9-plex bead array for immune gene expression analysis in the black tiger shrimp, *Penaeus monodon***<br>Sopacha Arayamethakorn, Nitsara Karoonuthaisiri and Wanilada Rungrassamee<br>*BIOTEC, NSTDA, Thailand* |
| 13.45-14.00 | **[S5O-10] Environmental-friendly antiviral microalga *Chlamydomonas reinhardtii* producing dsRNA**<br>Patai Charoonnart, Metha Meetam, Colin Robinson, and Vanvimon Saksmerprome<br>*Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, Thailand* |
| 14.00-14.15 | **[S5O-11] Controls of shrimp Vibrio diseases through the use of inhibitors of bacteria biofilm formations**<br>Chumporn Soowannayan and Pattanan Yatip<br>*BIOTEC, NSTDA, Thailand* |
| **Session VII: Genetics, Omics and Biotechnology**<br>**Chair: Assist. Prof. Dr. Kanda Kamchoo, Vice-chair: Dr. Jareeporn Ruangsri** | |
| 14.15-14.30 | **[S7O-09] Complete genome of the first and novel shrimp pathogenic Shewanella sp. TH2012 isolated from Early Mortality Syndrome (EMS) outbreak shrimp in Thailand**<br>Anuphap Prachumwat, Piyanuch Wechprasit, Kallaya Sritunyalucksana and Siripong Thitamadee<br>*BIOTEC, NSTDA, Thailand* |
| 14.30-14.45 | **[S7O-03] Single Nucleotide Polymorphism (SNP) in molt-inhibiting hormone 1 gene and its association with growth parameters of the black tiger shrimp *Penaeus monodon***<br>Puttawan Rongmung, Piamsak Menasveta, Sirawut Klinbunga and Bavornlak Khamnamtong<br>*BIOTEC, NSTDA, Thailand* |
| 14.45-15.00 | **[S7O-10] Masquerade-like protein is involved in TSV resistance in Pacific white shrimp *Penaeus vannamei.***<br>Premruethai Supungul, Sureerat Tang, Wisarut Junprung, Anchalee Tassanakajon, A<br>*BIOTEC, NSTDA, Thailand* |
| 15.00-15.30 | *Coffee break* |
| 15.30-15.45 | *Closing Ceremony* |

# Poster Session

**Saturday 13th October 2018**

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| **Session I: Marine Production Systems** | |
| **S1P-01** | **Growth promotion by prebiotic in whiteleg shrimp (*Litopenaeus vannamei*) (Boone,1931)**<br>Muthukumarasay Srinivasan, Krittika Mandal and Ramalingam Vinothkumar<br>*CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, India* |
| **Session II: Inland Production Systems** | |
| **S2P-01** | **The application of aquatic worms to dispose bottom waste of catfish pond**<br>Prachaub Chaibu, Sirichat Soonthornvipat and Namped Prakobsin<br>*Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sansai, Chiang Mai, Thailand* |
| **Session V: Health and Environment Management** | |
| **S5P-01** | **Identification and treatment of Fusarium isolated from black spots on the cuticle of Pacific white shrimp *Litopenaeus vannamei***<br>Phimsucha Bunphimpapha, Orasa Muesantad and Sage Chaiyapechara<br>*Biotec, NSTDA, Thailand* |
| **S5P-02** | **The clinicopathological evaluation of red hybrid tilapia (*Oreochromis* sp.) within 48 hours post-*Streptococcus iniae* and *Streptococcus agalactiae* challenge in the presence of heat stress**<br>Nurhani M.N., Ain-Mirzani, A.R., Jamil, M.S. and Sabri, M.Y.<br>*Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia* |
| **S5P-03** | **Screening of atinomycetes from pond bottom soil against pathogenic bacteria in aquatic animal**<br>Mahattanee Phinyo, Kotchanan Jantong and Wilasinee Inyawilert<br>*Department of Agricultural Science, Faculty of Agriculture, Natural Resources, and Environment, Naresuan University* |
| **S5P-04** | **Rapid and sensitive visual detection of EMS/AHPND bacteria using loop-mediated isothermal amplification combined with colorimetric gold nanoparticle probe**<br>Jantana Kampeera, Narong Arunrut, Sarawut Sirithammajak, Wansadaj Jaroenram and Wansika Kiatpathomchai*<br>*Biotec, NSTDA, Thailand* |
| **Session VI: Economics, Livelihood and Policies** | |
| **S6P-01** | **Red tilapia cage culture: A comparative analysis of technical efficiency for selected aquaculture farms in The Philippines and Thailand**<br>Joseph Christopher C. Rayos<br>*National Fisheries Research and Development Institute, Philippines* |
| **S6P-02** | **Effects of a probiotic mixture on the growth performance and health status of white shrimp, *Litopenaeus vannamei***<br>Ann-Chang Cheng, Shao-Yang Hu, Chiu-Shia Chiu and Chun-Hung Liu<br>*Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung, Taiwan* |
| **S6P-03** | **Study on the immunoregulation pathway of white shrimp, *Litopenaeus vannamei* after the oral-administration of probiotic *Bacillus subtilis* E20**<br>Yu-Chu Wang, Chia-Chun Chi, Chun-Hung Liu and Shinn-Pyng Yeh<br>*Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung, Taiwan* |

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| **S6P-04** | **Bacterial community characterization in intestine of the white shrimp, *Litopenaeus vannamei* after an oral-administration of synbiotic, *Lactobacillus plantarum* plus galactoo ligosaccharide**<br>Truong-Giang Huynh, Yo-Ju Wang, Chiu-Hsia Chiu, Chun-Hung Liu and Shieh-Tsung Chiu<br>*Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung, Taiwan* |
| **Session VII: Genetics, Omics and Biotechnology** | |
| **S7P-01** | **Development of one-tube nested PCR method for the detection of *Enterocytozoon hepatopenaei* (EHP) in shrimp.**<br>Panyisa Potibutr, Sirintip Dangtip, Ornchuma Itsathitphaisarn, and Sage Chaiyapechara<br>*Biotec, NSTDA, Thailand* |
| **S7P-02** | **Characterization of culturable bacteria isolated from white shrimp *Litopenaeus vannamei* postlarvae and rearing water from biofloc systems**<br>Mongkhol Phantura, Phimsucha Bunphimpapha, Panyisa Potibutr, and Sage Chaiyapechara<br>*Biotec, NSTDA, Thailand* |
| **S7P-03** | **Propagation and purification of ISKNV from Asian sea bass (*Lates calcarifer*) using Grunt Fin (GF) cell line.**<br>Sarocha Jitrakorn, Warachin Gangnonngiw, Triwit Rattanarojpong, Ha Thanh Dong and Vanvimon Saksmerprome<br>*Biotec, NSTDA, Thailand* |
| **S7P-04** | **Effect of Bacillus amyloliquefaciens TOA5001 as a potential probiotic on whiteleg shrimp (*Litopenaeus vannamei*)**<br>Kentaro Imaizumi, Sasiwipa Tinwongger, Hidehiro Kondo and Ikuo Hirono<br>*Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Tokyo, Japan* |

**Dr. Alice Joan G. Ferrer**
Professor, University of the Philippines Visayas (UPV), Philippines
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Alice Joan G. Ferrer is a Professor of Economics at the University of the Philippines Visayas (UPV) and also UP System Scientist I. She completed her BA Economics-Psychology at UPV and her Masters and PhD in Economics from the School of Economics, University of the Philippines. At present, she is the Vice-President of the Asian Fisheries Society (2015-2019), Constitution Committee Coordinator of the Gender in Aquaculture and Fisheries Section (GAFS) of the AFS. and the Coordinator of the Asian Fisheries Social Science Research Network Section of the AFS. In the Philippines, she is the National Deputy Director of the Economy and Environment Group Philippines, and the Executive Director of the Western Visayas Health Research and Development Consortium of the DOST-Philippine Council for Health Research and Development.

She is trained in Health Economics, Population Economics, and Environmental Economics but her research interests are in a number of areas including health economics, policy analysis, health policy, fisheries social science, health social science, gender, environmental economics, and peace and conflict.

To date, she has finished 42 research projects and has 53 publications including journal articles, a book, and chapters in books. A number of her researches have been used in updating health and environmental policies in the Philippines.

Her awards include outstanding performance as Associate Professor in 2007 and as Professor in 2017, and as best mentor in health research in a regional health research conference. She has also received various awards as a researcher including best poster presentation during the Global Health Forum in 2007 and during the national conference on Women in Fisheries, best paper in 2018 from Journal of World Aquaculture Society, and outstanding achievement in research in 2008 and in 2018 from UPV. She has received the highest award, Gold Medal Award, from the Asian Fisheries Society in August 2016 for her contribution to the society. She has also received an award from her hometown in the field of Science and Technology in May 2018.

**Mariculture Park Program in the Philippines:**
**Challenges and the Way Forward**

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**Abstract**

Mariculture plays a crucial role in national and local economies, and its sustainable development is important. The presentation focuses on the development, current status, and potential of mariculture parks after 15 years of the Mariculture Park Program of the Philippines using seven mariculture parks in three regions in the country as case studies. The presentation identifies the main concerns in mariculture operation and a number of factors on why the expected benefits of the Mariculture Park Program have not been fully realized so far. It will also present information on the profitability of mariculture operation, and the extent to which mariculture production is providing jobs to local community members. Recommendations for improving the implementation of the mariculture park program, profitability of mariculture operation, and community participation are provided. It will end with an update on the policy response of the government.

**Dr. Sirawut Klinbunga**
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Dr. Sirawut Klinbunga received a degree of Bachelor of Science (Cum laude) in Aquatic Science from Burapha University in 1988 and continued his post-graduate studies at the Institute of Aquaculture, University of Stirling, UK during 1991-1996. After graduation, he has joined National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA) as a researcher since September 1996. Dr. Klinbunga started his career at Marine Biotechnology Research Unit (MBRU) which is a BIOTEC satellite unit located at Faculty of Science, Chulalongkorn University. He was appointed as the first Director of Agricultural Biotechnology Research Unit, BIOTEC in October 2010 and subsequently, a Director of Animal Biotechnology Research Unit since 2015-present.

His research interests are development of molecular genetic markers to improve the culture and management efficiency of economically important marine species including shrimp, oysters, abalone, crabs and sardines. He has extended the research on molecular mechanisms of ovarian development in the giant tiger shrimp (*Penaeus mondon*) and development of molecular markers for genetic selection of *P. mondon* and Pacific white shrimp *Litopenaeus vannamei*. Dr. Klinbunga is a reviewer of more than 60 international journals and was one of the editorial broad members of Developmental and Comparative Immunology (DCI; 2008-2012) and Journal of World Aquaculture Society (JWAS; 2015). He has published 111 peer-reviewed papers in international journals.

**Genetics and biotechnologies for sustainable aquaculture of the black tiger shrimp *Penaeus monodon***

Sirawut Klinbunga[1*], Bavornlak Khamnamtong[1,2] and Piamsak Menasveta[3,4]

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**Abstract**

The black tiger shrimp *Penaeus monodon* is one of the most important cultivated shrimp species. Reduced spawning potential and low degree of maturation of *P. monodon* in captivity crucially prohibit several possible applications including development of effective breeding programs of this species. Determining molecular mechanisms involving female broodstock quality (i.e. maturation potential) can be applied to promote reproductive performance of domesticated stocks. Accordingly, the use of molecular genetic markers that allow selection of broodstock with a high potential for reproductive success would be useful for the shrimp industry. In addition, the lack of high quality broodstock of *P. monodon* has been proposed to cause wide size variation of cultured shrimp at harvest. Thus, genetic improvements for increasing growth rate and size uniformity are key breeding objectives for this species. However, the fundamental controls of growth in penaeid shrimp are poorly understood. Identification of molecular markers that allow selection of juveniles and broodstock with high growth rates are being sought to increase the efficiency of genetic improvement in *P. monodon*. Here, the basic research about identification and characterization of genes/proteins differentially expressed in different stages of ovarian development of *P. monodon* is illustrated. Further studies for development of single nucleotide polymorphism (SNP) markers associated with reproduction and/or growth of domesticated *P. monodon* are discussed.

**Dr. Ikuo Hirono**
Professor, Laboratory of Genome Science, Tokyo University of Marine Science and Technology, Tokyo, Japan
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Prof. Ikuo Hirono obtained his PhD from Kagoshima University in 1993, did a postdoctoral fellowship (JSPS) at Tokyo University of Fisheries in 1993-1994 and was a research scholar at Hopkins Marine Station, Stanford University in 1998. He was Assistant Professor, Tokyo University of Fisheries from 1994-2002, and Associate Professor, Tokyo University of Fisheries, Tokyo University of Marine Science and Technology from 2002-2009. He is a Professor, Tokyo University of Marine Science and Technology from 2009. He is co-editor in chief of Fish and Shellfish Immunology. He has over 340 publications in several international journals. His main research area is shrimp immune system and development of fish DNA vaccines. His team He has many of students in Thailand, Philippines, Vietnam, Indonesia, Singapore, India, South Korea, China, and Columbia.

Japanese Government Committee member

Committee of aquatic medicine (2003~), Committee of veterinary medicine Japan (2005~)

The chair of Committee of aquatic medicine Japan (2011~)

Member of academic societies:

1. The Japanese Society of Fisheries Science
2. The Japanese Society of Fish Pathology
3. International Society for Fish and Shellfish Immunology
4. The Japanese Biochemical Society
5. Asian Fisheries Society
6. American Society for Microbiology
7. The Japanese Society for Marine Biotechnology
8. The Molecular Biology Society of Japan
9. The American Society for Biochemistry and Molecular Biology

Journals

Co-editor in chief of Fish and Shellfish Immunology

Editorial board member of Journal of Fish Diseases

Editorial board member of Review in Fisheries and Fish Biology

**Aquatic vaccines in Japan and studies on fish DNA vaccines**

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**Abstract**

Infectious diseases are serious problems in aquaculture. Vaccination is the most powerful tool to prevent the diseases. There are 16 different types of approved and commercialized fish vaccines in Japan. I will talk the rules and regulations on the use of aquatic medicine in Japan, licensing of aquatic medicine in Japan. I will also introduce studies on development of fish DNA vaccines against RNA and DNA virus and bacterial diseases in Aquaculture. Evaluation of efficacy of vaccine is important for development of vaccines. I will introduce some studies of transcriptome analysis of marine fish.

# Oral Presentation

**Flying fish resourses from southeast coast of India**

Muthukumarasay Srinivasan, Ramalingam Vinothkumar and Jaya praba

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**Abstract**

Indian marine fishery occupies the third position globally and strengthens nation's economic status through receiving foreign exchange. India has 8129 km of stretched coastal line, which has 0.5 million $km^2$ of continental shelf and 2.02 million $km^2$ of Exclusive Economic Zone (EEZ). Flying fish fishery contributes about 1063 tones (0.0336%) of the annual production of 3.16 million tons in 2012. The flying fish fishery is an important seasonal fishery on the east coast of India extending from Chennai to Point Calimere along Coromandel Coast. Beyond this region there is no organized fishery for this species though they have recorded in small numbers elsewhere along the east coast and rarely along the west coast in the month of August. In this study, feeding and breeding biology of *Exocoetus volitans* were carried out based on the gut analysis and gonadosomatic index (GSI). Proximate composition of two flying fishes, *Cypselurus spilopterus* and *Hirundichthys coromandelensis* were also examined. The results showed that *E. volitans* fed mainly on crustaceans, copepods, and mollusks, suggesting that the fish is a carnivorous feeding habit supplemented by a wide variety other food items such as siphonophara, chaetognatha, fish scales. The GSI of *E. volitans* showed that the fish breed from May – July in Cuddalore coast area. Fecundity estimates ranged from 5,032 to 12,010 eggs per female. Least square regression analysis showed that fecundity increased linearly as a function of body length, and weight. Proximate composition study revealed that *Cypselurus spilopterus* had higher protein (20.45%), carbohydrate (0.856%), and lipid (3.6%) contents when compared with *Hirundichthys coromandelensis*. It may be due to the species, size, sex, feeding habit, season, reproductive status etc. The protein content of these two fish species were more or less similar to the protein content of tunas, sardines and mackerel.

Keywords: *India, Flying fish, Feeding, Breeding biology, Proximate composition*

**Culture of Indian pompano, *Trachinotus mookalee* in sea cage in India - A new potential candidate fish for coastal aquaculture and mariculture**

Sekar Megarajan, Ritesh Ranjan, Biji Xavier, Suresh Relangi Durga., Shubhadeep Ghosh, Boby Ignatius and Achamveetil Gopalakrishnan

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**Abstract**

Cage culture of Indian pompano using hatchery produced seeds was carried out in the Bay of Bengal, Visakhapatnam, India. The seeds (2.5±0.02 g and 5.25± 0.04 cm in length) were stocked at 35 nos/$m^3$ in 6 m diameter HDPE cages. Initially fingerlings were fed at 10% of body weight (BW) with diet containing 45% crude protein (CP) and 10% fat twice a day. Fishes were maintained at same stocking densities until they reachedan average BW of 280±0.5 g and thereafter, fishes were stocked into two different stocking densities i.e. 15 and 20 nos/$m^3$ in two different 6 m HDPE cages. Henceforth, fishes were fed with low valued finfishes (sardine, Indian scad and tilapia) at 8-10% of BW twice a day for a culture period of another six months. The study observed that during the initial six months of culture period fishes grew from 2.5±0.02 g to 280±0.5 g with an average FCR of 1.0:1.29 on artificial pelleted feed. In the next six months of culture, fishes stocked at 15 and 20 nos/$m^3$ reached to 769 and 478g with FCR of 1:4.98 and 1:7.48, respectively. The SGR for 15 and 20 nos/$m^3$ were 1.83% and 1.69% per day, respectively with an average survival of 93.6%. The study revealed the fish grows moderately, with good feed acceptance for both pellet & low value fishes in cages. This study is the first of its kind on culture of hatchery produced Indian pompano in cages and it paves the way for further expansion in mariculture and coastal aquaculture production.

Keywords:*Indian pompano, Candidate species, Cage culture, Indian water, Stocking density*

# Growth performance of hilsa (*Tenualosa ilisha*) at varying stocking densities in floating cages at Ukai reservoir, India: A maiden effort

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**Abstract**

The present study forms the first global report on cage farming of hilsa (*Tenualosa ilisha*), which is considered the "king of all fishes" in the eastern part of India and Bangladesh and have attained an iconic status in the region. Six circular 6 m diameter HDPE cages were used for evaluating the growth performance of *T. ilisha* stocked at varying densities in cages installed at Ukai reservoir, Gujarat, India during 2017.Cages were installed using single point revolving mooring system at a water depth of 5 m with net depth of 3.5 m and were spaced 20 m apart. Water quality at the cage site was optimum for cage farming. Mesh sizes of outer and inner nets were 25 mm and 12 mm respectively. Fishes were collected from the wild using mahajal (dragnet) during the early hours and evening hours of the day and were stocked in cages after prophylactic treatment. Initial size of stocking was 127.92±1.38 mm and 19.21±0.18 g. Various stocking densities tested were 8, 16and 24 numbers/$m^3$ in duplicate and the duration of culture was for six months. Fishes were fed approximately 5-7% of biomass twice daily with floating pellets (35% protein and 12% fat). At the lowest stocking density, fish reached 208.67±2.82 mm and 70.75±1.90 g with 47.60% survival rate. In the moderate stocking density, fish attained 205.08±2.79 mm and 64.50±1.88 g with41.73% survival rate. At the highest stocking density, fish reached 197.42±2.12 mm and 60.50±2.04 g with 27.99% survival rate. Growth was allometric and did not differ with stocking density. Average production from the lowest, moderate and highest stocking densities were 26.67 kg, 42.63 kg and 40.23 kg. Weight gain %, weight increment per day and specific growth rate (SGR) were 268.3, 0.28 g and 0.71 at stocking density of 8 numbers/$m^3$; 235.76, 0.25 g and 0.66 at stocking density of 16 numbers/$m^3$ and 214.94%, 0.23 g and 0.62 at stocking density of 24 numbers/$m^3$. Hence, it is concluded, that with more or less similar growth performance at all the three stocking densities, the moderate stocking density of 16 numbers/$m^3$ is superior because of higher production.

Keywords:*Hilsa, Tenualosa ilisha, Cage culture, Growth, Stock density*

**Cage culture of orange spotted grouper, *Epinephelus coioides* off Visakhapatnam Coast: A novel initiative in India**

Ritesh Ranjan, Sekar Megarajan, Biji Xavier, Relangi Durga Suresh, Shubhadeep Ghosh, Imelda Joseph and Gopalakrishnan Achamveetil

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**Abstract**

The present study describes the first trial on cage farming of orange spotted grouper, *Epinephelus coioides* in Indian waters stocked from hatchery produced seeds. Two 6 m diameter HDPE floating cages were installed in the Bay of Bengal off Visakhapatnam coast, Andhra Pradesh, India. The cages were stocked with orange spotted grouper at the rate of 12 and 6 nos/$m^3$having an average length of 12.2±0.28 cm and weight of 30.70±2.34 g. The fishes were initially fed upon floating feed pellet containing 40% protein and 10% lipid at 4-6% of body weight for 3 months. Subsequently, low value fishes were fed to the stocked fishes at 7-10% body weight twice a day. The fishes stocked at 6 nos/$m^3$ and 12 nos/$m^3$ had grown to a size of 1475±49.57 g and 1420±100.31g, respectively with average specific growth rate of 0.97%/day after 13 months of culture period. The percentage survival at both stocked densities was very high with an average percentage survival of 97.85%. Feed conversation ratio during the initial culture period (3 months) was 1.0:1.46 and 1: 6.7 during later culture period. Production of 16.30 kg/$m^3$ and 8.40 kg/$m^3$ was obtained at 12 nos/$m^3$ and 6 nos/$m^3$ stocking densities, respectively. In the present experiment, various growth and survival parameters were not found to be significantly different ($P$<0.05) between the two stocking densities. Even the FCR was found to be similar. However, further experimentation needs to perform with higher stocking densities for optimizing cage culture of orange spotted grouper in Indian waters.

Keywords: *Orange spotted grouper, Cage culture, Growth potential, Indian water*

# R & D efforts in harnessing cage farming technology to increase fish production through mariculture in India

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## Abstract

The fishery sector is a major foreign exchange earner in India's economy and fisheries contribute to about 1.4 % of the Indian GDP and 5% of agricultural GDP. The sector supports livelihood options for about 15 million people in India. The marine fishery resources of the country include a coastline of 8129 km with an Exclusive Economic Zone (EEZ) of 2.02 million km$^2$. The marine fish production in India during 2017 was estimated at about 3.84 million tonnes, which is more than 70% of the harvestable potential. To enable India to take advantage of the opportunity of the vast coastline and other water resources, innovations capable of overcoming supply-side constraints will play a critical role. Mariculture has tremendous potential to impart growth of the fishery industry as well as in generating income and employment. Innovative technology such as cage farming is a more productive system in comparison to farming in ponds - a farmer doing cage farming produces 70 times more production than his counterpart practicing onshore farming in ponds. Policy gaps that limit the development of mariculture in India are being remedied by drafting national mariculture policy for the country for an earlier implementation. Cage farming lessons from other countries with the most successful stories in the technology and support services could enormously increase mariculture productivity and exports in India. The first sea cage was launched in Bay of Bengal off Visakhapatnam coast during May 2007. The initial cage versions were 15 m diameter HDPE cages and of late, the size has been modified to 6 m. These indigenous cages have been found to be successful in the different maritime states along the Indian coast on a series of demonstration farming during 2009-12. Cost effective GI and HDPE cages have been designed for low investment farming operations. Based on different criteria the ideal species for cage culture are: Asian seabass *Lates calcarifer*, cobia *Rachycentron canadum*, pompano *Trachinotus blochii*, *T. mookalee* and orange spotted grouper *Epinephelus coioides* for which hatchery technology has been developed. Among crustaceans, lobster fattening in sea cages has been proved successful. Sea cage farming was carried out by CMFRI in Gujarat, Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh and Odisha. The government of India has been promoting cage farming by blue revolution schemes through developmental agencies like National Fisheries Development Board and maritime states of the country.

Keywords: *Sea cage culture, Asian seabass, Cobia, Pompano, Lobster*

**Growth performance of Asian seabass *Lates calcarifer* in open water cage farms in Kerala, South West coast of India**

Imelda Joseph, Shoji Joseph, Boby Ignatius, Reema George, Binoy Bhaskaran, and K.M. Venugopalan

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**Abstract**

With an annual production of about 1000 tonnes from nearly 400 cages, Asian seabass *Lates calcarifer* is leading in open water cage culture in Kerala, Southwest coast of India. Majority of the farms are spread along three districts of Kerala State *viz.,* Ernakulam, Thrissur and Alappuzha. The spatial mapping of the cages was also carried out during the present study. Cages fabricated with Galvanized iron frame of varying dimensions ranging from 2 x 2 x 2 $m^3$ to 6 x 6 x 4 $m^3$ are being used for farming sea bass in Kerala. The grow-out period for sea bass fingerlings (8-10 cm size) in cages ranges from 6 to 8 months and the most preferred harvest size is 1 kg. The stocking density for fingerlings was 40 to 150 nos/$m^3$ which have been culled to 15 to 35 nos/$m^3$ on reaching 100 g and above. The growth measurements were recorded at fortnightly intervals along with water quality parameters. Fingerlings were fed with pelleted floating feed, containing 35-45% protein. While fish above 50g were fed using low-value bycatch fishes or shrimp. The specific growth rate (SGR) was recorded as 1.68%, 1.105% and 1.302% for 4 x 4 x 3 $m^3$ cages. The average daily growth rate (ADGR) was observed to be ranging from 3.14 to 3.93g. The salinity of the culture sites varied from 0 to 25 ppt, pH from 6.5 to 7.5 and temperature from $25^0$C to $30^0$C. The maximum weight recorded per fish in 8 months of culture in a cage was 2 kg. However, majority of the fish were harvested on attaining 1 to 1.2 kg. The survival ranged from 50 to 80%. The FCR was 1:4 on feeding with low value fish. The production per cage ranged from 200 kg (cage of 2 x 2 x 2 $m^3$) to 2500 kg (6 x 6 x 3 $m^3$). The farm gate price of seabass ranged from US$5.7 to 9-2 $kg^{-1}$. The marketing strategy included on farm sale by arranging harvest festivals, live fish sale at different locations in the cities or by bulk sale to nearby markets. The economics worked out has shown that there was 80 to 120% profit in cage farming of seabass in the open waters of Kerala, southwest coast of India.

Keywords: *Cage farming, Asian seabass Lates calcarifer, Open water, Growth performance*

**Survival, growth and production of Asian Seabass, *Lates calcarifer* in high volume sea cage at Marine Farm at Karwar, Karnataka**

Jayasree Loka[1], Philipose K.K.[1], Praveen Dube[1], Sanjeev Deshpande[1], Navanath Kumbhar[1], Imelda Joseph[2] and Gopalakrishnan, A[2]

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[2]ICAR-Central Marine Fisheries Research Institute, Post Box No. 1603, Ernakulam North P.O Kochi-682 018.Kerala, India

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**Abstract**

An experiment on the survival, growth and production of the Asian seabass, *Lates calcarifer* in 10 m diameter HDPE cage at Marine farm of Central Marine Fisheries Research Centre at Karwar during 2016-2017 was carried out. Nursery reared Asian seabass each weighing 100 g on an average were stocked in the cage with an initial biomass of 1 kg $m^{-3}$. The fish were fed with oil sardine at 8% biomass of fish and growth parameters were monthly recorded. The water quality parameters, such as temperature, salinity, pH, dissolved oxygen, nutrients (phosphate, nitrate, silicate, ammonia and nitrite) were also monitored at cage and reference sites at monthly intervals and found no significant ($P$>0.05) variation between sites. The growth rate (6.67% $day^{-1}$) and survival (84%) were found higher than that recorded earlier in 6 m diameter cages (3.2 % $day^{-1}$and 60% respectively). The production of Asian seabass after 150 days of grow-out culture was 8.8 $kgm^{-3}$. The present study revealed that culture of *L. calcarifer* in HDPE cages is advantageous if initial stocking weight is above 100 g and cage is of high volume.

Keywords:*Asian seabass, Growth, HDPE cages, Marine cage farming, L. calcarifer, Production*

**Participatory farming demonstration of orange spotted grouper (*Epinephelus coioides*) at Kollam District of Kerala, India**

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**Abstract**

Orange spotted grouper (*Epinephelus coioides*) is one of the most important and suitable species for cage farming in Asia-Pacific region. In India, grouper farming gained its momentum after the successful seed production of the orange spotted grouper from Visakhapatnam Regional Centre of ICAR-CMFRI. Seeds from Visakhapatnam Centre were airlifted and reared in nursery at Vizhinjam Research Centre, till they reach suitable size for stocking. The seeds were transported and stocked in 5 rectangular cages with effective volume of 18 $m^3$ each, installed in coastal water bodies of Kollam district. The first group of cage was at Chemmakkad where the fish were fed with low value fishes and pellets and the second group was at Prakkulam where only low value fishes were used as feed. The salinity ranged from 15 to 26 ppt and the temperature ranged from 25 to 30°C. Dissolved oxygen and other parameters measured were within the safe range throughout the culture. The stocking density was 10 nos/$m^3$ and size of fish was 11.5 cm. Fish were grown to an average size of 1.42 kg in 10 months culture period (from September 2017 to June 2018) in second group where only low value fish was used for feeding. Average growth was only 1.1 kg where both pellet and low value fishes were used. Uniform growth was observed in low value fish fed group whereas differential growth was observed and grading was done at two times in the first group. Two times mortality was observed after turbulent weather, followed by isopod infection in both the group of cages. The average price obtained at farm level was Rs. 400/kg.

Keywords: *Cage culture, Orange spotted grouper, Epinephelus coioides, Kollam, Kerala*

**Stock and mass culture of *Bestiolina similis* (Sewell, 1914) - a promising copepod live feed for marine finfish larviculture**

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**Abstract**

Calanoid copepods are the most abundant zooplankton of the sea and form the major food for fish and fish larvae. *Bestiolina similis* is an important and popular species in the copepod family Paracalanidae with very small naupliar stages and reported as suitable for feeding fish larvae. The species, used in this study, has been isolated from the plankton samples collected from Vizhinjam coast and pure culture is being maintained in the stock culture tanks. Naupliar stages of *B. similis* ranged from 65 - 125 µm in length and width ranged from 40 - 95 µm. This is the suitable size range for feeding atresial type of fish larvae of groupers and snappers. *B. similis* grow well in feed mixture of *Nannochloropsis salina* and *Isochrysis galbana. B. similis* possess all the essential qualities of an ideal live feed. This species is hardy, tolerant to wide range of salinity and temperature. *B. similis* is a productive species with the fecundity range from 20-45 eggs/day. Within 15-20 days it can reach highest density in normal mass culture conditions. *B. similis* generally occupy the entire water column evenly; hence this is ideal for feeding even week fish larvae. The stock and mass culture of the species has been standardized at Vizhinjam Research Centre. Experimental trials indicated that *B. similis* perform equally well like *Parvocalanus crassirostris* for first feeding of fish larvae.

Keywords: *Bestiolina similis, Live feed, Copepod culture, Stock and mass culture*

**Identification of SNP in ribosomal protein S6 and ribosomal protein S6 kinase and their association with growth traits of the black tiger shrimp Penaeus monodon**

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**Abstract**

Single nucleotide polymorphism (SNP) of *ribosomal protein S6* (*Rps6*) and *ribosomal protein S6 kinase* (*Rps6k*) in domesticated 3-month-old juveniles of the black tiger shrimp (*Penaeus monodon*) were identified by PCR-direct DNA sequencing. A single SNP was found in each of the amplified $PmRps6_{309}$ (C>$T_{229}$), $PmRps6k_{355}$ (A>$G_{178}$) and $PmRps6k_{515}$ (C>$A_{445}$) gene segments. A rapid method for genotyping of a C>$T_{229}$ SNP in $PmRps6_{309}$ was successfully developed using a gel-based bidirectional PCR amplification of specific alleles (gel-based Bi-PASA). In addition, a SNP detection based on PCR amplification of specific alleles using real-time PCR (real-time PCR-based PASA) was also successfully developed for detection of C>$T_{229}$ of $PmRps6_{309}$, A>$G_{178}$ of $PmRps6k_{355}$ and C>$A_{445}$ of $PmRps6k_{515}$. Association analysis between SNP genotypes and growth parameters (average body weight, BW; total length, TL; hepatopancreatic weight, HPW and hepatosomatic index, HSI) of SNP3A juveniles were examined. For C>$T_{229}$, juveniles carrying a C/$C_{229}$ genotype had a significant greater average BW, TL and HPW than those exhibiting a C/$T_{229}$ genotype ($N$ = 150, $P$ < 0.05). For A>$G_{178}$, SNP3A juveniles with an A/$A_{178}$ genotype had a significantly greater average BW, TL and HPW than those with the A/$G_{178}$ genotype ($N$ = 131, $P$ < 0.05). In contrast, shrimp exhibiting different genotypes of a C>$A_{445}$ SNP did not show significantly different growth parameters ($N$ = 87, $P$ > 0.05).

Keywords: *SNP, Rps6, Rps6k, DNA sequencing, Bi-PASA, Real-time PCR-based PASA*

**Association between single nucleotide polymorphisms (SNPs) of X-box binding protein 1 and growth of the black tiger shrimp *Penaeus monodon***

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**Abstract**

Molecular markers that allow selection of juveniles and broodstock with high growth performance are useful for the shrimp industry. Here, single nucleotide polymorphism (SNP) in *X-box binding protein 1* (*PmXbp1*) of the black tiger shrimp (*Penaeus monodon*) was examined. Association between genotypes of *PmXbp1* and the average body weight and total length of a 3-month-old domesticated stock of *P. monodon* (SNP3A sample established from one female and two males; average body weight and total length = 12.32 ± 0.40 g and 11.30 ± 0.12 cm, $N = 162$) were tested using single-strand conformational polymorphism (SSCP) analysis. The average body weight and total length of juveniles carrying pattern A (corresponding to a $T/T_{477}$ SNP; 15.07 ± 0.76 g and 12.14 ± 0.21 cm, $N = 44$) was significantly greater than that of juveniles carrying pattern B (corresponding to a $T/C_{477}$ SNP; 11.37 ± 0.44 and 10.99 ± 0.14 cm, $N = 116$). The relative expression levels of *PmXbp1* in the hepatopancreas of juveniles carrying different SSCP/SNP genotypes were significantly different (A < B; $P < 0.05$). In addition, the expression level of *PmXbp1* in shrimp exhibiting a greater growth performance (22.08 ± 0.90 g and 13.60 ± 0.17 cm, $N = 11$) was significantly lower than that with a lesser growth performance (5.00 ± 0.31 g and 8.60 ± 0.34 cm, $N = 8$) ($P < 0.05$). Subsequently, SNP polymorphism of *PmXbp1* in the 7th generation of domesticated *P. monodon* was investigated and a $G>A_{456}$ SNP was found by PCR-direct sequencing. A rapid technique for detection of this SNP was developed based on PCR amplification of specific alleles using real-time PCR (real-time PCR-based PASA). Association analysis was tested in four G7 families of 5-month-old juveniles reared in the same concrete pond. Results indicated that juveniles exhibiting a $G/G_{456}$ genotype possessed significantly greater average body weight and total length (15.38 ± 0.40 g and 11.66 ± 0.12 cm, $N = 204$) than those carrying a $G/A_{456}$ genotype (12.75 ± 1.16 g and 10.96 ± 0.31 cm, $N = 32$, $P < 0.05$).

Keywords: *SNP, Penaeus monodon, Xbp1, Growth*

**Reproductive organs of triploid black tiger shrimp, *Penaeus monodon***

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**Abstract**

Triploid induction of *Penaeus monodon* was performed successfully by blocking of the second polar bodies at 8-min post-spawning. The condition of treatment was cold shock at 8 °C with duration of 10 minutes. To investigate the shrimp's chromosome number, flow cytometry was applied at age of 4 months. Shrimp gonad histology and RNA sequencing were examined at age of 1 year. The results showed spermatogenesis abnormality in male reproductive organs of triploid shrimp. Testis contained many seminiferous tubules that produce a large number of spermatogonium and primary spermatocytes while secondary spermatocytes, spermatid and spermatozoa were sparse. Vas deferens contained few abnormal spermatozoa, and spermatophore did not contain spermatozoa. Female reproductive organ (ovary), showed normal morphology. It contained previtellogenic, vitellogenic and cortical rod oocytes. However, RNA sequencing analysis revealed down-regulation of vitellogenin gene cluster in ovary of triploid shrimp. It is possible that triploid *P. monodon* have abnormal reproductive organs.

Keywords: *Histology, Reproductive organs, Triploid, Cold shock, Penaeus monodon*

**A combination of fresh feeds enhances sperm quality of domesticated male black tiger shrimp (*Penaeus monodon*)**

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**Abstract**

Poor reproductive maturation of male captive black tiger shrimp is one of the major problems for shrimp industry. Fresh feeds were commonly used as maturation diet. This study examined effects of fresh feed combinations on sperm quality of male captive broodstock. Five shrimp groups were fed with different feeds: grow-out pellet, broodstock pellet, squids, polychaetes and a combination of polychaetes and squids. Growth rate, spermatophore weight, and total sperm count were measured after a 4-week feeding trial. All three fresh feed groups significantly improved growth rate, spermatophore weight and sperm count when compared to those of pellet groups. The analysis of nutrients and fatty acid profiles of the feeds is needed for future study to identify proper feed composition for successfully enhancing sperm performance and maturation of male captive black tiger shrimp.

Keywords: *Feed combination, Sperm performance, Black tiger shrimp, Penaeus monodon*

**Study on nutrition compositions of protein hydrolysates and a possibility of using them as protein sources for shrimp feed, *Penaeus vannamei***

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**Abstract**

Shrimp aquaculture is one of the economically important industries of Thailand. Feed cost is the major cost of shrimp culture. Up to date, the main ingredient of shrimp feed is fish meal. Declining of fish meal production resulted in the increasing cost of fish meal and shrimp feed. There were many attempts to use other protein sources to substitute fish meal in the shrimp feed. This study aimed to search for an alternative protein resources to substitute fish meal in shrimp feed. Selected raw materials, trash fish, shrimp head and soybean were by product. *Lactobacillus plantarum* was used to hydrolyse trash fish, shrimp head, squid, frozen krill and soybean while baking yeast was used for soybean. In order to reduce the hydrolysate cost, pineapple juice was used as media for *Lactobacillus plantarum* (TISTR 541). Raw materials were grounded and mixed with *L. plantarum* at $1x10^9$ CFU/ml and 1% of baking yeast. The mixing was set for 1 min every hour. At day 11, hydrolysates were filtered and freeze-dried and nutrition compositions were analysed. Average protein percentage of squid and soybean hydrolysate increased in contrast with the other hydrolysates. Total amino acids and attractant amino acids (Ala, Asp, Glu, Gly) of squid, krill and soybean hydrolysates were higher than those of the raw materials. Total unsaturated and n-3 fatty acids in krill hydrolysate were the highest. Saturated, unsaturated and n-6 fatty acids in soybean meal hydrolysate increased compare to soybean meal. Before the fermentation, n-3 in soybean meal could not be detected but it was found in soybean meal hydrolysates. The nutrient compositions of protein hydrolysates could be improved by adjusting the processing conditions, period, and the amount of the bacteria.

Keywords: *Trash fish, Krill, Squid, Shrimp head, Soybean, Yeast, Lactobacillus plantarum*

**Effect of herbal plants supplemented diet on the growth of a commercial carp, *Labeo rohita***

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**Abstract**

This study was conducted to evaluate the effect of herbal plants supplemented diets on the growth performance; total length and body weight of *Labeo rohita*. The fingerlings of *L. rohita* (mean body weight 5.07 ± 1.21 g; mean total length 6.01 ± 0.95cm) were fed on 5% herbal plants supplemented diet for 60 days. Three herbal plants: Amla (*Phyllanthus emblica*) (T1), Marshmallow (*Althaea officinalis*) (T2) and Peelak / Peelu plant (*Solanum nigrum*) (T3) were used. The treated feed was given to the fish at the rate of 2% body weight. Our results indicated that *L. rohita* fingerlings exhibited significant increase in total length and body weight ($P < 0.05$). The maximum increase in total length (6.57 ± 0.11cm) (107.70%) was in T3 and the lowest increase in T1 (5.06 ± 0.11 cm) (82.95 %) in T1. The maximum body weight was gained in T3 (28.41 ± 0.43 g) (560.07%) and lowest weight gain (16.42 ± 0.25 g) (323.69 %) was in T1. It may be concluded that the addition of herbal plants especially *Solanum nigrum* and *Althaea officinalis* in fish feed, can act as an important growth promoter.

Keywords: *Growth, Weight gain, Plants extract, Feed*

**Efficacy of *Pueraria candollei* extract to digestive system and growth performance in marble goby (*Oxyleotris marmorata*)**

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**Abstract**

*Pueraria candollei*, in Thai as white kwao krua, was reported high levels of phytoestrogens; miroestrol and deoxymiroestrol. In human, phytoestrogens have huge benefit in term of nutritional supplement and pharmaceuticals. In this study, we produced its two extracts with aqueous base (WKK-A) and ethanol base (WKK-E). Compositions in our extract products were identified. For indirect ELISA to measuring miroestrol, WKK-A had 23.23 ± 1.39 ug and WKK-E had 52.23 ± 4.47 ug. HPLC analysis showed that WKK-A mainly consisted of daidzin, glycitin, genistin and malonyl genistin. Whereas WKK-E mainly consisted of malonyl daidzin and malonyl glycitin. For experiment, juveniles were fed with blood worm (N), commercial feed (C) and commercial feed supplemented with 0.5 % extracts. Digestive tract and growth parameters (WG, FE, SGR) were performed at 4, 8 and 12 weeks after feeding trial. The histology of digestive tract and 4 digestive enzymes (amylase, lipase, trypsin and chymotrypsin) were investigated. Protein profile was revealed by 2D–PAGE. It illustrated that enzyme activities showed no different in all experimental groups. Whereas growth parameters and intestinal villi histology were gradually better than control groups (N and C). Therefore, we concluded that our WKK extracts have helpful to increasing growth in marble goby. This will be benefited to develop growth enhancing feed for marble goby and in other fish feeds as well.

Keywords: *Pueraria candollei, Marble goby, Digestive system, Growth, Muscle growth*

# Growth performance and microbiota of Pacific white shrimp *Litopenaeus vannamei* reared in Biofloc systems using different carbon sources

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**Abstract**

Biofloc rearing system has several advantages including efficiency of nitrogenous waste treatment and improving nutrition and health of cultured animals. However, its potential in manipulating the microbiota have yet to be explored. This research examined effects of biofloc using three carbon sources (molass, cassava starch, and rice bran) on growth, water quality and microbiota fingerprinting in post larvae15 (PL15) of the Pacific white shrimp (*Litopenaeus vannamei*) compared with recirculating aquaculture system (RAS). The trial was conducted in triplicate with 100 shrimp per tank for 28 days. Results clearly indicated that growth performance and microbiota patterns (using automated ribosomal intergenic spacer analysis) were affected by the rearing system and carbon sources. Shrimp reared in biofloc treatments had significantly higher final weight and survival rate, and better feed conversion ratio than that in the RAS system ($P < 0.05$). Among the biofloc treatments, shrimp in the rice bran source treatment had the best growth performance but resulted in the poorest water quality parameters as shown by high total bacterial count and ammonia nitrogen levels. Microbiota in both shrimp intestines and rearing water in the RAS system was significantly different than that of the biofloc groups (ANOSIM, $P< 0.0005$). Specific bacteria (according to ARISA operational taxonomic unit) appeared to be associated with different carbon sources for biofloc. Further analysis of the microbiota using next generation sequencing (NGS) of the *16S rRNA* gene reveal the detailed differences among treatments. The potential for biofloc system in manipulating the microbiota and promoting growth performances is useful to increase the culture efficiency of *L. vannamei* in the future.

Keywords: *Biofloc system, Pacific white shrimp, Microbiota, ARISA*

**Development of LAMP technique for detection of scale drop disease virus in Asian seabass**

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**Abstract**

In this study, we developed a rapid detection for scale drop disease virus in Asian seabass (*Lates calcarifer*) by a loop-mediated isothermal amplification (LAMP) technique.The LAMP reactions were performed at the optimized temperature of 65°C for 60 minutes to amplify SDDV-Adenosine Triphosphatase (*ATPase*) gene. The reaction mixture of 25 µl consisted of 0.2 µM outer primers (F3 and B3), 2 µM inner primers (FIP and BIP) and loop primers (LF and LB), 1.6 mM of dNTP mix, 0.8 M betaine, 6 mM $MgSO_4$, 8U of *Bst* DNA polymerase, 1x of the supplied buffer. The limit of detection when using total DNA from SDDV infected Asian seabass DNA as template was 100 times more sensitive than nested PCR technique. The nucleic acids of 12 other shrimp and fish pathogens were not amplified by this LAMP technique, indicating that it was specific for SDDV. Application of the new test will be useful in assessing the prevalence and impact of SDDV infections on the production of Asian seabass cultured in Thailand.

Keywords: *Loop-mediated isothermal amplification (LAMP), Scale Drop Disease Virus (SDDV), Asian seabass*

**Attempt protection from *Streptococcus iniae* infection using incorporated feed and top-dressed feed vaccines in red hybrid tilapia (*Oreochromis* sp.) fingerlings**

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**Abstract**

Intensification of tilapia farming to achieve higher productivity has resulted in an increase of susceptibility of the fish towards diseases. Streptococcosis is one of disease in concerned as it had caused high economic losses in aquaculture worlwide. Vaccination would be an ideal method to prevent disease outbreak and it can be done through oral vaccination. Although this method is economical, the effectiveness is still questionable. This study was conducted to study the efficacy of incorporated feed vaccine and top-dressed feed vaccine against *Streptococcus iniae* using bacterin in tilapia fingerlings. Group 1 was vaccinated with the incorporated-feed vaccine; while Group 2 was vaccinated with top-dressed feed vaccine and Group 3 serves as the control. Serum, body mucus and gut lavage from each fish group was collected weekly and subjected to indirect-ELISA to assess the antibody response. All fish were challenged in week 4 and the protective capacity was observed for 14 days post-challenge. Results from indirect ELISA showed that there is significant different ($P < 0.05$) between vaccinated group and unvaccinated group. In addition, the protective immunity of vaccinated group was excellence following challenge trial as compared to an unvaccinated group based on the development of clinical sign of streptococcosis. In general, oral vaccination of feed-based vaccine confers protective immunity to a certain extend. However, it can be improved to increase its effectiveness with some modifications.

Keywords: *Red hybrid tilapia, Streptococcus iniae, Incorporated feed vaccine, Top-dressed feed vaccine, ELISA*

**Development of a multiplex recombinase polymerase amplification (RPA) assay for rapid and sensitive detection of $VP_{AHPND}$ and EHP in shrimp**

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**Abstract**

Shrimp aquaculture used to be a profitable industry until disease outbreaks occurred worldwide, particularly in Thailand. Recently, the unique isolate of *Vibrio parahaemolyticus* ($VP_{AHPND}$), causative agent of acute hepatopancreatic necrosis disease and *Enterocytozoon hepatopenaei* (EHP), causative agent of hepatopancreatic microsporidiosis are the major pathogens of common penaeid species cultured commercially. Here, we developed a multiplex recombinase polymerase amplification (RPA) assay for the rapid and simultaneous detection of $VP_{AHPND}$ and EHP in shrimp. The primers were designed from AHPND toxin and spore wall genes to specifically amplify the DNA fragments of 140 and 200 bp of $VP_{AHPND}$ and EHP, respectively. The optimal condition for the multiplex RPA assay was 38°C for 30 min. By gel electrophoresis, it was found that the limit of detection (LOD) for this method was $10^4$ copies each of DNA plasmids containing $VP_{AHPND}$ and EHP targeted genes. There was no cross reaction with DNA templates derived from other pathogens commonly found in shrimp ponds. Further, to reduce the time and cost of instrumentation, a visual detection using lateral flow dipstick (LFD) will be developed for interpreting the multiplex RPA amplification results instead of the gel electrophoresis. The new multiplex RPA-LFD assay will reduce assay time and easy format for read the results since both pathogens can be diagnosed in a single reaction. It will be useful for the control of these shrimp pathogens for on-site field applications in Thailand and worldwide.

Keywords: *AHPND, EHP, Recombinase polymerase amplification, RPA, Multiplex detection*

**Parasites infection in seabass, *Lates calcarifer* (Bloch, 1790) in Earthen pond culture at Surat Thani province**

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**Abstract**

The Asian seabass *Lates calcarifer* (Bloch, 1790) is an economically important fish species in the tropical and subtropical regions of the Asia-Pacific area. Diseases are major problems affecting low production efficiency of Asian seabass in Thailand. In this study, species origin of ectoparasites and the infection rates in *L. calcarifer* cultured in earthen ponds were examined. Asian seabass cultured at Klongcha-nak Sub-district, Muang district, Surat Thani Province were monthly collected during August to December 2016 ($N = 20$ per month). Parasite availability was examined externally. In addition, internal organs (digestive tract, liver, gall bladder and spleen) of each fish were also dissected out and the appearance of parasites were also examined. For overall study period, 37% of examined specimens (37/100) were infected by at least one parasite. Two ectoparasites including *Laticola paralatesi*, monogenean parasite and *Trichodina compacta*, a protozoan parasite, were found on gills and skin, respectively. These parasites may cause irritation and inflammation of either the body surface or gill filament. The highest prevalence of infection, mean intensity and abundance were observed in December where the infection rate was 45 (9/20) and 55% (11/20) for respective parasites and *Trichodina compacta* (183.15 parasites per fish) was more abundance than *Laticola paralatesi* (5.8 parasites per fish). In contrast, endoparasite was not found in all examined specimens. Ectoparasite infection is one of the important factors affecting fish health and good management practice is required to promote the health status of cultured seabass.

Keywords: *Lates calcarifer, Laticola paralatesi, Trichodina compacta, Earthen pond culture*

**Development of 9-plex bead array for immune gene expression analysis in the black tiger shrimp, *Penaeus monodon***

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**Abstract**

A bead-based array technology can be applied as a tool for detecting multiple target genes in a single sample, which will reduce time, labor and cost of analysis. In this work, we developed a 9-plex bead-based array as an approach to detect the transcription levels of multiple immune-related genes in the black tiger shrimp. The oligonucleotide probes were designed with a length of 50 bases to target eight immune-related genes whose products function as antimicrobial peptides (*alf1*, *alf3*, *pen3a*, *crusPm7*), melanization (*proPO1*), pathogen recognition proteins (*lec*, *LGBP*), and lysozyme (*lyz*) and one internal control reference gene (*EF-1α*). Each gene-specific probe containing a 5'-amino linker and a C12 spacer was coupled to a carboxylated-magnetic fluorescently barcoded bead set. The bead array comprising nine sets of oligo-linked barcoded beads was evaluated for its specificity and multiplexity. The 9-plex bead array was applied to determine transcript levels of these immune genes in gills in the time-course *Vibrio harveyi*-challenged shrimp and the control shrimp groups. To validate results from the bead array, gene expression levels were determined by a real-time PCR technique. The gene expression patterns from real-time PCR analysis were consistent to those of the bead array. Our multiplex bead-based array assay was proven useful as a high-throughput tool to evaluate molecular effects of shrimp immune-related genes in different feed additives.

Keywords: *Bead array technology, Multiplex detection, Shrimp immune responses, Gene expression*

**Efficacy of dietary formalin-killed *Vibrio harveyi* to improve survival of the Pacific white shrimp through induction of granular-type hemocyte**

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**Abstract**

The goal of our study is to develop a strategy to improve shrimp survival rate after bacterial infection through immune priming phenomenon. Shrimp were oral administrated with different concentrations $10^8$ to $10^{12}$ cells/g feed of formalin-killed *Vibrio harveyi*-supplemented feed (FVh) for a period of 10 days before *V. harveyi* challenge. The group that received FVh $10^{12}$ cells/g feed showed improvement of survival rate to 40% post challenge, whereas the control group fed with a commercial feed showed 0% survival. The degree of protection was correlated with the significant induction of total hemocyte count (THC), granular cell count (GC) and the transcriptional level of cytosolic manganese superoxide dismutase (cMn-SOD). Taken together, our results suggest that primed-shrimp with FVh significantly improved the survival rate after challenge with *V. harveyi.* The knowledge from this study will lead us to design a strategic approach to control bacterial disease outbreak in shrimp industry.

Keywords: *Formalin-killed Vibrio harveyi, Oral administration, Vibrio harveyi, Total hemocyte count, Granular cell, Superoxide dismutase*

**Evaluation of antibiotic resistance profiling in EMS-causing *Vibrio parahaemolyticus***

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**Abstract**

*Vibrio parahaemolyticus* is the major cause of early mortality syndrome (EMS) in shrimp. Infected shrimp cannot uptake nutrients and show mortality up to 100% within 30 days. EMS-causing *V. parahaemolyticus* can be classified into 2 groups based on histological signs; acute hepatopancreatic necrosis syndrome (AHPND) and non-AHPND strains. Only AHPND strains produce PirAB toxins which damage hepatopancreatic and stomach tissues in shrimp. The use of antibiotics in aquaculture is common for prevention and treatment of diseases. A number of antibiotic-resistant *V. parahaemolyticus* strains are found and appear to increase in clinical and veterinary fields. The investigation of antibiotic resistance profiling is important for drug selection and estimation of antibiotic resistance incidents. Here, we determined antibiotic resistance profiling of EMS-causing *V. parahaemolyticus* and non-pathogenic *Vibrio* strain. 5HP and 2HP were selected as models for AHPND and non-AHPND strains, respectively. S02 was used for a non-pathogenic strain. Antibiotic resistance profiles of *Vibrio* spp. were determined by PCR-based method and disk diffusion. Five drug-resistant genes were tested by PCR. Twelve drugs were tested with 3 strains of *V. parahaemolyticus* by disk diffusion. The results showed that 3 strains of *V. parahaemolyticus* were PCR positive for chloramphenicol-resistant gene *catA1*. For disk diffusion, 5HP are resistant to ampicillin, penicillin G, and streptomycin. 2HP are resistant to penicillin G and streptomycin, and exhibits intermediate resistance to ampicillin and gentamycin. Non-pathogenic S02 is resistant to streptomycin and exhibits intermediate resistance to ciprofloxacin. Our data demonstrates evidence of antibiotic resistance in EMS-causing *V. parahaemolyticus* in Thailand.

Keywords: *Vibrio parahaemolyticus, EMS, AHPND, Antibiotic resistance*

**Environmental-friendly antiviral microalga *Chlamydomonas reinhardtii* producing dsRNA**

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**Abstract**

Using of specific double-stranded (ds) RNA to trigger RNA inference machinery has been proved its high effectiveness for shrimp viral diseases protection. However, delivering system is a bottleneck of implementing this technology. Chloroplast transformation of edible microalga *Chlamydomonas reinhardtii* was exploited as an alternative dsRNA-production platform since its photosynthesis capability could be used for being transformation marker. This research aimed to study feasibility of using *C. reinhardtii* chloroplast for producing dsRNA in order to make antiviral feed. Double-stranded RNA expression cassette for chloroplast transformation consisted of two convergent *psaA* promoters covering shrimp yellow head viral (YHV) gene which was then cloned into vector carrying photosynthetic restoring *psbH* gene. The recombinant plasmid was transformed into *psbH* deficient *C. reinhardtii* strain and selected successful transformed cell by selective media. After purified dsRNA from late-log phase transgenic culture, RT-qPCR suggested 16.0±0.9 ng specific dsRNA could be produced. Protection efficiency assay of transgenic microalgae was performed on post-larval shrimp. Post-larval shrimp received dsRNA-expressed algal cells ($5x10^5$ cells/ml seawater) 3 days prior to YHV infection had 50% survival at 8 day-post infection (dpi) while non-specific and positive controls showed 100% mortality. RT-PCR using viral specific primers revealed deviation of YHV-infected level in all groups with infection percentage of dsRNA-expressed algae group was at 55.6 ± 11.1%, while those of positive and non-specific groups were 88.9 ± 11.1% and 100.0 ± 0.0%, respectively. These results presented success of using microalgae to produce antiviral dsRNA in aquaculture, and this production platform is environmentally friendly without antibiotic resistance gene contamination.

Keywords: *Chlamydomonas reinhardtii, Double-stranded RNA, Shrimp diseases, Yellow head virus, Chloroplast transformation*

# Controls of shrimp Vibrio diseases through the use of inhibitors of Vibrio bacteria biofilm formations

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**Abstract**

Many bacteria, including *Vibrio* pathogens of shrimp, need to colonize and/or form biofilms in hosts or the environment to cause disease. Thus, one possible control strategy for shrimp vibriosis is biofilm inhibition. With this objective, ginger extract and extract from the Japanese fermented soybean, Natto (produced using *Bacillus subtilis var Natto* called B-Natto) were tested for growth and biofilm inhibition with *Vibrio harveyi* (VH) that causes luminescent shrimp disease and with *V. parahaemolyticus* (VP) 3HP that causes acute hepatopancreatic necrosis disease (AHPND). Using 96 well micro titer plates coated with 0.4% chitosan, we found that biofilm formation by both VH and VP was inhibited by both ginger and natto extracts, while growth in parallel broth cultures was not. When these extracts, whole Natto product or B-Natto cells were mixed with feed and given to the whiteleg shrimp *Penaeus vannamei* post larvae before immersion challenge with VP at $10^6$ cfu/ml, survival was significantly higher ($p \leq 0.05$) than for control shrimp given feed without these additives. Both extracts and natto supplemented feed were also found to promote shrimp growth while feed supplemented with *B. subtilis* cells alone did not. Further work was done based on previous contradictory reports that D-amino acids may signal biofilm disassembly and inhibit or degrade biofilms formed by the bacteria *B. subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Of 9 synthetic D-amino acids tested individually or in combination, none were found to inhibit cell growth or biofilm formation. Similar experiments conducted with three compounds known to occur in ginger extracts (6-gingerol, 8-gingerol, and 6-shogaol) revealed that 6-shogaol was the most potent biofilm inhibitor followed by the others in descending order. None were found to inhibit bacterial growth in broth. The results of this study suggested that the use of feed additives that inhibit biofilm formation may constitute a practical approach to reduce the negative impact of vibriosis and AHPND in shrimp aquaculture.

Keywords: *Vibriosis, AHPND, Biofilm formation, Biofilm inhibition, Fermented soybean, Bacillus subtilis, Feed supplement, Penaeus vannamei*

**A comprehensive study of shrimp acute hepatopancreatic necrosis disease (AHPND)**

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**Abstract**

Shrimp aquaculture is a globally important industry. However, serious shrimp diseases, including acute hepatopancreatic necrosis disease (AHPND) and white spot syndrome virus (WSSV), are currently threatening this industry and causing huge economic losses. To prevent disease outbreaks, basic research is essential to understand mechanisms underlying function in healthy shrimp and how they are altered in disease. We propose to use multidisciplinary studies combining transcriptomics, metabolomics and microbiomics for elucidating pathogen-host-environment interactions in shrimp. We expect to use this knowledge to establish a functional balance among shrimp, microbiome, environment and pathogen[s]. In this project, we are using cutting-edge techniques to develop new knowledge regarding mechanisms relating to AHPND outbreak in white shrimp culture. First, using comparative genomics, the pathogenesis of AHPND-causing *Vibrio parahaemolyticus* with a virulence plasmid was characterized. Critical changes in microbial diversity and bacteria species-to-species connectivity in shrimp stomach was first detected with microbiomics. In addition, the putative factor and mechanisms related to pathogenicity of AHPND-causing bacteria were revealed by transcriptomics, implicating migration of Pir toxin and *V. parahaemolyticus* across the epithelial barrier of the stomach into the hepatopancreas. An exaggerated immune response and metabolic shift may damage the hepatopancreas. Taking all things together, we propose a novel model involving cross-talk among shrimp immunity, AHPND-causing bacteria and gut microbiome.

Keywords: *AHPND*, *WSSV*, *Vibrio parahaemolyticus*, *Shrimp immunity*, *Microbiome*

**Impacts of climate change on the tilapia value chain from cage culture in Luzon, Philippines**

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**Abstract**

The impacts of major climate change hazards experienced by tilapia value chain players in Luzon, Philippines were analyzed. The impacts were estimated in terms of direct and indirect effects on the economic activities across key segments in the value chain. The direct effects involve either damage to operating inputs, fixed assets, and outputs or defensive expenditures incurred by players in trying to prevent or minimize the potential impacts or both. Indirect effects were captured through the cascade of impacts to other players from players directly affected, mainly through input and output prices. Using the value chain approach, the paper highlighted the economic viability of cage culture activities as well as the associated risks along the tilapia chain. Data were collected mainly through key informant interviews with key players in the production and trading nodes of the value chain, local government units, and Bureau of Fisheries and Aquatic Resources. Result pointed out that direct impacts are largest in the production segments of the value chain, i.e. input provision from ponds and production culture in cages. Under extreme weather conditions, losses among players manifested either through reduced volume in tilapia produced/traded or diminished value at times of excessive supply/harvest of smaller sized tilapia. While share of cage culture operators is the largest among chain players, they also bear the highest share in the risk and losses in the face of climate change hazards. Various adaptation strategies practiced by value chain players were also noted.

Keywords: *Climate change impacts, Value chain, Tilapia cage culture, Adaptation strategies*

**Willingness to accept compensation for the establishment of mariculture operation in selected coastal communities in The Philippines**

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**Abstract**

This paper focused on the willingness to accept (WTA) compensation for the reduction of coastal activities (i.e. fishing, gleaning, and recreation) due to the establishment of mariculture (fish cage/pen) operation. Data were collected from 785 small scale fishing and non-fishing households from villages inside and outside of the declared mariculture sites in seven municipalities in the Philippines in January to August 2015. Five bid amounts were drawn from 48 focus group discussions with 315 participants in the study sites and randomly assigned to the same number of survey participating households. Results showed that 47% were not supportive of mariculture and not willing to accept compensation while 53% were willing to accept compensation. When classified by type of household, 54 % of the 489 fishing households and 51 % of the 296 non-fishing households were willing to accept compensation amounting to, on average, Php186/day and Php182/day, respectively, for five years from the establishment of mariculture area. When the households were classified by distance to the mariculture area, no difference in terms of proportion (53 %) of both the 378 households within and 407 households outside the mariculture area and amount willing to accept as compensation (Php 189/day) was found. These imply that both the fishing and non-fishing households or those households living within or outside of the mariculture area see the impact of the mariculture on their activities. This has to be considered in the design mariculture areas to be established in the country.

Keywords: *Fish cages, Willingness to accept, Philippines*

**Evaluation of Malaysia marine fishery sustainability in strengthening national food security**

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**Abstract**

This study aims to present a document review of sustainable marine fishery in Malaysia to strengthen national food security. The increase in Malaysia population is parallel with world population growth that is expected to reach 9.6 billion by 2050. This situation needs a stable marine fisheries resources. However, the trend shows landing marine fishes around the world is increasing at a small scale or almost constant compared to the population growth. This study conducted content analysis on factors contribute to marine fisheries sustainability. The study presenting the major works by past literature, how their works could support future studies, and what aspects need to be enhanced for the marine fisheries sustainability. The comprehensive review has provided an in depth understanding on factors affecting sustainability of marine fisheries in Malaysia such as resource management, climate change and economic growth.

Keywords: *Fisheries economic, Food security, Marine fishery, Sustainability*

## Cage Culture of Monosex Tilapia for Food and Financial Security

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**Abstract**

In Thailand, cage culture is becoming common in canals, rivers, reservoirs and lakes mainly with the booming monosex tilapia farming. Monosex-male tilapia has several advantages i.e. it grows fast, does not breed, and can be stocked at high densities. More importantly, its meat has no Y-bones, which makes it suitable for making fillets for export. Most Thai farmers use standard size of cages either 5m x 5m or 6m x 4m with 2m in depth. They stock about 2,000 juveniles (30-40g)/cage. Farmers harvest about 1 ton in 5-6 month with the size ranging from 0.6 to 1 kg. A cage gives approximately US$1,000 net profit/crop. This cage culture technology expanded rapidly spreading even to other countries such as Bangladesh. About 4,000 cages were installed during 2005-2011. Recently, India is opening the door for cage culture in lakes and reservoirs. Its going to be a big boom there. In Vietnam, cage culture of tilapia is expanding. In Indonesia, Malaysia, Zambia, Zimbabwe etc. tilapia is grown in large-circular cages used by Salmon farms. Cage diameters often reach over 20m and production up to 100 tons/cage. Some countries are still skeptical in allowing full-fledge cage culture e.g. Nepal where fish are still not allowed to be fed with pellets thinking that it will pollute the water. This paper presents recent developments and contribution to food security, and also discusses about environmental issues, which are required as more cage culture is expected because aquaculture production needs to be double by 2050.

Keywords: *Monosex tilapia, Cage culture, Food Security, Environment*

**Effect of extenders and cryoprotectants on viability of spermatogonia and oogonia of striped catfish (*Pangasianodon hypophthalmus*)**

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**Abstract**

Cryopreservation of germ cell in fish would provide a useful tool to preserve genetic resources for further restoration via germ cell transplantation. The objective of this study is to develop a cryopreservation method for spermatogonia and oogonia of striped catfish (*Pangasianodon hypophthalmus*). The interaction between three extenders [Calcium free - Hanks' balanced salt solution (CF-HBSS), extender RT and Leibovitz's L-15 medium (L-15)] and two cryoprotectants [dimethyl sulfoxide (DMSO) and ethylene glycol (EG)] on the viability rate were examined. The immature testis or ovary was collected from juvenile fish and frozen by decreasing temperature at 1 °C/min until -80 °C. Subsequently, the frozen testis or ovary was transferred to liquid nitrogen. The cryopreserved testis was dissociated, and the viability of spermatogonia and oogonia were determined using trypan blue staining. The highest viability rate of cryopreservation of spermatogonia was achieved by using a combination of L-15 with DMSO. The highest viability rate of cryopreservation of oogonia was achieved by using a combination of CF-HBSS, extender RT or L-15 with DMSO.

Keywords: *Cryopreservation, Spermatogonia, Oogonia, Striped catfish, Pangasianodon hypophthalmus*

**Recombinant *Saccharomyces cerevisiae* expressing delta 6 desaturase of Nile tilapia (*Oreochromis niloticus*)**

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**Abstract**

Delta 6 desaturase (Δ6) is an enzyme involved in long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis. This study was aimed to produce recombinant *Saccharomyces cerevisiae* expressing Δ6 from Nile tilapia (*Oni-Δ6*). Three ubiquitous promoters including translational elongation factor (*TEF*), actin (*Act*) and phosphoglycerate kinase (*PGK*) promoters were compared the expression level to drive *Oni-Δ6*. Expression vectors carrying *Oni-Δ6* driven by *TEF*, *Act* or *PGK* promotors were constructed and transformed into *S. cerevisiae* to generate recombinant yeast RY-*TEFΔ6*, RY-*ActΔ6* or RY-*PGKΔ6,* respectively. For control yeasts, the recombinant RY-*TEF*, RY-*Act* or RY-*PGK* which contained each promoter (without *Oni-Δ6)* were produced. The results showed that, using RT-PCR, mRNA of *Oni-Δ6* were observed in these recombinant RY-*TEFΔ6*, RY-*ActΔ6* and RY-*PGKΔ6,* indicating that these recombinant yeasts could express the *Oni-Δ6*. Exogenous substrate C18:2n6 and C18:3n3 were used to determine the Δ6 activity. The recombinant yeast RY-*TEFΔ6*, RY-*ActΔ6* and RY-*PGKΔ6* exhibited the Δ6 activity by converting C18:2n6 into C18:3n6 and C18:3n3 into C18:4n3. The recombinant RY-*TEFΔ6* had highest Δ6 activity. No detectable of Δ6 activity in non-transformed yeast and the control recombinant (RY-*TEF*, RY-*Act* and RY-*PGK*). Therefore, recombinant *S. cerevisia*e expressing *Oni-Δ6* driven by *TEF* promoter have potential as a yeast factory for the sustainable production of LC-PUFAs.

Keywords: *Nile tilapia, Oni-D6, Recombinant yeast, ACT promoter, PGK, TEF*

**Single Nucleotide Polymorphism (SNP) in molt-inhibiting hormone 1 gene and its association with growth parameters of the black tiger shrimp *Penaeus monodon***

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**Abstract**

Molecular markers linked to commercially important phenotypes are useful and can be applied for selection of juveniles and broodstock with desired traits in breeding programs of economically important species. In this study, nucleotide sequences of *molt-inhibiting hormone 1* of *Penaeus monodon* (*PmMIH*) from five families of the fifth generation (G5) of domesticated *P. monodon* were sequenced and multiple aligned. Several SNP were found. Sequence analysis using the NEBCutter program suggested that a G>$T_{217}$ SNP can be simply analyzed by restriction analysis with *Bst*API. As a result, association analysis between *PmMIH* genotypes and the body weight of a domesticated G7 stock (four full-sib families of 5-month-old juveniles cultured in the same concrete pond) were investigated. Juveniles exhibiting a G/$T_{217}$ genotype (15.52 ± 0.42 g, $N$ = 161) had a significant greater average body weight than those exhibiting a G/$G_{217}$ genotype (13.73 ± 0.74, $N$ = 82). Subsequently, additional specimens from 19 full-sib families of G7 juveniles cultured in 5 different concrete ponds (4, 4, 4, 4 and 3 families/pond, $N$ = 60 for each pond) were further examined. Results were consistent when the data were combined to cover all examined samples (18.11 ± 0.63 g for G/$T_{217}$ juveniles and 15.90 ± 0.77 g for G/$T_{217}$ juveniles; $N$ = 314 and 159, respectively). Our results indicate the potential of SNP of *PmMIH* for differentiation of fast from slow growing juveniles in the selective breeding program of *P. monodon*.

Keywords: *SNP, Penaeus monodon, MIH, PCR-RFLP*

**Molecular mechanisms of reproductive maturation in the black tiger shrimp (*Penaeus monodon*) through transcriptomic analysis**

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**Abstract**

Sustainability of shrimp farming has always been threatened by poor reproduction in domesticated broodstock. To overcome the threat, several efforts have been made to decipher molecular mechanism necessary to improve reproductive maturation. Functional genomic studies have been employed to shed lights onto molecular mechanisms of desired traits in various organisms. However, with lack of genome sequence of a non-model organism like *Penaeus monodon*, transcriptomic analysis becomes a method of choice to overcome the challenge of functional genomics. This study will employ several transcriptomic techniques such as microarray and next-generation sequencing to reveal genes and pathways potentially relevant to reproductive maturation. Results from some case studies such as effects of eyestalk ablation in female shrimp and effects of polychaetes to enhance male reproduction will be overviewed.

Keywords: *Transcriptomics, Microarray, Pyrosequencing, Reproduction, Penaeus monodon*

**Identification of G-protein coupled receptor from transcriptomics of premolt Y-organ in mud crab, *Scylla olivacea***

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**Abstract**

Mud crab*, Scylla olivacea*, progresses the life cycle through the periodic shedding of the exoskeleton known as molting. Molting is complex mechanisms that are hormonally regulated: stimulated by steroid hormone (ecdysteroids) while suppressed by molt-inhibiting hormone (MIH). MIH is produced in the X-organ, stored and released from the sinus gland to suppress Y-organs for ecdysteroidogenesis. Previous studies suggest that the responsiveness of Y-organ to MIH needs to be considered because an increasing of intracellular $Ca^{2+}$ *via* the rapid-signaling pathway of ecdysteroids overrides effects of MIH. This circumstance cannot be occurred through the classical nuclear steroid receptors. It is hypothesized that the rapid ecdysteroid responses are mediated through putative ecdysteroid receptor G-protein coupled receptor (ErGPCR). Therefore, this study aims to isolate and examine the expression profile of *ErGPCR* in Y-organ of *S. olivacea*. Our transcriptome results in premolt Y-organ revealed that the levels of GPCR: moody-like and methuselah (Mth)-2 increase specifically in Y-organs from the premolt stage and those administered with 20-hydroxyecdysone. After molecular cloning, we have isolated two isoforms of *GPCR*: *SoGPCR1* consisting of 2,110 bp (557 aa: predicted Mw = 62.8 kDa) and *SoGPCR2* containing of 2,446 bp (463 aa, predicted Mw = 52.07 kDa). Real-time PCR analysis indicated that the levels of both *SoGPCR1* and *SoGPCR2* significantly increase in the premolt stage.

Keywords: *G-protein coupled receptor, Molt, Ecdysteroid receptor, Transcriptomics, Scylla olivacea*

# Development of microsatellite markers in the white scar oyster (*Crassostrea belcheri*) using next generation sequencing technology

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**Abstract**

The white scar oyster *Crassostrea belcheri* is one of economically important species in Thailand. To improve the management efficiency of *C. belcheri* production, appropriate molecular markers are needed to be developed. In this study, microsatellite markers were developed and characterized from the next generation sequencing (NGS) database of *C. belcheri*. In total, 60 microsatellite loci were tested and 50 loci (83.33%) generated the amplification products against the genomic DNA of *C. belcheri*. Of these, 22 loci (44%) were polymorphic and subsequently screened to test against genomic DNA bulks (10 individuals for each bulk) of *C. belcheri* collected from 7 different geographic locations (i.e. Chanthaburi, Trat, Surat Thani, Ranong, Trang, and 2 locations from PhangNga). Eighteen loci generated the positive amplification products and revealed polymorphisms among 7 geographic samples. All informative microsatellite markers preliminary screened through the bulk segregant analysis (BSA) provide a valuable genetic information to facilitate the further research on population genetics and stock management of *C. belcheri*.

Keywords: *Crossostrea belcheri, Illumina paired end sequencing, Microsatellite, polymorphism*

# Characterization of Insulin-degrading enzyme and association between its SNP and growth parameters of the Pacific white shrimp *Litopenaeus vannamei*

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## Abstract

The insulin-like hormone superfamily is composed of insulin, relaxin, and insulin-like growth factors which regulates cell growth and metabolisms. The presence of insulin-like proteins has been demonstrated in various invertebrates and their function as growth promoting or controlling factors has been reported in mollusks and insects. Here, the full-length cDNA of *insulin-degrading enzyme* (*Ide*) of Pacific white shrimp (*Litopenaeus vannamei*) was characterized. *LvIde* was 3145 bp in length containing an open reading frame (ORF) of 2946 bp corresponding to 981 amino acids. The deduced LvIde protein shared approximately 50% similarity and a zinc binding site compared to other known Ide proteins. The complete ORF of *LvIde* was cloned and *in vitro* expressed in *E. coli*. Recombinant LvIde protein (110 kDa) was expressed in a soluble form. It was partially purified by a single affinity column and the yield obtained was about 50 mg per liter of the culture. Further activity analysis revealed the ability to cleave the internally quenched fluorogenic peptide substrate of rLvIde protein. Primers for amplification of the *LvIDE* gene segment were designed. The PCR product of 332 bp were obtained against genomic DNA of *L. vannamei* juveniles. Nucleotide sequences of the amplified *LvIde* gene segment was determined. Four SNP including $A>C_{113}$, $G>T_{128}$, $G>del_{269}$ and $A>T_{294}$ were found. Interestingly a $A>T_{294}$ SNP resulted in an amino acid replacement from Lys to Asn. Association between $A>T_{294}$ SNP genotypes and growth-related parameters was examined in the domesticated sample where 2-month-old juveniles with a $T/T_{294}$ exhibited a greater average body weight and total length than those possessing $T/A_{294}$ and $A/A_{294}$ genotypes ($P < 0.05$).

Keywords: *Insulin-degrading enzyme, Protein expression, Growth, SNP, Litopenaeus vannamei*

**Differential expression of *X-box binding protein 1* following ammonia stress in Pacific white shrimp *Litopenaeus vannamei***

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**Abstract**

Super-intensive and intensive culture systems are currently used for farming of the Pacific white shrimp *Litopenaeus vannamei*. Ammonia is the main product from waste products and uneaten food and it causes oxidative stress in cultured shrimp through increasing reactive oxygen species (ROS). Here, effects of ammonia on the expression of *X-box binding protein 1* (*LvXbp1*), one of the key gene functionally related with endoplasmic reticulum stress (ER) stress, in two-month-old *L. vannamei* were examined. Juvenile shrimp (the body weight of 8-10 g) were treated with different concentration of ammonia (control, 10 and 20 mg-N/L) for 72 hours, Hemocytes and hepatopancreas of treated juveniles were collected at 0, 6, 12, 24, 48 and 72 hours post treatment (hpt; $N = 6$ for each group). The expression of hemocytes *LvXbp1* was not significantly different throughout the treatment period in the control group ($P > 0.05$) while that in juveniles treated with 10 mg-N/L ammonia was significantly increased at 24 and 48 hpt before returned to the basal level at 72 hpt ($P < 0.05$). More sensitive responses were observed in hepatopancreas. In the control group, the expression of *LvXbp1* in juveniles exposed to unchanged culturing water for 48 and 72 hours was significantly greater than at 0-24 hpt ($P < 0.05$). Its expression in 10 and 20 mg-N/L ammonia was significantly increased at 12-48 and 6-72 hpt ($P < 0.05$). Results clearly revealed dose-dependent responses of *LvXbp1* against ammonia in both hemocytes and hepatopancreas of *L. vannamei*.

Keywords: *Ammonia, Litopenaeus vannamei, Xbp1, Gene expression, Quantitative real-time PCR*

# Complete genome of the first and novel shrimp pathogenic *Shewanella* sp. TH2012 isolated from Early Mortality Syndrome (EMS) outbreak shrimp in Thailand

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**Abstract**

During the 2012-2013 searches for causative agents of early mortality syndrome (EMS) in Thai cultivated shrimp, unique isolates of *Vibrio parahaemolyticus* ($VP_{AHPND}$) were found predominantly in diseased shrimp samples, exhibiting pathognomonic hepatopancreatic lesions of acute hepatopancreatic necrosis disease (AHPND) resulted by $Pir^{vp}$ toxins. A novel *Shewanella* strain TH2012 was also isolated from EMS shrimp during the searches and demonstrated its virulence for shrimp accompanied by distinctive histopathology different from that of AHPND. Here, we report and analyze the full genome sequence of *Shewanella* sp. TH2012, another EMS causative agent and the first *Shewanella* isolate reported to be pathogenic to shrimp. The complete TH2012 genome contained 4,858,998 base pairs with putative 4,176 protein-coding genes and 127 RNA genes on a single circular chromosome and a single circular plasmid. Analyses of multilocus phylogenetic trees of concatenated four conserved house-keeping genes (*16S rRNA*, *mreB*, *atpA* and *rpoA*) and *in silico* DNA-DNA comparison suggested that TH2012 is a novel *Shewanella* species, separated from both type strains of its closely related species *S. litorisediminis* and *S. amazonensis*. Searches of the TH2012 genome revealed no homologs of $Pir^{vp}$ toxin genes. However, a number of other potential virulence factors and putative pathogenicity and resistant islands were identified in TH2012. Thus, the complete TH2012 genome sequence and its analyses provide an important resource for future development of specific and sensitive detection method and also make *Shewanella* sp. TH2012 a suitable candidate to test in co-challenge tests for possible enhancement of $VP_{AHPND}$ virulence.

Keywords: *Early mortality syndrome (EMS), Acute Hepatopancreatic Necrosis (AHPND), Shewanella sp. TH2012, Penaeus vannamei*

**Masquerade-like protein is involved in TSV resistance in white shrimp *Penaeus vannamei*.**

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**Abstract**

The shrimp farming industry contributes significantly to the Thai economy over $US 2 billion per year. However, infectious diseases continue to be a major problem of shrimp aquaculture. A selective breeding program of *Penaeus vannamei* to improve resistance to Taura syndrome virus (TSV) has been successfully established and provided a great benefit to the shrimp aquaculture industry. However, there are few when compare with economic importance because of lacking of knowledge of molecular mechanisms of immune responses in shrimp. Therefore, selective breeding program for disease-resistant shrimp has been developed by using DNA markers genetically linked to disease resistance. In this study, the DNA markers linked to the genes associated with TSV-resistant shrimp *P. vannamei* were developed. Masquerade-like protein (*Pv*Mas), an immune-related gene, was one of the candidate genes possibly associated with TSV-resistant shrimp. The correlation of the expression of *PvMas* transcript and the susceptibility/resistance to TSV was examined and the result showed high expression of this gene in TSV-resistant line. Moreover, TSV challenge test showed that TSV capsid gene (CP2) was expressed higher in *PvMas* knocked-down shrimp than the control shrimp. These findings suggest that *PvMas* involves in TSV defense mechanism of *P. vannamei*. Interestingly, we found an insertion (82-base) in intron of *PvMas* gene of TSV-resistant line. The genotype frequencies of susceptible and resistant groups of this DNA marker were analyzed in 120 shrimp from 2 families of TSV-resistant and 2 families of TSV-susceptible shrimp lines, respectively. The result revealed significant difference in genotype frequency between both lines ($P < 0.05$). This marker will be useful for the future marker assisted selection for disease resistant shrimp and for maintenance of the selected lines.

Keywords: *Shrimp, Penaeus vannamei, Masquerade-like protein, Taura syndrome virus, TSV, DNA marker*

# Poster Presentation

**Growth promotion by prebiotic in whiteleg shrimp (*Litopenaeus vannamei*) (Boone,1931)**

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**Abstract**

Prebiotics have the role of increasing growth rate, improve immune system as well as change the community of bacterial in gastrointestinal track. Many scientists have worked to optimize the dosage of supplementary prebiotics in feed to achieve better growth and survival rates. In the present study, *Litopenaeus vannamei* (PL 20) shrimps were reared for two months in a single tank with proper aeration and optimum water quality parameters before used in the experiment. Prebiotics was prepared and used with the feed. One whole potato contains 2-3 g of non-digestible fiber and 25-30 g of complex starchy carbohydrate. Two whole potatoes were boiled with skin and then grated and dried under sunlight for 3 days. Dried grated potatoes were then grinder and fine particles were sieved and collected. Weighed required amount of potato powder, mixed with 70% ethanol and shook for 15 hours. Then filtrates were evaporated for 30 minutes at 40$^{o}$C and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and mixed with feed using egg yolk as binder. The feed was dried for 10 minutes at room temperature. This prepared prebiotic diet was given to one group of shrimps. Another group of shrimps were cultured as the control to compare the measurement of weight, length, growth etc. between prebiotic-fed shrimps and control shrimps. In case of prebiotic-fed shrimp's growth was more than the control groups of shrimps. Also beneficial bacterial population in gastrointestinal tract was higher than the control group. Pathogenic bacteria populations in the GIT of control group of shrimps were higher than prebiotic-fed group of shrimps. The beneficial bacterial population helped the shrimps to digest the feed better by the help of producing enzyme. The prebiotic-feed played a role as a feed supplement which increased the growth rate of shrimps than normal feed. The details were discussed in this paper.

Keywords: *Prebiotics, Shrimp, Growth rate, Bacteria*

**The application of aquatic worms to dispose bottom waste of catfish pond**

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**Abstract**

The possibility study was conducted on using aquatic worms to dispose off wastes accumulated at the bottom of fish pond using recirculated aquaculture system (RAS) unit. A trial culture that made use of catfish and aquatic worm was designed to examine the effect of this combined species to the removal of the bottom pond wastes. Catfish with stocking at the density of 120 individuals per $m^2$ were each paired with different aquatic worm density treatments of 0, 300, 450, and 600 g of worms. All treatments were run in triplicates for 12 weeks. At the end of the experiment, the quality of water and bottom soil was studied. It was revealed that the combined culture of catfish and aquatic worms helped with the waste disposal in the pond and reduced the organic matter and ammonia content in the sediment and water respectively.

Keywords: *Aquatic worms, Catfish, Recirculated-Aquaculture System, Pond waste*

# Identification and treatment of *Fusarium* isolated from black spots on the cuticle of Pacific white shrimp *Litopenaeus vannamei*

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**Abstract**

Pacific white shrimp (*Litopenaeus vannamei*) is the most cultivated shrimp species in Thailand at present. While bacterial and viral diseases are the major concern during the juvenile pond growing stages, fungal diseases can also affect the cultured shrimp. The aims of this study is isolation and treatment of the fungi isolated from *L. vannamei* broodstock showing the black spot disease. Seven broodstock-sized *L. vannamei* showing black infected lesions on the cuticle at various parts of the body were collected. The portion of the wounds (cuticle and attached tissues) were aseptically dissected and observed using light microscope. Similar signs of infection with several hyphae-like structures protruding from the cuticle into the tissues underneath and brown pigmentation surrounding the hyphae were observed in all shrimp. Glucose Yeast Peptone (GYP) medium supplemented with ampicillin (250 µg/mL) and chloramphenical (100 µg/mL) was used to isolate the fungi. The 18S ribosomal RNA sequences of the representatives were determined. The closest matched of fungal isolates was *Fusarium oxysporum*. *Fusarium* is known fungal pathogen affecting the cuticle and gill of the shrimp. The symptoms are expanding wounds with melanization. Natamycin was applied for the possible treatment of the pathogenic agent. The minimum inhibitory concentration (MIC) of natamycin against cultured *Fusarium* was evaluated. The MIC of natamycin against *Fusarium* was 3.125 µg/mL. In addition, various concentrations of natamycin (0 - 25 µg/mL) were also tested against bacteria isolated from the pond water. The results indicated no inhibition effect of natamycin on growth of examined bacteria. Our results revealed the effective treatment of natamycin on *Fusarium* with the safe effect on beneficial bacteria in the culture system of *L. vannamei*.

Keywords: *Fungi, Fusarium sp., Pacific white shrimp, Litopenaeus vannamei*

# The clinicopathological evaluation of red hybrid tilapia (*Oreochromis* sp.) within 48 hours post-*Streptococcus iniae* and *Streptococcus agalactiae* challenge in the presence of heat stress

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## Abstract

The study emphasized on the clinical signs and the severity of lesions in the brain, gills and kidney of the Red hybrid tilapia *(Oreochromis* sp.*)* at 12 hours intervals within 48 hours post-*Streptococcus iniae* and *Streptococcus agalactiae* inoculation in the presence of heat stress. A total of 54 tilapia fish were equally divided into three groups. Group 1 and Group 2 were inoculated with $10^{10}$ CFU/mL of *Streptococcus iniae* and *Streptococcus agalactiae* respectively. A stress factor; heat stress was given by maintaining the temperature of water at 35°C. Group 3 served as the control group. Organ samples were subjected to evaluation of lesion macroscopically and microscopically. Both infected groups developed clinical signs of air gulping, reduced appetite, motionless and death, but at different point of time. Macroscopically, both groups showed lesions of congestion of kidney and gills, and haemorrhages at fins, tail and operculum at the same time. Meanwhile, softening of brain and eyes can be seen at the different time of infection. As for histopathology, the microscopic lesions observed in the organs include infiltration of inflammatory cells and congestion. In addition, vacuolation, degeneration of tubules and secondary lamellar changes were included in pathological changes in the brain, kidney and gills respectively. To compare between the groups, Group 1 revealed significant result in brain lesion at 12 hours compared to Group 2 and Group 3. No significant results were observed between Group 1 and Group 2. Apart from that, Group 1 also showed higher mortality rate compared to Group 2 with recorded mortality percentage of 83% and 44%, respectively. The results confirmed that the development of clinical signs and pathological lesions in both groups were more or less similar.

Keywords: *Oreochromis sp., Streptococcus iniae, Streptococcus agalactiae, Histology, Heat stress*

**Screening of atinomycetes from pond bottom soil against pathogenic bacteria in aquatic animal**

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**Abstract**

Actinomycetes are gram-positive bacteria regarding as important sources of bioactive metabolites and mostly found in soils. In this study, fifty-seven actinomycetes isolates were screened from 3 aquaculture pond bottom soil samples collected from 0, 10 and 20 cm depth on inorganic salts-starch agar (ISP2) and humic acid-salts vitamin agar media. In total, 25, 15 and 22 isolates were found from respective samples. All of the actinomycetes isolates were tested for the antimicrobial ability to inhibit fish pathogens including *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Streptococus agalactiae*. Results indicated that 8, 2 and 15 isolates acted against at least one pathogenic bacteria, respectively. Interestingly, the isolate A0_016 acted against all fish pathogens examined. Isolate A10_004 had the greatest activity against *A. hydrophila* while isolate A0_016 and A0_055 showed the greatest inhibitory activity against *P. aeruginosa*, *S. agalactiae*. The ratio values of their isolate were 2.07±0.18, 2.21±0.07 and 3.73±1.14, respectively.

Keywords: *Actinomycetes, Pond bottom soil, Pathogenic bacteria*

**Rapid and sensitive visual detection of EMS/AHPND bacteria using loop-mediated isothermal amplification combined with colorimetric gold nanoparticle probe**

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**Abstract**

Acute hepatopancreatic necrosis disease (AHPND) is a component cause of early mortality syndrome (EMS) of shrimp. The causative agent was found to be unique isolates of *Vibrio parahaemolyticus* ($VP_{AHPND}$) that contained a 69 kbp plasmid (pAP1) carrying binary Pir-like toxin genes $Pir^{vp}A$ and $Pir^{vp}B$. Here, we describe a rapid and sensitive approach for detection of $VP_{AHPND}Pir^{vp}A$ gene based on loop-mediated isothermal amplification (LAMP) combined with visual reading of LAMP products using a ssDNA-labeled nanogold probe (AuNP). The LAMP reaction was carried out at 65°C for 45 min followed by addition of the red AuNP solution and further incubation at 65°C for 5 min, allowing any LAMP amplicons present to hybridize with the probe. The presence of $Pir^{vp}A$ gene of the LAMP amplicons prevented an AuNP aggregation and a solution remained as red color of AuNP (positive result), while non-complementary targets cannot prevent AuNP aggregation, resulting in a visible color change to purple color (negative result) after addition of salt. The total assay time was approximately 50 min. The detection limit was 100 CFU which comparable to that of nested PCR method. There was no cross reaction with DNA templates derived from non-AHPND bacteria commonly found in shrimp ponds (including other *Vibrio* species).The new method significantly reduced the time, difficulty and cost for molecular detection of VPAHPND in shrimp hatchery and farm settings.

Keywords: *Acute hepatopancreatic necrosis disease (AHPND), Colorimetric, Early mortality syndrome (EMS), Gold nanoparticle, Loop-mediated isothermal amplification (LAMP)*

**Red tilapia cage culture: A comparative analysis of technical efficiency for selected aquaculture farms in The Philippines and Thailand**

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**Abstract**

The study analyzed the factors influencing technical efficiency of red tilapia production in the Philippines and Thailand. Total enumeration of red tilapia cage farms were surveyed using face-to-face interview. Data were collected through a structured questionnaire; the information was coded and analyzed through the use of both descriptive statistics and stochastic production frontier based on Cobb-Douglass production function. Technical Efficiency results showed that all fish farmers in the study areas were operating below the production frontier. Hence, there is a need to investigate extensively sources of inefficiencies in the socioeconomic variables and farm characteristics to increase production and efficiency. The maximum likelihood estimation of the stochastic production frontier shows that the mean technical efficiencies are 0.32 and 0.78 for Philippines and Thailand, respectively. In addition, the results show that the use of aerator has significant impact on technical efficiency. Result of the model revealed that red tilapia cage production is explained by area, feeds, and labor. Finally, suggestion was made based on the result to carry out further research on red tilapia aquaculture as related to food security and sufficiency. Farmers should expand the culture area and increase the amount of feeds given to red tilapia stocks to further increase production of red tilapia. Farmers should also consider use of aerator. This could help reduce production costs, increase farmers' income, as well as provide the much needed animal protein to consumers at an affordable rate.

Keywords: *Technical efficiency, Red tilapia, Aeration*

**Effects of a probiotic mixture on the growth performance and health status of white shrimp, *Litopenaeus vannamei***

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**Abstract**

A probiotic mixture containing *Lactobacillus pentosus* BD6, *Lac. fermentum* LW2, *Bacillus subtilis* E20, and *Saccharomyces cerevisiae* P13 was added in the diet of white shrimp, *Litopenaeus vannamei,* to evaluate its effects on growth performance and disease resistance of shrimp against *Vibrio alginolyticus*, and the probiotic efficiency of the mixed probiotics was compared to diets containing single probiotics. The probiotic efficiencies of the mixed probiotics for shrimp growth performance and health status improvement were better than those when using single probiotics. The probiotic mixture at a level of $10^8$ colony-forming units (cfu) (kg diet)$^{-1}$ significantly improved the growth performance and health status of shrimp, whereas doses of single probiotics were $10^9$ cfu (kg diet)$^{-1}$. After 56 days of the feeding trial, shrimp fed the diet containing the probiotic mixture at levels of $10^7$~$10^9$ cfu (kg diet)$^{-1}$ had higher survival after injection with the pathogen, *V. alginolyticus*, but $10^9$ cfu (kg diet)$^{-1}$ of single probiotics (except for *S. cerevisiae* P13) had to be administered to improve shrimp survival, which might have been due to increased immunity, including phenoloxidase activity, respiratory bursts, and lysozyme activity of hemocytes. The findings indicate that the multiple-probiotic could adequately provide probiotic efficiency for white shrimp, and a diet containing $10^8$ cfu (kg diet)$^{-1}$ probiotic mixture is recommended.

Keywords: *White shrimp, Probiotic mixture, Growth performance, Health status*

**Bacterial community characterization in intestine of the white shrimp, *Litopenaeus vannamei* after an oral-administration of synbiotic, *Lactobacillus plantarum* plus galactoo ligosaccharide**

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**Abstract**

This study were to discover intestinal microbiota of *L. vannamei* by using next generation sequencing (NGS) technology to evaluate the relationship between shrimp intestinal microbiota, and growth performance and health status. Results showed that 6 phyla, 11 classes, 19 orders, 30 families, 58 genera and 73 species with taxonomical names were assigned. The majority of OTUs were shared between synbiotic (SYN) and control shrimps composed of 37 OTUs. However, intestinal biodiversity analyses revealed that SYN-fed shrimp had a trend of higher species richness, evenness, and Shannon-Weaver index than those of the control shrimp, but no statistical significance. Interestingly, shrimp that were fed SYN diet improved colonization of *Lacto. plantarum* and reduced prevalence of *Vibrio harveryi* and *Photobacterium damselae* in the intestines. The findings indicate that the SYN are suitable to modulate the bacterial community of shrimp and could be used to control vibriosis in shrimp.

Keywords: *Litopenaeus vannamei, Intestinal microbiota, Synbiotic, Lactobacillus plantarum, Galactooligosaccharide*

# Study on the immunoregulation pathway of white shrimp, *Litopenaeus vannamei* after the oral-administration of probiotic *Bacillus subtilis* E20

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## Abstract

This study examined the mechanisms of action of *Bacillus subtilis* E20 in activating the immunity of shrimp via dietary administration. The white shrimp were firstly divided into two groups, one group was fed a control diet (a basal diet) and the other was fed an E20-containing diet (a basal diet with $10^9$ CFU $kg^{-1}$ of *B. subtilis* E20 added). After the 8-week feeding regimen, results from transcriptome analysis indicated a significant increase in immune-related gene expressions in the hepatopancreas of E20-fed shrimp, including superoxide dismutase (SOD), mitogen-activated protein kinase (MAPK), MAPK7, lysozyme (hypsozyme), and heat shock protein 70 (HSP70). On the other hand, we also found that glycosylation pathway-related genes in the E20 group increased significantly as compared with the control ($P < 0.05$), including glutamine fructose-6-phosphate aminotransferase (GFPT) for transfer of amino groups on the substrate, and UDP-*N*-acetylglucosamine-peptide *N*-acetylglucosaminyl transferase. In conclusion, *B. subtilis* E20 may improve the pathway of glycosylation, such as increase the content of glycosylated HSP70 protein in hepatopancreas, and then activating white shrimp immunity.

Keywords: *Bacillus subtilis E20, Litopenaeus vannamei, Immunity, Glycosylation, Heat shock protein 70*

# Characterization of culturable bacteria isolated from white shrimp *Litopenaeus vannamei* postlarvae and rearing water from biofloc systems

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## Abstract

In recent years, there have been significant interests in understanding the roles of microbiota relating to aquaculture, both on the animals and the rearing water. This research examined strategies to isolates different groups of bacteria from shrimp and rearing systems. Three different culture protocols were used to target different major groups of aquatic bacteria: Marine 2216 agar (non-selective media for heterotrophs), heat treatment (80°C for 10 min) following by plating on trypticase soy agar + 1.5% sodium chloride (for spore formers), and plate count agar (PCA) with kanamycin (for *Bacteroidetes*). Plates were incubated at 32°C for 24-48 hr. Colonies with morphology from each treatment were sub-cultured and further identify using *16S rRNA* gene sequencing. Sixty-three bacterial isolates from 3 target groups (23 heterotrophs, 30 spore-formers, and 10 *Bacteroidetes*) were collected from all treatments. Based on *16 rDNA* sequences, bacterial isolates from 4 phyla including *Proteobacteria, Firmicutes, Bacteroidetes,* and *Actinobacteria* were obtained. Phylum *Proteobacteria* including the genera *Aestuariibacter, Enterobacter, Halomonas, Leisingera, Pseudoalteromonas, Pseudomonas, Pseudoruegeria* and *Vibrio* were isolated from Marine 2216 agar. All isolates from the heat treatment method were *Bacillus* (19 species) with *Bacillus amyloliquefaciens* as the most numerous isolates. PCA+kanamycin plates allowed for isolation *Bacteroidetes* (5 genera: *Algoriphagus, Formosa, Maricauda, Tamlana,* and *Tenacibaculum*), *Actinobacteria (Microbacterium)* and some *Proteobacteria (Pseudomonas)*. Other characteristics of isolates such as antagonistic assay against shrimp bacterial pathogens *Vibrio harveyi* and *Vibrio parahaemolyticus* are being examined. The results showed an opportunity to study different groups of culturable bacteria in shrimp cultivation environment for future utilization.

Keywords: *16S rRNA, White shrimp, Culturable bacteria, Selective media*

**Propagation and purification of ISKNV from Asian sea bass (*Lates calcarifer)* using Grunt Fin (GF) cell line.**

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**Abstract**

Infectious spleen and kidney necrosis virus (ISKNV) has been classified as members of Megalocytivirus of the family Iridoviridae. The disease outbreaks from this Iridoviridae virus caused economic losses, due to mass mortality, in fin fish industry of China, Japan and South-East Asia. In addition to inflicting massive mortality in infected fish, infections of ISKNV have also been reported without causing clinical symptoms in various fish species at different age groups. The present study demonstrates isolation of ISKNV from Asian sea bass (*Lates calcarifer*) cultured in farm experiencing ~50% cumulative mortality in Vietnam. Crude ISKNV extract from clinical samples were confirmed by PCR prior subsequently propagating in Grunt fin (GF) cell, a commercial cell line. Cytopathogenic effect (CPE) could be obviously observed in crude extract co-cultured GF cell. The virus was purified from infected GF cells by ultracentrifugation and examined under transmission electron microscope (TEM). A particle of approximate 150-200 nm in diameter was observed. PCR sequencing of purified virus suggested 99% sequence similarity to ISKNV in the database. This established viral propagated model offers a practical tool for facilitating fish virus research and further development of vaccine technology.

Keywords: *ISKNV, Megalocytivirus, Grunt fin, Lates calcarifer, Vaccine*

**Effect of *Bacillus amyloliquefaciens* TOA5001 as a potential probiotic on whiteleg shrimp (*Litopenaeus vannamei*)**

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**Abstract**

Infectious diseases are one of the serious limiting factors in shrimp aquaculture. Recently, a disease called acute hepatopancreatic necrosis disease (AHPND) has expanded and caused severe damage to shrimp farming. *Bacillus* spp. are used as potential probiotics to deal with those problems. In this study, *B. amyloliquefaciens* TOA5001 (Toa Pharmaceutical Co., Ltd.) was evaluated. From the results of the cross-streak assay on agar plates, the production and secretion of antimicrobial substances by TOA5001 was confirmed. Spores of TOA5001 were then mixed with shrimp feed and fed to whiteleg shrimp (*Litopenaeus vannamei*) for the duration of two and four weeks. After feeding, challenge tests with the AHPND strain of *Vibrio parahaemolyticus* were conducted. Shrimp fed with TOA5001 showed significantly higher survival rate compared to the control group after challenging with *V. parahaemolyticus*. In addition, after two weeks of feeding, mRNA expressions in hepatopancreas were comprehensively examined by microarray analysis. The results showed that mRNA levels of immune-related genes between TOA5001-fed and the control group of shrimps were not significant different. Our results may suggest that *B. amyloliquefaciens* TOA5001 and the substance produced may have affected AHPND strain of *V. parahaemolyticus* and the infection was prevented. Presently, we analyze the effects of TOA5001 administration to intestinal microbiota in shrimp by *16S rRNA* gene-based metagenomic analysis.

Keywords: *Shrimp, Bacillus amyloliquefaciens, Probiotics, Early mortality syndrome/acute hepatopancreas necrosis disease (EMS/AHPND)*

**Development of one-tube nested PCR method for the detection of *Enterocytozoon hepatopenaei* (EHP) in shrimp.**

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**Abstract**

*Enterocytozoon hepatopenaei* (EHP) is an intracellular parasite causative agent of Hepatopancreatic microsporidiosis (HPM) in shrimp. Infection of this parasite in the hepatopancreas and midgut resulted in slow growth rate and consequently large economic loss to the farms. Currently, a two-tube nested PCR method to detect the spore wall protein (SWP) gene of EHP with a separate PCR was available. To stream line the detection process, minimize potential contamination, and include verification, this study aimed to develop a one-tube nested PCR method to detect EHP with an internal control. First, primer concentrations from both the first and nested PCR reactions were optimized. Appropriate internal controls among house-keeping genes (*18S rRNA*, *β-actin*, or *elongation factor*) were chosen, and their associated primer concentrations were optimized. PCR parameters including magnesium concentration, DNA polymerase concentration, and annealing temperature were then adjusted. PCR products were electrophoresed using 2% agarose gel, stained with ethidium bromide and observed under UV illumination for all steps. The results showed that the one-tube nested PCR method using *18S rRNA* gene as an internal control could detect $10^2$ plasmid copies of SWP-EHP genes. In addition, this PCR method did not cross react with ten other important shrimp pathogens and yielded the same results as a two-tube method based on field testing of 75 samples. In conclusion, one-tube nested PCR method can be used to detect SWP-EHP DNA that is faster, specific and minimize the chance for contamination than two-tube nested PCR method.

Keywords: *One-tube nested PCR, Enterocytozoon hepatopenaei, EHP, Pathogen detection, Shrimp*