DAA10 Handbook

DAA 10 - Back to Bali

10th Symposium on Diseases in Asian Aquaculture (DAA10)
28 August - 1 September 2017, Bali, Indonesia

Organised by:

Fish Health Section - Asian Fisheries Society
and
Indonesian Ministry of Marine Affairs and Fisheries

http://daa10.org/
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About this Handbook

Handbook of the 10th Symposium on Diseases in Asian Aquaculture (DAA10) 28 August – 1 September 2017 The ANVAYA Beach Resort, Kuta, Bali, Indonesia.

Citation:

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This volume is the pre-symposium compilation of abstracts. Although oral presentations were selected from submitted abstracts by three independent reviewers, the contents are not peer-reviewed and part from lay-out changes, the contents have been printed as received from submitting authors. Some abstracts are also need an extensive English editing. Please consult with the authors before using information contained in any of the abstracts. The Fish Health Section of the Asian Fisheries Society does not guarantee that this Handbook is without flaws and therefore disclaims any liability for any error, loss or other consequence which may arise from persons relying on the information in this publication. The symposium programme is correct of the time of the printing, however the organisers reserve the right to make changes where necessary.

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Publications:

1. Review papers by keynote speakers will be published in **Journal of the World Aquaculture Society**. https://www.was.org/View/Journal-of-the-World-Aquaculture-Society.aspx
2. Subject to review, papers presented by participants will be published as a special issue of **Fishes** journal. http://www.mdpi.com/journal/fishes/special_issues/10th_Symposium_Disease_Asian_Aquaculture

Contents:

Greetings from Chairperson of FHS-AFS

Ladies and Gentlemen,

Welcome to the 10thSymposium on Diseases in Asian Aquaculture (DAA10) the most popular triennial event of the Fish Health Section of the Asian Fisheries Society (FHS-AFS), held here in The ANVAYA Beach Resort, Kuta, Bali, Indonesia from 28 August to 1 September 2017. The event covers topics from classic parasitic, bacterial and viral diseases to emerging trends and cutting-edge research in aquatic animal health and its implementation into better biosecurity.

"Enhancing Aquatic Animal Health Research and Services through Public-Private Sector Partnerships" is the theme of DAA10. To research, to manage a disease, we need to work together.It doesn't matter, if you are from government or private sector, if you are PhD or you are farmer, if you are professor or you are student. We all should unite for a better health of aquatic animals and thus for better environment, animal welfare and human health. But also to achieve better food security and prosperity through responsible aquaculture.

This time DAA returns to the venue of its first event in 1990, Bali, Indonesia - to celebrate its 10th event. DAA always has been a longing event for not only people working on aquatic animal health but also people in other related fields. It gives the most updated information about disease situation, diagnostic, services and research. It is also an event for young researchers to show their proud work to the world.

Nowadays, disease is no doubt the biggest constraint in aquaculture. Even though aquaculture technology is very much improved, new diseases still happens. For last 5 years, Asian aquaculture sector has experienced two new severe diseases, Acute Hepatopancreatic Necrosis Disease in shrimp and Tilapia Lake Virus Disease in tilapia. They have causedhuge lossesto farmers and relatedservice sectors all over the world. Two young, newly graduated scientistsand their respective teams were first to successfully identify the causatives of these disease and guess what, they are both from Asia! We are very proud about that. I do believe that DAA is always a platform for knowledge and information exchange, for network making and for the development of aquatic animal health, especially in Asian countries where most of aquaculture products in the world come from!

From Bali (Indonesia), Thailand, the Philippines, Australia, Sri Lanka, Taiwan, India, and Vietnam now we are back to Bali to surf science in the sun. This event is proudly hosted by Fish Health Section of theAsian Fisheries Society and Ministry of Marine Affairs and Fisheries, Indonesiaand its Directorate General of Aquaculture. It could only happen with their hard work and well organization by local National OrganizingCommittee. Members of the International Scientific Committee have been working very focus and on time to select excellent papers to be presented in DAA10 in the form of oral and poster presentations. Committee for Student Award has identified well deserved students to grant them awards for travel. Executive committee members of FHS-AFS also contributed valuable ideas/information. Also the valuable support from our platinum sponsor: Blue Aqua; silver sponsor: NutriAd; sponsor all season: Hinabiotech, Kinglab, Evergreen, CJ and publication sponsor: World aquaculture Society, Fishes. For all these, I would like to, on behalf of FHS-AFS, thank you for together make DAA10 happen.

Thank you distinguished guests and thank you all participants, keynote speakers, oral and poster presenters for attending.Please enjoy the DAA10, its field trip and side events, but don't forget to enjoy the sunshine, the beautiful beach, the hospitality here in Bali.

Thank you,

Dr Phan Thi Van
Chairwoman FHS-AFS

Welcome Speech from Director General of Aquaculture

Indonesia is honoured to be selected as the host country of the 10th Symposium on Disease on Asian Aquaculture (DAA10), which also marked as the milestone of one of the biggest scientific events on research, diagnostics and services of aquatic animal health in Asian aquaculture. On behalf of the government of Indonesia, I warmly welcome all delegates to the DAA10 and sincerely thank the international communities, especially the Fish Health Section of the Asian Fisheries Society that have worked closely with the Directorate General of Aquaculture to prepare the DAA10 effectively.

Welcome back and high appreciation to the founders of the DAA Symposium on its presence, where Bali, Indonesia was where the first Symposium DAA1 in 1990 took place. Thereafter, DAA2-DAA9 were conducted in Asian countries: Thailand, (1993), Thailand, (1996), The Philippines (1999), Australia (2002), Sri Lanka (2005), Taiwan, (2008), India, (2011) and Vietnam, (2014). The DAA10 in Bali is an excellent momentum to refresh and accelerate the role of DAA10 in the management of aquatic animal health in supporting the development of regional and international aquaculture sectors. With the spirit of "Back to Bali and Surfing Science in Sun" as the tagline DAA10 will produce a prospective and productive output.

DAA10 will feature current fish health technology and information including Virology, Bacteriology, Parasitology, Immunology, Vaccinology, Biosecurity, Diagnostic and Shrimp Disease, among others, that will be presented by experts and researchers from the region and around the world. There will also be an international exhibition, which feature latest technology on fish health management and aquaculture. The symposium also provides a great opportunity for international partners to develop and expand the research collaboration, markets for aquatic products. Through this event, I believe that the problem of fish diseases that have become a constraint in the development of regional aquaculture can be addressed.

I really appreciate and sincerely thank sponsors for technical and financial support in succeeding the DAA10. My deep gratitude to all experts and researchers at the DAA10 Symposium for sharing and discussing important and useful scientific information on aquatic animal diseases and control measures.

Once again, I welcome and thank you all very much for your great contribution and collaboration in the DAA10.I wish you all a fruitful discussion at the symposium and a pleasure time during your visit in Bali.

Dr. Slamet Soebjakto
Director General of Aquaculture, MMAF.

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Donation Acknowledgements:

FHS-AFS thanks Prof Chu-Fang Lo for her generous donation of $1000. The donation has enabled FHS-AFS to provide stipend to five awardees of Student Travel Awards.

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Program at a Glance

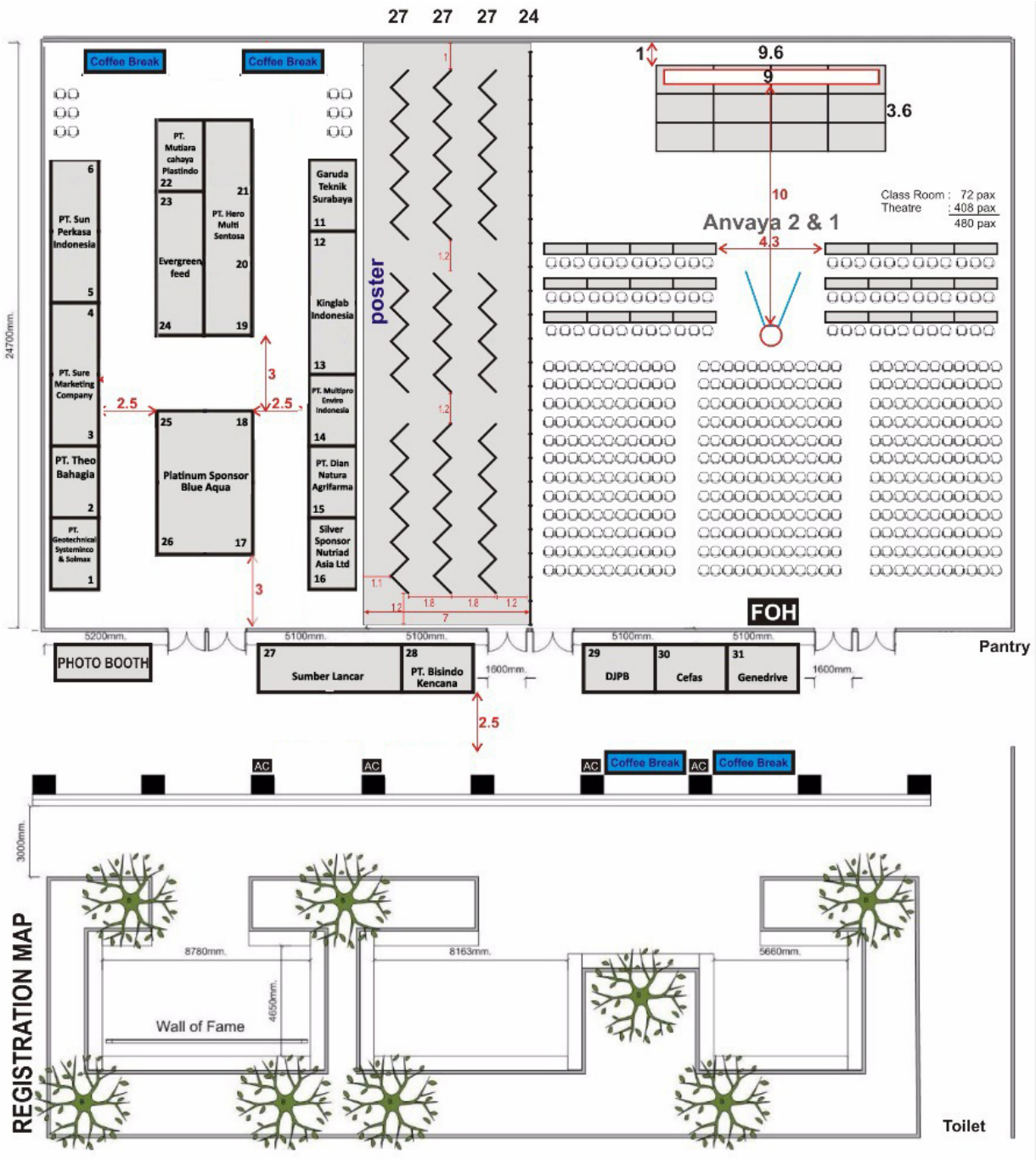
Date	Time	Program
Sunday, 27 August	17.00 - 21.00	Registration
Monday, 28 August	08.00 - 08.30	Opening of Exhibition
	08.30 - 09.00	Opening Ceremony
	09.00 - 09.30	Plenary Session
	10.00 - 12.30	Session 1. Recent advances in aquatic animal research
	13.30 - 17.00	Session 2. Virology
	17.00 - 17.15	Presentations of EoI to host DAA11 in 2020
	17.15 - 19.00	Welcome Reception (nibbles and drinks)
Tuesday, 29 August	09.00 - 14.30	Session 3. Bacteriology
	15.00 - 17.00	Session 4. Parasitology
	19.00 - 21.00	TGM11 (The 11th Triennial General Meeting of FHS-AFS)
		Free night in Bali for other participants
Wednesday, 30 August	09.00 - 15.00	Session 5. Shrimp diseases
	15.30 - 17.36	Elevator Pitch
	17.36 - 18.30	Poster Session
	19.00 - 21.00	Farmer class for members of Shrimp Clubs Indonesia
		Free night in Bali for other participants
Thursday, 31 August	09.00 - 10.30	Session 6. Immunology
	11.00 - 14.30	Session 6. Vaccinology
	15.00 - 17.00	Session 7. Biosecurity and diagnostics
	17.00 - 17.05	Presentation of upcoming ISAAH in 2018
	17.05 - 17.10	Presentation of upcoming DAA11 in 2020
	19.00 - 22.00	Gala dinner and conference closure
Friday, 1 September	AM-PM	Field trip

Social Program

Welcome Reception (Monday, 28 August 2017, 17.15 - 19.00)
Tickets: included in full symposium registration.
Nibble and drink will be served.

Gala Dinner (Thursday, 31 August 2017, 19.00-22.00)
Tickets: included in full symposium registration, $50 for additional ticket.
Dress code: smart casual, but national and traditional costumes are more than welcome.
Hosted by Indonesian Ministry of Marine Affairs and Fisheries.

Floorplan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Wall of Fame
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Toilet

Scientific Program Schedule

Sunday, 27 August 2017: Registration (17.00 - 21.00)	
Monday, 28 August 2017: Opening Ceremony	
07.00	**Registration**
08.00	**Opening of Exhibition**
08.30	**Opening Ceremony:** 08.30: Welcome remarks 08.33: Balinese welcome dance 08.40: Greetings from Chairperson of Fish Health Section - Asian Fisheries Society **Phan Thi Van** 08.50: Welcome speech and official opening by Director General of Aquaculture - MMAF **Slamet Soebjakto**
Monday, 28 August 2017: Plenary Session **Chair: Melba B. Reantaso**	
09.00	Aquatic Animal Health Management in Asia: Yesterday, Today and Tomorrow (AbstractID 249) **Rohana Subasinghe**
09.30 - 10.00	Coffee break
Monday, 28 August 2017: Session 1. Recent advances in Aquatic Animal Health Research **Chair and Co-Chair: Chu-Fang Lo and Matthew Neave**	
10.00	New Paradigms to Help Solve the Aquaculture Disease Crisis (AbstractID 212A) **Grant D. Stentiford**
10.30	Challenges for Controlling White Spot Disease (WSD) (AbstractID 195A) **Chu-Fang Lo**
10.45	The Pathobiome Concept: an Emerging View of Microbes and Disease (AbstractID 117A) **David Bass**, Ronny van Aerle, Lydia Doherty, Jamie Bojko, Dominique Chaput and Grant D. Stentiford
11.00	Advances in the Application of High-Throughput Sequencing in the Discovery and Characterisation of Novel and Emerging Aquatic Animal Pathogens (AbstractID 104A) **Ronny van Aerle**
11.15	Immune Response of Common Carp to Cyprinid Herpesvirus 3 (AbstractID 148A) **Matthew J. Neave**, Agus Sunarto and Kenneth A. McColl
11.30	The Impact of Quorum Sensing Signals on the Virulence of *Vibrio Campbellii* toward Different Crustacean Hosts (AbstractID 126A) **Pande Gde Sasmita Julyantoro**, Peter Bossier and Tom Defoirdt
11.45	Ethanolic Extract of Ginger and Three of Its Major Components Inhibit Biofilm Formation by *Vibrio harveyi* and *V. parahaemolyticus* in *In Vitro* Study and Extract Supplemented Feed Protects *Penaeusvannamei* Shrimp from AHPND (AbstractID 188A) **Chumporn Soowannayan** , ThitimaAnatamsombat, SasithornBoonmee, SukanyaPuckcharoen, Pattana Yathip, Ng Wing Keang, PatoomratanaTuchinda, BamroongMunyoo, NapasonChabang, BanlungNuengsaeng and MolruedeeSonthi.
12.00	Use of Marine Vessel Tracking System to Create a Contact Network of Aquaculture in Norway and the Implication for Risk-Based Surveillance and Zone Establishment (AbstractID 102A) **Saraya Tavornpanich**, Carola Sauter-Louis and Edgar Brun
12.15	Using Physiology to Optimise Water Quality and the Sustainability of Intensive Aquaculture (AbstractID 019A) **Robert P. Ellis** and R.W. Wilson
12.30 - 13.30	Lunch

Monday, 28 August 2017: Session 2 – Virology **Chair & Co-Chair: Kelly Batemanand Dewi Syahidah**	
13.30	Behavioural Fever Induced by Cyprinid Herpesvirus 3: When the Environment Makes the Difference Between Life and Death(AbstractID 247B) **Alain Vanderplasschen**
14.00	Koi Herpesvirus in Carp: The Tale of Two Countries (AbstractID 132B) **Agus Sunarto**, Matthew J. Neave, Angela Lusiastuti, Ayi Santika, Kei Yuasa, Arnon Dishon and Kenneth A. McColl
14.15	Integrated Analysis of mRNA and Viral miRNA in the Kidney of *Carassius auratus gibelio* Response to Cyprinid Herpesvirus 2 (AbstractID 129B) **Jianfei Lu**, Dan Xu and Liqun Lu
14.30	Carp Edema Virus: An Emerging Threat in Koi Carp, *Cyprinus carpio koi*, in India (AbstractID 196B) **Swaminathan, T.R.,** Kumar, R., Arathi, D., Pradhan, P.K., Basheer, V.S.and N. Sood
14.45	Emergence of Tilapia Lake Virus in Thailand (AbstractID 025B) **Win Surachetpong**, Puntanat Tattiyapong, Taveesak Janetanakit, Nutthawan Nonthabenjawan and Alongkorn Amonsin
15.00 - 15.30	Coffee break
15.30	Vero Cell Lines Expressing Nuclear Location Signals of *Penaeus merguiensis* Hepandensovirus: An Early Study (AbstractID 190B) **Dewi Syahidah,** J. Elliman, C. Constantinoiu and L. Owens
15.45	A New Member of Iridoviridae in Pacific White Shrimp (*Litopenaeus vannamei*) (AbstractID 064B) Liang Qiu, Meng-Meng Chen, Xiao-Yuan Wan, Chen Li, Qing-Li Zhang, Ruo-Yu Wang, Dong-Yuan Cheng, Xuan Dong, Bing Yang, Xiu-Hua Wang and **Jie Huang**
16.00	Discovery of A Novel Distant Relative of White Spot Syndrome Virus (WSSV1) from Wild Crabs in Disease Free Area (AbstractID 105B) **Kelly S. Bateman**, Ronny van Aerle, Just M. Vlak, Rose Kerr, Jamie Bojko, K. Fraser Clark, Sarah E. Stewart-Clark, Philip Byrne, Spencer J. Greenwood, David Bass and Grant D. Stentiford
16.15	The Role of Cell Surface ATP Synthesis of Shrimp in WSSV Infection (AbstractID 070B) **Yan Liang**, Menglin Xu, Xiaowen Wang, Xiaoxiao Gao, Jun-Jun Cheng, Chen Li and Jie Huang
16.30	Shrimp miR-10a is Co-Opted by White Spot Syndrome Virus (WSSV) to Increase Viral Gene Expression and Viral Replication (AbstractID 192B) **Jiun-Yan Huang,** Shih-Ting Kang, Chia-Ying Chu and Chu-Fang Lo
16.45	Large Scale Production of dsRNA-Producing *Chlamydomonas reinhardtii* and Its Use in Viral Disease Protection (AbstractID 033B) **Patai Charoonnart,** Metha Meetam, Julie A.Z. Zedler, Colin Robinsonand Vanvimon Saksmerprome
17.00	Welcome reception (nibble & drink)

Tuesday, 29 August 2017: Session 3 – Bacteriology **Chair & Co-Chair: Indrani Karunasagar and Desrina**	
09.00	Bacterial Pathogens, Horizontal Gene Transfer and Antimicrobial Resistance Taking Center Stage in Aquaculture?(AbstractID 243C) **Iddya Karunasagar**
09.30	Comparative Genomic Analysis of High Drug-Resistance *Aeromonas hydrophila* Induced By Doxycycline(AbstractID 024C) Guoliang Zhang, Ye Zhang, Hao Wang and **Liqun Lu**
09.45	Review of Antibiotic Resistance in Pathogenic Bacteria onStriped Catfish(*Pangasianodon hypophthalmus*) in The Mekong Delta, Vietnam(AbstractID 115C) **Dung Tu Thanh** and Quach Van Cao Thi
10.00	*SpaC*-type *Erysipelothrix* sp. Causing Diseases in Fish (AbstractID 013C) **Esteban Soto**,E. K. Pomaranski,S. R. Reichley,R Yanong, J. Shelley, D. B. Pouder, J. C. Wolf, K. V. Kenelty, B. Van Bonn, F. Oliaro, B. Byrne, K. A. Clothier,M. J. Griffin and A. C. Camus
10.15	Virulent Genes Determination of *Streptococcus agalactiae* Isolated from Malaysian Red Tilapia (*Orechromis spp.)* in Malaysia (AbstractID 056C) **Suphia-Amiera Sulaiman**, Nur-Nazifah Mansor, Siti-Zahrah Abdullah and Azzmer-Azzar Abdul Hamid
10.30 - 11.00	Coffee break
11.00	Assessing the Virulence of *Streptococcus agalactiae* Serotypela, Ib and III Using a Cohabitation Infection Model in Nile Tilapia (*Oreochromis niloticus*)(AbstractID 116C) **Anita Jaglarz**, William Leigh, Janina Costa and Kim D. Thompson
11.15	*Flavobacterium columnare* Recovered from Diseased Tilapia in Thailand is Taxonomically Distinct from the Type Strains (AbstractID 009C) **Pattanapon Kayansamruaj**, Ha T. Dong, Ikuo Hirono, Hidero Kondo, Saengchan Senapin and Channarong Rodkhum
11.30	Infectious Disease Caused by *Elizabethkingia*in Farmed Frogs in China (AbstractID 147C) **Ruixue Hu** and Zemao Gu
11.45	*Aeromonas veronii*Biovar Sobria Associated with Mortality of Riverine Ayu *Plecoglossus altivelis*in the Tama River Basin, Japan (AbstractID 211C) **Hisato Takeuchi**, Aki Namba, Kazutomo Hori, Daigo Inoue, Tomohiro Takase, Masako Sawazaki, Shosaku Kashiwada and Nobuhiro Mano
12.00	Epitheliocystis in Farmed *Pangasianodon hypophthalmus* is Associated with *Candidatus*Actinochlamydia Pangasiae Sp. Nov. Infection (AbstractID 227C) **Neeraj Sood**, Swaminathan, T. R., Verma, D. K., Yadav, M. K., Krishna, A. D. and Pradhan, P. K.
12.15	Characteristics of *Streptococcus dysgalactiae* Isolated from Different Farmed Fish Species and Expression of Immune-Related Genes During Its Infection (AbstractID 166C) **Thuy Thi Thu Nguyen**, Hai Trong Nguyen, Ming-An Tsai, Omkar Byadgi, Pei-Chyi Wang, Terutoyo Yoshida and Shih-Chu Chen
12.30 - 13.30	Lunch
13.30	Identification of A *Nocardia seriolae* Secreted Protein Targeting Host Cell Mitochondria and Inducing Apoptosis in FHM Cells (AbstractID 175C) Jianlin Chen,Yishan Lu, Honglian Zhang and **Liqun Xia**
13.45	Stress Response and Susceptibility to *Vibrio alginolyticus* Infection of Juveniles *Haliotis squamata* Cultured in Different Water Temperature (AbstractID 083C) **Ngurah S. Yasa**, Murwantoko, A.Isnansetyo, Niken S. N. Handayani, Gemi Triastutik and Lutfi Anshory
14.00	Chitosan Coated Ag/Zno Nanocomposite Against *Vibrio* sp (AbstractID 087C) **Vaseeharan Baskaralingam**, Anjugam Mahalingam and Iswarya Arokiadhas
14.15	Effect of Autochthonous Bacteria with Potential Probiotic Properties on Physiological Conditions of *Cyprinus carpio* Against Waterborne Lead Toxicity (Abstract ID 185C) **Sib Sankar Giri**, Jin Woo Jun, V. Sukumaran and Se Chang Park
14.30 - 15.00	Coffee break

Tuesday, 29 August 2017: Session 4. Parasitology Chair and Co-Chair: Supranee Chinabut and Maria Mercè Isern-Subich	
15.00	Control of Parasites – Does History Inform Our Future?(AbstractID 182D) **Andy P. Shinn**
15.30	Pathogenicity and Pathology of Parasite Marine Leech *Zeylanicobdella arugamensis* Infestation in Asian Seabass *Lates calcarifer* Under Laboratory Condition (AbstractID 171D) **Beng Chu Kua,** Leaw Yoon Yau and Noraziah Mat. Rashid
15.45	Searching for Infection Related Proteases on Yellowtail Skin Fluke *Benedenia seriolae* (AbstractID 058D) **Keigo Kobayashi**, Hirofumi Yamashita, Ikuo Hirono and Hidehiro Kondo
16.00	Synergistic Infection of the Ectoparasite *Ichthyophthiriusmultifiliis* and the Intracellular Bacterium *Francisellanoatunensis* Subsp. *Orientalis* in Red Tilapia (*Oreochromis*sp.) (AbstractID 213D) **Vuong Viet Nguyen**, Ha Thanh Dong, Saengchan Senapin, Nopadon Piraratand Channarong Rodkhum
16.15	Purification of Spores From the Microsporidian *Enterocytozoon hepatopenaei* and Viability Confirmation by Polar Tube Extrusion Assay Using Phloxine B (AbstractID 043E) **Diva January Aldama-Cano**, Piyachat Sangaunrut, Natthinee Munkongwongsiri, Ornchuma Itsathitphaisal, Rapeepun Vanichviriyakit, Kallaya Sritunyalucksana, Timothy W. Flegel, Jose Cuauhtemoc Ibarra-Gamez and Siripong Thitamadee
16.30	Epidemiological Holistic Approach on Fish Farming: A Cross Sectional Study on Prevalence and Risk Factors of Ectoparasite Infestation in Giant Gouramy *Osphronemous goramy* Farming in West Java, Indonesia (AbstractID 020D) **Dominico Caruso**, Taukhid, A.M. Lusiastuti, J.W. Slembrouck, Tri Barkah, M. Yuhanaand J.C. Avarre
16.45 – 17.00	Functional Feed Additives as Prevention of Parasitic Disease in Fish (AbstractID 233D) **Maria Mercè Isern-Subich**, Sam Ceulemans and Peter Coutteau
19.00 - 21.00	TGM 11 (11th Triennial General Meeting, FHS-AFS members only) Free night in Bali for other participants
Wednesday, 30 August 2017: Session 5. Shrimp Diseases Chair and Co-Chair: Celia Pitogo and Kallaya Sritunyalucksana	
09.00	A Future Vision for Disease Control in Shrimp Aquaculture (AbstractID 236E) **Tim Flegel**
09.30	Ten Things You Need to Know AboutEHP(AbstractID 214E) **Grant D. Stentiford**, David Bass and Bryony A.P. Williams
09.45	Genome, Virulence Factor, and Specific Molecular Diagnosis of the Microsporidian *Enterocytozoonhepatopenaei*(EHP)(AbstractID 041E) **Pattana Jaroenlak**, PiyachatSanguanrut, Bryony A. P. Williams, Dominic Wiredu-Boakye, Grant D. Stentiford, Timothy W. Flegel, Kallaya Sritunyalucksana and Ornchuma Itsathitphaisarn
10.00	A Genomic and Transcriptomic Aspect of the Emerging Shrimp Pathogen *Enterocytozoonhepatopenaei*(AbstractID 042E) **Anuphap Prachumwat**, Dominic WireduBoakye, Bryony A. P. Williams, Grant D. Stentiford, Pattana Jaroenlak, Ornchuma Itsathitphaisarn, Timothy W. Flegel and Kallaya Sritunyalucksana
10.15	Controlling EHP: From Molecular Insight to Farm Applications(AbstractID 015E) **Ornchuma Itsathitphaisarn**, Pattana Jaroenlak, Natthinee Munkongwongsiri, PiyachatSanguanrut, Bryony A. P. Williams, Grant D. Stentiford, Timothy W. Flegel and Kallaya Sritunyalucksana
10.30 - 11.00	Coffee break
11.00	Molecular Identification of AHPND Positive *Vibrio parahaemolyticus* in Cultured Shrimp of Bangladesh(AbstractID 124E) Md. MostaviEnanEshik, Nusrat Jahan Punom, Md. Munjur Hossain, Utpal Chandra Roy, Md Monwarul Islam, MstKhadiza Begum, Tahsin Khan, Mihir Lal Saha and **Md Shamsur Rahman**

11.15	Characterization and Comparative Genomics of AHPND-Causing *Vibrio parahaemolyticus* and *Vibrio campbellii* Isolates(AbstractID 031E) **Xuan Dong**, Dexi Bi, Hailiang Wang, Peizhuo Zou, Guosi Xie, Xiaoyuan Wan, Qian Yang, Yanping Zhu, Mengmeng Chen, Chengcheng Guo, Zhen Liu, Wenchao Wang, Liang Yan and Jie Huang
11.30	Discovery of An AHPND Disease Marker Gene in Infected Shrimp(AbstractID 193E) **I-Tung Chen**, Yun-Tzu Huang, Chia-Wei Lu and Chu-Fang Lo
11.45	Dynamics of the Shrimp Stomach Bacterial Microbiome in An AHPND-Infected Pond(AbstractID 045E) **Han-Ching Wang**
12.00	Application of Metagenomic Analysis to Biological Environment Monitoring of Shrimp Culture Pond(AbstractID 098E) **Yuki Midorikawa**, Tsubasa Uchino, Yoshinori Nomura, Motoyuki Nakane, Mamoru Sameshima, Hidehiro Kondo, Ikuo Hirono, Matthura Labaiden, Sataporn Direkbusarakom, Goshi Katoand Motohiko Sano
12.15	Transcriptome Analysis of Hepatopancreas and Stomach of AHPND Toxin-Resistant *Litopenaeus vannamei*(AbstractID 027E) **Sasiwipa Tinwongger**, Hidehiro Kondo and Ikuo Hirono
12.30 - 13.30	Lunch
13.30	Comparative Transcriptomics Reveal Specific Survival Mechanisms of Shrimp Infected with White Spot Syndrome Virus (WSSV)(AbstractID 094E) **Kallaya Sritunyalucksana**, Suparat Taengchaiyaphum, Jiraporn Srisala, Dararat Thaiue and Anuphap Prachumwat
13.45	Understanding the Molecular Basis of Susceptibility to WSSV in Shrimp (AbstractID 049E) **Rebecca S. Millard**[1], Verbruggen, B.[1], Bickley, L. K.[1], Bateman, K.S.[2], Stentiford, G.D.[2], Tyler, Charles R.[2], van Aerle, R.[2] and E.M. Santos[1]
14.00	Epidemiological Study: Risk Factors and Prevalence of WSSV and IMNV in Shrimp Ponds in Lampung Selatan (Lampung Province) and Banyuwangi (East Java Province), Indonesia(AbstractID 078E) **Arief Taslihan**, Maskur, Febriyanto, Bambang Hanggono, Mukti Sri Hastuti and Sugeng Raharjo
14.15	Multimodal Strategies to Control Diseases in Hatchery and Grow-Out Phases of Cultured Shrimp, *Penaeus monodon*(AbstractID 014E) **Mangalika Hettiarachchi**and K.R.P.S. Kumara
14.30	Sequencing of A Novel (Chequa Virus) from the Picornavirales in Redclaw Crayfish *(Cherax quadricarinatus*)(AbstractID 003E) **Kitikarn Sakuna**, Jennifer Elliman and Leigh Owens
14.45	Detection of the Lethal Bacterial Pathogen *Spiroplasma eriocheiris* in the Freshwater Prawn *Macrobrachium rosenbergii* in Thailand(AbstractID 047E) **Arnon Pudgerd,** Jiraporn Srisala, Rapeepan Pukmee, Ornchuma Itsathitphaisarn, Robin McIntosh, SudharmaChoosuk, Timothy W. Flegel, Kallaya Sritunyalucksana and Rapeepun Vanichviriyakit
15.00 - 15.30	Coffee break
15.30 – 17.36	Elevator Pitch
17.36 – 18.30	Poster Session
19.00 - 21.00	Farmer class for members of Shrimp Clubs Indonesia Free night in Bali for participants

Thursday, 31 August 2017: Session 6 – Immunology Chair & Co-Chair: Alexandra Adams & Motohiko Sano	
09.00	What We Know and What We Have To Study for Understanding Fish and Shrimp Immune System? (AbstractID 235F) **Ikuo Hirono**
09.30	Effect of Chronic Heat Stress Response on Hemocyte Transcriptome of *Penaeus vannamei* Challenged with *Vibrio parahaemolyticus* AHPND (AbstractID 210F) **Kunlaya Somboonwiwat**, Benedict A. Maralit, Phattarunda Jaree, Pakpoom Boonchuen, Warunton Luangtrakul and Anchalee Tassanakajon
09.45	Transcriptome Profiling of Cobia (*Rachycentron canadum*) Infected with *Photobacterium damselae* Subsp. *Piscicida* with an Emphasis in Immune Responses (AbstractID 035F) **Hung B. Tran** and Ta-Chih Cheng
10.00	Peculiar Expression of CD3-Epsillon in Kidney of Ginbuna Crucian Carp (AbstractID 100F) **Ryuichiro Miyazawa**, Norifumi Murata, Megumi Kurose, Yuta Matsuura and Teruyuki Nakanishi
10.15	Determination of Marron (*Cherax Cainii*)Haemocyte Cell Types, Morphometric Characteristics and Their Phagocytic Activity at Different Temperatures In Vitro (AbstractID 218F) **Bambang Widyo Prastowo,** Ravi Fotedar, Rima Caccettaand Ricky Lareu
10.30 - 11.00	Coffee break
Thursday, 31 August 2017: Session 6 – Vaccinology Chair & Co-Chair: Angela Lusiastuti & Pravan Pradhan	
11.00	Advances in Fish Vaccine Development – Prevention is Better than Cure (AbstractID 241F). **Alexandra Adams**
11.30	Effectiveness of Formalin-Killed Vaccines Against *Vibrio harveyi*Infection in Orange-Spotted Grouper (AbstractID 167F) **Hai Trong Nguyen**,Thuy Thi Thu Nguyen, Ming-An Tsai, Ya-Zhen E, Yi-Ting Wang, Pei-Chi Wang and Shih-Chu Chen
11.45	PLGA Microparticle Vaccine Encapsulated Formalin-Killed *Aeromonas hydrophila* Cells Against *A. hydrophila* Infection (AbstractID 135F) **Saekil Yun**, Sib Sankar Giri, Jin Woo Jun, Hyoun Joong Kim, Cheng Chi, Sang Guen Kim, Sang Wha Kim, Jung Woo Kang and Se Chang Park
12.00	Effect of DNA Vaccine on Maternal Immunity of Common Carp (AbstractID 172F) **Sri Nuryati**, Ardana Kurniaji and Alimuddin
12.15	Uronema-Vibrio Vaccine (UViVac) Enhances Protective and Humoral Responses in*Sparidentax hasta* (AbstractID 123F) **Azad, I.S**., H. Al-Gharabally and S. El-Dakour
12.30 - 13.30	Lunch
13.30	Immunization With Inactivated Germinated Zoospores of *Aphanomyces invadans* Provide Protection Against Infection in Rohu *Labeo rohita* (AbstractID 228F) **Pravan K. Pradhan**, Sood, N., Bhushan, C. K., Yadav, M. K., Arya, P., Kumar, U. and Rathore, G.
13.45	Immune Defense of Shrimp Gills through *Marsupenaeus japonicus* Gill C-Type Lectin (MjGCTL) (AbstractID 048F) **Rod Russel R. Alenton**, Keiichiro Koiwai, Rika Nakamura, Jumroensri Thawonsuwan, Hidehiro Kondoand Ikuo Hirono
14.00	MHC Class II Expression on Gill Epithelial Antigen Sampling Cells (AbstractID 030F) **Yumiko Nakayama**, Takuya Yamaguchi, Uwe Fischer, Hidehiro Kondo, Motohiko Sano and Goshi Kato
14.15	Health and Survival Enhancement of *Penaeus vannamei* Post Larvae by Means of Probiotic

Thursday, 31 August 2017: Session 7 Biosecurity and Diagnostics Chair and Co-Chair: Han-Ching Wang and Budi Prayitno	
15.00	Biosecurity: From Management Reaction to Strategic Planning (AbstractID 242G) **Victoria Alday Sanz**
15.30	Epidemiology and Biosecurity for Shrimp Farming Industry(AbstractID 062G) **Jie Huang**, Xuan Dong, Qing-Li Zhang, Xiao-Yuan Wan, Guo-Si Xie, Bing Yang, Xiu-Hua Wang, Yan Liang, Hua Xu, Chen Li and Xiao-Ling Song
15.45	Biosecurity Model to Control Parasitic Disease in Recirculation Aquaculture System (RAS) for Catfish (*Clarias Sp*) Nursery and Growing Out(AbstractID 168G) **Tri Wahyuni**, Ayi Santika, Yuani Mundayana, Mira Mawardi, Edward Schram, Herry, Murtiati, Ucu Cahyadi, Juyana, Ece Ridwan, Dodo Suganda, Dery Derianti, Jaelani and Restu Rahayu B
16.00	Detection and Management of Diseases in Ornamental Marine Fish (AbstractID 080G) **Dolores V. Baxa**, Khairunissa I, Lam C, Kurobe T, Teh S, Rapi S, Pfahl H, Williams SL and N Janetski
16.15	Rapid Diagnostic Test for Red Sea Bream Iridoviral Disease (RSIVD) in Grouper *Epinephelus* Sp. Based on Serological Co-Agglutination and Molecular Study(AbstractID 082G) Surya Amanu, Kuniasih and **Dwi Sulistiyono**
16.30	Role of Biosecurity in Addressing of Antibiotic Resistance Concerns(AbstractID 112G) **Barbara Montwill**
16.45 - 17.00	Biosecurity and Its Role in National Protection(AbstractID 071G) **James Forwood**
19.00 - 22.00	**Gala Dinner & Conference Closure:** (Details may be changed later) 19.00: Balinese welcome dance 19.10: Remarks by Director General of Aquaculture (*Slamet Soebjakto*) 19.15: Appreciation for Senior Members of FHS-AFS (*Melba Reantaso*) 19.30: Student Presentation Awards by Chair& Vice-Chairperson of FHS-AFS 19.35: Introduction to ExeCom of FHS-AFS 2017-2020 22.00: Closure

DAA10 Elevator Pitch Schedule

Wednesday, 30 August 2017: Elevator Pitch(15.30 - 17.36)
Chair & Co-Chair: Agus Sunarto & Eduardo Leano

No	Time	Pitcher	Paper Title and Abstract ID
1	15.30	Rajeev Kumar Jha	*Enterocytozoon hepatopenaei* (EHP) is the Causative Agent of White Feces Symptoms and Growth Retardation in *Penaeus vannamei* In Indonesia (119A)
2	15.33	Siti Subaidah	Identification andExpression of Genes Related to Growth,Molting, and ImmuneSystem ofLobster, *Panulirus*sp. (150A)
3	15.36	Insariani B. Santosa	White Spot Syndrome Virus (WSSV) Examination in Fish Quarantine and Inspection Standard Examination Laboratory (BUSKIPM) Indonesia (090B)
4	15.39	Desrina	Simultaneous Occurrence of Haplosporidia-Like Organism (HLO) and White Spot Syndrome Virus (WSSV) in Polychaetes from Shrimp Ponds in Central Java, Indonesia (179B)
5	15.42	Khumaira Puspasari	Genomic Classification of Betanodavirus, the Causative Agent of Viral Nervous Necrosis, in Indonesia (091B)
6	15.45	Hong Liu	Insight into IHNV in Chinese Rainbow Trout Aquaculture from Virus Isolated from 9 Provinces between 2009-2014 (138B)
7	15.48	Misato Mori	The Temporal Shifts in Genogroups of Infectious Hematopoietic Necrosis Virus (IHHV) Isolates in the North Kanto Region of Japan (110B)
8	15.51	YS Jiang	Detection Methods of Cyprinid Herpesvirus 2 Infection in Crucian Carp (*Carassius auratus gibelio*) via an VP72 Monoclonal Antibody (118B)
9	15.54	Kei Yuasa	Sensitivities of New Cell Lines from Carp *Cyprinus carpio* X Goldfish *Carassius auratus* Hybrid and From Ginbuna *Carassius auratus* Langsdorfii to Koi Herpesvirus (KHV) (133B)
10	15.57	Jifang Yang	Interactome Study Of *Scylla serrata* Reovirus Proteins (139B)
11	16.00	Orachun Hayakijkosol	Case Report of Bacterial Infections in a Redclaw Crayfish (*Cherax quadricarinatus*) Hatchery (036C)
12	16.03	Jie Li	The *Aeromonas salmonicida* in Cold Farming Fish in Shandong Province of China (046C)
13	16.06	Mira Mawardi	Identification Of *Aeromonas sobria* from Catfish, *Clarias sp*, in Sukabumi, Indonesia (141C)
14	16.09	Miftahul Fikar Ultira	*Edwardsiella ictaluri* Infections in Indonesian Farmed Catfish (239C)
15	16.12	Anita Jaglarz	Genomic Comparison of *Streptococcus agalactiae* Isolates Recovered from Streptococcosis Outbreaks in Tilapia (*Oreochromis niloticus*) (229C)
16	16.15	William Leigh	Evaluation of Primers to Detect *Streptococcus agalactiae* (234C)
17	16.18	Yani Lestari N	Current Status of Antimicrobial Resistance of Bacteria from Grouper In Indonesia (157C)
18	16.21	B. Somridhivej	Antimicrobial Resistance Surveillance in Commensal *Escherichia coli* from Goldfish Farm in Ratchaburi Province (223C)
19	16.24	Arif Zaenuddin	Efficacy and WithdrawalTime ofErythromycin in White Leg Shrimp (*Penaeus vannamei*)(151C)
20	16.27	Lutfi Anshory	Effectiveness of Powder Probiotic Cultured in Seawater With Molasses Against Vibrio in White Shrimp *Litopenaeus vannamei* (005C)
21	16.30	Fatmawati	Utilization of Ketapang (*Terminalia catappa*) Leaves to Control Vibriosis in Hybrid Grouper (*Epinephelus spp*) (156C)
22	16.33	Nur Amalin Nadia Mat Nasir	Differential Responses of *Vibrio alginolyticus* to Aqueous and Ethanolic of Plant Extracts (057C)

23	16.36	Wang Yu-Chi	Characterization and Effect of Lactic Acid Bacteria Isolated from Digestive Tract of Cultured Fish on the Growth, Immune Response and Resistance to *Streptococcus iniae* in Silver Perch (*Lates calcarifer*) (066C)
24	16.39	Zafar Iqbal	Import of Ornamental Fishes, Risks to Emerging Pet Fish Trade in Pakistan (039D)
25	16.42	Angela Mariana Lusiastuti	Case Study the Parasite Infection of Gyrodactylid Monogeneans in *Clarias gariepinus* (121D)
26	16.45	Tri Wahyuni	Utilizing Tropical Almond Plant (*Terminalia catappa* L) Leaves to Reduce Parasitic Disease in *Clarias* sp Nursery (155D)
27	16.48	Wasin Srirattanasart	New Host Records and a Checklist of Fishes Infested with *Transversotrema patialense* (Trematoda: Digenea: Transversomatidae) in Thailand (205D)
28	16.51	Tze Hann Ng	Transcriptome Analysis Reveals Genes and Potential AHPND Pathogenesis Pathway in *Litopenaeus vannamei* Stomach (044E)
29	16.54	Xupeng Hong	Identification and Pathogenicity of *Vibrio parahaemolyticus* Isolates and Immune Responses of *Penaeus* (*Litopenaeus*) *Vannamei* (Boone) (006E)
30	16.57	Rajeev Kumar Jha	*Photobacterium* spp. is a Pathogenic Agent of *Penaeus vannamei* (131E)
31	17.00	Mari Inada	Improvement of PCR Program for the Detection of Infectious Myonecrosis Virus (IMNV) (186E)
32	17.03	Dinamella Wahjuningrum	Herbs for Controlling Vibriosis in Shrimp Cultured in the Sea (209E)
33	17.06	Nishikanta Chakrabarty	Does Environmental Stress Affect Fish Immunity? An Empirical Investigation (001F)
34	17.09	Vanvimon Saksmerprome	Microalgal Technology for Production of Shrimp RNA-based Vaccines (007F)
35	17.12	Yuki Ikari	Development of a Monoclonal Antibody against Gill Epithelial Antigen Sampling Cells of Rainbow Trout (029F)
36	17.15	Indah Istiqomah	Protective Granuloma Controls Non-Motile *Edwardsiella tarda* Infection in Vaccinated Red Sea Bream *Pagrus major* (111F)
37	17.18	Maggie Huang	Effects of Replacement of Fish Meal With Yeast Nucleotides on Growth, Non-Specific Immunity and Intestinal Morphology of Pacific White Shrimp (*Litopenaeus vannamei*)(142F)
38	17.21	Bambang Widyo Prastowo	Phagocytosis by Differential Involvement of Marron (*Cherax cainii*) Haemocytes with Live or Heat-Killed *Vibrio mimicus* as Measured by Flow Cytometry and their Nitric Oxide Activity Studies (219F)
39	17.24	K. Koiwai	Evaluation of the Potential of Integrin ? Subunit as a Marker for Hemocytes in Kuruma Shrimp *Marsupenaeus japonicas* (021F)
40	17.27	Diah Nur Maulida	Effect of Whey Tofu on Phycocyanin of Microalgae *Spirulina platensis* as Candidate for Antioxidant as Immunity System (107F)
41	17.30	Yu Huang	Costimulatory Signals in a Teleost Fish: CD28 Interact with CD80 (177F)
42	17.33	Narong Arunrut	Development of Recombinase Polymerase Amplification Assay Combined with Lateral Flow Dipstick for Detection of Acute Hepatopancreatic Necrosis Disease in Shrimp (038G)
43	17.36	Jigang Chen	A Convenient Immunochromatographic Test Strip for Rapid Detection of *Scylla serrata* Reovirus (140G)

ABSTRACTS

Plenary Session

Abstract ID 249 (Keynote 1)

AQUATIC ANIMAL HEALTH MANAGEMENT IN ASIA: YESTERDAY, TODAY AND TOMORROW

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Aquaculture is still the fastest growing food producing sector in the world. Aquaculture is critical to meeting the global nutritional and food security needs. The demand for food fish is increasing and aquaculture is poised to bridge this growing supply demand gap in the coming years. Asia has been the largest producer of aquatic food in the world, contributing nearly 90 percent to the global total over the past two decades. Over the next two decades, Asia will not only be the major supplier of food fish, but also the major consumer of fish, making aquaculture even more important sector in Asia. Diseases are common to all aquatic species cultured in all environments and all production systems and practices. They can be more prominent in commercial aquaculture; however, experience shows that epizootic level disease outbreaks in aquatic organisms can also occur in natural environments such as rivers and lakes. Aquatic animal disease outbreaks are not new to Asia. As the main global producer of aquatic food, over the decades Asia experienced disease outbreaks in many commercially produced aquatic species. Adding to the burden, the Tilapia Lake Virus (TiLV) is now threatening the important tilapia industry in Asia. Owing to the recurrent disease outbreaks in Asian aquaculture over the past decades, there have been attempts to improve aquatic animal health management capacity in Asia. Numbers of qualified fish health scientists and experts have been increased and their contributions to disease diagnostics and control have been amplified. The veterinary capacity of the corporate private sector has also been increased significantly. While we see some parallels in aquatic production and aquatic health capacity in Asia, the rate of increase of aquatic production in Asia has clearly outpaced the rate of increase of aquatic animal health capacity in the region. Since Asia is expected to contribute the lion's share for bridging the ever-growing demand-supply gap of global aquatic food in coming decades, this presentation will attempt to discuss the importance of further improving Asian aquatic animal health management capacity to ensure that this expectation is fulfilled.

Abstract ID 248 (Keynote 2, withdrawn)

TILAPIA LAKE VIRUS - AN EMERGING THREAT TO THE TILAPIA INDUSTRY

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Tilapia consist one of the most important groups of farmed fish worldwide and serve as a primary protein source in the developing world. Being grazing fish, tilapia are also important for the maintenance of ecological systems and of aquaculture of other edible species. In recent years, significant mortality of wild and cultured tilapia has been observed in the Middle East, South America and Asia. Fish showed lesions in a variety of organs, including the skin, eye, brain, gastrointestinal tract, liver, spleen and kidney. Inflammation and necrosis in affected organs were also detected, as well as hepatocellular syncytial cell formation. The high mortality of tilapia was suspected of having a viral etiology. We have isolated a virus from diseased fish, named it 'tilapia lake virus (TiLV)', and established conditions to efficiently propagate it and to purify its virions. We revealed that TiLV is the etiological agent of the disease in tilapia by reproducing morbidity and mortality in naïve tilapia that were either injected with the virus or cohabitated with TiLV-injected fish; and by the repeated isolation of TiLV from experimentally infected, diseased fish. We demonstrated that TiLV virions are enveloped (using ether or chloroform sensitivity assays) and that its deproteinized genome is not infectious. Determination of the sequence of TiLV genome, allowed us to show that diseased tilapia in Israel and South America are infected by the same virus (by diagnostic PCR); that the viral genome is detected in affected organs (e.g. brains and livers) and in the nuclei of infected cells (using in situ hybridization assays); that the genome is made of a single-stranded RNA (by combining RT-PCR and nuclease sensitivity assays); that the full-genome of the virus consists of ten segments, each with an open reading frame (ORF) (by applying high-throughput sequencing); and confirmed the presence of peptides, predicted for these ORFs (determined by mass spectrometry). Nine segments (and their ORFs) show no significant sequence similarity to other known sequences, and one segment shows only weak sequence similarity to a polymerase subunit of influenza C virus. Altogether, these findings demonstrate that TiLV is an emerging virus, highly pathogenic to tilapia, which imposes a serious threat to the global tilapia industry. The fact that in experimentally-infectedtilapia, fish surviving the initial mortality were immuneto further TiLV infections, suggests that elicitation of a protective immune response against this pathogen may be achievable. The development of vaccines and rapid diagnostics for TiLV should contribute to disease containment strategies.

Session 1 – Recent Advances in Aquatic Animal Health Research

Abstract ID: 212A (Keynotefor Recent Advances in Aquatic Animal Health Research)

NEW PARADIGMS TO HELP SOLVE THE AQUACULTURE DISEASE CRISIS

Grant D. Stentiford[1], Kallaya Sritunyalucksana[2], Timothy W. Flegel[3], Bryony A.P. Williams[4], BoonsirmWithyachumnarnkul[3], Orn Itsathitphaisarn[3,5] and David Bass[1,6]
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By 2050, global production from aquaculture is set to at least double with well-managed fisheries expected to flatline or even decline over the same period. Aquaculture will make an increasingly important contribution to the future global diet. Forty years since the landmark FAO Technical Conference on Aquaculture, the implicit forecast for aquaculture to rival fisheries production has therefore been fulfilled. The Bangkok Declaration which followed recommended several key requirements for development beyond 2000; in particular that animal health be managed by cooperative action at national, regional and inter-regional levels as “an urgent requirement for sustaining growth”. Although significant progress has been made in identification, diagnosis, treatment and zone management of disease in certain sectors, recalcitrant issues have remained as significant barriers to expansion. Infectious microbial diseases continue to impose major yield-limiting effects on production with overall impact of these diseases exceeding $6bn per annum, rivalling in magnitude projected proportional losses experienced in terrestrial livestock sector due to diseases such as Foot and Mouth. In specific sectors (e.g. shrimp), disease losses may exceed 40 % of global capacity with emergent diseases have potential to collapse national/regional production. Such issues confirm disease as *the* major constricting factor for expansion of the aquaculture industry to 2050.Even more so than terrestrial systems, aquatic environments impose a constant risk of exposure to disease-causing pathogens. An historic poor knowledge of background microbial ‘diversity’ in aquatic farm systems leads to frequent emergence of previously unknown diseases, surprising farmers and creating shock in the wider value chain. Some of these issues remain restricted to certain regions or, go away relatively quickly. Others have potential for rapid spread to distant regions with trade and, impose long term (often decadal) economic and social effects, often in the world’s poorest farming communities. In order to break the cycle of emergence, spread, persistence and effect, new thinking is needed. A recent Newton-funded UK-Thai workshop broadly considered the challenge: How to detect potential emerging diseases earlier? How to understand and manage the background microbial diversity in aquaculture systems? How to stop the spread of diseases between farms, regions and nations? How to engage farmers in biosecurity? How to align surveillance activities with targeted research? How to embrace technologies for rapid detection and reporting of disease? How to use advances in animal breeding and genetics to improve disease resistance? How to share responsibilities for animal health between net producer and net consumer nations? These issues are tackled in a recent paper by our team and will be outlined in this talk. Marginal improvements which reduce the global burden of disease in aquaculture will convert to direct benefits for yield, profit, poverty alleviation and food security for producer nations. More significant interventions, including those which capitalise on automated detection of disease and even remote sensing applications have significant potential to mitigate against the most important yield-limiting production diseases and, to improve the insurability of the global aquaculture sector, promoting inward investment and assuring production targets to 2050 are met in a sustainable manner.

Abstract ID: 195A (Oral)

CHALLENGES FOR CONTROLLING WHITE SPOT DISEASE (WSD)

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White spot disease (WSD) is a disease that is very difficult to control. It not only has a very broad host range, but the causative pathogen, WSSV (white spot syndrome virus) replicates very rapidly in the host and it has lots of anti-host defense strategies. You might think that to control WSD, all you need to do is monitor and survey for its presence and then quarantine, or otherwise keep separate, the virus and its shrimp host. However, if you look at the history of this disease, you can see that, despite our best efforts, since its emergence ~25 years ago in East Asia, it has spread to become an almost global pandemic.During 2010 to 2012, WSSV caused severe mortalities in cultured penaeid shrimp in Saudi Arabia, Mozambique and Madagascar. In a 2013 study, Dr. Lightner's group suggested that the WSSV epidemics in these 3 countries were from a common source, and that this source was possibly the environment. This is quite depressing; it suggests that we need to look beyond diagnosis and quarantine. Right now, this is an enormous challenge. Our laboratory is always seeking new knowledge to prevent or minimize the effects of WSSV on shrimp. One intervention strategy currently being explored by us is genetic improvement of shrimp. We have applied a systems-biology approach to elucidate critical host factors for WSSV infection and replication, and we have used these genes to develop anti-WSSV genetic markers. These markers were than used to develop a screening platform for shrimp (farmed or wild) with resistance to WSSV. We have already identified four families of shrimp that are resistant to WSSV infection, and two of these families have already been bred to the 2nd generation by mating between siblings. When these shrimp were challenged with the virus, the WSSV-resistant shrimp all survived, and no virus genomic DNA copies were present in the shrimp. In contrast, all susceptible shrimp died (with a high virus load). This WSSV-resistance test on the second generation of two of the resistant families confirms that the virus resistant trait is heritable. Building on this achievement, the next step will be to develop shrimp lines that are both disease resistant and fast growing for use in the shrimp industry.

Abstract ID: 117A (Oral)

THE PATHOBIOME CONCEPT: AN EMERGING VIEW OF MICROBES AND DISEASE

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Recent studies have shown that pathogens, parasites, and related lineages are highly diverse in environmental and organismal matrices. While some of these are recognized disease causing agents, the role of others is less clear – they may be aymptomatic or contribute directly or indirectly to a cumulative effect on host health. The pathobiome idea is starting to replace a one-pathogen-one-disease paradigm, based on the concepts of a host organism being the focus of a diverse community of microbial symbionts, multiple infections being the norm rather than exception, and synergistic effects occurring between infecting microbes. This talk will present the results of recent studies investigating microbial diversity associated with a range of invertebrate and plant hosts, and review broader findings supporting the pathobiome concept, a further case study being the microbiome associated with disease outbreaks in aquaculture pond systems.

Abstract ID: 104A (Oral)

ADVANCES IN THE APPLICATION OF HIGH-THROUGHPUT SEQUENCING IN THE DISCOVERY AND CHARACTERISATION OF NOVEL AND EMERGING AQUATIC ANIMAL PATHOGENS

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Over the last decade, advances in high-throughput sequencing technologies have revolutionised biological research, making it possible for DNA/RNA sequencing of any organism of interest to be undertaken in most modern molecular laboratories. Sequencing approaches are now routinely used in the detection and characterisation of (novel and emerging) pathogens and/or to investigate host-pathogen interactions with the aim to develop effective disease treatment strategies. For the sequencing and characterisation of pathogens of interest, whole genome sequencing can be performed on isolated organisms. However, often it is not possible to culture these pathogens in the laboratory and in these cases, DNA/RNA extracted from infected host tissues can be sequenced using metagenomics approaches. High-throughput sequencing can also be used to investigate host-pathogen interactions by investigating (temporal) transcriptomic responses of both the host and pathogen, providing insight into the molecular mechanisms of infection, potentially leading to the discovery of novel opportunities for treatment and drug targets. In addition, pathogens in environmental samples (e.g. water or gut samples) can be identified using environmental DNA (eDNA)/metagenomics approaches. The promise that recent developments in sequencing brings to the field of invertebrate pathology is not devoid of technical challenges, including the need for better laboratory and bioinformatics strategies to sequence and assemble the genomes of pathogens within complex tissues or environmental samples, and the difficulties associated with the annotation of the large number of novel pathogens being discovered. An overview of the various methods and bioinformatics approaches, including challenges and opportunities for the discovery and characterisation of novel and emerging pathogens and studies on host-pathogen interactions will be presented.

Abstract ID: 148A (Oral)

IMMUNE RESPONSE OF COMMON CARP TO CYPRINID HERPESVIRUS 3

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Common carp, *Cyprinus carpio,* is a major pest species in Australian waterways, in some instances comprising 90% of all fish biomass. This results in the dislocation of native species, increased water turbidity, loss of aquatic vegetation, and alterations in zooplankton and benthic invertebrate diversity. On the other hand, carp are an important aquaculture species in many counties. *Cyprinid herpesvirus* 3 (CyHV-3) specifically infects carp and induces high mortality rates causing significant losses in aquaculture facilities, although it may also be a useful biocontrol agent in an Australian context. However, carp immune responses and mechanisms of resistance to CyHV-3 are not well understood. These data are important for predicting susceptibility changes in wild carp populations over time, for developing long-term control strategies, and for helping control CyHV-3 outbreaks in aquaculture. We used high throughput sequencing of carp messenger RNA (mRNA) during different phases of CyHV-3 infection to detect the gene expression dynamics of both host and virus simultaneously. During acute CyHV-3 infection, the carp host modified the expression of several thousand genes that were involved in a range of immune systems and detoxification pathways. These activated pathways indicated that a humoral immune response, rather than a cell-mediated response, was preferred by the carp. Interestingly, the type of immune response mounted by the carp may have been influenced by the virus itself through the expression of a captured interleukin-10 homologue, thereby favouring virus survival. In addition, many immune-related genes were duplicated in the carp genome, and often these were expressed differently across the infection phases. This genetic redundancy may allow immune-related genes to evolve more rapidly, possibly improving the ability of carp to develop resistance mechanisms. Finally, the humoral adaptive immune response in carp was examined by assembling immunoglobulin transcripts. The carp immunoglobulin repertoire significantly diversified during CyHV-3 infection, which was followed by the selection of high-affinity B-cells, indicating a developing humoral immune response. These findings will help improve the usefulness of CyHV-3 as a biocontrol agent, and inform efforts to prevent CyHV-3 outbreaks in aquaculture.

Abstract ID: 126A (Oral)

THE IMPACT OF QUORUM SENSING SIGNALS ON THE VIRULENCE OF *Vibrio campbellii* TOWARD DIFFERENT CRUSTACEAN HOSTS

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Quorum sensing systems have been reported to regulate the expression of virulence genes in many pathogenic bacteria, and many of them use different signal molecules to regulate virulence gene expression. The aquaculture pathogen *Vibrio campbellii* contains a three-channel quorum sensing system, with three different types of signal molecules (HAI-1, AI-2 and CAI-1, respectively) feeding a shared signal transduction cascade. In the present study, we investigated the impact of the signal molecules on the virulence of *V. campbellii* towards two different crustacean hosts, brine shrimp (*Artemia fransiscana*) larvae and larvae of giant freshwater prawn *Macrobrachium rosenbergii*. In this study we used *V. campbellii* wild type and quorum sensing signals molecule synthase mutants. Two different hosts, (gnotobiotic) brine shrimp and giant freshwater prawn were challenged by inoculating the rearing water with 10^5 CFU.ml^{-1} and 10^6 CFU.ml^{-1} *V. campbellii* respectively. Briefly, the shrimp were cultured in groups of 20 larvae in glass tubes containing 10 ml synthetic sea water (35g.l^{-1} Instant Ocean) and fed with an autoclaved suspension of *Aeromonas sp.* LVS3. The challenge test for giant freshwater prawn larvae was performed by distributing 25 larvae in glass cones containing 100 ml fresh autoclaved brackish water (12g.l^{-1} Instant Ocean). The larvae were fed daily with axenic *Artemia.* Larval survival was counted daily and the growth was monitored by determining the larval stage index (LSI). The results showed that AI-2 and CAI-1 deficient mutants were significantly less virulent to the brine shrimp larvae than the wild type, while the HAI-1 deficient mutant was as virulent as the wild type. On the other hand in the prawn larvae, the HAI-1 and AI-2 deficient mutants showed a significantly decreased virulence and the addition of the signal molecules together with the synthase mutants restored the virulence of the mutants. In contrast to the HAI-1 and AI-2 deficient mutants, significant mortality (similar to that caused by the wild type) was observed in prawn larvae challenged with the CAI-1 deficient mutant. Possible explanations for this are (1) that the signal molecules can have a different stability in different environments and thus potentially also in different hosts, (2) that the expression of signal molecule synthases and/or receptors might be different in different environments and (3) that there might be not yet identified signal transduction cascades that are not affected by all three signals. Finally, none of the strains affected growth of the surviving larvae. In conclusion, our results indicate that it is highly important to study the impact of quorum sensing regulation under conditions that are as close as possible to the clinical situation because pathogen can rely different signal molecules to control its virulence in different hosts.

Abstract ID: 188A (Oral)

ETHANOLIC EXTRACT OF GINGER AND THREE OF ITS MAJOR COMPONENTS INHIBIT BIOFILM FORMATION BY *Vibrio harveyi*AND *V. parahaemolyticus*IN *IN VITRO* STUDY AND EXTRACT SUPPLEMENTED FEED PROTECTS *Penaues vannamei* SHRIMP FROM ACUTE HEPATOPANCREATIC NECROSIS DISEASE

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Specific isolates of Vibrio bacteria that cause acute hepatopancreatic necrosis disease (AHPND) colonize and form biofilms on the chitin lined fore stomach of *Penaeus vannamei* shrimp host before releasing toxin into and destroy the hepatopancreas. To reduce or to control these bacterial biofilms, ethanolic extract of ginger (0.2 mg/ml and 2 mg/ml) was tested for its ability to inhibit or reduce the biofilm formations by 4 isolates of Vibrio bacteria, including the AHPND causing isolate *V. parahaemolyticus* 3HP, on a shrimp stomach model made of chitosan coated 96-well polystyrene plastic plates. The extract was found to significantly ($p<0.05$) inhibit biofilm formation by all four isolates but did not affect planktonic cell growth of the same bacteria cultured in uncoated plate in parallel experiment. To determine if the extract can protect shrimp post larva (PL), the life stage most affected by AHPND, in vivo experiments were conducted. In these experiments *P. vannamei* PL, were fed with either extract supplemented feed (0.2 mg/g and 2 mg/g feed) or un-supplemented feed for 7 day prior to infection with *V. parahaemolyticus* isolate 3HP by immersion in 10^6CFU/ml. The shrimp health and mortalities were monitored for 7 days. PLs that were fed with ginger extract supplement feed were found to have 40%-60% higher survival than those infected PLs that were fed un-supplemented feed. It was also found that the extract have no negative effect on the palatability of the feed nor on the shrimp growth. To identify the bioactive compounds in the extract, four major compounds commonly found in the extract were tested; three of these compounds were found to possess biofilm formation inhibition activities but not the planktonic cell growth inhibition activity. These results suggest that inhibitor of biofilm formation in ginger can reduce the effect of Vibrio infection in shrimp and can potentially be used as a feed additive to reduce effects of AHPND bacterial infection in shrimp.

Abstract ID: 102A (Oral)

USE OF MARINE VESSEL TRACKING SYSTEM TO CREATE A CONTACT NETWORK OF AQUACULTURE IN NORWAY AND THE IMPLICATION FOR RISK-BASED SURVEILLANCE AND ZONE ESTABLISHMENT

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In this study, the contact network among the aquaculture facilities was developed using real-time data from automatic identification system (AIS) of which any encounters of the marine vessels into the areas registered for aquaculture facilities have been automatically captured to a database and displayed in a real-time system. AIS data include current and historical records of the encounters along with identity of the encountered vessel, identify of the encountered aquaculture facility, information on vessel type, date and time of the encounter, type of the encounter, and area at which the encounter occurs. We used the AIS data to develop a network-based, disease spread model to simulate a spread of Infectious hematopoietic necrosis virus (IHNV) among aquaculture facilities in Norway, and evaluate different strategies for prompt detection of the virus incursion into salmon farms and marine environment. IHN is a notifiable fish disease to EU and OIE. The disease has never been diagnosed in Norway; therefore risk-based surveillance for early detection of the virus is important for disease prevention. The disease has been reported in fresh-water and marine farmed salmonids in Europe and North America. Outbreaks of IHN have resulted in substantial economic losses in farmed salmon, and the disease has also had an impact on wild populations. Transfer of live fish for stocking and slaughtering via wellboats is recognized as the key risk factor for disease emergence and spread of IHNV in salmonid production.The data generated from AIS system help to better understand the complexity of aquaculture network connected through marine vessels and its implications on disease spread. Outcome from evaluation of surveillance strategies reveals the most effective risk-based approach was by targeting on-growing farms that are most reachable or those with the high in-closeness centrality measure. Information obtained from such network connectivity is valuable to design risk-mitigation strategies to better prevent and control diseases of aquaculture. In addition, a community structure based on the network could facilitate the establishment of zone for surveillance and monitoring in relation with aquaculture activities.

Abstract ID: 019A (Oral)

USING PHYSIOLOGY TO OPTIMISE WATER QUALITY AND THE SUSTAINABILITY OF INTENSIVE AQUACULTURE

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Exponentially rising CO_2 is a global concern (currently 400 µatm), driving climate change and causing acidification of both marine and freshwater environments. Physiologists have long known that CO_2 directly affects acid-base and ion regulation, respiratory and metabolic function. More recently, many studies have demonstrated that elevated CO_2 projected for end of this century (e.g. 800-1,000 µatm) has additional, previously unforeseen, effects on sensory and nervous system functions of fish and invertebrates, negatively impacting behaviour, fitness, and survival. Increasing alongside the human population, rising CO_2 levels not only threaten aquatic ecosystems but also global food security. Despite this, elevated CO_2 is also intimately associated with intensive aquaculture. High stocking densities commonly used in aquaculture can elevate CO_2 levels beyond 10,000 µatm. Understanding the potential physiological implications of these extreme CO_2 conditions is thus crucial for the optimisation of intensive aquaculture practises, and ultimately for ensuring this sector is able to sustainably intensify production to meet the increasing global demand over the coming decades.The greatest yield-limiting pressure impacting the sustainability of worldwide aquaculture production is disease, imposing over $6billion in losses annually. It is thus surprising that the role of high CO_2, and related water quality issues, in disease outbreaks in aquaculture has not been investigated. There is evidence from climate change studies that shellfish suffer impaired immune function and altered host-pathogen interactions when grown under relatively mild increases in CO_2 level (~1,000 µatm), with elevated temperature also shown to increase the probability of disease outbreak. Thus, elevated CO_2 levels projected for end of the century are likely to have significant impacts on future coastal and offshore aquaculture production, even though these sites are considered to have “low” CO_2 levels currently (i.e. close to open ocean). However, with CO_2 levels routinely exceeding 5,000 µatm in various intensive aquaculture settings, including flow-through production, land-based recirculating aquaculture systems (RAS) and ponds, the potential implications of such suboptimal water quality for disease or adverse health outcomes are significant. Whilst high CO_2 is of relevance to both intensive aquaculture and aquatic acidification, traditionally these two connected fields have remained disparate. By outlining the emerging evidence from climate change research we highlight the importance of bringing these communities together, and of delineating a pathway for positive interaction that can direct future research for mutual benefit. This in turn will improve understanding of the negative impacts of CO_2, as well as enable the optimisation of aquaculture practices and animal welfare. The future challenge of managing disease in global aquaculture and the development of successful mitigation strategies are crucial to support an expanding and sustainable industry in the future. Understanding of the impact of water quality on organism physiology is imperative to enable this process.

Abstract ID: 119A (Poster with Elevator Pitch)

Enterocytozoon hepatopenaei (EHP) IS THE CAUSATIVE AGENT OF WHITE FECES SYMPTOMS AND GROWTH RETARDATION IN *Penaeus vannamei* IN INDONESIA

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A microsporidiun, *Enterocytozoon hepatopenaei* (EHP) and several Vibrio species, *Vibrio harveyi*, *Vibrio parahemolyticus* and *Vibrio vulnificus* were was successfully isolated and purified from the hepatopancreas and feces of infected *Penaeus vannamei*. A series of bioassay trials were conducted to optimize the challenge method and dose. After standardization, the Post Larvae, free from known pathogen including EHP, was challenged using co-habitation method. The challenged and not-challenged (control) shrimp were reared for 60 days. The feces and hepatopancreas of challenged group were detected positive to EHP by RT-PCR. The challenged shrimp showed of white feces syndrome and retarded growth. There was significant difference in size variation, survival rate and mean body weight were recorded between challenged and not-challenged group. The second set of trial was designed in the way that shrimp were challenged with EHP alone, Vibrio species alone, Vibrio species mixed in 1:1:1 ratio and EHP with *V. harveyi*, EHP with *V. parahemolyticus* and EHP with *V. vulnifucus*. The white feces symptoms and retarded growth only appeared in the groups challenged with EHP and EHP with Vibrio species. The EHP alone and EHP with *V. parahemolyticus* challenge were the most lethal. There was no white feces symptoms appearance in the groups challenged only with different *Vibrio* species or in combination. The obtained results showed that only EHP is the causative factor of white feces symptoms and retarded growth in Vannamei. The Vibrio species act as secondary pathogen and causes mortality. The lethality level of EHP reduces with the age of shrimp i.e. the appearance of symptoms were more prominent when shrimp were challenged at Post larvae stages as compared to juvenile (2 g and 5 g) and grow out (10 g) stage.

Abstract ID: 150A (Poster &Elevator Pitch)

IDENTIFICATION ANDEXPRESSION OF GENES RELATED TO GROWTH,MOLTING, AND IMMUNESYSTEM OFLOBSTER, *Panulirus*sp.

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Lobster hatchery cannot be done because there are still many obstacles. All these obstacles related to metabolism, physiology, reproduction, nutrition, hormone system, immune system and others. For that as a first step performed gene approach in order to obtain an overview of the system of hormones that affect the growth and immunity Lobster. Tests starting from the collection of stage nauplisoma/new larvae hatch, the larvae day 3, larvae day 6, seed size 5 cm, seed size 50 grams, and lobster adults, in fresh condition extraction of RNA and cDNA were analyzed using PCR with primer that has been designed previously. If the desired gene appears followed by real-time PCR analysis and sequencing. The results showed that the gene expression related to growth there is a tendency that the larger the size of the lobster higher the value of gene expression, but because of high gene expression MIH also slow the growth of lobster. While the expression of genes related to immunity showed that the gene expression of small size until adult is no increase, there is a tendency that low immunity, and that causing lobsters susceptible to disease and death. Similarities growth and immunity-related genes of lobster with other species were very low. From the results of this engineering can be applied several things that can boost feed use CHH expression, application of biotechnology to transfer genes GH, adding immunostimulan to improve immunity, and some testing related to feed, feed supplements, disease, and the environment.

Abstract ID: 008A (Poster)

DEVELOPMENT AND CHARACTERIZATION OF CELL LINE FROM GILL AND TAIL FIN FROM *Cyprinus carpio*

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Fish Quarantine and inspection standard examination laboratory (BUSKIPM) has been succesfully make cell line from gills and tail fins of *Cyprinus carpio*. The methode used was the wolf and Quimby (1976) that has been modified in the treatment of time, the turn of the media and media concentration (Sumiati T, *et al.,* 2009). The cell line from gills dan tail fins *Cyprinus carpio* grew very fast and heavy in medium L-15. In 2012, Characterization BUSKIPM cell line have been tested, the tested are 1. cell line from gills and tail fins *Cyprinus carpio* are free from mycoplasma; 2. The morphology of cell line from gills and tail fins *Cyprinus carpio* are epithel-like and fibroblast-like; 3. The number of cells/ml cell line from gill passages level 30 dan cell line from tail fin *Cyprinus carpio* level 14 are seeded with initial concentration of each 10^5 cells / ml in the flask 25 cm^3 and the result of population doubling time of cell line from gills *Cyprinus carpio* passages level 30 is on day 3 after seeding. While in the cell line from tail fin *Cyprinus carpio* passages level 14 on day 6; 4. Number of chromosomes cell line from gills *Cyprinus carpio* was 2n = 84 while on cell line from tail fin *Cyprinus carpio* was 2n = 100; 5. Multilevel trypsinasi proses with 3 level , a.) cell line from gills *Cyprinus carpio* passages level 23 concentration of 0.005% trypsin at 1st, 2nd and 3rd minute morphology of cells collected are epithelial-like and fibroblast-like. Cell line from tails fin *Cyprinus carpio* passages level 22 trypsin concentration of 0.005% in the 1st and 2nd minute morphology of cells collected are epithelial-like and fibroblast-like while in the 3rd minute only fibroblast-like. b.) cell line from gills *Cyprinus carpio* passages level 23 concentration of 0.01% trypsin at 1st and 2nd minutes morphology of cells collected are epithelial-like and fibroblast-like while in the 3rd minute only fibroblast-like. Cell line from tails fin *Cyprinus carpio* passages level 22 trypsin concentration of 0.01% in the 1st and 2nd minute morphology of cells collected are fibroblast-like while in the 3rd minute are epithelial-like and fibroblast-like.c.) cell line from gills *Cyprinus carpio* passages level 23 concentration of 0.015% trypsin at 1st minute morphology of cells collected is fibroblast-like while in the 2rd and 3 rd minutes morphology of cells collected are epithelial-like and fibroblast-like. Cell line from tail fin *Cyprinus carpio* passages level 22 trypsin concentration of 0.015% in the 1st minute morphology of cells collected are Epithel-like while in the 2nd and 3rd minutes are epithelial-like and fibroblast-like . Cell line from tail fin *Cyprinus carpio* passages level 22 trypsin concentration of 0.01% in the 1st and 2nd minute morphology of cells collected are fibroblast-like while in the 3rd minute are epithelial-like and fibroblast-like. The last last of passages cell line of BUSKIM from gills is 70 and from tail is 72. The preservation of cell lines is in 10% DMSO under -80°C for one year. Thawing will be done every 3 months to ensure the viability of the cell.

Abstract ID: 022A(Poster)

A Spirochete AS PUTATIVE PATHOGEN IN AKOYA OYSTER DISEASE

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Mass mortality events due to Akoya oyster disease (AOD) have been reported since 1994 in cultured Japanese pearl oyster, *Pinctada fucata martensii*. The disease causes the adductor muscle and mantle of affected oysters to turn a reddish-brown color. Histopathological changes in the diseased oysters were characterized by the marked infiltration of hemocytes and the collapse of the loose connective tissue. Although spreading pattern of the disease and transmission experiments suggest that the disease is infectious, the causative agent has not yet been identified. In a previous study using shotgun and 16SrRNA-targeted metagenomic analyses to identify the causative agent of AOD, we found that a bacterium closely related to the genus *Brachyspira* (phylum *Spirochaetes*) was the potential causative agent of AOD. To test this hypothesis, a penicillin administration test, PCR-based epidemiological assay, and immunostaining and in-situ hybridization observations of microbial cells were performed in this study. Penicillin bath-administration immediately after injections to naïve oysters with hemolymph of diseased animals inhibited AOD development. PCR primers were designed from a shotgun metagenomic sequence, which Blast searches revealedhad a high homology with *Spirochaetes* sequences. No amplification was observed in pearl oysters that were collected from an AOD-free area, but specific target amplicons were generated in pearl oysters affected with AOD. By *in situ* hybridization using specific 16S rRNA directed FISH probe or immunostaining using rabbit antiserums against *B.aalborgi* and *B.pilosicoli*, a *Brachyspira*-like bacterium was observed in smears of hemolymph from affected oysters, but not from healthy oysters. Since this bacterium was ubiquitously and specifically detected in AOD-affected pearl oysters, it is strongly suspected to be the causative agent of AOD.This work was supported by a grant from the Project of the Bio-oriented Technology Research Advancement Institution, NARO.

Abstract ID: 063A(Poster)

EFFECTS OF DIETARY SELENIUM LEVELS ON GROWTH, PHYSIOLOGICAL AND IMMUNE RESPONSES OF TILAPIA, *Oreochromis niloticus* × *O. aureus*

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The study aimed to estimate the dietary selenium (Se) requirements and its effects on physiological and immune responses for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Sodium selenite was added to the semi-purified basal diet at 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1 mg Se/kg diet providing 0.21, 0.27, 0.29, 0.44, 0.54, 0.60, 0.80 and 1.16 mg Se/kg diet. Total of 8 experimental diets, each diet was fed to triplicate groups of fish (mean initial weight: 1.12 ± 0.02 g) in a closed, recirculating rearing system for 8 weeks. Weight gain of fish fed the diet with 0.60 mg Se/kg diet was significantly higher (P<0.05) than that in fish fed diets with 0.21-0.29 mg Se/kg diet. Muscle Se concentration was higher in fish fed the diet with 1.16 mg Se/kg diet than that in fish fed diets with 0.21-0.29 mg Se/kg diet. Hepatic Se concentration and leukocyte superoxide production increased linearly with the increase of dietary Se supplementation. Hepatic Se-dependent glutathione peroxidase activity was higher in fish fed diets with = 0.80 mg Se/kg diet than all the other dietary treatments. Fish fed the diet with 0.21 mg Se/kg diet had higher hepatic thiobarbituric acid reactive substances (TBARS) value than fish fed the diet with 0.54 mg Se/kg diet. The results indicate that the adequate dietary Se concentration for growing tilapia estimated by weight gain and by linear regression of whole-body Se retention of the fish is 0.42-0.68 mg Se/kg diet. The study also suggests that the diet supplemented with adequate Se promotes physiological and immune responses.

Abstract ID: 161A(Poster)

ASSESSMENT OF LACTIC ACID BACTERIA AS PROBIOTIC AGAINST PATHOGENIC *Vibrio harveyi*

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Abundance of fish pathogenic microorganisms and frequently disease outbreaks have become one of major constraint in fish farming. Misuses of antibiotics as a prophylactic measures have produce the development of antibiotic resistance that cause adverse effect to the environment and animal health. As an alternate solution, introducing a beneficial microorganism called probiotic is getting much attention among researchers. In the present study, seven potential Lactic Acid Bacteria (LAB) were successfully isolated from intestine and liver of 15 tails of healthy juvenile seabass using MRS media. Through *in vitro* screening method using spot lawn and well-diffusion assays against *Vibrio harveyi*, the number of potential isolates was narrowed down to one and labelled as LAB3. The isolate was classified as gram positive with cocci shape. LAB3 also demonstrated ability to inhibit the growth of *V. harveyi* in liquid condition using co-culture assay. Moreover, the potential probiont able to grow at pH 2 until 10 and the optimum growth was observed at pH 7 within three hours of incubation in MRS+4% NaCl broth. Antibiotic sensitivity tests indicated that LAB3 was resistant to kanamycin, penincilin, gentamycin, tetracyline and streptomycin antibiotics. Preliminary *in vivo* assay showed LAB3 conferred significant protection to *Artemia* nauplii after challenged with *V. harveyi* at 10^5 CFUmL^{-1}. Thus, LAB3 showed great potential as probiotic and worth to be carried out for challenge assay using seabass larvae against vibriosis.

Abstract ID: 169A (Poster)

OOMYCETE AND ASCOMYCETE INFECTIONS IN MANTIS SHRIMP *Oratosquilla oratoria* AND THEIR POTENTIAL INVOLVEMENT IN THE DEPLETION OF WILD MANTIS SHRIMP POPULATIONS IN JAPAN

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Since the mid-1990s, the depletion of wild populations of mantis shrimp (*Oratosquilla oratoria*) has been reported across major shrimp fishing grounds in Japan. Fungal infections have been found in mantis shrimps in some coastal waters and are suspected to be a cause for the depletion of the populations. However, the infection levels of the pathogen have not been ascertained and their involvement in the depletion of the populations has not been evaluated. In the present study, we examined the infection of mantis shrimp with an ascomycete (*Plectosphaerella oratosquillae*)anda oomycete(*Halioticida noduliformans*), using microscopic and histological observations, a culture method, and molecular analyses, in several waters around Japan. The prevalence levels of *P. oratosquillae* and *H. noduliformans* were high, being 50%–100% at some sites. We also examined seasonal fluctuation in the prevalence of the fungal infections in mantis shrimps from Tokyo Bay, where the mantis shrimp fishery has collapsed. We discuss the impact of the ascomycete and oomycete on the mantis shrimp populations.

Abstract ID: 122A (Poster)

HIGH GENETIC CONNECTIVITY AND GENE FLOW OF NARROW-BARRED SPANISH MACKEREL (Scomberomorus commerson) FROM THE SOUTH CHINA, BALI AND JAVA SEAS

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Genetic connectivity and gene flow of narrow-barred Spanish mackerel (*Scomberomorus commerson*) from the South China, Bali and Java Seas was investigated using mitochondrial cytochrome *b* gene sequences. The nucleotide and haplotype diversities estimated among the gene sequences ranged between 0.012–0.014 and between 0.83–0.92, respectively. The phylogenetic analysis (Maximum Likelihood) of gene sequences indicated that the individuals sampled belong to a single taxonomic clade. The analysis of molecular variance (AMOVA) and F_{ST} values revealed non-significant variation among populations. Both Tajima's *D* and Fu's F_S statistics were non-significant indicating an effective large and stable population size. The results suggested that the *S. commerson* population has strong genetic connectivity and high gene flow in the study regions. In addition, mismatch distribution analyses and tests of neutrality evolution indicated that *S. commerson* populations had not undergone significant sudden population expansion recently. Hence, the current study suggests that Southeast Asian nations might need to consider cooperative management of *S. commerson* for sustainable use.

Abstract ID: 134A (Poster)

EFFECTS OF TAURINE SUPPLEMENTATION WITH PARTIAL REPLACEMENT OF FISH MEAL BY SOYBEAN MEAL ON RED SEABREAM (*Pagrus major*) UNDER LOW WATER TEMPERATURE

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This study was conducted to investigate the compensating effect of supplemental taurine in diets for two fish species on negative growth performance by fish meal (FM) replacement with soybean meal (SM). A FM based 2 diets were considered as positive and negative controls with and without taurine (FM-T and FM), and six other diets were formulated to replace FM by 20, 35 and 50% without taurine (SM20, SM35 and SM50) or with taurine supplementation at a level of 1% (SM20-T, SM35-T and SM50-T). Red seabreams averaging 108g were fed the experimental diets for 15 weeks. Weight gain (WG) and final body weight (FBW) were significantly higher in fish fed FM, FM-T, SM20-T and SM35-T than others while SM50 showed the significantly lowest values. However, FM-T group showed significantly highest WG and FBW. Feed utilization and survival were not significantly different among treatments. Hematological parameter, innate immunity and intestine and liver histology results will be discussed further.

Abstract ID: 173A (Poster)

SAXITOXIN CONTENT CAUSES PARALYTIC SHELLFISH POISONING (PSP) ON GREEN MUSSELS (*Perna viridis*) IN CIREBON WATERS

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Saxitoxin is one of the marine biotoxin and has been detected in filter-feeding bivalve molluscs such as oysters, mussels, scallops, and clams. This type of toxin is mainly produced by phytoplankton of the dinoflagellates group belonging to the genus *Pyrodinium*, *Alexandrium* and *Gymnodinium*. Saxitoxin cause paralytic shellfish poisoning (PSP) in humans, characterised by symptoms varying from a slight tingling sensation or numbness around the lips to fatal respiratory paralysis. The aim of this observation was to study the saxitoxin content in green mussels and sea water which is cultivated in Cirebon waters as an effort to provide food safety for human.This observation was conducted on December 15, 2016 in aquaculture area of green mussels in Cirebon waters. Sampling was conducted in 5 stations started from Gunung Jati sub-districts to Kapetakan sub-districts. The type of samples taken was green mussels and sea water. Saxitoxin content on green mussels meat was analyzed using ELISA method as well as in sea water samples testing and observation of phytoplankton identification and abundance in Sedgewick Rafter using microscope.The test results of saxitoxin content in green mussels at 1st to 5th station respectively were 0.66; 0.49; 0.60; 0.56; and 0.70 mg/kg. While the content of saxitoxin in water at 1st to 5th station respectively were 0.0061; 0.0076; 0.0053; 0.0029; and 0.0007 mg/L. The test results of saxitoxin content in green mussels are still below the regulatory limit (0.8 mg/kg). Bio Concentration Factor (BCF)of saxitoxin at 1st to 5th station respectively were 108.37; 64.73; 113.85; 193.10; and 1,029.41. *Pyrodinium* sp. was found from observation of phytoplankton and had the highest abundance in all stations. The abundance of *Pyrodinium* sp. at 1st to 5th station respectively were 7,031; 1,847; 16,761;6,818; and 10,795 ind/L. The presence of phytoplankton producing saxitoxin increase the risk of saxitoxin accumulation in the green mussels body and potential to harm human when they consumed it. Poisoning in humans whom consumption biotoxin contaminated green mussels could be happened if there areharmful plankton blooms. As precautionary measures, green mussels during harvest time need to be tested their saxitoxin content in order to ensure the quality assurance and food safety as well.

Abstract ID: 200A(Poster)

POLLUTION POTENTIAL BY OTC USE IN SEABASS (*Lates calcarifer* Bloch) FARMING

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Antibiotics in Indonesia nowadays is still as one of the additional substance at fish farming. It is still widely used to overcome the attack of bacterial diseases. Oxytetracycline is one type of antibiotic that is quite widely used and is a broad-spectrum antibiotic whose function can inhibit the growth of both gram-positive and gram-negative bacteria. The use of antibiotics through artificial feed is one of the most common ways farmers do for one of the most practical ways, but this method is predicted to worsen environmental conditions because of the potential pollution of antibiotics to the farming environment. The aims of this study is to know the concentration of Oxytetracycline exposed in the farming environment when doing treatment to fish. The fish for this test were Seabass (*Lates calcarifer* Bloch) with 75 - 100 gram weights. Those were divided into three ponds with 100 fish each of it, the dose of Oxytetracycline was 75 mg / kg body weight through artificial feed for seven days in a row. The Samples were water during experiment and the mixture of feces and wasted feed, taken for seven consecutive days per 24 hours after giving artificial feed. The method in this study was ELISA (Enzyme-linked Immunosorbent Assay). It was used to analyze the samples during experiment. The result showed that there is a concentration of Oxytetracycline in water media with 249.48 ppb - 496.50 ppb. While in the mixture of feces and wasted feed was 127.01 ppb - 162.28 ppb. That concentration of residual antibiotics will be a potential pollutant if it exposes to the environment.

Abstract ID: 203A (Poster)

REVIEW: HARMFUL ALGAL BLOOM IN LAMPUNG BAY (2012-2016)

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Harmful algal blooms (HABs) events are observed in several regions in the world including Indonesia that cause devastating impacts to coastal resources, local economies and public health. This review is intended to overview HAB events in Lampung Indonesia from 2012-2016. Human activities such as development of ponds, recreation, housing, harbor and deforestation have contributed to reduce environmental quality in Lampung Bay. Eutrophication has triggers blooming plankton some of which are harmful. Plankton species were reported present during HABs such as *Cochlodinium polykrikoides*, *Noctiluca scintillans*, *Pyrodinium bahamense*, *Prorocentrum* sp., *Pseudo_Nitzchia, Dinophysis caudata* and *Trichodesmium* sp.

Abstract ID: 206A (Poster)

THE USE OF SALINITY SHOCK, THERMAL SHOCK, AND FORMALDEHYDE DIPPING FOR SCORING TEST VALIDATION ON QUALITY DETERMINATION OF VANNAMEI SHRIMP PLS

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Determination for vannamei shrimp Pls quality was not well recognized by the user nor by the seed producer. Some methods for this purposes had been applied but it seems difficult to determine the scale of the Pls quality. The methods included measurement of body length, size uniformity, necrotic and ectoparasites occurrence, and stress test by means of thermal shock with 20 ^{0}C within 30 minutes, and 300 ppm for 60 minutes formaldehyde dipping, there was not found distinguished quality. Some trials were conducted to clarify the optimum level of salinity shock, thermal shock and formaldehyde (37% ingredient) dipping dose for PLs 10. The objectives of these trials were to determine the correlation of the treatments level (magnitude, dose and duration) to the survival rate (SR) effect that closed to 90 %. Thermal shock be done with 9, 12, 15, and 18 ^{o}C. Salinity shock be done with 0, 5, 10, and 15 ppt. Formaldehyde dipping be done with 300, 600, 900, and 1200 ppm. The survival rate of tested PLs observed every 15 minutes during 60 minutes. The survival rates of data on each treatments was analyzed using regression correlation. The results showed that the linear correlation for salinity shock described Y = 136.67 - 2.1889 X, the exposed duration required for the test with 0 ppt was about 21.3 minutes for about 90% SR. The temperature for thermal shock showed that the linear correlation Y= 105.00 - 0.5889 X. The temperature applied for 90% SR was 15 ^{o}C within 25.5 minutes exposed duration. The formaldehyde dipping test showed that the linear correlation was Y = 99.1667- 0.1222 X. The equation means the formaldehyde in 600 ppm for 90% SR should be exposed for 75.0 minutes.

Abstract ID: 207A (Poster)

DIETARY EFFECTS OF VARIOUS ANTIOXIDANT SUPPLEMENTS ON GROWTH, SURVIVAL, ANTIOXIDANT CAPACITY, IMMUNE RESPONSE, METABOLIC RESPONSEAND OXIDATIVE STRESS STATUS OF PACIFICWHITE SHRIMP (*Litopenaeus vannamei*)

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This study was aimed to determine the dietary effects of various antioxidant supplements on growth, survival, antioxidant capacity, immune response and oxidative stress resistance of Pacific white leg shrimp *Litopenaeus vannamei*. The seven supplements were astaxanthin (AX), vitamin A (VA), extract of *Quillaja saponaria* (QS), *Yucca schidigera* (YS), Nutrafito plus (NP, mix of QS and YS), leaves or seeds of *Moringa oliefera* (ML, MS). Each supplement was incorporated into a basal diet with the same diphenylpicrylhydrazyl (DPPH) antioxidant capacity. These 7 treatment diets and a control diet (C) without supplement were fed to the shrimp in triplicates for 12 weeks. Shrimp's growth, survival, antioxidant capacity, immune response and hypoxia stress resistance were evaluated. The treatments had no effect on survival. Shrimps fed with supplements had better growth performance than C fed shrimps. As compared to the control, the treatment diets resulted in higher antioxidant capacity, namely, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and immune response in respiratory burst (RB). AX shrimp had the highest and C shrimp the lowest antioxidant capacity in SOD, GPx and GR and resistance against hypoxia stress. Hypoxia stress increased shrimp's GPx and AST, but had no effects on SOD and ALT. The treatments exhibited their effect on RB when under no stress, however, hypoxia stress overrode the treatment effect, causing no difference in RB and total haemocyte count (THC). Hypoxia stress increased glucose (Gluc) and lactate (Lac) and decreased triglycerides (Trigs). When under stress, treatments with supplements resulted in lower Gluc, Trigs and Lac and lethal dissolved oxygen level and longer lethal time than the control. However, there were no differences in those metabolic responses among treatments of the supplement. In conclusion, those antioxidants and plant extracts enhanced shrimp's growth, antioxidant capacity and hypoxia stress resistance and stabilized their metabolic responses when under stress, however, only slightly affected their immune responses.

Abstract ID:240A (Poster)

LIGHT INDUCED SEQUENCE SPECIFIC GENE EDITING IN *Caenorhabiditis elegans* AS NEW MULTI-PURPOSE LIVE FOODS FOR AQUACULTURE

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Artemia is the live food mostly employed in aquaculture although several serious problems existed. These include expensive and very variable prices and important nutritional deficiencies. Model organism *Caenorhabiditis elegans* has been proposed as an ideal substitute or complementation to Artemia in larviculture. As a new medium for growing *C. elegans* has been developed to adopt aquaculture environment including sea water. However, genetic editing for using *C. elegans* to provide antigen for disease prevention for aquatic animal and optimization of their food nutrition as well as other specific purposes such as water quality sense-and-report has not been well developed. In this research, we used triplex-forming-oligonucleotide (TFO)-gold-nanoparticle conjugate as a vector and successfully achieved sequence specific targeting as evidenced by EMSA assay. We then assemble the conjugate with a photonic energy activated dual reactive species generation compounds to the 5'-end of the TFO to complete the assembly of artificial targeted light activated nanoscissor system (ATLANS) and demonstrated photo-activated double strand DNA scission precisely in the predesigned sequence site in an *E. coli* system harboring the plasmid containing the target sequence but not in the control plasmid upon illumination with a 1W 460nm LED for 15 minutes of exposure. Finally, we tested this light activated gene scission in a genetic modified *C. elegans* carrying enhanced green fluorescent protein (EGFP) gene in their somatic genome. We demonstrated successful deletion of the EGFP gene permanently in the genome level and completely know down the EGFP protein expression. We continuously monitor the EGFP signal in the ATLANS processed *C. elegans* and confirmed that even after 5 generations, the EGFP-knockout phenotype still existed. This study confirmed that we have achieved gene specific scission using photonic operated ATLANS system in both *E. coli* and *C. elegans*, which can be an attractive technology innovation to the aquaculture industry.

Session 2 – Virology

Abstract ID: 247B (Keynote for Virology)

BEHAVIORAL FEVER INDUCED BY CYPRINID HERPESVIRUS 3: WHEN THE ENVIRONMENT MAKES THE DIFFERENCE BETWEEN LIFE AND DEATH

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When infected by pathogens, endotherms and ectotherms can both increase their body temperature to limit the infection. Ectotherms do so by moving to warmer places, hence the term "behavioral fever". We studied the expression of behavioral fever by common carp infected by cyprinid herpesvirus 3 (CyHV-3) using multi-chamber tanks encompassing a 24°C-32°C gradient. We showed that carp maintained at 24°C all died from the infection, whereas those housed in multi-chamber tanks all survived as a consequence of their transient migration to the warmest compartment. As the expression of behavioral fever occurred only at an advanced stage of the disease, we hypothesized that the virus might delay this phenomenon in order to promote its replication. This hypothesis was proved correct, and the delay mechanism was found to rely on the expression of a soluble viral decoy receptor for Tnfα encoded by CyHV-3 ORF12. This conclusion relied on three complementary observations: (i) a CyHV-3 ORF12 deleted recombinant induced an early onset of behavioral fever in comparison to wild-type CyHV-3; (ii) ORF12 expression product binds and neutralizes carp Tnfα; and (iii) injection of anti-Tnfα neutralizing antibodies suppressed behavioral fever, and decreased fish survival in response to infection.This study provides a unique example of how viruses have evolved to alter host behavior to increase fitness. It demonstrates that behavioral fever in ectotherms and fever in endotherms are evolutionarily and functionally related through common cytokine mediators that originated more than 400 million years ago. Finally, this study stresses the importance of the environment in the host-pathogen-environment triad.

Abstract ID: 132B (Oral)

KOI HERPESVIRUS IN CARP: THE TALE OF TWO COUNTRIES

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Common carp (*Cyprinus carpio*) is an important food fish, valued at US$5.2 billion, in many countries of the world including Indonesia. However, in Australia, New Zealand and North America, carp is considered as an invasive pest species and a serious threat to natural biodiversity. Koi herpesvirus (KHV) is a lethal virus of koi and carp, devastating the industries worldwide. For example, following the first outbreak of KHVD in Indonesia in 2002, it took <4 years for the disease to spread throughout the whole country - from Sumatra in the west to Papua in the east - caused huge economic losses to the farmer. KHV may be seen as a dreaded pathogen in carp aquaculture or as a potential biological control (biocontrol) agent for pest carp. Safety and efficacy, two major concerns for a successful biocontrol virus, need to be taken into consideration before the use of any exotic biocontrol virus is considered. Indonesian C07 isolate is highly virulent in Australian carp and the isolate being assessed as a biocontrol agent for carp in Australia. Extensive host-specificity testing of 22 species of fish and other animals suggests that KHV is safe. The optimal water temperature range for KHV in fish is from 18-28 °C. However, in Australia, it is known that carp can be found where water temperatures are 4 °C in winter and possibly up to 40 °C in summer. Therefore, we need to develop temperature adapted mutants of KHV that can be used in these colder and hotter conditions. Whole genome sequencing of Indonesian KHV C07 identified 310 SNPs, indels and replacements, providing the ultimate fingerprint of the isolate and a basis for tracking the evolution of the virus once it is released as a biocontrol agent. To get insights into the virus-host relationship, a possible interaction between carp IL-12, carp IL-10 and khvIL-10 during the course of KHV infection were investigated. Transcriptomics analysis also indicates a developing adaptive immune response of carp against KHV infection. Understanding the virus-host interaction will benefit both countries. It may contribute to both the development of efficient antiviral vaccines, which are much need in Indonesia, and to the effective use of the virus as a biological control agent for carp in Australia. The work has provided the foundation for longitudinal studies to track the evolution of virus-host relationship following the release of KHV as a biocontrol agent. The initial high virulence of KHV will decline following its release in Australia, and therefore, the evolution of carp resistance to KHV will likely necessitate the future release of progressively more virulent strains of KHV. Modelling studies show a combination of virus biocontrol and genetic approaches is the most effective control method for carp in Australia. Therefore, we're not only investigating the use of KHV as a biocontrol agent, but also gene-editing and gene-drive as genetic approaches for the control of carp in Australia.

Abstract ID: 129B (Oral)

INTEGRATED ANALYSIS OF mRNA AND VIRAL miRNA IN THE KIDNEY OF *Carassius auratus gibelio* RESPONSE TO CYPRINID HERPESVIRUS 2

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miRNAs are small non-coding single stranded RNAs that play crucial roles in numerous biological processes. Vertebrate herpesviruses encode multiple viral miRNAs that modulate host and viral genes. However, the roles of viral miRNAs in lower vertebrates have not been fully determined. Here, we used high-throughput sequencing to analyse miRNA and mRNA expression pro?les of Carassius auratus gibelio in response to infection by cyprinid herpesvirus 2 (CyHV-2). RNA sequencing obtained 26,664 assembled transcripts, including 2,978 differentially expressed genes. Based on small RNA sequencing and secondary structure predictions, we identified 17 CyHV-2 encoded miRNAs, among which 14 were validated by Stem-loop qRT-PCR and 8 were validated by Northern blot. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the miRNAs-mRNA pairs revealed diverse affected immune signalling pathways, including the RIG-I-like receptor pathways and the JAK-STAT pathways. Finally, we presented 4 genes involved in RIG-I-like pathways, including host gene IRF3, RBMX, PIN1, viral gene ORF4 were negative regulated by CyHV-2 encoded miRNA miR-C4. The present study is the first to provide a comprehensive overview of viral miRNA-mRNA co-regulation, which might have a key role in controlling post-transcriptomic regulation during CyHV-2 infection.

Abstract ID: 196B (Oral)

CARP EDEMA VIRUS: AN EMERGING THREAT IN KOI CARP, *Cyprinus carpio koi*, IN INDIA

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The global ornamental fish trade relocates large quantities of live fish species between countries, and therefore can be a potential source for spread of exotic pathogens, particularly viral pathogens, which have been associated with high morbidity and mortality in fishes, in the importing countries. Carp Edema Virus (CEV) infection in fishes also known as koi sleepy disease (KSD), characterized by typical sleepy behaviour, enophthalmia, generalized oedematous condition and gill necrosis in koi carp. The affected juvenile carps congregate near the surface of pond, whereas the older fish tend to lie on the bottom of the pond and eventually die of anoxia and the mortality may reach 80–100 %. KSD was originally described from koi carps in Japan in 1974 and has been reported from many European countries including UK, France, Netherlands, Germany, Italy, Czech Republic, Austria and recently from India.In the present study a disease outbreak was reported in adult koi carp fishes showing 100% mortality (a total of 800 fish) within 8 days of stocking in Ernakulam District, Kerala, India. The clinical signs viz. anorexia, lethargy, skin erosions, enophthalmos and gill necrosis was observed. The histopathological examination of the affected fish revealed severe necrosis of gills, proliferation of the epithelial cells and adhesion of gill lamellae. Degenerative changes were observed in tubular epithelial cells of kidney. Spherical and electron dense virus particles were demonstrated in cytoplasm of gill epithelial cells of affected koi in TEM analysis. However, no virus particles were observed in spleen and kidney tissue sections. Molecular screening of tissue samples (gills, spleen and kidney) from affected koi revealed negative result for important diseases viz. KHV, SVCV, koi ranavirus and iridovirus. Gill tissue (37 samples) from affected koi carps was positive for CEV in nested PCR developed by Qyamatsu et al. (1997). The sequence analysis of 548 bp fragment from CEV-positive fish revealed greater than 96 and 98% sequence identity with the original sequences submitted by Oyamatsu et al. (1997) and Jung-Schroers et al.(2015). All 37 fish samples were re-tested with a second nested PCR assay (Matras et al. 2016) targeting 478 bp partial 4a gene sequence from CEV. Phylogenetic analysis of 478 bp fragment of 4a gene revealed high relatedness between the Indian koi carp CEV sequence and koi carp CEV sequences reported in UK, Poland and Japan. No cytopathic effect was observed in six cell lines following inoculation of filtrate from gill tissue homogenate of CEV positive koi, after 15 days of inoculation and even after five blind passages in cell lines derived from koi, goldfish and four other ornamental fish species cultured in India. In the survey carried out to detect presence of CEV from other locations in India, a total of 53 out of 320 koi carps (16.56 %) were positive for CEV in the first step, whereas additional 9 samples (2.8 %) were positive in the second-step PCR. Three sampling locations viz. Chennai, Madurai and Kolkata (hubs of ornamental fish farming in India) had higher prevalence levels of 31.58, 27.78 and 28.57 %, respectively. In the present study, screening of koi showing symptoms similar to sleepy disease from different locations revealed that CEV prevalence was widespread at all sampling locations. The trading apparently healthy koi may pose a threat of spreading CEV to unaffected regions, which emphasizes the need of precautionary approach to prevent its spread.

Abstract ID: 025B (Oral)

EMERGENCE OF TILAPIA LAKE VIRUS IN THAILAND

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The massive mortality of Nile and red tilapia due to an unknown etiology was investigated in Thailand from 2015 to 2016. The disease has been called tilapia one month mortality syndrome (TOMMS). In this study, we isolated the recently discovered tilapia lake virus (TiLV) in diseased tilapia in Thailand. The virus was propagated in the permissive cell line, with cytopathic effect (CPE) developing 3 to 5 days post-inoculation. Electron micrographs of infected cell culture and fish tissues showed round enveloped virions of 60 to 80 nm with characteristics very similar to those of *Orthomyxoviridae*. *In vivo* challenge of red tilapia confirmed that TiLV is the primary cause of TOMMS. Furthermore, we used RT-PCR to confirm the infection in inoculated cell line and experimental challenge fish. The histopathological findings of infected fish included inflammatory cells infiltration in the brain and massive degeneration of liver. To date, TiLV has been reported in Israel, Ecuador, Colombia, Egypt and Thailand. The spread of this emerging virus increases awareness that it is a potential threat to tilapia aquaculture in Thailand, Asia, and worldwide.

Abstract ID: 190B (Oral, Student)

VERO CELL LINES EXPRESSING NUCLEAR LOCATION SIGNALS OF *Penaeus merguiensis* HEPANDENSOVIRUS: AN EARLY STUDY

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Parvoviral diseases are emerging as a constant threat to penaeid culture due to their ability to cause slow growth and mass mortality of infected prawns. *Penaeus merguiensis* hepandensovirus (PmeHDV) (GenBank accession No. DQ458781) is a shrimp hepatopancreatic parvovirus (HPV), an Australian strain of the species Decapod hepandensovirus1, in the genus Hepandensovirus, subfamily Densovirinae. Densovirinae are intranuclear and require S-cells in their S-phase for all or most of all their replication and assembly. Transportation into and out from nucleus is allowed by the binding of nuclear location signals (NLSs) to Importins (Imp). A bioinformatical study on PmeHDV supported that the virus has putative NLSs that need to be tested. The present study aims to determine if of the three putative NLSs of PmeHDV are functioning by transfecting NLS-inserted-plasmid DNAs into mammalian cell culture (Vero) using a transfection reagent. Each plasmid has been synthetically constructed and inserted with each sequence of the putative NLSs and a fluorescent protein. The presence of the NLS in the cell nucleus and cytoplasm was screened under a fluorescent microscope. Findings: All transfected cells in our study demonstrates no noticeable differences within transfected-Vero cell cultures with desired NLSs genes. The overlay of visualization of transfected plasmids with sequential fluorochrome sets is presented in figure. It appears the NLSs are not functioning well as that the proteins are blocked at the nuclear membrane and not going across. In conclusion, our fluorescent study was not sensitive enough to detect differences in NLS-transfected-cells under different filters. The method used was ineffective in identifying the location of NLSs. In the future, the study of virus-host interaction using cell cultures as models remains a major challenge.

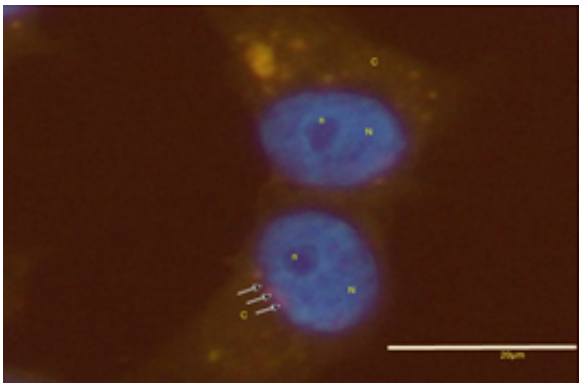

Figure: Visualization of transfected Vero cells with putative NLSs of PmeHDV 4 days-post transfection using combination of three fluorochromes (FITC-Green, DAPI, and Texas-Red). N: nucleus; n: nucleolus; c: cytocol; arrows: blocked proteins.

Abstract ID: 064B (Oral)

A NEW MEMBER OFIRIDOVIRIDAE IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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A newly discovered iridescent virus that causes severe diseases and high mortality in farmed white shrimp (*Litopenaeus vannamei*) in Zhejiang, China, has been verified and temporarily specified as shrimp hemocyte iridescent virus (SHIV). Histopathological examination of the diseased shrimp revealed typical features including basophilic inclusions and pyknosis in hematopoietic tissue and hemocytes in gills and sinus of hepatopancreas. Using viral metagenomics sequencing, we obtained partial sequences annotated as potential iridoviridae. Phylogenetic analyses using the amino acid sequence of major capsid protein (MCP) and ATPase indicated that a new iridescent virus, but it does not belong to five known genera of *Iridoviridae*. Transmission electron microscopy showed that the virus exhibited a typical icosahedral structure with a mean diameter of 158.6±12.5 nm (n=30) (v-v) and 143.6±10.8 nm (n=30) (f-f), with an 85.8±6.0 nm (n=30) nucleoid. Challenge tests of *L. vannamei* via intramuscular injection (im), per os and reverse gavage (rg) revealed the virus can cause 100% mortality in all challenged groups within 2 weeks. The median lethal times (LT_{50}) of the im, rg, and per os groups were 3.34±0.32 d, 5.69±0.48 d, and 8.11±0.81 d, respectively. The virus was purified from serum of hemolymph of experimentally infected shrimp and enveloped virus particles of 160.2 ± 7.0 nm (n=10) in diameter were observed on negative stained grids. The results of *in situ* hybridization showed that the hemopoietic tissue, gills, and hepatopancreatic sinus were positively reacting tissues. In addition, a nested PCR assay specific for SHIV was developed, and the PCR detection results revealed that *L. vannamei*, *Fenneropenaeus chinensis*, and *Macrobrachium rosenbergii* were SHIV-positive, indicating that a new threat is recognized in the shrimp farming industry.

Abstract ID: 105B (Oral)

DISCOVERY OF A NOVEL DISTANT RELATIVE OF WHITE SPOT SYNDROME VIRUS (WSSV 1) FROM WILD CRABS IN DISEASE FREE AREA

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White Spot Disease (WSD) is caused by White Spot Syndrome Virus (WSSV 1), which is a notifiable disease to the World Organisation for Animal Health (OIE) and has been responsible for massive losses in the shrimp farming industry. WSSV 1 is a large dsDNA virus and currently the sole member of the genus *Whispovirus* within a very distinctive DNA virus family, the *Nimaviridae*. Since this is a relatively newly recognised viral family, the International Committee on Taxonomy of Viruses (ICTV) acknowledge that this family is likely to expand as new viral taxa are discovered. Previous studies have identified viral infections in the European shore crab (*Carcinus maenas*), in particular B virus and Rod shaped virus of Carcinus maenas (RVCM), and it has been suggested that they may be ancestral forms of WSSV 1. Anecdotal evidence suggests that shrimp were fed with crab tissues prior to the initial outbreak of this disease. These viruses have been tentatively listed as putative members of the family *Nimaviridae*, however listings were removed due to lack of evidence as it had not been possible to compare these viruses directly with WSSV 1 isolates from penaeid shrimp farming regions. We have recently isolated a viral infection of wild caught shore crabs. Histologically and ultrastructurally, this infection appears to be very similar to WSSV 1. We have sequenced (Illumina MiSeq) and assembled the genome of this virus and, based on nucleotide and protein sequence homology and genome organization, conclude that it is related to WSSV 1. However, it is a different species compared to WSSV, and probably a member of a new Genus of the *Nimaviridae* Family. It is quite likely that further members of the *Nimaviridae* family exist in wild populations of decapod crustaceans and will be discovered as sequencing methods and sampling programmes are developed.

Abstract ID: 070B (Oral)

THE ROLE OF CELL SURFACE ATP SYNTHESIS OF SHRIMP IN WSSV INFECTION

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The intensification of shrimp farming has been accompanied with the development of many infectious diseases. White spot syndrome virus (WSSV), the only member of the genus *Whispovirus* of the family of *Nimaviridae*, has emerged globally as one of the most prevalent and lethal pathogen for Penaeid shrimp species since its first outbreak in 1992. The better understanding of its pathogenesis, especially the nature of virus–host interactions, could lead to a better understanding on how to control WSSV. Over the past a few years, evidences indicate that adenosine triphosphate (ATP) is an energy source for the binding, maturation, assembly, and budding process of many enveloped viruses. Our previous studies suggest that the F_1-ATP synthase beta subunit (ATPsyn β, BP53) of the shrimp *Litopenaeus vannamei* (*L. vannamei*) serve as a potential receptor for WSSV's infection. Further, BP53 was identified its location on the surface of shrimp hemocytes and gill epithelial cells by immunofluorescence assay and immunogold labeling technique. Interestingly, cell surface ATP synthesis was demonstrated by an *in vitro* bioluminescent luciferase assay. In order to clearly understand the pathway during WSSV infection, the binding proteins that could specifically bind BP53 were identified, which were two envelope proteins VP28 and VP37 of WSSV, and a host protein Astakine. By co-localization assay, bound VP37 on the cell surface was found co-localized with BP53 and shared a similar subcellular location on the outer surface of shrimp cells. While, the binding between BP53 and Astakine was specifically competed by WSSV-VP37. These results suggested that BP53, presenting on cell surface, likely served as one of the receptors for WSSV infection in shrimp. Correspondingly, WSSV appears to disturb the host energy metabolism through interacting with host ATPsyn β during infection. This work firstly showed that host ATP production is required and consumed by the WSSV for binding and proceeds with infection process.

Abstract ID: 192B (Oral)

SHRIMP MIR-10A IS CO-OPTED BY WHITE SPOT SYNDROME VIRUS (WSSV) TO INCREASE VIRAL GENE EXPRESSION AND VIRAL REPLICATION

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Host microRNAs (miRNAs) play many important roles in diverse biological mechanisms, especially in gene regulation. In this study, next-generation sequencing (NGS) technology was used to identify shrimp miRNAs from *Litopenaeus vannamei* that were differentially expressed during white spot syndrome virus (WSSV) infection. The most highly up-regulated shrimp microRNA was miR-10a, which is also highly upregulated in several types of human cancers. After confirming the expression level of miR-10a by Northern blot and quantitative RT-PCR, an *in vivo* experiment showed that the viral copy number was decreased in miR10a-inhibited shrimp. miR-10a targeted the 5' UTR of at least three viral genes *(vp26, vp28* and *wssv102*), and plasmids that were controlled by the 5' UTR of these genes produced enhanced luciferase signals in transfected SF9 cells. All of these results suggest that shrimp miR-10a is a host miRNA that enhances WSSV replication by targeting the 5' UTR of viral genes.

Abstract ID: 033B (Oral)

LARGE SCALE PRODUCTION OF DSRNA-PRODUCING *Chlamydomonas reinhardtii* AND ITS USE IN VIRAL DISEASE PROTECTION

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RNA interference (RNAi) triggered by viral specific double-stranded (ds)RNA has been extensively reported to efficiently control widespread shrimp viruses, for example WSSV, YHV, TSV and IHHNV. Nonetheless the problem of a suitable dsRNA delivery approach still needs to be tackled. We have successfully produced large amounts of dsRNA in *E. coli* and demonstrated its effectiveness as a viral protectant using inactivated whole cells in the feed. However, with rising public concern of using genetically modified organisms and one of the major drawbacks of the bacterial expression system is the use of antibiotic resistance cassettes, thus alternative dsRNA production systems need to be explored. Because we aim to introduce dsRNA by oral administration, microalgae are a noteworthy new host for dsRNA production. The potential for producing high-value compounds in *Chlamydomonas reinhardtii* using chloroplast transformation technology with glass beads has previously been shown. The Yellow Head Virus (YHV) is stillcausing huge losses in the shrimp aquaculture industry. The RNA-dependent RNA polymerase (RdRp) gene of YHV has been proposed as a dsRNA as it has been proven to efficiently target the YHV virus. For production of the dsRNA in *C. reinhardtii* a*psbH* deletion strain was used as a host for transformation, so that restoration of photosynthesis can be utilized for selection instead of antibiotics. Cultivation of *C. reinhardtii* strains containing the dsRNA-expressing cassette will be optimized and scaled up to maximize dsRNA amounts. Ultimately, the desired culture will be mixed with shrimp feeding pellet and studied for its potential to protect shrimp from the YHV virus.

Abstract ID: 090B (Poster &Elevator Pitch)

WHITE SPOT SYNDROME VIRUS (WSSV) EXAMINATION IN FISH QUARANTINE AND INSPECTION STANDARD EXAMINATION LABORATORY (BUSKIPM) INDONESIA

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Since 1995, white spot syndrome virus (WSSV) has spread to all center cultivations of shrimp in Indonesia. The mortality of WSSV can reach 100% in only few days after infection. WSSV causing economic and social losses quite large. The first occurrence of WSSV was in Taiwan in 1992. Fish Quarantine and inspection standard examination laboratory (BUSKIPM) is one of the technical implementation unit of Fish Qurantine. Several functions of BUSKIPM based on the regulation of maritime affairs and Fisheries Minister of the Republic of Indonesia number: 25/MEN/2011are: examination of quarantine pest fish and Proficiency Testing. WSSV examination in BUSKIPM consist of four (4) methods. There are 1. Conventional PCR. In Conventional PCR we are using primer WSSV-F1 & WSSV-R1 (1st step) and WSSV-NF & WSSV-NR (nested) (OIE, 2012). 2. Real Time PCR, In Real Time PCR we are using Kit IQ Real for WSSV 3, Histopathology test with hematoxylin-eosin staining. 4.Sequensing for determine strain of virus. In 2012 and 2016, BUSKIPM held proficiency test for WSSV. The proficiency test was conducted by technical implementation Unit (UPT) of Fish quarantine and stakeholder in Indonesia. Buskipm examination results in the year 2016-2017, there are 11 provinces positive WSSV by using methode PCR conventional or real time PCR. The provinces are Riau, Bangka Belitung Island, West Sulawesi, Southeast Sulawesi, Central Sulawesi, West Java, Jambi, Banten, Aceh, North Sumatra.

Abstract ID: 179B (Poster &Elevator Pitch)

SIMULTANEOUS OCCURRENCE OF HAPLOSPORIDIA-LIKE ORGANISM (HLO) AND WHITE SPOT SYNDROME VIRUS (WSSV) IN POLYCHAETES FROM SHRIMP PONDS IN CENTRAL JAVA, INDONESIA

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Polychaetes are common benthic invertebrate and natural food of the shrimp in the ponds. Wild polychaetes in the shrimp ponds may acquire various pathogens live in the pond environment and carry multiple infection. Wild polychaetes *Dendronereis* spp. and *Marphyssa* sp, were reported to be host and vector of WSSV respectively. However information on concurrence of WSSV infection with shrimp parasites in polychaetes from the shrimp pond is very limited. The objective of this study was to determine simultaneous occurrence of the white spot syndrome virus (WSSV) and haplosporidia in the polychaetes in the shrimp ponds. Polychaetes *Marphyssa* spp. (8 indiv) and *Dendronereis* spp (13 indiv) used in current studies were obtained from the shrimp ponds experienced white feces syndrome and WSSV outbreak within a month of sampling. Polychaetes were brought alive to the laboratory and individually kept in the plastic jars containing sterile brackishwater for 1-3 days. The fresh smear of coelomic fluid was obtained by making small incision on the body wall and the coleomic fluid was collected with pasteur pipets. The smear was stained with Giemsa (10%). Specimens for histopathological examination were preserved in Davidson's, processed and stained with Hematoxylin and Eosin (H&E). The remains of polychaetes were stored at -20 ^{o}C freezer to be used in PCR analysis to detect WSSV. .WSSV was detected in 13 out of 15 polychaetes tested. Haplosporidia like organisms (HLO) were seen in body fluid smear of *Marphyssa* sp. and to a lesser extent in the *Dendronereis* spp. The HLO observed at various stages, but mostly were multicellular plasmodia and some spores were released from plasmodia. Histological examination confirmed the findings of fresh smear. Plasmodia containing multinucleated spores were observed in coeloemic cavity and gut lumen, parapodia and epithelial layer of gut mucosal, causing hyperplasia of the epithelial cells in the mucosal layer. Basophilic multinucleated spores were also present in the epithelial lining of the *Dendronereis* spp. with less pathological effect than that found in the *Marphyssa* sp. Since this study is at early stage, the significance of this finding is still unknown. More in-depth study is on going.

Abstract ID: 091B (Poster &Elevator Pitch)

GENOMIC CLASSIFICATION OF BETANODAVIRUS, THE CAUSATIVE AGENT OF VIRAL NERVOUS NECROSIS, IN INDONESIA

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We have amplified by reverse transcription-polymerase chain reaction (RT-PCR) and sequenced a 502-bp fragment covering the variable region of the coat protein gene of fish nodaviruses infecting grouper in some regions of Indonesia. Eight new isolates from grouper and one isolate from bawal bintang (*Trachinotus blochii*) were identified and their sequences were combined with sequences in the literature to produce phylogenetic relationships data of Betanodavirus. Phylogenetic analyses were performed according to the maximum parsimony and neighbor-joining methods.Our results support the monophyly of fish nodaviruses. Moreover, they confirm the subdivision of fish nodaviruses into five main clusters, tiger puffer nervous necrosis virus (TPNNV), striped jack nervous necrosis virus (SJNNV), berfin flounder nervous necrosis virus (BFNNV), red-spotted grouper nervous necrosis virus (RGNNV) dan turbot nodavirus (TNV), in agreement with the previously suggested phylogeny of the genus Piscinodavirus. All the Indonesian isolates in this study were clustered in the group of the red-spotted grouper nervous necrosis virus (RGNNV). Those isolates were divided at least into three group of RGNNV. The first group were consisted of TGNNVGondol_49_03/13 (*E. fuscogutattus* from Gondol, Bali), *E.polyphakeion*NNVSitubondo_34_03/13 (*E. polyphakeion* from Situbondo, East Java), EpinephelusNNVLampung_157 (source *Epinephelus spp.* from Lampung, South Sumatra) and BawalBintang_180 (*T. blochii*) betanodavirus isolates. Second group were consisted of GGNNVAceh_32_02/13 (*E. tauvina* from Aceh), EpinephelusNNVBima_68 (*Epinephelus spp*. from Bima), and EpinephelusNNVGorontalo_98 (source *Epinephelus spp.* from Gorontalo, North Sulawesi) betanodavirus isolates. Third group were consisted of GGNNVMedan_33_02/13 (source *E. tauvina* from Medan, North Sumatra) and EpinephelusNNVP Pinang_81 (*Epinephelus spp.* from Pangkal Pinang, Sumatra).

Abstract ID: 138B (Poster &Elevator Pitch)

INSIGHT INTO IHNV IN CHINESE RAINBOW TROUT AQUACULTURE FROM VIRUS ISOLATED FROM 9 PROVINCES BETWEEN 2009-2014

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The fish pathogenic rhabdovirus infectious haematopoietic necrosis virus (IHNV) causes substantial losses in Chinese coldwater aquaculture. IHNV was reported in China in 1985 and has since undergone considerable spread in China. In this study, phylogenetic analyses of the mid-G sequences of 62 strains isolated from 9 provinces in China (Liaoning, n = 33; Jilin, n = 3; Heilongjiang, n = 3; Yunnan, n = 2; Sichuan, n = 3; Hebei, n = 5; Gansu, n = 5; Shandong, n =1; Beijing, n = 1; unknown location, n = 6) enable determination of the evolution and spread of the IHNV in China since the first report. Sequence comparisons revealed 11 different sequence types. The results of phylogenetic analyses showed that most of Chinese IHNV isolates are belong to Nagano subgenotype, J genotype. The results suggest that there are co-circulating lineages of IHNV present within specific areas of China. More important, we first report the MA and MN subgenotype, M genotype in China. Further, the database allows us to analyze the pathway of distribution in China over time. The results suggest that in most of the cases, spread of IHNV was related to movement of infected egg or fish. The data further demonstrate that knowledge of the sequences is required to determine the source of infections in different farms.

Abstract ID: 110B (Poster &Elevator Pitch)

THE TEMPORAL SHIFTS IN GENOGROUPS OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) ISOLATES IN THE NORTH KANTO REGION OF JAPAN

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Infectious hematopoietic necrosis virus (IHNV), a rhabdovirus that causes acute disease in salmonids in western North America, was first described in hatchery-reared sockeye salmon *Oncorhynchus nerka* in 1953 (Rucker et al., 1953) and identified in 1969 (Amend et al., 1969). Thereafter, this virus has spread following movement of salmonid fish and eggs to European and Asian countries, and is currently subdivided into six genogroups worldwide on the sequence of glycoprotein (G) gene: the North American M, U and L genogroups, the European (E) genogroup, and the Asian M, U, JRt-Shizuoka (S), and JRt-Nagano (N) genogroups (Emmenegger *et al.*, 2000; Kurath *et al.*, 2003; Nishizawa *et al.*, 2006; Mochizuki *et al.*, 2009; Kim *et al.*, 2016). In Japan, IHNV belong to U genogroup was introduced from Alaska, USA in the 1970s (Kimura & Yoshimizu, 1991; Yoshimizu, 1996), and Japanese isolates after 1980s were diverged to U, S and N genogroups (Mochizuki *et al.*, 2009). However, there are no information on the temporal shift in each IHNV genogroup found in Japan.In the present study, we confirmed that IHNV isolates collected in the North Kanto region from the 1980s to 2010s were classified into U, S and N genogroups, based on the phylogeny of Japanese IHNV G gene. Furthermore, those in U genogroup were subdivided into two distinct subtypes, U2 and U3 supported by a high bootstrap value (> 70%). In 2000 and up to the present, 87% of the total isolates at North Kanto region was belonged to the N genogroup, and the diversities of nucleotide and educed amino acid in N genogroup were the highest in genogroup/subtype found in this study. Currently, although it is found that there are reinfection with IHNV in the many salmonids farms in Japan, the phenomenon may be related to the changes in antigen mutation with genetic divergence of IHNV in N genogroup. Further study should investigate the variations of pathogenicity and antigenicity in each IHNV genogroup/subtype found in this study.

Abstract ID: 118B (Poster &Elevator Pitch)

DETECTION METHODS OF CYPRINID HERPESVIRUS II INFECTION IN CRUCIAN CARP (*Carassius auratus gibelio*) VIA AN VP72 MONOCLONAL ANTIBODY

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Cyprinid herpesvirus II (CyHV-2) is the main pathogen responsible for causing hematopoietic necrosis disease in *Carassius auratus gibelio*. Although many nucleic acid-based diagnostic methods have been applied, no stable and sensitive immunological diagnostic approaches have been reported. In this study, to detect CyHV-2 in clinical samples using immunological methods, recombinant VP 72, encoded by the CyHV-2 *ORF72* gene, was used as a capture antigen to identify blood and tissues infected with CyHV-2. First, *ORF72* gene was amplified from the CyHV-2 genome and cloned into a PGEX-4t-3 expressing vector to produce recombinant VP 72 in *Escherichia coli*. The purified recombinant VP 72 was used as an immunogen to prepare monoclonal antibodies. The Western blotting assays revealed that the monoclonal antibody could specifically identify the recombinant VP 72, and then VP 72 was successfully identified in infected fish tissues. Furthermore, an immunohistochemical protocol and a blood smear method were established to detect CyHV-2 in carps. The results indicate that the monoclonal antibody against VP 72 could be utilized as an effective detection tool for hematopoietic necrosis disease in *Carassius auratus gibelio*.

Abstract ID: 133B (Poster &Elevator Pitch)

SENSITIVITIES OF NEW CELL LINES FROM CARP *Cyprinus carpio* x GOLDFISH *Carassius auratus* HYBRID AND FROM GINBUNA *Carassius auratus langsdorfii* TO KOI HERPESVIRUS (KHV)

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Until now, several cell lines from carp/koi have been developed for isolation or proliferation of koi herpesvirus (KHV). However, sensitivities of existent cell lines from carp/koi to KHV are often too low to isolate the virus from fish. In this study, we attempted to develop cell lines from carp x goldfish hybrid and ginbuna as well as from koi, and evaluated their sensitivities to KHV. In addition, susceptibility of those fish to KHV were also tested. Five healthy fish including one carp/goldfish hybrid, one ginbuna and three koi of different strains (Kohaku, Hikari and Sanke) were used for the development of cell lines. A small part of the dorsal fin of each fish were sampled without sacrificing the fish to develop new cell lines in MEM supplemented with 10% bovine serum. After 20 passages of sub-culture, sensitivities to KHV of each cell line and CCB cells (a standard cell line for KHV) were evaluated by measuring $TCID_{50}$ of KHV NRIA0301. The fish used for the cell line development were exposed to KHV to evaluate their sensitivities to the virus. A small part of fins of each fish were sampled at 0, 1, 2, 3, 4 and 7 days post viral exposure (dpe) to quantify KHV DNA by a real-time Taqman PCR (Gilad et al., 2004) and to detect KHV mRNA by mRNA specific RT-PCR (Yuasa et al., 2010). Viral isolation with CCB cells were also conducted to detect infectious virus from fish at 4 and 7 dpe. $TCID_{50}$/mL of cell lines from the hybrid (HBF-2), ginbuna (GBF-1), Kohaku (KohF-1), Hikari (HikF-1) and Sanke (SanF-1) were $10^{6.3}$, $10^{5.8}$, $10^{5.3}$, $10^{4.6}$, $10^{5.6}$ and $10^{5.8}$, respectively. In the experimental infection, the hybrid and the ginbuna showed no clinical sign through the period of experiment, although the three koi were dead or moribund due to KHV infection at 7 dpe. KHV DNA increased in the koi, but not in the hybrid and the ginbuna. KHVmRNA was detected in the koi from 1 to 7 dpe and in the hybrid from 1 to 4 dpe, but not from the ginbuna. KHV was isolated from the koi at 4 and 7 dpe and the hybrid at 4 dpe, but not from the ginbuna. In summary, HBF-1 from the hybrid and GBF-1 from the ginbuna showed higher sensitivity to KHV than any cell lines from koi, although the hybrid and the ginbuna, from which the cells were established, showed low susceptibility to KHV. HBF-1, which is more sensitive to KHV than CCB cells, has a potential to be a new standard cell line for KHV isolation.

Abstract ID: 139B(Poster &Elevator Pitch)

INTERACTOME STUDY OF *Scylla serrata* REOVIRUS PROTEINS

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Abstract: *Scylla serrata* reovirus (SsRV) is one of the most prevalent viral pathogens of the mud crab (*S. serrata*). The virus represents an unassigned novel genus in the Reoviridae family, and contains 12 double-stranded RNA genomic segments. Here, we identify the SsRV structural proteins by tandem time-of-flight mass spectrometry (MS/MS), and use yeast two-hybrid to analyze the possible associations between the 11 SsRV proteins. MS/MS analysis showed that proteins VP1, VP3, VP6, VP9, VP11 and VP12 are structural proteins. Of the 121 combinations among the 11 SsRV proteins which accounted for 5 possible interactions, 2 binary and 5 self associations were identified to be stable and functional within the yeast environment. The 2 binary interactions comprised VP11&VP6 and VP11&VP12, while the self association comprised VP1&VP1, VP3&VP3, VP4&VP4, VP11&VP11 and VP11&VP12. None of these interactions have been documented previously for SsRV.

Abstract ID:002B (Poster)

EPIDEMIOLOGY STUDY OF VIRAL NERVOUS NECROSIS (VNN) IN MALAYSIAN GROUPERS: SEQUENCE ANALYSIS STRATEGIES AND QUASISPECIES DETERMINANT'S

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Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) is considered to be a serious disease of several marine fish species, characterized by significant losses associated to vacuolating lesions of the central nervous system and the retina. In Malaysia, VNN has been isolated from groupers, seabass, red snappers, cobia and golden pomfret. Betanodaviruses causes 100% mortality in larval stages while lower losses have been reported in juveniles and adult fish. Due to the unsystematic surveillance and reporting system, including risk factors, source of VNN infections; early detection method together with control and prevention strategies in Malaysia are not fully determined yet. VNN is reported to be vertically transmitted and it is still unclear how the virus is transmitted from broodstocks to fry/fingerlings, and the inconsistencies of detection in the broodstocks itself, especially during breeding time (self-observation). Since it is difficult to obtain and culture grouper broodstocks, hence, early control and prevention should be applied to ensure the broodstocks are protected against this disease. Thus, we propose to study the epidemiology of VNN in broodstocks and cultured grouper with a focus on prevalence, risk factors together with its genome characterization. These information may provide basis for the development of detection methods, vaccine or other control and prevention strategies. The samples (complete life stages) will be collected from associated grouper farms in Malaysia. In this study, risk factors for VNN infections such as age, species, life stages and water quality parameters will be determined. The use of invasive and non-invasive sampling methods will be evaluated as well. The genotypes and prevalence of VNN (seasonally) can be determined in this study. The confirmation of VNN genome sequence will provide information useful for understanding the evolution and divergence of the virus as well as towards the potential vaccine development.

Abstract ID:026B(Poster)

IDENTIFICATION OF EGCG AS A POTENTIAL AGENT FOR BLOCKING INFECTION OF GRASS CARP REOVIRUS

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Grass carp reovirus (GCRV), the representative strain of *Aquareovirus C*, serves as a model for studying the pathogenesis of aquareoviruses. Previously, epigallocatechin gallate (EGCG) was shown to inhibit orthoreovirus infection; the aim of this study was to test its potential in blocking infection of GCRV.We provided evidence to show that adhesion to CIK(*Ctenopharyngodonidellus* kidney) cell surface by GCRV particles was inhibited dose-dependently by EGCG, as well as the crude extract of green tea. We also evaluated the safety of EGCG and green tea extract to CIK cells, which supported EGCG as a promising compound that may be developed as a plant-derived small molecular therapeutic agent against grass hemorrhagic disease caused by GCRV infection. As the ligand for the 37/67-kDa laminin receptor (LamR), EGCG's blocking effect on GCRV attachment was associated with the binding potential of GCRV particles to LamR, which was implied from a VOPBA assay.

Abstract ID: 077B (Poster)

MOLECULAR DETECTION OF CHANNEL CATFISH VIRUS (CCV) IN CAGE-CULTURED *Pangasius hypophthalmus* IN MALAYSIA

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Pangasius hypophthalmus (Sauvage, 1878) is one of the valuable catfish species extensively cultured in Southeast Asia. In Malaysia, the production of *Pangasius* spp. showed ten-fold increased in 2000 (1,625.21 tonnes) to 2011 (10,891.51 tonnes). However, disease is always a major constraint causing almost 30% mortalities in cage-cultured *Pangasius* spp. especially in Pahang River, Temerloh. An epidemiological study based on histopathological findings and molecular detection revealed multiple infections of bacteria and virus. The previous studies demonstrated the presence of channel catfish virus (CCV) in striped river catfish which normally considered as host specific to channel catfish. Thus, the aim of this study was to determine the prevalence of CCV infection in cage-cultured *P. hypopthalmus* in Temerloh. A pair of newly designed primers and OiE reference primers were used in this molecular screening. A total of 110 cage-cultured *P. hypophthalmus* were randomly sampled between April and September 2016 in Temerloh. All samples did not show any sign of CCV infection. However, molecular detection revealed contrast outputs where the use of OiE reference primers showed negative results in all samples, but the new set of primer targeting ORF27 detected the presence of CCV at 27% prevalence. Further confirmation was done by sequence analysis using NCBI-BLASTn. The CCV-positive amplicon sequences were compared with Genbank database which showed 100% identity (589bp) to Ictalurid herpesvirus 1, strain Auburn 1 (GenBank Acc. No. M75136.2). Hence, these findings further support the documentation on the presence of CCV in *P. hypophthalmus* in Malaysia. Although the significant impact of this virus is still unknown, further investigation should be carried out to understand the etiology of the virus in *Pangasius* spp.

Abstract ID: 130B (Poster)

SUMO CONJUGATING ENZYME 9 (UBC9) BANDS TO GRASS CARPREOVIRUS OUTER-FIBER PROTEIN AND FACILITATES VIRAL REPRODUCTION

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Reoviruses are potential anticancer agents due to their ability to induce cell death in tumor cells. Grass carp reovirus (GCRV) is one of the best characterized models on reovirus pathogenesis *in vitro*. However, there is little known about how SUMOylation affects reovirus pathogenesis. The SUMO conjugating enzyme 9 (Ubc9) determines the targets of SUMOylation. Here, the protein interactions between reovirus outer fiber proteins, specifically GCRV-104 VP55, and Ubc9 were probed using a yeast two-hybrid system. The N-terminal coiled-coil domain of VP55, containing a single lysine residue, was responsible for the interaction between VP55 and Ubc9 in yeast. In solid phase binding assays, a single amino acid mutation (K87R) prevented Ubc9 from binding to VP55. Overexpression of Ubc9 enhanced GCRV-104 infection efficiency, and knockdown of Ubc9 in CIK cells inhibited viral replication, which suggested that Ubc9 was a proviral factor. Furthermore, Ubc9 was shown to bind outer fiber proteins from type II GCRV, avian reovirus and mammalian reovirus in yeast. To our knowledge, this is the first study to show that Ubc9 binds to reovirus outer-fiber proteins and likely contributes to efficient orthoreovirus replication.

Abstract ID: 197B (Poster)

DETECTION OF NEW GENOTYPE STRAIN OF CYPRINID HERPES VIRUS -2 FROM INDIA

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Cyprinid herpesvirus 2 (CyHV-2) is a causative agent of the acute haematopoietic necrosis disease in farmed goldfish (*Carassius auratus* L.) and gibel carp (*Carassius auratus gibelio* Bloch). In 1992-1993, CyHV-2 was first reported as the cause of epizootics in juvenile goldfish in Japan. Later haematopoietic necrosis disease reported in cultured goldfish in the USA, Taiwan, Australia, New Zealand, UK and China. Recently, in 2015-2016 the disease has been reported from farmed goldfish in India. The presence of CyHV-2 in goldfishes was confirmed using PCR, histopathology and transmission electron microscopy. However, the presence of less number of virus particle was not detectable using previously mentioned primers. Hence, new set of primers, based on major capsid protein, were designed and successfully amplified. The PCR reaction using designed primers were able to detect the presence of virus particle from 0.1 pg DNA (DNA mix of fish and virus.). Till date only two different genotypes of CyHV-2 viz, SY-C1 (China, 289 365 bp) and ST-J1 (Japan, 290 304) have been reported, which share 98.8% homology. The variations between genotypes include single-nucleotide mutations, insertions, deletions, and rearrangements. In the present study primer pairs CyHVGF4–CyHVGR4 and CyHVGF24–CyHVGR24 were used to detect CyHV-2 strains from India. Analysis of nucleotide sequences amplified using revealed that Indian CyHV-2 was same as SY-C1. Based on complete genomic nucleotide sequence of SY-C1 and ST-J1 a different set of primer was designed for further confirmation of the identity of Indian CyHv-2. The sequence analysis revealed that the Indian CyHV-2 had high homogeneity with ST-J1. Nucleotide sequence analysis further showed single-nucleotide mutations, insertions, deletions in nucleotide sequence of Indian CyHV-2 compared to the other two CyHV-2 strains. Analysis of the sequences from different strains of CyHV-2 gave confounding result about the identity of Indian CyHV-2. Hence, whole genome sequencing of Indian CyHV-2 is required which will provide accurate knowledge about genome size, nucleotide composition and number of ORF's. It will strengthen future studies on the pathogenesis of CyHV-2 in farmed goldfish and its health management in India.

Abstract ID: 224B (Poster)

DETECTION OF MEGALOCYTIVIRUS FROM FRESHWATER ORNAMENTAL FISH IN THAILAND

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The virus belonging to the genus megalocytivirus has been found in several types of freshwater ornamental fish namely gourami, cichlids and poeciliids cultured in Thailand. Although non-specific clinical signs were observed in megalocytivirus-infected fish, the virus damaged several internal organs such as spleen, kidney and liver. Major capsid protein (MCP) gene from the virus was detected using PCR assay and DNA sequencing. Phylogenetic analysis has been performed to access viral genotype. PCR-positive samples exhibited large number of inclusion bodies in some organs, including spleen, kidney and gonad which revealed histophatological changes caused by megalocytivirus. These finding confirm the infection of megalocytivirus in Thai freshwater ornamental fish.

Abstract ID: 225B (Poster)

THE PATHOLOGY OF MEGALOCYTIVIRUS-INFECTED FISH

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Megalocytivirus, one genus of fish virus, causes systemic infectious affect in wide varieties of marine and freshwater fish including many ornamental fish species. Megalocytivirushas caused mortalities in infected fish which can be significant in some species while many ornamental fish species can be infected without specific clinical signs. Although the virus can be detected by PCR techniques, histopathology is another appropriate diagnostic test to reveal viral infection in fish. The results from this study exhibited a clinical sign from megalocytivirus infected fish such gourami, cichlid and poeciliids family similar to many other infectious diseases as caused by bacteria or other viruses. They may also alike disease sign caused by environmental toxins or water quality problem. For necropsy, infected fish were often observed to have damage of visceral organ as pale or dark color of liver, kidney, gastrointestinal tract, gonads, heart, gill or splenomegaly. Some fish may have haemorrhagic fluid within body cavity. Histopathological examination from positive PCR samples showed hyperplasia and eosinophilic granular cell in gill as common occurrence, while the most distinctive finding is the presence of hypertrophic basophilic cell or inclusion body in the kidney, spleen, liver and other organs.

Abstract ID: 010B(Poster)

DEVELOPMENT OF FISH CELL LINE SUSCEPTIBLE FOR ISKNV ISOLATED FROM VIETNAMESE SEABASS

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Infectious spleen and kidney necrosis virus (ISKNV) is a causative agent of severe fish disease worldwide, particularly in Asian regions. In addition to inflicting massive mortality in infected fish, infections of ISKNV have been reported without causing clinical symptoms in various fish species at different age groups. While this virus is capable of inflicting mass mortalities at early stages of development in tilapia, some tilapia fry survived the virulence of the viral infection, and later developed a chronic infection without clinical signs. We suspect that thermal stress is an important environmental parameter that could trigger viral virulence in asymptomatic host carrier for successful invasion, growth, reproduction and transmission. The study aims to culture ISKNV strains collected from naturally diseased barramundi (*Lates calcarifer*) in Vietnam. First, viral infectivity assay in Grunt Fin cell (GF) will be designed to establish the cell line susceptible ISKNV, which will be indicated by the cytopathic effect (CPE) and PCR. Viral stock will then be prepared for molecular characterization under the stressful thermal conditions in both fish cell culture and animal model. Ultimately, the established cell line susceptible for ISKNV can be a platform for future vaccine development against this virus.

Abstract ID: 016B(Poster)

DETERMINATION OF THE ANTIGEN RECOGNIZED BY ANTI-RSIV MONOCLONAL ANTIBODY (M10)

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Red sea bream iridoviral disease (RSIVD) is listed by OIE. The disease causes great economic loss of aquaculture in Asian countries. RSIV may spread easily through the marine environment because of its wide variety of host species. Therefore, a simple and rapid diagnostic method, such as immunofluorescence antibody test (IFAT), would help to control the disease. An anti-RSIV monoclonal antibody (MAb M10) has been developed for the practical use of IFAT for RSIV-infected fish in Japan. However, the target (antigen) of MAb M10 has not yet been determined. In the present study, we carried out an epitope mapping by using the phage display method to identify the antigen. Genomic DNA of RSIV (genotype II) was sheared by sonication and size-fractionated by agarose gel electrophoresis. DNA fragments within the range of 100—600 bp were excised from the gel, and then the fragments were randomly inserted into the T7 phage vector to construct the phage-displayed RSIV peptide library. After three rounds of panning against MAb M10, the concentrated phage clones were grown on an agarose plate with the host cell of *E. coli* BLT5403. The plaque-lift assay was then conducted to select the phage clones that were recognized by MAb M10. A total of 18 phage clones were successfully selected by the assay, and were subjected to DNA sequencing. As the result of the sequencing, partial fragments of laminin EGF repeat including protein (LERIP) gene of RSIV was detected from all of the selected phage clones. This result suggests that anti-RSIV MAb M10 recognizes LERIP as its antigen. This work was supported in part by JSPS KAKENHI Grant-in-Aid for Exploratory Research number 16698811 from the Ministry of Education, Culture, Sports, Science and Technology; Research and Development Projects for Application in Promoting new policy of Agriculture Forestry and Fisheries from the Ministry of Agriculture, Forestry and Fisheries.

Abstract ID: 079B (Poster)

REACTIVATION OF CYPRINID HERPESVIRUS 2 (CYHV-2) IN ASYMPTOMATIC SURVIVING GOLDFISH

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Herpesviral hematopoietic necrosis caused by cyprinid herpesvirus 2 (CyHV-2), has affected the commercial production of goldfish *Carassius auratus* and gibelio carp *C. auratus gibelio*. Overt infection of the virus mostly occurs when the water temperature ranges from 15 to 25°C. The source of infection could be the virus carrier fish, which survived the infection and permit the virus harboring inside persistently or latently. The virus reactivation in fish may occur under stressful conditions including maturation, resulting in the shedding of virus from the fish into the rearing water. To determine if the reactivation can occur in asymptomatic goldfish surviving the infection, the presence of CyHV-2 genome was investigated in the spleen, kidney, heart, brain, gill and fin dissected from survived fish. PCR specific to CyHV-2 was done on tissues directly after dissection from fish and on those after being incubated in medium 199 at 25°C for 5 days. Although there were no virus DNA detected in each tissue freshly dissected from the 5 asymptomatic fish, 4 out of 5 fish turned positive for the PCR after the *in vitro* incubation. In addition, the presence of CyHV-2 genome was also examined in the surviving fish which has been subjected to stress by temperature change and the injection with anti-ginbuna IFN-γs rabbit antibodies and dexamethasone. Four out of 5 fish showed positive virus DNA detection from the spleen, kidney, brain, heart or gills of the fish injected with anti-ginbuna IFN-γs antibodies and dexamethasone. In contrast, 2 out of 5 fish were positive for the virus onlyin the spleen or brain in the corresponding control group. The virus detection rate and positive tissues in the temperature fluctuated group were not so different from those in the temperature constant group. These results indicated that the stress treatment increased the virus over detectable level in fish tissues, suggesting that the asymptomatic surviving fish can be a carrier of CyHV-2 and the virus may reactivate under the depressed immune system. Further study is needed to identify the main factor(s) in fish immune system which suppresses virus growth in asymptomatic surviving fish.

Abstract ID: 089B(Poster)

GENOME ANALYSIS OF APOXVIRUS, CAUSATIVE VIRUS OF ATYPICAL CELLULAR GILL DISEASE OF AYU*Plecoglossus altivelis*

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Atypical cellular gill disease (ACGD) caused by a poxvirus, designated as *Plecoglossus altivelis* poxvirus (PaPV), has given serious losses in ayu aquaculture in Japan. The disease frequently occurs from spring to summer when rearing water temperature ranges from 17-20°C. The manifestation includes gill congestion, formation of atypical basophilic enlarged cells in gill epithelium, and fusion of secondary gill lamellae, but it may be influenced by coincidental bacterial infection. The virus has not been succeeded in culturing *in vitro* so far, and the nature of the virus is still largely unknown. In this study, we investigated the genome DNA sequence of PaPV to help understanding characteristics of the virus and developing diagnostic tools, counter and prophylactic measures including a vaccine. The gills of dead ayu due to ACGD were homogenized, and PaPV was partially purified by sucrose density gradient centrifugations. After DNA extraction, DNA library was prepared using Illumina TruSeq Nano DNA Library Preparation kit, and DNA sequencing was performed with Illumina MiSeq. Read sequences were assembled using Virus TAP pipeline. Gene prediction, homology search using predicted amino acids sequences, and phylogenetic analysis were carried out. As the result of read assembly, three large scaffolds of 185,012 bp, 115,605 bp, and 57,161 bp containing predicted genes that showed significant homology (E values, $<10^{-5}$) with the poxvirus genes were obtained. GC content of each scaffold was 28.2%, 28.4%, and 28.2%, respectively, which corresponded with low GC contents of most of poxviruses. Ninety-five genes were annotated in the scaffold of 115,605 bp. Fifty-eight genes of them showed homologies with genes of salmon gill poxvirus (SGPV) which causes a gill disease in Atlantic salmon, and the identities were from 21% to 56%. Phylogenetic analysis using predicted protein sequences of 13 genes conserved in all viruses of *Poxviridae* showed that PaPV formed a cluster with SGPV and was placed on the root of the Chordopoxvirinae, which infect vertebrates.

Abstract ID: 143B(Poster)

DETECTION AND PHYLOGENETIC ANALYSIS OF CARP EDEMA VIRUS IN KOI (*Cyprinus carpio haematopterus*) IN THE REPUBLIC OF KOREA

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Koi sleepy disease (KSD) was first reported in Japan in the 1970s and had been serially reported inmany countries in Europe recently. KSD occurs in koi (*Cyprinus carpio haematopterus*) and common carps (*Cyprinus carpio*) when water temperature changes in spring or fall. It is caused by carp edema virus (CEV) which is included in the family *Poxviridae*. The disease has high mortality of 80~100% and since it shows latent infection, potential risk of spreading through international trade is higher. So KSD is being paid attention due to the possibility of massive economic loss in koi and carp industry. Fish die mainly due to anoxia in this disease, which is resulted by club-shaped gills with massive infiltration of inflammatory cells to interlamellar space. In this study, KSD by CEV infection was detected and phylogenetically analyzed for the first time in Korea. Twenty-five koi were randomly selected and bought from a whole sale market. From the next day of their introduction to the fish tanks, all koi started to show lethargic behavior lying on the bottom and died within 20 days, showing 100% mortality. In all koi, the progress of their clinical signs was observed and *post mortem* necropsies were done including histopathological and clinical examinations. PCR detection was also done with gill tissues. As a result, all were diagnosed as KSD by CEV infection based on their clinical signs, examinations and PCR results. Phylogenetic analysis of 4a protein partial sequences of CEV was done. The result indicated that CEV from Korea was more closely related to that from the UK and Poland than from Japan, though Japan is geologically closer. In this regard, special attention to this disease is required not only around European countries but also in East Asian countries since this disease can induce massive economic loss in global trade of koi and carps.

Abstract ID: 181B(Poster)

DEVELOPMENT OF A HIGHLY PERMISSIVE CELL LINE FROM SPOTTED KNIFEJAW (*Oplegnathus punctatus*) FOR RED SEA BREAM IRIDOVIRUS

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Red sea bream iridoviral disease (RSIVD) has been reported in more than 30 cultured marine fish species and causes severe economic damages to mariculture in Japan. Currently, there is no cell line to propagate RSIV efficiently although grunt fin (GF) cell line is often used for the isolation and propagation of RSIV. In the present study, we developed a cell line (SKF-9) from spotted knifejaw (*Oplegnathus punctatus*),which is highly susceptible to red sea bream iridovirus (RSIV). SKF-9 cells mainly consisted of epithelial-like cells and cell doubling time at 25 °C was approximately 1-2 day(s). Cytopathic effect (CPE) characterized by rounding and enlargement was clearly observed in RSIV-infected SKF-9 cells. The amount of viral production of SKF-9 cells was approximately 100 times greater than that of GF cells. The viral titer was not decreased through passages using SKF-9 cells, whereas a considerable reduction in the titer was observed when GF cells were used. On the other hand, control of appropriate passage numbers (<50) of the cell line was important to maintain the high permissiveness of SKF-9.Mortality rate was 100% when culture supernatant of RSIV propagated with SKF-9 cells was injected into spotted knifejaw with dilutions of 10^{0}, 10^{-5}, and 10^{-6}. Thus, the SKF-9 cell line can be a useful tool for research of RSIV and for vaccine production.

Abstract ID: 194B(Poster)

DEVELOPMENT OF CELL CULTURES TO PROPAGATE CRUSTACEAN VIRUSES

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Growing animal cell cultures, including mammalian and insect cell cultures *in vitro* has been exceptionally an alternative tool for animal experimentation, biotechnological applications and pathological investigation. Due to the lack of crustacean cell line to be used for studying crustacean viral diseases, fish, mammalian and insect cell cultures from different species have been investigated for their susceptibility to some crustacean viruses. The challenge to find out alternative cell cultures to study crustacean viruses is still widely open as the previous publications tent to show negative growth of the examined viruses. This paper aims to summarizing attempts to grow several cell cultures (mammalian and insect cells) in vitro in our lab at JCU Townsville Australia, to gain understanding of the growth of some crustacean virus isolates from Australia. Adopting methods described in some publications, the African green monkey kidney (Vero) and mouse macrophage (RAW-Blue ISG) cell lines, and insect cell lines from mosquito (C6/36) and moth (Sf9) have been examined for their permissively to some crustacean viruses. After growing the cells, viruses were inoculated in vitro to the cell cultures and incubated for several days to observe the cytopathic effect (CPE) using HE stains and fluorescent assays. Molecular studies (PCR) have also been conducted using designed primers for each virus to confirm the infectivity and the growth of the viruses. HE stains shown negative CPE and the fluorescent results was not clearly identifying the success of viral entry to the cell nuclear. In general, our study tent to show negative growth of some crustacean viruses both in the mammalian and insect cells cultures. As the crustacean virus strains are develop, crustacean aquaculture will face and deal with new diseases in the future, the development of alternative cell cultures in vitro to grow different crustacean viruses should be continued.

Session 3 – Bacteriology

Abstract ID: 243C (Keynotefor Bacteriology)

BACTERIAL PATHOGENS, HORIZONTAL GENE TRANSFER AND ANTIMICROBIAL RESISTANCE TAKING CENTER STAGE IN AQUACULTURE?

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Though a wide spectrum of pathogens ranging from acellular viruses to multicellular parasites affect cultured aquatic animals, viral pathogens have attracted most attention due to heavy losses caused to shrimp aquaculture. But the recent emergence AHPND (acute hepatopancreatic necrosis disease) causing *Vibrio parahaemolyticus* has shifted attention to bacterial pathogens. *V. parahemolyticus* is an autochthonous estuarine organism widely distributed in brackish waters globally, but AHPND causing strains contain virulence genes in a plasmid, which is a mobile genetic element. The discovery of this plasmid carrying PirAB virulence genes in other *Vibrio* species confirms the mobile nature of this plasmid and focuses attention on horizontal gene transfer and emergence of new pathogens. This is not the first time that plasmids have been reported to carry virulence genes in pathogens of aquatic animals. Virulent strains of *V. anguillarum* harbour a plasmid carrying genes encoding iron-sequestering factors. Changing ecosystems in aquaculture systems may see more such pathogens emerging. Antibiotic resistance in bacteria associated with food animals is attracting attention from both animal health as well as public health perspective. The United Nations General Assembly resolution, action plan by UN agencies like FAO and WHO on antimicrobial resistance emphasizes the importance tackling this issue from one health perspective. While some bacteria have innate resistance against certain antibacterial agents, acquired resistance needs more attention due to their rapid spread across bacterial genera. Antimicrobial resistance determinants are mostly present in mobile genetic elements like plasmids, transposons, integrons or bacteriophages and acquisition of such genetic determinants may be favoured in certain ecological niches. Understanding molecular ecology of resistance determinants and factors that favour their spread may help developing strategies to minimize the public health risk of antimicrobial resistance.

Abstract ID: 024C (Oral)

COMPARATIVE GENOMIC ANALYSIS OF HIGH DRUG-RESISTANCE *Aeromonas hydrophila* INDUCED BY DOXYCYCLINE

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Pathogenic *Aeromonas hydrophila* strains cause persistent outbreaks of motile *Aeromonas* septicemia in warm-water fishes worldwide. Doxycycline, one of the second generation tetracyclines, has been employed in fish farming to fight *A. hydrophila* infections due to its broad-spectrum and comparably lower cost. However, progressively increasing resistance of *Aeromonas* strains to doxycycline poses as a serious cause of concern. Here, drug-resistant *A. hydrophila* AH10 was induced and selected through consecutive culture (1:50 dilution) in Mueller-Hinton Broth(MHB) supplemented with increasing concentration of doxycycline. Then the bacterial cultured in the presence of 25μg/mL doxycycline was spread onto LB agar plate, 5 colonies, named AH101, AH102, AH103, AH104 and AH105, were randomly selected and subjected for drug-resistance analysis. Minimal inhibitory concentrations(MIC) shows that the MIC of AH101, AH102, AH103, AH104 or AH105 was 100 times higher than the original strain AH10. All 5 strains show strong cross resistance to sulfonamids and amides. We sequenced all 5 strains and performed comparative genomic analysis of these draft genomes with 9 *A.hydrophila* complete genomes from GenBank. Pan-genome analysis revealed that the size of the pan-genome was 4730 genes, and there are 3056 genes(core) shared among the 14 strains,there is no specific single nucleotide polymorphisms(SNP) and insertion-deletion(INDEL)were identified in any functional gene locus, but significant copy number variation(CNV) and structure variation(SV) were identified among the genome of AH10 mutated strains. There is a great difference of drug-resistance genes in different strains.

Abstract ID: 115C (Oral)

REVIEW OF ANTIBIOTIC RESISTANCE IN PATHOGINIC BACTERIA ONSTRIPED CATFISH(*Pangasianodon hypophthalmus*)IN THE MEKONG DELTA, VIETNAM

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Striped catfish (*Pangasianodon hypophthalmus*) have been freshwater fish that have high economic value, and are popularly cultured in the Mekong Delta, Vietnam. The intensification and expansion in the production has been coupled with an increase in bacterial fish diseases and the need for treatment with antimicrobials. *Aeromonas hydrophila* and *Edwardsiella ictaluri* are established to be important fish pathogens. Tetracyclines [i.e., tetracycline (TE), oxytetracycline (OTC)], and phenicols (i.e., florfenicol) and sulfonamide (i.e., trimethoprim, sulfamethoxazole) are among the most widely used antimicrobial compounds in aquaculture, and they have been used extensively to control bacterial diseases. The emergence of antimicrobial-resistant (AMR) bacteria in striped catfish farming has increased considerably during the last 15 years. The review present the general picture of molecular mechanisms related to multiple antibiotic resistance (MAR) bacterial isolates. The development and spread of AMR bacteria and antimicrobial resistant genes (ARGs) are also discussed. Class 1 integrons harboring different combinations of the resistance gene cassettes dihydrofolate reductases (*dfrA1*), aminoglycoside adenyltransferases (*aacA4, aadB*), rifampin ADP-ribosyl transferase (aar2), metallo-betalactamase (blaVIM-1) and hypothetical proteins (orfC) were detected in bacterial isolates. In addition, other genes responsible for resistance to tetracycline (*tet*), genes resistant to sulfonamide (*sul1* and *sul2)* and florfenicol resistant gene (*floR)* were also detected in these class 1 integron-positive bacterial isolates. In the conjugation experiments, *A. hydrophila* and *E. ictaluri* can transfer their resistance genes to *Escherichia coli*. In generally, the use of antibiotics in fish farming should be discouraged, the developments of vaccines and immunostimulants incorporated in the feed and general preventive health efforts have to be urgently considered.

Abstract ID: 013C (Oral)

spaC-type *Erysipelothrix* sp. CAUSING DISEASES IN FISH

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Since 2012, *Erysipelothrix* sp. bacteria positive for surface protective antigen C,*spaC* have been associated with disease and mortality in ornamental fish. Infections occur at temperatures of 24 – 30°C and often correlate with breeding, harvest, or shipping. The most consistent clinical finding has been ulcerative stomatitis. Histologically, findings can include facial cellulitis, necrotizing dermatitis and myositis, and disseminated coelomitis with abundant intralesional Gram-positive bacterial colonies. Sixteen *Erysipelothrix* sp. isolates identified phenotypically as *E. rhusiopathiae* were recovered from diseased fish. Koch's postulates were fulfilled by intracoelomic injection and bath exposure challenges in zebrafish, *Danio rerio* and tiger barbs, *Puntius tetrazona.* Similar histopathological changes were observed in laboratory infected fish when compared to those infected under natural conditions. Additionally, the infectivity and virulence of *E. rhusiopathiae* isolates displaying the *spaA* and *spaB* genes were compared to *Erysipelothrix* sp.*spaC* isolates using the zebrafish model. Twenty-one days post-challenge, 87.5% (14/16) of the *Erysipelothrix* sp.*spaC* infected fish succumbed to mortality, whereas only 6.25% (1/16) of the *E. rhusiopathiaespaA* died. No *E. rhusiopathiae spaB* or PBS injected controls die. The *Erysipelothrix* sp. isolates from ornamental fish were compared phenotypically and genetically to *E. rhusiopathiae* and *E. tonsillarum* isolates from aquatic and terrestrial animals from multiple facilities. Biochemical profiles, antimicrobial susceptibilities, repetitive extragenic palindromic PCR fingerprints(rep-PCR), virulence factor profiles, surface protective antigen (Spa) typing, as well as 16S rRNA and *gyrB* sequencing demonstrated isolates from diseased fish were largely clonal and divergent from *E. rhusiopathiae* and *E. tonsillarum* isolates from normal fish skin, marine mammals and terrestrial animals. This study supports previous work citing the genetic variability of *Erysipelothrix* spp. *spa* types and suggests that isolates from diseased ornamental fish may represent a genetically distinct species. Additionally, the results support our hypothesis that the *spaCErysipelothrix* sp. is significantly more virulent than fish mucosal associated *E. rhusiopathiae* isolates *spaA* and *spaB*. Our proposed laboratory controlled challenge model can be used to investigate prophylactic and therapeutic protocols against *Erysipelothrix* sp. infection in fish.

Abstract ID: 056C (Oral, Student)

VIRULENT GENES DETERMINATION OF *Streptococcus agalactiae* ISOLATED FROM MALAYSIAN RED TILAPIA (Orechromis spp.) IN MALAYSIA

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The presence of *Streptococcus agalactiae* causing streptococcosis in caged-cultured Malaysian Red Tilapia (*Orechromis* spp.) in Malaysia are very prominent until the recent years. The outbreak causing major losses to the farmers mainly during the hot season (between April and July) with the mortality reaching 50% to 100%. The aim of this research is to study the diversity of *Streptococcus agalactiae* virulent genes isolated locally in Malaysia for better understanding towards the prevention of such outbreaks. Local isolates from previous epidemiological studies (2006 – 2008) and much recent cases of diseased tilapia in a total of 60 isolates was obtained for this study. The molecular studies were conducted to determine the biotype and identification of virulent genes of the locally isolated *Streptococcus agalactiae*. The profiling of the virulent genes was attained thru multiplex assay (multiple template PCR reaction). The virulent genes (bac, scpB, lmb, cspA, cylE, hylB, fbs_A and fbs_B) profiling from this study are required in identifying the biotype of the local isolates. From the profiling results, all the local isolated shows positive amplification of bac gene, cspA gene, cylE gene, hylB gene, fbs_A and fbs_B gene. Negative amplification was observed for lmb and scpB gene. The presence of only bac gene and the absence of scpB and lmb genes demonstrate the similarities of the local isolates with Biotype 1 Streptococcus agalactiae molecularly. Virulent genes profiling on local *Streptococcus agalactiae* virulent genes shows that 100% of the local isolates share the identity of Biotype 1. *Streptococcus agalactiae* Biotype 1 is categorized by the β-haemolytic reaction on blood agar whereas Biotype 2 display non-β-haemolytic reaction on blood agar. Epidemiologically, Biotype 1 *Streptococcus agalactiae* is very pathogenic in Malaysia as infection may occur from hatchery until grow-out stage in the production cycle.

Abstract ID: 116C (Oral)

ASSESSING THE VIRULENCE OF *Streptococcus agalactiae* serotypeIa, Ib AND III USING A COHABITATION INFECTION MODEL IN NILE TILAPIA (*Oreochromis niloticus*)

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Streptococcusagalactiae, also known as Group B Streptococcus (GBS), is a Gram-positive pathogen that infects a broad range of hosts, most notably humans, cattle and fish. Different serotypes have been described for *S. agalactiae* (i.e. Ia, Ib, II to IX), but only 3 of these have been associated with disease in fish, namely serotype Ia, Ib and III. These cause disease in a range of fish species living in warm freshwater, marine or estuarine environments, particularly in tilapia. Two biotypes of the bacterium have been recovered from fish, biotype 1 and biotype 2. Biotype 1 from fish includes serotypes Ia and III, are represented by CC7 and CC283 respectively. They have a biochemical profile similar to GBS recovered from humans, they are β-haemolytic and are found primarily in Asia. Biotype 2, previously referred to as *S. difficile* or *S. difficilis*, is serotype Ib represented by CC552. It is non-haemolytic and less metabolically active and slower growing than Biotype 1. This serotype is found in both Latin America and Asia. The aim of the present study was to assess the virulence of twelve *Streptococcus agalactiae* isolates representing four of each serotype (Ia, Ib and III), using a co-habitation model in Nile tilapia (*Oreochromis niloticus*). The virulence of each *Streptococcus* isolate was tested in naïve tilapia, (40 fish per tank in duplicate tanks) when cohabiting with varying amounts of shedders.The shedders were infected with either a high (10^6 CFU/ml) or a low (10^2 CFU/ml) dose of bacteria. Mortality was recorded daily and at least 10% of dead fish were sampled from each tank. The pathogen was re-isolated from the brain of dead fish and specific mortality confirmed using a specific agglutination test on re-isolated bacteria.Antibody titres of fish surviving at the end of the challenge were determined by ELISA, and the quantity of *S. agalactiae* in the brain of dead fish was determined by qPCR. The main clinical signs of *S.agalactiae*in infected fish were septicaemia and meningoencephalitis. Clear differences were evident in the mortality kinetics between and within the three serotypes (presented as % cumulative mortality). The relationship between antibody response, mortality kinetics and bacterial load in the brain with respect to the virulence of the isolate will be discussed.

Abstract ID: 009C (Oral)

Flavobacterium columnare RECOVERED FROM DISEASED TILAPIA IN THAILAND IS TAXONOMICALLY DITSINCT FROM THE TYPE STRAINS

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Flavobacterium columnare is one of the most important bacterial pathogens in freshwater fish responsible for massive outbreaks in aquaculture settings worldwide. The bacteria were highly regarded for their intraspecies genotypic diversity corresponding to geographical and host origins. In the recent study, genome characteristics of *F. columnare* strains (n = 5) collected from columnaris-associated tilapia in Thailand were investigated using Illumina's MiSeq Next-generation sequencing and compared to those of the type strains. Thai strains were mainly categorized as genomovar II and only one strain was genomovar I/II. Among these, two genomovar II (strain 1214 and NK01) and I/II (strain 1215), recovered from red tilapia (*Oreochromis* sp.) and Nile tilapia (*O. niloticus*), harbored larger genome sizes (3.44 - 3.52 Mb) and higher numbers of protein-encoding genes (3031- 3101 CDS) comparing to other *F. columnare* strains. Bacterial phylogeny inferred from multilocus sequence analysis (MLSA) and concatenated SNPs of core genome indicated that strain 1214, NK01 and 1215 were distantly related to other bacterial strains. Digital DNA-DNA hybridization (dDDH) analysis between emphasized dissimilarity between strain 1214, NK01, 1215 and type strains as the estimated dDDH values were as low as 27.7-30.4%. Collectively, we hypothesized that taxonomic delineation of three uniquely tilapia-originated strains (1214, NK01 and 1215) from Thailand required further revisions.

Abstract ID: 147C (Oral, Student)

INFECTIOUS DISEASE CAUSED BY *Elizabethkingia* IN FARMED FROGS IN CHINA

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Frog farming, as a large proportion in aquaculture has been practiced in many Asian countries, especially in China. The black-spotted frog *Pelophylax nigromaculatus*which is endemic to East Asia is one of the most popular farmed frogs in south-central China in the last three years for its good dietary and medicinal properties. Since 2016, epidemic disease mainly characterized by severe neurologic dysfunction has occurred in many separate black-spotted frog farms in China and has been creating huge economic loss to the frog industry. To figure out the pathogenesis of this disease, a total 213 abnormalblack-spotted frogs were collected from seven separate farms in Hunan, China, during MaytoOctober 2016. After euthanasia, a routine necropsy and histopathology were performed. Bacteria isolation, microscopic parasites examination, PCR test for fungus and viruses were conducted for etiology detection. Histopathologic examination demonstrated chronic severe meningitis with denatured incrassated meninges. Inflammatory infiltrates, moderate multifocal gliosis and perivascular cuffing were observed in the cerebellum.Bacterial infections (190/213) were confirmed in the etiological examination. Results oftest for fungus and viruses were negative. Although Myxosporidia (9/213) and some protists were observed in the microscopic observation, they were not accounted as the etiology. Among all the identified isolates, 90%were identifiedas*Elizabethkingia miricola* according to 16S rRNA gene and gyrB gene.Thepathogenicity of *E. miricola*was been verified by experimental challenges: intramuscular injection, immersion infection and cohabitation with infected frogs. As *Elizabethkingia* was reported to be occasionallyassociated with human clinical infections, whole-genome sequencing analyses is underway to characterize this amphibian isolate.We described the first infection of *E. miricola*inblack-spotted frogs. As we know, this was not the first report of *Elizabethkingia* infection in frogs in China. *Elizabethkingia meningoseptica*as another important member in the *Elizabethkingia* genus was reported as a pathogen for tiger frog in Hainan, China (Xie 2009). The clinical signs of the two *Elizabethkingia* infections have some similarities with each other. These studies indicate that *Elizabethkingia* is an emerging infectious pathogen in frog farming, and whether *Elizabethkingia* infect aquatic animals need to be further studied.

Abstract ID: 211C (Oral)

Aeromonas veronii BIOVAR SOBRIA ASSOCIATED WITH MORTALITY OF RIVERINE AYU *Plecoglossus altivelis* IN THE TAMA RIVER BASIN, JAPAN

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Ayu (*Plecoglossus altivelis*) is an amphidromous osmerid fish with 1-year life cycle. Since this fish is one of the most important species for fresh water fisheries and recreational fishing, large numbers of seed-production or wild ayu (captured from lake, rivers and sea coasts) are released annually into rivers to enhance natural stock.On the other hand, there have been many reports on the bacterial infections in ayu, such as vibriosis, motile aeromonad disease, cold-water disease and *Edwardsiella ictaluri* infection.Of these, cold-water disease and *E. ictaluri* infection are causing serious damages to ayu in many rivers of Japan.In July 2016, a gram negative, motile, short rod bacterium was isolated from dead ayu captured when mass mortality of riverine ayu occurred in tributary of Tama River basin, and the isolates were identified as *Aeromonas veronii* by 16S rRNA sequencing.*A. veronii* is generally ubiquitous opportunist pathogens, and the bacterium has been reported as causative agent of epizootic ulcerative syndrome (EUS) in cultured freshwater fish farm. In contrast, there have been few reports on diseases occurrence caused by *A. veronii* in wild fish. In the present study, we investigated the characterization and the pathogenicity of isolates from diseased wild ayu and showed that a new aeromonas diseases occurred in riverine ayu. All isolates obtained from diseased ayu showed the same characteristics by API 20E test (profile no. 7004124) except for 1 isolates (7006124), and these isolates were classified as biovar sobria according to results of arginine dihydrolase (ADH) and ornithine decarboxylase (ODC).Regarding phylogenetic analysis of gyrB gene, the isolates formed a different cluster from reference or past strain (isolated from riverine ayu captured in Tama River basin in 2012 and 2014) of *A. veronii*.Additionally, pathogenicity against ayu of the isolates was confirmed by experimental infection using immersion method with bacterial suspension (approximately 10^7 CFU/mL). These results conclude that mass mortality of riverine ayu found in tributary of Tama River in July 2016 was caused by motile aeromonad disease with *A. veronii* biovar sobria.

Abstract ID: 227C (Oral)

EPITHELIOCYSTIS IN FARMED *Pangasianodon hypophthalmus* IS ASSOCIATED WITH *Candidatus* ACTINOCHLAMYDIA PANGASIAE SP. NOV. INFECTION

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Epitheliocystis, an emerging bacterial disease reported from over 90 species of freshwater and marine fishes, is worldwide in distribution. It is primarily caused by members of phylum Chlamydiae, but recently β- and γ-proteobacteria have also been implicated. The farmed species are considered to be at higher risk of getting the infection due to higher stocking densities and greater stress, in comparison to wild counterparts. In the fishes, the gills are the primary target organs but, sometimes, the skin may also be affected. In the present study, histopathological examination of gills of farmed *Pangasianodon hypophthalmus* with history of continuous mortality, revealed the presence of large basophilic inclusions between the secondary lamellae which were typical of epitheliocystis. In addition, concurrent infection with trichodinids, flagellates and monogenea was also observed in gill sections. The presence of chlamydial DNA in affected gills was confirmed by amplification and sequencing of signature specific as well as nearly full length 16S rRNA gene. BLAST-n analysis of signature sequence and near full length 16S rRNA gene from epitheliocystis agents revealed 96% similarity with *Candidatus* Actinochlamydia clariae. The phylogenetic analysis of the sequences revealed that epitheliocystis agents from *P. hypophthalmus* belong to genus *Candidatus* Actinochlamydia under family Actonchlamydiaceae. On the basis of phylogenetic relationships of the bacterial genotypes with other taxa within order Chlamydiales, it is proposed that epitheliocystis agents from *P. hypophthalmus* be known as *Candidatus* Actinochlamydia pangasiae. The present case is the first report of epitheliocystis from India and in a new fish host, *P. hypophthalmus*.

Abstract ID: 166C (Oral, Student)

CHARACTERISTICS OF *Streptococcus dysgalactiae* ISOLATED FROM DIFFERENT FARMED FISH SPECIES AND EXPRESSION OF IMMUNE-RELATED GENES DURING ITS INFECTION

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Lancefield group C *Streptococcus dysgalactiae*, an emerging pathogen in fish, caused high mortality and economic loss in many farmed fish species worldwide, especially in Taiwan and Japan.Despite its increasing clinical importance, little information is available about the bacterium and host immune response following the infection. In the present study, 79 *S. dysgalactiae* isolates from different aquatic animal sources (cobia, grey mullet, loach, sturgeon, tiger-skinned frog, amberjack, yellowtail, and pompano) were discriminated using pulsed-field gel electrophoresis (PFGE), examined for the presence of putative virulence factors and antimicrobial resistant genes by polymerase chain reaction (PCR). Furthermore, three representative strains isolated from cobia were randomly chosen from their distinct PFGE profiles for virulence screening and expression of immune-related genes during the infection was determined by qRT-PCR assay. The results indicated that PFGE with SmaI or ApaI digestion displayed 19 different pulsotypes each, reflecting a genetic diversity among isolates from different sources. All examined strains harbored the virulence-associated genes *sagA*, *NAPlr*, and α-*enolase*, whereas 76/79 (96.2%) and 77/79 (97.5%) harbored *spegg* and *sof*, respectively. PCR analysis of the most common tetracycline and macrolide resistance genes demonstrated that isolates carried *tet*(M) (14/79, 17.7%), *tet*(S) (12/79, 15.2%), *erm*(B) (5/79, 6.3%), and *mef*(A) and *msr*(D) (3/79, 3.8%). Isolates harboring one or more resistance genes showed resistance to drugs by the disk diffusion method. In virulence test, isolates having different PFGE pulsotypes from cobia exhibited distinct virulence with the highest mortality presented in S2A2 group (87.5%), moderately in S1A1 (12.5%) and no mortality was observed in S3A3 (0%). Cobia challenged with the highest virulent *S. dysgalactiae* strain displayed an early significant up-regulation of pro-inflammatory cytokines in head kidney, liver, and spleen. Together, obtained results from this work might facilitate a clearer understanding regarding with genetic characteristics of piscine *S. dysgalactiae* and host inflammatory defense during its infection.

Abstract ID: 175C (Oral)

IDENTIFICATION OF A *Nocardia seriolae* SECRETED PROTEIN TARGETING HOST CELL MITOCHONDRIA AND INDUCING APOPTOSIS IN FHM CELLS

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Nocardia seriolae, a facultative intracellular bacteria, is the main pathogen of fish nocardiosis. A bioinformaic analysis based on genomic sequence of *N. seriolae*strain ZJ0503 showed that ORF3141 encoded a secreted protein which may target mitochondria in host cell, and ORF3141 has a signal peptide (MKLLNPRGFGLVCASAAVAAGLMLAG) at the N-terminate. So far the function of this protein and its homologs are totally unknown. In this study, we experimentally tested the bioinformatic prediction on this protein. Mass spectrometry analysis of extracellular products from *N. seriolae* showed that ORF3141was a secreted protein, and subcellular localization of ORF3141-GFP fusion protein revealed that the green fluorescence proteins co-localized with mitochondria while ORF3141sig-GFP(with the signal peptide deleted) fusion proteins were evenly distributed in the whole cell of FHM cells. It referred that the N-terminate signal peptide was important for mitochondria-targeting. Notably, the expression of ORF3141 changed the distribution of mitochondria from perinuclear halo into lumps in the FHM cell. In addition, apoptosis assay suggested that apoptosis was induced in FHM cells by the overexpression of *N. seriolae* ORF3141. Thus, ORF3141 is a *N. seriolae* secreted protein targeting host cell mitochondria and inducing apoptosis in FHM cells. It may help the host cell survival and immune escape of *N. seriolae* by participating the cell apoptosis regulation and play an important role in the pathogenesis of *N. seriolae*.

Abstract ID: 083C(Oral)

STRESS RESPONSE AND SUSCEPTIBILITY TO *Vibrio alginolyticus* INFECTION OF JUVENILES *Haliotissquamata* CULTURED IN DIFFERENT WATER TEMPERATURE

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Increasing water temperature can be a significant physical factor affecting stress for Abalone, *Haliotis squamata*which is known as Indonesian Tokobushi. This study were aimed to determine the effect of water temperature on stress response of *H. squamata* and susceptibility to *V.alginolyticus* through pallial sinus injection. Abalone juveniles with shell length and weigh of 32.97±1.83mm and 5.13±0,83g, respectively were cultured in 30°C and 34‰ sand filtered and UV threated seawater, which were then injected with 100µl *Vibrio alginolyticus* ($1.6x10^5$ cfu $abalone^{-1}$) in phosphate buffer saline and then transferred to the tanks at temperatures of 28,30,32and 34°C. All abalones transferred to 34°C died by 12 h after treatment. The mortality of *V.alginolyticus*-injected abalone reared at 28 and 32°C was significantly different. Abalone juveniles cultured in 34‰ seawater at 30°C which were then transferred to 28, 30, 32 and 34°C were examined for swiveling rate, falling rate, survival rate, mucus production, total haemocyte count (THC), and phenoloxidase activity to *V. alginolyticus* infection after 12, 24, 48 and 96 h. The swiveling rate, falling rate, survival rate and mucus production increased significantly as the increasing of water temperature whereas phenoloxidase activity and THC decrease significantly at 24 hours after transfer to 32 and 34 °C. It was conclude that transfer of *H. squamata* juveniles from 30 to 28 °C did not resulted any stress responses otherwise transfer to 32 and 34°C increased their stress responses and reduced their resistance to *V. alginolyticus* infection.

Abstract ID: 087C (Oral)

CHITOSAN COATED AG/ZNO NANOCOMPOSITE AGAINST *Vibrio* sp

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Shrimp aquaculture has witnessed for tremendous growth worldwide because of an increasing demand for nutrient enriched shrimp food. *Vibrio* species has been considered as a primary and opportunistic pathogen and one of the most virulent bacterial causative agents for the large-scale mortality in all stages of cultured penaeid shrimps. In aquaculture, various approaches have been processed already to control the pathogenic *Vibrio* strains by antibiotics but results in the development of resistant bacterial strains, and therefore much effort is required to find ways to formulate new types of safe and cost effective biocidal agents. The present study investigates,the antibacterial and antibiofilm potential of chitosan coated Ag/ZnO nanocomposite against *Vibrio* Sp which causes mass mortality in cultured shrimps. Despite their biocmpatability, biodegradability and low toxicity chitosan receives much attention in the field of aquaculture. In the present study, a linear biopolymer chitosan was synthesized from chitin present in the shell waste of *Portunus pelagicus*. Chitosan coated Ag/ZnO (CS/Ag/ZnO) nanocomposite was synthesized and characterized by UV-vis spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Scanning electron Microscopy (SEM). The CS/Ag/ZnO nanocomposite exhibited potent antibacterial activity against *Vibrio* strains which includes *Vibrio vulnificus* (ATCC 27562) and *Vibrio alginolyticus* (ATCC 17749). Agar disc diffusion assay revealed that, 12.5 mm and 13.3 mm clear zone was formed around the disc loaded with least concentration of CS/Ag/ZnO nanocomposite (10 µg/ml) respectively. Also, CS/Ag/ZnO nanocomposite effectively inhibited the biofilm growth of gram negative *V. vulnificus* and *V. alginolyticus* at 30 µg mL^{-1} assesed through crystal violet assay. The antibiofilm property of CS/Ag/ZnO nanocomposite specifies that, it had the potent to interrupt the biofilm architecture of both *V. vulnificus* and *V. alginolyticus*by disturbing the cell adhesion and polysaccharide matrix of matured biofilm. Moreover, Confocal laser scanning microscopy (CLSM) showed CS/Ag/ZnO nanocomposite collapse biofilm architecture which leads to loosening and reduction of bacterial colonies and reduce thickness of biofilm from 40 µm to 19 µm. This was further confirmed by exopolysaccharide (EPS) quantification and cell surface hydrophobicity (CSH) assay which suggests that, the disturbance in EPS matrix by CS/Ag/ZnO nanocomposite leads to structural integrity of biofilm and bacterial adhesion to hydrocarbons. Based on EPS quantification assay, 61 % and 64 % of EPS quantity was significantly reduced in *V. vulnificus* and *V. alginolyticus* respectively. Aside, *in vitro* cytotoxicity of CS/Ag/ZnO nanocomposite against shrimp haemocytes was appraised and the results showed reduced toxic effect.Thus our study concludes that, CS/Ag/ZnO nanocomposite is the promising candidate to be used as biomaterial agent against bacterial infections without any toxicity risk in aquaculture industries.

Abstract ID: 185C(Oral)

EFFECT OF AUTOCHTHONOUS BACTERIA WITH POTENTIAL PROBIOTIC PROPERTIES ON PHYSIOLOGICAL CONDITIONS OF *Cyprinus carpio* AGAINST WATERBORNE LEAD TOXICITY

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Lead (Pb) is an immunotoxicant for both animal and human health. It has potential adverse effects on aquatic organisms. Lead ends up in water bodies from the atmosphere and soil thus affect the aquatic organisms. The objectives of the present investigation were: (i) to screen the intestinal autothchonous bacteria of *Cyrprinus carpio* for their potential probiotic activity against lead toxicity, (ii) protective effect of dietary administration of potential probiotics on the physiological conditions of *C. Carpio* against waterborne lead toxicity. A total of 107 lactic acid bacteria (LAB) from the intestinal sample of healthy common carp were isolated. Of those, 41 LAB isolates which were Gram-positive and catalase negative, evaluated for Pb-binding properties. Among those, 7 LAB (P2, P6, P7, P9, P16, P19, and P22) exhibited comparatively higher Pb binding ability (>15% removal), were screened through further studies such as Pb tolerance and *in vitro* probiotic characteristics. Strain P16 displayed significantly better *in vitro* probiotic properties such as acid and bile tolerance, antioxidative capacity, and adhesion to fish mucus. Therefore, P16 identified as *Lactobscillus reuteri*, and chose for *in vivo* investigation. *C. carpio* fingerlings were divided into four groups: control, *L. reuteri* P16 only, Pb only, and Pb-plus-*L. reuteri* P16. The Pb-plus-*L. reuteri* group was exposed to waterborne Pb (1 mg/L) for 5 weeks and fed with *L. reuteri* P16 supplemented diet (10^8 cfu/g). Dietary *L. reuteri* promoted ($P<0.05$) the growth performance and prevented the death of Pb-exposed fish. The Pb exposure altered the haemato-biochemical parameters (WBC, RBC, hemoglobin, total protein, cholesterol, triglycerides, aspartate aminotranferase, alanine aminotransferase, etc.) in fish blood, which was reversed by dietary administration of *L. reuteri*. Further, Pb exposure reduced the activities of SOD (superoxide dismutase) and GPx (glutathione peroxidase), and increased MDA (malodialdehyde) level in fish blood, but these parameters remained unaffected in *L. reuteri* supplementation group. Moreover, expressions of TNF-α and IL-1β were significantly decreased in the head-kidney of Pb-exposed fish group, thus implicated the NF-κB signaling pathways. However, *L. reuteri* P16 supplementation reversed the expression of TNF-α and IL-1β in fish. Thus, the results of present study suggest that *L. reuteri* P16 may be a novel feed supplement against Pb toxicity in carp culture.

Abstract ID: 036C (Poster &Elevator Pitch)

CASE REPORT OF BACTERIAL INFECTIONS IN A REDCLAW CRAYFISH (*Cherax quadricarinatus*) HATCHERY

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Redclaw crayfish, *Cherax quadricarinatus*, is a native tropical freshwater crayfish in northern Australia and has become an important aquacultured animal in many countries. Viruses, bacteria and parasites have been found and caused massive losses in the redclaw hatcheries. Many bacteria were cultured from redclaw larvae in the hatchery and they were identified using the commercial API and Biolog systems. 16S rRNA, *rpo*D and *aro*A primers were used in PCR identification. A number of locally-isolated bacteria were selected and used as probiotic candidates in this study. Gram-negative bacteraemia presented in the hepatopancreatic tissue of redclaw crayfish accompanied with inflammatory cell infiltration. *Aeromonas hydrophila* was identified with both bacterial identification systems including PCR identification. *A. hydrophila* caused high mortality in the stage 2 larvae in the hatchery. The commercial probiotics containing *Bacillus* species and probiotic candidate bacteria(*Acinetobacter genospecies* 6, *Acinetobacter grimontii* and *Chryseobacterium balustinum*) did not inhibit the development of the *A. hydrophila* - associated bacteraemic disease. The evidence of an infection by bacteria common in freshwater that enter after hatching and affected predominantly the hepatopancreas of the larvae indicated the necessity of at least attempting to control the most obvious sources of the bacteria. This directly led to changes in hatchery management regarding better control of the microbiological quality of the incoming water.Also, developing new probiotic bacteria or bacteriophages may be effective in controlling bacterial infection in the future.

Abstract ID: 046C (Poster &Elevator Pitch)

THE *Aeromonas salmonicida* IN COLD FARMING FISH IN SHANDONG PROVINCE OF CHINA

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Aeromonassalmonicida is a pathogen that infecting a variety of fish cultured marine and brackishwaters. In recent years, the infection of *A. salmonicida* became more and more frequently in cold farming fish in Shandong province of China. In this research, we investigated the *A.salmonicida* disease in Shandong province as turbot (*Scophthalmus maximus*), Atlantic salmon (*Salmo salar*) and sablefish (*Anoplopoma fimbria*). Epidemiology results showed that *A. salmonicida* is the major disease in cultured Atlantic salmon and sablefish. Without vaccination, the calculated mortality of cultured Atlantic salmon and sablefish were over 70%. In cultured turbot bacteria disease in winter, the infection of *A.salmonicida* were over 50%. Over 50 strains of *A.salmonicida* were isolated from diseased fish, and identified by 16s rDNA sequence and biochemical profile using ATB identification system with API ID20E. 16 of these strains were further identified by sequence analysis of *gyrB*, *rpoD*, *recA* and *dnaJ*. The phylogenetic trees derived from sequences grouped the isolates with *Aeromonas* type strains, and these strains were all belong to *A.salmonicida* subsp. *masoucida*. The chemotherapeutant sensitivity test showed all the strain could resist penicillin, amoxicillin, streptomycin, cotrimoxazole, metronidazole and erythromycin. The present study indicated that *A. salmonicida* subsp. *masoucida* could infect cold farming fish in Shandong province, and the results provide supports for disease control in farming fish.

Abstract ID: 141C (Poster &Elevator Pitch)

IDENTIFICATION OF *Aeromonas sobria* FROM CATFISH, *Clarias sp*, IN SUKABUMI, INDONESIA

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Isolation and identification of *Aeromonas sobria* on Clarias sp from National Center forFreshwater Aquaculture, Sukabumi pond area has conducted in September 2016. A sobria is an important pathogen freshwater fish. The aim of this study is to identification of bacterialdiseases that causes mortality in catfish culture. Total of 140 samples comprising liver, kidney, spleen and tissue were collecting from 35 fish. Bacterial isolated in NA and BHIAmedium then incubated at 28 oC for 24 hours. The identification is based on several testingwhich are morphology and motility test, physical test, biochemical test, histology, API 20Ekit and Vitex (Biomerieux). The result obtained from the study of isolation and identificationof the bacteria in bacteria fish disease. Distribution of A. Sobria infection among four organsexamined indicated that A. Sobria was isolated most frequently from tissue (25%); liver(9.28%); kidney (8.57%) and spleen (3.57%)with significant difference among organs.Sensitivity test result using tetracycline (30ug) and Chepalotin (KF) 30ug were 9.09 mm and 9.24 mm, both of them are resistant. The survival rate of the fish were 68,71%. Finally, asthe case for any infectious fish pathogen, there is limited information about infection of A.Sobria in catfish culture in Indonesia and hence further study to have comprehensiveinformation on the agent is forwarded.

Abstract ID: 239C (Poster &Elevator Pitch)

*Edwardsiella ictaluri*INFECTIONS IN INDONESIAN FARMED CATFISH

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The bacterium *Edwardsiella ictaluri* continues to cause disease outbreaks in farmed catfish species globally, heavily impacting sustainability of USA *Ictalurus punctatus* (Hawke and Khoo, 2004; USDA, 2003) and Asian *Pangasianodon hypophthalmus* (Crumlish et al. 2002). Aquaculture production in Indonesia has steadily increased by average 8.5% per year reaching 4.5 million tonnes in 2015 (FAO, 2015). The intensification of freshwater catfish has significantly contributed towards the total volume produced and remains important for the rural economies in Indonesia. However, mass mortalities and morbidity have been reported in these farming systems. The cause of the mortalities may be multifactorial with bacterial infections contributing. The aim of this study was to investigate the role of *E. ictaluri* in the Indonesian catfish farm losses.Catfish farms in Jambi Province, Central Sumatra reporting fish losses were visited in April-May 2017, and samples taken for bacterial recovery and histopathology using routine diagnostic methods. Tissues samples were aseptically taken into 10% neutral buffered formalin, fixed and then wax embedded tissue sections stained with haemotoxylin and eosin for pathology. Bacterial recovery was performed by aseptically streaking a loopful of kidney, liver and spleen tissue onto tryptone soya agar and selective *E. ictaluri* agar plates, incubated at 28^{o}C and purified, prior to performing identification and characterisation tests including antibiogram. Data was also collected on the farm history and disease outbreak and analysed for detection of risk factors associated with the fish losses. Whilst a range of bacterial species were recovered, the dominant species was *E. ictaluri isolated* from both Pangasius and Clarias species presenting with gross clinical signs of disease including multifocal areas of necrosis in the liver, kidney and spleen. The bacterial strains were homogenous phenotypically and biochemically to each other as well as with known clinical isolates from other outbreaks in Vietnamese Pangasius species. This presentation will discuss the role of *E. ictaluri* in the Indonesian catfish sector, compare with other catfish outbreaks globally as well as consider risk factors for disease outbreaks in these Indonesian systems. The impact of this study will inform on biosecurity measures to control disease outbreaks, particularly from *E. ictaluri* in Indonesia.

Abstract ID: 229C (Poster &Elevator Pitch)

GENOMIC COMPARISON OF *Streptococcus agalactiae*ISOLATES RECOVERED FROM STREPTOCOCCOSIS OUTBREAKS IN TILAPIA (*Oreochromis niloticus*)

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Streptococcusagalactiae, also known as Group B Streptococcus (GBS), is a Gram-positive pathogen of tilapia (*Oreochromis niloticus*), and is responsible for sever mortalities in farmed fish. We have previously assessed the virulence of eight *S. agalactiae* isolates representing four serotype Ia and four serotype Ib isolates using a co-habitation model in Nile tilapia. Clear differences were evident in the mortality kinetics between and within the two serotypes. The aim of the present study was to perform a genomic comparison between the eight *S. agalactiae* isolates to identify candidate genes that might be associated with differences in pathogenicity between the two serotypes, and genes associated with virulence within the serotypes. Sequencing, de novo assembly and annotation of each of the eight *S. agalactiae* genomes was performed by Edinburgh Genomics sequencing facility (University of Edinburgh, UK). The genomes were sequenced using an Illumina GAIIx, employing a paired-end 150bp read of 12 TruSeq Nano libraries (550 base insert size). The *de novo* assembly was performed for each genome, followed by genome annotation in with PROKKA software. The genome sequences were further compared with multiple genome alignment (Mauve) and BLAST Ring Image Generator (BRIG) software. The comparative analysis of these genomes is currently ongoing and the results of this work will be provided in more detail in the poster. The information obtained from this work will provide a better understanding of the disease process between the two *S. agalactiae* serotypes and possible targets for the control of streptococcosis in tilapia aquaculture.

Abstract ID: 234C(Poster &Elevator Pitch)

EVALUATION OF PRIMERS TO DETECT *Streptococcus agalactiae*

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Streptococcosis is highlighted as a major disease problem for the tilapia aquaculture industry. The two main bacterial species associated with disease outbreaks are *Streptococcus agalactiae* and *Streptococcus iniae*. As well as being responsible for disease in tilapia, *S. agalactiae* is also an important pathogen in humans and cattle. The bacterium has a global spread in multiple hosts and extensive genetic lineages. Due to the importance of *S. agalactiae* as a pathogen, there are many published PCR primers which have largely been designed for the detection of *S. agalactiae* from dairy and human samples. The design of primers for PCR to confirm species identity of *S. agalactiae* can be problematic, with many primers designed for this purpose also cross reacting with *S. iniae*. The ability to differentiate between *S. agalactiae* and *S. iniae* ininfected samples derived from the aquaculture industry is much more important than from samples collected from other host species; as both species of bacteria are likely to be isolated from infected fish, cause very similar disease symptoms and are morphologically similar, therefore they are easily confused. *S. iniae* is unlikely to be isolated from humans and has not been found in cattle, so differentiation between these species of bacteria is not so important in samples collected from these host species. The aim of the present study was to design a set of primers, based on the *groEL* gene of *S. agalactiae* thatis specific for *S. agalactiae* in PCR, can detect a diverse range of *S.agalactiae* isolates from different hosts and it is capable of discriminating between *S. agalactiae* and *S. iniae*. The chosen *SagroEL2* primers were shown to be epidemiologically sensitive to *S. agalactiae.* Ninety seven isolates of *S. agalactiae*, representing 11 clonal complexes, produced a very clear product of the correct size with this primer set. This demonstrates the value of these primers, not just for use in aquaculture, but also in other fields were *S. agalactiae* needs to be identified. The primers were shown to have high analytical sensitivity, detecting low levels of *S. agalactiae* DNA, between $1x10^{-5}$ and $1x10^{-6}$ ng/µl of reaction mixture or 10-100 copies of genomic DNA per reaction. This shows that the assay is sensitive over a wide range of DNA concentrations and is likely to produce a positive result even in the presence of a very low number of bacteria. The assay was shown to produce a clear product with every isolate of *S. agalactiae* grown in broth culture, and also with DNA extracted from infected tilapia brain. The high analytical sensitivity of the assay is particularly important when diagnosing the early stages of disease outbreaks as sampled fish may only have low levels of bacteria growing in the organs sampled. Finally, as it is essential that primers designed for aquaculture purposes do not cross react with *S. iniae* the *SagroEL2* primer pair was tested with 9 isolates of *S. iniae* chosen from different geographical locations and no cross-reaction was observed.

Abstract ID: 157C (Poster &Elevator Pitch)

CURRENT STATUS OF ANTIMICROBIAL RESISTANCE OF BACTERIA FROM GROUPER IN INDONESIA

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Based on decree of the Minister of Marine and Fisheries of the Republic of Indonesia Number 52/KEPMEN-KP/2014 on Classification of Drugs Fish, there are three classes of antibiotics that are allowed to be used during the aquaculture process i.e..quinolone groups (enrofloxacine), tetracycline class. The side effects of drug used in fish is potentially harmful human consumption and environmental safety. Based on the potential for these side effects, monitoring of resistance should be done. The purpose of this research is to find out the status of microbial resistance pathogens in fish and shrimp to fish medicines such as quinolone class, tetracyclines and erytromycin. We collected several bacteria from a working area of BADC Situbondo. Parameter used in this study were inhibition zone diameter and the minimum inhibitory Concentration (MIC). Kirby Bauer test method used for inhibition zone diameter. Method for MIC used to measual the smolest concentration to inhibit the growth of bacteria.. OTC bioassay test was done by soaking and injection of the appropriate dose MIC for humpback grouper MICThe study concluded that 1) all test bacteria resistant to 30 ppm OTC 2) Erytromysin can be still used to control the *V parahaemoliticus* and *V. vulnificus*, except to isolate 1 Situbondo (*V. algynoliticus*) 3) Enrofloxacin can not be used to control *V parahaemoliticus* but can be used to control *V. algynoliticus* . and *V. vulnificus* 4) OTC can be used to control the *V. vulnificus* dose of 50 ppm by deeping for 24 hours and repeated 5 consecutive days and injection method. Survival rate of fish treated with OTC were 88.89% (deeping method), 72.22% (injection method) and 31.25% (control).

Abstract ID: 223C (Poster &Elevator Pitch)

ANTIMICROBIAL RESISTANCE SURVEILLANCE IN COMMENSAL *Escherichia coli* FROM GOLDFISH FARM IN RATCHABURI PROVINCE

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The incidence of antimicrobial resistance in the ornamental fish industry is a concern. The potential exists that antimicrobial resistance could be passed along with aquaculture waste or fish trade. The purpose of this study is aimed to investigate the impact of goldfish farming on antimicrobial resistance. The water samples were collected from goldfish farm effluents and canals in Ratchaburi Province. *Escherichia coli* was isolated from water and tested its antimicrobial sensitivity using disc diffusion method. The following twelve antibiotics were tested: Amoxycillin, Ampicillin, Chloramphenicol, Enrofloxacin, Erythromycin, Imipenem, Nitrofurantoin, Oxolinic acid, Oxytetracycline, Streptomycin, Sulphamethoxazole/Trimethoprim, and Vancomycin. The results showed that *E. coli* isolated from goldfish farm effluents and canals revealed high level of resistance to Ampicillin, Amoxicillin, and Erythromycin. *E. coli* isolated from goldfish farm effluents were sensitive to Chloramphenicol, Enrofloxacin, and Sulfamethoxazole/Trimethoprim. Result also revealed that *E. coli* presented multi-resistance to the tested antibiotics. In this study, the multi-resistance varied between one to six antimicrobial agents. In conclusion, water from canal provides potential risk of antimicrobial resistance than water from aquaculture which may indicates the development of antimicrobial resistance resulting from integrated users. The proper antimicrobial usage and good aquaculture practice could reduce potential risk of antimicrobial resistance.

Abstract ID: 151C (Poster &Elevator Pitch)

EFFICACY AND WITHDRAWALTIME OFERYTHROMYCIN IN WHITE LEG SHRIMP (*Penaeus vannamei*)

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Antibioticsandantibacterialis one typeof fish drugs usedfor thetreatment of varioustypes offish diseasescausedby bacteria. However,due to limitedknowledge andunderstanding of theusersin the fieldsotheir useis alsointended for the treatmentof fishdiseasescaused byviruses andfungi. Even mostfarmersbelieve thatuntil now have notfound a drugother types of fishthat rivalsthe effectiveness ofthe use of antibioticsandantibacterialsin the treatment ofvariousdiseasesof fish. AntibacterialErytromycinis onethat is stillusedup to this time.Withdrawltimetesting aims toobtain downtimedrugsafety limitharvestingon vanname shrimpfeedcontaining Erytromycin50and 100mg/kgbiomass ofshrimp/dayfor 21days. Samplingconducted after thediscontinuance of theerytromycin. Shrimpmeat sampleswere analyzed usingELISAtechnique. After 21 daysgivingamixedfeederythromycin, erythromycinconcentrationsfound inshrimpmeatonly20.897ppbto50 mg/kg and31.082ppbtoa dose of 100mg/kg, much lower thanthe maximum limitof 200ppberythromycin.Efficacytestingaimed to findthe right doseofmedication usefish. At the end oftesting hasdonereisolationbacteria fromshrimporgans, from the hepatopancreas, wheretypicallymost numerousbacteria.On thepositive controlcontainedvibriocoloniesof 2.5x 10^4, ata dose of 50contained1.0x 10^3colonies, whileata dose of 100there is nocolony todilution10^2 and10^4.The conclusion ofthis studyisanantibacterialErytromycinrelativelyrapiddegradationof the body ofthe shrimp. Withdrawaltime of erytromycinis 14daysvanameshrimp. This is toprovideassurancethat theresidueerytromycinisbelow the thresholdrequired by theshrimpexport destinationsarethe European Union, the UnitedStates andJapan. Efficacyoferythromycinis at dose of 100mg/kg body weight, where noonevibriocoloniesafter the last dayof treatment inshrimphepatopancreasdilution10^2.

Abstract ID: 005C (Poster &Elevator Pitch)

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

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In the case of bacterial desease, vibriosis was the chief problem particularly in the stages of the shrimp's life history. Vibrio infection can lead to necrosis, melanisation, even mass mortality of shrimp. An alternative method to prevent it by using probiotic. Probiotic can be found in powder form because powder probiotic can make it long term to keep and stability in quality. However, some cases indicated that vibrio in ponds was still high althought the powder probiotic has been applied. The objective of this study was to evaluate the effectiveness of powder probiotic cultured in seawater with molasses against vibrio. White shrimp (*Litopenaeus vannamei*) was captive in four of 60.000 m^3 ponds. Total of 0,1 ppm powder probiotic cultured in seawater with 0,2 ppm molasses as carbon source. The data analized was probiotic colony growth in probiotic culture, vibrio colony growth in ponds, and water quality of ponds for one month. The result of probiotic culture showed instead of probiotic colony, dominant colony growth was vibrio with 93% *Vibrio parahaemolyticus*. The seawater wasn't steril so it still contain vibrio, and probiotic was defeated. The result of vibrio colony growth in ponds showed probiotic can controlled green vibrio but still can't defeated yellow vibrio. Green vibrio can be pressed until $1x10^1$ cfu/mL to $1x10^2$ cfu/mL while yellow vibrio stood in $1x10^3$ cfu/mL to $1x10^4$ cfu/mL. The result of water quality of ponds was ammonia can be reduced between 0,06 ppm to 0,56 ppm but nitrite was still high. It means that activity of probiotic was not finished to nitrate. Based on this study, it can be concluded that powder probiotic cultured in seawater with molasses still ineffective contribution to defeated against vibrio in white shrimp (*L. vanname*), in case of seawater that used to culture was directly taken from the sea. Seawater with sterilized treatment would be recommended before applied in probiotic culture to clear it from vibrio.

Abstract ID: 156C (Poster &Elevator Pitch)

UTILIZATION OF KETAPANG (*Terminalia catappa*) LEAVES TO CONTROL VIBRIOSIS IN HYBRID GROUPER (*Epinephelus spp*)

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Vibriosis is a bacterial disease on the cultivation of fish, including grouper, which can reduce the sale value even cause death in acute vibrio infection. Use of chemicals, drugs, antibiotics, for this is still a mainstay for the fishery in the management of fish health, but these treatment have a negative impact their antibiotic residues. Disease control strategy at this time is directed to use herbal products because it does not lead to residues in fish body and does not pollute the marine environment. Ketapang (*Terminalia catappa*) is one kind of plant that can be used as an herbal remedy that has been shown to have antibacterial substance. In this research, Invitro and in vivo test ketapang leaf extracts to determine their effect in controlling vibriosis. In this research that the water extract of Ketapang has potential as an antibacterial against Vibrio alginoliticus and Vibrio vulnivicus. Water extract of ketapang leaves has inhibitory zone (10.33 ± 0.58 mm) greater against Vibrio vulnivicus than with the methanol extract of ketapang leaves (8.33 ± 0.58 mm) at a concentration of rough weight of 100 mg / mL. MBC test results can inhibit the growth of bacteria *Vibrio vulnivicus* at a concentration of 40 mg / ml of methanol extract of ketapang leaves of 2.9×10^9 CFU / mL to 2.0×10^2 CFU / mL. While the treatment of infection with *Vibrio vulnivicus* with 200 ppm water extract of ketapang leaves has survival rate of 57.14%, while 42.86% of control.

Abstract ID: 057C (Poster &Elevator Pitch)

DIFFERENTIAL RESPONSES OF *Vibrio alginolyticus* TO AQUEOUS AND ETHANOLIC OF PLANT EXTRACTS

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Herbal plants are known for their non-biodegradable and biocompatible properties. Some of these herbs have anti-viral, anti-fungal and anti-bacterial characteristics. A screening was conducted using aqueous and ethanolic extract of *Azadirachta indica, Curcuma longa, Zingiber officinale,Cymbopogon citratus* and *Tetracera indicia* to evaluate their antibacterial sensitivity against *Vibrio alginolyticus*. The modified disc diffusion method (Bauer et al, 1966) was used in this study. Six mm, sterile discs were loaded with the extract and placed on the inoculated plate. Aqueous *Tetracera indica*(9mm) showed the highest inhibition, followed by ethanolic *Zingiber officinale* (7mm) extract. Low inhibition zone were observed in *Azadirachta indica and Cymbopogon citratus.* No inhibition zone were observed in *Curcuma longa*. This results shows that herbs can be use in prophylaxis against bacteria with further study.

Abstract ID: 066C (Poster &Elevator Pitch)

CHARACTERIZATION AND EFFECT OF LACTIC ACID BACTERIA ISOLATED FROM DIGESTIVE TRACT OF CULTURED FISH ON THE GROWTH, IMMUNE RESPONSE AND RESISTANCE TO *Streptococcus iniae* IN SILVER PERCH (*Lates calcarifer*)

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Several lactic acid bacteria were isolated from digestive tract of cultured fish were selected based on their antibacterial activity against *Streptococcus iniae*. Four LAB strains exhibiting highest reduction of *S. iniae* were identified as *Weissella cibaria* MFIC2 and SPIC2 and *W. paramesenteroides* MFIA1and SPI1 based on the nucleotide sequence of their 16S rDNA. They were investigated for their capabilities to survive at pH 2.0, 2.5, 3.0 and in the presence of 0.2, 0.3 and 0.4% bile salts, in addition to their sensitivity against 5 selected antibiotics. All these LAB strains were able to survive in low pH and bile salt conditions at pH 2.5 and 0.3% bile salt for 2 h. Moreover, all these LAB strains were sensitive to examined antibiotics. After 2-week feeding trial with these LAB strain supplemented diet, silver perch (*Lates calcarifer*) larvae and juveniles exhibited significant differences ($p < 0.05$) in relative growth rate (% RGR) compared to the control group fed with non-supplemented diet, especially *Weissella cibaria* SPIC2 supplemented diet. Two innate immune response parameters, respiratory burst activity of blood leukocytes and plasma lysozyme activity, were significantly enhanced in the group fed with *W. paramesenteroides* SPI1 for 2 weeks. Feeding the fish with the *W. paramesenteroides* SPI1supplemented diet for 14 days before challenging them with *Streptococcus iniae* could reduce the mortality rate of the fish from 60%(in control group) to 30%(in treated). The lactic acid bacteria count of digestive tract in each feeding trial group were about 4.64×10^4 CFU / ml and no lactic acid bacteria was detected in the control group. In conclusions, four LAB strains isolated from digestive tract of cultured fish showed antibacterial activity against *S. iniae*, acid-bile tolerance and antibiotic susceptibility. LAB strains supplemented diet can also enhance the growth rate and two innate immune responses in silver perch. In addition, these LAB strains can also colonized in fish digestive tract and reduce the mortality in silver perch after *S. iniae* infection. Based on its origin and beneficial effect on growth, innate immune response and disease resistance, these four LAB strains may be potential candidates for use as a natural and safe immunostimulant and biocontrol agent in silver perch against *S. iniae* infection.

Abstract ID: 012C(Poster)

INTERACTION OF *Francisella sp.* WITHTILAPIACELL LINE

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Francisella noatunensis subsp. *orientalis* (*Fno*) (syn. *F. asiatica*) is an emergent warmwater fish pathogen and the causative agent of piscine francisellosis. Although *Fno* causes septicemia and can live extracellularly in infected tilapia (*Oreochromis* spp.), the early interaction of *Fno* with vasculature endothelium is unknown. In the present study, we examined the interaction of wild-type *Fno* (WT) and two *Fno* knockout [intracellular growth loci C (Δ*iglC*) and pathogenicity determinant protein A (Δ*pdpA*)] strains with the endothelial *O. mossambicus* bulbus arteriosus cell line (TmB) at 25°C and 30°C. Addtionally, we present a novel in vitroco-culture method to investigate the interactions between planktonic and mature*Fno* biofilms with TmB. Similar amounts of WT, Δ*iglC,* and Δ*pdpA* attached and were detected intracellularly after 5h of incubation at both temperatures; however temperature affected attachment and uptake. While significantly greater amounts of *Fno* (WT, Δ*iglC,* and Δ*pdpA*) were detected intracellularly when TmB cells were incubated at 30°C, bacteria attached to TmBs at greater levels at 25°C. Only WT *Fno* was able to replicate intracellularly at 25°C, which resulted in *Fno* mediated cytotoxicity and apoptosis at 24 and 72 h post-infection. WT *Fno* incubated at 30°C as well as Δ*iglC,* and Δ*pdpA* incubated at 25°C and 30°C were all defective for survival, replication, and the ability to cause cytotoxicity in TmB. Moreover, attachment, internalization, and cytotoxicity induced by planktonic *Fno* was significantly greater than those in the biofilm counterpart. Taken together, these results demonstrate that temperature plays a vital role for *Fno* intracellular survival, persistence and cytotoxicity. The current findings provide insight into the pathophysiology of francisellosis in tilapia.

Abstract ID: 051C(Poster)

BACTERICIDAL ACTIVITY AND GROWTH FITNESS OF *Vibrio anguillarum* EXPRESSING A CONSTITUTIVE TYPE VI SECRETION SYSTEM

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The type VI secretion system (T6SS) was recently shown to modulate quorum sensing and the stress response in *Vibrio anguillarum* serotype O1 strain NB10. It is not known whether there is a functionally active T6SS in other serotypes of *V. anguillarum*. Here, homologues to T6SS cluster VtsEFGH and hemolysin coregulated protein (Hcp) encoding genes were found to be prevalent and conserved in clinical isolates of *V. anguillarum* from fish, including four O1 and five non-O1 serotype strains. Unexpectedly, only the non-O1 serotype strains expressed VtsE-H and Hcp under laboratory and marine-like conditions, in contrast to the serotype O1 strains. This suggested that the *V. anguillarum* non-O1 serotype strains tested have constitutive expression of T6SS. Examination of a representative non-O1 strain, MHK3, showed that Hcp production was growth phase dependent and that maximum Hcp production was observed in the exponential growth phase. Moreover, Hcp production by MHK3 was most active under warm marine-like conditions. Further examination of MHK3 and serotype O1 strain M3 revealed a correlation of the constitutive expression of T6SS with bactericidal activity against *Escherichia coli* and *Edwardsiella tarda* and with growth advantages in stress conditions (0.2% and 5% NaCl, 10°C and 37°C). The work presented here suggests that the constitutive expression of T6SS provides *V. anguillarum* with advantages in microbial competition and growth fitness in marine environments.

Abstract ID: 054C (Poster)

FIRST DETECTION OF *Edwardsiella ictaluri* (PROTEOBACTERIA: ENTEROBACTERIACEAE) IN A WILD AUSTRALIAN ECOSYSTEM

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The bacterium *Edwardsiella ictaluri* is considered to be one of the most significant pathogens of farmed catfish in the United States of America, and has also caused mortalities in farmed and wild fish in many other parts of the world. Wild fish populations in Australia are considered free of this and many other diseases that impact fish elsewhere; although the bacterium has previously been detected in imported ornamental fish and native catfish held in Australian aquarium facilities, which may present a vector for invasion. A risk-based sampling model was constructed and wild catfish from 15 sites across the continental expanse of northern Australia were tested for *E. ictaluri*. The bacterium was isolated in eight Wet Tropics tandan (*Tandanus tropicanus*)from the Tully River, Queensland, and results were confirmed using conventional biochemical tests, and DNA sequencing. This is the first report of *Edwardsiella ictaluri* in wild fish on the Australian continent.

Abstract ID: 055C(Poster)

BACTERIAL COMMUNITIES IN CAGED-CULTURED *PANGASIUS HYPOPHTHALMUS* IN PAHANG RIVER, MALAYSIA

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Pangasius spp. is a popular freshwater fish in Malaysia and consists of 36.7 percent of total freshwater aquaculture production. The production of *Pangasius* spp. in Malaysia showed tenfold increased from 1,625.21 tonnes in 2000 to 10,891.51 tonnes in 2011. However, Pangasius culture has been reported as substantial outbreak of disease due to multiple infections of bacteria and virus causing almost 30% mortalities of fishes in Pahang River. Among *Pangasius* sp., the most common candidate species for Malaysian aquaculture is *Pangasius hypophthalmus.* Based on above perspectives, a study was conducted to identify bacterial infection in *P. hypophthalmus* in Pahang River, Malaysia. Bacterial communities in fish, ambient water and sediments were cultured on Tryptic Soy Agar (TSA). The samples were incubated at 30°C for 18 hours and Gram stained accordingly. The bacterial communities were identified using Biochemical test and API kit. The findings of the study reveals the prevalence of following bacteria: *Aeromonas hydrophila, Photobacterium damselae, Plesiomonas shigelloids* and *Pseudomonas fluorescens.* Among them, *A. hydrophila* has shownthe highest prevalence followed by *P. damselae, P. fluorescens* and *P. shigelloids.* Statistical analysis showed the significant relationship between *P. damselae* and *P. fluorescens* in *P. hypophthalmus (p<0.05)* in between fish, water and sediment. This is perhaps due to the conducive environment of the bacteria surrounding the cultured *Pangasius hypophthalmus*.

Abstract ID: 075C(Poster)

INVESTIGATION ON THE GROWTH RATE AND HEMOLYTIC ACTIVITY UNDER DIFFERENT TEMPERATURE AND SALINITY OF*Streptococcus iniae*ISOLATED STRAINS FROM INFECTED CULTURE FARMS IN TAIWAN

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Streptococcosis is one of important bacterial diseases of farmed fish in Taiwan. *Streptococcus* bacteria are common on the fish and environment along with numerous other types of bacteria, as part of the normal flora. *Streptococcus iniae* is a major bacterial pathogen of cultured fish, including fresh water and seawater species. It causes significant economic losses in the aquaculture industry in all over the world.*Streptococcus iniae* was Isolated from infected silver perches, tilapia, and giant groupers cultured in the southern of Taiwan. Those samples were collected from different temperature and salinity usually. Identified by PCR with specific primer to as *S. iniae*. And screened out the different strains by the results of RAPD. We cultivated *S. iniae* in different salinity and temperature to analyze their growth characteristics, and compared the growth characteristic, the hemolytic activity in different strains. The results showed that the strains growth best in low salinity and high temperature. The results of hemolysis assay also demonstrated that bacteria cultured in lower salinity and higher temperature had higher hemolytic activity. However, no hemolytic activity was showed to the blood of perch and tilapia. In toxicity test, the zebrafishes were infected with higher or lower hemolytic activity *S. iniae* strains in high or low dosage by IM injection. The results showed that the differences among different dosage were only on morbidity time of infected fishes. The strains of weak pathogenicity and low dosage caused high mortality rate in suitable environment.

Abstract ID: 088C (Poster)

CRUSTACEAN IMMUNE PROTEIN β-GBP AGAINST AQUATIC BACTERIA

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Infectious diseases pose one of the most important threats to thriving aquaculture. The maintenance of vast numbers of fishes crowded together in a small area provides an opportunity for the development and spread of infectious diseases. In this crowded fishes were stressed and more sensitive to diseases, where bacterial diseases are a primary constraint to such practices due to relatively unnatural environment. Bacteria belonging to the genus *Vibrio* and *Pseudomonas* are important aquaculture pathogens which severely affect the production rate of aquatic fishes. Aquacultural output should be increased several fold to meet the rising demands for fish in the near future. Preventing bacterial diseases is thus become a major challenge that the aquaculture industry faces. Use of antibiotics and sanitizers are often ineffective in preventing aquatic diseases because resistant strains are rapidly evolving. In this study, we have investigated the antibiofilm potential of β- 1, 3-glucan binding protein against two aquaculture associated pathogens namely, Gram negative *Vibrio harveyi* (HQ693274) and *Pseudomonas aeruginosa* (HQ693275) which was isolated from aquatic environments. β- 1, 3-glucan binding protein (β-GBP) was purified from the heamolymph of rice field crab *Paratelphusa hydrodromus* by affinity column chromatography using immobilized laminarin. The purified β-GBP is a monomeric protein appeared as a single band with molecular weight of approximately 95 kDa in SDS-PAGE analysis. MALDI-TOF/TOF analysis revealed that, 95 kDa purified protein was matched with β- 1, 3-glucan binding protein of crayfish *Astacus lepidodactylus* with 36 % similarity. β-GBP inhibited the biofilm formed by *V. harveyi* and *P. aeruginosa* which revealed through confocal laser scanning microscopy analysis. The results displayed that, the control cells showed highly complexed multilayered cells and strong affinity to their substratum while the biofilm treated with β-GBP showed disrupted membrane and collapsed micro colonies. The antibiofilm property of β-GBP specifies that, it had the potential to disrupt the biofilm architecture by disturbing the cell adhesion and polysaccharide matrix. The β-GBP showed antiadhesive activity by inhibiting the initial step of biofilm formation by preventing the attachment of bacterial cells. The efficiency of the β-GBP in disrupting cytoplasmic membranes was determined by performing the ortho-nitrophenyl-β-D-galactoside (ONPG) assay. These results revealed that, the β-GBP effectively collapse cytoplasmic membranes and thereby the β -galactosidase was released into the reaction medium which leads to death of the bacterial cells. Thus, our findings were reliable and β-GBP was proved to be an important biocidal agent as it decreases the burden of multi drug resistant bacteria in aquaculture.

Abstract ID: 095C(Poster)

IDENTIFICATION OF *Acholeplasma laidlawii* GROWTH CURVE ISOLATED FROM OLIVE FLOUNDER, *Paralichthys olivaceus*

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Acholeplasma laidlawii is a small bacterium, categorized into *Mycoplasmasp*. which lacks the cell wall. Even though the biological importance was not revealed, it was reported that some bacteria of *Mycoplasmaspp*. live in hosts such as plants and animals, including the marine organisms. So far, no case reported about the virulence of *Acholeplasma* sp. infected in fish, but the inflammation accompanying skin ulcer caused by *Mycoplasma* implies that the *Acholeplasmalaidlawii* also may infected in fish and assumed to cause similar symptoms. In this study, *Acholeplasma laidlawii* 16S rDNA partial sequence was cloned and determined using degenerate primers designed from nucleotide sequences of the other 11 *Acholeplasmasp*. registered in Gene bank database. Also, *Acholeplasma laidlawii* was cultured in PPLO broth containing 10% horseserum and 100 of ampicillin, Optical Density at 600 nm (OD600) was measured at every 6 hours, and the growth curve confirmed that the stationary phase reached at OD600 is about 0.8.

Abstract ID: 103C(Poster)

IDENTIFICATION OF VIBRIOSIS AND STREPTOCOCCOSIS DISEASES ON TILAPIA "SALINA" IN KARAWANG, INDONESIA

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Nile tilapia "SALINA" is tilapia that have tolerant and optimum growth with salinity range 20-25 ppt. It was new strain that can be adapted to improve the idle pond in Indonesia. Tilapia culture on brackishwater and sea water was prone to vibriosis and streptococcosis diseases. The goals of this study were to isolate and identificate the *Vibrio* spp. and *Streptococcus* spp. that infected to Nile tilapia "SALINA". Local bacterial strain was isolated from Nile tilapia farming in Karawang, West Java, Indonesia. Identification of bacteria was accomplished by PCR, using 16S rRNA primers, which revealed the 1,500 bp PCR product. The PCR product was direct sequenced and analized using BLAST homology. The 16S rRNA sequence code 15SA analysis confirmed that the local bacteria was 97% similarity with *Streptococcus agalactiae* strain 15-92MPnew.The16S rRNA sequence code 16SI confirmed that have query cover 91% with *Streptococcus iniae* Dan 1 16S ribosomal RNA gene. The16S rRNA sequence code VP confirmed that have query cover 93% with *Vibrio parahaemolyticus* MS27. The16S rRNA sequence code VF confirmed that have query cover 91% with *Vibrio fluvialis* CIFAMVIFL01. The results showed that the majority of bacteria caused Vibriosis were *Vibrio parahaemolitycus* and *Vibrio fluvialis*. The majority of bacteria caused streptococcosis were *Streptococcus iniae* and *Streptococcus agalactiae*.

Abstract ID: 096C (Poster)

POTENTIAL USE OF PLASMA TECHNOLOGY FOR CONTROLLING DISEASE AND WATER QUALITY MANAGEMENT IN AQUACULTURE

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Maintenance of good water quality and prevention of pathogens propagation are of most concerns in intensive aquaculture. In recent years, non-thermal plasma has been introduced as an antimicrobial agent in water treatment, but few information on its application on aquaculture is still remained. Therefore, this study was conducted to examine the efficiencies of the air- and oxygen-plasma water in inhibition of endemic aquaculture pathogens, water quality improvement, as well as their influences on survival of aquacultured species, including giant freshwater prawn, *Macrobrachium rosenbergii*, orange-spotted grouper, *Epinephelus coioides*, and white shrimp, *Litopenaeus vannamei*. The results showed that air-plasma water had a great antibacterial activity but high toxicity to juveniles of giant freshwater prawn, *M. rosenbergii*. However, oxygen-plasma water was less toxicity to aquatic animals, good ammonia-N and nitrite-N removals, and efficient inhibition the growth of pathogenic bacteria. It is concluded that the oxygen-plasma water could be used to maintain water quality and to decrease the risk of disease in aquaculture.

Abstract ID: 108C(Poster)

EFFECT OF β-GBP-ZNO NPS ON IMMUNE RESPONSE OF TILAPIA

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Aquaculture is expected to satisfy the growing world population's demand for food. Nonetheless, one of the major threats to aquaculture is the infectious diseases which cause mass mortality in aquatic fishes. Among that, bacterial infection has become a major impediment to aquaculture due to its well surveillance of bacteria in aquatic bodies. In this regard, fish culturists try to face this problem by applying antibiotics in order to improve the immune status of fishes against bacterial diseases. Consequently, a fish culturist seeks various compounds to boost up or strengthen the immune system of fishes and those compounds were called as immunostimulants. In addition, searching for new delivery systems is required to improve the administration of immunostimulants. Therefore the use of nanodelivery systems has been proposed as an alternative strategy. The present attempt was made onto investigate the potential of *Portunus pelagicus* β-1, 3 glucan binding protein based zinc oxide nanoparticles (*Pp*β-GBP-ZnO NPs) supplemented diet on growth, immune response and disease resistance of *Oreochromis mossambicus*. β-GBP was purified from the haemolymph of *P. pelagicus* through affinity column chromatography using Sephadex G-100 matrix. MALD-TOF analysis was performed to confirm the purified target protein was β-GBP and the resultant spectrum was read through MS-FIT search engine. MALDI-TOF/TOF analysis revealed that, 100 kDa purified protein was matched with β- 1, 3-glucan binding protein of crayfish *Astacus lepidodactylus* with 36 % similarity. β-GBP based zinc oxide nanoparticles (*Pp*β-GBP-ZnO NPs) were synthesized through co-precipitation method. *Pp*β-GBP-ZnO NPs particle size and shape were evaluated via TEM which reveals spherical and hexagonal shaped particles with 20-50 nm size. Moreover, FTIR measurements clearly depict out the presence of various functional group of *Pp*β-GBP which may acts as a hydrolyzing agent for zinc oxide nanoparticles synthesis. *Pp*β-GBP-ZnO NPs was supplemented at 0.001%, 0.002% and 0.004% to a commercial diet, which was fed to triplicate groups of *O. mossambicus* for 30 days. Fishes fed with *Pp*β-GBP-ZnO NPs express significant increase in weight, growth and feed conversion ratio. During feeding trial, cellular immune responses (myeloperoxidase activity, lysozyme activity and reactive oxygen activity) and humoral immune responses (complement activity, antiprotease activity and alkaline phosphatase activity) were increased significantly in fishes fed with *Pp*β-GBP-ZnO NPs than control. *Aeromonas hydrophila*, one of the most common bacteria in freshwater fishes which cause mass mortality in intensive freshwater fish culture. In the current attempt, *Pp*β-GBP-ZnO NPs was evaluated for its biofilm inhibition potential on *A. hydrophila* (ATCC 7966). Confocal laser scanning microscopy analysis displayed that, the preformed biofilm thickness of *A. hydrophila* was significantly reduced to 12 µm at the concentration of 50 µg/ml of *Pp*β-GBP-ZnO NPs than control. Moreover, after 30 days of post feeding, fishes were challenged with aquatic fish pathogen *A. hydrophila* (1×10^7 cells ml^{-1}) and the result of challenge study was observed with reduced mortality rate of fishes fed with *Pp*β-GBP-ZnO NPs. Thus from our observation, *Pp*β-GBP-ZnO NPs supplemented diet have a potential to strengthens the immune system of *O. mossambicus*.

Abstract ID: 109C(Poster)

DETERMINE VIRULENCE GENE OF *Streptococcus iniae* CAUSING DISEASES ON CULTURED FISH IN THE MEKONG DELTA, VIETNAM

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Streptococcus iniae is one of emerged pathogens and causes serious loss in fish species throughout the world. The purpose of this study was to determine the virulence of *Streptococcus iniae*, causing disease on cultured fish farms in the Mekong Delta, Vietnam. A total of 08 *S. iniae* isolates causing "dark body" disease on climbing perches (*Anabas testudineus*) in intensive farms and 08 *S. iniae* isolates causing exophthalmia, hemorrhage on Nile tilapia (*Oreochromis niloticus*) in cage culture, were examined. Conventional and the API 20 Strep system, PCR and 16S rDNA gene partial sequencing were used to identify and confirm the causative agents of the disease in the study. *In vitro* studydetermined the virulencegene of *Streptococcus iniae*, specific primers PGM-F and PGM-R to amplify phosphoglucomutase virulence genes (*pgm* gene) of 16 *S. iniae* isolates were used to confirm *S. iniae* in the study with PCR products were 1865 bp in length, gene sequences 88-97% similarity to phosphoglucomutase gen (*PgmA*) of *S. iniae* isolate in NCBI under accession number AY846302.1. *In vivo* study, experimental enges were carried out during 14 days. Results showed that most of challenged fish were observed the similar clinical signs as the natural infection. The high mortality rates of both ONBT02 *S. iniae* isolate (Nile tilapia) and ATHG36 *S. iniae* isolate (climbing perches) presented at LD_{50} values $2.34x10^4$ and $1.81x10^4$ cfu/ml, respectively. Comparison of *S. iniae* strains of different virulence, both at a phenotypic and genotypic level should help to obtain better insights in the virulence factors of this micro-organism.

Abstract ID: 114C(Poster)

INVESTIGATION ON THE DISEASE SITUATION OF KOI CARP (*Cyprinus carpio*) IN SEVERAL KOI FARMS IN THE MEKONG DELTA

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An investigation about pathogen infection on Koi carp (Cyprinus carpio) was conducted in the period from January to November 2016 in several koi farms the Mekong Delta provinces, Vietnam. The study purpose to determine the infection status on Koi carp and provide additional information for the study subsequent research. A total of 128 disease Koi carp at the stage from 1 to 6 months, weighing from 15 to 80 gram, were collected. Fish specimens were observed for clinical signs, examined for parasites and bacteria isolated. The clinical signs including reduced feeding, lethargy swimming, red spots on the body, red-abdominal fluid and visceral hemorrhage. Results on parasitic examination had indicated 5 genera of parasites, namely Myxobolus sp., Trichodina sp., Dactylogyrus sp. and Metacercariae and Argulus sp.; found mostly in the skin and gills of the infected fish. The prevalence ranged from 14-72.3%, in which Trichodina sp. showed the highest intensity (3-28/40X) and Argulus sp., the lowest (2/10X). Remarkably, results on bacterial identification also isolated 32 strains of Aeromonas bacteria from internal organs of the affected fish. Conventional and rapid identification systems, PCR were also used to identify the causative agents of the disease. Meanwhile, histological section of gills infected by Myxobolus sp. with severed lamellar hyperplasma and chondrodyslasma of cartilage were also observed. In generally, hemorrhagic disease and Myxobolus sp. infection were the most two common diseases that caused the highest rate of infection and high mortality on the Koi carp in this investigation.

Abstract ID: 152C(Poster)

THE CONTROL OF FISH BACTERIAL DISEASE WITH BETEL LEAF EXTRACTS

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Betel leaf extracts have some natural antimicrobial. This extracts has been used as antimicrobial agent in some aqua farmer in Indonesia for an alternative method to control fish diseases. This study aims to determine the effects of betel leaf extract on fish pathogenic bacteria in-vitro. Three kinds of solvents were used in betel leaf extracts. There were alcohol, n-hexane and ethyl-acetate. About 100 g of betel leaf simplicia were diluted by 1000 ml of solvents in stainless container cap for 24 hours maceration. This extracts were filtered with 0.42 µm paper filter and then evaporated at 50^{O}C during 30 minutes in 50 rpm with vacuum rotary evaporator. This concentrated extracts were used to antimicrobial testing. The pathogenic bacteria for in-vitro testing were from Gram-negative bacteria such as *Aeromonas hydrophila*, *Vibrio alginolyticus*, *Edwardsiella tarda* and Gram-positive bacteria as*Staphylococcus cohnii*.The in-vitro test of herbal extract from betel leaf againts Gram-negative and Gram-positive pathogenic bacteria indicated that the betel leaf extract could inhibited the growth of pathogenic bacteria with diameter mean of inhibition zone were more than 14 mm. The solvents variations were not significantly different ($p > 0.05$) to diameter inhibition zone of *V. alginolyticus*, *A. hydrophila* and *S. cohnii* but significantly different ($p < 0.05$) to *E. tarda*.

Abstract ID: 153C(Poster)

ANTIBIOTICS TEST IN FISH PATHOGENIC BACTERIAL RESISTENCE

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The use of antibiotics to control fish diseases could induce the side effects such as resistance pathogenic bacteria, antibiotic residues in fishery products and the environment, and also detrimental to human health as a consumer. The observation of pathogenic bacterial resistance to antibiotics intended to determine the status or level of resistance some bacteria in fish/ shrimp and the environment against the antibiotics. Methods of these activities include the collection of pathogen isolates from fish and shrimp organ. These samples collected from several areas of aquaculture in South Lampung regency, Banten and several regencies / cities in West Java province. Isolation, identification of bacteria and testing of bacterial resistance use the method of disk difusion by sensidisk antibiotics enrofloxacine (5 ug), tetracycline (30 ug) and oxytetracycline (30 ug). The test results showed that from each 45 isolates bacteria tested: 15.6% resistance, 13.3% intermediate and 71.1% sensitives to enrofloxacine. For Tetracycline: 24.4% resistance, 26.7% intermediates and 48.9% sensitives. Whereas for the status of Oxytetracycline: 20% resistance, 42.2% intermediates and 37.8% sensitives.

Abstract ID: 158C(Poster)

SCREENING OF ISOLATED POTENTIAL PROBIOTIC FROM MUD-AQUACULTURE IN CENTRAL JAVA INDONESIA WITH MOLECULAR BASED

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*Aeromonas hydrophilla*is still a big concern as a problem in intensive fresh water fish culture in Indonesia. An alternative solution to solve the aquaculture problem which use probiotics. Its usually live microorganisms which when administered in adequate amounts confer a health benefits on host. The aim of this research was to find out the potensial probiotic candidates against *A. hydrophilla*which identified based on the 16S rDNA gene sequences. This research combined between exploratory in the field and experimental methods. Potensial probiotic candidates diversity were isolated from Boyolali, Klaten and Banjarnegara Regency, Central Java, Indonesia. A total of 54 isolates bacteria were isolated frommud of aquaculture medium, and cultured with TSA, TSB (merck) and GSP medium Out of 54 isolates only 8 isolates exhibited pathogen *A. hydrophilla* activity. It just three promising isolates were identified with molecular based method. Based on 16S rDNA sequence analysis,isolate CBL20, CBjL13 and CBjL15 were closely related to *Bacillus methylotrophicus* strain XJAJ2 (100%.), Bacillus amyloliquefaciens strain CGPY3 (96%), *Bacillus subtilis* strain 118-907R (99%).

Abstract ID: 159C(Poster)

VIBRIO PHAGES FROM HAEMOLYMPH AS BIOCONTROL AGENTS

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Vibrio parahaemolyticus,a commensal of aquatic foodstock like paneid shrimp has gained the interests of researchers in the recent years due to the emergence of opportunistic pathogenicity in this organism leading to tremendous commercial losses. The routine use of antibiotics in an attempt to curb opportunistic pathogens is likely to result in the emergence of antimicrobial resistance and accumulation of antibiotic residues in the meat which would be consumed. Bacteriophages are natural co-inhabitants with bacteria in aquatic environments and play an important role in maintaining the ecosystem equilibrium.Three virulent phages of *Vibrio parahaemolyticus* were isolated from 80 haemolymph samples. A cocktail of the phages showed an activity on 82 (> 80%) of the *Vibrio parahaemolyticus* isolated from the same geographical region of isolation (n=100).The host range of these phages was also performed on a panel of other 35 isolates representing *Vibrio parahaemolyticus* from across the globe. The three phages together showed lytic activity on 20 (57%) of the global isolates. These phages may be used as a suitable biocontrol method for preventing outbreaks due to *V. parahaemolyticus.*

Abstract ID: 165C(Poster)

FIRST ISOLATION AND IDENTIFICATION OF *Edwardsiella ictaluri* FROM Catfish,*Pangasius* sp, CULTURED IN CIRATA LAKE,INDONESIA

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In January 2016 there were reported rampant mortalities of freshwater aquaculture catfish.*Edwardsiella ictaluri* is primarily recognized as a disease causing pathogen in aquaculture fish species which is known to cause an economically important bacterial infections hence affecting productivity of aquaculture enterprises in many regions across the globe. 51 tissue samples (three from each fish) comprising spleen, kidney and liver were collected from 17 fish. Distribution of *E. ictaluri* infection among the three organs examined indicated that *E. ictaluri* was isolated from kidney (29,4%), spleen (25,4%) and liver (13,7%). The characteristic of tissue samples are showedabnormal sizes and abnormal color including white nodules. Samples were identified by conventional biochemical test,polymerase chain reaction (PCR) and sequencing analysis. Phylogenetic analysis showed that bacteria was closely related to *Edwardsiella ictaluri*strain that is accession numberKF907129.1 with 95% similarity.Sensitivity test results of bacteria using tetracycline (30ug) and Chepalotin (KF) 30ug were 20.57mm and 22.39mm, both of them are susceptible. There is limited information on *E. ictaluri* of fish in Indonesia and rather than further study to have comprehensive information on the agent is forwarded.

Abstract ID: 174C(Poster)

SUBCELLULAR LOCALIZATION AND PRELIMINARY FUNCTION STUDY OF A SECRETED PHOSPHOLIPASE C FROM *Nocardia seriolae*

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Fish nocardiosis is a chronic systemic granulomatous disease, and *Nocardia seriolae* is the main pathogen. But the pathogenesis and virulence factors of *N. seriolae* are not fully understood. A phospholipase C (PLC), which likely to be secreted and target host cell mitochondria, was found by the bioinformatics analysis on the whole genome sequence of *N. seriolae*. In order to determine the subcellular localization and study the preliminary function of PLC from *N. seriolae*, the gene cloning, secreted protein identification, subcellular localization and apoptosis detection of *N. seriolae* PLC were carried out in this study. The results showed that PLC was a secreted protein by mass spectrometry analysis of extracellular products from *N. seriolae*. Subcellular localization of PLC-GFP fusion protein revealed that the green fluorescence was dot distribution near the nucleus and did not co-localize with mitochondria. In addition, apoptosis assay suggested that apoptosis was induced in FHM cells by the overexpression of *N. seriolae* PLC. The subcellular localization and preliminary function study of PLC from *N. seriolae* may lay the foundation for further study on the function of this gene and promote the understanding of the virulence factors and pathogenic mechanism of *N. seriolae*.

Abstract ID: 215C(Poster)

ISOLATION OF *Aeromonas* BACTERIOPHAGES FOR PRELIMINARILY CONTROL THE HEAMORRAGE IN STRIPED CATFISH (*Pangasianodon hypophthalmus)*

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The hemorrhage on striped catfish farming in Vietnam with the two main causative agents, *Aeromonas hydrophila* and *A. dhakensis,* have been resulted in a significant loss for farmers annually. Biological control of pathogenic bacteria using bacteriophages has attracted significant attention as an effective way to treat such pathogens. The pathogenic *A. hydrophila* and *A. dhakensis* species, isolated in striped catfish in the Mekong Delta,*Pangasianodon hypophthalmus* from 2012 to 2014, were used as hosts for isolation and characterization of bacteriophages. The five different groups of *Myoviridae* bacteriophages against *A. hydrophila* and *A. dhakensis* were successfully isolated from waste and fish pond's water and the first reported in Vietnam. The characterizations of bacteriophages and the phage typing will be further carried out in order to be able to apply phage therapy in striped catfish.

Abstract ID: 232C(Poster)

INVESTIGATION OF DISEASED CHANNEL CATFISH, *Ictalurus punctatus,* COLLECTED FROM CAGE CULTURED FARM AT UTTARADITH PROVINCE, THAILAND

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Investigation of diseased channel catfish, *Ictalurus punctatus* collected from cage cultured farm at Uttaradith Province, northern part of Thailand. The diseased fish revealed hemorrhage, deep ulcer on body and resulting of dead. Thirty fishes were sampling for diseased diagnosis. Trichodinids and mongenean trematode were observed on gills and skin. The bacteria were then isolated from lesion, liver, spleen and kidney and identified base on biochemical characteristic analysis. The bacteria were *Aeromonas hydrophila*, *Aeromonas sobria* and *Edwardsiella tarda*. Virus (CCVD) is negative. Histopathological observations on catfish infected naturally revealed lymphocyte infiltration in muscle and focal necrosis, hyperplasia, edema, and swelling of the gill lamellar epithelium. The liver sections revealed lymphocyte infiltration and focal necrosis. The kidney of diseased fish exhibited ischemic type tubulopathy, necrosis of nephritic tubules, hyperplastic hematopoietic tissue, hemosiderin deposition, and edema. These results indicated that such bacteria were the causative of disease, however, the study of pathogenesis have to study in further.

Abstract ID: 237C(Poster)

DEVELOPMENT OF A RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR RAPID DETECTION OF FRANCISELLA NOATUNENSIS SUBSP. ORIENTALIS

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Francisella noatunensis sub.sp. *orientalis* (*Fno)* is an emerging bacterial pathogen representing a major threat to tilapia aquaculture globally. Its detection by conventional techniques is a major diagnostic challenge. Several diagnostic methods have been developed, but are not suitable for field diagnosis. We report the development of a novel quantitative real-time isothermal recombinase amplification assay (RPA) for rapid detection of *Fno*. The developed assay showed high analytical sensitivity and specificity when tested with plasmid standard, genomic DNA (gDNA) of various bacteria and clinical samples. The performance of the assay was comparable to a published real-time PCR with more tolerance to reaction inhibitors and quicker results in approximately 6 min. The reported RPA assay represents a promising point-of-care detection assay for pond-side *Fno* detection and might be useful for surveillance and early outbreak warning.

Abstract ID: 238C (Poster)

OCULAR VIBRIOSIS IN CAGE CULTURED SNUBNOSE POMPANO *Trachinotusblochii* IN THE PHILIPPINES

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Occurrence of exophthalmia was documented among pompano (*Trachinotus blochii*) broodstocks cultured in floating net cages in SEAFDEC/AQD's Igang Marine Station, Guimaras, Philippines. Gross examination of affected fish revealed mild to moderate infestation of caligid parasites (*Lepeophtheirus spinifer*)causing mild to severe hemorrhaging of the body surface, fins and eyes. A Gram-negative bacterium was isolated in pure culture from the eyes and brains of affected fish (average body weight [ABW]: 1.5±0.45 kg). Based on biochemical characteristics and sequence of the16S rRNA, the causative bacteria isolated from the diseased fish were identified as *Vibrio harveyi*. The strain *V.* harveyiTbE0901 was selected as a representative isolate for the infection bioassay. Pompano juveniles intraperitoneally injected with *V. harveyi*TbE0901 at inoculum doses of 10^8, 10^6, 10^5 and 10^4 c.f.u. $fish^{-1}$ resulted in 100%, 70%, 50%, and 25% mortalities, respectively, with some moribund fish exhibiting abdominal distension and exophthalmia. In addition, intravitreal injection of pompano juveniles with 20 µl of *V. harveyi* at an inoculum dose of 10^2 c.f.u. eye^{-1} resulted in exophthalmia within 1 week after injection. In both injection route of experimental infection, *V. harveyi* was reisolated from the kidneys, brains, and eyes of moribund fish. On the contrary, mortality and concomitant exophthalmia was not noted in fish intraperitoneally and intravitreally injected with saline (control). Bath infection of pompano juveniles with 10^8 c.f.u. ml^{-1}*V. harveyi* did not likewise induce mortality or abnormal signs such as exophthalmia. Taken together, results of pathogenicity tests clearly indicate that mechanical injury inflicted by *L. spinifer* is a crucial step responsible for the inadvertent entry and subsequent proliferation of *V. harveyi* in the eye of pompano, thereby propelling the induction of exophthalmia and consequential systemic bacterial infection of the brain and other vital organs.

Session 4 – Parasitology

Abstract ID: 182D (Keynotefor Parasitology)

CONTROL OF PARASITES – DOES HISTORY INFORM OUR FUTURE?

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Yes! A priori assumptions and experiences are critical in the management and control of parasitic infections. Previous lessons learned can help accelerate the lead in to the development of novel control strategies but more critically can increase the probability of a treatment being effective while at the same time minimise the potential associated risks. Although there are examples of entirely novel approaches being employed in aquaculture, for the most part they are rare. Instead, improvements in parasite control are a result of incremental improvements on current and historical methods. Unfortunately, there are “no one size fits all” approaches and the strategies employed must be calculated by considering the infinite variety of circumstances and the intracacies peculiar to each case. However, broad principles can be applied across the range of farms to ensure success. In this talk, we will take a closer look at the evolutionary development of a range of treatment strategies, prophylaxis methods and farm management practices and, where possible, use case studies to highlight the importance of certain considerations, where they are frequently overlooked and, what the repercussions of such oversights might be. For chemical treatments, which for many are invariably the first choice for the management and control of parasite infections, we shall discuss the importance of: understanding the physiology of the host; identifying the parasite you want to treat and having a comprehensive understanding of its life-cycle; the culture environment; and, the physical and chemical properties of the treatment to be used – specifically its mode of action, uptake, distribution and residency time. When administering bath treatments, for example, we will demonstrate the importance of understanding the organic loading of the culture environment, the size and biomass of the aquatic animals to be treated and how this can affect treatment efficacy. To temper the importance and need for specific chemical-based treatments, we will demonstrate that through proper farm husbandry practices and an understanding of parasite life-cycles, how certain parasite populations can be managed. Where possible, we shall provide examples of the perils of misdiagnosis, improper veterinary advice and, poor management including the consequences of ignorance, tardiness, denial and procrastination in implementing a treatment.

Abstract ID: 171D (Oral)

PATHOGENICITY AND PATHOLOGY OF PARASITE MARINE LEECH *Zeylanicobdella arugamensis* INFESTATION IN ASIAN SEABASS *Lates calcarifer* UNDER LABORATORY CONDITION

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The ectoparasite marine leech *Zeylanicobdella arugamensis* had caused a significant impact on the marine farmed fish economically in Southeast Asia. In Malaysia, the leech had infested 14 species of marine farmed fish and usually associated with secondary infection such as scale drop disease. One approach of managing marine leech infestation issue was to determine the minimum number of marine leech that trigger action to be taken to prevent further damages on the fish. Thus, the aim of this study was to determine the pathogenicity and pathology caused by marine leeches in Asian seabass under laboratory condition. Five groups of healthy Asian seabass were exposed to different number of marine leech (0, 1, 10, 30 and 70) for seven days. Each group consists of individual Asian seabass fingerling with eight replicates. Replicates 5 to 8 from each group were used for histology. Gross observation showed dark body colour, passive movement, hemorrhage and scale drop on infested fish with 10 leeches onward. From day 3 onwards, 50% of the fish infested with marine leech showed clinical signs such as hemorrhage and ulcer with mortality ranged from 20% to 40% in the group with 10 leeches onward. A higher mortality (60%) was observed on day 7 in the group with 30 and 70 leeches. Pathology on the muscle of infested fish showed hyperplasia even with only one leech. Other pathology signs include an increase of mucous, hyperplasia in epithelium cells and increase in the number of pigmentation in fishes exposed to 10 marine leeches onwards. The result showed that Asian seabass fingerling infested with 10 leeches onward were capable of causing injuries and diseases to the host. On the other hand, infestation by one marine leech on the fish can cause hyperplasia without killing the fish up to 7 days. This information obtained can help farmers to response promptly once they have discovered leeches in their cages and provides a better understanding on the damages caused by marine leech. It can also be used in the development of husbandry strategies to avoid the dissemination of marine leech parasitic disease at cages.

Abstract ID: 058D (Oral, Student)

SEARCHING FOR INFECTION RELATED PROTEASES ON YELLOWTAIL SKIN FLUKE *Benedenia seriolae*

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Yellowtail aquaculture production is the biggest in Japan. Skin fluke disease is caused by *Benedenia seriolae* infection on yellowtail skin surface. Farmers treat skin flukes using fresh water bath and by changing nets regularly to remove attached eggs. However, these treatments are tedious for farmers and stress to the fish. Thus new protective method is required. Morphological and genetical classification, relationships with environmental conditions, and some treatment methods have been studied on *B. seriolae*. However, there are a few reports on genes of *B. seriolae*. Recent study reports in proteases play important roles in initial infection to mammals in *Schistosoma*, another Platyhelminthes. In this study, we investigated *B. seriolae* genes transcriptome analysis to identify various protease and protease activity was measured.*B. sriolae* were collected at Ehime prefecture in Japan. An infected yellowtail was bathed in fresh water for a few minutes, and dettached *B. seriolae* were collected and stored in RNA*later* at 4°C and -80°C for protease asssay. Total RNA were extracted from a *B. seriolae*. cDNA was synthesized using TruSeq Stranded mRNA Library Prep Kit for Next Generation Sequencing. Illumina MiSeq was used to obtain the cDNA sequences, and obtained reads were assembled by TRINITY program, assembled contigs were annotated by Blast2GO program. To reveal the protease activity of *B. seriolae*, gelatin and casein zymography assays were conducted using homogenate of thirty individuals of *B. serolae*. Twenty five µg total protein was used for the assay.From NGS analysis, 2,307,736 reads, 27,374 contigs and 11,548 annotated contigs were obtained. Serine protease, cysteine protease, aspartic protease and metalloprotease were identified. Two protease bands could be detected under 3.5% NaCl and non-NaCl conditions in zymography gels. However clearer bands were detected in 3.5% NaCl condition, indicating a stronger activity. Among four types of protease inhibitors (serine protease, cysteine protease, aspartic protease and metalloprotease) were contained in reaction buffer. The serine protease and metalloprotease inhibitor inhibited the activity, indicating that these proteases are serine protease and metalloprotease. These results indicated that *B. seriolae* possess types of proteases were serine proteases and metalloproteases mainly works.

Abstract ID: 213D (Oral, Student)

SYNERGISTIC INFECTION OF THE ECTOPARASITE *Ichthyophthiriusmultifiliis* AND THE INTRACELLULAR BACTERIUM *Francisellanoatunensis* subsp. *orientalis* IN RED TILAPIA (*Oreochromis* sp.)

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Francisellanoatunensis subsp. *orientalis* (*Fno*) and *Ichthyophthiriusmultifiliis* (*Ich*) are deadly infectious pathogens in farmed tilapia especially during cool season when the water temperature dropped to a range of 23-28 °C. We hypothesized that parasitism of the ectoparasite (*Ich*) might enhance susceptibility of red tilapia to the intracellular bacterium *Fno*. To prove the hypothesis, the experiment was designed as follows. Natural *Ich*-infected tilapia (9 ± 6 trophonts/fish gills; 4 ± 3 trophonts/fish skin) were distributed into 2 groups with 20 fish each (10 fish/replicate). One group was exposed to *Fno* by immersion method while the other served as control group. In parallel, two groups of *Ich*-free tilapia were challenged to only *Fno* in the same manner. The results showed that cumulative mortality in the *Fno* single infection was 25 ± 7 % whereas 100% in the coinfection treatment. No mortality was observed in both control groups (*Ich*-infected and *Ich*-free fish). The coinfected fish revealed typical signs and histopathological manifestations of francisellosis and ichthyophthiriasis. This study revealed synergistic effect of the *Ich* and *Fno* infection in red tilapia (*Oreochromis* sp.). Thus, farming management of fish to be free from the *Ich* ectoparasite might reduce risk of Francisellosis and probably other bacterial diseases in farmed tilapia.

Abstract ID: 043E (Oral, Student)

PURIFICATION OF SPORES FROM THE MICROSPORIDIAN ENTEROCYTOZOON HEPATOPENAEI AND VIABILITY CONFIRMATION BY POLAR TUBE EXTRUSION ASSAY USING PHLOXINE B

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The microsporidian *Enterocytozoon hepatopenaei* (EHP) is a rapidly spreading, emerging pathogen of cultivated penaeid shrimp in Asia that causes growth retardation, and it is believed to be endemic in the Australasian region. Control of EHP infection is currently focused on preventative measures but unfortunately, no proven methods are currently available for treatment of infected shrimp. In order to understand this pathogen, potential treatment methods like laboratory challenge models based on feeding of infected hepatopancreatic tissue or cohabitation of uninfected and infected shrimps separated by mesh cage or netting have been carried out. In this study, we have developed the EHP purification method by using Percoll gradient centrifugation. The viability of the purified spore have been evaluated by its polar tube extrusion with Phloxine B staining. Once we were able to obtain active spores, we exposed them to different physical and chemical treatments to identify factors that inhibited the extrusion of the polar tube, thus becoming a possible treatment to inhibit the spread of the pathogen. These studies were aimed to generate more understanding of this pathogen as well as establishing innovative strategies to reduce its viability and potential infectivity in shrimp farms.

Abstract ID: 020D (Oral)

EPIDEMIOLOGICAL HOLISTIC APPROACH ON FISH FARMING: A CROSS SECTIONAL STUDY ON PREVALENCE AND RISK FACTORS OF ECTOPARASITE INFESTATION IN GIANT GOURAMY *Osphronemous goramy* FARMING IN WEST JAVA, INDONESIA

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Ectoparasites are a dangerous threat for aquaculture, especially for intensified production. The prevalence, intensity of infestation and spatial distribution of parasites in fish depend on both ecological and epidemiological patterns very complex to identify. Lot of risk-factors are intrinsic to the farms practises and to the biotic and abiotic factors and holistic epidemiological approach is required to highlight the risk factors. In order to get insight into the description, prevalence, epidemiological features and risk factors of ectoparasite infestation in *O. goramy* farming, a two-step cross-sectional study was realized in Bogor district (West Java). In a first step, a descriptive data set was collected from 71 farms through a structured questionnaire proposing more than 180 different putative risk factors divided into 5 sub-questionnaires. These sub-questionnaires included information about farmers, fish, farm characteristics, fish feeding and health management. In a second step, a shorter questionnaire for complementary information was realized. Concurrently, 326 fish (6.9 to 25 cm of Total Length) were randomly sampled and water of the corresponding rearing ponds was analysed. More than 21,000 ectoparasites belonging to 12 species were detected from the skin and the gills and 97.2±2.2% of fish showed at least one parasite. Nine of these parasitic species has a prevalence over to 5% in fish sample and some of them may be a serious concern for fish health. The highest prevalence (67.8±5.1%) was observed for a myxosporea *Henneguya sp n.* in gills, and *Trichodina sp., Piscioodinium sp* and *Ichthyophytirius multifilis* had also a high prevalence. The larval trematode *Centrocestus formosanus* (21±4.5%) was identified based on morphological appearance and confirmed by sequencing. Moreover, molecular investigations also revealed the presence of other Heterophyidae and larval trematodes, which could not be characterized yet. As attested by health assessment index (HAI) and Fulton's condition factor of autopsied fish, parasite's load observed here did not seem to exert a negative impact on fish. Nonetheless, the high prevalence of ectoparasites, the strong segmentation of production chain of *O. goramy* in the country associated to the increasing intensification, may facilitates spreading and the increasing severity of parasitic infestation in fish-farms. Dataset from questionnaires were analysed throughout several univariate analysis and logistic binomial regression that in order to select risk factors for each species of ectoparasite, Finally, Odds Rate (OR), the attributable risk and the attributable proportion have been calculated for selected risk factors–. This study highlight that in farms of *O. goramy*, the ectoparasite species may have shared and species-specific risk factors, it suggests possible ways how to reduce parasite prevalence in farm, and it emphasis how holistic epidemiology approach may contribute into improvement of health management in small scale fish farming.

Abstract ID: 233D (Oral)

FUNCTIONAL FEED ADDITIVES AS PREVENTION OF PARASITIC DISEASE IN FISH

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The aquaculture industry is expected to continue to expand and fulfill the increasing global needs for fish and shellfish in the coming years. However, as the industry grows and intensifies, biological plagues are becoming more critical factors affecting profitability of aquaculture producers. All commercial aquaculture fish species worldwide suffer from a variety of parasites which often cause important economic losses. Parasites affect all fish species, including highly industrialized species such as salmon, bulk freshwater species such as tilapia as well as new species of marine fish such as yellowtails and tunas.Gill and skin flukes are causing increasing problems in fish farms, such as *Sparycotyle crisophrii* in Mediterranean seabream *Sparus aurata*, or gyrodactilids in tilapia worldwide. A wide diversity of protozoan ectoparasites infests freshwater fish, including white spot, *Trichodina spp…* Endo-parasites such as digenean worms, acanthocephalan, to coccidians, mixosporidians, and microsporidians (*Eimeria spp, Enteromyxuum spp*) attack the digestive system and internal organs. The traditional approach to combat fish parasites, based on the use of chemicals and some therapeutics once the parasite outbreak is detected, is increasingly hampered by the development of resistance and the increasing restrictions on the use of chemicals. Health promoting feed additives have already become a standard ingredient in premium brands of salmon and many other fish species feeds designed to reduce the impact of parasites. A wide range of additives with different mode of actions are currently offered to fish including yeast extracts, phytobiotics, probiotics, prebiotics, organic acids and their derivates. Functional feeds containing gut health promotors deliver with every meal an adequate concentration of natural compounds which can work through multiple mechanisms to reduce the success of the parasitic infestation. Natural compounds with anti-parasitic activity can work directly on gut parasites or reach the blood and/or mucus to affect ecto-parasites, whereas immune modulators can change the composition and thickness of the mucus.This presentation will review the latest advances in the research and development of health promoting feed additives, both in laboratory and field conditions, showing their efficacy to boost fish health and reduce the economic impact of parasites on fish production. A number of case studies will be presented, including the prevention of gill flukes and endoparasites in Gilthead seabream *Sparusaurata* and European seabass *Dicentrarchuslabrax*.

Abstract ID: 039D (Poster &Elevator Pitch)

IMPORT OF ORNAMENTAL FISHES, RISKS TO EMERGING PET FISH TRADE IN PAKISTAN

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Pakistan is ranked 54th in the world to import ornamental fishes and this trade is increasing gradually. However, at the same time the risk of introduction of pathogens along fish is also rising. The present study describes parasitic infection in imported ornamental fishes. Seven species of ornamental fish, *Carassius auratus* (double tail and single tail goldfish), *Astronotus ocellatus* (red tiger Oscar), *Cyprinus carpio* (koi carp),*Balantiocheilos melanopterus* (silver shark), *Osphronemus goramy* (giant gourami), *Pterophyllum scalare* (angelfish), *Pangasianodon hypophthalmus*(blue line shark) were examined for parasitic infections. The clinical signs associated with infected fishes were eroded scales, skin lesions, damaged fin and pop eye. Low to high level infections was observed in fishes studied. Five groups of parasites: protozoan (*Trichodina* sp. *Tetrahymena* sp. *Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*), monogeneans (*Dactylogyrus* spp. *Gyrodactylus* spp.), digeneans (unidentified metacercariae), molluscan (glochidium larva) and crustacean (*Argulus foliaceus*) were found infecting the skin, fin and gills of these fishes. Dactylogyrosis was the most prevalent diseases found in all seven species of fish. Whereas, protozoans were found only in *C. auratus* and *A.ocellatus*. The pop eye in *O. goramy* and glochidium larva on gills of *B. melanopterus* is reported first time from Pakistan. At the same time the occurrence of bacterial and viral infections in these fishes cannot be rules out. The indiscriminate trade of diseased ornamental fishes into Pakistan must be checked through implementation of import regulations. Pre-quarantine and quarantine are important bio-security measures and both must be ensured. These steps may help to minimize the chances of transmission of pathogens and their spreading in the local fish fauna of Pakistan. In future, it may also reduce the probable risk involved in pet fish trade in the country.

Abstract ID: 121D(Poster & Elevator Pitch)

CASE STUDY THE PARASITE INFECTION OF GYRODACTYLID MONOGENEANS IN Clarias gariepinus

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The aim of this research was to explore the parasitic infection of gyrodactylid monogeneans from the skin and fins of *Clarias gariepinus*. The methods were included of sampling, microscopic examination, morphometric measurement and analysis, DNA extraction, PCR amplification, visualization, sequencing and data analysis. Gyrodactylid monogeneans was identified using median hook morphology and by sequencing the nuclear ribosomal DNA internal transcribed spacer (ITS) 1 and 2. Based on morphometric and molecular characterization gyrodactylid monogeneans specimens were described as *Macrogyrodactylus congolensis* and *Gyrodactylus rysavyi*. The different morphometric analysis reported as body length and body width *of M. congolensis* were 2526,99 (2112,00–2878,43) µm and 362,31 (251,99–499,44) µm three times longer and wider than *G. rysavyi* 894,91 (812,66–981,81) µm and 93,65 (82,14–10,55) µm. The hamulus total length, hamulus point length, hamulus shaft length and hamulus root length of *M. congolensis* were 388,35 (343,03–433,59) µm, 97,16 (74,25–119,06) µm, 297,38 (197,74–351,84) µm and 188,17 (140,67–236,83) µm. Length of the anterior lateral arm of the ventral bar, length of posterior central arm of the ventral bar, total length of the ventral bar and width of the ventral bar of *M. congolensis w*ere 89,62 (79,64–112,69) µm, 20,61 (14,09–29,04) µm, 147,04 (132,53–155,76) µm and 110,43 (82,30–125,37) µm. Total length of the marginal hook, length of the marginal hook handle and length of the marginal hook sickle of *M. congolensis* were 86,28 (68,53–101,83) µm, 75,37 (61,52–93,65) µm and 12,36 (11,65–12,95) µm respectively. PCR analysis showed an expected band of 1.023–1.025 bp nucleotides in length. Phylogenetic analysis showed that gyrodactylid monogeneans was closely related to *Gyrodactylus rysavyi* species with 99% similarity. This was indicating a competitive exclusion between gyrodactylid monogeneans which *G. rysavyi* was found to reproduce more rapidly and to expand its microhabitat at the expense of the slow growing and slightly proliferating *M. congolensis* and then it suggested there was infra population crash of *G. rysavyi* and *M. congolensis.*The growth of *G. rysavyi* was dominated on the skin of fish.

Abstract ID: 155D (Poster & Elevator Pitch)

UTILIZING TROPICAL ALMOND PLANT (*Terminalia Catappa* L) LEAVES TO REDUCE PARASITIC DISEASE IN *Clarias* sp NURSERY

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Clarias sp is one of favorite fish in Indonesia. Along with increasing production, encourage the increasing needs of seeds demand. But on the other hand, nurshery of *Clarias bathracus* still faces obstacles to parasite attacks especially when temperature is below 23°C. Utilizing *Terminalia catappa* leaves can reduce parasitic attack that has been widely utilized for traditional medicine for fish and also for human. The primary content of Terminalia catappa is Tanin that can make water brown and also can act as antimicrobial and prevent parasitic attact. In this reasearch, we use C*larias* seed (1 – 2 cm size) that be nursed in tarpauline tank (1x1x2m^3 size) with density about 500 fish/m^3 for 1 month period. The treatment are designed by giving dried catappa leaves about 75 g/m^3 (A); giving wet catappa leaves about 75g/m3 (B), and control (C). Catappa leaves put into the water two days before stocking fish. Each treatment is replicated (duplo). Monitoring of parasite and water quality is conducted every week. The result show that survival rate for A is 88,1% ± 1,98%; B is 27,8% ± 2,55%; and C is 40% ± 1,5%. This highest SR in A also supproted in parasite data that dried catappa treatment can reduce parasite attact for Trichodina sp in skin about 78% (from intensity 31±29 ind to 7±0 ind); Trichodina sp in gill about 39% (from intensity 35±2 ind to 21±6 ind); Ichtyophtirius in skin about 100% (from intensity 13±6 ind to 0±0 ind); Ichtyophtirius in gill about 100% (from intensity 1±0 ind to 0±0 ind); and Dactylogyrus in gill about 100% (from intensity 2±0 ind to 0±0 ind). The result of parasite in B show that wet catappa leaves can decrease for Trichodina sp in skin about 45% (from intensity 31±29 ind to 10±11 ind); Trichodina sp in gill about 44% (from intensity 35±2 ind to 19±22 ind); Ichtyophtirius in skin about 99% (from intensity 13±6 ind to 0±0 ind); Ichtyophtirius in gill about 100% (from intensity 1±0 ind to 0±0 ind); and Dactylogyrus in gill about 100% (from intensity 2±0 ind to 0±0 ind). The result of parasite in C show that there are decreasing and increasing parasite in kontrol : Trichodina sp in skin decrease about 68% (from intensity 31±29 ind to 10±11 ind); Trichodina sp in gill increase about 68% (from intensity 35±2 ind to 59±16 ind); Ichtyophtirius in skin decrease about 21% (from intensity 13±6 ind to 10±13 ind); Ichtyophtirius in gill increase (from intensity 1±0 ind to 1±1 ind); and Dactylogyrus in gill increase (from intensity 2±0 ind to 2±2 ind). Based on this results, it shows that dried Terminalia catappa is more effective than wet Terminalia catappa to reduce parasitic disease in catfish nurshery. During testing, temperature range is quiet low which is in range about 21,7 – 23,3°C and pH range is about 6,6 – 7,63.

Abstract ID: 205D (Poster & Elevator Pitch)

NEW HOST RECORDS AND A CHECKLIST OF FISHES INFESTED WITH *Transversotrema patialense* (TREMATODA: DIGENEA: TRANSVERSOMATIDAE) IN THAILAND

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The digenetic trematode *Transversotrema patialense* is known as ectoparasite. It is found underneath the scales of several fish species. This study aims to provide supplementary information of its host. The parasitological survey was conducted at Omkoi district, Chiang Mai province, Thailand during August to November 2015. *T.patialense* was found from *Pethia stoliczkana* with the prevalence and mean abundance were 44.44 and 2.89, respectively. This finding provides the new host record for *T. patialense*. The morphology and host list of this parasite in Thailandare discussed herein.

Abstract ID: 032D (Poster)

MARINE LEECH INFESTATION IN CULTURED HYBRID GROUPERS

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Grouper is an important fish species due to high price both in domestic and international market. Several hybrid groupers have been released and can be accepted by farmers. A major production constraint in grouper culture is mortality due to disease. One of disease of grouper in sea cage is leech. The objectives of this study are to determine the prevalence and intensity of leech on hybrid grouper cultured in sea cage at Buleleng waters, to know its life cycle at laboratory condition and to identify based on morphological and molecular characteristics. Sampling was conducted from 7 cagesand composed 14 populations of Cantik and Cantang hybrid groupers. The presence of leech is done by unaided observation from 36 fishes in each population. Life cycle was observed by rearing adult leech in laboratory, counting the fecundity and observation the development of eggs until adult. The identification was conducted based on the morphological and nucleotidessequence of cytochrome oxidase sub unit I (COI) for molecular method. The result showed that hybrid grouper at sea cage is infected by leeches at prevalence and intensity up to 100% and 21.2 leeches/fish. The prevalence and intensity were varied depended on the cages, populations, species and size of fishes. During 3 days rearing, the adult leech could deposit 10.9 eggs in average with 600 µm – 800 µm in diameter. Twelve days were needed for the new egg inside the cocoon to develop into juvenile under 24-25 °C at 34 ppt of salinity. It took another 9 days for the juvenile leeches to grow to mature adults. Identification based on morphological characteristic showed that this fish parasite was belonged to *Zeylanicobdella arugamensis*. This result alsosupported by analysis on COI nucleotides sequence that showed 100% homology with *Z. arugamensis* (accession number KY 441721.1), whereas with*Aestabdella abditovesicu*lata (accession number DQ414300.1) only showed 90% homology.

Abstract ID: 136D (Poster)

RECENT ADVANCES IN THE RESEARCH OF MICROSPORIDIOSIS IN CULTURED SHRIMP AND CRAB IN CHINA

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Abstract: Microsporidia comprise a large phylum of obligate intracellular eukaryotes that are fungal-related parasites, which have gradually infected cultured shrimp and crab in China in recent years. Microsporidiosis caused by four microsporidian parasites in cultured shrimp and crab in China have been carried out in our laboratory. *Ameson sp.* infected swimming crab (*Portunus trituberculatus*) and *Vesicspora sp*. infected ridgetail prawn (*Exopalaemon carinicauda*) have been first reported in our laboratory, which infected skeletal muscle cells and caused musculature albinism, while *Enterocytozoon hepatopenaei* infected white Pacific shrimp (*Litopenaeus vannamei*) and *Hepatospora eriocheir* infected Chinese mitten crab (*Eriocheir sinensis*) infected mainly hepatopancreas. (1) The microsporidan (*Ameson sp.*) found in *P. trituberculatus* did not possessed the sporophorous vesicle structure. The special microvillus projections which presented on the surface of spore exosporium shaped radial. Mature uninucleate spores were almost oval-shaped, approximately 1.8±0.11μm ×1.4±0.08 μm, with 9 coils of polar ? lament which aligned regularly in single rank. Sixexisting forms of mature spores were observed within host cells and the host extracelluar matrix. And in the laboratory artificial infection under muscle injection and oral infection with crab were successful. (2) The microsporidan(*Vesicspora sp.*) infected ridgetail prawn has sporophorous vesicle containing eight oval-shaped uninucleate spores and measured 5.4±0.55 (4.4-6.6μm). The mature and fresh spore was approximately 2.3±0.25μm×1.5±0.19 μm with 9-10 polar ? lament coils which aligned irregularly in two ranks. (3) The epidemiological investigation of *Hepatospora eriocheir* infected Chinese mitten crab (*Eriocheir sinensis*) was conducted. The relationship between the microsporidan infection and chronic necrosis of hepatopancreas of Eriocheir sinensis was studied. (4) The epidemiological investigation of *Enterocytozoon hepatopenaei* infected white Pacific shrimp was carried out. The correlation between the microsporidan infection and shrimp white feces/slow growth was investigated.

Abstract ID: 137D (Poster)

EFFECTS OF DISSOLVED OXYGEN CONCENTRATION AND PHOTOPERIOD ON THE DEVELOPMENT AND EXCYSTMENT OF TOMONTS OF *Crytocaryon irrains*, THE CAUSATIVE AGENT OF CRYPTOCARYONIASIS IN MARINE TELEOSTS

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Cryptocaryoniasis of marine teleosts is caused by *Cryptocaryon irritans*, an obligatorily parasitic ciliate. The tomont stage （cystic stage） of the parasite has very unique biological characteristics; the development of tomonts is suppressed by low oxygen concentration and the excystment of theronts (invasive stage) from tomonts has a circadian rhythm. However, the characteristics has not been fully understood. In the present study, we examined the suppression of the tomont development by low oxygen in detail using the whole mount staining technique of tomonts. Additionally, we examined the effect of photoperiods on the circadian rhythm in the excystment. Based on our present results together with previously reported results, we discuss the potential involvement of the disappearance of thermocline in water columns and tidal cycles in the frequent outbreaks of cryoptocaryoniasis in autumn and after typhoons in floating net cage farms in Japan.

Abstract ID: 183D (Poster)

PRELIMINARY STUDY ON CASES MASS MORTALITY OF AFRICAN CATFISH*Clarias sp*INFECTED BY *Macrogyrodactyluscongolensis*

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African catfish is important freshwater species in Indonesia. National Center of Freshwater Aquaculture (NCFA) at Sukabumi has responsibility to improve quality and produce broodstock. Since 2016, there are three cases of mass mortality of *Clarias sp* that reared on earthen ponds that used as brood stock production at NCFA. In each case, the mortality can reach 50-80% of population, within a period of 14-30 days. The preliminary study of case mortality on *Clarias* spwas conducted on February 2016-April 2017. The aim of this study is to determine gross pathology, histopathology, examine parasit, identification of parasite based on morphplogy and molecular approaches. Fishes were sampled and observed on the presence of parasite and clinical signs followed by preparation for histological analysis. The parasites were collected for morphological examination under microscope and fixed for molecular identification based on 18S rRNA sequences. Clinical signs examination showed that fishes were lethargy, float to surface water, hemorrhage, jaundice, crepitus on muscle. The gross pathology findings showed white to gray focus necrosis and some visible jaundice of the liver; the gills look pale; spleen swollen. Histopathology examinationshowed congestion, hydropic degeneration, fatty infiltration and bilirubin accumulation in liver; pulp depletion in spleen, necrosis in the renal tubules and glomerular dilatation, and desquamation epidermis. Microscopy observation showed that two species of Gyrodactylidae monogeneans were abundantly found from fishes. Alignment analysis using BLAST on the sequences of 18S rRNA showed that bigger monogenean had 99% homology with *Macrogyrodactylus congolensis*(Acession number HF5486680,1), and smaller monogenean had 995 homology with *Gyrodactylus rysavyi* (Acession number FR850680.1). The morphometric analysis reported different morphometric body length and body width *of M. congolensis* 2526,99 (2112,00–2878,43) µm and 362,31 (251,99–499,44) µm three times longer and wider than *G. rysavyi* 894,91 (812,66–981,81) µm and 93,65 (82,14–10,55) µm. The mass mortality of fish (size 300-400 g/each) was occurred when *M. congolensis*infected at intensity ranging from 69 to 85 parasites/fish. We predicted that infection of *M. congolensis*as a cousative agent of catfish mass mortality.

Abstract ID: 226D(Poster)

ESTABLISHMENT OF *Perkinsus beihaiensis* ISOLATE FROM THE MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) IN JAPAN

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The genus *Perkinsus* consists of seven parasitic species of bivalves and gastoropods, and three of them, *P. marinus* in the eastern oyster, *P. olseni* in the venus clams and Australian abalones and *P. qugwadi* in Japanese scallop, are recognized as significantly important pathogens for fishery production. In Japan, *P. olseni* and *P. honshuensis* have been reported from the Manila clam (*Ruditapes philippinarum*), however, presence of the other *Perkinsus* species in Japanese water remained totally unknown.Recently, we conducted field surveys to examine parasite fauna in Tokyo Bay, and histologically found *Perkinsus* cells in the gill and the connective tissues around the digestive gland of the Mediterranean mussel. Infection of *Perkinsus*sp.in the Mediterranean mussel was also confirmed by RFTM assay, the conventional and specific diagnostic method for the genus *Perkinsus*, and subsequent DNA sequence and *in situ* hybridization identified this *Perkinsus* in the mussels as *P. beihaiensis*.Cases of infection with *P. beihaiensis* were reported from oysters (*Crassostrea* spp.) and pearl oysters (*Pinctada* spp.) in countries other than Japan, and it was supposed that *P. beihaiensis* may pose economic problems for industrially important bivalve species in Japan. Thus, this study aimed to establish isolates of *P. beihaiensis* for its pathobiological studies.In order to establish *P. beihaiensis* isolate, gill tissues were excised from infected mussels and then inoculated into an *in vitro* proliferation medium, JL-ODRP2A. At three months later, proliferation of a single type of cell was observed. Although its morphology was apparently different from the proliferative stage, trophozoite, of other *Perkinsus* spp., cells in JL-ODRP2A developed to prezoosporangia after culture in RFTM, and the cells were identified as *P. beihaiensis* with similarity of DNA sequence from Brazil, confirming that *in vitro* isolates of *P. beihaiensis* were successfully established. This is the first report of the establishment of *in vitro* cultures of *P. beihaensis*. Currently, these isolates are used for experimental infection to assess the infectivity and pathogenicity to other bivalve species.

Abstract ID: 050D (Poster)

STUDY OF INFESTATION WITH EXTERNAL AND INTERNAL PARASITES IN KOI *Cyprinus carpio* L. IN ORNAMENTAL FISH CENTERS OF TEHRAN

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Koi carp is a beautiful and popular aquarium fish that lives in freshwater. Koi is a color variety of common carp, Cyprinus carpio L. This ornamental fish, originating from Japan, has been bred in Iran since 2002. In addition to its beauty, this fish is one of the most resistant species of cyprinidae. However due to the poor management of propagation and culture centers throughout the country, this fish is subjected to various contaminations, especially parasites infestation. This study focuses on studying internal and external parasites of this fish in Iran. In 2016, 250 healthy-looking ornamental koi fish were collected from 10 ornamental fish centers of Tehran, Iran, randomly. Then these fish were transferred to the ornamental Fish Clinic, Veterinary faculty, University of Tehran, in order to detect the probable external parasites. At first the samples were investigated macroscopically. Macroscopic examination revealed presence of Lernea (Lernea cyprinacea) (3%) and Argulus sp. (2%) in some cases. Then, wet smear was prepared from the skin, fin of the above mentioned fish and they were studied microscopically. Microscopic investigations showed infestation with some external parasites including Trichodina spp. (14%), Dactylogyrus spp. (16%), Gyrodactylus spp. (10%) and Epistylis sp. (5%). For treatment of rest of infected fish in propagation centers, short-term salt bath (20 g/lit for 30 min) was used and because of the low number of Argulus sp. and Lernea sp. they were removed by forceps. After one month, these fish were examined again and no sign of infection was observed.

Abstract ID: 068D(Poster)

SYSTEMIC MYCOSIS AND HISTOPHAGOUS CILIATE CO-INFECTION IN A CAPTIVE SCALLOPED HAMMERHEAD SHARK (*Sphyma lewini*) IN TAIWAN

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A severe mycotic and ciliated protozoa co-infection occurred in a captive scalloped hammerhead shark *Sphyrna lewini* in Taiwan. Animals died after a behavioral abnormality. Gross findings included cutaneous lesions were characterised by skin erosions or ulcers of the caudal fin, lateral line and head in the shark. The fungus was observed and cultured from cutaneous lesions of the head and lateral line system. The fungal isolate was identified as *Fusarium solani* based on the nucleotide sequences of internally transcribed spacer (ITS) region. Histological sections of the cutaneous lesions, heart and stomach revealed slender, branching, septate fungal hyphae. The ciliated protozoa were also observed from gill, heart, spleen, liver and stomach. *F. solani* produced significant necrosis in the heart and cutaneous lesions, while ciliated protozoa were also associated with tissue changes in the heart and gill. This is the first report of *F. solani* and ciliated protozoa co-infection in captive scalloped hammerhead shark.

Abstract ID: 198D(Poster)

REPORT OF MASS MORTALITY IN JUVENILE MEKONG GIANT CATFISH (*Pangasianodon gigas*) CULTURE IN CHIANG MAI PROVINCE, THAILAND

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Mekong giant catfish (*Pangasianodon gigas*) is an important gigantic freshwater fish. In order to enhance the stock in natural resources, the aquaculture becomes necessity. During the culture of juvenile Mekong giant catfish in 2015, the mass mortality was occurred. Therefore, the aim of this study is to report the pathogen found from juvenile Mekong giant catfish culture in Chiang Mai. Moribund fish samples were examined for external pathogen and histopathology. The fish samples exhibited apparent clinical signs as white patch on their bodies, tails and fin rot, opaque eyes, and cream and brown color tufts on skin and fins with 80% mortality rate. Wet smear examination discovered parasitic protozoans (*Trichodina* sp., *Epistylis* sp.), columnaris bacteria, and water mold. The histopathology sections demonstrated epithelial oedema of the secondary gill lamellae, hemorrhage and degeneration of dermal, and hematopoietic tissues. These effects indicated the juvenile Mekong giant catfish was susceptible to common pathogens. Based on this information, it will be useful for disease control and prevention in Mekong giant catfish cultured in Chiang Mai.

Abstract ID: 231D(Poster)

IN VITRO EFFICACY OF BRONOPOL, FORMALIN AND SODIUM CHLORIDEAGAINST *Acanthamoeba* sp. ISOLATED FROM OSCAR, *Astronotus ocellatus*

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Three common chemicals used in aquaculture, bronopol, formalin and sodium chloride (NaCl) were tested for their efficacies against *Acanthamoeba* sp. AAHRI 0001 *in vitro*. The purified amoebae suspensions containing of $5x10^4$ cells/mL were inoculated to each different concentration of bronopol, formalin and NaCl. The growth and survival of the amoebae were examined after exposed to each chemical for 30 min, 1, 2 and 24 h. The results indicated that *Acanthamoeba* sp. AAHRI 0001 was strongly resistant to bronopol, when exposed to 25-200 for 30 min, 25-100 ppm for 1 h, 25-50 ppm for 2 h and 25 ppm for 24 h. The strain was also resistant to formalin, when exposed to 50-1,000 ppm for 30 min, 50-250 ppm for 1 h, and 50 ppm for 2 and 24 h. In addition, the strain also grew when exposed to NaCl for 1 h at 1-5% NaCl, and for 2 and 24 h at 1-3%. The results of the *in vitro* screening tests performed here may be useful indicators of the effectiveness of chemicals when used for anti-amoebial growth. These chemicals may have a wide range of applications to control the infections when applied on the farms either against the pathogen directly or short immersion for the fish.

Session 5 – Shrimp Diseases

Abstract ID: 236E (Keynote for Shrimp Diseases)

A FUTURE VISION FOR DISEASE CONTROL IN SHRIMP AQUACULTURE

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Aquaculture is the fastest growing animal production sector. For penaeid shrimp, culture production already exceeds that from the capture fishery and it is a major source of year-round export income from many tropical countries. Viruses and bacteria account for the majority of disease losses for shrimp farmers. Viral pandemics in the mid 1990's and more recently a bacterial pandemic from 2009 to 2015 have led to the conclusion that future, sustainable shrimp aquaculture will depend on the development of more efficient and biosecure production facilities cultivating genetically improved, disease tolerant, specific pathogen free (SPF) stocks. A major requirement for development, maintenance and use of SPF stocks in aquaculture is dependent on pathogen surveillance and prevention of diseases. When protective measures fail and diseases occur in production ponds, there are only a few, approved and practical therapeutic methods available for use with bacterial pathogens and none, so far, for viral pathogens. To improve existing methods of prevention and therapy and to develop new ones, research is being carried out on the nature of shrimp-pathogen interactions. Promising results have been obtained at the laboratory level for possible applications involving use of immunostimulants, RNA interference and endogenous viral elements. Some of these promising new directions will be reviewed.

Abstract ID: 214E (Oral)

TEN THINGS YOU NEED TO KNOW ABOUT EHP

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Enterocytozoon hepatopenaei (EHP) is a microsporidian pathogen of penaeid shrimp. In the modern shrimp industry, it bears the hallmarks of an emergent disease (appearing in a population for the first time or, rapidly increasing in incidence or geographic range) and has rapidly established in farmed populations, at least across Asia. In the context of emergence, it is useful to consider the conditions that have facilitated emergence, both from a host and pathogen perspective. EHP resides in an increasingly replete family of gut-infecting microsporidians infecting crustaceans, fish and mammals (incl. humans). It is closely related to *Enterocytozoon bieneusi*, a human pathogen unknown before the HIV/AIDS epidemic of the 1980s. In fact, *E. bieneusi* is the only 'terrestrial' member of the family at present, proposing that this group has its origins in water. Members of the family are known to exploit immune-compromised hosts, associated with environmental, genetic and co-infection drivers. Their ability to colonise the host gut and, to form disease, may then be related to perturbations in the wider gut microbiome. Some are known to transmit between invertebrate and vertebrate hosts, exposing potential for multi-trophic transfer in the wider family. Recent work focussed on the genome of EHP, *E. bieneusi* and other members of the family has revealed a much-reduced gene complement, with key elements of the eukaryotic metabolic machinery missing. In some cases, family members occupy the host nucleus, pushing further their ability for intricate association with the host cell. We are yet to fully understand how these organisms exploit the host cellular machinery for their own replication and transmission. From a taxonomic perspective, our improved understanding of potential diversity across hosts and biomes is driving a need for better molecular diagnostics and systematics. Many human microsporidian infections currently go undetected or undiagnosed (using molecular tools). Furthermore, infections in animals and humans may unhelpfully be brigaded under generic terms (such as 'microsporidiosis') which hamper strategies to understand their lifecycles and, to control them. In this talk, I will place EHP in to context with wider work on microsporidians and particularly, the apparent emergence of the Enterocytozooidae as pathogens of the weakened.

Abstract ID: 041E (Oral, Student)

GENOME, VIRULENCE FACTOR, AND SPECIFIC MOLECULAR DIAGNOSIS OF THE MICROSPORIDIAN *Enterocytozoon hepatopenaei* (EHP)

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Hepatopancreatic microsporidiosis (HPM), a newly emerging disease caused by *Enterocytozoon hepatopenaei* (EHP), has become a major disease in the Asian shrimp industry. EHP has been reported to cause slow growth in both black tiger shrimp (*Penaeus monodon*) and white-legged shrimp (*P. vannamei*) resulting in significant economic losses. Unfortunately, treatment against EHP infection is yet available due to the lack of the insight into the parasite infection mechanism. Therefore, a genomic study of EHP was carried out to identify potential pathogenic genes of EHP. Among many putative virulence factors identified through the genomic study, this investigation aims to characterize how a spore wall protein (SWP) called EhSWP contributes to the pathogenesis of EHP. In other microsporidia, SWP has been reported to function in both host cell adherence and structural maintenance. To investigate localization and function of EhSWP in attachment of spores to host, antibody against EhSWP has been produced for immunofluorescent analysis (IFA). IFA result revealed that EhSWP is localized on the surface of mature spores. However, subcellular localization needs to be further investigated by immunoelectron analysis (IEM). If the EhSWP is located in the exospore layer, it may provide a new marker for the development of an immunogenic, pond-side EHP diagnostic kit. In addition, this species-specific SWP has already been used as a marker to improve the specificity of EHP diagnosis. Taken together, the insight from the functional characterization of the aforementioned protein may lead to a pathway to control EHP infection.

Abstract ID: 042E (Oral)

A GENOMIC AND TRANSCRIPTOMIC ASPECT OF THE EMERGING SHRIMP PATHOGEN *Enterocytozoon hepatopenaei*

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The emerging pathogen *Enterocytozoon hepatopenaei* (EHP) causes hepatopancreatic microsporidiosis (HPM) that currently reaches an epidemic in Asian cultivated shrimp. In EHP-infected juvenile Pacific whiteleg shrimp *Penaeus vannamei,* the associated growth retardation becomes evident only after the second month rearing in grow-out ponds without other gross signs during the earlier months. As a single-celled eukaryotic intracellular parasite confined to tubule epithelial cells in shrimp hepatopancreas, EHP expectedly exploits its host directly within shrimp cytoplasm. Unfortunately, knowledge of such exploitation mechanisms remains largely unknown, but it is essential for parasite control and prevention. To gain insights on how EHP interacts with shrimp, we investigated both genome and transcriptomes of the parasite. A 3.10-Mb draft genome obtained by whole genome shotgun sequencing of genomic DNA from purified EHP spores showed gene content shrinkage and genomic compactness. Importantly, key characteristics of the EHP genome included (1) the absence of almost all of enzymes in core carbon metabolic pathways, i.e., glycolytic pathway, pentose-phosphate pathway and trehalose metabolism, which produce ATP via oxidative phosphorylation, (2) no evidence for ATP generation by other alternative pathways, but (3) presence of large paralogous protein families of putative transporters of nucleotides, multidrug (ATP-binding cassette transporters), amino acids, and choline, suggesting EHP's dependency on salvaging energy and nutrient molecules from shrimp cells. Produced by the RNA sequencing (RNA-Seq) technique, two transcriptomes of EHP-infected shrimp hepatopancreas at light and heavy infection levels were obtained from juvenile *P. vannamei* in a cohabitation challenge assay. Both light and heavy infection samples produced similar EHP transcriptomic profiles and revealed highly expressed genes in ribosomal protein complexes, histone complexes, translation and transcription processes, chaperones and a large fraction of hypothetical proteins. Interestingly, all four paralogs of nucleotide transporters, presumably capable of transporting ATP, were expressed but at different levels, suggesting that EHP likely utilizes all four paralogs to mediate ATP uptake during its life cycle in shrimp cells. It is hoped that the information on the EHP genome and transcriptomes will lead to development of novel strategies to control infections or reduce virulence.

Abstract ID: 015E (Oral)

CONTROLLING EHP: FROM MOLECULAR INSIGHT TO FARM APPLICATIONS

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Hepatopancreatic microsporidiosis (HPM) caused by the microsporidian, *Enterocytozoon hepatopenaei* (EHP) is currently a serious pathogen causing growth retardation in cultured shrimp across the Australasian region. The lack of treatment for EHP combined with its transmission via environmentally resistant spores facilitates its spread. To control the spread of EHP, research in our laboratory is divided into two areas. The first focuses on molecular and biochemical investigations to characterize its mechanisms of pathogenesis. From our whole genome study, one virulence factor is a spore wall protein (SWP) gene that we have used as the basis of a new and more specific EHP diagnostic method. Further investigations are underway into the role of SWP and other virulence gene that might be vulnerable targets for EHP control. The second area of research focuses on identification more immediate, practical and urgently needed methods to control EHP. To this end, we have identified conditions for purification of living spores of EHP and for induction of their polar tube extrusion, an event that initiates EHP entry into the host cell cytoplasm. Thus, abortive extrusion inactivates spores, preventing infection. At the same time the living, purified spores can be used for threshold infection studies, for infection trials with potential carriers, for quantitative inactivation tests using various disinfectants and other reagents, etc. Under this umbrella, we have also developed a cohabitation laboratory challenge model to facilitate further research on possible methods to block horizontal transmission in shrimp ponds and to treat already-infected shrimp.

Abstract ID: 124E (Oral)

MOLECULAR IDENTIFICATION OF AHPND POSITIVE *Vibrio parahaemolyticus* IN CULTURED SHRIMP OF BANGLADESH

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Acute Hepatopancreatic Necrosis Disease (AHPND), also called Early Mortality Syndrome (EMS), is a recently emergent shrimp bacterial disease caused by strains of *Vibrio parahaemolyticus* that contain a unique virulent plasmid, resulted in substantial economic losses since 2009. AHPND has caused severe mortalities up to100% in farmed populations of marine shrimp *Penaeus monodon*. The purpose of this work was to identify and characterize the pathogenic strain of *V. parahaemolyticus* causing EMS in cultured shrimp using classical and molecular techniques. To conduct this work, shrimp samples were collected from three different locations of south-west shrimp farming region of Bangladesh *viz.* Sadar Upazilla of Satkhira district, Mongla and Morrelganj Upazilla under Bagerhat district. The shrimp samples were processed for microbial load count and to isolate *V. parahaemolyticus* strains. Besides classical microbiology, molecular approaches (16S rRNA gene sequencing, pathogenic gene PCR using AP3 and AP4 primers) were performed to identify the pathogenic strains of *V. parahaemolyticus* causing AHPND in cultured shrimp.Shrimp suffering from AHPND showed significant atrophy of hepatopancreas (HP), pale to white hepatopancreas due to pigment loss in connective tissue capsules and guts with discontinuous or no contents. In this study, TBC, TVC on TCBS and on HiChrome showed little variation of AHPND affected shrimp. TBC was found highest (6.37×10^8 cfu/g) in shrimp of Satkhira Sadar Upazilla, whereas highest number of Total *Vibrio* on TCBS agar (2.40×10^7 cfu/g) was found in shrimp of Mongla Upazilla. In this study, among 46 isolates, representative eighteen isolates were checked for the species-specific detection of *V. parahaemolyticus* using *ldh* primers; and *tdh* primers were used for the detection of human pathogenicity of *V. parahaemolyticus*. Detection of *ldh* gene fragment in the isolates showed positive result for *ldh* but isolates were negative for human toxigenic gene *tdh*. The representative isolates were also subjected to 16S rRNA gene sequencing and were identified as *V. parahaemolyticus*. Multiple sequence alignment was performed to find out the polymorphic sites among the sequenced strains with considering 1386 bp nucleotides where 1.15 % dissimilarities were observed. Phylogenetic analysis also confirmed the taxonomic position of the isolates as *V. parahaemolyticus*. On the other hand, 18 *V. parahaemolyticus* isolates were further characterized to check the AHPND positivity using AP3 and AP4 primer. Twelve isolates showed positive result for AP3 and fourteen isolates for AP4 primer that indicate those isolates were AHPND positive which caused EMS in cultured shrimp. This study also reported that all the representative strains of AHPND positive *V. parahaemolyticus* were resistant to Gentamycin whereas all the strains showed sensitivity to Chloramphenicol, Nalidixic Acid, Nitrofurantion and Tetracycline.This report also agreed with the mortalities of shrimp that occurred within 30 days after stocking in shrimp farms of three different south-west regions of Bangladesh which caused by AHPND positive *V. parahaemolyticus*; and to the best of our knowledge, this is the first report of this shrimp pathogen in Bangladesh.

Abstract ID: 031E (Oral)

CHARACTERIZATION AND COMPARATIVE GENOMICS OF AHPND-CAUSING*Vibrio parahaemolyticus*AND*Vibriocampbellii*ISOLATES

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Acute hepatopancreatic necrosis disease (AHPND) is a severe shrimp disease that has emerged and been causing heavy losses to the global shrimp farming industry since 2010. Initially, *Vibrio parahaemolyticus* that becomes highly virulent by acquiring a unique AHPND-associated plasmid carrying *pirAB*^{vp} (VP_{AHPND}) is regarded as the only known pathogen responsible for the disease. Rare cases of AHPND caused by *Vibrio* species other than *V. parahaemolyticus* were reported. This study compared an AHPND-causing *V. campbellii*(VC_{AHPND}) and a VP_{AHPND} isolate from the same AHPND-affected pond in June of 2013 from diseased *L. vannamei* in Guangxi, China. Both strains are positive for the virulence genes *pirAB*^{vp}. Immersion challenge test with *Litopenaeus vannamei* indicated the two strains possessed similar pathogenicity. Complete genome sequencing showed that the *pirAB*^{vp}-bearing plasmids in the two strains were highly homologous, and they both shared high homologies with plasmid pVA1, the reported *pirAB*^{vp}-bearing plasmid, indicating that interspecies transfer of the pVA1-type plasmids might have occurred. Novel variations likely driven by IS*Val1* in the genetic contexts of the *pirAB*^{vp} genes were found in the two strains. Moreover, the VC_{AHPND} isolate additionally contains multiple antibiotic resistance genes. We hypothesize that future transfer of *pirAB*^{vp} into multidrug-resistant hosts will cause a more critical situation. This study provides timely information for better understanding of the causes of AHPND and molecular epidemiology of *pirAB*^{vp} and also appeals for precautions to encounter the dissemination of the hazard genes.

Abstract ID: 193E (Oral)

DISCOVERY OF AN AHPND DISEASE MARKER GENE IN INFECTED SHRIMP

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To identify disease markers from AHPND-infected shrimp, we used next-generation sequencing (NGS) to differentially analyze the transcriptome in the hepatopancreas of healthy control shrimp (*Penaeus vannamei*) versus shrimp that were severely infected with AHPND. Of the 91,587 contigs that were *de novo* assembled from the cDNA, 23,674 were identified as protein-coding genes. We found a total of 1,350 differentially expressed genes (>2 fold, $p<0.01$) between the severe infection group and the control group. Of these, 1,135 genes were upregulated and 215 genes were downregulated during pathogen infection relative to the control. Subsequently, we selected the top 20 most significantly upregulated genes and designed specific primers in order to validate their expression profiles in the AHPND-infected and uninfected shrimps with RT-qPCR. We identified one candidate AHPND disease marker that was consistently highly expressed in the transcriptome analysis as well as in the RT-qPCR analysis in the infected shrimps. We therefore conclude that this is a unique and persistent gene which can be used to distinguish between the non-diseased and AHPND-infected shrimps. This gene thus provides an important disease marker for the detection of AHPND in shrimp hepatopancreas.

Abstract ID: 045E (Oral)

DYNAMICS OF THE SHRIMP STOMACH BACTERIAL MICROBIOME IN AN AHPND-INFECTED POND

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Acute hepatopancreatic necrosis disease (AHPND), an emerging disease affecting shrimp in China and Southeast Asia, is caused by *Vibrio parahaemolyticus* (VP) with extra-chromosomal elements that produce a virulent toxin and damage shrimp hepatopancreas. However, whether all AHPND disease outbreaks are caused by VPonly, or with involvement of other agents, remains unclear. We speculated that a destabilized microbiota community in either the shrimp or their pond seawater may predispose to an outbreak of AHPND. Therefore, we used a standardized approach to monitor salt water shrimp grow-out ponds from 21 to 36 days after stocking with postlarvae. Each day, 10 shrimp were collected and individually tested for disease markers. Using a culture-independent metagenomics approach involving next generation sequencing technology (NGS), AHPND-associated microbiomes in shrimp stomach and cultured pond water were characterized. In total, our multivariate analysis examined individual microbiomes from 37 shrimp and 25 pond seawater samples from a Vietnamese shrimp pond that ultimately succumbed to an outbreak of AHPND. The stomach microbiome clustered into 2 distinctive groups by principal coordinate analysis, which predicted AHPND-associated microbiome changes during the AHPND outbreak. Instead of VP abundance being the critical factor for AHPND pathogenesis, there were third party microbiota that may be potential AHPND biomarkers. Altered bacterial metabolism of stomach microbiota may alter the host environment and increase VP virulence. The present metagenomics study shed some light on AHPND pathogenesis by documenting and analyzing changes in shrimp stomach microbiomes.

Abstract ID: 098E (Oral, Student)

APPLICATION OF METAGENOMIC ANALYSIS TO BIOLOGICAL ENVIRONMENT MONITORING OF SHRIMP CULTURE POND

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In penaeid shrimp farming, biological environment including plankton and bacteria considered to be critical on the productivity. It is, however, still unclear what kind of plankton and bacteria are suitable for shrimp culture. Since most plankton and bacteria are hard to cultivate in artificial media, it is impossible to determine the fauna in the environment by the cultivation methods. Therefore, we focused on metagenomic analysis which can detect the whole organisms in the sample by analyzing sequences of the whole DNAs extracted from the sample using a next generation sequencer. To apply the technology to the shrimp culture, basic data are needed. In this study, we studied on the basic conditions for sample preparation and analysis, as a trial application, of the planktonic and bacterial fauna in rearing water of shrimp farms.Rearing water was collected from a Kuruma shrimp *Marsupenaeus japonicus* culture pond in Kumamoto, Japan and the plankton and bacteria were trapped on polycarbonate membrane filters using a filtration system. After DNAs were extracted from the filters, partial sequences of 18S rRNA gene for eukaryotic cells and 16S rRNA gene for bacterial cells were amplified by PCR. Then, DNA sequences of the amplicons were obtained using the next generation sequencer Illumina MiSeq. Claident (http://www.claident.org) was used to perform clustering and homology search of the sequences followed by bioinformatic analyses. Comparing DNA extraction methods from planktonic and bacterial cells, we found CTAB method was most suitable. Minimum sample volume was estimated to be 20 mL of rearing water. Metagenome analysis was performed on whiteleg shrimp *Litopenaeus vannamei* culture ponds in Suratthani, Thailand. Rearing water were collected from 3 farms in 2015 and 1 farm (same as 2015) in 2016. The number of operational taxonomic unit (OTU) was 30-47 in planktonic fauna and 75-128 in bacterial fauna in the samples. Using beta-diversity and non-metric multidimensional scaling (NMDS) for composition similarity, samples were plotted in two-dimensional tables. Result of plotting for bacterial fauna, almost samples were placed far each other, but two samples which collected from the different farms in the same season were in relatively close position. For planktonic fauna, all samples were plotted far each other. This result suggested that each sample had different biological composition, even the samples collected from the same farm in 2015 and 2016. When extracted bacterial genera accounting for over 5% in the composition, we found *Synechococcus* was included in all samples. In plankton, no generawere commonlydetected over 5% occupation level. Thus, metagenomic analysis enable us to investigate whole biological environment of shrimp culture pond water. We further intend to investigate on the ponds with emergence of acute hepatopancreatic necrosis disease (AHPND) for development of reference organisms related to “healthy pond”.

Abstract ID: 027E (Oral, Student)

TRANSCRIPTOME ANALYSIS OF HEPATOPANCREAS AND STOMACH OF AHPND TOXIN-RESISTANT *Litopenaeus vannamei*

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AHPND, acute hepatopancreatic necrosis disease is an outbreak in shrimp farming, caused by a unique strain of *Vibrio parahaemolyticus* or another Vibrio species which carrying plasmids encoding Vp_PirAB-like toxins. The Vp_PirA- and B-like toxins are characterized as virulence factors and both is necessary to cause mortality in shrimp. In previous study, we discovered these toxins are heat stabile and formalin-resistant. Feeding shrimp with formalin-killed cells (FKCs) of AHPND-causing *V. parahaemolyticus* strain caused 100% mortality in a week post feeding. Nevertheless, feeding of FKCs AHPND strain to 200 *L. vannamei,* the four shrimps survived post 2 weeks-feeding. To identify genes associated with AHPND-toxin resistance in *L. vannamei*, hepatopancreas and stomach tissues were collected for cDNA library construction and performed RNA sequencing by Next-Generation Sequencing. In addition, the apparently healthy shrimp and shrimp immersed with AHPND-causing strain were used as control and infected groups, respectively. RNA sequences of all groups were generated *de novo* assembly and analyzed by Trinity software. From 6 libraries, the number of transcripts was 94,135 with a N50 of 1,777 bp. The differential gene expression analysis between control and infected groups versus resistant group, revealed total of 302 and 140 differentially expressed genes in hepatopancreas and stomach. The most highly expressed genes in resistant group were related to immune response, include antimicrobial peptides, proteinases/proteinase inhibitors, pattern recognition proteins and others. The differentially expressed genes were randomly selected to quantify the mRNA expression level by qPCR. The mRNA level of gene homologous to anti-lipopolysaccharide factor AV-R (ALF_AV-R) was significantly different in hepatopancreas of resistant group, not stomach. This results suggest that the expression of ALF_AV-R gene might associate with shrimp resist to AHPND. However, the further studies are necessary to elucidate this assumption and investigate the relative genes causing AHPND resistance.

Abstract ID: 094E (Oral)

COMPARATIVE TRANSCRIPTOMICS REVEAL SPECIFIC SURVIVAL MECHANISMS OF SHRIMP INFECTED WITH WHITE SPOT SYNDROME VIRUS (WSSV)

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White spot syndrome virus (WSSV) is until now the major shrimp virus that causes mass shrimp mortality during the infection period. In this study, disease outbreak caused by WSSV infection has been introduced in the laboratory condition. More than 80% of shrimp died during the first 5 day after WSSV injection. There was about 5-10% of shrimp survived with apparently healthy until 30 days after injection. The purpose of this study is to better understand a mechanism involved in survival from WSSSV infection of these shrimp. Detection of WSSV replication demonstrated high levels of WSSV infection in the WSSV moribund, less in the survivor, and none in the control group with buffer injection. Transcriptomic analysis using shrimp cuticular epithelium tissue was performed in comparisons between the survivors, moribund and control. The unique transcripts with high frequencies found only in the survivors have been characterized. A large group of transcripts with unknown functions were demonstrated. We have selected some genes for further validation and showed that they are involved in the WSSV survival mechanism. Information retrieved from the study will enable us to design an appropriated strategy to control WSSV infection in shrimp farm.

Abstract ID: 049E (Oral, Student)

UNDERSTANDING THE MOLECULAR BASIS OF SUSCEPTIBILITY TO WSSV IN SHRIMP

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White spot syndrome virus (WSSV) is the major pathogen of shrimp culture, causing global annual losses in the region of $1bn USD. Understanding host-pathogen interactions is crucial for the development of disease treatments and sequencing techniques are increasingly being employed to address this challenge. In this study, we aim to identify the molecular pathways associated with susceptibility to WSSV in the Pacific whiteleg shrimp (*Penaeus vannamei*). To do so, we are establishing the temporal transcriptional changes in response to WSSV infection, and the role of microRNAs in regulating host and pathogen gene expression. We will compare these data with an existing dataset on the temporal transcriptional and microRNA expression changes in a crustacean (European shore crab, *Carcinus maenas*) infected with WSSV that is highly resistant to WSD. In this way we expect to be able to identify individual genes and pathways associated with susceptibility and resistance to WSSV.To address this aim, shrimp were divided into two treatment groups and injected with either SPF- (specific pathogen free) or WSSV-shrimp homogenates. Four shrimp from each treatment were sampled at 3h, 6h, 9h, 12h, 24h and 36h post-injection, and the gills were dissected and frozen at -80^{o}C until analysis. Total RNA was then extracted from gills (n=48) and mRNA libraries (Illumina) and small RNA libraries (Nextflex) were prepared and sequenced using the Illumina HiSeq 2500.These datasets are currently being analysed to identify individual genes, miRNAs and molecular pathways that are modified as a result of WSSV infection. WSSV responsive pathways in the shrimp and in the resistant shore crab will be compared using an existing dataset generated using the same methodologies. Through this work we hope to identify mechanisms of susceptibility and resistance, and identify potential gene targets for disease prophylactics in the future.

Abstract ID: 078E (Oral)

EPIDEMIOLOGICAL STUDY: RISK FACTORS AND PREVALENCE OF WSSV AND IMNV IN SHRIMP PONDS IN LAMPUNG SELATAN (LAMPUNG PROVINCE) AND BANYUWANGI (EAST JAVA PROVINCE), INDONESIA

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Shrimp aquaculture become an interesting business in Indonesia. Lampung and East Java considered as largest producers shrimp in Indonesia. Among shrimp species cultivated by farmers, vannamei was dominated, like in Banyuwangi, 99.85% shrimp production was vannamei. Problems encountered during crops was diseases, mainly due to WSSV and IMNV. An epidemiologal study: risk factors followed by prevalence study was conducted within two years, covered Lampung Selatan (Lampung province) and Banyuwangi (East Java province) as represent for shrimp producers area in Indonesia. Farm were selected as epidemiological unit, shrimp pond was selected as sample unit. Real time quantitative PCR selected to perform diagnoses of the samples. Totally 1,836 samples were collected and analysed, for risk factors and followed by prevalence. Sample were represent intensive and extensive shrimp pond in the two studed area, taken proportionally. Some risk factors were identified as responsible for outbreak for WSSV and IMNV were pond don't fertilized the pond (P=0.0217), pond don't desinfect water (0.0101), don't screened the fry (P=0.0257), seepage (P=0.0595), and water exchange (P=0.0001). Result of risk factor were dessiminate to farmers for further make improvement of their pond. Based on prevalence data, WSSV tend to decline to zero in intensive shrimp ponds in Banyuwangi on November 2015 and December 2016. In extensive shrimp ponds, however WSSV still unmanageable causing mass mortality, may due to biosecurity were not implemented properly. Contrary to WSSV, IMNV prevalence were show tend to increase in both of Lampung area and in Banyuwangi. In intensive shrimp ponds, eventhough biosecurity were implemented, intensive shrimp ponds still get infected. Case of IMNV in extensive ponds in Banyuwangi were low, compare to intensive shrimp ponds, due to shrimp pond collapsed during the first month of cultivation. In Lampung, IMNV tend to increase both intensive and extensive shrimp pond. In May 2016, IMNV prevalence reach as high as 53.3% in extensive shrimp pond in Lampung. High prevalence in extensive shrimp pond may due to farmers use seed from infected local broodstock (back yard hatchery). However occurence of IMNV in intensive shrimp ponds may due to environment was infected by the virus. Sterilizing water may not effectively destroyed IMNV in the environment. Further study still require, especially to eliminate the virus, especially IMNV. Strictly screen PL for those two virus recommended to suppress prevalence.

Abstract ID: 014E (Oral)

MULTIMODAL STRATEGIES TO CONTROL DISEASES IN HATCHERY AND GROW-OUT PHASES OF CULTURED SHRIMP, *Penaeus monodon*

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Investigations were carried out to find out two multimodal strategies, respectively for hatchery phase and grow-out phase of cultured *Penaeus monodon* inSri Lankatocontrol WSV disease, MBV disease and different types of vibriosis caused by pathogenic *Vibrio*. Four hatcheries each were arranged as experimental and control groups to collect data over two production cycles. Prevention of pathogenic contaminations through brood stocks, through equipments, culture facilities and rearing water of maturation and larval rearing tanks, through feed offered to brood stocks and larval stages were in the multimodal strategy tested. Proper disinfection of equipments and culture facilities, selection of apparently healthy brood shrimp only (collected from natural populations), transporting them individually in separate polythene bags containing UV treated sea water, activated carbon, EDTA, and Tris HCl buffer, individual primary quarantining and screening individually for white spot virus (WSV) and monodon baculo type virus (MBV), taking WSV and MBV free shrimp only for secondary quarantining, treating water in maturation tanks with a locally produced probiotic/ bioaugmenter containing a locally isolated strain of *Bacillus subtilis*, feeding brood shrimp with squid flesh and beef liver, using brood shrimp free of WSV, MBV and pathogenic *Vibrio* to produce larvae, rearing the larvae in UV treated water with the same probiotic/ bioaugmenter and feeding the larvae with indoor reared algae and disinfected *Artemia* nauplii proved to be successful in producing post larvae free of WSV, MBV and pathogenic *Vibrio* with significantly higher survival (81.3 %; $P < 0.05$) compared to the survival (65.2%) recorded for control hatcheries managed under prevailing practices in Sri Lanka.Sixteen grow-out ponds each were arranged as experimental and control groups to collect data over two production cycles. Prevention of pathogenic contaminations through viral carriers and pond sediments, through post larvae, through externally contaminated water were in the multimodal strategy tested. Two months of shut down period, proper disinfection of pond bottom and culture water, arrangement of crab fence and bird lines, stocking with post larvae free of WSV, MBV and pathogenic *Vibrio*, zero water exchange, feeding young shrimp with probiotic incorporated feed while treating the culture water with the same *B. subtilis* proved to be successful in producing healthy, marketable size shrimp (free of WSV, MBV and pathogenic *Vibrio*), free from human pathogenic bacteria and antibiotic residues; mean body weight achieved by shrimp at the end of production cycle (27.9 g) was significantly higher with significantly higher mean survival (94%) and mean harvest (5285 Kg ha^{-1}; $P < 0.05$) compared to those values recorded for control ponds. Application of the relevant multimodal strategy for the relevant phase could increase the cultured shrimp production in Sri Lanka in a sustainable manner with indigenous *Penaeus monodon* as the species.

Abstract ID: 003E (Oral, Student)

SEQUENCING OF A NOVEL (CHEQUA VIRUS) FROM THE PICORNAVIRALES IN REDCLAW CRAYFISH *(Cherax quadricarinatus*)

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A novel virus in cultured red claw crayfish was first observed in Queensland, Australia in 2014. The virus caused high cumulative mortality under stress and significant economic losses with no obvious external clinical signs. In microscopic sections, the muscles were brittle and friable. This virus was firstly suspected to be a member of Nodaviridae because nuclear pyknosis in muscle and nerve cells were found, similar with other nodaviruses. Several attempts of sequence dependent PCR with both consensus and degenerate primers related to Nodaviridae and other RNA viruses such as Togaviridae and Dicistroviridae were unsuccessful. However, icosahedral/sphere shaped capsomeres approximately 14 nm in diameter were identified under transmission electron microscope (TEM) with a buoyant density of 1.1 g/cm^3 in sucrose.RNA next-generation sequencing (NGS) of a control and infected crayfish produced approximately 500 million readable sequences with 35.58 mean quality score. The transcriptomereads were firstly mapped using Trinity and the 500,000 contigs obtained were then searched for viruses using NCBI Protein database (Blastp). Subsequent analyses showed that a novel virus (Chequa virus) with a length of 9,951bp was a member of the Picornavirales and found only in the infected crayfish.

Abstract ID: 047E (Oral, Student)

DETECTION OF THE LETHAL BACTERIAL PATHOGEN *Spiroplasma eriocheiris* IN THE FRESHWATER PRAWN *Macrobrachium rosenbergii* IN THAILAND

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Spiroplasma eriocheiris, a motile intracellular bacterium, was first isolated from haemolymph of the Chinese mitten crab *Eiocheir sinensis* with tremor disease in 2011. It was also reported to infect some of freshwater and marine crustaceans including the crayfish *Procambarus clarkii*, the giant freshwater prawn *Macrobrachium rosenbergii*, the oriental river prawn *Macrobrachium nipponensis* and the whiteleg shrimp *Penaeus vannamei*. The infections of *S. eriocheiris* in all species of crustaceans have been described as systemic. In this study, we describe unique lesions in the hepatopancreas and other tissues of *M. rosenbergii* infected with *S. eriocheiris*. The most prominent lesions in hematoxylin-eosin stained tissue sections of moribund *M. rosenbergii* were basophilic to mixed esinophilic/basophilic intracellular inclusions in inter-tubular cells of the hepatopancreatic tissue. Some smaller, less prominent and scattered eosinophilic/basophilic nodules were also seen in the heart muscle, skeletal muscle, connective tissues, and hematopoietic organ. Using transmission electron microscopy technique, we found the inter-tubular lesions in the hepatopancreatic tissue containing cells with cytoplasmic colonies of un-walled bacterial cells with diameters of ~150 nm. PCR using group-specific primers for the SSU rRNA was performed in the infected tissues followed by cloning and sequencing. The result revealed 99.9% sequence identity to the matching sequence of *S. eriocheiris*. *In situ* hybridization assays using a probe specific to *S. eriocheiris* showed positive signals in the hepatopancreatic inter-tubular spaces corresponding to the regions of the unique lesions shown in hematoxylin-eosin stained tissue sections. The prominent eosinophilic to basophilic cytoplasmic inclusions in cells occupying the hepatopancreatic inter-tubule spaces can serve as a strong histological indicator for infections with *S. eriocheiris* in *M. rosenbergii*. This information would be useful in facilitating the early identification of *S. eriocheris* infections in *M. rosenbergii.*

Abstract ID: 044E(Poster &Elevator Pitch)

TRANSCRIPTOME ANALYSIS REVEALS GENES AND POTENTIAL AHPND PATHOGENESIS PATHWAY IN *Litopenaeus vannamei* STOMACH

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Acute hepatopancreatic necrosis disease (AHPND), originally termed early mortality syndrome (EMS), is an emerging bacterial disease of shrimp blamed for enormous economic losses in Asian shrimp production since it was identified in 2009. The cause of AHPND is *Vibrio parahaemolyticus* (VP) with a unique AHPND-associated plasmid pVA1 encoding a virulent toxin (Pir^{VP}). Based on our pioneering studies, we formulated the following hypothesis: AHPND causing VP strains initially colonize and replicate in shrimp stomach, compromising integrity of the stomach barrier, leading to bacteria or toxins migrating across the epithelial barrier to reach the hepatopancreas. Shrimp were immersed in virulent or nonvirulent VP strains (5HP and S02, respectively) or TSB medium (control). Samples of shrimp stomach collected 24 hours post immersion (hpi), total RNA was recovered and subjected to next-generation sequencing. A transcriptome (37, 678 annotated transcripts) was generated (NextSeq 500 sequencing system) and a whole-transcriptome analysis pipeline was done, using the ContigViews web server (http://www.contigviews.bioagri.ntu.edu.tw). Based on comparison to the non-virulent S02 group, a gene-to-gene network of major genes involved in or correlated with AHPND caused by the virulent 5HP strain, was built. Twenty-three major correlated-genes were used to generate a relationship map. Based on KEGG mapping of these major correlated-genes, a potential mechanism of AHPND pathogenesis, consistent with our hypothesis, was identified. In conclusion, our comprehensive transcriptome analysis detected host genes or potential pathways apparently involved in AHPND pathogenesis.

Abstract ID: 006E (Poster &Elevator Pitch)

IDENTIFICATION AND PATHOGENICITY OF *Vibrio Parahaemolyticus* ISOLATES AND IMMUNE RESPONSES OF *Penaeus* (*Litopenaeus*) *Vannamei* (Boone)

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Five different *Vibrio parahaemolyticus* strains (SH8, SH108, SH58, AH5 and GD10) isolated from the hepatopancreas of moribund shrimp in farms of mainland China were identified and capable of inducing massive mortality of *Penaeus* (*Litopenaeus*) *vannamei*. The immersion challenge results with five isolates indicated variance of virulence, while only GD10 caused massive sloughing of tubule epithelial cells which was recognized as the most significant symptom of AHPND. Differences in immune responses were detected of *P. vannamei* during 48 hrs post infection (p.i.) by injection or immersion challenge with *V. parahaemolyticus* (SH8, SH108 and GD10) isolates. When injected SH8 and SH108 isolates, the expression of lysozyme (*LSZ*) showing statistically significant up-regulation at 16 and 48 h p.i. and that of Toll-like receptors (*TLR*) showed statistically significant up-regulation at 48 h p.i. When immersion challenge with the GD10 isolate, *TLR* were up-regulated after 8 h p.i. challenge with 10^4 cfu·ml^{-1}; however, *LSZ* was down-regulated when challenged with 10^3 cfu·ml^{-1}. The results suggested that *LSZ* and *TLR* serve as crucial molecular markers of innate immunity in shrimp against *V. parahaemolyticus* infection. *LSZ* is a vital marker for acute bacterial infection, while *TLR* as a crucial marker for chronic infection.

Abstract ID: 131E (Poster &Elevator Pitch)

Photobacterium spp. IS A PATHOGENIC AGENT OF *Penaeus vannamei*

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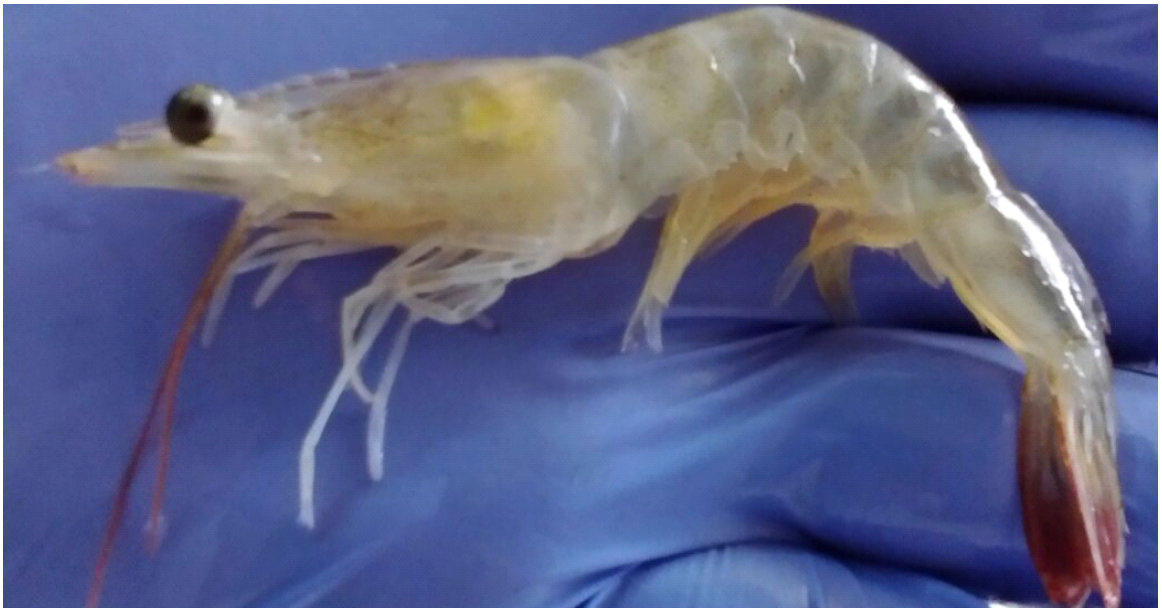
A new emerging pathogen called *Photobacterium* spp. isolated from a moribund *Penaeus vannamei* in a heavy mortality ponds. The dead and moribund shrimp showed typical white muscle appearance i.e. at 2-3rd segments and at 5-6th segment, as same as symptoms of Infectious Myonecrosis Virus (IMNV) of shrimp. The bacteria was isolated from the muscle of shrimp and purified and sequenced at First Base, Singapore using BLAST method. The BLAST confirmed that its *Photobacterium* spp., partial sequence of which matched 100% with *Photobacterium leiognathi* NB09002and *Photobacterium mandapamensis* (ATCC 33981). A series of bioassay trials using immersion method of challenge were conducted to determine the pathogenicity level in *P. vannamei*. The amount of *Photobacterium* applied in the pathogenicity tests were four doses i.e. log 8 CFU/mL; log 7 CFU/mL; log 6 CFU/mL and log 5 CFU/mL in 4-5 g of shrimp in 3 replicates. There was significant rate of cumulative mortality achieved in the group with higher dose of *Photobacterim*, groups log 8 achieved 71.9 %; group log 7 achieved 58.5 %; group log 6 achieved 43.9 % and group log 5 achieved 31.1 % after 5 days of challenge. The shrimp showed typical white muscle appearance (Figure attached). There was no mortality recorded in the negative control group. In the second trial, three kinds of bacteria *Photobacterium* sp., *Vibrio harveyi* and *V. parahemolyticus* of it dose i.e. log 8 were challenged by immersion method to 4-5 g of shrimp in three replicates each. The cumulative mortality on day 5 of challenge of each group were recorded as followed, *Photobacterium sp.* 70%, *Vibrio harveyi* 6.7% and *V. parahemolyticus* 30%. There was no mortality in negative control group. The trials confirmed that Photobacterium species was the most lethal among the bacterial species tried. It's a new threat to the aquaculture industry.



Abstract ID: 186E (Poster &Elevator Pitch)

IMPROVEMNET OF PCR PROGRAM FOR THE DETECTION OF INFECTIOUS MYONECROSIS VIRUS (IMNV)

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Infectious myonecrosis (IMN) caused high mortality in the white leg shrimp (*Litopenaeus vannamei*) and was specified as an OIE-listed disease. In the current OIE manual, a commercial kit is recommended to conduct one-step RT-PCR for the detection of IMNV. However, the reagent kit is no longer sold in Japan. We therefore tested different programs for RT-PCR using SuperScript® III One-Step RT-PCR System, which is different from the one that is recommended in the OIE manual for the diagnosis of the disease. When SuperScript III was used for one-step RT-PCR, the detection efficiency was clearly higher using the protocol of 3-step PCR after reverse-transcription at 55 °C than the protocol of the OIE manual, which was two-step (shuttle) PCR after reverse-transcription at 60 °C. For the reverse transcription, degradation of the enzyme at the higher temperature may have caused a reduction in the detection sensitivity in the RT-PCR for the OIE program, because the temperature of 60°C is the upper limit of the range recommended for SuperScript III for reverse transcription (45-60°C). For subsequent PCR programs, the total time for extension (45sec including annealing) in each cycle of the 2-step PCR might not enough compared with the total time of 75sec for annealing and extension in the 3-step PCR. In any case, the 2-step (shuttle) PCR program does not seem to be suited for RT-PCR using SuperScript III. The manufacturer recommends a general 3-step PCR program for this enzyme, whereas a 2-step PCR program is recommended by the manufacturer of the rTth, which is the suggested enzyme in the OIE manual. The result suggests that the 3-step PCR program with SuperScript® III is useful for the diagnosis of IMN.

Abstract ID: 209E (Poster &Elevator Pitch)

HERBS FOR CONTROLLING VIBRIOSIS IN SHRIMP CULTURED IN THE SEA

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Vaname shrimp cultivated in the sea has a potential stress and disease caused partly by the vibriosis bacteria. Therefore, the shrimp immune systems need to be improved by giving turmeric and bitter. Enhancement of shrimp immunity should consider the administration period, since the shrimp does not have an adaptive memory cell in the immune system. The aims of this study are to determine the administration period of the mixture of turmeric-bitter extract (2:1) dose 6 mL kg^{-1} feed to prevent disease caused by *Vibrio harveyi* infection and to improve the growth of shrimp vaname. The study was conducted with four treatments, (1) the control, without turmeric-bitter extract (2) a mixture of turmeric-bitter extract (2:1) dose of 6 mL kg^{-1} feed the first week, (3) a mixture of turmeric-bitter for two weeks with a one-week rest, (4) a mixture of turmeric-bitter every day for 4 weeks. The study was conducted in the field scale and laboratory studies (only for challenging test). The results from field scale after treatment by administration of a turmeric-bitter mixture in two weeks rest one week, and every day for four weeks, showed the best of survival, specific growth rate and the growth of absolute length of vaname shrimp ($P<0.05$). The laboratory study showed that the survival, immune response in the form of *total hemocyte count* (THC), phagocytic activity, and phenoloxydase, respiratory burst better than the positive control ($P<0.05$). From the both fields and laboratory results, the turmeric-bitter extract was able to control vibriosis caused by administration two weeks rest one week.

Abstract ID: 052E (Poster)

THE EFFECT OF VIRGIN COCONUT OIL ON GASTROINTESTINAL *Vibrio spp.* GROWTH IN PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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The virgin coconut oil (VCO), extracted from fresh coconut milk by cold extraction method was investigated its effect of growth inhibitory on *Vibrio* spp. in gastrointestinal tract of Pacific white shrimp (*Litopenaeus vannamei*). Three treatment groups were used, including a control group (feed without VCO), group fed with 3% VCO-mixed feed and group fed with 6% VCO-mixed feed.The samples were collected at 48 and 96 hours after feeding. The results indicated that the population of *Vibrio* spp. in CFU/ml were decreased in shrimp fed with VCO-mixed feed. Moreover, the population of *V. alginolyticus* showed significantly lower ($p < 0.05$) growth in shrimp fed with VCO-mixed feed than the control group. However, there were no significant different between shrimp fed with 3% and 6% VCO-mixed feed. The study suggested that VCO could be a potential to inhibit the growth of *Vibrio* spp. in the healthy shrimp and this is the alternative of shrimp culture to reduce the using of chemicals and antibiotics in production cycle.

Abstract ID: 060E (Poster)

PROTECTION OF MANGROVE LEAF EXTRACT (*Acanthus ilicifolius*) TOWARD TIGER PRAWN (*Penaeus monodon*) FRY INFECTED BY V*ibrio harveyi* AT VARIOUS SALINITIES

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This research was aimed to investigate the protection performance of *Acanthus ilicifolius* leaf extract at various salinities to prevent bacterial infection (*Vibrio harveyi*) of tiger prawn. Tiger prawn fry (*Penaeus monodon*) of PL-7 was cultured at 15, 23 and 30 ppt salinities then submerged in various mangrove leaf extract for 30 minutes. The mangrove leaf extract were crude, ethyl acetate fraction at concentration of 200, 450, and 750 mg/L, and n-buthanol fraction at concentration of 100, 200, 300 mg/L. On the third day experimental prawns were challenged by *Vibrio harveyi* at concentration of 10^6cfu/mL. Variables obsereved were survival rate (SR), total bacterial count in the prawn (TPC) and in experimental media, total haemocyte (HC), and Relative precentage of survival (RPS) at day 7 and 14 respectively. Experimental prawn that submerged in n-buthanol fraction 300 mg/L and salinity 15 ppt demonstrated the best results in; SR, TPC in media, RPS, and THC at both day 7 and 14. While ethyl acetate fraction at 700 mg/L and 23 ppt demonstrated the best results in TPC from the prawn in day 3 but not in day 7. It can be concluded that 300 mg/L n-buthanol fraction of mangrove leaf demonstrated the best protection to prawn when it was cultured at 15 ppt salinity.

Abstract ID: 065E (Poster)

CROSS-SECTIONAL STUDY OF *Enterocytozoon hepatopenaei* (EHP) INFECTION IN CULTURED WHITELEG SHRIMP (*Litopenaeus vannamei*)

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Enterocytozoon hepatopenaei (EHP) is a microsporidian parasite that affects shrimp hepatopancreas and was reported in *Penaeus monodon*, *Litopenaeus vannamei* and *Marsupenaeus japonicas*. It has been reported that the affected shrimp often showed slow growth without mortality. This has become an emerging problem for shrimp farmers recently. A cross-sectional study of EHP's prevalence was conducted in two whiteleg shrimp (*L.vannamei*) ponds in Perak, Malaysia. Sampling of shrimps was done on day of culture (DOC) 29 involving gross observation, water quality and Polymerase Chain Reaction (PCR) analysis. Examination of 60 shrimps from two farms showed 46.7% having slow growth with average weight of 2.8g each at DOC 29. Analysis of water quality showed the parameters were at optimum level except for ammonia and ferum. Both parameters showed higher readings; ranging from 1.37 to 1.42 mg/L and 1.14 to 2.09 mg/L; respectively. Detection of EHP using IQ2000 EHP kit showed 3.3% of the shrimps were positive. This study showed presence of EHP in both ponds with low prevalence although percentage of shrimps showing slow growth is high. The root cause of slow growth has not been determined in this study. Therefore, further studies are needed.

Abstract ID: 084E (Poster)

ENVIRONMENTAL RESERVOIRS OF THE EMERGENT PARASITE *ENTEROCYTOZOONHEPATOPENAEI* (EHP)

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Hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) is the pathogen of major concern in Asia at this time. High prevalence of EHP was not realized until an epidemiological study of EMS outbreaks was carried out in Thailand during year 2013-2014. The results revealed an unexpected overall pond prevalence of 119/196 = 61% that prompted an immediate warning and follow-up studies to characterize EHP which will help to design an effective strategy to control infection in shrimp. Our study aims to find potential reservoirs for EHP and to remove them from the culture system. The availability of the spore wall protein PCR (SWP-PCR) method established in our laboratory makes it possible to screen not only cultured shrimp but also suspected carriers and environmental samples for EHP with good assurance that false positive results will not be obtained from closely related microsporidians. The preliminary studies found the positive PCR for EHP infection in the natural feed including blood worm, sand worm, oyster and artemia cyst. The on-going study is to examine that whether they are mechanical or infectious carriers by in situ hybridization. Recently, the success in establishment of EHP spore purification and preparation of active EHP spore have been reported. Confirmation of EHP carriers will be demonstrated by feeding of the active spore to the suspected carriers such as artemia and polychaete. Knowledge about the environmental reservoirs will help with in the implementation of biosecurity strategies in the industry and, in the academic area, a better understanding of host-parasite interactions.

Abstract ID: 113E (Poster)

IMPACT OF CS/ALG MICROSPHERES ON AQUATIC PATHOGEN

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Aquaculture emerging as the fastest growing food producing industry in the globe and is established itself as high protein resource to fulfil the demand for food consumption. However, outbreaks of various bacterial diseases resulting in annual economic losses to the aquaculture industry estimated at billions of dollar worldwide. Among diverse types of aquatic microorganisms, bacteria cause serious economic effect in shrimp culture. Vibriosis is one of the major bacterial disease which causes mass mortality in cultured shrimp. The practice of antibiotics and chemotherapeutics to combat Vibriosis caused by *Vibrios* Sp has the risk of generating resistant pathogens, bioaccumulation and environmental pollution. To limit the use of chemicals and antibiotics, good farm management is highly recommended. In terms of treatment, chemicals and antibiotics should be evaluated to establish recommended doses and withdrawal periods, otherwise alternative treatments should be developed. Hence, the current study was focused to explore the antibacterial and antibiofilm activity of chitosan-alginate (CS/ALG) microspheres against aquatic pathogen *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio vulnificus*. Chitin was extracted from the shell waste of blue swimmer crab *Portunus pelagicus*, followed by synthesis of chitosan from chitin deacetylation. Here, chitosan-alginate (CS/ALG) microspheres with narrow size distribution were fabricated by ionically cross linking method using Ca^{2+} ions as agents for polymer solidification. The physicochemical properties of CS/ALG microspheres, such as surface morphology and size were studied by Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) and the functional group interactions were studied by Fourier transform infrared (FTIR) spectroscopy. TEM and SEM analysis showed that, CS/ALG microspheres were spherical in shape with smooth surfaces, size was 50-100 µm. The formation of CS/ALG microspheres was observed through alterations in the vibration modes of chitosan (CS), alginate (ALG) and CS/ALG microspheres (functional groups) by FTIR spectroscopy. The synthesized CS/ALG microspheres were tested for its antibacterial and antibiofilm potential against *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*. Agar disc diffusion method was primarily employed for the analysis of antibacterial activity of CS/ALG microspheres which exhibited appreciable antibacterial activity at the concentration of 5-20 µg, by significantly inhibit the growth of all tested bacteria. Furthermore, light microscopy and confocal laser scanning microscopy (CLSM) analysis were employed for determining the antibiofilm potential of CS/ALG microspheres on *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*. Inverted light microscopy analysis exposed that CS/ALG microspheres inhibited the bacterial biofilm formation at 40 µg. CLSM analysis clearly depict that, CS/ALG microspheres have an potential to disturb and loosening the architecture of bacterial biofilm and thereby reduce the thickness of biofilm at 40 µg/ml concentration. Overall, our findings underlined that, synthesized CS/ALG microspheres has high antibacterial and antibiofilm activity against *Vibrios* Sp and this synthesis route can be further exploited for overcoming the infection of aquatic pathogens.

Abstract ID: 125E (Poster)

STUDY OF WHITE FECES DISEASE OUTBREAK ON VANNAMEI SHRIMP POND AND PREVENTION TECHNIQUES

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White feces disease on vannamei shrimp farming has now lowered the productivity of shrimps vaname. Exploration study on some farms infected to determine the cause and techniques for the control and treatment has been done and can prevent infections of white feces disease, Study exploration method is done by observing water quality include the content of organic matter (TOM); total bacteria and vibrio; the stability of the plankton, as well as the identification of species of bacteria in diseased shrimp. The results showed that shrimp disease of white feces disease there are several in the intestinum are species of bacteria *Vibrio alginolyticus, Vibrio parahaemolyticus* and *Vibrio vulnificus.* Pond water contains vibrio bacteria dominance more than 12% of the total bacteria. Tottal and organic matter (TOM) more than 250 ppm. WFD olso outbreak affer water color removed become dark blue or dark green. The preventive measure to avoid WFD infection be done to improve water quality by controlling the organic matter, plankton and maintain the stability of the application of probiotics to balance settings C: N: P ratio. Mechanical fasting and application form of natural antibiotic feed additive from garlic extract (alicyn) and vitamins to improve the effectiveness of the incoming material on shrimp digestion. Fasting technique, alicyn and vitamins application, controlling vibrio dominance less than 10% with the application probiotics well as the disposal of sludge pond bottom and water changes to reduce content of organic matter could be for prevent disease. Ponds production be maintained on the survival rate of > 70% with average daily growth rate (ADG) 0.11-0.25 g.

Abstract ID: 127E (Poster)

ANTIBACTERIAL ACTIVITY OF SPONGE ASSOCIATED-FUNGI AGAINST VIBRIOSIS AGENT IN FISH AND SHRIMP CULTURE

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Vibriosis is one of the common diseases in fish and shrimp culture which caused by bacteria from the genus *Vibrio* such as *V. harveyi* and *V. alginolyticus*. This diseases causes big losses because it could increase of mortality ratio in fish and shrimp culture. Since the use of antibiotics to overcome it has been banned by several countries, the exploration of natural resources as an anti-vibriosis agents is urgently needed. The aims of this research were to screen antibacterial activity of several sponge associated-fungi, obtain the crude extract and its antibacterial activity against two vibriosis agents, and to detect NRPS genes. Among 8 sponge associated-fungi, there was only *Trichoderma asperellum* isolate MT02 which showed antibacterial activity against *V. harveyi* and *V. alginoliticus*. It produced crude extract amount 0.0204±0.00102% (b/v%). The widest inhibition zone against *V. harveyi* was 10±0.111803 mm shown by concentration 1000 µg/mL with no significant different with other concentratrion ($P<0.05$). On the other hand, it succesfully inhibited *V. alginoliticus* with inhibition zone was 12.2±0.158114 mm by concentration 1000 µg/mL. According to genes detection, fungus *T. asperellum* MT02 was known to has NRPS genes. The ability of producing antibacterial activity of *T. asperellum* MT02 was suspected as result of the exisitance of NRPS genes.

Abstract ID: 128E (Poster)

DETECTION OF *Enterocytozoon hepatopenaei*(EHP) IN*Penaeus vannamei*IN INDONESIA

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Recently, a disease caused by *Enterocytozoon hepatopenaei* (EHP) has infected vaname shrimp cultured in Thailand, Vietnam, China and India. That disease causes a slow growth and smaller size of shrimp during harvesting. This study aimed to know the diagnosis technique for hepatopancreatic microsporidiasis caused by EHP in vaname shrimp.Samples of vaname shrimp were taken from several shrimp pond areas such as Situbondo, Bali and Lampung. Hepatopancreas was extracted and examined using Polymerase Chain Reaction (PCR) and realtime qPCR. Wet mount was also conducted using the fresh hepatopancreas sample and observed under microscope. Histology was also done with another hepatopancreas sample. Polymerase Chain Reaction (PCR) analysis either single step method or nested method showed the same sensitivity. Based on realtime PCR analysis, the number of microsporidia EHP in white feces reached 24.000 copy per microliter and it was a highest number compare to another samples. Wet mount analysis with Giemsa staining showed a huge number of EHP spores in hepatopancreas. Histology with H&E revealed a group of spores in the tubule cell in hepatopancreas has been released into hepatopancreas lumen and also a prespore of EHP found in the hepatopancreas tubule cell. This study concluded that detection of EHP in vaname shrimp can be conducted using several methods such as Polymerase chain reaction (PCR) either conventional or realtime qPCR, wet mount preparat and histology.

Abstract ID: 216E (Poster)

PRESENT STATUS OF SHRIMP DISEASES IN WHITE SHRIMP *Litopenaeus vannamei*IN COASTAL DISTRICT OF ANDHRA PRADESH, INDIA

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Vannamei shrimp culture developed intensively with upgraded technology for higher and successful production. In many occasions shrimp culture is affected by various diseases and experienced in loss of crop or reduced the production level by various reasons. Intensive and semi intensive aqua farming accompanies several disease problems often due to opportunistic pathogens as evident from general aquaculture. *L. vannamei* is an exotic species and culturing both in freshwater and saline waters in Andhra Pradesh. The viral outbreaks are minimal in low saline waters compared to the high saline waters with the best management practices. In this context, a study was conducted in 287 shrimp culture ponds for one year from February, 2016 to January, 2017 in Nellore district of Andhra Pradesh, The major cultural issues recorded were Less Seed (PL) survival, Slow Growth – High FCR, White Spot Syndrome (WSSV), Black Gill Disease (BGD), Running Mortality Syndrome (RMS), Loose Shell Syndrome (LSS), White Faecal Syndrome (WFS), White Muscle Disease (WMD) and body deformity (cramping). The disease outbreaks ranged from 50%-94% having a great loss on investments.

Abstract ID: 222E (Poster)

PATHOGENICITY COMPARISON OF *VIBRIO PARAHAEMOLYTICUS* FROM LOCAL ISOLATES ON WHITE LEG SHRIMP (*Litopenaeus vannamei*)

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The aim of this research is to understand the comparison of pathogenicity of *Vibrio parahaemolyticus* isolate from Karawang and isolate from Cilacap towardWhiteleg shrimp (*Litopenaeus vannamei*). Both isolates was collected from ponds with WFD (*White Faeces Disease)* outbreak. The pathogenicity test of *Vibrio parahaemolyticus* was performed by intramuscularly injection with concentrations of 1 x 10^7, 1 x 10^6, 1 x 10^5, and 1 x 10^4 cells / ml on whiteleg shrimp (9 - 12 grams). Observation of bacterial pathogenicity was based on clinical symptoms, Lethal Dose 50 (LD50) according to Reed & Muench method and histopathology observation. The results showed that there was no difference in pathogenicity based on clinical symptoms and histology. Nevertheless it had differences in LD50 values and death time. The mortality in shrimp were injected with isolates from Karawang was faster and more rapid compared to isolates from Cilacap. The highest percentages of mortality at concentrations of 1 x 10^7 cells / ml and 1x10^6 cells / ml are showed in Karawang isolate, 100% and 83% respectively. There was no mortality at concentrations of 1 x 10^5 cells / ml and 1 x 10^4 cells / ml on both isolates. The LD50 value was obtained at concentration 3, 99 x 10^5 cells / ml for isolate from Karawang, while isolate from Cilacap was obtained at concentration 1 x 10^6 cells / ml. Based on LD50 value, Karawang isolate more pathogenic than Cilacap isolate. Whereas clinically and histopathologically both isolates did not show any differences.

Abstract ID: 081E (Poster)

EPIDEMIOLOGICAL STUDY REVEALS AHPND IS A PART OF SHRIMP EARLY MORTALITY SYNDROME (EMS)

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A correlation between the known shrimp pathogens and early mortality syndrome (EMS) in shrimp have been investigated. The 196 Thai shrimp ponds were randomly selected prior to stocking and subsequently analyzed from 19/08/2013 to 23/04/2014. Among those, 29 ponds (14.8%) were found to be EMS ponds, which shrimp mortality was observed before 35 days post stocking in the earthern pond and 167 ponds (85.2%) were non-EMS ponds, which no shrimp mortality observed until 35 days post stocking. By using PCR and histological examination, white spot syndrome virus (WSSV), VP_{AHPND} (acute hepatopancreatic necrosis disease-inducing *Vibrio parahaemolyticus*), *Enterocytozoon hepatopenaei* (EHP) and aggregated transformed microvilli (ATM) could be found in both non-EMS and EMS ponds, but with different prevalence. Higher prevalence of WSSV and VP_{AHPND} were found in the EMS ponds, compared to the non-EMS ponds suggesting that WSSV and VP_{AHPND} can be the causes of shrimp EMS. In contrast, higher prevalence of EHP and ATM were found in the non-EMS pond, compared to the EMS ponds suggesting that EHP and ATM are the chronic infection or pathological condition and might not directly involve with shrimp EMS. Histological examination of shrimp hepatopancreas tissues from both EMS and non-EMS ponds demonstrated different patterns and can be divided into 5 groups including (1) ponds with no HP pathology in at 35.2% prevalence (69/196); (2) ponds showing collapsed HP tubule epithelia at 25.0% prevalence (49/196); (3) ponds positive for AHPND by histology and/or PCR at 21.4% prevalence (42/196); (4) ponds showing HP bacterial lesions at 14.8% prevalence (29/196); (5) ponds exhibiting EMS but without HP lesions at 3.6% prevalence (7/196). Only 62.1% of the EMS ponds fell within the AHPND group. These results suggest us that AHPND is the only part of EMS. Further studies should focus on identification of the other causes of shrimp EMS.

Abstract ID: 097E (Poster)

USING THE *Bacillus subtilis* E20-FEREMENTED SOYBEAN MEAL (FSBM) AND AN ANTIMICROBIAL PEPTIDE DERIVED FROM *B. subtilis* E20-FSBM TO IMPROVE INTESTINAL IMMUNITY AND PREVENT VIBRIOSIS IN WHITE SHRIMP, *Litopenaeus vannamei*

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The aims of this study is to evaluate the effects of *Bacillus subtilis* E20-fermented soybean meal (FSBM) and an antimicrobial peptide (AP), HTSKALLDMLKRLGK, derived from *B. subtilis* E20-FSBM on the intestinal immunity of white shrimp and its disease resistance against *V. parahaemolyticus* (VP). A protective effect in shrimp against VP infection was recorded in shrimp fed the diet containing 15% fish meal replaced by FSBM as well as for shrimp fed a diet containing AP (62.5 μg/g). For intestinal immunity assay, shrimp fed the diet containing FSBM had higher immune-related gene expression, including anti-lipopolysaccharide factor1, peritrophin, crustin and penaedin 3 in the intestinal tissues, but not the shrimp fed the diet containing AP. It is considered that the FSBM improve shrimp mortality after oral VP challenge might due to the AP and some other immune activators in FSBM, and the results also demonstrated that *B. subtilis* E20-FSBM could be a biofunctional ingredient to prevent vibriosis in shrimp aquaculture.

Abstract ID: 099E (Poster)

ISOLATION AND IDENTIFICATION OF INFECTIOUS *Aeromonas veronii* FROM THE ORNAMENTAL SHRIMP *Caridina babaulti*

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The aim of this study was to identify the bacterial agent that resulted in mass mortality of the ornamental shrimp *Caridina babaulti* culture. The diseased shrimp displayed anorexia, lethargy, whitish musculature, and mortality.Eight strains of *Aeromonasspp*. were isolated from diseased shrimp and evaluated on the basis of morphological, physiological, biochemical characteristics and molecular analysis. A gram-negative, facultatively anaerobic, rod-shaped, spore and viscosity forming bacterium identified as *Aeromonas veronii* was isolated from diseased *C. babaulti*.The results showed that the isolates belonged to a single species that grew in 0 to 3% NaCl, at 4 to 40 °C on TSA (tryptic soybean agar) medium. Furthermore, the isolates showed positive reactions with β-galactosidase, arginine dihydrolase, gelatinase and cytochrome-oxidase. Identification of ISM01 (1 of 8 isolates) wasconfirmed by polymerase chain reaction (PCR) assay and the 16S rDNA sequence performed 97.8% similarity to *A. veronii*. In addition, the mortality of shrimp reached 70.0% after 96h of test challenges. The sections of histopathology displayed the tissue necrosis and aggregation of hemocytes in the muscles as well as detachment of epithelial cells from the basement membranes of affected hepatopancreatic tubules of infected shrimp.

Abstract ID: 184E (Poster)

EFFECTIVENESS OF USE OF PROBIOTIC BACTERIA ON THE ABUNDANCE OF *VIBRIO SP* BACTERIA WITH WHITE FECES DISEASE AND ITS EFFECT ON VANAME SHRIMP (*Litopenaeus vannamei*) PRODUCTION

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This study aims to determine the effectiveness of probiotic bacteria applications in shrimp ponds, the relationship of abundance of *Vibrio* sp bacteria with White Feces Disease (WFD) and provide information related to the spread of WFD disease in Bali Province. The research method is survey method. Primary data collection is done by distributing questionnaires, interviews, observation and focus group discussions. Secondary data collection is obtained from various related reports. Data analysis used quantitative descriptive analysis and multiple linear regression analysis. The hypothesis in this study is *Vibrio* sp bacteria as a trigger of WFD disease in vaname shrimp in ponds.Shrimp production data from the Province of Bali in 2016 significantly decreased. The second cycle of 2016 maintenance of vaname (*Litopenaeus vannamei*) shrimps in the pond units Karangasem, Buleleng, Jembrana and Denpasar found many white feces disease (WFD). The survey results showed that 7 (seven) from 14 (fourteen) units of the company's own ponds and individuals experienced the problem of Infectious Myonecrosis Virus (IMNV) disease continued white feces disease. *Vibrio* sp bacteria was recorded to exceed the optimal limit on each pond affected by WFD. Decrease in bacterial abundance along with the disappearance of WFD disease in shrimp. Mass mortality of shrimps occurs when handling is not appropriate and slow. The loss of farmers is very significant due to WFD disease. Good water quality management, application of biosecurity and appropriate use of probiotics as recommendation materials to avoid WFD disease.

Abstract ID:244E (Poster)

KEY ROLE OF GLUTAMINE METABOLISM FOR WSSV REPLICATION

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White spot syndrome virus (WSSV) can cause huge economic losses, providing the impetus to determine its pathogenesis. We reported that WSSV induced a Warburg effect and glutaminolysis during viral genome replication (12 hpi). There are two pathways of glutaminolysis: an α-KGDH-mediated oxidative pathway and an IDH1 or 2-mediated reductive pathway. In the present study, gene expression of both α-KGDH and IDH1 were increased during WSSV infection. Moreover, WSSV genome copies and VP28 gene expression were significantly decreased after silencing α-KGDH, IDH1 and IDH2. Therefore, we inferred that these enzymes may have critical roles in both pathways for WSSV replication. To further confirm the flux of carbon sources during glutaminolysis, stable isotopic labeling was used to track metabolic fluxes in WSSV-infected shrimp. Using [U-^{13}C]-glutamine as tracer, both the amount and production rate of labeled-metabolites in the TCA cycle were significantly increased during WSSV genome replication (12 hpi). In conclusion, at genome replication (12 hpi), gene expressions of related enzymes were induced and there were increased amounts and production rates of metabolites to replenish the TCA cycle to support WSSV replication.

Abstract ID:245E (Poster)

THE INVOLVEMENT OF *Enterocytozoon hepatopenaei*(EHP) IN RETARDED GROWTH SYNDROME IN *Penaeus vannamei*

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Asian shrimp industry witnesses a gradual increase of production in the last decade of twenty century. Since 2003, however, it has been faced to an issue named monodon slow growth syndrome (MSGS) or retarded growth which was first discovered in *Penaeus monodon* culture in Thailand. The causative agent of slow growth syndrome has not been understood even though multiple pathogens have been detected in the shrimp suffered from slow growth syndrome. Apart from viruses, a new microsporidia called *Enterocytozoon hepatopenaei* (EHP) has been also detected in hepatopancreatic (HP) cells of suffered shrimps. EHP has been detected in hepatopancreas from retarded growth *P. monodon* and *P. vannamei* and it is referred as a causative agent of retarded growth syndrome anecdotally. Hence, this study was conducted to elucidate the role EHP in retarded growth syndrome by feeding SPF shrimp with EHP-infected hepatopancreases. The result showed that the EHP-challenged shrimp grew slower than control shrimp.

Abstract ID:246E (Poster)

DEVELOPING A WHITE SPOT DISEASE MOLECULAR SURVEILLANCE SYSTEM FOR ASIA AND THE PACIFIC REGION

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White spot disease (WSD) in farmed penaeid shrimps, was first identified in East Asia (Japan, Taiwan and China) in 1992-93. Subsequently, it was detected in a number of major shrimp producing countries, including Thailand, Vietnam and India, causing an unprecedented aquatic epizootic. Although the adoption of control measures including improved biosecurity, the use of specific pathogen free (SPF) brood stock and a shift to the less susceptible white shrimp *(P. vannamei*) have greatly reduced the impact of the disease, it remains a major threat to the industry and outbreaks continue to occur.An important technique when investigating outbreaks of infectious disease is genotyping, as this can provide clues as to the country or region of origin, as well as the most plausible pathway of introduction. For WSD, the main genotyping technique has been the determination of the "variable number tandem repeats" ("VNTR"). Nevertheless, although the method has been applied to a number of outbreaks, in few instances has it resulted in epidemiological insight as to origins and pathways of introduction, mainly due to the technique lacking discriminatory power. To overcome the limitations of VNTR typing, and to make genotyping more useful for outbreak investigations, the AAHL Fish Diseases Laboratory is investigating the use of whole genome sequencing (WGS) using next-gen sequencing (NGS) methodologies. The intention is that once the WGS methodology is validated, that we will then develop a web-based "WSD Molecular Surveillance System" which will integrate both sequence and outbreak data. This is intended to be a regional initiative, allowing project partners throughout Asia and the Pacific Region to share data and thus assist in both local and regional control of WSD.

Session 6 – Immunology & Vaccinology

Abstract ID: 235F (Keynote for Immunology)

WHAT WE KNOW AND WHAT WE HAVE TO STUDY FOR UNDERSTANDING FISH AND SHRIMP IMMUNE SYSTEM?

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For understanding of fish and shrimp immune system, molecular tools including mRNA, gene and genome sequences, antibody for distinguishing blood cells and immune-related proteins, cell lines, etc are necessary. In case of fish, Genome sequence information of at least 13 aquaculture species such as Asian sea bass, Atlantic, cod, Atlantic salmon, blue fin tuna, carp, channel catfish, Japanese flounder, puffer fish, rainbow trout, sole, tilapia, and turbot have been reported in several international journal by 2016. Using these published information, we can find our interesting immune-related genes from our target fish. Now, finding a homolog of immune-related genes from our target fish species is not difficult. However, some fish species immune-related genes are existed as multi-copies due to multiplication of their genome. Some of homologues of mammalian immune-related genes in fish have a different function (including gene expression pattern) of those of mammal. If we can have a nucleotide sequence of a studying molecule, we can make an antibody against an interesting protein by using small synthesized peptide and/or recombinant protein using *E. coli* system for characterization of studying molecule in protein level. This protein study is also not difficult to conduct and not expensive now. In some fish species, we can use cell lines and/or primary cultures of several immune-related cells such as B cell, T cell, macrophage, etc. For understanding more in detail of fish immune system, we have to conduct a functional study of each immune-related molecules. In case of shrimp immune system, unfortunately, we do not have enough information of their genome and genes. Because, we know that shrimp has unique genome and it is difficult to complete genome analysis. There are so many unique repeat sequences in their genome. In addition, there is no closely related organism of shrimp in model animal. So, it is not easy to find a homolog of known immune-related molecule from shrimp. We could collect many of mRNA sequences of shrimp. However, about half of them have no significant similar to reported sequences in public databases. So, it is difficult to analysis which is immune-related molecules and genes. We can use RNAi technology for study on shrimp immune-system. However, RNAi may give a non-specific effect to shrimp immune-system. So, we need more study about RNA in shrimp. We still do not have a cell line of shrimp. In addition, we do not have any of hemocytes markers of shrimp. We have to characterize/ study genome, transcripts, hemocytes markers, and cell lines for understanding shrimp immune system.

Abstract ID: 210F (Oral Student)

EFFECT OF CHRONIC HEAT STRESS RESPONSE ON HEMOCYTE TRANSCRIPTOME OF *Penaeus vannamei* CHALLENGED WITH *Vibrio parahaemolyticus* AHPND

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An organized system of resistance to *Vibrio ssp.* related to heat stress is believed to exist in shrimp but our understanding on the complex links of stress and immune response is still limited. Previously, our challenge experiments showed that 5-minute chronic heat stress for a period of 7 days significantly increased survival against acute hepatopancreatic necrosis disease (AHPND) infection. To explore the global expression of genes related to immunity under heat stress and identify putative genes under this specific condition, RNA-seq analysis of the transcriptome in shrimp haemocyte under heat stress and *Vibrio parahaemolyticus* AHPND (VP_{AHPND}) infection was done. Next generation sequencing and de novo assembly generated a reference library with 205, 137 isotigs and differential expression profiles between 6 libraries. Approximately 47, 401 sequences (23.11%) were annotated by BLASTX against Swiss-Prot and 11 to 78 genes were detected to be differentially expressed in several pairwise comparisons. Real-time qPCR analysis confirmed the expression profiles of selected genes validating our RNA-seq analysis' power of detection for differentially expressed genes. Among them, we found that Relish small isoform gene was VP_{AHPND}-responsive genes but did not play role in the increased percent survival in the heat treatment shrimp. Some significant heat-stress modulated immune factors in VP_{AHPND}-infected shrimp identified such as aminopeptidase O, lipoprotein aminopeptidase and aminomethyltransferase were considered as novel immune genes. Interestingly, aminopeptidase is implicated in immune priming in some organisms and has been reported as a receptor for *Bacillus thuringiensis* CryIA(c) toxin. In conclusion, this transcriptomic data provides preliminary clues to critical heat-modulated immune genes and the overall molecular response of shrimp hemocytes under heat stress in conjunction with *V. parahaemolyticus* AHPND infection.

Abstract ID: 035F (Oral Student)

TRANSCRIPTOME PROFILING OF COBIA (*Rachycentron canadum*) INFECTED WITH *Photobacterium damselae* subsp. *piscicida* WITH AN EMPHASIS IN IMMUNE RESPONSES

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Cobia, *Rachycentron canadum*, is one of the most valuable aquatic species in Taiwan. Given to the rapid development of this industry, the fish was subjected to *Photobacterium damselae* subsp. *piscicida* (Pdp), which is a causal agent of photobacteriosis. However, the molecular mechanisms underlying the fish defense are not yet fully understood. In the present study, the transcriptomic profiles of livers and spleens from Pdp-infected and non-infected cobia were obtained for the first time by Illumina-based paired-end sequencing method with a focus on immune-related genes. Totally, 164,882 unigenes with high quality were obtained in four libraries (PBS_liver, Treat_liver,PBS_spleen and Treat_spleen). Following Pdp infection, 7,302 differentially expressed unigenes from liver and 8,600 differentially expressed unigenes from spleen were identified, including 3,269 up-regulated unigenes and 4,033 down-regulated unigenes in liver, and 2,828 up-regulated and 5,772 down-regulated unigenes in spleen. Results suggested MyD88-independent pathway could be a response against bacteria, as testified by up-regulation of proteins involved in this pathway. However, a remarkable finding was negative regulation of complement components and the increased expression of IL-10, which are characteristic for an inadequacy of immune responses. In summary, this study not only characterized several putative immune pathways to Pdp acute infection but also provides a better understanding of the molecular response to photobacteriosis in cobia.

Abstract ID: 100F (Oral Student)

PECULIAR EXPRESSION OF CD3-EPSILLON IN KIDNEY OF GINBUNA CRUCIAN CARP

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TCR/CD3 complex is composed of the disulfide-linked TCR-αβ heterodimer that recognizes the antigen as a peptide presented by the MHC, and non-covalently paired CD3γε- and δε-chains together with disulfide-linked ζ-chain homodimers. The CD3 chains play key roles in T cell development and T cell activation. In the present study, flow cytometric (FACS) analysis revealed lower expression of CD3ε in the CD4-1^{+} and CD8α^{+} T lymphocytes of head- and trunk-kidney, while CD3ε was expressed at the normal level in T lymphocytes from thymus, spleen, intestine, gill and peripheral blood. Both qPCR and Western Blot analyses also showed that CD3ε expression was lower in head- and trunk-kidney than other tissues. To be interested, expression of CD3ε increased after 24 hours in *in vitro* culture in head- and trunk-kidney T cells. We also examined the expression of ZAP-70 as well as CD3ε, both of which are well known T cell markers. FACS analysis showed that the fluorescence intensity of CD3ε positive cells is lower than that of ZAP-70 positive cells in kidney lymphocytes. Furthermore, percentages of positive cells and their fluorescence intensity increased for both CD3ε and ZAP-70 after 24 hours in *in vitro* culture in head- and trunk-kidney. Lower expression of CD3ε was also found in goldfish and carp kidney T lymphocytes. These results indicate that kidney lymphocytes express lower level of CD3ε at both mRNA and protein levels. Here we discuss this phenomenon from the point of function of teleost kidney as lympho-hematopoietic tissue.

Abstract ID: 218F (Oral Student)

DETERMINATION OF MARRON (*Cherax cainii*) HAEMOCYTE CELL TYPES, MORPHOMETRIC CHARACTERISTICS AND THEIR PHAGOCYTIC ACTIVITY AT DIFFERENT TEMPERATURES IN VITRO

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The purpose of this study is to identify and morphologically characterise the haemocyte cell types from marron, *C. cainii*, and to identify which haemocyte types were involved in the phagocytosis of pathogens. In addition to morphological observations using a light microscope (LM) and transmission electron microscope (TEM), we also employed flow cytometer (FCM). Three major *C. cainii* haemocyte types were identified by LM, TEM and FCM. Determination of haemocytes by LM based on the number, size of the cytoplasmic granules and N:C ratio. These cells were hyaline (HCs), small granular (SGCs), and large granular (LGCs) cells. Three haemocyte types were also observed by TEM based oncell and nucleus size, granule diameter, number of cytoplasmic granules per cell and N:C ratio. Haemocyte populations were successfully resolved by FCM into forward scatter (FSC), which measures relative cell size, versus side scatter (SSC), which measures cell granularity with data plots via scatter parameter gating. Three event clusters were observed in the typical hemogram of FSC versus SSC. Gates were drawn around each event cluster, which were provisionally classified as region of SGCs, LGCs and HCs in one parameter dot-plots in log scale. Morphometric analysis was conducted by TEM on *C. cainii* haemocytes to quantify various cellular features. Some morphological features varied between haemocyte types and were also influenced by temperature. Hyaline cells were the smallest cell compared to SGCs and LGCs. There was no significant difference in cell size between SGCs and LGCs, either at 20 or 30 °C. There was a trend in diminished cell size at the higher temperature but this was only significant for the LGCs population. Hyaline cells had the highest N:C ratios while LGCs had the lowest while also possessing significantly smaller nuclei compared to HCs and SGCs. There was no significant difference in granule size between the haemocytes type but LGCs and SGCs possessed abundant, small round granules, significantly greater numbers than HCs. Total haemocyte counts and differential haemocyte count was count using FCM. THC increased with higher temperature, going from 1.9 x 10^6/ml at 20 °C to 4.9 x 106/ml at 30 °C. LGCs increases out of proportion as the THC rises with temperature, and both other types decrease in representation. However, the most abundant haemocyte at all temperatures was HCs, followed by SGCs and LGCs. This results is according to LM observation, which are HCs were the most abundant cells (50%) followed by SGCs (29.2%) and LGCs (20.8%). In order to identify which cell types were involved in the phagocytosis of pathogens, an *in vitro* phagocytosis assay was performed with live and heat-killed *V. mimicus* and heat-killed *E. coli*, incubated for 2, 4 and 8 hours at 20 and 30 °C and observed using TEM. All the haemocyte types underwent drastic morphological changes after the inoculation with bacteria. The alterations were more evident in haemocytes inoculated with live *V. mimicus*. The major morphological alterations found in all haemocyte types were an increase of multi vesicular structures and pinocytic activity, and clustering of mitochondria. Changes to phagocytic activity and haemocyte morphology appeared to be stimulated by bacteria and temperature.

Abstract ID: 241F (Keynote for Vaccinology)

ADVANCES IN FISH VACCINE DEVELOPMENT - PREVENTION IS BETTER THAN CURE

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Vaccination is important for the prevention and control of diseases in aquaculture and has been shown to significantly reduce the use of antibiotics in fish. Although the number of vaccines that are commercially available has expanded in recent years, vaccines are still not available for many pathogens and some existing vaccines do not perform well. In addition, the optimal strategy for their use in aquaculture has still to be established.The most critical step in developing an effective vaccine is identification of potentially protective antigens and confirming their protective response in the host species. Consideration needs to be given to the type of pathogen (*e.g.* bacterium, virus, parasite), type of vaccine developed (whole cell, recombinant, DNA etc.), fish species, adjuvant used, and the optimal method of administration (injection, immersion, oral, booster) for use in aquaculture. In addition, a standardised experimental pathogen challenge model needs to be available or developed to test vaccine efficacy in the host species.A number of case studies will be presented to illustrate key points in fish vaccine development and strategies for use. This will include characterisation and selection of bacterial isolates from given serotypes/genotypes to include in traditional whole cell vaccines. Technologies will also be described for the identification of specific antigens for recombinant, DNA or peptide vaccines, and the difficulties encountered in developing vaccines against parasite diseases or for new fish species discussed. The majority of commercial fish vaccines are still administered by injection while more practical immersion and oral vaccination strategies (*i.e.* via the mucosal route) need improvement, thus advances in administration methods and alternative strategies will also be presented.

Abstract ID: 167F (Oral, Student)

EFFECTIVENESS OF FORMALIN-KILLED VACCINES AGAINST *Vibrio harveyi*INFECTION IN ORANGE-SPOTTED GROUPER

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Vibrio harveyi is a major bacterial pathogen that causes the systemic disease vibriosis in many commercially important fish. In grouper, this fatal disease leads to massive deathsand dramatic economic loss that threaten the grouper culture industry; therefore,effective vaccine is needed to control the pathogen. In this study, we evaluated the immune response and protective efficacy of vaccines containing *V. harveyi* formalin-killed cells (FKC) formulated with plasmid constructs harboringCpG ODN 1668 (p60CpG) or metabolizable ISA763 AVG adjuvants. Our results indicated that the antibody titers were significantly elevated in the vaccinated grouper as early as two weeks after immunization. A pivotal observation was that the vaccines highly protected grouper from a homologous *V. harveyi*strain challenge with relative percentage survival values of 96.2% in the FKC + p60CpG vaccinated and 100% in the FKC + ISA763 AVG-vaccinated fish. Vaccinated grouperalso demonstrated strong cross-protection against a heterologous bacterial isolate challenge. These results indicate that the *V. harveyi* inactivated vaccines are promising candidatesand CpG ODN 1668 1668 is a potential adjuvant that can be further developedfor vaccines against *V. harveyi*.

Abstract ID: 135F (Oral)

PLGA MICROPARTICLE VACCINE ENCAPSULATED FORMALIN-KILLED *Aeromonas hydrophila* CELLS AGAINST *A. hydrophila* INFECTION

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Control and prevention of disease is a high priority in aquaculture, and vaccination is important to prevent outbreaks. Here, poly (D,L-lactide-co-glycolic acid) (PLGA) microparticles (MPs)approximately 36 µm in diameter were used to encapsulate and deliver *Aeromonas hydrophila* formalin-killed cells (FKC) as an antigen, and the innate and adaptive immune responses of cyprinid loaches and common carp were assessed following vaccination. Fish were divided into three groups (A, B, C). Total antigen of 0.1 ml vaccine was adjusted by 2 x 10^8 CFU and injected via intraperitoneal route. Group A was vaccinated with 0.1 ml of PLGA vaccine, group B was with 0.1 ml of FKC vaccine and group C was with 0.1 ml of sterile PBS. All three groups were challenged with *A. hydrophila* and challenge dose was lethal dose (LD_{50}). Loaches and carp were then challenged with A. hydrophila at 12 and 20 wpv, and 10 and 14 wpv, respectively, and relative survival rates were calculated. For both fish species, the curve of antibody titer over time was shallower in the PLGA group than the FKC group and the PLGA groups demonstrated higher survival rates at all time points. Relative expression of IL-1β and TNF-α mRNA was significantly upregulated in the PLGA group at 2 and 4 wpv. Moreover, PLGA-MP vaccination increased relative mRNA levels of lysozyme C and IgM, which were significantly higher than those observed with FKC treatment at 2 wpv and 4, 6, and 8 wpv, respectively. In conclusion, PLGA-MP vaccines have the potential to induce longer and more potent immune responses than FKCs alone, and protect both cyprinid loaches and common carp with greater efficiency.

Abstract ID: 172F (Oral)

EFFECT OF DNA VACCINE ON MATERNAL IMMUNITY OF COMMON CARP

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Koi herpesvirus (KHV) is a virus that infects various stadia of carp. Based on previous research, the virus can be prevented through vaccination using anti-KHV DNA vaccine. This DNA vaccine can protect the cyprinids from KHV infection. However, the efficacy of DNA vaccine on the maternal immunity and its protection to broodstock and larvae has not been studied yet. Therefore, it is necessary to investigate the effect of anti-KHV DNA vaccine on the maternal immunity of common corp broodstock. This study aimed to evaluate anti-KHV DNA vaccine in the immunity performance of broodstock and fry. Fish vaccination was done by intramuscular injection at 30, 45, 60 days pre-breeding and unvaccinated fish was used as the control. Challenge test was performed on fish fry at the age of 7, 14, 21 and 28 days by one hour immersion in 10-3 KHV filtrate dilution and the infected fish was observed for 21 days. Serum collection was done in broodstock every 15 days for 75 days post vaccination and in fry every 7 days after hatching for 28 days. The results showed that vaccination had affected broodstock haematology as shown by the improved leucocytes and phagocytic activity since day 30 and the decrease of erythrocytes, hemoglobin and hematocrit until day 45. Antibody titer was detected to protect the broodstock which was observed since day 30 after vaccination, and different vaccination timing resulted in different transfer of immunity to the larvae. The fish fry survival and specific growth rate in vaccinated fish were significantly higher than that of the control ($P<0.05$). It can be concluded that KHV DNA vaccine could improve the maternal immunity of common carp broodstock and protect the fry against KHV infection.
Key words: koi herpesvirus, maternal immunity, DNA vaccine, common carp

Abstract ID: 123F (Oral)

URONEMA-VIBRIO VACCINE (UViVac) ENHANCES PROTECTIVE AND HUMORAL RESPONSES IN *Sparidentax hasta*

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Uronema-Vibrio bivalent vaccine (UViVac) was evaluated for dose optimization as an attempt to apply the vaccine developed by KISR in sobaity (*Sparidentax hasta*) aquaculture for improved diseases resistance and hence, lower fish losses. The Vaccine was applied through feed at a concentration of 10^6 cells/g of feed for 10, 20 and 30 days after an initial β-glucan-fed immunostimulation for 20 days. The glucan application in 50d-old larvae significantly improved larval survival (97.6%) against a survival of 92.8 % in the unstimulated larvae. The stimulated larvae also showed increased antibody response. Vaccination for 20 days with a dose of 10^6 cells/g of feed produced positive impact on the humoral response of the fish which was significantly higher than that of the other vaccination groups. There was no significant enhancement in the protective response with the extension of vaccine feeding.The serum and mucus properties showed that all the vaccinated fish had elevated levels of antibodies against both *Vibrio spp* and live *Cryptocaryonirritans.* The combination challenge produced the relative percent survival (RPS) rates of 72%, while the control fish suffered mortalities with survival rates of 10%. The phagocytic ability of head kidney cells in the vaccinated (all groups) fish was enhanced significantly and the parasite immobilization was higher than that in the control fish.

Abstract ID: 228F (Oral)

IMMUNIZATION WITH INACTIVATED GERMINATED ZOOSPORES OF *Aphanomyces invadans* PROVIDE PROTECTION AGAINST INFECTION IN ROHU *Labeo rohita*

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Infection with *Aphanomyces invadans* is one of the most destructive diseases of freshwater fishes. Indian major carps, the major cultured species of the Indian sub-continent are highly susceptible to this disease. In the present study, inactivated (3% paraformaldehyde) germinated zoospores of *A. invadans* were used as antigen for evaluating the effect of immunization, in conjunction with and without adjuvant Montanide™ ISA 763 A VG (Seppic). For the experiment, 160 numbers of rohu (74 ± 12 gm size) were divided into 4 groups with 40 fish in each group. The experimental groups were immunized intraperitoneally with adjuvant, inactivated germinated zoospores and inactivated germinated zoospores mixed with adjuvant, separately whereas the control group was injected only with PBS. After 21 days of first immunization, the fish in experimental groups were given a booster dose, similarly. After 7 days of booster dose, the fish were challenged with zoospores (1000 no./fish) of *A. invadans* and observed for a period of 4 weeks and level of protection was determined in terms of relative percent survival (RPS). There was 66% RPS in the group immunized with inactivated germinated zoospores mixed with adjuvant. Further, histopathological examination of the surviving fish indicated that the lesion area was restricted to the site of infection with well developed granulomas. On the other hand in the adjuvant control group as well as group of fish injected with inactivated germinated zoospore, although there was delayed mortality in comparison to control, all the challenged fish succumbed to infection. These preliminary findings indicate that inactivated germinated zoospores of *A. invadans* could be explored for use as one of the vaccine candidates.

Abstract ID: 048F (Oral Student)

IMMUNE DEFENSE OF SHRIMP GILLS THROUGH *Marsupenaeus japonicus* GILL C-TYPE LECTIN (MjGCTL)

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Gills of penaeid shrimp act as the gateway between internal and external environment, functioning in gas and ion exchange, filtering out harmful biotic and abiotic factors. To the immune system, gills are known to merely assist mechanically through the removal of trapped foreign materials during molting. Here, we demonstrate that gills are also equipped with immune molecules such as the gill C-type lectin MjGCTL of *Marsupenaeus japonicus.* Predicted to be a secreted protein, MjGCTL was hypothesized to be secreted on the gill surface mucous. Supporting this hypothesis are our findings that the gill mucous was also observed to cause bacterial agglutination like the recombinant (r)MjGCTL, and likewise by the detection of MjGCTL by western blot in gill mucous. Evidence confirming that this agglutination activity is caused by MjGCTL was demonstrated by the inhibition of agglutination ability of gill mucous upon neutralizing MjGCTL with its specific antibody. Using lactose-agarose beads, MjGCTL was purified from gills, where similar protein functions with rMjGCTL were observed in the purified MjGCTL protein. Phagocytosis assay by flow-cytometry with PKH67-labelled *Streptococcus agalactiae* revealed that MjGCTL can act as an opsonin, increasing the phagocytic rate. Transcripts of MjGCTL was found to be 11-fold higher in shrimp immersed in pathogenic strain of *Vibrio parahemolyticus* compared to those in non-pathogenic. *In vivo* functional analysis of MjGCTL was done by silencing MjGCTL by RNAi followed by challenge test by immersion using a low bacterial concentration. Results showed silencing MjGCTL made shrimp more vulnerable to infection reducing survival to 20% at 7 days post-infection. Also, significant increase in bacterial load from 6 to 12 hours post-immersion with *V. parahaemolyticus* was observed in both gills in hemocytes of MjGCTL-silenced shrimp. These are evidences that shrimp gill is not only a physical, but also a biochemical barrier lined with immune molecules such as MjGCTL.

Abstract ID: 030F (Oral Student)

MHC CLASS II EXPRESSION ON GILL EPITHELIAL ANTIGEN SAMPLING CELLS

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Bath-vaccination is a fish mucosal vaccination method which can dramatically reduce working cost in aquaculture. We previously reported that vaccine antigens are taken up by gill epithelial antigen sampling (GAS) cells during bath-vaccination. Further, we reported that the GAS cells expressed almost all genes related to MHC class II (MHCII) antigen presentation pathway in transcriptome analyses. In this study, we aimed to further explore MHCII antigen presentation by GAS cells after bath-vaccination. Rainbow trout was immersed into water containing *Aeromonas salmonicida* subsp. *salmonicida* (*A.s.s.*) bacterin stained with Syto61 fluorescent dye. After bath-vaccination, the gill epithelial cells were collected, stained with the lectin *Ulex europaeus* agglutinin 1 (UEA1), and subjected to flow cytometric analysis and flow sorting. Each of three cell populations observed (negative cells, UEA1?*A.s.s*?; macrophages, UEA1?*A.s.s*?; GAS cells, UEA1?*A.s.s*?) were sorted and RNA was extracted. Expression levels of genes involved in MHCII antigen presentation pathway (MHCIIα, MHCIIβ, Cathepsin B, Cathepsin L, Cathepsin S, invariant chain, and GILT) were analyzed in each cell population by quantitative RT-PCR. In the second experiment, rainbow trout gills were immersed into RPMI1640 containing Syto61-stained *A.s.s.* bacterin. The gills were then incubated in a flesh medium, and were sampled at 0 h, 1 h and 3 h after the incubation. Then, epithelial cells were isolated from the gills and were stained with UEA1 and an anti-MHCII monoclonal antibody (MAb) followed by flow cytometric analysis. In addition, immersed gills were also processed for the frozen sections stained with UEA1 and anti-MHCII MAb. All analyzed genes, MHCIIα, MHCIIβ, Cathepsin B, Cathepsin L, Cathepsin S, invariant chain, and GILT were strongly expressed in GAS cells and macrophages. MHCII expression on the surface of GAS cells were confirmed by flow cytometry, and the percentage of MHCII positive GAS cells increased with time; 9.0% at 0 h, 21.5% at 1 h, and 25.5% at 3 h after exposure to the bacterin. Furthermore, UEA1?MHC II?epithelial cells were frequently observed at the base of the secondary gill lamellae on the frozen sections. Taken together, these results suggest that GAS cells can uptake and directly present bacterial antigens via MHCII in the gill epithelium of rainbow trout.

Abstract ID: 162F (Oral)

HEALTH AND SURVIVAL ENHANCEMENT OF *Penaeus vannamei* POST LARVAE BY MEANS OF PROBIOTIC AND FEED ADDITIVE APPLICATION WITHIN EARLIER REARING PERIOD

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The use of probiotics in aquaculture seems increase since the last few decade. Water quality improvement was the dominant expected reason. For shrimp hatchery the probiotic application is a challenge to understand. Two experiments was conducted to know the efficacy of some commercial probiotic and feed additive in order to improve the survival and health status vannamei shrimp larvae. Four trials with 3 replication each was design to determined the effect of feed additive and probiotic, solely and combined to the survival and healthy of reared larvae. The first experiment for earlier larval stage to obtain PL-6 (15 days), vannamei nauplius from breeding unit were stocked in 12 unit of 500 L capacity of fiberglass tank with density about 46.000 pcs/tank. The standard procedure for good hatchery practices applied on the larval rearing processes. The 2nd experiment (simultaneously) was rearing of PL-4 stage to obtain PL-10 (7 days), stock with density about 7.800 pcs/tank.The both experiments comprised of 4 treatments i.e. (1) Combined application of additive feed and probiotic; (2) Probiotic without additive; (3) additive without probiotic; and (4) without probiotic nor additive as control. Commercial feed additives powder such as vitamin C (IMMUNE-CE TM) and mineral compound (MINTECH FEED™) applied with 8 g/kg feed; the commercial probiotic powder (EPICINE D™) inoculated to the rearing water as activated cells, with 7.5 x 10^7 cells/tank. The effect of the treatments was analyzed for the water quality (pH, NH3-N, DO, Vibrio density), intestinal condition, absolute growth rate, and survival rate. At the end of the rearing some shocking conditions were challenged to the survive PLs such as hypothermal shock and osmotic shock. The result show the application of probiotic and feed additive during the PL rearing (2nd experiment) was not significant different with the control treatment, but the application in the 1st experiment show high significant. The use of probiotic, feed additive, and combined treatment showed increase the survival rate by 52%, 29.4 and 94% respectively. The effect of probiotic application on water quality was indicated on reducing NH3-N concentration, but was not able to control *Vibrio spp.* density in rearing water. The observation of the digestive organ, survival due to osmotic and thermal shock showed that the probiotic and feed additive application in larvae rearing was able to enhance the health status.

Abstract ID: 001F(Poster &Elevator Pitch)

DOES ENVIRONMENTAL STRESS AFFECT FISH IMMUNITY? AN EMPIRICAL INVESTIGATION

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The immune system of both humans and animals is, no doubt, resists the toxic effects of pollutants. The exposure of animals to xenobiotics produces a variety of responses and the survival of the organism depends upon efficiency of the endocrine function. Untreated sewage discharged from the municipal and agricultural activities adds high concentration of carbon-rich organic material, heavy metals to the pollutants loads .We attempted to study firstly the effects of pollutants available in rivers Ajoy, Ganga in India and their confluence zone on fish physiology. Second investigation of change in fish diversity indices due to presence of pollutants are taken into study. The air-breathing teleost Channa punctatus available in these rivers are exposed to sub-lethal concentration of mercuric chloride, cadmium chloride, phenol, ammonia and mixture of these four for 48 hours to determine the effects of these pollutants on fish hypothalamo-pituitary–thyroid axes and also on carbohydrate metabolism. Inhibition of head kidney peroxidase enzymes is usually associated with a decrease in iodide peroxidase activity and blood thyroxine and tri-iodothyronine levels. An alteration in the head kidney acid phosphatase activity indicates changes in the lysosomal membrane characteristics. The stabilization of lysosomal membrane may be explained by reduction of head kidney lysosomal protease activity, which is essential for thyroxine and tri-iodothyronine release from the follicular cells of the head kidney. The elevated guaiacol or non-iodide peroxidase activity has a role in the detoxification of pollutants. Fishes are found to be hyperglycemic with a concomitant depletion in the hepatic glycogen content and increase in glucose-6-phosphatase activity on 1st and 2nd days of exposure to pollutants. Fish diversity is high in Ganga and Ajoy and relatively meagre at confluence zone of Ajay and Ganga where pollution level is high .Variation of species in three different zones indicate that altered water quality support diverse fish fauna. The cellular damage in the inter-renal tissues, haemopoietic tissues necrosis, shrinkage of capillaries in the glomeruli and increase in Bowman's space are noticed. Hepatopancreas accompanied by karyolysis, apoptosis and necrosis, derangement of the pancreatic acini's are observed. The secretion of adreno-cortical cells is mainly concerned with the organism reaction to stress. The cellular damage in the inter-renal tissue points to a lowered production of cortisol with overall decrease in the capacity of the fish to fight stress. Analysis of the available data suggest that pollutants cause the depletion of energy resources and disturbs the metabolic pathway by the adverse effects on thyroid function, carbohydrate metabolism, histopathological lesions in the hepatopancreas and kidney of Channa punctatus. We could *suggest* that application of β-D-glucans through immersion, dietary inclusion or injection may enhance many types of immune responses, resistance to bacterial and viral infections and to environmental stress in many fish species.

Abstract ID: 007F(Poster &Elevator Pitch)

MICROALGAL TECHNOLOGY FOR PRODUCTION OF SHRIMP RNA-BASED VACCINES

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RNA interference (RNAi) is highly effective for combating viral pathogens by using sequence-specific double-stranded (ds)RNA designed to knockdown the key viral genes. The application of this technology in small-scale tests suggests the potential of RNAi in mitigating shrimp viruses that cause massive mortality and controlling slow growth-associated virus, that lead to profit losses due to small size but not mortality. The key challenge is developing a low-cost production and oral delivery system for dsRNA that can be adopted by the shrimp industry. Edible microalgae offer an attractive solution in this regard. The project involves engineering *Chlamydomonas reinhardtii* microalgaevia two transformation methods to produce oral RNA-based vaccines. First, bioengineering *C. reinhardtii* by nuclear transformation with the developed expression vector is shown to successfully produce dsRNA targeting the lethal yellow head virus, and its oral application provides specific viral inhibition in shrimp. Second, another transformation technique is carried out to produce marker-free transgenic lines for dsRNA production in the *C. reinhardtii* chloroplast. Production of dsRNA is expected to be best achieved in the algal chloroplast since it lacks any RNAi machinery for dsRNA processing allowing high-level accumulation of dsRNA within the organelle. Given that viral target sequence is available, the microalgal dsRNA expression system should be applicable to combat with the virus of interest in the future. We have recently initiated partnership with algal researchers from UK to investigate downstream processing for whole-cell algal vaccinesand the potential ofthe algal system as a viable pathogen protection technology for the shrimp aquaculture.

Abstract ID: 029F(Poster &Elevator Pitch)

DEVELOPMENT OF A MONOCLONAL ANTIBODY AGAINST GILL EPITHELIAL ANTIGEN SAMPLING CELLS OF RAINBOW TROUT

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Bath-vaccination is a promising technique to reduce working cost dramatically in fish vaccination. However, the mechanisms for uptake and immune recognition of antigens on the mucosal surfaces of fish are largely unknown. We previously reported that vaccine antigens are taken up by gill epithelial antigen sampling (GAS) cells during bath-vaccination of rainbow trout. So far, GAS cells were identified by the binding ability to a lectin, *Ulex europaeus* agglutinin 1 (UEA-1), and their characteristic of taking up the inactivated bacteria *Aeromonas salmonicida* subsp. *salmonicida* (*A.s.s.*). In this study, we aimed to develop a monoclonal antibody (MAb) against GAS cells for more simple identification of the cells, and further characterized the GAS cells using the MAb. BALB/c mice were intradermally injected with 1.0×106of UEA-1 positive cells separated from gill epithelium of rainbow trout. Hybridomas were generated by conventional techniques, and the culture supernatants were first screened using flow cytometry with live UEA-1 positive GAS cell that had taken up *A.s.s.* bacterin. MAb-positive cells were further sorted by flow cytometry and their smears were stained by the May-Grünwald-Giemsa method. Furthermore, the MAb was subjected to immunofluorescence assay on frozen gill sections fixed with acetone or paraformaldehyde (PFA), and on paraffin sections of gills fixed with Davidson's solution. A MAb, designated as 2B4-1 specifically bound to UEA-1^{+}*A.s.s.*$^{+}$ GAS cells. Flow cytometric analysis showed that 2B4-1 positive cells accounted for 31.1% in the gill epithelial single cell suspension, with high-side scatter. As expected, 2B4-1 positive cells were highly granulated as seen by May-Grünwald Giemsa staining. MAb 2B4-1 was suitable not only for staining of frozen section, but also for staining of paraffin section. In those sections, cells stained with MAb 2B4-1 were often observed in the basement of the secondary lamellae. Thus, MAb 2B4-1 is a powerful tool to study the role of GAS cells in mucosal immune system of gill.

Abstract ID: 111F(Poster &Elevator Pitch)

PROTECTIVE GRANULOMA CONTROLS NON-MOTILE *Edwardsiella TARDA* INFECTION IN VACCINATED RED SEA BREAM *Pagrus major*

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Edwardsiellosis of red sea bream Pagrus major is caused by the intracellular non-motile (atypical) *Edwardsiella tarda*. The disease was characterized by the presence of granulomatous inflammations or granuloma in fish tissue. We previously demonstrated effectiveness of the inactivated bacterin in protecting red sea bream from the disease which lasts for at least 4 months post infection. In the acute stage of infection, the bacterial growth inside phagocytes in vaccinated fish was reduced but with no total bacterial clearance. In the present study, further investigations of the protection mechanism in vaccinated fish in the late stage of infection were done by the histopathological or immunohistochemical techniques. Focal accumulation of phagocytic cells in necrotic tissue foci as the initial stage of granuloma formation was detected after 4 days of infection in the posterior kidney, spleen and hepatopancreas of the vaccinated as well as non-vaccinated fish. Granulomas at different stages were found in all organs, particularly posterior kidney, after 2 weeks of infection. Based on the composition, developmental stages of granulomas in red sea bream were classified into 3 major types: developing granulomas, protective granulomas or non-protective granulomas. High abundance and final shrinkage of the protective granulomas were mostly found in the vaccinated fish. Meanwhile, continuous presence of non-protective granulomas with spreading antigen were dominant in the non-vaccinated fish. In addition, melanomacrophage centers (MMC) (granulated and degranulated) were found in large amounts in the organs and associated with more than half of the granulomas developed in the vaccinated fish. Accordingly, it is concluded that granuloma formation process is very critical for bacterial clearance in red sea bream edwardsiellosis. Protective granulomas are developed in vaccinated red sea bream for final clearance of atypical *E. tarda* infection. The MMC is thought to be another factor contributing in the protection conferred by the vaccination.

Abstract ID: 142F(Poster &Elevator Pitch)

EFFECTS OF REPLACEMENT OF FISH MEAL WITH YEAST NUCLEOTIDES ON GROWTH, NON-SPECIFIC IMMUNITY AND INTESTINAL MORPHOLOGY OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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An 8-week feeding trial was conducted to evaluate the effects of replacement of fish meal with yeast nucleotides (NT) on growth, non-specific immunity and intestinal morphology in Pacific white shrimp (*Litopenaeus vannamei*). Four isonitrogenous and isolipidic practical diets were formulated to contain 0%, 1%, 3% and 5% of a yeast-originated NT mixture; the replacement levels of fish meal with the NT diets were 0%, 8.7%, 24% and 40%, respectively. A total of 480 shrimp with an average initial body weight of 1.86 ± 0.01 g were randomly allocated into four groups, with four replicates per group and 30 shrimp per replicate. The results indicated that weight gain rate (WGR) and specific growth rate (SGR) were significantly affected by the replacement of fish meal with NT, but significant differences in survival rate among treatments were not observed under the conditions tested. Shrimp fed the diet containing 5% NT had a significantly higher WGR, SGR and protein efficiency rate (PER) than those fed the 1% NT diet, and the lowest feed conversion ratio (FCR) occurred in the 5% NT group. Crude protein of whole shrimp was significantly higher in the 5% NT group compared with controls. The replacement of fish meal with NT significantly affected total protein and triglyceride, and the activities of aspartate aminotransferase and alanine aminotransferase, in serum. The activities of serum phenoloxidase (PO) and lysozyme (LZM) were significantly increased in shrimp fed the diet containing 5% NT. Total nitric oxidesynthase activity increased with increased NT supplementation, but the effect was not significant. The expression of alkaline phosphatase (ALP) and LZMwas significantly up-regulated in the 3% NT group. Overall, it suggests an enhancement effect of the NT diet on the non-specific immune response of shrimp. Lastly, shrimp fed with the 3% NT diet had a significantly higher intestinal fold height (hF) and fold width (wF) than those fed the control diet; microvillus height was significantly increased in the 5% NT group. In summary, this study showed that a shrimp diet supplemented with 5% yeast NT gave the best performance in terms of WGR, SGR, PER and FCR. Some enzymes, such as PO and LZM, were also significantly increased. On the other hand, the 3% NT diet resulted in a higher expression of certain genes (ALP, LZM) and better intestinal morphology parameters.

Abstract ID: 219F(Poster &Elevator Pitch)

PHAGOCYTOSIS BY DIFFERENTIAL INVOLVEMENT OF MARRON (*Cherax cainii*) HAEMOCYTES WITH LIVE OR HEAT-KILLED *Vibrio mimicus* AS MEASURED BY FLOW CYTOMETRY AND THEIR NITRIC OXIDE ACTIVITY STUDIES

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Treatment with live *V. mimicus* resulted in an increase in phagocytosis for all three cell types compared to no treatment (minimum p value of <0.0009), and a general trend of increased phagocytosis with time. There was a statistically significant difference at all time-points between HCs and both SGCs and LGCs (minimum p value of <0.0004) and no statistical difference between SGCs and LGCs. The highest levels of phagocytosis were seen at 8 hours but these failed to attain significance from 2 and 4 hours, within each cell type: for LGCs, this was largely due to a high level of sample variation. When treated with heat-killed *V. mimicus*, the pattern of phagocytic activity by the haemocytes was similar to that seen for live *V. mimicus* treatment. Again, there was a statistically significant difference between treatment and no treatment (p<0.0001) and between HCs and both SGCs and LGCs at all time-points (minimum p value of <0.05) and only statistical difference between SGCs and LGCs at 8 hours treatment (p<0.03). The comparison between live and heat-killed *V. mimicus* treatments for each cell type revealed that there was only statistical significance in phagocytic activity between treatment and no treatment and between treatment types (i.e. live vs heat-killed) for HCs alone (minimum p value of <0.004 across the three time-points). Although there was a trend for higher phagocytic activity due to the heat-killed treatment at 2 and 4 hours for SGCs and LGCs, both treatments resulted in similar phagocytic levels at 8 hours. In *C. cainii* haemolymph, live *V. mimicus*-stimulated NO production was significantly higher at 20 and 25 °C compared with 30 °C (p<0.0001). However, with respect to the timing of treatment, incubation with live *V. mimicus* did not produce any variation in the concentration of NO implying that response is over by 2 hours. In addition, treatment of *C. cainii* haemocytes with live *V. mimicus* caused a significant increase in nitrite production with respect to each control group. The NO levels in *C. cainii* haemocytes after exposure by heat-killed *V. mimicus* at 20 and 25 °C was significantly higher than at 30 °C (p<0.0001). Incubation with heat-killed *V. mimicus* did not result in any variation in the concentration of NO, with regard to the time points noted except for heat-killed at 30 °C which showed an increase at 8 hours (p<0.01). For the control groups, NO production was relatively stable over the experiments however, their mean values at lower temperature were higher than at 30 °C (p<0.0001). Nitric oxide production in the control group was lower than heat-killed *V. mimicus*-stimulated NO production. Both live and heat-killed *V. mimicus* showed similar patterns in NO stimulation, with heat-killed *V. mimicus* showing slightly higher levels than live *V. mimicus* for the 20 °C and 25 °C treatments. Both 20 and 25 °C incubation temperatures demonstrated significantly higher NO concentrations compare to 30 °C (p<0.0001). Even though NO concentration was higher for 20 °C it was not significantly different from 25 °C. With respect to time-points, there was little difference in NO concentration for live and head-killed *V. mimicus* except for the heat-killed, 30 °C treatment.

Abstract ID: 021F (Poster &Elevator Pitch)

EVALUATION OF THE POTENTIAL OF INTEGRIN ? SUBUNIT AS A MARKER FOR HEMOCYTES IN KURUMA SHRIMP *Marsupenaeus japonicus*

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In crustaceans, including shrimp, circulating hemocytes contribute both cellular and humoral immune responses such as phagocytosis, encapsulation, melanization, opsonization, etc. Conventionally, crustacean hemocytes are characterized into three sub-populations, and different sub-populations are considered to function differently. Up to now, however, the hemocyte characterization have been based on morphology observed under the microscope. Therefore, establishment of molecular markers to distinguish hemocytes is required for the further immunological studies. Our previous study revealed that the mRNA of *Marsupenaeus japonicus* integrin alpha subunit (Mj-intg?) is strongly accumulated in phagocytic hemocytes compared with total hemocytes. In this study, we have evaluated the potential of Mj-intg? as a marker for hemocytes.Firstly, full length mRNA of Mj-intg? was obtained by RACE-PCR based on the previous RNA-seq result. Secondly, mRNA level of Mj-intg? were detected by RT-PCR in various organs and in phagocytic hemocytes. Furthermore, a recombinant protein of a specific portion of Mj-intg? was produced by *Escherichia coli*, which was used to produce rabbit antiserum against recombinant Mj-intg?. Antiserum reactivity against Mj-intg? was confirmed by Western-blot analysis. Finally, localization of Mj-intg? was detected by immunostaining in both total and phagocytic hemocytes. Mj-intg? consists of 1,111 amino acids residues and has a signal peptide, four integrin? domain and a trans-membrane region. mRNA level of Mj-intg? were detected in total hemocytes, hearts and lymphoid organ, as well as phagocytic hemocytes. By Western-blot analysis, antiserum reacted against 80-90 kDa molecules from hemocytes, which is considered as the light chain of Mj-intg?. By immunostaining, 60-70 % of total hemocytes and 80-90 % of phagocytic hemocytes were positive for Mj-intg?. Moreover, signal pertaining to Mj-intg? was detected on the surface of hemocytes. Using Mj-intg? as basis, hemocytes were divided into two groups: Mj-intg?-positive and –negative hemocytes. Analysis of hemocytes based on Mj-Intg? as a molecular marker may pave the way for functional characterization of shrimp hemocytes.

Abstract ID: 107F (Poster &Elevator Pitch)

EFFECT OF WHEY TOFU ON PHYCOCYANIN OF MICROALGAE *Spirulina platensis* AS CANDIDATE FOR ANTIOXIDANT AS IMMUNITY SYSTEM

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Phycocyanin of *Spirulina platensis* has been investigated as antioxidant. *S. platensis* need optimum nutrients to produce phycocyanin. Whey Tofu is a material with enough nutrients for *S. platensis.* The aim of this research is to know effect of whey tofu on phycocyanin of *S. platensis* and antioxidant activity of phycocyanin. The use whey tofu is according this dosage : 4 ml/L, 5 ml/L, 6 ml/L. Culture *S. platensis* for 6 days and measurement of water quality, then extraction of phycocyanin by buffer phosfat Ph 7 and antioxidant test with with DPPH method. The result of this research show that dosage of whey tofu 4ml/L give the highest phycocyanin with 0.15 mg/ml and best antioxidant activity with 3.19 mikrogram/ml as the lowest IC 50. Water quality of this research is in good range for growth of *S.platensis* with range between 25,33 – 30°C for temperature; Ph 9.08 – 9.35 ; salinity 25-27 ppt; oxygen dissolved 6.25-6.95 mg/L ; nitrate 1.54 – 3.7 mg/L and phosphate 0.008-0.973 mg/L. From this research show that dosage of whey tofu 5ml/L can increasing phycocyanin of *S.platensis.* More research show that dosage of whey tofu 5ml/L can increasing phycocyanin of *S.platensis.* More research is needed about relation between harvest time and phycocyanin and then about reaction of phycocyanin for fish and crustaceae.

Abstract ID: 177F(Poster &Elevator Pitch)

COSTIMULATORY SIGNALS IN A TELEOST FISH: CD28 INTERACT WITH CD80

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In mammals, T cell activation requires a combination of signals, one of which is provided by the interaction of CD28 with CD80 molecules. In this study, we cloned a CD28 molecule (On-CD28) and a CD80 molecule (On-CD80) from Nile tilapia (*Oreochromis niloticus*). Sequence analysis showed On-CD28 protein is composed of 218 residues, and consists of a signal peptide at the N-terminus, an extracellular region, a transmembrane domain and a cytoplasmic region. The proline-based motif (^{117}TYPPPL^{122}), which is similar to the MYPPPY motif in mammals and essential for CD28 binding to B-7 ligands. On-CD80 protein is composed of 298 residues, which is consists of a extracellular Ig variable region-like domain, an Ig constant region-like domain followed by a TM domain and a cytoplasmic tail. And the residues Y64, Q66, D70, E119 and K133, which are critical for the interaction between mammalian CD80 and CTLA-4, are conserved in On-CD80. In healthy tilapia, expression of On-CD28 and On-CD80 transcripts were both mainly detected in immune relevant organs, especially in lymphocyte. Moreover, there was a clear time-dependent expression pattern of On-CD28 and On-CD80 after infected by *Streptococcus agalactiae*. Signi?cantly, Yeast Two-Hybrid showed that On-CD28 could interact with On-CD80. These ?nding indicates that the capacity to modulate T cell activation is a primordial function that has been conserved both in ?sh and mammalian CD28 interact with CD80.

Abstract ID: 004F (Poster)

DEVELOPMENT OF LOCAL ISOLATE VACCINE OF Streptococcus SP. TO PREVENT STREPTOCOCCOSIS IN JICA TILAPIA STRAIN (Oreochromis sp.)

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Sumatra Island is one of tilapia production area in Indonesia, with JICA tilapia as dominant strain. This strain is production of Sungai Gelam Freshwater Aquaculture Development Centre (FADC), result of cooperation with Japan Government (JICA). JICA strain is popular in tilapia farm at Sumatera since commercial release at 2004 until today (2016). Fish health problems in JICA tilapia production that can't overcome is streptococcosis caused by *Streptococcus* sp. bacteria. This disease effect high mortality and failed harvest. Preventive solution suggested by the government using vaccine, which is currently Streptococcus vaccine available commercially. Effective disease control with vaccine could be achieve, if vaccine that we use have high compatibility with pathogen agent in the farm. This is because bacteria have many variations and biochemical characteristics, although in one species. Commercial vaccine usually using strain bacteria that frequently outbreak and had high pathogenicity. Therefore, often using commercial vaccines for controlling streptococcosis disease at tilapia production area in Sumatra Island unsuccessful. Vaccine from local isolate would be more effective to control this bacterial disease. Sungai Gelam FADC conducted vaccine development that obtained from *Streptococcus* bacterial disease attack at tilapia production area, Sungai Batanghari, Jambi in 2015. Isolate taken from sick tilapia, once refine, identified as gram positive bacteria *Streptococcus agalactiae*. Furthermore vaccine synthesis conduct with heat killed method to obtain bacteria antigen, after that stored in PBS liquid. For measure effectiveness, vaccine tested to JICA tilapia (weight 3.60± 0.17 gr, and 6.53 ± 0.10 cm total length). Vaccine injected via intramuscular (IM), challenge test with active bacteria conducted after 21-day post vaccine. Challenge test with *Streptococcus agalactiae* (1.5×10^5 cell/ml) injection for three trial groups (30 fish each, with 3 replicate); 1) Local isolate vaccine; 2) Commercial *Streptococcus* vaccine for tilapia; 3) non-vaccine (PBS injection/control). Observations for a week post challenge test obtained the survival of fish each group; commercial vaccine 76,67 %, local isolate 78,04 %, and non-vaccine 18,89 %. Relative percent survival better for local isolate vaccine (72,83%) compare to commercial vaccine (71,07%). While for fish daily growth rate percentage, no significant different in all groups trial.

Abstract ID: 037F (Poster)

GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSES OF COBIA (*Rachycentron canadum*) ENHANCED BY *Scutellaria radix* WATER EXTRACT AGAINST *Photobacterium damselae* subsp. *piscicida.*

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Cobia meat contains a higher of EPA and DHA that become a good candidate for amarine protein source. As well as another aquaculture species, cobia culture was also vulnerable to the disease resistance that caused mass mortality of fish which caused economic losses. Plant extract has been several years studied on the fish culture to enhance the growth performance of fish, immunostimulant to enhance the fish immune responses and to treat and prevent fish disease with greater accuracy than a chemotherapeutic agent. The active compound of plant extract was primarily active in the digestive tract or the sensory organs associated with feeding behavior. *Scutellaria radix* has been listed in a Traditional Chinese Medicine (TCM) are used to enhance the growth performance, innate immunity and disease resistance of cobia against *Photobacterium damselae* subsp. *piscicida.* Fish diet containing different concentration of *S.radix* water extract (0%, 0.5%, 1%, 2%, 3% and 5%) were feed to the cobia for 30 days in three replicate. Humoral immune responses, growth performance and gene expression of fish were examined at 7, 14, 21, and 30 days of feeding and the challenged was done on the 30th day. The result of this study showed that 1% concentration *S.radix* extract added on the fish feed, can enhance the growth performance with lower of feed utilization (FCR 0.71). In addition, the humoral immune responses of cobia such as MPO, PO, lysozyme activity was significantly increased. Of the gene expression, TLR-9, IL-1β, IgM, and TNF-α in the spleen were significantly increasing on the cobia feed with 1% *S.radix* water extract. Since all of these cytokines are play and important role in the innate immune system, the up-regulating of humoral responses and gene expression of the cobia becomes the main reason for the higher survival rate of cobia in the treatment group compared with control after challenged by bacteria pathogen. The transcription of the immune-related gene was higher in the IgM followed by TNF-α, IL-1β, and TLR-9A. Plant extract has been confirmed can enhance the innate immune responses such as macrophages, monocytes, granulocytes and humoral elements of immune. The result of this experiment suggests that 1% concentration of *S.radix* added to the cobia fed can enhance the growth performance, humoral immune responses, gene expression, and increasing the disease resistance in cobia against *Photobacterium damselae* subsp. *piscicida* infection.

Abstract ID: 074F (Poster)

EVIDENCE OF AN ADAPTIVE HUMORAL IMMUNE RESPONSE IN THE ANCIENT ASIAN AROWANA (*Scleropages formosus*)

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The Asian Arowana (*Scleropages formosus*) is an ancient fish that can be traced back to the Cretaceous period around 145 million years ago. Asian Arowana belongs to fishes of the order Osteoglossiformes and is the earliest type of bony fish that can only be found in freshwater habitats. An IgM-like immunoglobulin molecule was isolated and purified from the serum of mature Asian Arowana. The purified immunoglobulin was further characterised using specially raised mouse polyclonal antibody against the Arowana IgM-like immunoglobulin in optimised immunoassays. The electrophoretic profiles of the immunoglobulin heavy and light chains were determined using sodium dodecyl sulfate-polycrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Immunoreactivity of the Arowana IgM-like immunoglobulin was then determined using the western blot assay. The polyclonal anti-Arowana IgM-like immunoglobulin antibody was also employed to investigate the humoral response of the Asian Arowana over a period of 35 days. Juvenile Arowana were immunised intraperitoneally with an inactivated bacterin and the Arowana immunoglobulin levels were determined using indirect ELISA. This investigation provides evidence for an adaptive immune response against a bacterial pathogen in the Asian Arowana.

Abstract ID: 076F (Poster)

EFFECTS OF *Bacillus sp.* SW1-1 AS DIETARY ADDITIVES FOR GROWING OLIVE FLOUNDER *Paralichthys olivaceus*.

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This study was conducted to investigate dietary *Bacillus sp.* SW1-1 on growth performance, feed utilization, hematological parameter, innate immunity and disease resistance for growing olive flounder. A basal diet (without Bacillus sp.) was considered as a control and two other diets were prepared by adding Bacillus sp. into the basal diet at 0.25% and 0.5%. Quadruplicate groups of olive flounder (average initial body weight, 153±1g) were fed one of the experimental diets to apparent satiation twice daily for 17 weeks. At the end of the feeding trial, weight gain, specific growth rate and feed utilization were not significantly different among them. Survival rate was numerically increased by the supplementation of *Bacillus sp.* SW1-1. However, significantly higher disease resistance was observed in fish group fed 0.5% diet compared to the fish group fed the control diet. Also, we found that the supplementation of *Bacillus sp.* SW1-1 in diets significantly increased innate immunity of olive flounder. The detailed results on hematological parameter and innate immunity will be discussed further.

Abstract ID: 120F (Poster)

EFFICACY EVALUATION OF IFN-Γ ON INNATE IMMUNE RESPONSE IN *Labeo rohita*

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Interferon-γ (IFN-γ) systematizes a diverse array of cellular response through transcriptional regulation of immunologically relevant genes against several intracellular pathogens like *Edwardsiella tarda* (*E. tarda*). The present experiment investigated the effect of administrating IFN-γ plasmid construct on two enzymatic pathways *viz.* glycolysis and cholinergic anti-inflammatory pathway in *Labeo rohita* (*L. rohita*) by evaluating lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) enzyme activities respectively. The LDH and AChE enzymes are important marker for defense mechanism of the host against microbial invasion. In addition, the expression kinetics of Mx gene transcript in response to the IFN-γ administration was also studied. In this context, an *in vivo* experiment was set up, in which *L. rohita* (20±6.6g) were immunized (IM injection) with IFN-γ plasmid construct and a subsequent booster dose was applied after 15 days of initial immunization. Simultaneously, a control group (injected with PBS) and a naïve group were maintained for determining the effect of IFN-γ plasmid construct. Both the treatment group and the positive control group were eventually challenged with *E. tarda* (8.7×10^5 Cfu / fish) 35 days post immunization. Serum and kidney tissue were collected at different time intervals for enzymatic assays and gene expression studies respectively. The enzymatic assays displayed higher LDH and AChE activities after the initial treatment with the plasmid construct when compared with the control group. However, the enzyme activities gradually subside to normal range post booster immunization due to pre-sensitization with the same construct. Likewise, the Mx gene transcript also showed similar trend in expression pattern post treatment. But after the bacterial challenge, Mx gene transcript showed significant upregulation at 24 and 48 hour post challenge (hpc) infection stages, and remained high until 96 hpc in contrast with the lower level of expression in the control group. Moreover, the LDH and AChE activities significantly decreased after bacterial challenge irrespective of groups but their level of activity varies. From these findings, it can be inferred that IFN-γ plays a pivotal role in defence mechanisms of the host against *E. tarda* infections.

Abstract ID: 145F (Poster)

HEMOCYANIN OF *Litopenaeus vannamei* AGGLUTINATES *Vibrio parahaemolyticus* AHPND (VP_{AHPND}) AND NEUTRALIZES VP_{AHPND} TOXIN

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Acute hepatopancreatic necrosis disease, AHPND, caused by a specific strain of *Vibrio parahaemolyticus,* results in great loss of global shrimp production. However, the knowledge on defense mechanism against this disease is still limited. Previously, suppression subtractive hybridization (SSH) was performed to identify differentially expressed genes from white shrimp *Penaeus vannamei* hepatopancreas upon *V. parahaemolyticus* AHPND infection at the early stage: 3 and 6 hours post infection (hpi) and the late stage: 48 hpi. Hemocyanin is the most abundant gene identified from SSH library. In this study, various hemocyanin subunits such as hemocyanin, hemocyanin subunit L1, L2, L3, and L4 were analyzed for the expression level upon *V. parahaemolyticus* AHPND infection and in response to challenge with crude toxin of *V. parahaemolyticus* AHPND (VP_{AHPND} toxin) by qRT-PCR. Hemocyanin was highly up-regulated at 3 hpi. Hemocyanin subunit L2 and L3 were up-regulated at the early phase of VP_{AHPND} toxin injection and all subunits were down-regulated at 48 hpi. Native hemocyanin protein was purified from shrimp hemolymph and used for functional study. Upon incubation with purified hemocyanin *in* vitro, agglutination of *V. parahaemolyticus* AHPND could be observed. Injecting hemocyanin along with *V. parahaemolyticus* AHPND into shrimp decreased the bacterial counts in the hemolymph while *in vitro* treatment of *V. parahaemolyticus* AHPND with hemocyanin reduced the amount of bacteria quantified by plate count technique. Moreover, pre-incubation of hemocyanin and AHPND toxin before injection into shrimp resulted in the decrease of cumulative mortality of shrimp when compared to the control. Our result indicated that upon *V. parahaemolyticus* AHPND infection the expression of hemocyanin was induced. Then, it agglutinates invaded *V. parahaemolyticus* AHPND and neutralizes AHPND toxin in shrimp.

Abstract ID: 146F (Poster)

*Penaeus monodon*VIRAL RESPONSIVE PROTEIN 15 (*PM*VRP15) INVOLVED IN REGULATION OF HEMOCYTE HOMEOSTASIS

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The viral responsive protein 15from black tiger shrimp *Penaeus monodon* (*Pm*VRP15) is highly up-regulated and mainly expressed in shrimp hemocyte upon white spot syndrome virus (WSSV) infection.Suppression subtractive hybridization (SSH) of *Pm*VRP15 knockdown WSSV-challenged shrimp identified the hemocyte homeostasis associated protein (*Pm*HHAP) as the highest up-regulated gene and transglutaminase II (TGSII) as the highest down -regulated gene. Quantitative real time RT-PCR showed that *Pm*HHAP gene was 195–fold up-regulated and TGSII was 11–fold down-regulated in *Pm*VRP15 knockdown shrimp at 24 h post -WSSV infection. To characterize *Pm*VRP15 function, the relationship between either *Pm*HHAP or TGSII with *Pm*VRP15 was determined. *Pm*HHAP, an inhibitor of apoptosis, is a key player in controlling hemocyte homeostasis. In *Pm*VRP15 knockdown shrimp that has extremely high expression of *Pm*HHAP during WSSV infection, the caspase-3 and -7 activity did not change suggesting that *Pm*VRP15 was not involved in hemocyte apoptosis. Whereas, TGSII has been reported that to play an important role in inhibition of hematopoietic tissue differentiation or hemocyte production in crustacean. That is decrease of TGII gene expression in *Pm*VRP15 knockdown shrimp after WSSV infection might lead to an increase in amount circulating hemocyte. As expected, the total circulating hemocyte number of *Pm*VRP15 knockdown shrimp was significantly decreased at 24 and 48 hours post WSSV infection. This result indicates that *Pm*VRP15 possibly plays a regulatory role on hemocyte homeostasis in shrimp.

Abstract ID: 154F (Poster)

Aeromonas hydrophila VACCINE APPLIED IN CATFISH CULTURE

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Fish health and environment management on dissemination program for fish farmer in aquaculture society is the basic principle in the development of fish health management system. This study purposed to determine effectiveness of *Aeromonas hydrophila* vaccine on growth, survival and feed conversion ratio (FCR) of catfish (*Clarias* sp) culture. The study was conducted from March to May 2015 held on "Mina Sejahtera", a group of fish farmer, located in Desa Umbul Tanjung nearby the SIFHE. Vaccination method performed by immersion. About 2,500 of 5-6 cm catfish juveniles were vaccinated with 30 ml of *A. hydrophila* vaccine in 10 liters of water during 15-30 minutes. The other juveniles with the same number were unvaccinated. About 15 m x 10 m x 1 m pond was used to catfish culture with static water system. The pond was divided into 2 groups. There were vaccinated and unvaccinated groups (control). The feed was fed about 2-3 times per day with dose 5% of biomass. Specific growth rate (SGR) was observed every 2 weeks. Survival rate (SR) and FCR were observed on the last observation. The results showed that SGR in length (P = 0.004) and weight (P = 0.007) were significantly different between vaccinated and control with mean 1.97% and 1.78% in length and 6.07% and 5.53% in weight respectively. Survival Rate (SR) for vaccinated juveniles and control were 94.48% and 85.68% respectively. The FCR for vaccinated and control were 1.17% and 1.42%. Vaccine has been providing good results indirectly to fish farmer's income and controlling fish disease in catfish culture.

Abstract ID: 220F (Poster)

IMMUNOLOGICAL ASSESMENT OF MARRON (*Cherax cainii*) HAEMOCYTES TO BACTERIAL LIPOPOLYSACCHARIDE AT DIFFERENT TEMPERATURE IN VITRO USING FLOW CYTOMETRIC ANALYSIS

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Marron (*Cherax cainii*) is, one of the world's largest freshwater crayfish, native to Western Australia. *C. cainii* is recognised as a potential aquaculture species due to their attributes. Recently, *C. cainii* was introduced into some countries around the world as a commercial aquaculture species. Lipopolysaccharide (LPS), glycolipids found in the outer leaflet of the outer membrane of Gram-negative bacteria, was able to activate the pro-phenoloxidase system and also strong stimulators of many crustacean species haemocytes in vitro. In this study, the in vitroeffects of LPS from *Escherichia coli* with concentrations at 0.25 and 0.5 mg/ml on the *C. cainii* haemolymph were assessed flow cytometrically using forward- and side-scatter light parameters using changes in differential haemocyte counts (DHC). Moreover, it also investigates the differential effects of temperature acclimatization (20, 25 and 30°C) on LPS tolerance. The haemocyte subpopulations of *C. cainii* are divided into hyaline (HCs), semigranular (SGCs) and large granular (LGCs) cells.Treatments with LPS at the lowest temperature, 20 °C, resulted in the earliest change in population profile: consisting of a low-moderate change starting from 0 hours, although the only difference was with the LPS at 0.25 mg/mL. By 4 hours both LPS treatments resulted in overall profile changes compared to the control, to the moderate, progressing to high by 8 hours. There was little difference between both LPS treatments. There was moderate change in the haemocyte profiles for the treatments incubated at 25 °C only from 2 hours onwards, and only for the 0.5 mg/mL LPS treatment. Interestingly, the profiles were consistent for 2 to 8 hours. The 30 °C incubations resulted in profile change only starting at 4 hours however, they were moderate and high for 0.25 and 0.5 mg/mL LPS compared to control, with only a low-moderate change between them. The highest change was seen for the 30 °C incubations for 8 hour. From the heat map, the overall patterns of change due to LPS treatments compared to controls and each other can be seen clearly: there was similarity between the 20 and 30 °C incubations with both LPS treatments being moderate to high compared to control, at 4 and 8 hours, but with little difference between LPS treatments. This contrasted with treatments at 25 °C, where most of the change, moderate, was only seen for the 0.5 mg/mL LPS treatment. The changes in profile were due to changes in the 3 haemocyte cell types, relative to each other. Whenever there was a change in profile, this corresponded to an increase in HCs with a concomitant decrease in SGCs and LGCs. The exception was the 0.25 mg/mL treatment at 20 °C, where an increase in HCs resulted in a small increase in LGCs and a decrease only in SGCs, not including the 8 hour incubation.

Abstract ID: 221F (Poster)

THE EFFECTS OF LIVE OR HEAT-KILLED *Vibrio mimicus*AS INFLAMMATORY STIMULUS ON MARRON (*Cherax cainii*) HAEMOCYTES AT DIFFERENT TEMPERATURE IN-VIVO USING FLOW CYTOMETRIC ANALYSIS

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The Gram-negative bacterium, *Vibrio mimicus*, was use as a model pathogen for the present study. Outbreaks resulting from *V. mimicus* have been reported in Australia for several years. *Vibrio mimicus* was the predominant organism isolated from systemic infections of cultured crayfish and was responsible for stock mortalities. In this study, the *in-vivo* effects of live and heat-killed *Vibrio mimicus* on the marron *Cherax cainii* haemolymph were investigated using changes in differential haemocyte counts (DHC) and to investigate the effects of bacterial infection with respect to different temperatures. Flow cytometry was used to evaluate changes.Treatments with live *V. mimicus,* at the lowest temperature 20 °C, resulted the greatest change, although only at low to moderate levels for overall haemocyte profiles. There were only minor changes between heat-killed treatments and controls. Most of the change for the live bacteria treatment was seen in the HCs (decrease) and LGCs (increase) early on, with the opposite response for HCs at 24 and 48 hours and primarily a reduction in SGCs. The greatest change in haemocyte profiles were seen at 25 °C. This change was mostly seen for the live *V. mimicus* treatments, resulting in moderate high levels of changes. In this treatment, HCs increased in proportion while both SGCs and LGCs decreased and this was consistent for 8 to 48 hours. Again, the heat-killed *V. mimicus* resulted in a change compared to control. The treatments at 30 °C incubations was the least changed, with only a low to moderate change in profile at 8 hours for the heat-killed treatment. However, they were low for both *V. mimicus* treatment compared to control, with only a low to moderate change between them. From the heat map, the overall patterns of change due to *V. mimicus* treatments compared to controls and each other can be seen clearly: there was similarity between the 20 and 30 °C incubations with both *V. mimicus* treatments being low to moderate compared to control, at 8 and 24 hours, but with little difference between *V. mimicus* treatments. This contrasted with treatments at 25 °C, where most of the change was only seen, but only for the live *V. mimicus* treatment.

Abstract ID: 018F (Poster)

INFLUENCE OF BODY SIZE ON SUSCEPTIBILITY TO FUNGAL INFECTION CAUSED BY *Ochroconis humicola* IN MARBLED ROCKFISH, *Sebastiscus marmoratus*

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Ochroconis infection caused by *Ochroconis humicola* was reported in juvenile stages in Japanese aquaculture marine fish but not in adult. Therefore, body size of fish may affect their susceptibility *to Ochroconis* infection. Group of marbled rockfish, *Sebastiscus marmoratus* were separated according to body size, small (2.3-2.6cm), medium (5.5-6.6 cm) and large (7.5-8.5cm). Each size class was experimental challenged with conidia *Ochroconis humicola* NJM 1503 by two different trials. For the first trial, head part of fish was gently scratched and 10^5 cells of conidia suspension was dropped on the scratched part. Intraperitoneal injection of the 10^5 cells conidia suspension was performed for second trial. Small fish in both trials were died within 30 days. After 30 and 56 days, medium and large fish in first and second trial were sacrificed respectively. Mortality in both trial of large fish was not observed until the sampling day. Infected fish from first and second trial showed ulceration on the head part and abdomen respectively. Head part was collected for the first trial where spleen, kidney and liver in the second trial. These fish samples were routinely embedded in paraffin and sectioned at 5µm. The serial sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) reaction and Schmorl method. Histopathologically, in the first trial, large number of hyphae was observed in the brain tissues of infected small and medium fish while absent in large fish. In the second trial, large number of hyphae was clearly seen in the spleen, liver, adipose tissues and kidney. These hyphae showed positive reaction with PAS and Schmorl method, thus indicated the presence of melanin in the hyphal walls. Severe inflammatory responses associated with granuloma formation was observed in the infected fish. Based on the results, rockfish with size smaller than 7.5cm was susceptible to *Ochroconis* infection.

Abstract ID: 023F (Poster)

PROTEOMIC ANALYSIS WITH 2D-PAGE OF LIVER PROTEIN EXTRACTED FROM JAPANESE FLOUNDER AFFECTED WITH *Edwardsiella piscicida*

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Edwardsiella piscicida, formerly classified as *E. tarda*, is the causative agent of edwardsiellosis in the Japanese flounder *Paralichthys olivaceus*. Abscesses develop in the kidney or liver in the infected fish with the bacterium. In early stages of infection, liver cells undergo hypertrophy before the bacterial invasion and the abscess formation become evident in the organ. However, the direct cause of the hypertrophy is still uncertain. In the present study, we conducted proteomic analysis of hypertrophied liver cells of diseased fish by comparative two-dimensional gel electrophoresis (2D-PAGE).Immersion challenge with the bacterium was performed to prepare hypertrophied livers of Japanese flounder. Two groups of fish (n=10/group) were immersed in either 20 L of sea water containing 8.0×10^7CFU/mL of *E.piscicida* or an equal volume of sea water without the bacterium for 30 min. Each group was reared in a 60 L aquarium filled with 30 L of running seawater at 22^{O}C. Five days after the challenge, the liver was excised from each fish, and subjected to protein extraction and histopathology. In the 2D-PAGE analysis, the proteins in each extraction were separated by isoelectric focusing with the IEF strip gel (pH range 3-10), and then the strip gel was applied to SDS-PAGE. Protein of each spot was quantified by the image analysis software PDQuest. The proteins wereidentified with MALDI-QIT-TOF mass spectrometry. When the livers were sampled, enlargement of the organ were confirmed in the challenged group, and hypertrophy of hepatocytes in those livers were observed with histopathology. Larger amounts of proteins of the challenged fish than those of the controls were found in 62 spots in the 2D-PAGE. Among these were staphylococcal nuclease domain-containing protein, heat shock protein 90β, endoplasmin, apolipoproteins, transferrin, ferritin, peroxiredoxin, aspartate aminotransferase and lipocalins. The increased production of those proteins may be related to the hypertrophy of liver cells in Japanese flounder infected with *E. piscicida*.This work was supported in part by JSPS KAKENHI Grant-in-Aid for Exploratory Research number 16K07882 from the Ministry of Education, Culture, Sports, Science and Technology.

Abstract ID: 040F (Poster)

DISTRIBUTION OF MHC CLASS Ⅱ POSITIVE CELLS IN JAPANESE FLOUNDER, *Paralichthys olivaceus*

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MHC class Ⅱ molecule is expressed on cell surface of antigen－presenting cells, which interacts with T cell receptor during antigen presentation. This molecule consists of a heterodimer of α chain and β chain. Here we investigated distribution of MHC class Ⅱ positive cells in various organs of Japanese flounderby *in situ* hybridization. Paraffin sections were prepared from gill, intestine, an anterior part of head (proboscis), spleen, kidney and liver. *In situ* hybridization was performed using a digoxigenin－labeled cRNA probe for MHC class Ⅱ α chain of Japanese flounder. MHC class Ⅱ positive cells could be detected in the gill epidermis, epithelium and lamina propria of the intestinal mucosal tissue, epithelium of the nasal cavity, parenchyma in the marginal region of the spleen, tubular epithelium and tissue stroma of the kidney. By contrast, it could not be detected in the liver. These results suggested that antigen－presenting cells of Japanese flounder are located in the lymphoid organs and the mucosal－associated lymphoid tissues (MALTs).

Abstract ID: 059F (Poster)

DEVELOPMENT OF PEPTIDE ANTIBODY FOR IMMUNOGLOBULIN DETECTION IN FISH

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Antibody responses are one of the key features for adaptive immunity. Detection of antibody is normally performed by using antibody specific to IgM of each fish species. However, there are many economically important fish species which have been cultured in Japan. To reduce the cost and time for development of antibody and detection of immune responses in many fish species, tools available for detection of antibody responses in various fish species are needed. In this study, two synthetic peptides (fish IgH-1 and fish IgH-2) were designed based on the conserved sequence of fish immunoglobulin heavy chain including Japanese flounder *Paralichthys olivaceus*, seabream *Pagrus major*, yellowtail *Seriola quinqueradiata*, carp *Cyprinus carpio* L., rainbow trout *Oncorhynchus mykiss*, hybrid sturgeon *Huso huso* x *Acipenser ruthenus* and banded houndshark *Triakis scyllium*. The synthetic peptides were used for peptide polyclonal antibodies (anti-fish IgH-1 and anti-fish IgH-1) production.The peptide antibody specificities were determined by Western blotting and enzyme-linked immunosorbent assay (ELISA). Anti-fish IgH-1 antibody showed reactivity to IgM of Japanese flounder, seabream, yellowtail, carp, rainbow trout and hybrid sturgeon under reducing and non-reducing condition of Western blotting. Anti-fish IgH-2 antibody reacted to IgM of seabream, yellowtail and rainbow trout under reducing condition. However, under non-reducing condition, anti-fish IgH-2 antibody solely reacted to IgM of rainbow trout. ELISA results showed that antibody titer was not detected in all fish species tested by using anti-fish IgH-1 and anti-fish IgH-2 antibodies. These results indicate the application of anti-fish IgH-1 peptide antibody for detection of immunoglobulins in various fish species by Wester blotting.

Abstract ID: 067F (Poster)

ORF 136 ENCODING MEMBRANE PROTEIN OF CYPRINID HERPESVIRUS 2 AS A PROMISING CANDIDATE FOR DNA VACCINE AGAINST GOLDFISH HVHN

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The Cyprinid Herpesvirus 2 (CyHV-2) is the agent of herpesviral hematopoietic necrosis (HVHN). It causes widespread mortality in the goldfish *Carassius auratus* and Gibelio carp *C. auratus gibelio* aquaculture industry. It was previously reported that a formalin inactivated vaccine was moderately effective to reduce the mortality of fish experimentally infected with CyHV-2. We previously reported the importance of serum neutralizing antibodies to protect fish from the virus. To develop more effective control measures, we focused on DNA vaccines which have the potential ability to induce both humoral and cell-mediated immunities in fish. In this study, we tried to determine the membrane proteins of CyHV-2 that can react with fish serum antibodies as targets for developing DNA vaccines. It was previously reported from the full sequence of CyHV-2 genome that encodes 17 type 1 membrane proteins. Open reading frames encoding these proteins from CyHV-2 SaT-1 isolate were amplified and cloned into bacterial expression vector pET-32a(+) which contains the six histidine tag by homologous recombination of competent *E.coli* strain ME9783. The genes were inserted whereby the histidine tag was at the C-terminus of the gene. After confirming the successful insertion of some genes by colony PCR and sequencing, they were purified and transfected to *E. coli* strain BL21. The transformed *E. coli* were then induced for protein expression in 0.5mM IPTG. Proteins of 3 constructed plasmids, pORF 30, 136 and 153C, were successfully expressed and confirmed by Western blotting with anti-His antibodies. To check the reaction with serum antibodies of goldfish surviving in CyHV-2 infection, the 3 recombinant proteins were transferred onto PVDF membrane after SDS-PAGE then exposed to the serum. After that, the bands were visualized with anti-ginbuna IgM mouse monoclonal antibody (B12) and anti-mouse Ig goat IgG conjugated with HRP. A band was observed for pORF 136 at the range of ~32kDa molecular weight, including the thioredoxin (Trx) fusion tag (~12kDa) and histidine tag (~1kDa). Since the homologous protein of koi herpesvirus (CyHV-3) ORF 136 was determined as one of the structural proteins, CyHV-2 ORF 136 protein is expected to be structural. The ability for fish antibodies, which are found in the serum, to recognize the protein expressed by ORF 136 shows that it could be a promising candidate for a DNA vaccine against HVHN of goldfish.

Abstract ID: 069F (Poster)

CHANGES IN KIDNEY LEUKOCYTE COMPOSITION WITH MATURATION AND AGING IN AYU *Plecoglossus altivelis*

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Ayu *Plecoglossus altivelis*is an annual fish species which is economically important in Japanese freshwater fisheries. Various infectious diseases, however, cause severe damages in the fish production. The prevalence of pathogenic bacteria such as *Flavobacterium psychrophilum* and *Edwardsiella ictaruli* appears quite high in ayu during summer to autumn, since their immune activities remarkably decrease with maturation and aging. The maturation of ayu is controlled by the photoperiod, and long-day treatment can suppress maturation process. In this study, we aimed to investigate the change of kidney leukocyte composition with/without long-day treatment in ayu.Ayu were reared under 24 h electric light period (aged-immature) or under the natural light period (aged-mature) over 9 weeks. Gonad-somatic index (GSI) was measured and used as an indicator for the maturation. Spleen was collected at 6 weeks after the long-day treatment and gene expression analyses of leukocyte marker genes, IgM, IgD, IgT, CD3ε, and CD8α were performed by real time PCR. Spleen collected at the start of the long-day treatment was used as a control group. Trunk kidney leukocytes were isolated at 8 weeks after the long-day treatment using Percoll centrifugation and were analyzed by flow cytometry with anti-ayu Ig monoclonal antibody (MAb), anti-ayu phagocyte (monocyte and neutrophil) MAb and anti-ayu thrombocyte MAb. Furthermore, infection experiments were conducted by intraperitoneal injection of *F. psychrophilum* (2.7×10^4 CFU / fish) into the fish from aged-immature group and aged-maturegroup. The average of GSI of the control fish and the aged-immature fish was less than 1%, whereas that of aged-mature fish was over 7% in males and 12% in females. There were no significant differences in IgM, IgD and IgT gene expression level between the control fish and aged-immature fish. Whereas, the gene expression levels of IgM, IgD and IgT were significantly lower in aged-mature fish than in aged-immature fish. CD3ε gene expression level was significantly lower in aged-immature fish and aged-mature fish, compared with the control fish. However, no significant difference was observed in CD8α gene expression levels among the three experimental groups. Flow cytometry showed that percentage of the Ig positive cells was lower in aged-mature fish than in aged-immature fish. In contrast, the percentage of phagocytic cells was higher in aged-mature fish than in aged-immature fish. The percentage of thrombocyte was similar between both groups. Furthermore, the cumulative mortality rate was 100% in the aged-mature fish, whereas it was 10% in the aged- immaturefish. These results suggest that the B cell amount and antibody production was mainly suppressed by maturation, but the Th cell ($CD3\varepsilon^+$ $CD8\alpha^-$) amount decreases with aging. In addition, the $CD8\alpha^+$ cytotoxic T cells are probably less susceptible to maturation and aging in ayu.

Abstract ID: 073F (Poster)

INFLAMMATORY RESPONSE WITH MULTI-NUCLEAR GIANT CELLS IN AYU, *Plecoglossus altivelis*, REARED AT LOW WATER TEMPERATURE

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Ayu reared at high water temperature for a certain period of juvenile stage showed poor development of thymus. Since the thymus is responsible for T cell development, rearing at high water temperature may affect the immune response of ayu. In this study, we analyzed the inflammatory response of ayu reared at low water temperature or high water temperature for a certain period of juvenile stage.Ayu (about 1.6g BW) were reared at 12°C (low water temperature) and 18°C (high water temperature) for about 2 months. At the end point of rearing under the controlled temperature, thymus of 5 fishes from each group were sectioned every 200µm, and then the cross-sectional area of thymus tissues on each section were measured with Image J software to estimate thymus volume. Continuously, fish was kept up to about 20.5g BW under natural water temperature. Inflammatory response was induced by intramuscular inoculation with zoospores of *Aphanomyces invadans* NJM1507. Gross appearance and histopathology of the lesion were observed 10 days after the inoculation. The thymus volume ratio (thymus volume / body length) of fish reared at 12°C was significantly higher than that of fish reared at 18°C. Among the fish infected with *A. invadans*, exposure of hyphae on the body surface was not observed in some of the fish reared at 12°C, but all of the fish reared at 18°C exposed hyphae on their body surface. The cross sectional area of hyphae in the lesion was also significantly smaller in the fish reared at 12°C than that of fish reared at 18°C. Inflammatory cells appeared around the hyphae of *A. invadans* in muscle tissue of the lesion. Multi-nuclear giant cells were conspicuously observed in fish reared at 12°C. By contrast, epithelial cells were conspicuously observed in fish reared at 18°C. These results suggested that ayu reared at low water temperature develops the multi-nuclear giant cells in inflammatory response against *Aphanomyces* infection, playing an important role in suppressing the hyphal growth.

Abstract ID: 086F (Poster)

Edwardsiella tarda-INDUCED CELL DEGENERATION IN JAPANESE FLOUNDER NEUTROPHILS

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Edwardsiellosis is an infectious disease caused by *Edwardsiella tarda* which is a gram-negative bacterium and damages many kind of aquaculture fish species including Japanese flounder *Paralichthys olivaceus*. A remarkable symptom of the disease is severe hepatitis with multifocal abscesses. The abscesses are comprised of dead cells which originate from numerously infiltrated neutrophils and the adjacent hepatocytes. In this study, we examined cell degeneration in Japanese flounder neutrophils exposed to *E. tarda*. The hepatitis was induced by experimental infection using *E. tarda* NJB0401 and then histological observation was performed about neutrophils accumulated in the liver. The majority of neutrophils showed cell degeneration such as nuclear condensation and fragmentation, cell swelling and shrinking, and loss of the plasma membrane at the early stage of cellular accumulation. By using TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP-digoxigenin nick end labeling) analysis, cell death involving nuclear DNA cleavage was detected in the accumulated neutrophils.The effect of *E. tarda* on cell degeneration of Japanese flounder neutrophils was analyzed *in vitro*. Sixteen hours after the mixture of naïve neutrophils collected from the kidney and *E. tarda* NJB0401, the cells were stained with Giemsa, propidium iodide and annexin V. The majority of neutrophils were morphologically normal and kept the permeability of their plasma membrane. The exposure of phosphatidylserine during apoptosis was not detected on cell surface of the neutrophils. Therefore, the cell degeneration of neutrophils in edwardsiellosis may be caused by the inflammatory factors in the physiological and immunological response to *E. tarda* infection.

Abstract ID: 093F (Poster)

EFFICACY AND SAFETY OF PISCIVAC™ IRIDO SIAGAINST *Streptococcus iniae* AND RED SEABREAM IRIDOVIRUS (RSIV) INFECTIONS IN RED SEABREAM *Pagrus major*

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Red seabream (*Pagrus major*) is an economically important marine fish species in Japan. The natural outbreaks of *Streptococcus iniae* and red seabream iridovirus (RSIV) cause massive mortalities and severe economic losses in red seabream culture. Effective vaccines for protection of red seabream against the two diseases with a single injection are not yet available. Here we evaluated efficacy and safety of Piscivac™ Irido Si, the bivalent formalin-killed vaccine, against *S. iniae* and RSIV in red seabream. In laboratory trials, intraperitoneally vaccinated fish were protected against challenges with *S. iniae* and RSIV as early as 3 days post vaccination (dpv) with relative percent survival (RPS) of 100% and 68.6%, respectively. The protection was also observed in fish reared at 15 °C, with RPS of 89.3% and 87.9% after challenging the fish with *S. iniae* and RSIV at 14 dpv and 10 dpv, respectively. Furthermore, intramuscular vaccination effectively protected fish against *S. iniae* (RPS of 100% at 14 dpv) and RSIV (RPS of 100% at 10 dpv). No adverse effects were observed in fish injected with five doses of vaccine during 14 days of observation. The vaccine conferred protection at least 2 months against RSIV infection, and at least 6 months against *S. iniae* infection in the field trial. These results indicate that Piscivac™ Irido Si is effective and safe for protecting red seabream against infections from *S. iniae* and RSIV.

Abstract ID: 101F (Poster)

THE EFFECTS OF TEMPERATURE ON IFN-REGULATED ANTIVIRAL MECHANISM IN ORANGE-SPOTTED GROUPER

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The impact of climate change influences the rearing temperature and poses a fundamental threat to the global aquaculture. As fish are poikilothermic animals, the innate immune system is deeply modulated by temperature. To assess the impact of temperature on antiviral response in grouper, the antiviral molecules involved in interferons (IFNs) related signal transduction pathway are applied to investigate the grouper antiviral responses at different temperature. In this study, we evaluated the antiviral response of orange-spotted grouper at different temperatures through investigating thermo-sensitivity of antiviral Mx protein. We firstly identified the regulatory motifs in three Mx promoters containing heat shock transcription factor 1 (HSF1) binding sites, which 6, 4 and 11 of them found in Mx1, Mx 2 and Mx 3 promoter, respectively. Furtherly, to evaluate the temperature effects on Mx promoter activities, promoter sequences were linked to a luciferase gene and the resulting constructions were transfected into grouper fin cells (GF-1 cells). Luciferase activity was measured post incubation at 20°C, 28°C and 36°C for 24 h. It shows that elevated temperature can potentiate the activity of three Mx promoters. Therefore, it suggested temperature modulation might influence Mx isoforms expression and lead to differential regulation during antiviral response. Moreover, grouper type I IFN recombinant protein was used to analyze its duration or bioactivities at different temperature. Here, this study will demonstrate the comparative studies on the related molecules involving in IFN-regulated antiviral pathway upon temperature modulation, it will help to elucidate the effect of temperature on the antiviral defense and provide information of the disease control strategy and development of fishery management under the impact of climate change.

Abstract ID: 149F (Poster)

DEEP SEQUENCING-BASED TRANSCRIPTOME PROFILING ANALYSIS OF SCALLOP EXPOSED TO MARINE TOXIN

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Harmful algal blooms (HABs), caused by pollution of water bodies and global climate change, can result in ecological and economic losses in coastal areas as they cause mass mortality of cultivated animals from the algal toxins they produce. The diarrhetic shellfish poisoning (DSP) toxins include okadaic acid (OA), which is the main marine toxin responsible for the DSP that causes gastrointestinal symptoms in humans following consumption of contaminated bivalves. Scallops are a cosmopolitan family of bivalves, some of which are widely farmed by the aquaculture industry for food and have important economic value. The bay scallop (*Argopecten irradians*) was introduced and has been cultured in the coastal provinces of China for more than 30 years, and now bay scallop farming is also suffering from HABs. Scallops accumulate toxins in their tissues to a greater extent as they have a low metabolic rate.To elucidate the toxicological mechanism of OA exposure on the immune system, we investigated differentially expressed genes (DEGs) and transcript abundance in bay scallop gill tissue after exposure to 500 nM of OA for 48 h using the deep-sequencing platform Illumina HiSeq. De novo assembly of paired-end reads yielded 55,876 unigenes. In total 55,876 unigenes,3,204 and 2,620 genes were identified as significantly up- or down-regulated, respectively. With DEGs, Gene Ontology (GO) was classified and functional enrichment for DEGs. GO has three ontologies: molecular function, cellular component and biological process, performing functional enrichment respectively, which the genes were related to the 20 terms including “cellular process”, “immune system process”, “metabolic process”, “catalytic process” in the OA-exposed bay scallop. In addition, KEGG assignments were used to classify functional annotations of the identified genes to further understand the biological functions of the genes. Among the differentially expressed genes, 7,214 were assigned to 41 pathways in the KEGG database, with 20 significantly enriched KEGG pathways. Moreover, 10 selected DEGs were validated using qPCR.Altogether, these results will provide a resource for subsequent gene expression studies regarding marine toxins exposure and the identification of OA biomarkers to monitor the aquaculture of scallop.

Abstract ID: 176F (Poster)

A NK-LYSIN FROM *Oreochromis niloticus* ENHANCES ANTIMICROBIAL DEFENSE AGAINST BACTERIAL PATHOGEN

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NK-lysin is an anti-microbial and anti-tumor protein produced by cytotoxic T lymphocytes and natural killer cells in mammals. However, information on the natural function of ?sh NK-lysin is limited, although NK-lysin genes have been identi?ed in several teleost species. In this study, we identi?ed a ?sh NK-lysin (termed OnNKl) in Nile tilapia (*Oreochromis niloticus*) and analyzed its expression, antibacterial property, and biological effect on pathogen infection. OnNKl is composed of 161 residues and shares 31–62 % overall sequence identities with other teleost NK-lysin. OnNKl possesses a Saposin B domain and six conserved cysteine residues that in mammals are known to form three intrachain disul?de bonds essential to antimicrobial activity. Expression of OnNKl mRNA was detected in multiple tissues and was upregulated by bacterial infection and polyI:C stimulation in a time dependent manner. Meanwhile, recombinant OnNKl protein could signi?cantly inhibit the growth of *Streptococcus agalactiae* in vitro. When OnNKl was overexpressed in tilapia, following bacterial infection, the pathogen loads in the tissues of OnNKl-overexpressing ?sh were signi?cantly lower than those in control ?sh. These results indicate that OnNKl plays important roles during anti-microbial defenses.

Abstract ID: 178F (Poster)

BIOLOGICAL CHARACTERIZATION, EXPRESSION, AND FUNCTIONAL ANALYSIS OF NILE TILAPIA(*Oreochromis niloticus*)TRAF6

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Nile tilapia (*Oreochromis niloticus*) is a crucial economic fish that has been plagued by *Streptococcus agalactiae* infections for many years. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is a crucial adaptor molecule of the interleukin-1 receptor/Toll-like receptor (IL-1/TLR) superfamily, which can trigger downstream signaling cascades involved in innate immunity. In this study, the full-length cDNA of (*On-TRAF6*) was cloned from *O. niloticus*, with an open reading frame of 1716bp, which encoding a polypeptide of 571 amino acids. The predicted amino acid sequence of On-TRAF6 contained the characteristic motifs of TRAF proteins, including a Zinc finger of RING-type, two Zincfingers of TRAF-type, and a MATH (meprin and TRAF homology) domain. Multiple sequence alignment revealed that On-TRAF6 shares high level of identity with TRAF6s from other species, but shares a relatively high level of identity with those of other fishes (64-98%). In healthy tilapia,the *On-TRAF6* could be detected in all the examined tissues and the highest expression level in the spleen. Moreover, there was a clear time-dependent expression pattern of *On-TRAF6* after immunized by formalin-inactivated *S. agalactiae* and the expression reached the highest level at 3 h in intestine, 6 h in the brain, 24 h in the headkidney, 48 h in the kidney and spleen, respectively. Over-expression of On-TRAF6 activated NF-κB strongly in HEK293T cells. These findings indicated that On-TRAF6 may play an important role in the immune response to intracellular bacteria in Nile tilapia.

Session 7 – Biosecurity and Diganostics

Abstract ID: 242G (Keynotefor Biosecurity)

BIOSECURITY: FROM MANAGEMENT REACTION TO STRATEGIC PLANNING

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From the point of view of aquaculture production, biosecurity includes all the measures taken to reduce the economic impact of diseases. Such measures should correspond to a sanitary strategy that needs to be defined specifically for each facility, species and culture system and within the sanitary status of a region. Such strategy has different layers, starting from the international and national frame which includes international agreements, national legislation and its enforcement. The next layer is the selection of the type of animal to be farmed, its health status (SPF versus not SPF) as well as their susceptibility/tolerance/resistance to disease. The type of animal used will be defined based on the culture system and endemic pathogen situation where animals will be raised. Eventually, the third layer includes all the sanitary measure taken during the culture period which will range from pond preparation, disinfections, water quality parameters, animal health monitoring and surveillance programs. A fundamental tool for biosecurity is the access to diagnostic capacity. Shortage of field diagnostic tools and increased reliance on remote diagnostic services often lead to misinterpretation of disease outbreak as these rarely consider the triggering effect of environmental conditions. Furthermore, the increase in the use of PCR and the loss of histopathological expertise is contributing to poorer understanding of the disease process and therefore less effective measures to control and respond to disease outbreaks. A proper biosecurity strategy should allow to move from the reaction of the farmer to solve emergency situations to a cost effective strategic plan to prevent the outbreaks of diseases.

Abstract ID: 062G (Oral)

EPIDEMIOLOGY AND BIOSECURITY FOR SHRIMP FARMING INDUSTRY

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Shrimp Farming industry in China has fast developed for more than 3 decades and become an important aquaculture industry producing about 1.9 million tons of shrimp in 2015. However, threats of diseases, such as white spot syndrome, reddish body syndrome, covert mortality syndrome, early mortality syndrome, slow growth syndrome, etc., emerge in the industry and cause significant losses every 8—10 years. Pathogens including white spot syndrome virus (WSSV), *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (VP_{AHPND}), *Enterocytozoon hepatopenaei* (EHP), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and yellow head virus (YHV) have been demonstrated for their prevalence in the industry. New pathogens, including covert mortality nodavirus (CMNV), shrimp hemocyte iridescent virus (SHIV), YHV genotype 8 (YHV-8), and *Vibrio campbellii* causing AHPND, etc. were found in farmed shrimp. Coinfections with two or more pathogens were frequently detected and increase difficulty for confirmative diagnosis. Multiple pathogens, emerging diseases, and complexity of occurrences in shrimp farming industry require urgently a comprehensive resolution for disease control and health management. Biosecurity concept was introduced in shrimp farming and hatcheries. We have deeply discussed the risk analysis based biosecurity concept and distinguish it with the concepts of disease control and health management or health aquaculture. Five biosecurity grades based on implementation of biosecurity measures were suggested, which include BSG1 (Biosecurity grade 1): diagnosis based treatment; BSG2: surveillance based prevention; BSG3: risk analysis based control; BSG4: systemic disease freedom; BSG5: official certificated compartment. Each biosecurity grade is standalone system and can be upgraded to next one, which is distinct from Palić's biosecurity steps to reach a disease free compartment (BSG5). Farms can consider to achieve which biosecurity grade based on their background, conditions, management, capability, and investment. Biosecurity plans for breeding centers, hatcheries or nursery farms is in developing based on practices in the collaboration of some hatcheries. Twelve principle measures for biosecurity in shrimp farms were recommended and will be implemented in shrimp farms.

Abstract ID: 168G (Oral, Student)

BIOSECURITY MODEL TO CONTROL PARASITIC DISEASE IN RECIRCULATION AQUACULTURE SYSTEM (RAS) FOR CATFISH (*Clarias sp*) NURSERY AND GROWING OUT

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Application of Recirculation Aquaculture System (RAS) in catfish nursery and growing out can increase the productivity of freshwater aquaculture ponds by improving the water quality through water treatment and water re-use. RAS is built for nursery and growing out *clarias* sp, from 5-7cm (2 g) size until consume size (100g/fish) in two fish tank (3l x 2w x 1h)m^3 size. RAS is supported with sedimentation tank that consist of 3 fiber (0,8l x 1,35w x 0,7h)m^3 size and trickling filter that consist of 40.000 pieces bioballs that spread out to 8 drum (diameter 0,6m and height 0,9m) which are arranged stacked up with 4drums at the bottom and 4 drums above. Biosecurity model in RAS concerns in screening disease of seed in quarantine before stocking to fish tank and also attends in preventing the introduction of disease through good sanitation in RAS system. Monitoring of parasitic disease from fish is conducted every 2 weeks and monitoring of water quality is conducted every week. The screening disease of seed in quarantine can decrease the parasitic intensity of *Tricodina* sp 87%, *Ichtyioptirius* sp 82%, *Dactylogirus* sp 83%, and *Gyrodactilus* sp 80%. Application of good sanitation in a whole of nursery and growing out catfish period can minimize the value of parasitic intensity in RAS, such as *Trichodina* sp about 0,4 ± 0,5 individu (range 0 – 1,5); *Ichtyioptirius* sp about 0,8 ± 1,1 individu (range 0 – 3); *Dactylogirus* sp about 2,6 ± 1,1 individu (range 1 – 5,5); and *Gyrodactilus* sp about 3,4 ± 3,1 individu (range 0,5 – 9). Water quality result show that temperature range is about 22,0 – 24,5°C; pH range 6,27 – 7,68 ; DO range 0,51 – 5,2 mg/L ; and NH_3-N range 0,0 – 0,04 mg/L. Survival Rate of *clarias* sp nursery in RAS is about 70 – 85% and survival rate of *clarias* sp growing out in RAS is about 90 – 96%.

Abstract ID: 080G (Oral)

DETECTION AND MANAGEMENT OF DISEASES IN ORNAMENTAL MARINE FISH

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UC Davis and Mars Symbioscience developed a partnership in recent years to help address the importance of marine resources in building foundations for food security and livelihood sources to small island communities in Spermonde Islands, South Sulawesi in Indonesia. In this region, marine resources are declining due to overexploitation of fisheries, destructive practices, and coastal development. These anthropogenic activities have been adversely impacting fisheries resources, marine biodiversity, and fishery livelihoods. Sustainable culture of ornamental marine species (OMS), such as seahorse and clownfish, has been established as one mechanism to provide alternative income opportunity to small fish farmers in Sulawesi. Culturing high value species are additionally aimed at rebuilding wild populations. Infectious diseases however, are a major limiting factor in various phases (larval, broodstock) of OMS culture where water quality, stocking density, nutritional adequacy, poor husbandry, and other risk factors commonly encountered in captive conditions are deemed, altogether, to predispose fish vulnerability to microbial infections. Transboundary movements of OMS potentially harboring infectious agents may promote disease transfer across species via supply chains of the local and global ornamental fish trade warranting development of screening measures to limit disease transmission. This presentation will focus on diseases of OMS and their management to potentially provide impetus at developing field-based disease diagnoses methods targeting remote and resource-limited labs in Indonesia and other developing countries. The overarching goal is to determine whether an existing disease diagnosis system (certification) for transboundary movement of marine ornamental fishes aligns with measures to address the threats of emerging infections and to cope with current challenges and expectations associated with responsible mariculture operation. At risk is the marine ornamental fish trade, a lucrative industry generating an estimated annual revenue of approximately 450 million USD globally, which could suffer from the adverse economic impacts of global transmission and spread of infectious diseases.

Abstract ID: 082G (Oral, Student)

RAPID DIAGNOSTIC TEST OF RED SEA BREAM IRIDOVIRAL DISEASE (RSIVD) IN GROUPER *Epinephelus* sp. BASED ON SEROLOGICAL CO-AGGLUTINATION AND MOLECULAR STUDY

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Red sea bream iridoviral disease (RSIVD) infection is known as acontagious disease in the marine aquaculture commodities mainly on grouper (*Epinephelus* sp.) cause a highly mortality rate. Symptoms of disease were weak, darker skin and swollen spleen of fish.Aim of study was to create and apply a rapid diagnostic test and supported by a molecular analysis. Field trials on a mass mortality outbreaks was identified in the city of Tanjungpinang, Indonesia. Serum anti RSIV was obtained by immunizingof the vaccine RSIV intraperitoneally with graded doses per week was 0.5 ml, 1 ml, 2 ml and 3 ml, to boost antibody titers. In the fifth week, serum washarvested via the auricular vein, serum was purified to obtain immunoglobulin G then was coupling with protein A of*Staphylococcus aureus*at the same volume (kit co-agglutination RSIVD). Field samples of spleen were taken from the normal fish and suspected fish then crushed and suspended with PBS pH 7.2, and centrifuged at 8.000 rpm for 15 minutes. Fifty microliterof kit co-agglutination RSIVD and 50 µl of spleen supernatant were reacted on the sterile glass object. The results showed sandyagglutination after 10 minutes for positive infected spleen, and no agglutination in the samples of healthy fish (negative) as well as in control with PBS (negative). Confirmation testing by polymerase chain reaction (PCR) using primer forward 1-F (5'-CTC-AAA-CAC-TCT-GGC-TCA-TC-3') and reverse 1-R (5'-GCA-CCA-ACA-CAT-CTC-CTA-TC-3') had 570 bp of band. Sequencing results showed the similarity of 99% identity with *Megalocytovirus* strain RSIV. Testing with kit co-agglutination RSIVD had the advantages such as cheap, fast and an accurate in diagnosing the disease red bream iridoviral (RSIVD).

Abstract ID: 112G (Oral)

ROLE OF BIOSECURITY IN ADDRESSING OF ANTIBIOTIC RESISTANCE CONCERNS

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The last three decades of continuing worldwide expansion in the farming of aquatic animals placed a biosecurity in a different perspective. An introduction of new technologies, species, increased movement of animals are only a few factors the might be associated with biosecurity risks. Most farms depend on trade for various inputs such as broodstock, post-larvae/fingerlings, and feed. Each of these inputs represent a potential pathway by which pathogens can enter farming operations and create conditions for emergence of new or reoccurrence of diseases and production loses. The use of veterinary medicine products might be required to prevent and treat disease outbreaks, assure healthy stocks and maximize production. The appropriate antimicrobial treatments can be one of the effective management responses to emergencies linked to infectious epizootics. However, the unrestricted use of antibiotics in aquaculture can contribute to the emergence of resistance which is very often managed by replacing an antibiotic that had become ineffective with a new class of antibiotic to which bacteria is susceptible. Worldwide, antimicrobial resistance is recognized as a significant issue affecting animal health, welfare, and production outcomes. Minimizing the conditions in which resistant bacteria can spread in aquatic animals and the responsible use of veterinary medicines are essential components of the biosecurity program implemented on the farm. Farm biosecurity together with adequate import and export controls can lead to the development of successful aquaculture industry as a reliable source of safe seafood product.

Abstract ID: 071G (Oral)

BIOSECURITY AND ITS ROLE IN NATIONAL PROTECTION

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Biosecurity has played a critical role in reducing risk and placing Australia as one of the few countries in the world to remain free from the world's most severe agricultural diseases. While our geographical isolation has played a key role in maintaining this status, the objective is now to maintain this status in an environment of increasing global trade while meeting our international trade agreement obligations.The Department of Agriculture and Water Resources determines the biosecurity requirements for new market access requests through a science-based Biosecurity Import Risk Analysis (BIRA). A BIRA considers the biosecurity risk for a commodity, and determines the risk management measures to apply across the biosecurity continuum (pre-border, at the border and post-border) that reduce the risk to an acceptable level and meet Australia's appropriate level of protection (ALOP), as determined by the BIRA. Australia considers three aquatic animal commodities 'high risk'; green (uncooked) prawns, live aquatic animals, and products containing salmonids. To manage the biosecurity risk with these commodities off-shore, pre-border risk management measures are implemented. This involves an evaluation of the structure of the exporting country's competent authority (CA) and its ability to control imports, undertake health surveillance, pre-export testing if required, report occurrences of aquatic disease, regulate processing facilities, and certify exports.Competent authority evaluations facilitate a consistent approach between trading partners, and help to build close relationships. They provide confidence in the CA's ability to meet Australia's strict biosecurity requirements, including the ability to implement equivalent measures to address risk. If required, the department employs further risk management measures at the border, and recommends post border controls to reduce the likelihood of establishment of disease.Management of biosecurity risk off shore requires effective communication across the entire biosecurity continuum to ensure that any weaknesses can be quickly identified, and a review undertaken to ensure Australia's ALOP continues to be met.The department's approach to off shore management of biosecurity risks using CA evaluations will be discussed, using raw salmonid materials as an example. How these evaluations contribute to Australia meeting its strict import requirements will be outlined.

Abstract ID: 038G(Poster &Elevator Pitch)

DEVELOPMENT OF RECOMBINASE POLYMERASE AMPLIFICATION ASSAY COMBINED WITH LATERAL FLOW DIPSTICK FOR DETECTION OF ACUTE HEPATOPANCREATIC NECROSIS DISEASE IN SHRIMP

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The recent outbreak of acute hepatopancreatic necrosis disease (AHPND) infection in the shrimp aquaculture industry and its severe mortalities in farmed penaeid shrimp have newly listed of World Organization for Animal Health (OIE) to declare as the international standards for animal health and zoonoses for safe international trade. In this study, accelerated detection method for the AHPND were developed based on the recombinase polymerase amplification (RPA) method. The developed method included the use of species-specific biotinylated primers to amplify RPA amplicons, which were simultaneously hybridized to specific FAM-labeled DNA probes and following lateral flow dipstick (LFD) assay. Under the optimal conditions, 30 min at 38°C including amplification and hybridization step followed by 5 min LFD resulted for visualization step at the LFD test line. Detection limit of RPA-LFD assay (10^4 CFU) was comparable to other commonly-used method for 1-step PCR for the same target sequence using amplicon detection by gel electrophoresis. DNA templates extracted from 12 bacterial isolates commonly found in shrimp ponds (including Vibrio species but excluding *V. parahaemolyticus*) all gave negative results with both the RPA-LFD assay and conventional PCR methods. The new RPA-LFD assay also have the advantages of reduced assay time and easy format for read the results. Use of this field-friendly method to screen for AHPND bacteria in environmental samples, broodstock feeds, feces from broodstock, post-larvae before stocking shrimp ponds and suspect shrimp under cultivation should help to reduce the probability of AHPND outbreaks.

Abstract ID: 140G(Poster &Elevator Pitch)

A CONVENIENT IMMUNOCHROMATOGRAPHIC TEST STRIP FOR RAPID DETECTION OF Scylla serrata REOVIRUS

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Scylla serrata reovirus (SsRV) is one of the most prevalent viral pathogens of the mud crab (*S. serrata*), which is an important aquaculture species in east China. A rapid and specific test for SsRV diagnosis at the site of a suspected outbreak is crucial for the implementation of control measures to minimize economic losses. Herein, an immunochromatographic strip for SsRV detection was developed. Monoclonal antibody 5D4, raised against SsRV p29, the most abundant viral capsid protein, was used as a capture antibody, while rabbit anti-SsRV polyclonal antibody was used as a detector antibody at the T line, and Rabbit anti-mouse IgG antibody was used as the capture antibody at the C line. Although the strips were ~1000-fold less sensitive than the one-step RT-PCR assay, they provide rapid results without a requirement for specialized equipment or professionally trained personnel.

Abstract ID: 011G(Poster)

WATERLIFT AS SOLUTION OF TOXIC MATERIAL IN WHITE SHRIMP *Litopenaeus vannamei* CULTURE CAUSED BY SUSPENDED SOLID

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White shrimp *Litopenaeus vannamei* farming was growing rapidly ranging from intensive to super-intensive systems. These developments pose a problem, especially in terms of suspended solids in container cultivation. Suspended solids contained in the remainder of feed such as nitrogen, phosphorus, organic matter, and hydrogen sulfide, could be toxic for shrimp.The simple and cheap method to solve it was waterlift. Principle of waterlift was carried suspended solid by flowing water aquaculture into a special tank to precipitate suspended solids by gravity, then drain the water back to container cultivation. The purpose of this test was to determine the effectiveness of waterlift as solution of toxic material in white shrimp *L. vannamei* culture caused by suspended solid. White shrimp cultured in two ponds of 30,000 m^3 (waterlift and control). Waterlift tank mounted on the center of the pond and connected to the aeration system. The analyzed data was the value of suspended solids and water quality (NH_3 and NO_2). The suspended solids test results showed that in the waterlift pond, suspended solids was reached an average of 84 ppm. This value is lower than the control with an average of 128 ppm. The water quality test results showed that in the waterlift pond, NH_3 was reached an average of 0.14 ppm and NO_2 with an average of 0.466 ppm. NH_3 value still higher than the control with NH_3 average of 0.07 ppm. Probiotics in the control change NH_3 to NO_2. However, the value of NO_2 waterlift pond lower than the control with NO_2 average of 0.742 ppm. Based on test results, it was concluded that waterlift effectively reduce toxic material in white shrimp *L. vannamei* culture caused by suspended solid.

Waterlift tank

Table 1. Result of waterlift and control tanks parameters

No	Parameters	Waterlift	Control
1	Suspended Solid (ppm)	84	128
2	NH_3 (ppm)	0.14	0.07
3	NO_2 (ppm)	0.466	0.742

Abstract ID: 017G(Poster)

THE USING OF MACROALGAE *Gracilaria spinosum* FOR PHOSPHATE REDUCTION IN PHYTOREMEDIATION OF ARTIFICIAL WASTE

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Improvement of fisheries, particularly shrimp culture will cause a large amount of nitrogen (N) and phosphorus (P) waste into natural waters. Commonly, the waste is flowing to the beach and the bay, if it take constantly would promote eutrophication. The efforts should be made to reduce through the application of phytoremediation technology. Phytoremediation is a technology that used plants to reduce or even eliminate the presence of contaminants in soil and water. The aims of this research to examine the impact of the use of seaweed *Gracilaria spinosum* in reducing phosphate on artificial waste. Methods of testing is done with the introduction of *Gracilaria spinosum* with different biomass on artificial waste (dissolving phosphate fertilizer / TSP and urea on sea water). Tank filled with 80 liters of artificial wastewater introduced by macroalgae, treatment A (control without macroalgae), B (250 g), C (500 g), D (750gr) and E (1000 g). Based on the results it appears that *glacilaria is* excellent for used as an artificial waste phytoremediation agent in reducing phosphate, where the highest reduction since D1 in treatment E followed by D, C, B and A (control). In the treatment E since D1 drastically decreased concentration of 8.9 ppm (D0) to 1.71 ppm (D1) then tends to stagnate until 0.23 ppm (D10). Likewise in treatment D, where a decline of 2.90 ppm (D1) - 0.68 ppm (D10), this is due to the availability of phosphate in artifial wastewater decreased, while the reducing power of phosphate by *Gracilaria spinosum* biomass still available. In contrast on B and C treatments which since D1 - D10 phosphate concentration decreased gradually, in B gained 5.96 ppm (D1) - 1.29 ppm (D10) as well as treatment C was obtained 4.28 ppm (D1) - 0.69 ppm (D10). The phenomenon in treatment B and C, in line with the states that the absorption of phosphorous or other nutrients are in excess of the amount required to grow known as luxury consumption. The ability to absorb and store nutrients than necessary is also a competitive advantage. It is quite interesting on the control that phosphate reduction also significantly in D5, where 8.09 ppm (D5) to 4.54 ppm (D4) continued to decline further to 2.37 ppm (D10). This condition is inseparable from the role of natural phytoplankton grow with the appearance of a greenish color, a common condition that nutrient and sunlight irradiation has triggered the growth of phytoplankton. It seems different on other treatment that no phytoplanktongrow, because availability of nutrients used for macroalgae grow. Data for the daily growth rate on *Gracilaria spinosum biomass* showed *that t*he highest on treatment B is associated with the availability of nutrients and space competition. Finally we take conclusions that the role of macroalgae *Gracillaria spinosum* quite effective in reducing phosphate in wastewater aquaculture.

Abstract ID: 106G(Poster)

DECENTRALISED MOLECULAR DIAGNOSTICS AND REMOTE DATA REPORTING FOR MANAGEMENT OF DISEASE IN GLOBAL AQUACULTURE

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Disease is widely acknowledged as the prominent bottle-neck to achieving global food security and poverty alleviation targets relating to aquaculture with annual losses exceeding US $6bn. High profile diseases in the $15bn shrimp industry include those caused by White Spot Syndrome Virus (WSSV 1) and the bacterial pathogen implicated in Acute Hepatopancreatic Necrosis Disease (AHPND). These pathogens alone are associated with annual losses of approx. $3bn per annum. Decentralised diagnostic testing, and collation of regional data to inform risk management, have been identified as key requirements in the large and growing Asian aquaculture sector. The project presented here is to validate a point of need diagnostic device, i.e. equipment that can be taken into the field and utilised to collect regional data. Genedrive® is a small footprint molecular diagnostics platform capable of rapid sample processing (SP) and the sensitive and specific detection of pathogens in <90 minutes. It combines proprietary ?hybrid' thermal engine technology with bespoke consumable elements designed for detection of the pathogen(s) of interest. Two-step SP and an ultra-simple, ?single-button' operation allows for the operation of the equipment by un-skilled operatives, with minimal training. The technology has high potential in other settings where rapid pathogen detection is required and where centralised laboratory infrastructure is poor. We are currently working to test and validate Genedrive® against gold standard diagnostics for WSSV 1 and AHPND applied to penaeid shrimps. In addition, we are developing a bespoke smartphone app to interface with Genedrive® and to transmit field data to a centralised data repository for subsequent analysis. Shrimp disease has been used to demonstrate proof of concept for the application of this technology and the centralised reporting model can now be implemented for many other applications. The formation of an accurate, low-cost diagnostic and integration with user-technology reporting of data has the potential to revolutionise disease management in global aquaculture and will contribute directly to poverty alleviation and global food security associated with aquaculture.

Abstract ID: 201G(Poster)

APPLICATION THE COMPARATIVE C_T METHOD TO DIAGNOSE VIRAL INFECTION

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The viral disease still be a serious problem in aquaculture, even in some cases can cause death cultivation up to 90%. The presence of virus infection can be diagnosis using molecular technics, before the appearance of clinical sign.Two different methods of determining quantitative of target genes using molecular technics, there are absolute and relative quantification.Relative quantification method presents the quantity data by comparing C_T value,this method is very easy to perform and without requiring a calibration curve for each test.The purpose of this activity is to review the formula of the comparative C_T method and apply these calculations in laboratory test.The method used are (1) determination the comparative C_T method. (2) Make a standard curve as a reference.(3) Determine quantity of virus without making the standard curve.The equation of the comparative C_T method is $2^{-\Delta\Delta CT}$.The concentration of virus genes obtained was 48.440 copies.

Abstract ID: 230G(Poster)

SURVEILLANCE PROGRAMME FOR AQUATIC ANIMAL DISEASES IN INDIA

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Disease surveillance for knowing the occurrence and distribution of endemic pathogens and rapidly detecting new/exotic pathogens is essential for effective health management. Realizing the importance, India has been implementing a National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) in 16 selected states and 2 Union Territories of the country having aquaculture importance through a network of 25 national/state fisheries organisations. The programme is funded by National Fisheries Development Board, Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Government of India and is being coordinated by ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow, India. The major emphasis of the programme has been to strengthen the passive disease surveillance in the country, so that the disease outbreaks are reported and investigated. In this direction, about 350 awareness programmes involving more than 12,000 fish farmers and state fisheries officers have been organized during last four years. Similarly, during the period 31 training programmes have been organised to strengthen the diagnostic capability of the fisheries officers. In addition, for sensitizing the project investigators of collaborating centres, an Orientation Training Workshop involving international resource persons and an International Symposium on Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture. An Epidemiology School on Aquatic Animal Diseases was also organised by Prof. K.L. Morgan, University of Liverpool, UK. Under the programme about 115 selected districts covering over 1100 farms are being regularly monitored. Each selected farm is visited twice per crop for collection of samples and screened for selected pathogens. It is important to note that, important pathogens viz., spring viremia of carp virus, koi herpes virus, infectious pancreatic necrosis virus, viral haemorrhagic septicemia virus, yellow head virus and taura syndrome virus have not been detected till date. A national database on aquatic animal diseases is also being developed under the programme. Moreover, for investigating new disease outbreak, there is a provision for constituting emergency response team comprising scientists from different institutes for disease investigation. Till date, such teams have been effective in dispelling speculations and misconceptions regarding occurrence of the AHPND in the country, investigating large-scale mortalities in goldfish in Hooghly District, West Bengal and for understanding the spread of infectious myonecrosis (IMN). Four new pathogens viz., cyprinid herpesvirus-2, carp edema virus, *Enterocytozoon hepatopenaei* andIMNV have been detected during the period. Moreover, *Perkinsus olseni* has been detected in a new host i.e. green mussel, *Perna viridis* for the first time. In order to follow uniform protocols for diagnosis, a Diagnostic Manual for Aquatic Animal Diseases of National Concern for all the prioritized diseases has been published. Furthermore, diagnostic capability has been developed for OIE-listed and emerging pathogens of finfish and shellfish. The implementation of NSPAAD has helped in knowing the distribution and detecting new pathogens in the country. The programme has also helped in improving reporting of aquatic animal diseases to international organisations viz., NACA and OIE. The work presented here is compilation of results received from all the collaborating institutes of NSPAAD.

Abstract ID: 053G (Poster)

BUOYANCY DISORDERS OF FRESH WATER ORNAMENTAL FISH

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In fish, buoyancy is controlled by the amount and distribution of gas within the swim bladder. Buoyancy disorders are common in goldfish (*Carassius auratus*) in particular and affected goldfish often present following a sudden onset and are found lying on the bottom of the tank or pond, or floating at the surface. Most of them that have positive buoyancy are suffering from skin damage. During 2009 to 2012 eighteen freshwater ornamental fish including 6 Flower horn fish (hybrid cichlid), 5 fancy goldfish (*Carassius auratus*), 4 Oscars (*Astronotus ocellatus*),1 Moray eel (*Gymnothorax tile*),1 koi(*Cyprinus carpio* Koi), and 1 Redtail Catfish (*Phractocephalus hemioliopterus*), with buoyancy disorders were referred to the Department of Aquatic Animal Health, Veterinary Faculty, the University of Tehran. These fishes had different buoyancy disorders. Some of them sank to the bottom (negative buoyancy), some were floating at the top (positive buoyancy), and some were listing or rolling. After clinical examination, dorso-ventral (DV) and lateral (L) radiographs were taken for all of them. Sonography was performed for 7 of them. Samples for pathological studies were taken from 8 fish and fixed in 10% buffered formalin and sections were stained with H& E. Radiological studies showed that redtail catfish, koi, moray eel, 2 oscars, 2 flower horn fish, 2 goldfish had over-inflation of their swim bladder. 2 gold fish had displacement of swim bladder. One goldfish had intestinal tympany. 4 flower horn fish had rupture of the swim bladder. Sonography showed poly cystic liver and fluid accumulation in 2 cases and renal tumor in 2 other cases. Autopsy showed koi, one oscar and one flower had fluid accumulation on their swim bladder. Autopsy and pathological findings showed that one case had cystic kidney and 2 cases had cystic liver and two other cases had renal tumor. One goldfish that had intestinal tympany and one flower horn fish that had rupture of the swim bladder were treated and could swim normally.

Abstract ID: 072G (poster)

ASSESSMENT OF DRUG RESIDUE IN TISSUES AND IMMUNOLOGICAL RESPONSES OF GIANT FRESHWATER PRAWN, *Macrochium rosenbergii,* AFTER OXOLINIC ACID TREATMENT

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Oxolinic acid (OA) is the only antimicrobial drug allowed to be used in crustacean in Taiwan, and the main target is to treat the *Aermonas hydrophila*, *Pseudomonas* sp. and *Vibrio* sp. infection. OA administrated into diets at 50 mg /kg of body weight /day to treat decapods for five days is recommended in Taiwan, and furthermore, after the treatment, withdrawal period of 30 days is necessary. In recent, the drug residue of products in aquaculture had been seriously monitored to protect the human health and prevent environmental pollution. The rules of "The veterinary drug residue limits in foods" declared that the maximum residue limit of OA was 0.1 mg/kg detected in muscle of decapods. Therefore, in the present study, *Macrobrachium rosenbergii* fed with OA containing diets at 0 and 50 mg /kg of body weight /day for 5 days and those followed with 30 days of withdrawal period were used to evaluate the drug residue in tissues and the effects on immunological responses of prawn including total haemocyte count (THC), phenoloxidase (PO) activity, respiratory bursts (RBs) and phagocytic activity (PA). The results showed that the residue of OA levels in muscle, hepatopancrea and haemolymph were 0.095±0.007, 0.080±0.008, and 0.038±0.007 mg/kg, respectively, of prawns fed with OA containing diets 50 mg/kg of body weight /day for 5 days of feeding trial. Moreover, OA level in muscle, hepatopancrea and haemolymph decreased to 0.0024±0.0001, 0.0017±0.0001 and 0.0020±0.001 mg/kg after 30 days of withdraw period respectively. The significantly decreased THC and PA only observed in prawns fed with OA containing diets for 1 day of feeding trial, and after that, no significant difference was revealed during the feeding trial. PO activity of prawns fed with OA containing diets for 3 days significantly decreased followed with the significantly increased PO activity post feeding for 5 days, and during the withdraw period, no significant difference in PO activity was shown. For RBs, there was no significant difference during the feeding trial. The results showed that *M. rosenbergii* fed with OA containing diets at

Abstract ID: 170G (poster)

ENVIRONMENTAL MONITORING SHRIMP ON VANAME ON THE NORTH COAST OF BALI

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The north coast of the island of Bali from Grokgak area, Tejakula and Kubu is an area of shrimp farms are still intensive vaname in Bali province with a production capacity of three regions was around 1800-2000 tonnes / year. Farms that operate will implement intensive shrimp farming to super-intensive, this is because all the farms in the north coast of the island of Bali using the equipment and materials for the cultivation of such equipment waterwheel, turbine, automatic feeder on each container cultivation. Artificial feed manufacturers, the addition immunostimulan form of probiotics, vitamins and minerals. The purpose of monitoring activities in shrimp farmers is to know the influence of the environment on vaname shrimp farming in the northern island of Bali. Supervision is carried out in ten shrimp farmers Vaname that have constraints decline in production with their mostly white Feces Disease (WFD) which marked their feces shrimp white float on the water surface of pond, before going on the water color changes suddenly from bright green to dark green, womb dissolved oxygen and pH values fluctuate, the value of the cultivation medium alkalinity water continued to decline in future shrimp farming. This condition occurs in shrimp ponds vaname with a density of 200-300 fish / m2 with a very intensive feeding at day fifty to seventy-five days. Pond fish farmers has been making efforts on the size of the total harvest is still below the market, or by feeding mixed with activated charcoal and gastrointestinal bacteria such as Lactobacillus sp and Bacillus subtilis. The conclusion of the supervision of the neighborhood is the instability of the environmental conditions of water from the cultivation of high-density pattern, feeding uncontrolled diseases cause white feces disease (WFD).

Abstract ID: 202G (Poster)

DIAGNOSIS OF FITOPLANKTON DENSITY IN INTERMEDIET SCALE WITH OPTICAL DENSITY METHOD USING UV-VIS SPECTROPHOTOMETERFOR *Skeletonema sp*

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The cultivation of vaname shrimp is any problem of low quality fries due to feed mismatches used in larval maintenance. This problem can be anatomical or physiological abnormalities can occur due to feeding which is low or inadequate nutritional content. Phytoplankton is a natural food that an important role as the basis for the nutrition fulfillment in the early life of larvae of vaname shrimp. Therefore plankton supplies in quantity and absolute quality must be met in the process of vaname shrimp farming. This activity aims to perform calculations on the density of Skeletonema phytoplankton cells, performed by two methods are *Counting Chamber* method using hemacytometer and *Optical Density* method by using spectrophotometer.To see the relationship between the absorbance value and the number of cells, the absorbance data obtained from the *Optical Density* method using the spektofotometer will be compared with the calculation of cell counts using the *Counting Chamber* method. The result of calculation using *Optical Density* method is following equation $y = 0{,}0148e^{0{,}1142x}$ with value $R^2 = 0{,}9093$. While counting using *Counting Chamber* method is following equation $y = 0{,}6e^{0{,}2427x}$ with value $R^2 = 0{,}9221$. From these results it can be seen that the pattern of increase with two different methods show similar results. This indicates the prediction of the number of plankton by using optical density method can be applied with good results.

Abstract ID: 204G (Poster)

IMNV PREVENTION WITH BIOSECURITY APPLICATIONON VANAME SHRIMP POND IN SEPAYUNG PLAMPANG, SUMBAWA, WEST NUSA TENGARA

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The attack of IMNV virus (Infectious Myonecrosis Virus) has become common in ponds in Sumbawa regency, especially in Plampang sub-district which is bay area. The attack of this disease has caused considerable losses to farmers in the area, because it resulted in mass deaths on shrimp are maintained. So commonly this IMNV virus attacks to cause farmers in the region become reluctant to cultivate vaname shrimp again.This IMNV attack generally attacks the traditional ponds in the subdistrict of Plampang, Sumbawa regency, this occurs in addition to the conditions of the bay area, so the disease spreads easily, also caused the farmers have not using bioscurity principles.The purpose of this activity is to know effectivity of the application of Biosecurity in controlling IMNV disease. The method used is the case study of bioscurity application in the pond belonging to the Suka Maju group, and Tirta Jaya of Sepayung Village, Plampang Sub-district, Sumbawa Regency, NTB.The results obtained from this activity is, the pond that has implement of biosecurity is not infection by IMNV disease, while the pond around that have not implement of Biosecurity is still detected of IMNV disease.

Abstract ID: 217G (poster)

DEVELOPMENT OF DUPLEX PCR ASSAY FOR RAPID DETECTION OF MARINE PATHOGENIC *Vibrio alginolyticus*

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Due to the risks of high mortality and infection spread, it is essential to diagnosis method that could prevent and control bacterial diseases in aquaculture. In this study, a newly developed duplex PCR has been developed based on the ompK and dnaJ genes sequences represent a valid alternative molecular approach for specific and rapid detection of major pathogen that cause vibriosis, *Vibrio alginolyticus*. The present study aimed to develop duplex PCR assay for species-specific identification of *V. alginolyticus* by amplification of the ompK and dnaJ genes. Artificially detection of *V.alginolyticus* in *Artemia* nauplii and water culture were also conducted to evaluate the sensitivity of this method. A mixture of two pairs of primer specificity was tested in the same PCR reaction for optimization of developed duplex PCR assay yielded DNA fragments of 846 and 144 bp belonging to *V. alginolyticus,* respectively. Our results show the detecting capability of the duplex PCR from crude DNA was at 10^2 and 10^3 cells/ml. The efficacy of the assay were clarified using artificially infected *Artemia* and water culture which a clear PCR bands of 846 bp and 144 bp were generated from *Artemia* homogenates and water culture infected with *V. alginolyticus*. The newly duplex PCR which developed in this study for the species-specific identification of *V. alginolyticus*, was proved to be highly specific for the detection of *V. alginolyticus* without the time-consuming bacterial cultivation step.

Notes

Exhibitors Company Profiles

Blue Aqua International Pte Ltd. 17, 18, 25, 26

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Kinglab is specialized distributor and sole agents of laboratory equipment and scientific instrument since 2006 for aquaculture and fishery marketplace in South East Asia. With offices in Singapore and Jakarta, Kinglab have strong networking and partnership with well-known manufacturer and researcher in aquaculture industry from the farm to the folk. We also provide genetic testing for aquatic disease such as WSSV, IMNV, AHPND, EHP, TiLV and seafood safety under ISO 17025 certified-laboratory.

PT.MUTIARACAHAYA PLASTINDO 22

Raya KarangPilang No.33
Surabaya,East Java
INDONESIA

Tel : (031) 7663989 / 7661354
Email : yenny@mutiaracahaya.com
Web : www.mutiaracahaya.com

YennyBudianto
Stephen Neotanto

PT.MutiaracahayaPlastindo established in 1995 is a manufacturing company that specializes in Plastic packaging and paper lamination.Our products are PP film grade,LLDPE film grade,HDPE film grade and paper lamination.The whole products are food grade.

PT.MutiaracahayaPlastindo using the latest technologies machinery which is established it self as a regional leader in plastics.We have set upour business to meet the increasing demands for plastic packaging in Indonesia.

After operating more than 20 years,we continue to improve production capabilities by providing one of the featured products are Plastic Geomembrane HDPE and LDPE which is using machinery 3 (three)layers.We also provide the best human resources and laboratory testing of internal and external.We believe that we can provide the best service to meet your needs such as in the use of our plastic on pond cultivation of biological,saltponds,coating or covering landfills,waterreservoirs,drainagechannels,etc.

The advantages of using our geomembraneproducts :

1. Warranty (5 year warranty for a thickness of 0.5 mm)
2. Size can be adjusted depending on custom fields / ponds
3. Easy handling because it does not use heavy duty equipment when lowering or holding the plastic on the land / pond
4. Installation by our technician
5. Repairing free as long as under warranty,if there is damage caused because our mistake (for example : plastic fragile / brittle before 5 years,welding is not strong)

We will continue innovating in for supporting the farmers to increase the production with a practical and efficient aquaculture

New Product/Service:

PP,LLDPE,HDPE film grade; HDPE AND LLDPE Geomembrane; Mulch Plastic; Uv Plastic; Polybag

PT TheoBahagia 2

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Daniel Karuniawan
Soegijanto

Company description/Company Profile

We are a manufacture company which produce paddle wheel aerator. We are the first and the only one factory of paddle wheel in Indonesia. We started production since 2 years ago. And now we have thousands of loyal customers all over Indonesia from Aceh to Papua. We have been supplying thousands of hectares of ponds in all corners of Indonesia.
Our vision is to be a blessing to all our product users through excellent quality products and the prosperity of many farmers.

PT. BisindoKencana/Bisley 29

WismaSlipi4th Fl., JalanLetjen S. ParmanKav. 12, Jakarta 11480

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*Email:*epramudya@bisley.biz or fkamadjaja@bisley.biz
Web: www.bisley.biz
Contact Person: Edwin Pramudya (08121860838) or FranciscusKamadjaja (0815 1600661)

PT. BisindoKencana/Bisley is one of the leading marketer and distributor of quality ingredients, raw materials and chemicals for nutraceutical, food, feed, personal care, water treatment, metal, construction, refractories/ceramic, agrochemical, plastic, rubber and other industries. The company was established in Australia in 1955.
Our offices and warehouses are strategically located at Australia, New Zealand, Singapore, Indonesia, Thailand, Malaysia and Middle East. They cover our business activities at more than 30 countries.
Our winning aspiration isto be the leader in contributing *material value* for our Customers and Principals beyond simple price, by leveraging the quality of our people, our market knowledge, technical expertise and logistical capability to better*understand*and*address*their needs

New Product/Service:

Created in 1863, Solvay is a global company driven by proud and committed chemists. With our historical anchorage in Europe, our products serve diversified markets worldwide, from consumer goods to aquaculture, with one main aim to improve quality of life and customer performance.

Solvay is developing peracetic-based, environmentally-friendly, and sustainable disinfectant for aquaculture industry. The registered trademark is Aqualisan

SURE Marketing Company, Inc. 3,4

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Contact Persons: Mr. Jing Tabayoyong / Mr. Teddy Gunawan
Mr. Agus Setiawan / Mr. Jerome Tabayoyong

We are *co-exhibiting* with **PT. GeneCraft Labs** as our Partner Company.

SURE Marketing Company, Inc. is a **leading** Supplier and worldwide *exclusive* Distributor of innovative biotechnology products that find applications in aquaculture and food safety.

Our **GenePasQ®** Shrimp Disease DNA Test Kit based on pioneering and ***patented*** technologies developed **in JAPAN** can ***simultaneously*** detect 4 Shrimp diseases like *EMS / AHPND, IHHNV, WSSV* and *EHP.* After PCR, you can **visually judge** with a high degree of accuracy and specificity the existence of the target genes using the ***PAS-STH*** / DNA Chromatography method. Gel electrophoresis is ***not*** needed.

We have recently introduced our **GenePasQ®** Shrimp Disease ***DNA*** Test Kit to selected **Southeast Asian** countries and shortly to other markets *worldwide.*

Our Japanese Partners are currently working on other Genetic Testing Kits for Shrimp and Fish Diseases caused by ***RNA* viruses** as well as a ***Shrimp vaccine.***

PT. GeneCraft Labs is a **leading** life science & biotechnology instruments and reagents Supplier in INDONESIA. They provide a ***complete range*** of life science products and services to the life science research, applied testing, human identity & forensics, food safety testing, biomedical and healthcare sectors *especially to the molecular diagnostics sector.*

New Products / Service:
GenePasQ® *DNA* Test Kit for *EMS / AHPND, IHHNV, WSSV* and *EHP* **GenePasQ®** *RNA* Test Kits for Shrimp and Fish Diseases
GenePasQ® Shrimp Vaccine *(under development)*
GenePasQ® Quick Bath QB-0224B *Portable* **PCR** Machine **GenePasQ®** Quick Mobile QM-0204A *Mobile* **PCR** Machine Biotechnology Instruments and Reagents, Laboratory Equipment, etc.

PT SUN PERKASA INDONESIA 5, 6

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PT SUN PERKASA INDONESIA was established by Tai Yih Sun Taiwan in Jakarta, Indonesia at 2005. Our company is the only official Authorized Agent and Stockist of Tai Yih Sun brand in Indonesia which specializes in manufacturing Aquaculture products. Our main products are **Tai yih Sun**:

- Paddle Wheel Aerator
- Submersible Pumps
- Roots Blowers

PT SUN PERKASA INDONESIA also provides for other aquaculture and industrial products manufactured in Taiwan such as:

Chuan Fan

- Ring Blowers
- Turbo Blowers
- Radial Blowers
- Cooling Fans
- Sirroco Fans

Back Port

- Paddle Wheel
- Ring Blowers