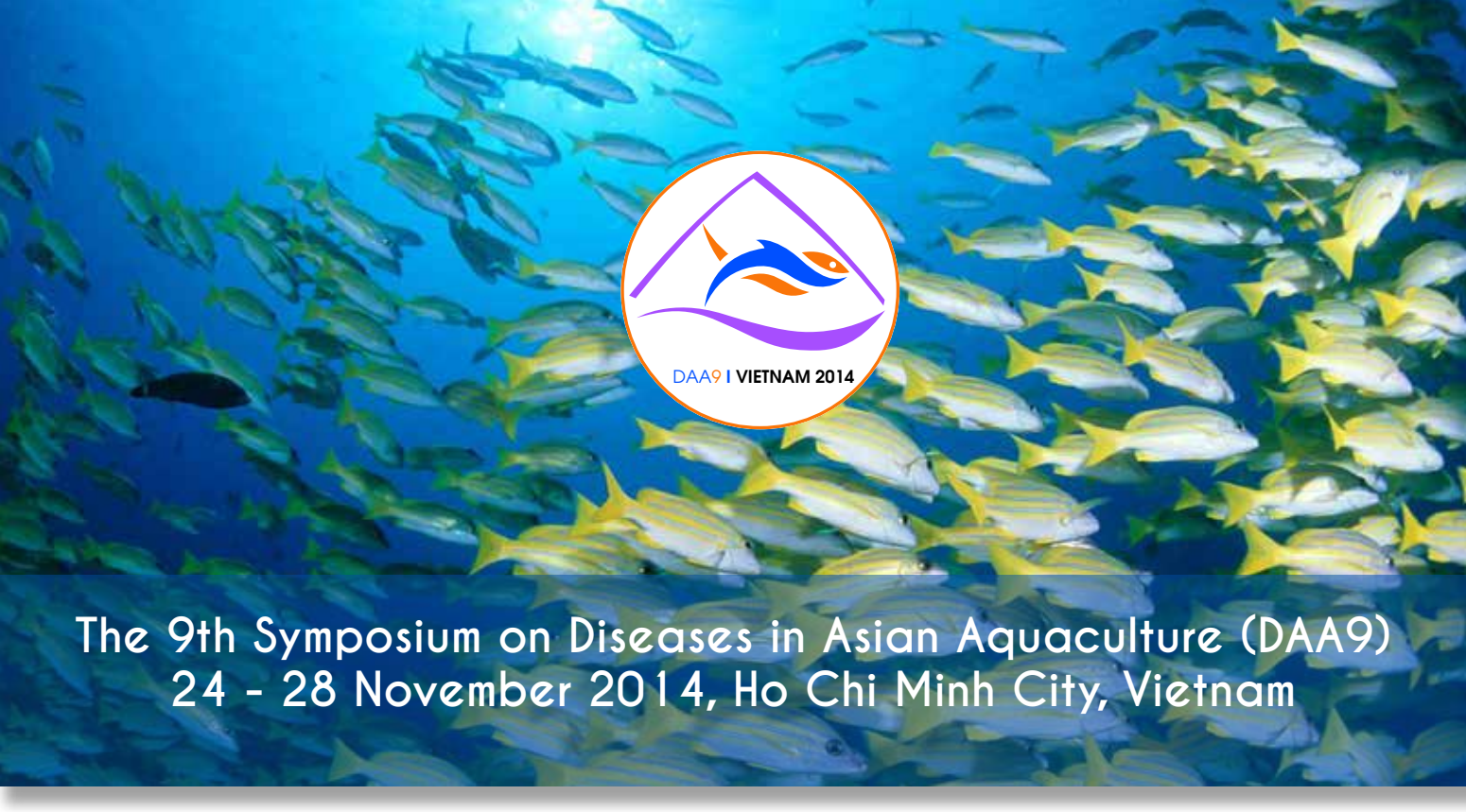

The 9th Symposium on Diseases in Asian Aquaculture (DAA9)
24 - 28 November 2014, Ho Chi Minh City, Vietnam

BOOK OF ABSTRACTS

Organized by

Fish Health Section (FHS) of the Asian Fisheries Society (AFS)
And
Department of Animal Health,
Ministry of Agriculture and Rural Development

DAA9 | VIETNAM 2014

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Welcome Message of Director General of Department of Animal Health of Viet Nam.

Vietnam is honored to be selected as the host country of the 9th Symposium on Disease on Asian Aquaculture (hereinafter referred to as the DAA9 Conference). This is one of very important evidence indicating that international communities, the Fish Health Section of the Asian Fisheries Society have appreciated and recommended the Vietnam Department of Animal Health to host the DAA9 Conference.

On behalf of the government of Vietnam with approval of the Ministry of Agriculture and Rural Development, as the Director General of the Vietnam Department of Animal Health and the CVO of Vietnam, I warmly welcome all delegates to the DAA9 Conference. I sincerely thank the international communities, especially the Fish Health Section of the Asian Fisheries Society have worked closely with the Vietnam Department of Animal Health to prepare effectively the DAA9 Conference.

DAA9 Conference is good opportunity for scientists, managers, businesses and students who are working and learning about aquaculture and aquatic animal health in Asia and around the world to share the important and useful research results and experiences in the prevention and control of aquatic diseases. This is also a great opportunity for businesses to promote aquaculture activities, trading of aquatic and aquatic products, aquatic feed, aquatic veterinary medical products, science and technology in aquatic animal health. The conference also provides a great opportunity for the Vietnam and international partners to develop and expand the research collaboration, markets for aquatic products and veterinary medical products.

I really appreciate and sincerely thank sponsors and supporters for both on technical and financial support to be used for the DAA9 Conference. My deep thanks to all scientists who are key speakers, submitters of abstracts and posters at the Conference DAA9 which is believed to be a very good open forum 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 preparing the DAA9 Conference.

I do expect you all will have pleasure time and a successful conference during your visit in Vietnam.

My best wishes to all delegates.

Dr. Pham Van Dong
DAH Director General, CVO.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

Greetings from FHS (AFS)

As the present Chairperson of the Fish Health Section of the Asian Fisheries Society I am very pleased to welcome all of you, on behalf of the FHS Exe-Com (2011-2014) and the LOC (Department of Animal Health, MARD, Vietnam) to the 9th Symposium on Diseases in Asian Aquaculture (DAA9). The seeds for FHS idea was sown in the form of a network (Asian Fish Health Network-AFHN) in the year 1985 through an IDRC supported project. Fish Health Section (FHS) was born as a formal section of the Asian Fisheries Society (AFS) in the year 1989 and the 1st Symposium on Diseases in Asian Aquaculture (DAA1) was conducted in Bali, Indonesia in 1990. Since then there has been no looking back. The section has grown from strength to strength largely because of the commitment of several fish health researchers in the region. This year happens to be the Silver Jubilee year for FHS and all of us can look back on our collective accomplishments with great sense of pride and joy. The vision and mission of those who started the DAA series in a humble way in 1990 in Bali, Indonesia and moved through Phuket, Thailand (1993), Bangkok, Thailand (1996), Cebu, The Philippines (1999), Gold Coast, Australia (2002), Colombo, Sri Lanka (2005), Taipei, Taiwan (2008), Mangalore, India (2011) and now Ho Chi Minh City, Vietnam (2014) needs to be continued, nurtured and carried forward by younger fish health researchers from the region. Three aquatic diseases, EUS in 1980's, WSD in 1990's and now AHPND have contributed immensely to fostering a long lasting networking among aquatic animal health professionals in the region. The region as a whole has excelled in aquatic animal health research over the last 25 years and this is illustrated by the excellent collaborations that are being sustained between aquatic animal health professionals of Asia Pacific and reflected in the quality of science outputs from the region. Today FHS has become a sustainable organization promoting shared learning, networking and providing benefits for members and the wider public interested in/wanting improved fish health.

The DAA9 event attracted over 260 high quality research submissions from Asia-Pacific and other parts of the World. The growing interest in emerging diseases (AHPND), diseases of fast developing aquaculture commodities like tilapia and catfish and fish immunology is very evident in the DAA9 scientific program. We hope the 5 day scientific program will excite, stimulate and motivate you all and contribute to further strengthening the networking, shared learning and bondage amongst aquatic animal health researchers. It is my duty to sincerely thank the Department of Animal Health under the leadership of Dr Pham Van Dong, several FHS senior mentors, the Event Management Company and FHS Exe-Com for their support and contributions to the organization of DAA9.

Best wishes

Dr. Chadag Vishnumurthy Mohan
FHS Chairperson (2011-2014)
Senior Scientist Aquaculture
WorldFish HQ
Penang, Malaysia.

DAA9 COMMITTEES

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The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

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DAA9 papers will be published as a special online issue in the Japanese Society of Fish Pathology Journal "Fish Pathology". Our Thanks to JSFP

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

Scientific Program Schedule (Oral Paper Presentations)

Abstract ID	Time	Paper Title	Presenters
24 Monday: DAA9 Opening Session			
	08h30-10h00	Formal Opening Ceremony: Detailed Program to be Provided	
419	10h00-10h30	Key Note Presentation 1: Managing Health in Aquatic Production: an Achievable Challenge in Meeting the Future Demand for Food Fish?	Rohana Subashinge
99	10h30-11h00	Key Note Presentation 2: Status and future perspectives of vaccines for industrialised fin-fish farming	Kjersti Gravningen
	11h00-11h30	Group Photo and Coffee Break	
24 Monday: Session-Biosecurity Compliance			
404	11h30-11h50	The European Union Reference Laboratory (EURL) system for aquatic animal health – structure, function and output	Stentiford, G.D.
355	11h50-12h10	Improving aquatic animal health in Asian Aquaculture: the role of OIE	Ingo Ernst
410	12h10-12h30	Evalutation of aquatic disease control strategies: catchment versus country based zones and compartments	Art Jonkers, Kieran Sharkey and Kenton Morgan
153	12h30-12h50	Ornamental fish importation — Australia's new approach to managing biosecurity risks	Yuko Hood and Ramesh P. Perera
	12h50-14h00	Lunch	
179	14h00-14h20	Establishing freedom from aquatic animal disease	Edmund J Peeler
149	14h20-14h40	IBIS: International Biosecurity Intelligence System for Early Warning, Better Planning, Rapid Response	Geoff Grossel, Sam Hamilton, Mark Burgman
418	14h40-15h00	Approaches in livestock animal health research and management	Phil Toy
	15h00-15h30	Coffee Break	

24 Monday: Session-Parasitic Diseases			
406	15h30-15h50	Parasite invasion: Their economic impact on Asian mariculture	Andrew P. Shinn, Jarunan Pratoomyot, James E. Bron, Giuseppe Paladini, Esther E. Brooker and Adam J. Brooker
172	15h50-16h05	Swimming, excystment pattern and distribution aspects of theronts of parasitic ciliate Cryptocaryon irritans	Kah Hui How, Kosuke Zenke, Tomoyoshi Yoshinaga
259	16h05-16h20	Infestation of marine leech, Zeylanicobdella arugamensis on farmed fish in Malaysia	Kua Beng Chu, Kamisa Ahmad and Nur Ashikin Arbi
277	16h20-16h35	A Study of Wet Tropics tandan Tandanus tropicanus (Welsh, Jerry & Burrows 2014) from the Bloomfield River, Queensland, Australia.	Erin Kelly, Susan Gibson-Kueh, Brendan Ebner, James Donaldson, Terry White, RongchangYang, David Morgan and Alan Lymbery
144	16h35-16h50	Efficacy of ginger-based treatments against infection with Gyrodactylus turnbulli in the guppy (Poecilia reticulata (Peters)).	G. Levy, D. Zilberg, G. Paladini and S. Fridman
166	16h50-17h05	Response to the detection of Perkinsus olseni in farmed New Zealand paua, Haliotis iris	Jen Brunton, Brian Jones, Rissa Williams, Edwin Ainley, Erin Breen
123	17h05-17h20	Host-parasite interaction and recent developments in Argulus vaccinomics	PK Sahoo,Banya Kar, Amruta Mohapatra, J Mohanty, P Jayasankar
	18h00-19h30	Opening Reception	
25 Tuesday: Session-Shrimp EMS/AHPND			
276	08h30-08h45	EMS/AHPND: a game changer for the future development of aquaculture	Timothy W. Flegel
420	08h45-09h00	Documentation of a Unique Strain of Vibrio parahaemolyticus as the Agent of Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) Affecting Penaeid Shrimp with Notes on the Putative Toxins	Donald V Lightner
262	09h00-09h15	Recent advances in the newly emergent acute hepatopancreatic necrosis disease (AHPND)	Chu-Fang Lo, Chung-Te Lee, I-Tung Chen, Yi-Ting Yang, Han-Ching Wang

194	09h15-09h30	Characterization of virulence factor of AHPND Vibrio parahaemolyticus which is the causative agent of shrimp disease	Sasiwipa Tinwongger, Jumroensri Thawonsuwand, Janejit Kongkumnerd, Reiko Nozaki, Hidehiro Kondo, and Ikuo Hirono
193	09h30-09h45	Identification of an insertion sequence related to deletion/ insertion of the potent toxin genes of acute hepatopancreatic necrosis disease (AHPND) in Vibrio parahaemolyticus	Yuki Nochiri, Sasiwipa Tinwongger, Reiko Nozaki, Hidehiro Kondo and Ikuo Hirono
183	09h45-10h00	ZOT-proteins occur in conjunction with E-family virulence factors in AHPND-causing Vibrio parahaemolyticus associated with either of three prophage elements in their genome	Eng Huan Ung, Kwai Lin Thong, Sh Min Yew, Siew Woh Choo, Wei Yee Wee
311	10h00-10h15	Serotypes, genotypes and virulence genes of Vibrio parahaemolyticus causing acute hepatopancreatic necrosis disease in southern Thailand	Jetnaphang Kongrueng, Mingkwan Yingkajorn, Supansa Bunpa, Natthawan Sermwittayawong, Varaporn Vuddhakul
229	10h15-10h30	An AP1, 2 & 3 PCR Positive non-Vibrio parahaemolyticus bacteria with AHPND histopathology	Kwai Lin Thong, Eng Huan Ung, Siew Woh Choo, Sh Min Yew, Wei Yee Wee, Kien Pong Yap
	10h30-11h00	TEA BREAK	
339	11h00-11h15	A microbial perspective on Acute Hepatopancreatic Necrosis Disease (AHPND) outbreaks in shrimp farming	Patrick Sorgeloos, Peter Bossier, Geert Rombaut, Peter De Schryver
102	11h15-11h30	Ecological approaches in controlling the Acute Hepatopancreatic Necrosis Disease	Loc Huu Tran, Kevin Fitzsimmons, Donald Lightner
335	11h30-11h45	Risk factors associated with EMS/ AHPND occurring in culture shrimp in Thailand	Visanu Boonyawiwat and Jiraporn Kasornchandra
254	11h45-12h00	Pesticides used in shrimp farms in the mekong delta, Vietnam: are they associated with acute hepatopancreatic necrosis syndrome?	Dang Thi Hoang Oanh and Truong Quoc Phu
252	12h00-12h15	Vibrio parahemolyticus associated with shrimp mortalities in India do not have characteristics of AHPND strains	Indrani Karunasagar, Krishna Kumar, Vijaya Deekshit, Juliet Raj, Praveen Rai, B. Shivanagowda and Iddya Karunasagar

272	12h15-12h30	Metagenomic analysis of bacteria in the hepatopancreas of cultivated shrimp exhibiting early mortality syndrome (EMS) in Thailand and Vietnam	Kallaya Sritunyalucksana, Anuphap Prachumwat, Siripong Thitamadee, Truong Hong Viet, Jiraporn Srisala, Timothy W. Flegel
	12h30-13h45	LUNCH BREAK	
401	13h45-14h00	Time-saving and specific methods with high sensitivity for detecting acute hepatopancreatic necrosis disease (AHPND)	Hai-Liang Wang, Yu-Juan Wang, Hao-Lin Yang, Na Wang, Jie Huang
110	14h00-14h15	Loop-mediated isothermal amplification combined with colorimetric nanogold for detection of bacterial isolates causing acute hepatopancreatic necrosis disease	Rungkarn Suebsing, Narong Arunrut, Jantana Kampeera, Piyachat Sanguanrat, Porranee Proespraiwong, Veerapat Kamamnuaysuk, Rapeepat Mavichak, Siripong Thitamadee, Wansika Kaitpathomchai
318	14h15-14h30	Biofilm formation by Vibrio species from shrimp including those that causes AHPND	Chumporn Soowannayan, Zeus Elumba, Karla Jessica Arida, Pattanan Yatip
25 Tuesday: Session-Fish Viral Disease			
304	14h30-14h50	Latency of koi herpesvirus (KHV) in koi and carp: implications for disease transmission and control	Agus Sunarto, Kenneth A. McColl, Mark StJ. Crane, Peter J. Walker
302	14h50-15h10	VNN: A Challenge to Mariculture in the Arabian Region with a Special Reference to Kuwait	Azad I.S. and K.A.Elah
161	15h10-15h25	Singapore grouper iridovirus ORF075R functions as a coupling protein during viral assembly	Zhu Yi, Liu Yang, Wang Fan, Wu Jinlu, Hew Choy Leong
	15h25-15h45	TEA BREAK	
283	15h45-16h00	Different expression profiles of Interleukin 11 (IL-11), Intelectin (ITLN) and Purine nucleoside phosphorylase 5a (PNP 5a) in crucian carp (Carassius auratus gibelio) in response to Cyprinid herpesvirus 2 and Aeromonas hydrophila	Patarida Podok, Lijuan Xu, Dan Xu, Liqun Lu
133	16h00-16h15	Emerging Ranavirus infections in ornamental and cultivable fishes of India	Riji John K, Rosalind George M, Kar Devashish and Waikhom Gusheinzed
	16h15-17h30	**POSTER SESSION**	Poster Viewing with Authors

26 Wednesday: Session-Shrimp WSD			
413	08h30-09h00	On the transmission of WSSV in aquaculture systems	Just M Vlak and Mart C.M.de Jong
131	09h00-09h15	On the role of polychaetes [Dendronereis spp.] in transmission of white spot syndrome virus in shrimp ponds	Desrina, Just M. Vlak, Slamet B. Prayitno, Johan A.J. Verreth and Marc C.J. Verdegem
88	09h15-09h30	WSSV achieves successful replication by triggering an invertebrate Warburg effect via activation of the PI3K-Akt-mTOR pathway	Han-Ching Wang
214	09h30-09h45	Two anti-apoptotic proteins of white spot syndrome virus that bind to an effector caspase of the black tiger shrimp Penaeus monodon	Tareerat Lertwimol, Pakkakul Sangsuriya, Kornsunee Phiwsaiya, Amornrat Phongdara, Chuenchit Boonchird, Timothy W. Flegel, Saengchan Senapin
198	09h45-10h00	Susceptibility and pathogenicity of White Spot Disease (WSD) in non-model crustacean host taxa from temperate regions	Kelly S Bateman, Michelle Pond, Grant D Stentiford
230	10h00-10h15	Mode of antiviral action of Penaeus monodon anti-lipopolysaccharide factor isoform 3 (ALFPm3)	Kunlaya Somboonwiwat, Pakpoom Boonchuen, Thanachai Methatham, Phattarunda Jaree, Anchalee Tassanakajon
268	10h15-10h30	Silencing protein kinase in shrimp: An insight on its role in the immune system	Maria Violeta Tare, Aiko Shitara, Hidehiro Kondo, Ikuo Hirono, Mary Beth Maningas
	10h30-11h00	COFFEE BREAK	
201	11h00-11h15	Study the role of glucose transporter 1 (Glut1) in white spot syndrome virus(WSSV) infection	Huai-Ting Huang, Li-Li Chen
136	11h15-11h30	A quantitative approach to disease transmission of white spot syndrome virus: seasonal effects in shrimp ponds	Tuyen N. Xuan, Thao T. Thanh, Just M. Vlak, Mart C.M. de Jong
174	11h30-11h45	Effects of WSSV and bio-security on shrimp farming in Bangladesh	Partho Pratim Debnath, Ben Belton, Manjurul Karim, Hendrik Jan Keus, Chadag Vishnumurthy Mohan

151	11h45-12h00	Towards the commercial use of double-stranded RNA for the prevention of shrimp viral diseases	Thitiporn Thammasorn, Pakkakul Sangsuriya, Saengchan Senapin, Parinyachat Somchai, and Vanvimon Saksmerprome
	12h00-13h00	**POSTER SESSION**	Poster Viewing with Authors
	13h00-14h00	LUNCH BREAK	
26 Wednesday: Session-Fish Immunology			
73	14h00-14h15	A successful case of phage therapy in aquaculture practice	Toshihiro Nakai, Yasuhiko Kawato, Takuto Tamada, Se Chang Park, Gang Joon Heo
160	14h15-14h30	Immune responses of Yellowtail kingfish (Seriola lalandi) to Photobacterium damselae subsp. damselae, Vibrio anguillarum, and Vibrio harveyi	Cecile Dang1, Sam Hair2, Jo Bannister1, Paul Hillier1, Fran Stephens1, Nicki Buller2 and Gavin Partridge3
121	14h30-14h45	Immunogenicity of formulating inactivated vaccine with Toll-like receptor 9 agonist CpG oligonucleotides and alum on cobia (Rachycentron canadum)	Omkar Byadgi; Ta-Chih Cheng
138	14h45-15h00	Characterization of IκBa, Rac2 and Rab21 as specific innate immune genes during persistent infection of Cyprinid herpesvirus 2 in crucian carp (Carassius auratus gibelio)	Dan Xu, Siyao Xia, Weichen Qiu, Patarida Podok, Liqun Lu
317	15h00-15h15	Protective immunity of recombinant FBSA and A-Enolase in tilapia (Oreochromis niloticus niloticus L) against Steptococcus agalactiae.	Ting Yi*, Yan-Wei Li*, Liang Liu, Xi-Xi Xiao, An-Xing Li§
	15h15-15h45	COFFEE BREAK	
282	15h45-16h00	Effect of high-concentration ascorbic acid supplementation on disease resistance and some innate immune responses in rainbow trout	Aki Namba,T. Ishikawa, T. Yokozuka, T. Yabu, N. Mano, M. Sawada, T. Nakanishi
163	16h00-16h15	Alpha Ject Panga 1-The first vaccine against Edwardsiella ictaluri in striped catfish (Pangasianodon hypophthalmus) in Vietnam	Thanh Pham Cong, Tung Vo Thanh, Khanh Nguyen Quang, Nhi Duong Van, Dung Truong Thanh, Kjersti Gravningen
417	16h15-16h30	Functional recombinant type I interferon of medaka fish produced using mammalian cell line	Shun Maekawa1, Takashi Aoki1, 2*, Han-Ching Wang1, Chu-Fang Lo3, 4, Haruko Takeyam5, Jun-ichi Hikima6 and Masahiro Sakai6

328	16h30-16h45	Immune response in carps to nanoparticle based delivery of recombinant outer membrane protein of Aeromonas hydrophila	Saurabh Dubey., Sangeetha .M. S., M.N.Venugopal, Indrani Karunasagar
	16h45-17h45	**POSTER SESSION**	Poster Viewing with Authors
	18h30-20h30	10th TGM of FHS (AFS)	
27 Thursday: Session-Tilapia and Catfish Diseases			
267	08h30-09h00	Major Diseases and health management in most commontly cultured species in the Mekong River Delta, Vietnam	Dang Thi Hoang Oanh
412	09h00-09h25	Disease outbreaks in pangasius catfish farming: past, present and future	Mags Crumlish
145	09h25-09h40	Dermocystidium, Streptococcus parauberis, liposarcoma: new disorders in ornamental fish in Israel	Dina Zilberg, Nitzan Reiss-Hevlin, Angelo Colorni and Michal Ucko
209	09h40-09h55	Identification and pathogenicity investigation of Chryseobacterium indologenes in red tilapia (Oreochromis sp.)	Apirat Weerapornprasit1 ,Nopadon Pirarat2 and Channarong Rodkhum1*
208	09h55-10h10	Francisellanoatunensis subsp. orientalis as the causative agent of visceral granulomas disease in cultured red tilapia (Oreochromis sp.) in Thailand	Vuong Nguyen, Ha Dong, Nopadon Pirarat, Saengchan Senapin, Channarong Rodkhum
103	10h10-10h25	Outbreak of polycystic liver in red hybrid tilapia, Oreochromis niloticus (L.) x Oreochromis mossambicus (Peters)	M Ismail-Salihin, A Siti-Zahrah, M Zamri-Saad, and MNA Amal
	10h25-11h00	COFFEE BREAK	
333	11h00-11h15	Francisellosis in tilapia	Kim Thompson, University of Stirling
218	11h15-11h30	Putative virulence gene profiles and pathogenicity of Flavobacterium columnare isolated from red tilapia (Oreochromis sp.)	Le Dinh Hai, Dong Thanh Ha, Nopadon Pirarat, Channarong Rodkhum
264	11h30-11h45	Bacterial disease and management of Nile Tilapia (Oreochromis niloticus) cage cultured in Chi River in Northeastern Thailand	Panarat Phadee, Weena Koeypudsa and Kishio Hatai

234	11h45-12h00	Load and composition of the bacterial microbiota of tilapia (Oreochromis niloticus) cultured in earthen ponds in the Philippines	Rolando Pakingking Jr., Peter Palma, Roselyn Usero
162	12h00-12h15	Genome sequencing and comparative analysis of four strains of Edwardsiella ictaluri from the Mekong Delta.	Are Klevan, Vo-Thanh Tung, Nguyen Truong Phuc, Tran Duy Phuong, Stine Therese Sjaatil, Anja Nygaard, Marianne Bordevik and Kjersti Gravningen
	12h15-13h00	POSTER SESSION	Poster Viewing with Authors
	13h00-14h00	LUNCH	
27 Thursday: Session-Shrimp Immunology			
405	14h00-14h30	Crustacean immunity; hemocytes are most important for immunity.	Kenneth and Irene Söderhäll
257	14h30-14h45	Functional elucidation and characterization of MrFH in Macrobrachium rosenbergii using RNA interference	Joseph Carlo V. Vergel
118	14h45-15h00	Application frequency of dietary Vibrio harveyi lipopolysaccharide on growth and white spot syndrome virus resistance of Penaeus monodon post larvae	Josette Emlen Genio
270	15h00-15h15	Interplay between white spot syndrome virus and shrimp Penaeus monodon melanization immune response	Pakkakul Sangsuriya, Saengchan Senapin, Anchalee Tassanakajon, Piti Amparyup
	15h15-15h45	COFFEE BREAK	
27 Thursday: Session-Marine and Other Aquatic Diseases			
351	15h45-16h10	Tail-rot and scale drop disease in asian seabass, Lates clacarifer: a review	LEONG Tak Seng
287	16h10-16h30	Diseases of barramundi or Asian seabass, Lates calcarifer Bloch – current knowledge and management strategies.	Susan Gibson-Kueh
178	16h30-16h45	Description and quantification of mortality in finfish marine aquaculture in Northern Vietnam	A.S. Boerlage, K.V. Nguyen, J. Davidson, V.T. Phan, B.N. Thanh, L.T. Dang, K.L. Hammell

281	16h45-17h00	Epidemiological survey of pathogenic bacteria among ayu and other feral fishes in a river	Hisato Takeuchi, Motoyuki Hiratsuka, Hiroki Oinuma, Takanori Ishikawa, Takehiro Ryuu, Nobuhiro Mano
213	17h00-17h15	Relationship between the protozoan parasite Perkinsus olseni and a decrease in Manila clam resources in Japan	Tsukasa Waki, Tomoyoshi Yoshinaga, Miki Takahashi, Tatsuya Eki and Jun Shimokawa
28 Friday: Session-Diagnostics			
332	08h30-09h00	Evolution of rapid diagnostic technologies -What does the future hold for aquaculture	Alexandra Adams
305	09h00-09h15	The problem of emerging diseases – the role of microscopy and histopathology as an integrating component in disease diagnosis	Stephen W. Feist
158	09h15-10h00	A novel immunomagnetic reduction assay for dection of fish iridovirus in groupers	Y.F. Hung, S.Y. Yang, H.Y. Chou, J.L. Wu, M.W. Lu
329	10h00-10h15	Development of monoclonal antibody based farmer level diagnostics for aquatic pathogens in India	Shankar K.M. , Naveen Kumar B.T. and Abhiman P B
310	10h15-10h30	Evaluation of diagnostic tests for detection and identification of spring viraemia of carp virus in imported ornamental fish.	John Hoad, Nick Moody, David Cummins, Mark Crane
	10h30-11h00	COFFEE BREAK	
80	11h00-11h15	Bacteriophages as an indicator for prediction of bacterial disease occurrence in aquaculture	Takuto Tamada, Indah Istiqomah, Hirofumi Yamashita, Yasuhiko Kawato, Emi Sugaya, Shintaro Urasaki, Kohei Ohta and Toshihiro Nakai
184	11h15-11h30	Detection of concatemeric DNA as an indicator of koi herpesvirus infection	Yasuhiko Kawato, Kei Yuasa, Yoshiko Shimahara, Norihisa Oseko
312	11h30-11h45	ShrimpGPAT: a gene and protein annotation tool for knowledge sharing and gene discovery in shrimp	Anuphap Prachumwat, Sirintra Vaiwsri, Parpakron Korshkari, Timothy W. Flegel, Sudsanguan Ngamsuriyaroj, Burachai Sonthayanon

245	11h45-12h00	The genome and occlusion bodies of marine Penaeus monodon nudivirus (PmNV, also known as MBV and PemoNPV) suggest that it should be assigned to a new nudivirus genus that is distinct from the terrestrial nudiviruses	Yi-Ting Yang, Guang-Hsiung Kou, Chu-Fang Lo
164	12h00-12h15	In vivo and in silico comparison of fish-associated and non fish-associated subtypes of Streptococcus agalactiae	Christian M. J. Delannoy, Ruth N. Zadoks, Margret Crumlish, David Rodgers, Frederick A. Lainson, Hugh W. Ferguson, Jimmy Turnbull and Michael C. Fontaine
	12h15-13h00	POSTER SESSION	Poster Viewing with Authors
	13h00-14h00	LUNCH BREAK	
28 Friday: Session Husbandry and Management			
239	14h00-14h15	Calcium is important for survival of prawn infected with Macrobrachium rosenbergii nodavirus	Tanatchaporn Utairungsee, Ratchanok Sirikharin, Idsada Mungsuntisuk, Anuphap Prachumwat, Timothy W. Flegel, Kallaya Sritunyalucksana
236	14h15-14h30	Expression of virulence genes in Harveyi clade vibrios in relation to their virulence towards gnotobiotic brine shrimp	H.A. Darshanee Ruwandeepika, Tom Defoirdt, T.S.P. Jayaweera, Indrani Karunasagar and Peter Bossier
300	14h30-14h45	Dietary effects of various antioxidant supplements on growth, survival, antioxidant capacity, immune response, metabolic response and oxidative stress status of Pacific white shrimp (Litopenaeus vannamei)	Laila M. Gallego, Yew-Hu Chien
288	14h45-1500	Heat Shock Protein 70 (Hsp70): a crucial molecular chaperone for thermotolerance and pathogenic Vibrio resistance in the Brine shrimp Artemia	Yeong Yik Sung, Mimi Iryani Mat Taib, Peter Bossier, Patrick Sorgeloos, Thomas H. MacRae
260	15h00-15h15	Involvement of Laminin receptor protein in shrimp hemocyte homeostasis	Walaiporn Charoensapsri, Pakkakul Sangsuriya, Tareerat Lertwimol, Warachin Gangnonngiw, Kornsunee Phiwsaiya, Timothy W. Flegel, Saengchan Senapin

	15h15-15h30	COFFEE BREAK	
286	15h30-15h45	Efficacy of herbs against Streptococcus infection in different Tilapia sp: Review	Gabriel A. Ataguba, Manoj T. Kamble, Samuel Omeji, Txomin Azpeitia, Nguyen T. Duc and Seema V. Medhe
279	15h45-16h00	Genetic characterisation of multidrug resistant Citrobacter spp. isolated from septicaemic fresh water ornamental fish	S.S.S.De S. Jagoda, Karim Honein, D.P.N. De Silva, Appudurai Arulkanthan, Shigeharu Kinoshita, Hideki Ushio, Shuichi Asakawa
147	16h00-16h15	Selection of probiotic bacteria and study of their inhibitory activity against pathogenic Vibrio spp. for Larval Asian Seabass, Lates calcarifer Bloch culture	Z.H Zakaria, M.Y Jasmin, M.Y Ina-Salwany, F.M.I Natrah, Murni Karim
128	16h15-16h30	Antibacterial activity of rampe, Pandanus amaryllifolius against a pathogenic strain of Aeromonas hydrophila and efficacy of rampe root extract in controlling A. hydrophila infections in guppy, Poecilia reticulata	K. R. Nagahawatte, M. Hettiarachchi
119	16h30-16h45	Characterization of the potential probiotics, Bacillus spp. isolated from striped catfish (Pangasianodon hypophthalmus, Sauvage, 1878) in Vietnam	Ho Thi Truong Thy, Nguyen Huu Thinh, Nguyen Nhu Tri, Ong Moc Quy, Korntip Kannika, Prapansak Srisapoome, Sasimanas Unajak, and Nontawith Areechon
271	16h45-17h00	AHL-Lactonase from Bacillus licheniformis DAHB1 inhibits Vibrio biofilm formation in vitro and reduced zebrafish mortality against Vibrio parahaemolyticus infection	Vaseeharan Baskaralingam
	18h30-LATE	DAA9 CLOSING DINNER	

Scientific Program Schedule (Poster Presentations)

ID	Abstract/Paper Title	Authors
Parasitic Diseases-POSTER SESSION		
200	Zoonotic trematode parasites of two cultured fish species in Vietnam: implications for epidemiology	Dang Thuy Binh, Arne Levsen, Vu Dang Ha Quyen
146	The effect of garlic, ginger and turmeric on survival of the fish parasites Cryptocaryon irritans and Tetrahymena sp.	Ji-Hyun Kim, Tamar Sinai, Sophie Fridma and, Dina Zilberg
108	Parasitic isopod infection in the hatchery and its presence in the sharks and rays caught in Sabah waters	Yen Thing Chong, Kishio Hatai, Julian Ransangan
101	Prevalence and pathogenesis of nematode (Philometra) infection on ovaries of food fishes of Bay of Bengal.	Gopalakrishnan Ayyaru and Selvakumar Periyasamy
250	A Record of family Clinosomatidae from wild guppies (Poecilia reticulata in Sri Lanka	W.M.H.K. Wijenayakea, S. Sripriya
115	Phylogeny of zoonotic parasites in fresh and brackish water fish in Vietnam	Dang Thuy Binh, Arne Levsen, Nguyen Nguyen Thanh Nhon, Vu Dang Ha Quyen, Tran Quang Sang
233	Euclinostomum sp. infection in Trichopsis and Betta Fish in Thailand	K. Phiwsaiya, S. Senapin, P. Laosinchai*, C. Kowasupat, P. Ruenwongs and B. Panijpan
190	Comparison of pathogenic potential of Anisakis simplex (sensu stricto) and Anisakis pegreffii third stage larvae in experimental rats	Chan Hyeok Jeon, Jeong-Ho Kim
324	Morphological and molecular identification of myxosporean parasite found in skeletal muscles of kuhlii loach, Pangio kuhlii (Valenciennes, 1846) collected from Thailand	Thitiporn Laoprasert, Rodjawan Jodchaiyaphum, Mark Andrew Freeman,
406	Parasite invasion: Their economic impact on Asian mariculture	Andrew P. Shinn1,2, Jarunan Pratoomyot3, James E. Bron2, Giuseppe Paladini2, Esther E. Brooker2 and Adam J. Brooker2
Shrimp EMS/AHPND- POSTER SESSION		
246	Acute hepatopancreatic necrosis disease in shrimp cultured in Mekong Delta of Vietnam	Le Hong Phuoc, Nguyen Van Hao
284	PCR Determination of Bacteria Vibro parahaemolyticus from Reported EMS/ AHPND Cases in Farmed Whiteleg Shrimp (Litopenaeus vannamei) in Malaysia	Iftikhar Ahmad, A. R., Kua, B. C, Irence, J., Siti-Zahrah, A., Norazila, J., Suphia, A.S.
402	Virulent Vibrio parahaemolyticus is proven as the agent of acute hepatopancreatic necrosis disease (AHPND) affecting Litopenaeus vannamei in China	Hai-Liang Wang, Yu-Juan Wang, Xiao-Yuan Wan, Jie Huang
217	Effectiveness of betel leave (Piper betle) and lemongrass (Cymbopogon citratus) extracts on challenged whiteleg shrimp, Litopenaneus vannamei with Vibrio parahaemolyticus that caused AHPND	Kua Beng Chu, Ahmad Iftikhar AM, Siti-Zahrah A, Nik Haiha N.Y, Fadzilah Y and Irence J
206	Prevalence and risk factors of Early Mortality Syndrome (EMS) in shrimp farms in Rayong and Chantaburi provinces, Thailand	J. Kasornchandra, V. Boonyawiwat, S. Yaemkasem, T. Chaweepak
289	PCR Detection of Early Mortality Syndrome in Litopenaeus vannamei and Penaeus monodon in Central Luzon, Philippines	Irma Dabu, Jalizah Jaira Lima, Ikuo Hirono, and Mary Beth Maningas
332	Involvement of Pir toxin of Vibrio parahaemolyticus in inducing acute hepatopancreatic necrosis disease in shrimp	Chung-Te Lee, I-Tung Chen, Yi-Ting Yang, Lien-I Hor, Chu-Fang Lo
327	Using multiple sequence alignment to find specific sequences that can distinguish between AHPND-causing and non-AHPND-causing strains of Vibrio parahaemolyticus	I-Tung Chen, Yi-Ting Yang, Chung-Te Lee, Yun-Tzu Huang, Chien-Yu Chen, Lien-I Hor, and Chu-Fang Lo
Fish Viral Disease-POSTER SESSION		
203	Origin of Korean fish viruses based on phylogenetic analysis	Wi-Sik Kim*, Jong-Oh Kim and Myung-Joo Oh
168	Complete genome sequence of nervous necrosis virus (NNV) isolated from sevenband grouper (Epinephelus septemfasciatus) in Korea	Jong-Oh Kim, Jae-Ok Kim, Si-Woo Kim, Wi-Sik Kim and Myung-Joo Oh
165	Effect of adding goldfish kidney extract to cell culture medium on the growth of goldfish hematopoietic necrosis virus	Tomoya Shibata, Azusa Nanjyo, Masato Saito, Keisuke Yoshii, Takafumi Ito, Teruyuki Nakanishi, Takashi Sakamoto, Motohiko Sano
152	Introduction of research on Cyprinid herpesvirus 2 infection emerged in China	Li Yu, Xiaocong Zheng, Peng Jia, Junqiang He, Jinjin Wang, Xiujie Shi, Wensheng Lan, Liguo Liang, Lu Wang, Hong Liu*, Jun Xie*
113	Binding of grass carp reovirus to the 37/67 kDa laminin receptor is necessary for efficient viral entry in vitro	Hao Wang, Patarida Podok,Jiale Li,Dan Xu, Liqun Lu
415	Full-length sequencing of CyHV-3 genomes directly from their host reveals mixed infections	Saliha Hammoumi1, Ayi Santika2, Zakki Zainun2, Tatiana Vallaeys3, Christophe Klopp4, Jean-Christophe Avarre1

148	Detection of Japanese eel endothelial cells-infecting virus (JEECV) in the Japanese eel Anguilla japonica, living in natural habitats	Sachiko Okazaki, Hisaya Manabe, Tsutomu Omatsu, Sinobu Tsuchiaka, Toshihiro Yamamoto, Seinen Chow, Takuro Shibuno, Kazutoshi Watanabe, Shin-ichi Ono, Hiroshi Kuwada, Tetsuya Mizutani

Shrimp WSD- POSTER SESSION

309	Transmission of white spot syndrome virus (WSSV) in vaccinated shrimp Penaeus vannamei	Ngo Thi Ngoc Thuy, Mark P. Zwart, Just M. Vlak, Mart C.M. de Jong
298	Effect of different salinities (5, 20, 37ppt) at low temperature (22oC) on WSSV infected shrimp	Eleonor A. Tendencia, Roel H. Bosma, Johan A.J. Verreth
296	Present status of wild caught Penaeus monodon broodstock and prevalence of wssv disease in Bangladesh	*Sheikh Aftabuddin, M. Monwar Parvez, M. Ashraful Hoque,
263	Targeting essential genes to mitigate WSSV infection by RNA interference	Mary Beth B. Maningas, Jassy Mary S. Lazarte, Rod Russel R. Alenton, Hidehiro Kondo, Ikuo Hirono
126	Glutaminolysis is important for replication of White Spot Syndrome Virus (WSSV)	Chun-Yuan Li, Han-Ching Wang
95	Effect of Astragalus Polysaccharide on the anti-infection of White Spot Syndrome Virus (WSSV) in Procambarus clarkia	HONG Xu-peng , LU Hong-da,ZHANG Qing-hua, XIA Si-yao,TANG Jia-jin,DING Zheng-feng,XUE Hui,TANG Jian-qing
256	In vivo efficacy study to evaluate the effective dose of VirusnipTM Aqua for horizontal transmission of White Spot Syndrome Virus in Pacific white shrimp Litopenaeus vannamei	Apichaya Taechavasonyoo, Nontawith Areechon, Sasimanas Unajak
255	Determination of the effective concentration of Organic Releasing Chlorine (VirusnipTM Aqua) against White Spot Syndrome Virus in Pacific white shrimp Litopenaeus vannamei	Apichaya Taechavasonyoo, Nontawith Areechon, Sasimanas Unajak
240	Persistence of Penaeus stylirostris densovirus delays mortality caused by white spot syndrome virus infection in black tiger shrimp (Penaeus monodon)	Sarocha Jitrakorn, Boonsirm Witchayachamnarnkul, Pattira Pongtippatee, Timothy Flegel, Vanvimon Saksmerprome
232	Development of a simple and cost-effective WSSV diagnostic kit for the Philippine shrimp aquaculture industry	Pocholo Mari Arabit, Amalea Dulcene Nicolasora, Patrick Ellis Go, Ricardo Balog, Mudjekeewis Santos, Christopher Marlowe Caipang and Mary Beth Maningas.
333	Bioinformatic prediction of WSSV-host protein-protein interaction	Shihao Li, Fuhua Li, Zheng Sun, Hui Yang, Jianhai Xiang

Fish Immunology-POSTER SESSION

400	Generation and evaluation of virulence attenuated mutants of Edwardsiella tarda as vaccine candidates to combat edwardsiellosis in flounder (Paralichthys olivaceus)	Zhao-Lan Mo , Jie Li, Gui-Yang Li, Jie Huang
71	Vaccine development for atypical Edwardsiella tarda infection in red seabream Pagrus major	Indah Istiqomah, Yasuhiko Kawato, Emi Sugaya, Hirofumi Yamashita, Toshihiro Nakai
175	Exploring the serine protease substrate involved in cell-mediated immunity in fish	Yuta Matsuura, Takeshi Yabu, Hajime Shiba, Tadaaki Moritomo, Teruyuki Nakanishi
242	Identification of immune-related genes from leukocyte subpopulations in ayu Plecoglossus altivelis altivelis	Goshi Kato, Tomokazu Takano, Wataru Kai, Motoshige Yasuike, Yoji Nakamura, Atushi Fujiwara, Takamitsu Sakai, Tomomasa Matsuyama, Natsumi Sano, Chihaya Nakayasu
280	Host responses to Aphanomyces invadans: a comparison between resistant and susceptible fish	Pradhan P. K., Yadav M. K., Verma D. K., Sood N., Punia P. and Jena J. K.
316	Identification of vaccine candidate proteins from Cryptocaryon irritans by proteomics analysis	Yong-Zhan Mai , Yan-Wei Li , Rui-Jun Li , Wei Li , An-Xing Li *
171	Functional vaccine of streptococcosis for Nile tilapia Oreochromis niloticus Linn. based on the serotypes of Streptococcus agalactiae in Thailand	Nontawith Areechon, Korntip Kannika, Sasimanas Unajak, Prapasak Srisapoome, Ikuo Hirono, and Hidehiro Kondo
117	Molecular characterization and antigenicity of outer membrane protein (OMP) of Vibrio alginolyticus isolated from diseased Tiger grouper (Epinephelus fuscoguttatus)	Nehlah Rosli, Ina Salwany Md Yasin, Murni Karim, Nur Nazifah, Siti-Zahrah Abdullah
315	Resistance genes to antimicrobial drugs in tilapia pathogen, Streptococcus agalactiae	Puttharat Baoprasertkul, Channapha Sakseepipad, Nudthapol Kaenchan and Temdoung Somsiri
135	Effect of Astragalus membranaceus and Rooibos (Aspalathus linearis) herbal extracts on non-specific immune response and disease resistance of barramundi (Lates calcarifer)	Galina Jeney, László Ardó, Tibor Feledi, Guojun Yin, Lourens de Wet, András Rónyai, Zsigmond Jeney
294	Effect of Moringa oleifera on non-specific immune response of Nile tilapia (Oreochromis niloticus) Chitralada strain and disease resistance against Streptococcus agalactiae biotype 2	Manoj T. Kamble, Wenresti G. Gallardo
211	Hyperosmotic infiltration in vaccination of atlantic salmon (salmo salar) against yersinia ruckeri	Thu D Nguyen, Barbara F Nowak and Andrew R Bridle

100	Immunostimulatory effects of herbal bioconditioners on tiger grouper (Epinephelus fuscoguttatus) against *V. parahaemolyticus* infection	Romi Novriadi, KB Haw
130	The influence of dietary fungal-derived β-glucan on immune function and immune gene expression in Pangasianodon hypoophthalmus challenged with Edwardsiella ictaluri	Wanna Sirimanapong*, Alexandra Adams, Ei Lin. Ooi, Michaël Bekaert, Bertrand Collet, John B Taggart, James E. Bron, Darren M Green, Michael J. Leaver and K.D. Thompson
134	Effect of feeding common carp (Cyprinus carpio) broodstock with different feeds on the fatty acid content of larvae and stress resistance of fry	László Ardó, Ágnes Adorján, Tibor Feledi, Zsuzsanna J. Sándor, András Rónyai, István Dankó, István Csengeri, Galina Jeney
205	Iinvestigation of inshore hagfish adaptive immune response focused on immune related gene and variable lymphocyte receptors (VLRS)	S.P Im, J.S Lee , S.W Kim, Y.L Kim, J.E Yu, J.M Lazarte and T.S Jung
186	Identification of cytokine homologues in giant grouper Epinephelus lanceolatus and tiger grouper Mycteroperca tigris using next generation sequencing data	Hidehiro Kondo, Kittipong Thanasaksiri, Paiboon Bunlipatanon, Patcharee Soonsan, Ikuo Hirono
141	Phosphoglycerate kinase enhanced immunity of the whole cell of Streptococcus agalactiae in tilapia,Oreochromis niloticus	Shih-Chu Chen*, Yi-Ting Wang, Hsing-Yen Huang, Ming-An Tsai, Pei-Chi Wang, and Bo-Huang Jiang,
140	Molecular cloning of orange-spotted grouper (Epinephelus coioides) CXC chemokine ligand 12 gene and characterization of its expression in response to nodavirus infection	Chee-Shin Chua, Hao-Ping Lin, Young-Mao Chen, Tzong-Yueh, Chen1
107	Transcriptional regulation of orange-spotted grouper (Epinephelus coioides) myostatin gene promoter correlated with nodavirus infection	Chao-Fen Lin, Chih-En Lo, Young-Mao Chen, Tzong-Yueh Chen
98	Functional analysis of interferon in orange-spotted grouper (Epinephelus coioides)	Tzong-Yueh Chen, Young-Mao Chen
90	Molecular cloning and characterization of heat shock transcription factor 1 isoform genes from orange-spotted grouper (Epinephelus coioides) exposed to temperature changes and nodavirus infection	Ting-Yu Wang, Young-Mao Chen, Tzong-Yueh Chen
407	Identification and expression analysis of two important molecules in TLR signal pathway: IRAK4 AND TRAF6	Ting Yi, Yan-Wei Li, Liang Liu, Xi-Xi Xiao, An-Xing Li
408	Protective immunity of recombinant FBSA and A-enolase in tilapia (*Oreochromis niloticus niloticus* L.) against *Steptococcus agalactiae*	Ting Yi, Yan-Wei Li, Liang Liu, Xi-Xi Xiao, An-Xing Li
409	Identification of vaccine candidate proteins from Cryptocaryon irritans by proteomics analysis	Yong-Zhan Mai , Yan-Wei Li , Rui-Jun Li , Wei Li , An-Xing Li

269	In vitro assessment of cellular immunomodulatory activities of Centella asiatica (Linn.) and Portulaca oleracea (Linn.) extracts in the Nile tilapia, Oreochromis niloticus (Linn.).	Maria Rexie Jimenez, Elena Catap

Tilapia and Catfish Diseases-POSTER SESSION

224	Study Edwardsiellosis in clown knifefish (Chitala chitala) in the Mekong Delta, Vietnam	Tu Thanh Dung and Tran Thi My Han
341	Induction of disease susceptibility in Streptococcus agalactiae infected Nile tilapia (Oreochromis niloticus) due to the elevation of water temperature	Pattanapon Kayansamruaj, Nopadon Pirarat, Ikuo Hirono, Channarong Rodkhum
325	The presence of virulence genes in Aeromonas hydrophila isolated from striped catfish (Pangasianodon hypophthalmus)	Hien Nguyen, Lan Ma, Phuong Vo, Phuong Ngo, Thuy Nguyen, Cuong Ngo, Tuyen Nguyen, Phuoc Le
261	Isolation and characterization of Streptococcus dysgalactiae from mudskipper (Pseudapocryptes elongatus) cultured in the mekong delta of Vietnam	Nguyen Thu Dung, Dang Thi Hoang Oanh
220	Status of Antibiotic resistance in Edwardsiella ictaluri and Aeromonas hydrophila from stripe catfish farming in the Mekong Delta, Vietnam	Tu Thanh Dung and Quach Van Cao Thi
291	Identification and characterization of Flavobacterium columnare isolated from Nile Tilapia (Oreochromis sp.) and striped catfish (Pangasionodon hypophthalmus) in Thailand	Channarong Rodkhum, Dong Thanh Ha
154	Characterisation of Edwardsiella ictaluri isolates recovered from natural infections in Pangasianodon hypophthalmus in Vietnam	Nguyen Ngoc Phuoc, Wanna Sirimanapong, Randolph Richards, Mags Crumlish
244	Novel genetic characterization of Flavobacterium columnare isolated from diseased red tilapia (Oreochromis sp.) in Thailand	Dong Thanh Ha1, Benjamin LaFrentz2, Nopadon Pirarat3, Channarong Rodkhum1*
295	Histopathology of Fusarium sp infected with striped catfish (Pangasianodon hypophthalamus)	Pham Minh Duc and Dang Thuy Mai Thy
226	Diversity of tetracycline resistance genes in Aeromonas hydrophila and Edwardsiella ictaluri from the striped catfish farmed in the Mekong Delta, Vietnam	Quach Van Cao Thi, Tran Tien Luc and Tu Thanh Dung
223	The presence of class 1 integrons in Aeromonas hydrophila causes disease on the striped catfish (Pangasianodon hypophthalmus) farmed in the Mekong Delta, Vietnam	Quach Văn Cao Thi, Huynh Thi Diem Trang and Tu Thanh Dung

212	Characterization of a mutant of Edwardsiella ictaluri lacking the gene coding a component of type VI secretion system	Chang Wei, Tsubasa Uchino, Hidehiro Kondo, Ikuo Hirono, Takahiro Nagai, Toshihiro Nakai, Takashi Sakamoto, Motohiko Sano
340	Molecular characterization of Streptococcus agalactiae strains isolated from diseased tilapia.	Anita Jaglarz, , Scott Waddell, Janina Z. Costa, Christian M.J. Delanoy
330	Epidemic of Bacterial Diseases in Nile tilapia (Oreochromisniloticus)	Varinee Panyawachira, Channapha Sakseepipad, Pongkochthron Sriwilai
93	Genome analysis of Streptococcus dysgalactiae 12-06 isolated from amberjack, Seriola dumerili	Naoto Yoshimura1 , Daisaku Oinaka1 , Issei Nishiki2 , Toshiaki Itami1 and Terutoyo Yoshida1

Shrimp Immunology-POSTER SESSION

199	Development of white spot syndrome virus (WSSV) vaccine using shrimp glucose transporter 1 (Glut1)	Hoi-Ling Chan,Huai-Ting Huang, Li-Li Chen
278	Antiviral from YHV infected shrimp can reduce YHV infection	Nipaporn Kanthong, Warachin Gangnonngiw, Timothy W. Flegel
334	Characterization of anti-lipopolysaccharide factor isoforms in penaeid shrimp and their potential applications	Fuhua Li, Shihao Li, Shuyue Guo, Jianhai Xiang
303	Inside a shrimp's gut: its bacterial community, pathogenesis and immunity	Soonthornchai W., Chaiyapechara S., Angthong P., Wongsrirattanakul O., and Jiravanichpaisal P.
308	Characterization of Cytokine Homologue Gene, IL-17, in Kuruma Shrimp Marsupenaeus japonicus: Gene Expression and Genomic Analysis	Mari Inada, Masahiro Sakai, Toshiaki Itami
307	Molecular Cloning and Expression Analyses of Multifunctional Cytokine, TGF-β Family, in Kuruma Shrimp Marsupenaeus japonicus	Mari Inada, Toshifumi Yui, Vu Duc Hanh, Masahiro Sakai, Toshiaki Itami
306	Cytokine Homologue Genes, VEGF, MIF and Astakine, in Kuruma Shrimp Marsupenaeus japonicus: Simulation of 3D Structure, Gene Expression Analysis during WSSV Infection and Gene Knockdown	Mari Inada, Toshifumi Yui, Masahiro Sakai, Toshiaki Itami
266	Production and application of dsRNA in probiotics bacteria for antiviral defense in shrimp	Thitiporn Thammasorn, Parinyachat Somchai, Sarocha Jitrakorn, Soraya Chaturongakul, Vanvimon Saksmerprome
238	Melanization reaction products of shrimp display antimicrobial properties against their major pathogens	Piti Amparyup, Walaiporn Charoensapsri, Anchalee Tassanakajon

202	Localization of VP28 protein on baculovirus envelope and its oral vaccination displayed enhanced protection against white spot syndrome virus in Penaeus monodon	Syed Musthaq S, Selvaraj Madhan, Sahul Hameed A.S., Jimmy Kwang
197	Characterization of copper zinc superoxide dismutase isoform 5 (MjCu/Zn SOD-5) gene, a lineage-hemocyte of kuruma shrimp Marsupenaeus japonicus	Mai Nam Hung, Hidehiro Kondo, Ikuo Hirono
195	Identification of genes induced by non-specific double-strand RNA in kuruma shrimp Marsupenaeus japonicus	Mami Komatsu, Benedict Maralit, Shiro Itoi, Haruo Sugita, Hidehiro Kondo, Ikuo Hirono
188	In vivo study of potential bacterial probionts against pathogenic effect of Vibriosis in artemia culture	Murni Karim, Nurul Syazwani, Mohamad Pipudin, Ina Salwany Md Yasin
336	Protection against white spot syndrome virus infection in Penaeus monodon by oral administration of VP28-Bacillus subtilis	Tran Thi Tuyet Hoa, Hong Mong Huyen, Pham Kien Cuong and Phan Tuan Nghia

Marine and Other Aquatic Diseases-POSTER SESSION

338	Vibriosis resistance and wound healing in onion- and ginger-fed brown-marbled grouper, Epinephelus fuscoguttatus exposed to acute stress and infectious challenge	Edgar C. Amar, Mary Jane S. Apines-Amar, Joseph P. Faisan, Jr.
169	An unknown 'Skin ulcer disease' of rainbow trout reared in seawater in Korea	Si-Woo Kim, Jong-Oh Kim, Jea-Ok Kim, Wi-Sik Kim and Myung-Joo Oh
142	Characterization of the Outer Membrane Protein (OMP) of Vibrio alginolyticus and Vibrio vulnificus Isolated from Diseased Grouper (Ephinephelus sp)	Mohd Jolharry, Nik Haiha, Zamri Saad, Nur Nazifah
204	Isolation of Vibrio vulnificus biotype 1 from disease outbreaks on cultured tiger grouper (Epinephelus fuscoguttatus, Forsskal, 1775)	Jumreonsri Thawonsuwan, Jiraporn Kasornchandra, Patchare Soonsane, Chantana Keawtapee
111	Isolation of pathogenic bacteria from diseased marine fishes	Ahmad Baihaqi, Nik Haiha,N.Y., Zamri-Saad, M., and Siti-Zahrah, A.
139	Isolation and molecular confirmation of Flavobacterium columnare from natural outbreak of columnaris disease in farmed catla, Catla catla (Hamilton), and comparative sequence analysis of 16S-23S rDNA intergenic spacer region among its genomovars	Dev Kumar Verma & Gaurav Rathore
326	Incidence of Vibrio cholerae non-O1 from plankton and seafood samples in Tamil Nadu, INDIA.	Baskaran Athmanathan, Dinesh Kumar Subaraj, Sureka Indrajith, Velmurugan Meganathan, Jennifer Emelda and Sathiyamurthy Karuppanan*

285	The development of a grading tool for 'Big Belly' histopathology	Norfalina Mohamed Rosli, Susan Gibson-Kueh, Professor Philip Nicholls & Diana Chee
274	Disease outbreak in cage cultured cobia (Rachycentron Canadum) associated with Streptococcus dysgalactiae	Ming-An Tsai, Terutoyo Yoshida, Pei-Chyi Wang and Shih-Chu Chen
243	Prevalence and antimicrobial susceptibility of ESBL-producing Escherichia coli isolated from fish in the Mekong Delta	Nguyen Thi Ngoc Hon, Tran Thi Tuyet Hoa, Nguyen Thanh Phuong and Tatsuya Nakayama
216	Detection of a partial homology pathogen of abalone herpesvirus associated with abalone chronic mortality	Chen YW, Chang PH, Chen MS, Kuo ST
215	Comparison of the susceptibilities of the Manila clam, Ruditapes philippinarum, and the hard clam, Mercenaria mercenaria, to Perkinsus olseni	Kousuke Umeda and Tomoyoshi Yoshinaga
210	Bacteriophage as a prophylactic agent for vibriosis infection caused by Vibrio parahaemolyticus in oysters	Jin Woo Jun, Sae Kil Yun, Hyoun Joong Kim, Cheng Chi, Toshihiro Nakai, Se Chang Park
170	Parasitic and shell diseases of abalone (Haliotis asinina) in the Philippines	Gregoria Erazo-Pagador, Rolando V. Pakingking, Jr. , Haydee Rose B. Dumaran
94	Atypical Lactococcus garvieae isolated from previously vaccinated yellowtail Seriola quinqueradiata	Daisaku Oinaka, Naoto Yoshimura, Yutaka Fukuda, Azumi Yamashita, Shintaro Urasaki, Yoshinobu Wada and Terutoyo Yoshida
92	Induction and characterization of a lysogenic bacteriophage of Lactococcus garvieae isolated from marine fish species	Truong Dinh Hoai, Terutoyo Yoshida
182	Comparative genomic analysis of Candidatus Xenohaliotis californiensis detected in abalone species Haliotis spp. in Japan	Toyohiro Nishioka
323	Overview of aquatic disease impacts throughout the ASEAN Community	Andrew P. Shinn, Leonardo Galli, Pikul Jiravanichpaisal, Oranun Wongsrirattanakul, Nattawadee Wattanapongchart, Wimonthip Jarupheng and Don Griffiths
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ABSTRACTS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

KEYNOTE PRESENTATIONS

ID 419:

Managing Health in Aquatic Production: an Achievable Challenge in Meeting the Future Demand for Food Fish?

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According to FAO estimates, to feed the world in 2050, agricultural output, originating from crops, livestock and fisheries, including aquaculture, must increase by over 60 percent. Foods derived from aquatic resources have a significant role to play across the food supply and value chain, linking ecosystems, economic development and human wellbeing. Since the contribution of capture fisheries to global food fish supplies has levelled off, aquaculture production has taken over as a major supply factor. Aquaculture makes valuable contributions to the local, national and regional economies through goods and services sold on the domestic and export markets. It contributes significantly to alleviating poverty and increasing food and nutrition security.

According to the World Bank, the total fish supply from both capture and aquaculture by 2030 would be around 187 million tonnes. If fish consumption patterns do not change significantly, fish prices should not significantly increase by 2030, thus maintaining current trends of aquaculture production growth, there would be enough fish to feed the growing population by 2030. However, since people would tend to consume more fish as their incomes grow, countries' per capita fish consumption are unlikely to remain constant at the level in 2007. According to recent FAO estimates, considering the potential impacts of global population growth and the global income growth on fish consumption, the world fish demand is expected to reach 261 mt in 2030. Since this additional demand has to be satisfied by aquaculture production, the future food fish supply from aquaculture should be increased significantly. From the past experience it is clear that the producers will always attempt to bridge the supply and demand gap, even forgetting the issues and concerns of sustainable production.

Historically, disease has proved a major constraint to efficient production in many aquaculture systems. Major improvements in the understanding of the aetiology and epidemiology of fish diseases have been made in recent years and aquaculture producers in many countries have dramatically improved their husbandry practices with greater focus now on fish health and welfare. Control of many serious infectious diseases has been achieved through new therapeutics and vaccines. However, new disease problems are emerging, and previously rare diseases becoming much more prevalent, so continued vigilance and solution development is required. This paper endeavors to analyze if the global aquatic animal health management capacity is adequate to address the potential threats to future aquaculture production, in meeting the demand for food fish in the coming decades.

ID99:

Status and future perspectives of vaccines for industrialised fin-fish farming

Kjersti Gravningen, Bjørn Erik Brudeseth, Knut-Egil Løkling, Are Klevan, Børge Nilsen Fredriksen, Marianne Bordevik, Karine Lindmo, Nils Steine and Rune Wiulsrød

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Fin fish farming is developing from extensive to highly intensive industrial scale productions. Production of fish in high-density culture conditions requires effective vaccines in order to control persistent and emerging diseases. Vaccines have made significant positive impacts, such as reduced usage of antibiotics. This was clearly demonstrated when vaccines were first introduced for Atlantic salmon in the late eighties and early nineties, resulting in a rapid decline of antibiotics usage. This review will focus on current vaccine applications for farmed industrialized fish species, both fresh water and marine fish species globally. The presentation will focus on use of licensed commercial vaccines in fin fish farming, as well as future trends for fish vaccines development and vaccination.

BIOSECURITY COMPLIANCE

ORAL PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID 404:

The European Union Reference Laboratory (EURL) system for aquatic animal health – structure, function and output

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Within the European Union, aquatic animal health surveillance and management is under the auspices of National Reference Laboratories (NRLs) designated by Competent Authorities of individual Member States. In addition, European Union Reference Laboratories (EURLs), designated by the European Commission (EC), assist with the delivery of quality-assured diagnostics and reporting of listed pathogens, by Member State NRLs. Specific EURLs (and NRLs) exist for Finfish, Molluscan and Crustacean Diseases in Denmark, France and the UK, respectively. All of these EURLs operate according to criteria stipulated within EC Directive 2006/88 and various associated Regulations (for example, see www.crustaceancrl.eu). EURLs offer a direct source of expert advice to the EC, coordinate methods for the diagnosis of listed pathogens across NRLs, supply control reagents for such testing, and organize comparative testing to ensure cross-EU quality assurance in diagnostic capacity. An additional role in performance of confirmatory testing on the request of Member States or the EC, and a targeted research programme for emerging issues, facilitates the development of the EURL as an expert centre. In this talk, I will summarize the operation of the EURL for Crustacean Diseases and how aspects of the established model for aquatic animal health surveillance and management within Europe may be transferred to operate within Asia. In addition, I will discuss the output of a recent UKTI-funded workshop on aquatic animal health in Asia and the potential for a Decentralized Testing and Reporting Model (DTRM), using in-field diagnostics, and remote data reporting to centralized epidemiological centres, for aquatic health surveillance and management within Asian aquaculture.

ID355:

Improving aquatic animal health in Asian aquaculture: the role of the OIE

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Aquaculture is characterised by rapid change that is unprecedented in the history of animal production. Production volume is increasing, new species are being farmed for the first time, species are being farmed in new geographic areas, domestication and genetic improvement are being pursued, new production technologies are being developed and considerable product volumes are traded internationally. These features of aquaculture present unique challenges for managing aquatic animal health now and into the future.

The World Organisation for Animal Health (OIE) is an intergovernmental organisation with a mandate from its 180 members to improve animal (including aquatic animal) health and welfare worldwide. The OIE fulfils its mandate by carrying out the following missions:

- Ensuring transparency of the world animal disease situation
- Collecting and disseminating veterinary scientific information, and in particular information on animal disease prevention and control methods
- Securing the sanitary safety of international trade in animals and animal products
- Defining and supporting good governance of veterinary services (and aquatic animal health services)
- Promoting animal welfare.

This presentation will highlight OIE activities that are particularly relevant to addressing the challenges presented by a rapidly changing aquaculture industry and for improving aquatic animal health in Asian aquaculture. Some challenges that will be discussed include emerging diseases, antimicrobial resistance, and aquatic animal welfare.

ID410:

Evaluation of Aquatic Disease Control Strategies: Catchment versus Country Based Zones and Compartments

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Objectives

The OIE animal health code uses concepts of zoning and compartmentalisation as strategies for disease control. These concepts are based upon isolation and movement restrictions, inherited from terrestrial animal health control codes. Aquatic population are rarely defined by land barriers. Simply stated, water transcends national boundaries; the Mekong runs through 6 countries (China, Myanmar, Laos, Thailand, Cambodia and Vietnam), marine fish are also able to migrate between nation's territorial waters. Intuitively one would expect control strategies based on river catchments to be superior to those based on country borders.

Methods

We use a geographically explicit network simulator, based on the location and movement of fish between farms in England and Wales. We assume that the counties are the equivalent of land based countries and compare control based on zones and compartments defined by river catchments with those defined by counties.

Results

We show that catchment based control is superior to country based control.

Conclusion

The application of zoning and compartments to international aquatic disease control requires a new paradigm based on transnational, catchment based, organisations rather than national boundaries.

ID153:

Ornamental fish importation — Australia's new approach to managing biosecurity risks

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Australia's current biosecurity controls for importation of ornamental fish focus on post arrival quarantine. Various reviews, including a recent import risk analysis of freshwater ornamental fish on megalocytivirus identified a range of shortcomings. These shortcomings include that the current post-arrival inspection and quarantine isolation do not fully manage risks associated with fish that are sub-clinically infected and that the current system does not adequately address risks posed by emerging diseases.

To better meet its biosecurity obligations, the Department of Agriculture is reforming to explore the potential for changes to the current system to place greater emphasis on managing biosecurity risks off-shore at source. At the heart of the new approach will be an on-arrival fish health monitoring system that is able to verify on-going compliance by overseas authorities in meeting Australia's import requirements. Data collected by the monitoring system will provide for evidence-based decisions to address problems through government-to-government and industry channels. This could include the restriction or suspension of imports from high risk sources when non-compliance is not remedied. Importantly, the new approach will reward compliant exporters and importers, and allow the department to channel its financial and human resources to areas of greatest biosecurity risk.

This biosecurity reform initiative is complex. Although some elements of the new system can be introduced in the shorter term, full implementation of the reforms including the fish health monitoring program is likely to take some time.

Extensive resources and consultation with a range of stakeholders will be required to effect this change. Stakeholder consultation on the reforms has begun and preliminary trials to test the operational feasibility of the health monitoring program are being conducted. Our energies are currently focussed on developing a statistically sound sampling and testing framework for the monitoring program.

Details on the current status of the fish health monitoring program and challenges being faced in its operational implementation will be discussed.

ID179:

Establishing freedom from aquatic animal disease

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Asian countries need to consider whether establishing freedom from listed (e.g. white spot syndrome virus - WSSV) or unlisted (e.g. early mortality syndrome) diseases is a viable policy to improve aquatic animal production (through lower disease costs) and expand export markets. Disease freedom underpins international regulations to minimise disease spread through trade. In this paper we review decision-making by governments on disease freedom and draw lessons from recent European experiences. Surveillance to determine disease prevalence and geographic distribution help determine the technical feasibility and cost of eradication. In Europe the eradication of pathogens in wild populations (e.g. mollusc diseases), or in a latent state (e.g. koi herpesvirus), has rarely been successfully completed. In the absence of disease, or following eradication, surveillance to demonstrate freedom is required by the World Organisation for Animal Health (OIE). The development of risk-based surveillance (promoted by EU aquatic animal health legislation) and scenario-tree modelling has allowed standards for disease freedom to be output-based (e.g. 95% confidence with 2% design prevalence). Efficiency savings can be made but the design of output-based surveillance requires more sophisticated epidemiological modelling, compared with traditional approaches. The OIE requires that biosecurity (notification, surveillance and import requirements) is in place to maintain disease freedom. The costs of biosecurity (and the control of disease if reintroduced) need to be weighed against the potential benefits of disease freedom. In the EU no country has sought freedom from WSSV and the EU gave up its claim to freedom from epizootic ulcerative syndrome. Presumably, the costs of achieving freedom (e.g. restrictions on imports) were judged to outweigh potential benefits, but no formal economic analyses were undertaken. Expertise in epidemiology, risk assessment and economics is needed to assess cases for disease freedom, and compare them to alternative disease control strategies. There are strong arguments for technological assistance to developing countries so they can meet OIE standards, establish the necessary biosecurity infrastructure, and thus participate more fully in international trade and safeguard aquatic animal health. Furthermore, improving disease control in countries which are major exporters of aquatic animal produce reduces the likelihood of disease spread across political boundaries.

ID149:

IBIS: International Biosecurity Intelligence System for Early Warning, Better Planning, Rapid Response

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The Australian Government Department of Agriculture plays a central role in facilitating import and export of goods to and from Australia, whilst managing the risks to the environment, animal, plant and human health. Intelligence gathering and analysis on emerging and re-emerging pest and disease threats are critical to ensure that these risks are managed effectively.

In the past, most of our intelligence gathering and analysis activities were resource intensive manual processes that required individuals to access and scan a vast volume of scientific, industry, and other sources including websites such as; ProMED, HealthMap and PestLens. Although these approaches provide accurate information, to effectively manage biosecurity risks with imported animal and plant products, timely information of greater depth and breadth, together with strategic intelligence analysis is required.

This presentation describes the results of a Centre of Excellence for Biosecurity Risk Analysis research project to develop the International Biosecurity Intelligence System (IBIS; www.biointel.org). IBIS is automated software that gathers near real-time open-source information to develop strategic intelligence on terrestrial/aquatic animal and plant pests and diseases from the World Wide Web. Emerging issues and trends are identified automatically by IBIS or by the user community and are analyzed using both human and computer together. IBIS is open for anyone to join, and has attracted an international network of users to promote cross-sector intelligence analysis, crowd-sourced analysis and information sharing.

ID 418:

Successful approaches for the development of vaccines and diagnostic assays for livestock: lessons for aquatic animal health management.

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Vaccines and diagnostic assays play a crucial role in the prevention and control of infectious disease. Vaccines are one of the most effective control measures ever devised for infectious diseases, and have been instrumental in the global eradication of two diseases (smallpox, rinderpest). Diagnostic assays have multiple applications including clinical use, epidemiological tools and surveillance. Although each disease and livestock species has unique characteristics, there are generic elements that can be applied to the development of vaccines and diagnostic assays. These serve to expedite the process of development. This presentation will provide an overview of the generic approaches used in the development of these technologies, and illustrate how these are being applied within our program – the Animal Health Flagship of the Livestock and Fish CRP of the CG.

FISH PARASITIC DISEASES

ORAL PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID406:

Parasite invasion: Their economic impact on Asian mariculture

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Obligate or opportunistic parasitic pathogens can have a major impact on global finfish and shellfish aquaculture and can represent a key constraint to production, sustainability and economic viability. These parasitic infections and their impacts can, broadly, be either unpredictable/sporadic or predictable/regular. While these infections may result in the direct loss of stock and incur costs associated with the control and management of infections once established, for predictable infections there are also costs associated with mitigation, prophylactic treatment and management. Estimating the true cost of each parasite event though is frequently complicated by a complex interplay of numerous factors in each episode that can extend from the direct losses in production to the wider, downstream socio-economic impacts on livelihoods and satellite industries associated with the primary producer.

In this study, we review the major marine and brackishwater aquaculture production industries throughout Asia and provide estimates of the economic cost on some notable parasite-related mortality events and on some of the lessons learned. For this we will draw on both historical and contemporary events impacting on the key aquaculture species reared in Asia (*i.e.* the top fish (n=20), molluscan (n=15), crustacean (n=10), ascidian (n=1) and holothurian (n=1) species). The estimates that will be provided relate to the loss of stock at the point in the production cycle when the disease event occurred and are assumption led where details in the original case report were missing. It is anticipated that by reviewing past and ongoing parasite-based events, we can provide baseline information contributing to risk assessments for new aquaculture-based enterprises and in the development of robust biosecurity practices, which can help to mitigate against and/or minimise the potential impacts of parasite-mediated disease in aquaculture.

ID172:

Swimming, excystment pattern and distribution aspects of theronts of parasitic ciliate *Cryptocaryon irritans*

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Cryptocaryon irritans is a ubiquitous parasitic ciliate which poses a major economic threat to the marine fish aquaculture. A wide variety of treatment methods against the theront and tomont stage of this parasite was described but control over Cryptocaryoniasis is still not possible. Therefore, preventive measures should be applied against multiple life- stages of this parasite to provide a higher efficacy in minimizing disease occurence. Therefore, in this study, the swimming ability, excystment sequence and distribution of theronts were investigated in attempt to develop a physical control strategy. In the assessment of the swimming ability of theronts via microscopic observations and video analysis, the amount of swimming theronts decreased down to 48.1% at six hours and subsequently 6.1% at 12 hours post-excystment. Theronts also displayed an excystment pattern of which most theronts were released during the dark period. Examination of vertical distribution of theronts suggested that theronts were distributed about 5 cm above the substrate. This suggested that theronts had low upward swimming ability. The assessment of swimming ability, video footages and vertical distribution indicated that theronts possessed limited mobility. The excystment pattern of theronts may also be controlled by photoperiod, where most theronts were released during the dark period of the day. Based on these observations, countermeasures such as increasing the water flow in a culture tank during the release period may be effective in reducing infection. Additionally, a combination of several methods against multiple stages of *C. irritans* might result in higher efficacy in minimizing the occurrence of Cryptocaryoniasis.

ID259:

Marine leech, *Zeylanicobdella arugamensis* infestation on farmed fish in Malaysia

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The high infestation of marine leech, *Zeylanicobdella arugamensis,* in farmed fish can cause serious injury and may lead to secondary infection. The infestations would spread and result in mortality leading to economic loss to the farmers if preventive measures were not taken timely. In Malaysia, an unidentified marine leech was reported in wild and cultured groupers (*Epinephelus malabaricus*) with prevalence of 0.4 and the reported mortality cases associated with marine leech has been increasing over the past twenty years. The present study was performed to determine the prevalence of marine leech infestation on farmed fish particularly in floating cages in Malaysia. The results of marine leech infestation over three years studies (2012-2014) on 12 species of marine fish cultured in floating cages showed prevalence ranging from 1 to 100%. Tiger grouper (*E. fuscoguttatus)* and four types of hybrid groupers *(E. fuscoguttatus x E. lanceolatus, E. fuscoguttatus x E. coioides, E. fuscoguttatus x E. tukula, E. coioides x E. lanceolatus x E. coioides)* showed high prevalence (100) while giant grouper (*E. lanceolatus*) and green grouper *(E. coioides)* showed lower prevalence ranging from 16.67 to 66.07. Apart from grouper species, asian seabass (*Lates calcarifer)*, snapper (*Lutjanus spp)*, golden pompano(*Trachinotus blochi)* and cobia *(Rachycentron canadum)* showed variable prevalence ranging from 5 - 83.88, 16.67 – 37.88, 16.07 - 35 and 1- 5 respectively. The high prevalence of marine leech in present study provides information that reflects the increasing mortality cases associated with marine leech infestation in floating cages. It also showed the continuity of the marine leech life-cycle in the culture system as well as recurrence of marine leech infestation even after common practices (freshwater or formalin bath).

ID277:

A Study of Wet Tropics tandan *Tandanus tropicanus* (Welsh, Jerry & Burrows 2014) from the Bloomfield River, Queensland, Australia.

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Nineteen *Tandanus tropicanus* were sampled from the Bloomfield River, Queensland in May 2014, for examination by haematology, bacteriology and histology. *T. tropicanus* leukocytes were essentially similar, morphologically, to those described in other catfish species. Bacteria isolated from the kidney of only two catfish were identified as *Aeromonas veronii, Aeromonas jandaei* and *Bacillus/Lactobaccilus* species. The absence of significant pathology suggests an opportunistic bacterial infection. All fish showed varying degrees of mild to moderate acute renal tubular epithelial degeneration, including hydropic changes, hyaline eosinophilic cytoplasmic droplet accumulation and the occasional nuclear pyknosis and karyolexis. These acute degenerative changes are possibly cold-stress induced. The epithelia of renal collecting ducts of all but one fish were dominated by large cells with foamy alcianophilic cytoplasm and basally displaced nuclei, likely mucus cells. Nematodes were observed within hepatic veins and occasionally hepatic parenchyma of eight fish, in association with little tissue inflammation or damage. Similar parasites were observed in the intestine of one fish. Sequence and preliminary phylogenetic analysis of PCR products carried out on selected wax block tissues with these helminth parasites using an ITS primer suggest that these parasites are closely related to *Contracaecum* spp., and may be a previously unreported species.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID144:

Efficacy of ginger-based treatments against infection with *Gyrodactylus turnbulli* in the guppy (*Poecilia reticulata* (Peters)).

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Monogenean infections of commercially farmed fishes are responsible for significant economic losses and existing chemical theurepeutants, often stressful to the fish, pose associated risks. As part of a recent trend to move towards the use of alternative, natural, plant-based remedies for commonly occurring aquaculture related diseases, the efficiency of ginger (*Zingiber officinale)* was investigated against the monogenean parasite *Gyrodactylus turnbulli* in the guppy (*Poecilia reticulata*). Ginger contains bio-active compounds which are known to have broad-spectrum prophylactic and therapeutic properties with reported anti-bacterial, immunostimulatory and anti-helminthic function in humans and animals.

In vitro trials revealed the clear anti-parasitic effects of ginger. Ethanolic and aqueous extracts prepared from freeze dried ginger were tested. A positive correlation was seen between concentrations of both extracts and time to death of parasites, with ethanolic extract being more efficient; at 75 and 200 ppt aqueous ginger extract parasites died at 65.6 ± 2.8 mins and 1.8 ± 0.2 mins respectively and at 5 and 40 ppt ethanolic extract parasites died at 26.1 ± 0.7 and 4.9 ± 0.3 mins respectively (n = 15-30 parasites per treatment).

Bathing *G. turnbulli*-infected fish in ethanolic ginger extract (*i.e.* 5 and 7.5 ppt for 90 and 30 mins respectively; n=3, 10 fish per treatment) significantly reduced infection prevalence and intensity when compared to the water and ethanol controls. The higher concentration (*i.e.* 7.5 ppt) proved equally effective as praziquantel, the conventionally used chemical treatment for this disease. Fish appeared to be completely cleared of the infection following both treatments. Oral treatment of *G. turnbulli*-infected guppies with diets supplemented with 10 and 20% ginger powder were tested, however treatment proved to be ineffective. The findings presented in this study demonstrate that immersion in ginger extract offers an effective, practical and natural alternative treatment against monogenean infection in fish.

ID 166:

Response to the detection of *Perkinsus olseni* in farmed New Zealand paua, *Haliotis iris*

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The protozoan parasite *Perkinsus olseni* was first confirmed in New Zealand in 2000, and was found to infect four species of wild bivalves from the Waitemata Harbour, in Auckland, New Zealand northwards. In 2013 it was confirmed in samples of the blackfoot paua (New Zealand abalone *Haliotis iris),* which was the first time it has been found in this host species. In July 2013, staff at a commercial aquaculture facility noticed blackfoot paua with mantle retraction and nodules during harvest, which prompted notification to the Ministry for Primary Industries (MPI) where laboratory investigation identified *Perkinsus* as a suspect cause. New Zealand's Ministry for Primary Industries Animal Health Laboratory verified the presence of *Perkinsus* sp. by histopathology, and confirmed *P. olseni* by real-time PCR and genetic sequencing. This was the first detection of *P. olseni* in this abalone species, which is endemic to New Zealand, and the first detection of *P. olseni* from a farmed shellfish population in New Zealand. MPI then responded by carrying out epidemiological analysis relating to this event. The findings of this work indicated there was no elevated mortality in the affected farm and no stock had been recently moved onto the farm. The affected farm is a semi-closed land-based system, and is located within the same region of New Zealand that *P. olseni* has previously been detected. MPI and the Paua Industry Council worked collaboratively to determine if *P. olseni* was present in wild populations of New Zealand paua. To do this Licensed Fish Receivers (LFRs) for wild-harvested paua were contacted and provided a fact sheet on *P. olseni* highlighting clinical signs so they could record and report any harvested wild caught paua with observed clinical signs. To date, no LFRs have reported seeing any sign of *Perkinsus* while processing wild caught paua. In March 2015, wild paua were harvested from within an area where *P. olseni* had previously been detected in bivalves as part of a detection survey for *P. olseni*, and a small number tested positive for *Perkinsus* by Rays fluid thioglycollate culture media. No physical signs of infection with Perkinsus were observed during dissection of these paua indicating that wild paua appear to be sub-clinically infected with *P. olseni*.

ID123:

Host parasite interaction and recent developments in *Argulus* vaccinomics

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The branchiuran ectoparasite *Argulus* has emerged as a major threat to freshwater aquaculture worldwide. In a country like India, the total loss due to argulosis can mount to the magnitude of 29524.40 INR (US$615)/ha/year. Owing to the massive economic loss incurred due to incidences of argulosis, development of control measures against these parasites becomes imperative. Vaccines are cost-effective and environment friendly alternative to traditional therapeutics and are used for long term protection. The development of vaccines for control of argulosis will follow a regime beginning with the study of host-parasite molecular interactions which include knowledge of the parasite contributing factors (parasite proteins, secretions, mechanical abrasion etc.) as well as the host contributing factors (molecules of innate and adaptive immunity and other responses), and finally ending in the characterization and validation of vaccine formulations. It has been shown by transcriptional analysis that *Argulus siamensis,* the most prevalent *Argulus* sp. of the Indian aquaculture system, modulates the immune response of its host rohu carp, *Labeo rohita* by down regulation of many immune molecules. However, the up-regulation of inflammatory genes in skin and IgM in kidney indicates the presence of protective response of the host. The transcriptome data of *A. siamensis* generating 46,352 contigs is the stepping stone to venture into development of anti-argulosis vaccines. Functional categorization of these transcripts into biological pathways is proving to be invaluable for vaccine research. A few candidates viz., development associated proteins like P0 proteins, moult hormones and AMPs have been selected from important physiological pathways of the parasite to be targeted for protective immunity. With reverse vaccinology, it is now possible to access all the proteins encoded by an organism using transcriptome data and computational analysis. In silico prediction tools are used to screen proteins on basis of characteristics like cellular localization and protective antigenicity. These candidates are then developed into recombinant proteins and validated by vaccination trials. Vaccinomics approaches hold promise for development of effective vaccine against argulosis in near future.

FISH PARASITIC DISEASES

POSTER PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID200:

Zoonotic trematode parasites of two cultured fish species in Vietnam; implications for epidemiology

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Fish-borne zoonotic parasites have received increased attention in Southeast Asia due to the steadily expanding aquaculture in the region. In a recent study, the trematode metacercariae of two cultured fish species from the Mekong Delta (striped catfish, *Pangasianodon hypophthalmus*; n=416) and Khanh Hoa Province (climbing perch, *Anabas testudineus*; n=35), have been investigated. Parasite identification is based on morphology and molecular analysis since any further epidemiological assessments rely on accurate species identification.

So far, 7 trematode species (metacercariae) have been recorded, of which 4 species (*Clonorchis sinensis*, *Haplorchis taichui, H. yokogawai*, and *Centrocestus formosanus*) are already known from cultured *P. hypophthalmus*. Here, the species occurred at low prevalence and intensity, ranging 1.52 – 6.34% and 1.86 –11.2, respectively. The preferred site was the skin and the subcutaneous flesh. The other three trematode species (*Centrocestus* sp., *Haplorchis* sp., *Metagonimoides* sp.) were recorded in the skin/gills/flesh of cultured *Anabas testudineus*, with prevalence and intensity ranging 8 – 60% and 2.5 – 7.47, respectively. Comparison of the 28S rDNA sequence with available sequences in GenBank revealed 97.9% similarity with *Centrocestus caninus*, 97.7% similarity with *Haplorchis taichui*, and 98.3% similarity with *Metagonimoides oregonensis*. Although the trematode species recorded so far occur at low prevalence and intensity, they are all zoonotic parasites and must thus be regarded potential consumer hazards. This is especially true for *C. sinensis*, *Haplorchis* spp. and *Centrocestus* spp. that seem primarily to infect the flesh of the fish.

ID146:

The effect of garlic, ginger and turmeric on survival of the fish parasites *Cryptocaryon irritans* and *Tetrahymena* sp.

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Cryptocaryon irritans and *Tetrahymena* sp. are protozoan parasites of teleosts. Infection in commercially farmed fishes can result in significant economic losses. *C. irritans* is commonly treated with toxic chemicals and there is currently no available treatment for *Tetrahymena* sp. Garlic (*Allium sativum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) are well-known spice plants which are known to possess anti-microbial and anti-parasitical properties. The current work was aimed to test the potential of these plants as treatment against *C. irritans* and *Tetrahymena* sp., by analyzing their anti-parasitic effect *in vitro*.

Analysis of parasites' survival following exposure to aqueous extracts of the selected plants (1 gram of freeze-dried powdered plant per 10 mL of DDW) was carried out by: (i) direct microscopic observation, and (ii) colorimetrically by analysing mitochondrial activity using the MTT assay [based on the reduction of 3-(4,5-dimethyl-2-thiazol-2-yl)-2,5-diphenyl-2h-tetrazolium bromide (MTT) in live cells]. The latter analysis was carried out only for *Tetrahymena* sp. Formalin was used as positive control.

Results revealed that exposure to garlic and ginger extracts, but not to turmeric extract resulted in immobilization of the parasites. The effect was dose-dependent, as the time to immobilization decreased with an increase in extract concentration. Garlic was the most effective, immobilizing *Tetrahymena* and *C. irritans* theronts and trophonts within 30, 15 and 15 minutes, respectively, at a concentration of 40 ppt. Ginger extract immobilized *C. irritans* theronts and trophonts within 60 and 75 minutes, respectively, at a concentration of 300 ppt, but did not affect *Tetrahymena*. Results of the MTT assay were in line with the immobilization analysis as garlic application at 40 ppt reduced mitochondrial activity of *Tetrahymena*, yet ginger produced no such effect.

These findings demonstrate the potential of garlic and ginger as a natural alternative to currently used chemical treatments for *C. irritans* and as a potential treatment against *Tetrahymena* sp. infection in fish. Analysis of additional solvents for determining anti-parasitic potential of ginger and turmeric is planned.

ID108:

Parasitic isopod infection in the hatchery and its presence in the sharks and rays caught in Sabah waters

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The distribution and impacts of many parasitic isopods to natural and aquaculture fish species have been extensively studied in many parts of the world. Nevertheless, such information is lacking in Malaysia especially the parasitic crustacean isopod from the family Gnathiidae. Recently, an infestation of the parasite occurred in one of the hatcheries in Sabah. It affected the broodstock of Tiger grouper, Epinephelus fusoguttatus; Napoleon wrasse, Cheilinus undulatus and Asian seabass, Lates calcarifer. Such infestation was blamed for the spawning failure of the fish broodstocks in the hatchery. A thorough examination on the morphological characteristics of the parasites has brought the conclusion that it was Caecognathia coralliophila. Moreover, further investigation was done on the wild elasmobranches. It showed that the juvenile stage of the parasitic isopod can also be recovered from the market specimens of elasmobranches especially the Blue-spotted stingray, Dasyatis kuhli; Sharp nose stingray, Himantura gerrardi; and Hammerhead shark, Sphyrna sp. At present, the study showed that the juvenile stage of the parasitic isopod, Gnathiidae can be recovered from gill filaments of both natural and cultured fish specimens. However, we anticipate more findings about the parasite especially the identification of the juvenile parasites once the present study completed.

ID101:

Prevalence and pathogenesis of nematode (Philometra) infection on ovaries of food fishes of Bay of Bengal.

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The factors influencing recruitment pattern in marine fishes are complex and poorly understood. The recruitment variation appears to be driven by a combination of factors, such as environmental and oceanographic process (Munch and Conover, 2000). The parasitism and disease are also an important factor to be considered in deciding the recruitment pattern of the fishes. Nematodes represent one of the most important groups of metazoan parasites of vertebrate. Some of them are known to be the agents if serious disease of domestic and wild animals including fish. In fish, the nematodes of the genus Philometra are parasitizing the gonads on many marine fishes of Atlantic, Pacific and Indian Oceans. During recent years, particularly in connection with the quickly developing marine culture fisheries, increasing attention has been paid to research on the impact of Philometrid nematodes parasitizing on ovary of fishes. They cause serious damage to the fish ovaries and affect the reproduction of wild and cultured fish hosts of economically important. The Philometra are the viviparous and the description exists only for the female worms, since the males are microscopic in size. The potential effects of this nematode on the reproduction potential of marine fishes are unknown. The purpose of this study is to investigate the prevalence, intensity and effect of nematode (Philometra sp) on the ovaries of food fishes of Bay of Bengal.

ID250:

A Record of family *Clinosomatidae* from wild guppies (*Poecilia reticulata* in Sri Lanka

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Records of digenian parasites in freshwater fish of Sri Lanka are scarce except from two species reported from tilapia and common carp. Yellow grub disease, which has not been reported earlier in Sri Lanka, is a common fish decease reported from other regions of the world. The disease causative agent belongs to genus *Clinostomum*. This is the first time that yellow grub disease has been reported from wild guppies (*Poecilia reticulata*) in Sri Lanka. The parasite was identified from the samples collected from urban drainage canal in Seeduwa, Western province.

Random fish samples were collected with a scoop net from July 2013 to October 2013 in four occasions. Water quality parameters of the canal were measured at each visit. Parasites were stained with borax carming and observed under light microscope to study the morphology and anatomy.

The metacercaria stage of the parasite was found in the muscles immediately beneath the skin of the fish and is visible to naked eye. Five percent of the fish population (size ranged from 1.1 to 3.3cm) were infected. The number of parasites reported in a fish ranged from one to four. The parasites were creamy yellow in colour. Body is oval, elongated with truncated fore body and wider in gonadal region. Clearly visible oral and ventral suckers are present. Ventral sucker is larger than the oral sucker. Oral sucker is surrounded by a well-developed oral collar. Ooesophagus is very short and divides in to two immediately posterior to level of oral sucker, oesophageal bulb is present. Anterior testis is triangular in shape and slightly lobed. Cirrus sac is locates in the left anterior margin of anterior testis.

Morphological characteristics of the metacercaria larvae are closely related with *Cliostomum marginatum* (Rudolphi 1819) published in the literature. Genus *Clinostomum* has not been reported previously in Sri Lanka and this is the first record of the parasite from the country.

ID115:

Phylogeny of zoonotic parasites in fresh and brackish water fish in Vietnam

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Trematode metacercariae were examined from 5 fish species (striped catfish *Pangasianodon hypophthamus*, climbing perch *Anabas testudineus*, java barb *Barbodes gonionotus*, greater lizardfish *Saurida tumbil*, and mullet *Mugil cephalus* by morphological and genetic characters. A total of 14 trematode species were found, of which 5 species (*Clonorchis sinensis, Centrocestus formosanus, Haplorchis yokogawai, H. taichui* and *Bucephalus* sp.) were in striped catfish, 5 unidentified species (*Centrocestus* sp., *Haplorchis* sp., *Metagonimoides* sp. Heterophyidae sp1., and Heterophyidae sp2.) in climbing perch, *Haplorchis taichui* in *Carassus auratus*, 1 unidentified species *Tormopsolus* sp. in *Saurida tumbil*, and 3 unidentified species (*Procerovum* sp., *Stellachasmus* sp., *Clonorchis* sp.) in *Mugil cephalus*. Sequence differences of species ranged from 2.3% to 10.6% of 28S rDNA and from 1.5% to 35.7% of ITS1 rDNA. A phylogenetic tree was constructed based on 28S and ITS1 genes of ribosomal DNA using Maximum Parsimony, Maximum Likelihood and Bayesian Inference algorithms. The 28S rDNA phylogram showed the monophyly of studied metacerarian genera, except *Haplorchis* and *Procevorum*. Species of Opisthochidae (*Clornochis* spp.) were placed in the same clade as those from Heterophyidae. 2 unidentified species (Heterophylidae sp1. and Heterophyidae sp2.) showed close relationship with *Haplochis* and *Procevorum* species. The ITS1 phylogenetic tree resulted in the same unclear branching for *Haplorchis* and *Procevorum*. *Bucephalus* sp. (Bucephalidae) was clearly distinguished from all Heterophyidae species. Morphological and phylogenetic analyses are necessary for species identification and taxonomic positions of unidentified species.

ID233:

Euclinostomum sp. infection in Trichopsis and Betta Fish in Thailand

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Parasitic metacercariae, identified as *Euclinostomum sp.* on the basis of morphological characteristics and molecular data, were taken from three osphronemid fish *Trichopsis vittata*, *T. schalleri*, and *Betta imbellis*, in Thailand. Phylogenetic analysis based on a mitochondrial gene (cytochrome c oxidase I) and two nuclear genes (18S rDNA and ITS- internal transcribed spacer) of these *Euclinostomum* parasites indicated a clear distinction from those belonging to the *Clinostomum* genus. These are the first records of partial mitochondrial and nuclear DNA sequences of *Euclinostomum sp.*

ID190:

Comparison of pathogenic potential of *Anisakis simplex* (sensu stricto) and *Anisakis pegreffii* third stage larvae in experimental rats

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Anisakidosis is a human parasitic disease caused by infections with the nematodes belonging to family anisakidae. Anisakidosis occurs by ingesting the raw or undercooked fish harboring anisakid nematode third stage larvae, and most of infections are known to be caused by *Anisakis simplex* (sensu stricto). In Korean waters, 2 sibling species, *A. simplex* (s.s.) and *A. pegreffii* sympatrically exist. However, only *A. simplex* (s.s.) is known to cause anisakidosis. The possible difference in pathogenicity between *A. simplex* (s.s.) and *A. pegreffii* was studied using experimental rats. Live nematodes were isolated from chum salmon (*Oncorhynchus keta*) belly flaps and chub mackerel (*Scomber japonicus*) body cavity, and all of them were identified by PCR-RFLP after the experiment. Twenty four SD-rats were orally intubated with third stage larvae of these 2 sibling species, *A. simplex* (s.s.)(120 larvae) and *A. pegreffii* (120 larvae). The migration pattern of these larvae was observed 4 times in the euthanized rats during 24 hours. Recovery rate after 3 hours of oral intubation was 86.7% (26/30) for *A. simplex* (s.s.) and 73.3% (22/30) for *A. pegreffii*, and *A. pegreffii* was found more frequently in intestinal organs or abdominal cavity than *A. simplex* (s.s.)(66.7 versus 55.8%). Recovery rate of administrated larvae was reduced with the increased time after challenge. The penetration ability of larvae was assessed, based on their attachment to stomach wall and body cavity. The non-penetrated larvae were also enumerated in intestinal organs. Following 3 hours of oral infection with larvae, the penetration ability was 19.2% and 27.3% for *A. simplex* (s.s.) and *A. pegreffii*, respectively. Penetration rate was 66.7% for *A. simplex* (s.s.) and 93.8% for *A. pegreffii* after 24 hours of oral intubation. From these results, both *Anisakis* species are thought to be able to cause problems when ingested. In addition, other mechanisms are thought to be involved in the pathogenesis of anisakidosis because most clinical cases are known to be caused by *A. simpex* (s.s.). Additional research is required to reveal the mechanisms behind these differences in the pathogenic potential of these *Anisakis* species larvae in human anisakidosis.

ID324:

Morphological and molecular identification of myxosporean parasite found in skeletal muscles of kuhlii loach, *Pangio kuhlii* (Valenciennes, 1846) collected from Thailand

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Morphological and molecular studies of a histozoic myxosporean parasite found in the skeletal muscles of wild kuhlii loach *Pangio kuhlii* (Valenciennes, 1846) were performed, in order to identify the parasite. Sixty fish were collected monthly from September 2006 to August 2007 at the Klong Jarean, a tributary of Chanthaburi in Chanthaburi province, eastern part of Thailand. Based on a morphological study of fresh samples, the myxosporean was ovoid in shape. The spore had two symmetrical shell valves with a prominent sutural line. The spore had two almost round polar capsules. Each polar capsule contained a filament with 4-5 turns. The average spore width (14.38μu) was greater than the average length (8.69μu). The myxospores had typical features of the genera *Neomyxobolus* and *Cardimyxobolus* which are currently assigned to the family *Ortholineidae*. The overall annual prevalence of this myxospore was 40.6 % (292/720) with a monthly variation of between 8.30-81.70% of fish being infected. The lowest and highest prevalence was in October 2006 and March 2007 respectively. Phylogenetic analyses showed that the new myxosporean occupied a solitary branch but was well supported as a member of the Myxobolidae. It did not group with members infecting the urinary systems of fish, therefore we conclude that the new myxosporean belongs to the genus *Cardimyxobolus*, and recommend that the genus be moved to the Myxobolidae.

ID406:

Parasite invasion: Their economic impact on Asian mariculture

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Obligate or opportunistic parasite pathogens can have a major impact on global finfish and shellfish aquaculture and can represent a key constraint to production, sustainability and economic viability. These parasitic infections and their impacts can, broadly, be either unpredictable / sporadic or predictable/regular. While these infections may result in the direct loss of stock and incur costs associated with the control and management of infections once established, for predictable infections there are also costs associated with mitigation, prophylactic treatment and management. Estimating the true cost of each parasite event though is frequently complicated by a complex interplay of numerous factors in each episode that can extend from the direct losses in production to the wider, downstream socio-economic impacts on livelihoods and satellite industries associated with the primary producer.

In this study, we review the major marine and brackishwater aquaculture production industries throughout Asia and provide estimates of the economic cost on some notable parasite-related mortality events and on some of the lessons learned. For this we will draw on both historical and contemporary events impacting on the key aquaculture species reared in Asia (*i.e.* the top fish (n=20), molluscan (n=15), crustacean (n=10), ascidian (n=1) and holothurian (n=1) species). The estimates that will be provided relate to the loss of stock at the point in the production cycle when the disease event occurred and are assumption led where details in the original case report were missing. It is anticipated that by reviewing past and ongoing parasite-based events, we can provide baseline information contributing to risk assessments for new aquaculture-based enterprises and in the development of robust biosecurity practices, which can help to mitigate against and/or minimise the potential impacts of parasite-mediated disease in aquaculture.

EMS/AHPND ORAL PRESENTATIONS

ID 276:

EMS/AHPND: a game changer for the future development of aquaculture

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Outbreaks of acute hepatopancreatic necrosis disease (AHPND) of shrimp, also referred to unadvisedly as early mortality syndrome (EMS), began in China in 2009 and spread sequentially to Vietnam (2010), Malaysia (2011), Thailand (2012) and Mexico (2013) in succeeding years. The disease was characterized by massive sloughing of epithelial cells of the shrimp hepatopancraeas in the absence of any recognizable pathogen by light or electron microscopy. In early 2013, the causative agent was identified as a unique type of *Vibrio parahaemolyticus* that colonized the shrimp stomach where it produced soluble toxins capable of inducing HP cell sloughing. Current research indicates that virulence is variable among isolates and is governed by a large episomal plasmid. Tentative PCR detection methods (AP1 and AP2) that targeted the two plasmids were released in December 2013 and subsequent testing suggested approximately 96% sensitivity in detecting AHPND bacteria for method AP2. Occasional false positive PCR test results using AP2 are hypothesized to arise from mutant plasmids that lack the virulence factor. Thus, more sensitive detection methods were sought that would target the relevant toxins. In the interim in 2013 and up to June 2014, PCR testing with AP2 revealed that significant proportions of broodstock, post larvae, pond-reared shrimp and live shrimp feeds were contaminated with AHPND bacteria and presented a serious biosecurity threat. In June 2014 an improved AP3 detection method based on AHPND-toxin detection and with 100% sensitivity and specificity was released. This should help in identification of AHPND bacteria reservoirs to exclude from the shrimp culture system. The impact of AHPND disease outbreaks resembles that of white spot disease (WSD) during its first emergence as the most serious viral disease threat to shrimp farmers. That emergence was a game changer in giving strong impetus to programs for the development and use of domesticated, specific pathogen free (SPF) shrimp stocks for aquaculture and the promotion of their use in relatively biosecure production facilities. Successful development led to worldwide adoption of SPF stocks of the Pacific whiteleg shrimp *Penaeus (Litopenaeus) vannamei* as first choice for shrimp farmers. Fortunately, prevention of WSSV transmission could be achieved by elimination of potential viral carriers combined with appropriate water preparation in shrimp cultivation ponds stocked with post larvae derived from SPF stocks. These measures will not be as effective for prevention of AHPND because the causative agent is a free-living organism that can persist in marine water and sediments for very long periods even in the absence of carriers. Only closed shrimp culture systems have consistently succeeded in avoiding AHPND outbreaks. Thus, I believe that the advent of AHPND, like that of WSD, will serve as a game changer, necessitating a major change in the future direction of shrimp aquaculture towards totally closed systems. This should be adopted as an important national strategy by tropical countries like Thailand, to insure reliable and sustainable aquaculture production with minimal negative impact on the environment. Preliminary results with modular systems have shown that this goal is now achievable using new advances in technology and that capital investment can be recovered within 1 or 2 years. What is needed is a concerted and coordinated program to use the latest technologies to develop an optimum modular design that can be replicated on a large scale to reduce capital costs. Multidisciplinary input from scientists in many fields (e.g., aquaculture engineering, biochemical engineering, materials science, aquaculture biology, microbial ecology, aquatic animal health and nutrition, etc.) will be required. So will governmental support programs to provide farmer loans for capital investment once a successful standard model has been developed. Although initially designed to prevent AHPND for shrimp, the system would be applicable for cultivation of a wide variety of aquaculture species, giving farmers additional protection against market swings for particular cultured species.

ID 420

Documentation of a unique strain of *Vibrio parahaemolyticus* as the agent of Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) affecting Penaeid shrimp with notes on the putative toxins

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A new emerging disease in shrimp, first reported in 2009, was initially named Early Mortality Syndrome (EMS). In 2011, a more descriptive name for the acute phase of the disease was proposed as Acute Hepatopancreatic Necrosis Disease (AHPND). Affecting both Pacific white shrimp (*Penaeus vannamei*) and black tiger shrimp (*Penaeus monodon*), the disease has caused significant losses in Southeast Asian shrimp farms, and most recently in Mexico. AHPND was first classified as idiopathic because no specific causative agent had been identified. However, since March of 2013, the Aquaculture Pathology Laboratory at the University of Arizona (UAZ-APL) was able to isolate the causative agent of AHPNS in pure culture. Immersion challenge tests were employed for infectivity studies, which induced 100% mortality with typical AHPND pathology to experimental shrimp exposed to the pathogenic agent. Subsequent analyses showed that AHPND lesions which were experimentally induced in the laboratory and were identical to those found in AHPND infected shrimp samples collected from the endemic areas, including Mexico. Bacterial isolation from the experimentally infected shrimp enabled recovery of the same bacteria colony type found in field samples. We found a unique plasmid called pVPA3-1 in which the presumed toxins from the insecticidal related genes PirA and PirB were found.

ID262:

Recent advances in the newly emergent acute hepatopancreatic necrosis disease (AHPND)

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Acute hepatopancreatic necrosis disease (AHPND) is a severe, newly emergent shrimp disease caused by *Vibrio parahaemolyticus*. Through some unknown mechanism, certain strains of this common opportunistic marine bacterium became virulent and able to induce acute hepatopancreatic necrosis in infected shrimp. We sequenced the genomes of three AHPND-causing strains and one non-AHPND-causing strain, and then performed multiple sequence alignment with two reference strains, RIMD 2210633 and BB22OP. After invoking Mugsy for multiple whole genome alignment on the four draft genomes and two reference genomes, we retrieved 315 contigs that were found in all three AHPND-causing strains but not in any of the non-AHPND strains. Some of these AHPND-specific contigs showed homology to plasmids. Although this homology was very low, it nevertheless suggested that these contigs very probably also originated from a plasmid rather than from the *V. parahaemolyticus* chromosomal DNA. We next sequenced the purified plasmids from one of the AHPND-causing strains. De novo assembly yielded the complete sequences of two plasmids. We determined that the smaller plasmid (64,743 bp) was not related to AHPND. However, most of the AHPND-specific contigs, 120 of the original 315 contigs (the 120 contigs covered 90% of the complete plasmid sequence), could hit to the larger, 69,436 bp plasmid (designated pVA1). pVA1 also contained an operon that encoded homologues to the Photorhabdus Pir toxins, PirA and PirB. We show that the ability of *V. parahaemolyticus* to cause disease is abolished by the natural absence or experimental deletion of the plasmid-encoded pirAvp and pirBvp

ID194:

Characterization of virulence factor of AHPND *Vibrio parahaemolyticus* which is the causative agent of shrimp disease

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Acute Hepatopancreatic Necrosis Disease (AHPND) is caused by a unique strain of *Vibrio parahaemolyticus* that have a plasmid harboring virulent genes. Recently, we developed the PCR diagnosis method for AHPND *V. parahaemolyticus*. In our genome and plasmid sequences of *V. parahaemolyticus* AHPND strain, we found a region which encoded for homologues of insecticidal toxins known as Photorhabdus Pir toxin. We targeted this toxin gene for PCR diagnosis of AHPND *V. parahaemolyticus*. In this study, we characterized the toxin genes of AHPND *V. parahaemolyticus*. There were two toxin genes, toxin A and toxin B, on the plasmid. Toxin A and toxin B consisted of 110 and 438 amino acid residues, respectively. We constructed recombinant plasmid carrying toxins A and B. This plasmid was transferred from *E. coli* to *V. parahaemolyticus* non-AHPND strain N7 by mating method. N7strain which received toxin genes A and B (N7-ToxAB) was used to conduct challenge testswith white-leg shrimp, *Litopenaeus vannamei*. Shrimp mortality of N7-ToxAB group was 100% at 24 hr post challenge. We also selected a mutant strain which lost a part of toxin A and whole of toxin B gene (E1M). Furthermore, we made polyclonal antibodies against toxin B for further analysis of toxin B in protein level. The toxin B was detected in only AHPND strains. These results suggested that the most important virulence factors of AHPND *V. parahaemolyticus* are the homologues of insecticidal toxins. However, further studies are needed to investigate the mechanism of these toxin genes.

ID193:

Identification of an insertion sequence related to deletion/insertion of the potent toxin genes of acute hepatopancreatic necrosis disease (AHPND) in *Vibrio parahaemolyticus*

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Acute hepatopancreatic necrosis disease (AHPND) is a serious problem in shrimp aquaculture all over the world. Certain strains of *Vibrio parahaemolyticus* have been identified as causative agents of AHPND and their whole genome have already been sequenced. These strains possess a plasmid harboring 2 potent toxin genes, which are used for the PCR diagnosis. The region encoding toxin genes is composed of approximately 6 kbp, which exhibit terminal inverted repeats of about 1.2 kbp. The repeats encode insertion sequence (IS). The IS encodes transposase and is identical to other reported strains of *V. parahaemolyticus*. The non-virulent strains carrying the plasmid completely lack the toxin region, but possess an IS. Interestingly, we found that the virulent strains also possess the region lacking toxin genes but have a single IS. These results suggest that the IS might have transposase activity and involved in deletion and/or insertion of the toxin genes.

ID183:

ZOT-proteins occur in conjunction with E-family virulence factors in AHPND-causing *Vibrio parahaemolyticus* associated with either of three prophage elements in their genome

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The entire genomes of 8 *Vibrio parahaemolyticus* strains were sequenced including the 3HP CENTEX, Mahidol University, reference strain, 2 Vietnamese strains (one AP1,2,3 +'ve ; another AP1,2,3 –'ve) and 5 Malaysian strains (3 of which are AP1,2,3 +'ve with high, medium and low virulence respectively and 1 strain that is only AP3 +'ve and 1 which is all AP1,2,3 –'ve). We found rather interestingly that ZOT-proteins were always accompanied by the presence of E-family virulence factors wherever one or two types of three prophages were present in the genome. We name these P2, P3 and P7 and note that generally whenever prophage P3 is present, virulence is also higher. Interestingly also, the Vietnamese virulent strain has no prophage element but is the only strain that has a completely different virulence factor which is motB not found in the Thai 3HP or all the Malaysian strains. As no prophages were found, these may perhaps be plasmid transferred. The ZOT-proteins were all related to *Shewanella* species so historically, this virulence factor may have been phage-transfected from this species into *Vibrio parahaemolyticus*. Phages P2 and P3 have ZOT-proteins highly related to ones from *Shewanella marina* which coincidentally is a new species first reported in 2009 from Korea, the very same year when AHPND was generally recognized as a new epizootic syndrome in Hainan Island, China. ZOT or Zonular Occludens Toxin compromises the Occludins – a group of proteins that associate with different peripheral membrane proteins such as ZO-1 located on the intracellular side of plasma membrane, which anchor the strands to the actin component of the cytoskeleton. Thus, tight junctions join together the cytoskeletons of adjacent cells. ZOT's action of breaking up these tight junctions may be the cause of the 'sloughing off' of hepatopancreal tubular cells unique to AHPND.

ID311:

Serotypes, genotypes and virulence genes of *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease in southern Thailand

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The AHPND outbreak in five shrimp farms located in Songkhla and Pattani provinces in the south of Thailand were investigated for *Vibrio parahaemolyticus* using a PCR method targeted to the unique DNA sequences derived from the plasmid (AP2 primers) and the toxin gene (AP3 primers). Thirty three isolates were positive for both primers. However, all 63 and 66 isolates of clinical and environmental *V. parahaemolyticus* respectively obtained previously from 2008 to 2014 from the same area were negative. This signified that these strains were likely to be the cause of the outbreak of AHPND in this area. Most of positive samples were obtained from intestinal samples. All isolates were investigated for serotypes, genotypes, virulence genes and antibiotic susceptibility. All the AHPND isolates possessed a unique O antigen but small variations of the K antigens were detected from different farms. In addition, the DNA profiles of *V. parahaemolyticus* AHPND isolates were similar, but distinct from those clinical and environmental isolates. It is postulated that the causative agent of AHPND might have originated from one clone then slightly different serotypes subsequently developed.

ID229:

An AP1, 2 & 3 PCR Positive non-*Vibrio parahaemolyticus* bacteria with AHPND histopathology

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Vibrio parahaemolyticus is so far the only reported disease agent causing Acute Hepatopancreatic Necrosis Disease (AHPND). This paper presents evidence that another species of Vibrio can also produce AHPND histopathology. A total number of 180 Vibrio isolates were taken from shrimp showing signs of AHPND from East Malaysia, West Malaysia and Vietnam. Of this, 126 isolates were pntA and toxR PCR positive and 19 were AP1 and AP2 PCR positive using 3HP strain from CENTEX, Mahidol University as the positive control Reference Strain. Also, another 8 isolates were found to be AP 3 positive only. Challenge tests using Loc Tran's method showed a different degree of pathogenicity among all isolates tested. There is also a great degree of variability in biochemical characteristics among these strains as shown using API 20E and API 20NE panels. Based on Pulsed Field Gel Electrophoresis (PFGE), most of the strains were found to be closely related to 3HP (> 80% relatedness). One particular AP1, 2 & 3 PCR positive strain (also positive for IQ2000 PCR) was least related to 3HP at 69% relatedness and so we performed whole genome sequencing on this strain. Based on the 16S RNA sequences, this strain was closed associated with *Vibrio sinaloensis* (85% homology). Further genomic analyses reviewed that there are 12 Genomic Islands specific to this strain only. Therefore, this is fresh evidence that *V. parahaemolyticus* may not be the only Vibrio species capable of causing AHPND histopathology.

ID339:

A microbial perspective on Acute Hepatopancreatic Necrosis Disease (AHPND) outbreaks in shrimp farming

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Up to now, research on the AHPND has mainly been oriented toward studying its pathology and etiology, although efforts to develop strategies to prevent or remedy the disease are equally – if not even more – needed. In this, it will be required to not only focus on the causative agent of the disease, but to take the microbial community as a whole within the system into consideration. The three dimensional water matrix and sediment in shrimp ponds support *in situ* microbial growth by the presence of uneaten feed, faecal matter and nutrients and make that shrimp live in an environment with an exceptionally high microbial load. Current pond culture practices further make that these microbial communities very often include a large fraction of opportunistic pathogens (De Schryver et al., 2014) such as *Vibrio parahaemolyticus* causing AHPND. Based on the ecology of the causative agent, it seems that approaches with a focus on controlling the presence or activity of vibrios in general have a high chance of decreasing the risk of AHPND outbreaks. To establish a more sustainable shrimp production, there is a need for new microbial management strategies that focus on 'join them' and not the traditional 'beat them' approaches. To efficiently manage the microbiota in the system to minimize disease risk, a lot can be learned from research on intensive larviculture of several fish species where detrimental host–pathogen interactions are a normal phenomenon. It is argued that ecological theory could serve as a foundation for developing sustainable microbial management methods that prevent pathogenic disease in larviculture (De Schryver and Vadstein, 2014). Management of the water microbiota in larviculture systems according to ecological selection principles has been shown to decrease opportunistic pathogen pressure and to result in an improved performance of the cultured animals. We hypothesize that such an approach will proof its value for the shrimp culture business in the context of AHPND as well. Improving robustness of organisms is an alternative holistic approach to tackle the many infectious and non-infectious disease problems encountered in aquaculture. It encompasses improving the energetic and nutritional reserves, homeostatic capacities (osmotic regulation etc.), defense and immune systems, as well as the microbiota in and on the animals. All of these levels have an additional/synergistic effect on how well the cultured organisms can fight off pathogens and deal with environmental stress. One of the central concepts in this kind of stress management is hormesis (Calabrese and Baldwin, 2002). It is characterized by a low dose stressor inducing a response in an organism of which the net outcome is favorable. The search for products which allow animals to better deal with stress is one of the most obvious avenues to obtain improved production in a short term.

ID102:

Ecological approaches in controlling the Acute Hepatopancreatic Necrosis Disease

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Prior to searching for viable solutions for the Early mortality Syndrome (EMS) also know as the Acute Hepatopancreatic Necrosis Disease (AHPND), determining the routes of infection of EMS/AHPND that can infect the shrimp is a crucial step. Based on this principle, we should be able to determine how effective each proposed solution may be in practice. Our studies show that EMS/AHPND can transmit via shrimp oral route by ingestion of the agent in water, shrimp carcasses, and surfaces contaminated with the agent. Other observations show that there is a correlation between the outbreaks of the disease in a new farming region with the importation of new broodstock, suggesting that contaminated broodstock could be a source of pathogen for their offspring. Once the pathogen is introduced to a new farming region, it appears that the transmission from the stock first plays an important role. Once the pathogen is established in the farming environment, it seems that the accumulation of the pathogen can initiate the infection for the future crops, making it very difficult to avoid the disease. Thereby, in this case both vertical and horizontal transmission are as essentially equally important.Most of the shrimp farming areas of Viet Nam have now been hit by EMS/AHPND; therefore, we should consider both vertical and horizontal transmission of the disease for the disease prevention strategies. Suggestions for the disease control of the postlarvae include a set of measures of pathogen control in hatcheries, testing for broodstock and post-larvae, implementing regulations of quality control and testing for imported broodstock and post-larvae. Implementing measures to control horizontal transmission including appropriate pond preparation, having sufficient water surface dedicated for water treatment and reservoir, implementing farm biosecurity, eradicating accumulated pathogens by ploughing and drying pond bottom, fish-shrimp or rice-shrimp crop rotations, polyculture, and aging water in reservoir by using tilapia, protecting shrimp gut health by using probiotics can be done at the farms. Those measures appear to be efficient in suppressing and eradicating the pathogenic bacteria and enhancing the natural balance of the microbiota, thus minimizing the outbreak of the disease.

ID335:

Risk factors associated with EMS/AHPND occurring in culture shrimp in Thailand

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During 2012-2013, the retrospective study and perspective study aimed to investigate the risk factors related to outbreak of EMS/AHPND on farm and pond levels were performed in Rayong and Chantaburi provinces, Thailand. The retrospective study demonstrated the pond level risk factors were stocking of post larva from some source of hatcheries and increasing in total pellet feed at 1 month old of shrimp per 100,000 PLs, while increase water adding time was reducing the risk of EMS/AHPND. The farm level risk factor associated with area has been affected with EMS/AHPND outbreak. Hundred ponds of 27 farms were included in perspective study. Stocking post larva from some providing source, stocking older post larva into the pond and increasing in stocking density associated with increasing of the prevalence of EMS/AHPND. In the other way, nursery post larva in hapa and supplement with natural feed and probiotic during early time of stocking was excellent preventive measure. The source of post larva exhibited the strongest association with occurring of EMS/AHPND outbreak from both studies. So, the most possible reasons of this association might be the contamination of pathogen into the shrimp production line especially hatchery or nursery stages via live feed of brooder stock or larvae. Additional, inappropriate management practice in nursery farm might increase number and virulent of pathogen. However, breeding and genetics might have slightly effect for this problem but need to confirm. The good management practice i.e. post larva quality assessment, good preparing pond before stocking, natural feed enrichment in pond before stocking and early period after stocking, promote heterotrophic microbial to control optimum water quality and produce biofloc, control the number of *Vibrio parahemolyticus* in water and shrimp gut environment with probiotic and other natural extraction products were valuable manners for prevent the problem.

ID254:

Pesticides used in shrimp farms in the Mekong delta, Vietnam: are they associated with acute hepatopancreatic necrosis syndrome?

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Shrimp farming growth has led to increased use of drugs and chemicals for prevention and treatment of infectious diseases. Pesticides have widely been used as agriculture pesticide, wild crustaceans elimination in shrimp farming, treatment of water quality and reduction of diseases. During the past 4 years, there were new agents of insecticides, fungicides and herbicides were used to protect the rice fields or integrated rice-shrimp areas. AHPNS outbreak appeared in large region and seriously affected to shrimp farming, and pesticides were found in water and sediment in these shrimp ponds. These pesticides include Deltamethrin, Fenitrothion and Hexaconazole. Environmental studies was carried out to ascertain if AHPNS pathology will develop in shrimp held in water–sediment systems containing *Vibrio parahaemolyticus*, the causative agent of AHPNH, and pesticides. Results showed that combination of pesticides and bacteria increased mortality of shrimp. Shrimp exposed to pesticides without bacteria did not show typical EMS/AHPNS pathology.

ID252:

Vibrio parahaemolyticus associated with shrimp mortalities in India do not have characterestics of ahpnd strains

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Pure cultures of *V. parahaemolyticus* were obtained from hemolymph of moribund shrimp during an outbreak of mass mortality in east coast of India during October-November, 2013. The organism could not be found in hemolymph of healthy animals from farms in the east and west coast of India. All isolates were negative for virulence genes associated with human pathogenic strains of *V. parahaemolyticus* and also negative by PCR for genomic regions considered specific for *V. parahaemolyticus* strains associated with acute hepatopancreatic necrosis disease (AHPND), but were positive for T3SS1. The isolates showed genetic diversity as indicated by Random Amplification of Polymorphic DNA (RAPD). Challenge studies with representative isolates by immersion did not cause mortalities or histopathological changes in the experimental shrimps. In conclusion, the present study has demonstrated the association of *V. parahaemolyticus* with outbreaks of mortalities in white shrimp *L. vannamei* in the grow-out ponds. Further, the *V. parahaemolyticus* isolates did not match the characteristics of strains associated with EMS/AHPND and the results demonstrate that these strains are probably opportunistic pathogens of immunocompromised cultured *L. vannamei* under unfavourable environmental conditions and the results suggest that the disease outbreak was due to vibriosis rather than AHPND in India.

ID272:

Metagenomic analysis of bacteria in the hepatopancreas of cultivated shrimp exhibiting early mortality syndrome (EMS) in Thailand and Vietnam

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Since late 2009, the most threatening new disease in Asian shrimp aquaculture is characterized by acute and massive sloughing of hepatopancreatic (HP) tubule epithelial cells in the absence of clearly visible causative pathogens by light or electron microscopy. The condition has been named acute hepatopancreatic necrosis disease (AHPND). The metagenomic analysis was applied to determine whether any unique bacteria (culturable or un-culturable) were associated with AHPND. DNA was extracted from pooled HP tissue samples from normal and AHPND shrimp ponds and subjected to PCR amplification of small subunit ribosomal RNA (ssu rRNA) gene fragments using universal bacterial primers. Sequencing and bioinformatics analysis of approximately 176,674 fragments (66.9 million basepairs) from 14 AHPNS ponds (Thailand = 10 and Vietnam = 4) and 3 control ponds (Thailand = 2 and Vietnam = 1) resulted in a total of 19-125 operational taxonomic units (OTUs). There are 32 OTUs of six genera from four algorithms with a significant higher proportion of reads in EMS/AHPNS ponds than in normal ponds ($p < 0.1$, Student's t-test). These included the genera *Delftia* and *Ralstonia* from the order Berkholderiales and *Leifsonia* and *Rhodococcus* from the Order Actinomycetales, which are the bacteria known from aquatic environments but never previously reported as shrimp pathogens. The DNA primers specific for these bacteria were designed and confirmed the presence of these bacteria in the specimens used for metagenomic analysis. The sequencing of positive amplicons were confirmed. The on-going experiment is to isolate these bacteria from AHPND specimens and perform a bioassay to determine the association with EMS/AHPND.

ID401:

Time-saving and specific methods with high sensitivity detecting acute hepatopancreatic necrosis disease (AHPND)

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Acute hepatopancreatic necrosis disease (AHPND) has caused great economic losses in Asian countries including China. In this present study, 8 AHPND associated isolates of 12 *Vibrio parahaemolyticus* strains were confirmed based on the bacterial challenge test. An assumed virulent protein VPP19 with a molecular weight of 19kDa was isolated from the AHPND positive isolates and identified as a hypothetical protein by using MALDI-TOF-TOF analysis. Its coding gene *vpp19* was cloned from isolate 629002S01 and was 336bp. Targeting the gene, fluorescent based loop-mediated isothermal amplification (FRt-LAMP) method with a sensitivity of 2.6×10^{1}—2.6×10^{2} copies per reaction tube, a highly sensitive and quantitative detecting method for AHPND was established. Moreover, an on-site detection kit was developed according to the optimized FRt-LAMP reaction mixture, which was easily operated and finished in 60min. In addition, quantitative real-time PCR (qPCR) method could be a laboratory method for analyzing AHPND samples. When detecting 10 fold serial DNA dilutions, the detection limit using the VPP19-3 primer set was 2.6—2.6×10^{1} copies/reaction, while the minimum account detected by using F3/B3 primers was 2.6×10^{2} copies/reaction. All these methods could give efficient and reliable results in 90min. Therefore, highly specific and quantitative detection methods of AHPND in this study were presented, which are valuable tools for the detection and quantification of AHPND bacteria and samples and epidemiological studies.

ID110:

Loop-mediated isothermal amplification combined with colorimetric nanogold for detection of bacterial isolates causing acute hepatopancreatic necrosis disease

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Acute hepatopancreatic necrosis disease (AHPND) causes severe mortally in shrimp early after stocking shrimp ponds and is caused by unique isolates of *Vibrio parahaemolyticus*. To identify reservoirs in order to control the disease, we established a loop-mediated isothermal amplification (LAMP) assay combined with colorimetric nanogold (AuNP) for rapid, sensitive and inexpensive detection of this pathogen. To design the special set of six primers for our LAMP assay, we used the sequence of a plasmid DNA fragment from AHPND bacteria that was recently released together with its conventional 1-step PCR detection method that showed 100% specificity, 96.4% sensitivity, 100% negative predictive value and 97.4% positive predictive value for AHPND bacteria in tests using 37 AHPND and 27 non-AHPND isolates of *V. parahaemolyticus*. With DNA templates extracted from isolates of AHPND bacteria in the LAMP reaction mix at 65°C for 45 min, specific amplicons from the target sequence could be visually detected via hybridization at 65°C for 5 min with a red ssDNA-labled nanogold probe followed by a salt induced AuNP aggregation step (total assay time approximately 50 minutes). After salt induction, positive samples remained red while negative samples turned blue from nanogold aggregation and eventual precipitation. This new method was 100-times more sensitive (100 CFU) than the 1-step PCR detection method (104 CFU) for the same target sequence using amplicon detection by electrophoresis or spectrophotometry. DNA templates extracted from the bacterial isolates commonly found in shrimp ponds (including *Vibrio* species but excluding *V. parahaemolyticus)* all gave negative results with both the LAMP and conventional PCR methods. Using DNA templates from the same 37 AHPND and 27 non-AHPND isolates of *V. parahaemolyticus* described above, LAMP detection values for specificity, sensitivity, negative predictive value and positive predictive value for AHPND bacteria were the same as for the 1- step PCR method. Similarly, DNA extracts from shrimp infected with AHPND bacteria gave positive results, while extracts from shrimp infected with other pathogens did not. The new LAMP-AuNP assay for detection of AHPND bacteria significantly reduced the time, ease and cost for molecular detection. Use of thisfield-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.

ID318:

Biofilm formation by *Vibrio* species from shrimp including those that causes AHPND

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Currently, the most important shrimp bacterial disease that threatens all cultivated penaeid shrimp in Asia is acute hepatopancreatic necrosis disease (AHPND). It is included in the complex of diseases that constitute what is commonly called early mortality syndrome (EMS) in shrimp. AHPND is caused by unique isolates of *Vibrio parahaemolyticus* and is characterized by unique histopathology consisting of massive sloughing of tubule epithelial cells of the hepatopancreas (HP) in the absence of any accompanying pathogen. The AHPND bacteria are believed to colonize the shrimp stomach and release toxins that enter the HP. These toxins are coded by novel plasmids with the potential for high mutation and for transmission among bacterial isolates. Since it has been estimated that more than 80% of all human and animal diseases are associated with bacterial biofilms, we hypothesized that stomach colonization by AHPND bacteria would take the form of biofilms on its chitinous, cuticular lining. We describe several methods for culture and study *Vibrio* biofilms that mimic the stomach cuticle *in vitro*. Using laser confocal scanning microscopy with a variety of lectins for different sugar moieties, we could identify carbohydrates associated with biofilms in extracellular matrices. Using scanning electron microscopy, we found that biofilms varied by isolate for form, structure and thickness. For example, the most virulent AHPND isolate formed the thickest biofilm, as did a virulent, lysogenized (bacteriophage-containing) *Vibrio harveyi* isolate (very thick, structured biofilm) when compared to its non-lysogenized parent isolate (thin and flat biofilm). To gain a better understanding of how AHPND pathology is related to biofilm formation, we are using the immersion technique to test and compare the toxicity of cell-free culture preparations from AHPND bacteria grown in suspension or as biofilms. We are also testing the potential for protection against AHPND by use of reagents that prevent biofilm formation.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

EMS/AHPND POSTER PRESENTATIONS

ID246:

Acute hepatopancreatic necrosis disease in shrimp cultured in Mekong delta of Vietnam

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Acute Hepatopancreatic Necrosis Disease (AHPND) causes mass mortality in shrimp cultured in Mekong Delta. This study was conducted to determine the evolution of AHPND in shrimp cultured in pond and in laboratory conditions. The prevention solution is proposed based on these results. Histopathological method was used to analyse 51 shrimp samples collected periodically every 10 days and 36 samples collected at disease outbreak. The earliest and latest signs of necrosis appeared on day 17 and 77 after stocking respectively. The highest frequency of necrosis appearance was recorded from 20 to 45 days after stocking. Mortality was concentrated in the period of 19-31 days of age. All shrimp samples collected at outbreak showed high prevalence of necrosis and lead to early harvesting after 2-3 days of necrosis detecting. It is interesting that the high variation of necrosis rate have been recorded between ponds (9-90%). This result shows the severity of AHPND although only low necrosis rate was detected. The early harvesting has been applied in all shrimp ponds with signs of necrosis. Shrimp form the slightly infected pond can survive in the laboratory condition for one month when water parameters were controlled. Monotring of *Vibrio* sp. and *V. parahaemolyticus* in the water and in the shrimp body seems to be important in control of AHPND. Based on this result, the farmers can decide the solution at certain periods.

ID284:

PCR determination of bacteria *Vibro parahaemolyticus* from reported EMS/AHPND cases in farmed Whiteleg Shrimp (*Litopenaeus vannamei*) in Malaysia

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Bacteria *Vibrio parahaemolyticus* has been identified as the main pathogen in Acute Hepatopancreatic Necrosis Disease (AHPND) which caused high mortality in farmed whiteleg shrimp (*Litopenaeus vannamei*). Shrimp farmers in Malaysia encountered the occurrence of AHPND in late 2010 but mortality was only reported in early 2011. A total number of 12, 14 and 26 mortality cases were reported in 2011, 2012 and 2013; respectively. Confirmation of AHPND by histopathology showed 6, 5 and 19 positive cases in 2011, 2012 and 2013; respectively. Fifteen isolates of *V. parahaemolyticus* identified and confirmed AHPND by biochemical and positive AHPND pathology were randomly selected for further confirmation with PCR method. The PCR method consists of primer *tox*R, 3 sets of primers (AP1, AP2 & AP3) and commercial detection kit (EMS-2 from IQ2000). Detection using primer *toxR* showed 86.67% positive while no positive cases were detected using 3 sets of primers. However, EMS-2 commercial detection kit showed 13.33% positive. Analysis of the 16s rDNA sequences of 13 positive isolates with Maximum Likelihood method based on the Tamura-Nei model showed two major groupings: 1) isolates E3, E10 and E11, and 2) isolates E1, E2, E3, E4, E5, E7, E8, E9, E12, E13, E14 together with strain *V. parahaemolyticus* from ATCC (VP) and Thailand (TF). The results from phylogenetic tree showed 10 positive isolates of *V.parahaemolyticus* in group 2 were of the same strain *V. parahaemolyticus* from ATCC and Thailand, whereas isolates E3, E10 and E11 in group 1 were different. The study highlighted 2 groups of *V. parahaemolyticus* isolates from reported mortality cases that had caused AHPND during 2011 until 2013 in Malaysia.

ID402:

Virulent *Vibrio parahaemolyticus* is proven as the agent of acute hepatopancreatic necrosis disease (AHPND) affecting *Litopenaeus vannamei* in China

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Vibrio parahaemolyticus has been reported as the agent of acute hepatopancreatic necrosis disease (AHPND) affecting shrimp. Methods of bacterial etiology and histopathology were employed to determine the nature of AHPND-like shrimp from Guangxi Province, China. One *Bacillus* sp. (named AS03) and two *Vibrio parahaemolyticus* strains (S01 and AS02) were isolated from the hepatopancreas (HP) of the shrimp samples and identified using 16S rDNA sequence. An immersion challenge study satisfied to Koch's Postulates using experimental shrimp, *Litopenaeus vannamei* was proceeded to find out the certain agent. They were treated with the approximate bacterial density of 10^8 cells ml^{-1} cultured in TSB+. Upon the results of challenge tests, the cumulative mortality reached 100% of the shrimp treated with bacterial density of 3.22×10^8 cells ml^{-1}, while those raged from 58.3 to 100% when shrimp treated with the density of 2×10^8 cells ml^{-1}. Histology examination of the HPs of affected shrimp suffering the virulent *V. parahaemolyticus* strain S01 showed typical AHPND pathological sings, however, the hepatopancreatic tubules and their epithelial cells of shrimp treated with bacterial suspension of strain AS02 presented intact and normal structure. Therefore, only the virulent *V. parahaemolyticus* is the true agent of shrimp affected with AHPND in Guangxi, China.

ID217:

Effectiveness of betel leave (*Piper betle*) and lemongrass (*Cymbopogon citratus)* extracts on challenged whiteleg shrimp, *Litopenaneus vannamei* with *Vibrio parahaemolyticus* that caused AHPND

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Acute HepatopancreaticNecrosis Disease (AHPND) is a bacterial disease caused by *Vibrio parahaemolyticus*. AHPND has caused mortality from 40 to 100% while the remaining survived shrimps were associated with slow growth and mortalities at later day of culture. This study focused on the survival of the shrimps having received oral medicated diets (betel leaves (BL) and lemongrass (L) extracts) for 14 days and later challenged with bacteria *V. parahemolyticus* that had caused AHPND. A total of 60 SPF white shrimps with the age of 20 days of culture were obtained from the farmer. The shrimps were screened and tested negative for AHPND using EMS detection kit. They were divided into 3 groups (A, B & C) with 30 white shrimps in group A (normal pellet), 15 in group B (normal pellet mixed with BL) and another 15 in group C (normal pellet mixed with L). Prior to the challenge test, the shrimps from group A were further divided into two groups namely positive and negative control groups. Each group consists of 15 shrimps and they were separated into five per tank. The challenge test was conducted by immersion of five shrimps for 1 minute in a 1L aquaria tank containing *V. parahaemolyticus* suspension of bacterial density 1 x 10^8cells/ml. After 1 minute, the shrimps were transferred into a 2L aquaria tank contained *V. parahaemolyticus* suspension of bacterial suspension 1 x 10^6cells/ml and observed for the mortality within 24 hrs. The antimicrobial property results showed that extraction from betel has a moderate positive antimicrobial activity toward *V. parahaemolyticus* while a weaker antimicrobial activity for lemongrass extract. Challenged test with *V. parahaemolyticus* showed higher survival, 83.3% in shrimps received betel extract compared with 66.8% in positive control group and 33.3% in lemongrass group. Bacterial *V. parahaemolyticus* were detected in all the groups except negative control group after 24 hours observation using EMS-2 detection kit. This study show that betel extract has a good potential for treatment of bacterial diseases in shrimp and oral medicated with betel extract has a promising result in minimize occurrence of AHPND.

ID206:

Prevalence and risk factors of Early Mortality Syndrome (EMS) in shrimp farms in Rayong and Chantaburi provinces, Thailand

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The cross-sectional study aimed to investigate the prevalence and risk factors related to EMS/AHPND in shrimp ponds in Rayong and Chantaburi provinces, Thailand. Two hundred and thirty three ponds of 483 farms in Klang, Rayong and Na-Yai Am, Chantaburi provinces were selected for studying. The retrospective data included farm management, pond preparation, bottom pond cleaning, feeding management, source of post larva, etc. were collected during 1 January to 31 December 2012. The prevalence of EMS/AHPND and associated risk factors were analyzed by STATA 8.2 software program. Univariate logistic regression and multivariate logistic regression analysis were used to identify risk factors of EMS/AHPND at significant level at P-value < 0.05. The prevalence of EMS/AHPND of studied samples were 33.4% (95% CI=26.9-40.9%). From multivariate analysis, the pond level factors which significantly associated to increase risk of EMS/AHPND when presented were the stocking of post larva from some hatcheries and the increasing of total feed within 1 month period per 100,000 PLs after releasing PLs into the pond. On the other hand, increasing frequency of adding water into the pond could reduce risk of the EMS/AHPND. In addition, the occurrence of EMS/AHPND outbreak in nearby farm(s) was the farm level factor that increased more risk of disease in shrimp farm.

ID289:

PCR Detection of Early Mortality Syndrome in *Litopenaeus vannamei* and *Penaeus monodon* in Central Luzon, Philippines

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A newly emerging disease that causes mass mortality in shrimp is caused by *Vibrio parahaemolyticus* and is known as early mortality syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND). It affects different species of shrimp including the Pacific White Shrimp (*L. vannamei*) and Black Tiger Shrimp (*P. monodon*). It has been detected in China (2009), Vietnam, (2010), Malaysia (2011), Thailand (2012), and Mexico (2013). In the Philippines, however, AHPND is yet to be detected. Three sites were selected in Central Luzon: Bulacan, Bataan and Pampanga where samples were collected for detection of the disease. Polymerase Chain Reaction (PCR) was the method used for detection using shrimp that were positive for the *V. parahaemolyticus* as confirmed by a microbiological assay. Gene-specific primers for AHPND were utilized for the detection assay. All three sites tested positive for AHPND with the following percentage: 22.22% for *L. vannamei* in Bulacan, 73.33% for *L. vannamei* and 83.33% for *P. monodon* Bataan and 40% for *L. vannamei* and 20% for *P. monodon* Pampanga, respectively. This study is the first to confirm the presence of EMS in the Philippines. Awareness should be raised regarding the presence of this disease so that measures and prevention can be done and outbreaks can be abated.

ID332:

Involvement of Pir toxin of *Vibrio parahaemolyticus* in inducing acute hepatopancreatic necrosis disease in shrimp

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Vibrio parahaemolyticus, a Gram-negative bacterial species inhabiting warm brackish water, is a causative agent of gastrointestinalitis in human. However, in 2013, this organism was also identified as the pathogen that induced acute hepatopancreatic necrosis disease (AHPND) in white shrimp, *Penaeus vannamei*. To date, AHPND has spread to several countries in Asia and South America and caused huge losses to the shrimp farming industry. We have identified AHPND-specific sequences which were found to be located in a ~69 kb plasmid that was found in all of the AHPND strains that we studied. We noted that this plasmid contained a two-gene operon encoding a "*Photorhabdus*-insect related" (Pir) binary toxin homologue, *pirA* and *pirB*. When the entire *pir* operon was deleted by an *in vivo* allelic exchange method originally described in *Vibrio vulnificus*, the virulence was greatly impaired in shrimp. Histological examination also showed that this *pir* isogenic mutant was unable to cause any AHPND-like symptoms, while the wild type strain and the complemented strain both exhibited AHPND-causing ability. Our data therefore suggest that the Pir toxin in *V. parahaemolyticus* was critical in inducing AHPND. The pathogenic mechanism in which the Pir toxin is involved is currently being investigated.

ID327:

Using multiple sequence alignment to find specific sequences that can distinguish between AHPND-causing and non-AHPND-causing strains of *Vibrio parahaemolyticus*

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Genome sequencing of three AHPND-causing strains (3HP, 5HP and China) and one non-AHPND causing strain (S02) was conducted to identify specific sequences that could distinguish the AHPND-causing strains from the non-AHPND strain. Sequencing was performed by an Illumina Miseq sequencer, and the assembler Velvet was used for *de novo* assembly of the four raw read sets. After invoking Mugsy for multiple whole genome alignment of the four draft genomes and two *Vibrio parahaemolyticus* reference strains, RIMD 2210633 and BB22OP, the aligned sequences from the three pathogenic genomes were retrieved. A total of 315 specific contigs were found in all three AHPND-causing strains but not in any of the non-AHPND strains. We developed a PCR-based AHPND detection method was based on two of the largest AHPND-specific contigs, and also found that these differential sequences were all located on an AHPND-associated plasmid.

FISH VIRAL DISEASES
ORAL PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID304:

Latency of koi herpesvirus (KHV) in koi and carp: implications for disease transmission and control

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Cyprinid herpesvirus 3 (CyHV-3), also known as koi herpesvirus (KHV), is the aetiological agent of a lethal disease (KHVD) associated with mass mortalities in koi and common carp (*Cypinius carpio* L.), and reported in at least 30 countries. It has been suggested that global trade in sub-clinical infected fish is responsible for the rapid spread of KHVD. However, mechanisms of KHV transmission remain unclear. An experimental model was established in carp to demonstrate that persistent KHV infections in survivor fish have the characteristics of latent, non-productive infections, with the capacity to reactivate and transmit the disease to healthy fish. During acute infections, when fish were maintained at 22°C, viral genes were expressed abundantly, and infectious virus was produced in association with tissue damage, clinical disease and mortality. In fish maintained at a lower temperature (11°C), viral gene expression was absent or greatly reduced although viral DNA was present; infectious virus was not recovered and there was no evidence of disease. Productive replication was re-initiated following an increase in water temperature to 22°C, resulting in 45% mortality. Shedding of reactivated virus killed 75% of cohabitating naïve fish, indicating a potential risk for disease transmission.

The segmental distribution of skin lesions in KHV-infected carp led us to consider classical herpesviral infections in which virus may persist in nerve ganglia. This study suggested that cranial nerve, and dorsal root ganglia may be a site of for latent KHV. To obtain insights into the mechanisms of KHV latency, the virus-encoded interleukin-10 (IL-10) homolog was investigated. Homology modelling revealed that the khvIL-10 3D structure is similar to human and carp IL-10s. Targeted knockdown using a splice site-blocking morpholino indicated that khvIL-10 functions via the IL-10 receptor long chain (IL-10R1). Phylogenetic analysis suggested that khvIL-10 was derived from carp with subsequent benefits to the virus. Based on the expression profile of carp immune-related genes in each stage of KHV infection - acute, persistent and reactivation phases - we propose a possible interaction between carp IL-12, carp IL-10 and khvIL-10 during the course of viral infection.

Segmental distribution of skin lesions in KHV-infected carp

Possible interaction of carp IL-12, carp IL-10 and khvIL-10 during KHV infection

ID302:

VNN: A Challenge to mariculture in the Arabian region, with a special reference to Kuwait

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Aquaculture in the Arabian region is on the increase due to an emphasis on controlled production of seafood. Many marine fish species have been domesticated for obtaining high productivity. One of the problems faced is the susceptibility of farmed fish and shellfish to diseases under aquaculture conditions. Emergence of new diseases is posing threats to aquaculture development in the region.

Nodavirus infections of teleost fish, variously termed Viral Nervous Necrosis (VNN) or Viral Encephalopathy and Retinopathy (VER), is a serious disease particularly in larval and juvenile fish in grow-out stage. VNN was suspected to be the cause of grouper mortalities at the mariculture facilities of the Kuwait Institute for Scientific Research (KISR) in 2008 andconfirmed in similar disease cases in groupers in subsequent years as well as exhibit fish in the marine aquarium of the Scientific Centre of Kuwait in 2011 . Antibody-based and VNN-specific PCR techniques were used to confirm the presence of the causative nerve necrosis virus (NNV). As there are no effective treatment methods available for controlling VNN, several management measures have to be applied for a sustained production and containment of the disease. Production and/or procurement of quality seed or stocking material and establishment of healthy VNN-free broodstocks need to be addressed with a top priority. As the Arabian region is gearing up for large-scale production of marine fish through aquaculture, a well-planned integration of efforts by different countries in the region has to be initiated. Each country needs to understand the urgency to institute comprehensive disease management strategies.

ID161:

Singapore grouper iridovirus (SGIV) ORF075R as a coupling protein during viral assembly

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Singapore grouper iridovirus (SGIV) is a pathogen that causes significant economic losses in grouper aquaculture. The virus contains a dsDNA genome of approximately 140kb, and is predicted to encode 162 open reading frames (ORFs). ORF075R is an abundant viral structural protein. It has no significant sequence homology to any other known proteins, and its functional role remains elusive. This study demonstrated that suppression of ORF075R expression result in a significant reduction of viral yield in grouper embryonic cells, and that ORF075R has four distinct protein isoforms. Mass spectrometry further showed that these proteins are differentially phosphorylated during early to late stages of infection, and highly phosphorylated in matured viral particles. ORF075R is localized in the host cell cytoplasm, and concentrated in the viral assembly site. Lipid array assays showed that ORF075R binds to various phospholipids. In ORF075R-knockdown samples, viral particles without the inner lipid-bilayer were observed using electron microscopy. These observations suggest that multi-phosphorylation of ORF075R may play a coupling role between the inner lipid layer and capsid shell.

ID283:

Differential expression profiles of Interleukin 11 (*IL-11*), Intelectin (*ITLN*) and Purine nucleoside phosphorylase 5a (*PNP 5a*) in crucian carp (*Carassius auratus gibelio*) exposed to Cyprinid herpesvirus 2 and *Aeromonas hydrophila*

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Nterleukin 11(*IL-11*), Intelectin (*ITLN*) and Purine nucleoside phosphorylase 5a (*PNP5a*) play important roles in innate immunity. In a previous study to identify expressed immune-related genes, suppression subtractive hybridization (SSH) assays were used to characterize differentially expressed genes in crucian carp (*Carassius auratus gibelio*) infected with Cyprinid herpesvirus 2 (CyHV-2) in which *IL-11*, *ITLN* and *PNP5a* were identified to be the three most significantly up-regulated genes. In this study, the complete open reading frames (ORF) of *IL-11*, *ITLN* and *PNP5a* genes were cloned and sequenced. The full-length cDNAs of the three genes contained an ORF of 597, 945 and 882 bp, encoding a polypeptide of 198, 314 and 293 amino acids, respectively. Phylogenetic analysis indicated that the three genes shared high homology to other bonyfish species including zebrafish. Interestingly, the *ITLN* gene of crucian carp lacked a 10 aa peptide that was found in the C-terminal of other fish species. A real-time RT-PCR assay was developed to quantitatively examine their tissue distribution. This study showed that *IL-11*, *ITLN* and *PNP5a* were expressed at low levels in all of the tissues examined. To monitor the response of these genes to CyHV- 2 or *Aeromonas hydrophila* (*A. hydrophila*) infection, we determined the expression level of *IL-11*, *ITLN* and *PNP5a* in kidney at different time points after infection. Significant up-regulation of *IL-11*, *ITLN* and *PNP5a* was observed 72 h post-CyHV-2 injection ($P<0.01$), whereas significant up-regulation was observed as early as 6 h after infection with *A. hydrophila* ($P<0.01$). Our results demonstrated that host innate immune response to CyHV-2, at least in which *IL-11*, *ITLN* and *PNP5a* were involved, lagged behind that induced by *A. hydrophila*. It was suggested that CyHV-2 may suppress host innate response during early infection. The lack of a C-terminal peptide in crucian carp ITLN gene implied a possible evolutionary difference in function of this gene, which merit further investigations.

ID133:

Emerging *Ranavirus* infections in ornamental and cultivable fishes of India

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Ranaviruses come under the family *Iridoviridae* and are a group of genetically divergent viruses associated with mortality in reptiles, amphibians and fishes. Ranaviruses can cause severe acute systemic disease in fish with necrosis of kidney and spleen, and haemorrhage on the skin and internal organs. Epizootic haematopoietic necrosis virus of the genus *Ranavirus* has been designated as a notifiable disease of fish by the World Organization for Animal Health (OIE). All ranaviral diseases of amphibians are also notifiable to OIE. We investigated mortalities in koi and damselfish in South India and cultivable freshwater carps in north-eastern Indian states to determine aetiological cause(s). Fishes collected from north-eastern state of Manipur had ulcerative dermal lesions resembling EUS. Two viral agents associated with mortalities in koi and damselfish were isolated and characterised. Biophysical, biochemical and molecular characteristics of the viral agents isolated showed that the virus belonged to the genus *Ranavirus* of the family *Iridoviridae*. Ranaviral major capsid protein specific DNA was detected in one of 27 DNA samples generated from infected carps from Assam and eight out of ten infected fish samples from Manipur, in North-east India. Sequence analysis of a 279 bp fragment of one of the amplified DNA from fishes of North-east India showed 99 % homology with the major capsid protein gene of the koi ranavirus (KJ939444). Our results showed the presence of ranaviruses in fishes suffering mortalities in both freshwater and marine environments. Ranaviruses are known for their adaptive capabilities that facilitates host shifting between fish, amphibians and reptiles. Detection of these ranavirus in fishes of India raises concern, and calls for prompt prophylactic measures for containing their spread.

FISH VIRAL DISEASES POSTER PRESENTATIONS

ID203:

Phylogenetic origin of Korean fish viruses

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Aquaculture has grown rapidly in Korea in the past few decades. However, because of intensive culture and environmental pollution, several viral diseases have appeared. The etiological agents of these viral diseases are viral hemorrhagic septicemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV), nervous necrosis virus (NNV), aquabirnavirus (ABV) and megalocytivirus (MCV). In the present study, we investigated the phylogenetic origin of these Korean fish viruses. Phylogenetic analyses based on the viral nucleotide sequences of Korean and isolates from other locations worldwide suggest that the origin of Korean VHSV, NNV, MCV (from marine fish) and ABV (from marine fish) may have been from Korean coastal waters. In contrast, this study suggests that IHNV, MCV (from freshwater fishes) and ABV (from salmonid fish and eel *Anguilla japonica*) have most likely been introduced by the movement of contaminated fish and eggs from outside the country.

ID168:

Complete genome analyses of nervous necrosis virus (NNV) isolated from seven-band grouper (*Epinephelus septemfasciatus*) in Korea

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Seven-band grouper (*Epinephelus septemfasciatus*) is one of the most valuable cultured fish in Korea. However, recentoutbreaks of viral nervous necrosis (VNN) in farmed seven-band groupers have caused huge economic losses during the summer season. To address these serious economic losses, our group investigated several new approaches in disease control, such as live vaccine at low rearing temperature and Poly(I:C) administration. The potential of reverse genetic approaches in developing vaccines and better understanding viral life cycles were investigated. Knowledge on genome structure and complete nucleotide sequence of Korean NNV isolates are necessary before these vaccines and diagnostic tools can be developed. The genome of nervous necrosis virus (NNV) SGYeosu08 isolated from sevenband grouper (*E. septemfasciatus*) in Yeosu, Korea was cloned and analyzed. RNA1 which include encoding region for RNA dependent RNA polymerase and untranslated regions from both ends was analysed as 3,103 nucleotides in length,. The deduced amino acids of RNA1 showed greater than 98.0 % (98.0 - 99.4 %) identity with other Red-spotted grouper nervous necrosis virus (RGNNV), 88.3 - 88.7 % with BFNNV, 88.6 % with TPNNV, and 87.9 % with SJNNV. RNA2 which encode a coat protein was 1,433 nucleotides in length and the deduced amino acids showed greater than 99.1 % (99.1 - 100 %) identity with other RGNNV, 85.5 - 87.0 % with BFNNV, 81.5 % with TPNNV, 81.5 % with SJNNV, and 78.5 % with TNV. Phylogenetic analysis explicitly demonstrated the close relationship of SGYeosu08 with members of Red-spotted grouper nervous necrosis virus (RGNNV).

This study is the first to analyze and report the complete nucleotide sequence of a NNV genome from Korea. Genetic information on the Korean NNV would be useful to develop accurate and rapid diagnostic tools, and investigate reverse genetic approaches.

ID165:

Effects of goldfish kidney extract in cell culture medium on the growth of goldfish hematopoietic necrosis virus

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Herpesviral goldfish hematopoietic necrosis due to infection with goldfish hematopoietic necrosis virus (GFHNV = cyprinid herpesvirus 2) has caused significant economical losses in goldfish (*Carassius auratus*) culture in several countries including Japan, and recently become an obstacle in Prussian carp (*C. gibelio*) culture in China. Several studies report the difficulty in culturing GFHNV, due to total loss of infectivity after several passages. Ito et al. (2013) succeeded in serial propagations of GFHNV in cell culture, but the virus titer was low at 10^3 $TCID_{50}$/mL. This study investigated the effects of goldfish kidney extract on *in vitro* growth characteristics of GFHNV.

RyuF-2 cell line newly derived from the fin of goldfish Ryukin variety were used for virus propagation of GFHNV SaT-1 isolate. Addition of 0.2% healthy goldfish kidney extract to culture medium, MEM or M199 supplemented with 5% FBS, resulted in complete cytopathic effect (CPE) at 25°C within several days after inoculation and reproducible high titers of 10^{5-6} $TCID_{50}$/mL. The virus propogated using this protocol were virulent to goldfish (average BW 2.6g) by intraperitoneal injection. Using this protocol, GFHNV SaT-1 isolate grew well in RyuF-2 cells at 15, 20, 25 and 30°C but not at 35°C. Higher incubation temperatures resulted in earlier CPE developments, but culture at 30°C yielded lower virus titer than those at the other temparatures tested. Cell lines derived from goldfish (RyuF-2, GFF) and ginbuna (*C. langsdorfii*) (CFS) were highly susceptibility to GFHNV SaT-1 isolate, whilst cell lines (KF-1, CCB) from carp were only susceptible when using a medium containing goldfish kidney extract. In contrast, GFHNV SaT-1 isolate did not produce any CPE in EPC, FHM and BF-2 even with addition of the goldfish kidney extract. Thus, addition of the kidney extract can enhance the susceptibility of some cell lines. In conclusion, we recommend the propagation of GFHNV in RyuF-2 at 25°C using a culture medium with 0.2% goldfish kidney extract.

ID152:

Research on emerging Cyprinid herpesvirus 2 infections in China

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Cyprinid herpesvirus 2 (CyHV-2), also named Goldfish Haematopoietic Necrosis Virus (GFHNV), is the second herpesvirus reported in Cyprinids. It was a major pathogen of goldfish (*Carassius auratus*) in Japan, Taiwan, Australia, New Zealand, the UK, and the USA. In 2011 to 2012, CyHV-2 caused serious mortality of farmed Prussian carp (*Carassius gibelio*) in China. Clinical signs of the disease included lethargy, inappetence, gill hemorrhage, and haemorrhagic spots on body surfaces. Internal gross pathology included hyperaemia, hepatomegaly and splenomegaly. Spleens in affected goldfish showed extensive necrosis of white pulp and ellipsoids. Viral particles 90-170 nm in diameter were observed by electron microscopy. PCR assays with CyHV-2 specific primers were used to detect the virus in DNA extracted from supernatant of infected cells and tissue homogenates of brain, liver, spleen, and kidney from diseased fish. Sequencing and analysis of PCR products showed more than 98% nucleotide identity with published CyHV-2 sequences . Challenge trials conducted using intramuscular injections of tissue filtrates from diseased fish caused 100% mortality. Challenged fish showed similar clinical signs to those seen in infected wild fish,. The biological characteristics, pathogenicity and semi-lethal dose (LD50) of the virus were also investigated. Results showed that the virus were highly pathogenicfor Prussian carp at 15°C to 25°C, pH 5 to 10, and 0 to 10g L^{-1} salinity. However, the virus lost its pathogenicity after treatment with chloroform and ether. In addition, the virus was only pathogenic to goldfish (*Carassius auratus*) and Prussian carp (*Carassius gibelio*).

Loop-mediated isothermal amplification (LAMP) for this pathogen was developed, resulting in a set of six primers targeting terminase gene. Detection limits of 1.09×10^{-4}μg/μL was superior to conventional PCR and real-time PCR. No test cross reactions were obtained with 28 other viruses or bacteria commonly found in fish. Single-chain antibody fragment (scFv) against CyHV-2 was developed by phage display, as part of this study. Single-chain antibody fragment (scFv) is a fusion protein consisted of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, with a short linker peptide as connection.

ID113:

Binding of grass carp reovirus to 37/67 kDa laminin receptor necessary for viral entry *in vitro*

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A representative strain of Aquareovirus-C, Grass carp reovirus (GCRV), , is used as a model for studying viral pathogenesis. The aim of this study is to isolate and characterize the cellular protein used by GCRV to gain entry into *Ctenopharyngodon idellus* kidney (CIK) cells. A 37/67-kDa laminin receptor (LamR) was identified as the interacting partner for the outer capsid protein VP5 of GCRV through yeast two-hybrid screening in *Saccharomyces cerevisiae*. GCRV infections caused increased levels of membrane-associated LamR as detected in CIK cells by both an immunofluorescence assay (IFA) and Western blot analysis of membrane extracts. Both virus overlay protein binding assay (VOPBA) and co-immunoprecipitation (co-IP) assay demonstrated that GCRV particles specifically bind to LamR proteins. LamR knockdown by RNAi resulted in inability of GCRV to bind to surface of CIK cells. Furthermore, adhesion to the CIK cell surface by GCRV particles was inhibited dose-dependently by laminin proteins. These results collectively suggest that binding of GCRV to LamR was necessary for efficient viral entry *in vitro*.

ID 415:

Full-length sequencing of CyHV-3 genome directly from host reveals mixed infections

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Koi herpesvirus disease (KHVD) is an emerging disease that causes mass mortalities in koi and common carps (*Cyprinus carpio* L). Since its first report in the late 1990s, it has spread to many countries worldwide. Its causative agent, the Cyprinid herpesvirus 3 (CyHV-3, also known as koi herpesvirus - KHV), is a large double-stranded DNA virus that belongs to the family *Alloherpesviridae*, genus *Cyprinivirus*. Even though published literature on the pathogenesis of this virus is relatively abundant, little is known about its genomic diversity. In this context, we developed a new strategy for sequencing full-length CyHV-3 genome directly from their host tissues. Total genomic DNA extracted from carp gills was specifically enriched with CyHV-3 sequences through hybridization, to a set of nearly 20 million overlapping probes designed to cover the entire length of the genome, using KHV-J sequence (GenBank accession number AP008984) as reference. This protocol employing both simplex and 4-plex formats was tested on DNA extracted from six common carps collected from a KHV enzootic area in Lake Cirata, West Java in Indonesia (,. All sample combinations were sequenced using a HiSeq platform (Illumina) and genomes were reconstructed using varying mapping tools. Even though the rate of enrichment was directly correlated to the initial viral load, results revealed that complete genomes could be recovered from gill samples containing as little as 5,000 CyHV-3 gene copies, with a good coverage (>100x) of the entire genome. The six Indonesian viruses studied were found to belong to the Japanese lineage. In spite of a high variability at several tandem repeat loci, genetic diversity at the genomic scale was very low (<1%). The highest divergence (3.6%) was observed on ORF131, which encodes a putative membrane protein. Sequencing also highlighted the presence of mixed infections in a single fish. In conclusion, our strategy will enable in-depth analyses of *in vivo* viral sequences, and shed new light on the evolution patterns (genetic shifts, genetic drifts) of this deadly virus.

ID148:

Detection of Japanese eel endothelial cells-infecting virus (JEECV) in Japanese eel (*Anguilla japonica*) in natural habitats

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Viral endothelial cell necrosis (VECNE) is a serious problem in cultured eels. The predominant sign of this infection is congestion of gills in affected eels. VECNE is caused by Japanese eel endothelial cells-infecting virus (JEECV) which is a member of the polyomavirus family. The origin of JEECV is unknown. As the source of cultured eel is wild glass eels, we conducted a survey to determine the status of JEECV infection in Japanese eel in natural habitats. Ten Japanese eels each were sampled from brackish water areas in the Nakagawa river (NR), Tokyo and from fresh water areas in an anonymous river (AR) located several hundreds kilometers from Tokyo. All sampled eels were at the yellow stage of their developmental life cycle. PCR was performed on viral DNA extracted from gills, using JEECV screening primer sets reported by Mizutani *et al.* To determine the partial large , Nested PCR using newly designed primer sets D and E were used to screen for T antigen-like genomic region, considered to be potential pathogenic genome of JEECV. Two JEECV positive eels each were detected in both NR and AR samples, and found to carry mutations in their nucleic acid and deduced amino acid sequences using primer set E PCR. , This suggests that wild-type JEECV infects Japanese eels living in natural environment. Determination of the whole-genome sequences of these wild-type JEECV will be necessary as we progress our research on understanding these viruses and the disease they cause.

WSD
ORAL PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID 413

On the transmission of White Spot Syndrome virus in aquaculture systems

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White Spot Syndrome (WSD) is the major curse in shrimp aquaculture industry. The disease is caused by White Spot Syndrome Virus (WSSV), a large double-stranded DNA virus, accommodated in the family *Nimaviridae*. Virus infection results in high mortality in shrimp and major crop losses in sub-tropical areas around the world. Since its appearance on the scene in the mid-1990s in Southeast Asia, the disease has spread globally and is now a true viral zoonosis. A critical cue in the development of WSD as an epidemic has been the virulence and transmission characteristics of the virus. WSSV is characterized by genetic heterogeneity, i.e. the presence of multiple related genotypes, and by increase of virulence over time and space as a consequence of genotypic drift. The molecular pathogenesis of WSSV is being studied widely, but much less is known about the transmission of this virus. WSSV has a wide host range among crustaceans and even beyond, and this is only a problem for shrimp aquaculture, but also for the environment as it can affect the food chain. In qualitative terms the virus is transmitted per os, upon cannibalistic feeding or on detritus or through the gills by free floating viruses. Also vertical transmission has been demonstrated for shrimp i.e. via eggs. In the transmission the role of the environment and that of intermediate hosts where there is no disease and hardly any within-species transmission is not yet understood. Moreover, it appears that also in shrimp the virulence of the virus depends on the circumstances, i.e. conditions in the pond. The virus can also persist in the host in a sub-lethal or latent state, i.e. the virus replicates in tune with the host and resist the defense of the host, but can break out in response to environmental cues. Much less is known about the quantitative aspects of WSSV transmission, which are key to the development of an epidemic at a small scale (pond) but also at a large scale (between farms) in time and space, respectively. Parameters such as transmission rates are important to know, as they co-determine the intervention strategies to control WSSV. In this contribution the lessons learned will be reviewed and areas for the future identified.

ID131:

On the role of polychaetes [*Dendronereis* spp.] in transmission of white spot syndrome virus in shrimp ponds

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White spot syndrome virus (WSSV) is the most devastating viral pathogen in shrimp production systems. WSSV is a generalist virus and infects members of several families of decapod crustaceans. Currently, WSSV has been found to be associated with 26 species of brackish water and marine shrimp, 15 species of fresh water shrimp and crayfish, 55 species of crabs and 10 species of lobster. The listing also includes 10 species of non-crustacean organisms, including polychaetes (*Annelida*), such as *Dendronereis*, and algae. *Dendronereis* spp. is a ubiquitous Nereid polychaete resident in shrimp ponds in Indonesia and part of the shrimps' natural diet. Here we report on the possible role of *Dendronereis* spp. in the transmission of WSSV in shrimp pond systems. Field surveys in two research locations in Indonesia, Delta Mahakam (Kalimantan) and Semarang vicinity (Central Java), showed that association of WSSV with *Dendronereis* spp. is quite common, with a point prevalence of 44 ± 27% (± SD). WSSV was found to replicate in the gut of naturally-infected *Dendronereis* spp. as detected via immunohistochemistry using monoclonal antibodies to detect immunoreactive nuclei and via RT-PCR to detect the viral mRNA. This is the first evidence for a non-crustacean to be a natural replicative host for WSSV. WSSV was transmitted from naturally infected *Dendronereis* spp. to *Litopenaeus vannamei* (Boone 1931) through the oral route and further to new naïve shrimp showing that natural transmission of WSSV from polychaetes to shrimp is possible. In shrimp ponds WSSV infection in *Dendronereis* spp. correlated positively with *Dendronereis* spp. density, with proportion of WSSV infection in shrimp and the past incidence of white spot disease. Findings of the present study emphasize that resident benthic organisms in shrimp ponds, such as *Dendronereis* spp., can be a reservoir host of WSSV and may explain the persistence of WSSV in pond systems over time. However, further studies are required to obtain a better understanding of the relative importance of *Dendronereis* spp. in WSSV epidemiology in and beyond shrimp ponds. The significance of the findings may provide new insight on WSSV persistence in shrimp pond environments but also in white spot disease management.

ID88:

WSSV achieves successful replication by triggering an invertebrate Warburg effect via activation of the PI3K-Akt-mTOR pathway

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Metabolic rerouting into aerobic glycolysis (i.e. the Warburg effect) is beneficial to cancer cells and vertebrate cells infected by viruses for supporting profoundly increased demands for energy and building blocks. Although all knowledge regarding this metabolic alteration has been derived from vertebrates, our research was apparently the first to implicate the Warburg effect in virus-infected invertebrates. Using proteomics and metabolomics, in shrimp hemocytes, we documented global changes triggered by white spot syndrome virus (WSSV). At the WSSV genome replication stage, WSSV induced the Warburg effect. Several critical metabolic properties of the WSSV-induced Warburg effect were similar to the vertebrate Warburg effect, including increasing intermediates in glycolysis, the pentose phosphate pathway and glutaminolysis. In contrast, the Warburg effect was not apparent at the late stage of WSSV replication. Moreover, on the basis of *in vivo* silencing and drug treatments, the PI3K-Akt-mTOR pathway appeared to have a central role in triggering this WSSV-induced Warburg effect. In that regard, when shrimp were treated with inhibitors to suppress the WSSV-induced Warburg effect, even though the TCA cycle was subsequently up-regulated by WSSV, it could not satisfy viral requirements for genome replication. These findings lead to a strategy to select disease-resistant shrimp, namely those which do not have a profound increase in metabolism (i.e. Warburg effect) in response to WSSV infection.

ID214:

Two anti-apoptotic proteins of white spot syndrome virus that bind to an effector caspase of the black tiger shrimp *Penaeus monodon*

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Caspases or cysteine-aspartic proteases play essential roles in the apoptosis pathway. So far, two full-length caspase (*PmCasp* and *Pm caspase*) cDNA sequences have been identified from the black tiger shrimp *Penaeus monodon*. Previous works have shown that a protein from white spot syndrome virus (WSSV), namely AAP1 (also known as WSV390 or WSSV449), was found to bind and inhibit caspase activity of *Pm* caspase. This study searched more anti-apoptotic proteins from WSSV that could bind to PmCasp. We employed a full-length sequence of PmCasp and its large subunit (residues 55–214) as baits in the yeast two-hybrid approach. The results showed that WSSV134 and WSSV322 proteins bind to the large subunit of PmCasp protein. Co-immunoprecipitation in the Sf-9 system was then used to confirm the protein-protein interaction. The results demonstrated that WSSV134 and WSSV322 were immunoprecipitated by both large subunit and PmCasp proteins. By morphological observation and DAPI nuclear staining, Sf-9 cell apoptosis could be induced by PmCasp but not by the large subunit. Interestingly, anti-apoptosis activity of WSSV134 and WSSV322 was revealed by inhibition of Sf-9 cell apoptosis induced by PmCasp. WSSV449 that was included in the control experiments did not bind to PmCasp in the yeast two-hybrid assay. In addition, WSSV449 could not inhibit apoptosis induced by Pmcasp. The results revealed diversity in effector caspases and their viral protein inhibitors in *P. monodon*.

ID198:

Susceptibility and pathogenicity of White Spot Disease (WSD) in non-model crustacean host taxa from temperate regions

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Despite almost two decades since its discovery, White Spot Disease (WSD) is still considered the most significant known pathogen impacting the sustainability and growth of the global penaeid shrimp farming industry. Although most commonly associated with penaeid shrimp farmed in warm waters, the virus is also able to infect, cause disease and kill a wide range of other decapod crustaceans from temperate regions, including lobsters, crabs, crayfish and shrimp. Using principles laid down by the European Food Safety Authority (EFSA) we used an array of diagnostic approaches to provide a definitive statement on the susceptibility to White Spot Syndrome Virus (WSSV) infection in seven ecologically or economically important European crustacean species. Exposure trials based upon natural (feeding) and artificial (intra-muscular injection) routes of exposure to WSSV revealed universal susceptibility to WSSV infection in these hosts, but also that relative susceptibility varied significantly between species. We describe the pathogenesis of WSD in these hosts and compare this to the well documented disease progression profile of model penaeid shrimp hosts. The European shore crab (*Carcinus maenas*) was shown to display a lower susceptibility to White Spot Syndrome Virus (WSSV) when compared to other European decapod species. Despite showing signs of infection with WSSV, the shore crab appeared resistant to the development of disease and was highlighted as a possible asymptomatic carrier of the virus. W**e** compared *Carcinus maenas* individuals which had been injected with WSSV and then exposed to varying temperature stress conditions. Analysis of response to WSSV exposure suggested that crabs could be divided into two groups (high and low responders) according to differences in pathogenesis (histopathology) and relative viral replication (viral copies mg^{-1} tissue). The presence of both high- and low-responders in both the 'stressed' and 'non-stressed' exposure groups, and the non-significant relationship between viral copy number in individual crabs and their exposure group suggests that the response type was not dependent on the presence or absence of an external stressor but was more likely an inherent capacity within individual crabs.

ID230:

A proposed anti- white spot syndrome virus activity of *Penaeus monodon* anti-lipopolysaccharide factor isoform 3 (ALF*Pm*3)

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An antimicrobial peptide from *Penaeus monodon,* namely anti-lipopolysaccharide factor isoform 3 (ALF*Pm*3) is active against bacteria, fungi and a shrimp pathogenic virus, white spot syndrome virus (WSSV). To gain insight into its molecular mechanism on WSSV, previously, the yeast two-hybrid assay identified 5 proteins of WSSV (WSSV186, WSSV189, WSSV395, WSSV458 and WSSV471) as ALF*Pm*3-interacting proteins. Four of them were successfully expressed in *Escherichia coli*. The true interactions between ALF*Pm*3 with their interacting proteins were confirmed by *in vitro* pull-down assay. Furthermore, we demonstrated that pre-incubation of each rWSSV protein with rALF*Pm*3 could interfere the neutralization effect of rALF*Pm*3 on WSSV. The increase in the mortality rate of shrimp injected with a mixture of rWSSV proteins, rALF*Pm*3 and WSSV as compared to those injected with rALF*Pm*3 and WSSV was clearly observed. The results indicated that the ALF*Pm*3 performs its anti-WSSV action by binding to WSSV proteins. All ALF*Pm*3-interacting WSSV proteins were structural proteins mostly envelope proteins. Transmission electron microscopy showed that treating WSSV virions with rALF*Pm*3 caused the significant loss of viral envelope when compared to the untreated WSSV resulting in the loss of viral infectivity. Therefore, the direct interaction of rALF*Pm*3 on WSSV virions is a proposed mode of rALF*Pm*3 antiviral action.

ID268

Silencing protein kinase in shrimp: An insight on its role in the immune system

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In the elucidation of shrimp-WSSV interaction, recent studies have been focusing on several genes located within the genome of shrimps and WSSV which can be potentially linked to the complex interaction. In this study, open reading frames (ORFs) that encode 424-aa polypeptide for protein kinase (PK) was found in *Marsupenaeus japonicus* with sequences for *Penaeus monodon* and *Macrobrachium rosenbergii* also obtained. Phylogenetic analysis revealed that the three ORFs has 30% homology to WSSV-PK supported by a 86% bootstrap value; Gene expression revealed the PK gene in different organs of the shrimp. PK was ubiquitously expressed in all relevant shrimp organs. Gene knockdown of PK showed increased survival rates of 55% was observed after Day 7 p.i. compared to the control groups, PBS and GFP revealed by the mortality data; RT-PCR analysis indicated that silencing of the PK gene was partial accounting for its multiple copies in the shrimp's genome. It can be inferred that the PK gene has a role in the regulation of viral and bacterial infections.

ID201:

Study the role of glucose transporter 1 (Glut1) in white spot syndrome virus (WSSV) infection

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White spot syndrome virus (WSSV) is a large enveloped DNA virus, and it causes a serious disease that has led to severe mortalities of cultured shrimps in many other countries. In this study, we identified a surface protein of *Litopenaeus vanname*, named glucose transporter 1 (Glut1), which could interact with WSSV envelope protein VP53A. Sequence analysis revealed that Glut1 closely related a member of a large superfamily of transporters that function to transport the sugar. Even though this *Glut1* gene was far away from other organisms in evolutionary distance except for arthropod, we could found this *Glut1* gene in other shrimp species. Glut1 protein was 68 kDa in molecular weight and could be observed on the surface of the shrimp hemocyte. Glut1 was revealed to interact with WSSV envelope proteins and *Penaeus monodon* chitin-binding protein (PmCBP), which itself was identified to interact with WSSV infectome. *In vitro* and *in vivo* neutralization experiments could inhibit WSSV infection in primary cultured hemocytes and delay the mortality in shrimps challenged with WSSV. In this conclusion, we think Glut1 has relations with WSSV infectome. The cell line expressed Glut1 depleted more glucose of the medium than other control. Thus, we could confirm that Glut1 of *L. vanname* could transport glucose. Taken together, we speculate that WSSV enter the host by binding with Glut1.

ID136:

A quantitative approach to disease transmission of white spot syndrome virus: seasonal effects in shrimp ponds

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White spot syndrome virus (WSSV) can cause serious disease with a very high mortality in farmed *Penaeus monodon* shrimp. More WSSV disease outbreaks are seen in Vietnam in the rain season than in the dry season and this could well be due to deteriorating water quality parameters, particular fluctuating water temperature and lower salinity. This study characterized the dynamics of WSSV transmission in a shrimp culture pond during dry warm conditions outside the rainy (dry) season and colder and wet conditions during the rain season. A comparison was made of the disease transmission of WSSV and the mortality caused by WSSV in *P. monodon* juveniles between the two types of seasons. The experiments were conducted in an empty marine fish pond using group cohabitation in fine nets to investigate the transmission of WSSV. In the same pond, one experiment was done in the dry season and another one was done in the rain season. Each experiment included one group of nets, each net cohabitated five inoculated shrimp and five contact-exposed shrimp, and another group of nets containing only contact shrimp, as set up to calculate the reproduction ratio, the transmission rate parameter and mortality rate parameter. The important quality parameters of the pond water were measured twice daily. A PCR method was used to test for virus infection. The Generalized Linear Model and Kaplan-Meier regression model are used to estimate the transmission rate parameter of WSSV and mortality rate parameter due to WSSV infection of the shrimp, respectively. Two major quality parameters of the pond water, temperature and salinity, were more optimal and fluctuated less in the dry season compared to those in the rain season. Significantly higher transmission rate parameter of WSSV and significantly higher mortality rate parameter of infected shrimp were found during the rain season compared to those during the dry season. The observed chance in transmission and mortality was similar to estimates from experiments where only temperature was changed and opposite to the estimates when only salinity was changed. Hence, the observed higher WSSV associated problems during the rainy season could well be due to the fluctuating water temperature conditions.

ID174:

Effects of WSSV and bio-security on shrimp farming in Bangladesh

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Culture of shrimp mainly *Penaeus monodon* accounts a large proportion of Bangladesh's aquaculture industry by value, and is the country's second largest source of export earnings. But the shrimp industry of Bangladesh has encountered enormous problems due to the spread of diseases, particularly WSSV, and has incurred significant economic losses as a result. The major factors encouraging WSSV are the production of PL using wild brood and traditional farming systems with poor farm level bio-security. Between 2005 and 2013 WorldFish collected samples of brood, naupli and PL from hatcheries in Bangladesh and tested those using PCR techniques, as part of a program to supply WSSV free PLs to project farmers. Using one way ANOVA, a statistically significant difference was found (F (7, 64) = 15.374, p<0.001) between the percentage of WSSV positive wild brood by month of brood collection but it had no significant difference (p>0.5) with year, again significant difference was found (F (8, 63) = 3.7, p<0.001) between percentage of WSSV positive in nauplii and year, (F (7, 64) = 6.526, p<0.001) between percentage of WSSV positive in nauplii and month. A strong positive correlation (R = 0.743) was found among WSSV infected brood and WSSV infected nauplii, providing evidence of vertical transmission of WSSV. On an average every year almost 40% of hatchery produced PL was found WSSV positive considering positive nauplii batches from 2005 to 2013. This would be sufficient to contaminate almost the entire farming system, as 88% of farming area is under traditional management. Traditional management practices include multiple stocking and multiple harvest and poor management of water exchange. There is no means of identifying WSSV negative PL, except for testing provided by WorldFish, which only covers 5% of the total supply of PL. The farming system is therefore highly vulnerable to the effects of WSSD. Introducing SPF *P. monodon* brood and developing commercial PCR testing facilities as well as bio-secure farming practices could improve shrimp farming performance and country's economy. Again following standard hatchery operation protocol and producing disease free PL is evenly significant to develop shrimp industry of Bangladesh.

ID151:

Towards the commercial use of double-stranded RNA for the prevention of shrimp viral diseases

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RNA interference (RNAi) is a specific and effective approach for inhibiting viral replication by introducing double-stranded (ds)RNA to interfere with viral mRNA. Use of specific dsRNA under laboratory settings has been shown to reduce shrimp mortality induced by viruses, including, White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV). The present work is involved in the developments of large-scale production of dsRNA for farm operations. First, we developed a commercial-scale production of antiviral dsRNA in the bacterial system. Culture expression of dsRNA was optimized through "fed-batch fermentation" with Terrific medium (TB). Estimated yield of dsRNA from the cultivation is 80-100 mg/ 1 L culture that is at least 25 times higher than the yield obtained under a typical batch fermentation. Feed pellets containing the bacteria expressing dsRNA are prepared according to the method described by Saksmeprome *et al.* 2013, and antiviral efficacy of the feed are under investigation. In addition, we seek to replace the current use of *E.coli* for dsRNA production that has raised public health concerns. *Chlamydomonas reihardtii* (*C. reihardtii*) or green microalgae is of interest as an alternative dsRNA expression system because of its scalability and well-established transformation methodology. Production of transgenic microalgae expressing dsRNA and analysis of its efficiency against shrimp viruses will be explored for possible farm applications.

WSD
POSTER PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID309:

Transmission of white spot syndrome virus (WSSV) in vaccinated shrimp *Penaeus vannamei*

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It has been published that vaccines can provide shrimp limited protection against white spot syndrome virus (WSSV). In those studies, mortality was used as the only indicator of efficacy; whereas transmission, which is key to pond control of WSSV has not been considered. This study quantified the effect of a VP28 subunit vaccine on the transmission of virus in a population of *Penaeus vannamei* shrimp by a transmission experiment. In this experiment, pairs of shrimp were housed together – an infected shrimp together with an uninfected shrimp – and the infection chain was monitored. The survival rate and the basic reproduction ratio (R) (the average number of secondary infections caused by one typically infected individual in a fully susceptible population) were estimated to evaluate the effect of vaccination in shrimp. Survival was significantly higher for the vaccinated group than for the control, as expected based on previous results. However, a reduction of viral transmission by vaccination in shrimp could not be demonstrated as *R* estimates were not significantly different. These findings suggested that the use of VP28 subunit vaccine alone will not reduce transmission enough to prevent WSSV outbreaks under tested conditions.

ID298:

Effect of different salinities (5, 20, 37ppt) at low temperature (22°C) on WSSV infected shrimp.

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Whitespot syndrome virus (WSSV) continues to bring havoc to the shrimp industry. After more than 3 decades, there is still no guaranteed treatment against WSSV infection. Reported treatments give inconsistent results whichc ouldb e due to the interaction of the risk factors.Previous studies identified low temperature (22°C) and low salinity (5ppt) as WSSV risk factors. This study aimed to determine the effect of different salinities at low water temperature on WSSV infection in *Penaeus monodon*. Shrimps (n=300; average body weight=4g) were divided into 2 groups: one, the infected group, fed to satiation with WSSV positive shrimp carcass and the other, the control group, fed with commercial pellet. A day after the feeding treatment, the two groups were stocked in separate experimental tanks, at 12ind/tank, with different salinities (5, 20, 37ppt) and cultured at 22°C. Shrimp in both groups were fed daily with commercial pellet at 1% of shrimp biomass. No water change was implemented until termination at 144 hours post infection (hpi). Prior to stocking in separate tanks, 10 shrimp were sampled from each group for WSSV detection using nested PCR and QPCR. One shrimp, prefereably dead/weak was taken daily from each tank for WSSV analysis using QPCR. Water microflora, pH, and temperature were monitored daily. After the treatment, infected shrimps were WSSV positive; QPCR showed 3 copies/mg gill tissue. Analysis showed no significant difference in shrimp mortality and viral load among the infected group. WSSV load of those maintained at 20 ppt was 5.11 copies/mg gill; 37ppt with 5.04 copies/mg gill and 5 ppt with 4.55 copies/mg gill. Highest mortality was observed in infected shrimp maintained at 37ppt (50%), followed by those maintained at 20 ppt(44%); lowest at 5 ppt (33%). After the treatment, control shrimp were WSSV negative. Interestingly, at 144hpi, WSSV was detected in the control group. Among the control group, significantly higher viral load and mortality were observed in control shrimp maintained at 5 ppt. Viral load of control shrimp maintained at 5 ppt was 2.88 copies/mg gill; 1.26 copies/mg gills at 20 ppt, and 0.3 copies/mg gills at 37ppt. Mortalities were observed in the control group maintained at 5 ppt (19%) but not in those at 20 and 37ppt. Mortality was negatively correlate withsalinity and positively with the infection, water pH, and hours post infection; viral load was positively correlated with mortality, infection, and hours post infection. Uninfected shrimp became infected with WSSV at higher rates at lower salinities, but the source of infection was not detected. Mortality is affected by the viral load which is affected by the duration the virus has been in the shrimp body. At salinities of 5, 20 and 37ppt, infection may result in mortality if the viral load is ≥2.88 copies/mg gill. Shrimp with low viral load of ≤1.26 copies/mg gill exposed to 1 stressor may not result in mortality. Previous research recommanded not to stock in the cold months, but if the culture period falls on the cold months, it is recommended to maintain the shrimp at high salinities.

ID296:

Present status of wild caught *Penaeus monodon* broodstock and prevalence of wssv disease in Bangladesh

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Bangladesh shrimp hatcheries (n=56) are totally dependent upon wild broodstock. To know the present status of broodstock catching, their carrying facilities, prices and seasonal variation of white spot syndrome virus (WSSV) prevalence, a study was conducted from September 2012 to July 2013. Information's were collected from shrimp trawlers association, trawler skippers and crews, hatchery owners, intermediaries and personal experience on board and a shrimp hatchery. In total 21 shrimp trawlers out of 40 were engaged in catching broodstocks at a depth between 25 and 65 meter and the trawling time was 1.5 to 2 hours. The Kohinoor point, Elephant point and down of the St. Martin's Island i.e. West point are the most important area for the broodstock catching. The maximum 27,387 broodstock were caught in the month of January and minimum 1182 in September. At new Moon, the average price was about US $ 50.00 and during the full Moon, it was US $ 125.00. Present study observed that January to May was the peak season and June to September was an off-peak season for broodstock catching, but hatchery demand was high in May to July. Average lowest prevalence of WSSV in broodstock was 5% in September and the highest was 75% in June-July during the monsoon season in Bangladesh. The WSSV variation patterns for nauplii correlated with broodstock patterns with average lowest prevalence of 2% in September compared to the highest of 50% in July. Similarly, the average lowest prevalence of WSSV in post larvae was 0% in December and highest was 20% in July. The present study found that during the monsoon period low temperature and salinity have direct impact on the incidence of viral disease. This study indicates that the use of wild gravid females without quarantine and screening could enhance the disease incidence in shrimp PL production in Bangladesh.

ID263

Targeting essential genes to mitigate WSSV infection by RNA interference

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The inflicting diseases on today's aquaculture have wreaked havoc on the sustainable growth of the shrimp culture industry worldwide. In the Asia-Pacific countries, the industry reported annual losses of about 4 billion US dollars yearly. White Spot Syndrome Virus (WSSV) remains the most widespread and devastating infectious agent that hit the shrimp aquaculture industry worldwide. To date, there are no known effective strategies yet to combat WSSV infection. This study aimed to elucidate host-pathogen interaction through the functional study of both a viral and a host gene. Utilizing RNA Interference, we elucidated the function of VP9 gene of WSSV, and contig23 (c23) found in the shrimp genome identified to have high homology with WSSVORF-325. Four set-ups using Macrobrachium rosenbergii shrimps were prepared for treatment of VP9-dsRNA, c23-dsRNA, GFP-dsRNA, and PBS. Each shrimp treatment group was challenged with WSSV and survival rate was recorded. VP9-, c23-, and GFP-dsRNA injected shrimps showed a significant survival rate of 80%, 100% and 100%, respectively, in contrast to 20% of the PBS injected shrimps at 10 days post-infection (dpi). These results are corroborated with the re-infection survival assay and the RT-PCR analysis. This study identified VP9 to be a very good target gene for RNAi therapeutics in WSSV infection in shrimps.

ID126

Glutaminolysis is important for replication of White Spot Syndrome Virus (WSSV)

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Infection with the white spot syndrome virus (WSSV) can induce a metabolic shift in shrimp (resembling the "Warburg effect" in mammalian cells) during WSSV replication (12 h after infection). Using selective inhibition, we demonstrated that the WSSV triggered the Warburg effect through activation of the PI3K-Akt-mTOR pathway. In neoplastic cells, the Warburg effect is usually accompanied by activation of several metabolic pathways: pentose phosphate, aerobic glycolysis, synthesis of fatty acids and nucleotides, and glutaminolysis (glutamine is metabolized by glutaminase and glutamate dehydrogenase [GDH] to produce glutamate and α-ketoglutarate). However, glutaminolysis has apparently not been studied in WSSV-infected shrimp. Glutamate concentrations were decreased in plasma but increased in the hemocyte of WSSV-infected shrimp. As well, both GDH activity and α-ketoglutarate concentrations were increased. Therefore we hypothesized that WSSV stimulated the host to uptake more glutamate into the cell to trigger glutaminolysis, replenish the TCA cycle and generate building blocks. Moreover, based on *in vivo* gene silencing, WSSV gene expression (IE-1, vp28) and viral copy number were decreased but could be restored by replenishing α-ketoglutarate in GDH-silenced shrimp. In conclusion, WSSV infection triggered the Warburg effect through the PI3K-Akt-mTOR pathway; furthermore, this effect was accompanied by glutaminolysis (important for WSSV gene expression and virus replication).

ID95:

Effect of Astragalus Polysaccharide on the anti-infection of White Spot Syndrome Virus (WSSV) in *Procambarus clarkii*

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We studied the effects of anti-infection of White Spot Syndrome Virus (WSSV) by adding different concentrations Astragalus Polysaccharides (APS) to the basal diet in *Procambarus clarkii*. The healthy *P. clarkii* which was negative of WSSV by PCR detection were injected intraperitoneally, with 100μL WSSV, and fed with 0%, 0.2%, 0.4% and 0.8% of APS bait. After feeding 20d, the result revealed that when the positive control group mortality rate was 100±0%, the mortality of 0.2%, 0.4%, 0.8 % APS addition group were 86.67%±13.33%, 91.11%±7.70%, 73.33%± 17.64 %, respectively. In order to evaluate the effect of APS on visceral organs, such as gill, hepatopancreas, and myocardial tissue in *P. clarkii*, the tissue sections were observed. The results showed that there was significant pathological change like nuclear pyknosis, cell ruptured and arranged desultorily in the group of 0% APS bait, there were no obviously pathological change in the gills and hepatopancreas, but nucleoli appeared a certain degree of shrinkage, while the cells have not yet broken in the myocardial tissue in the group of adding 0.8% APS bait. This experiment showed that the survival rate can be increased to 26.67% when adding 0.8% APS bait compared with the positive control in *P. clarkii*. The APS can significantly improve the effect of resistance of *P. clarkii* to the WSSV infection, and it can be expected to receive good value in the actual aquaculture production.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID256:

In vivo efficacy study to evaluate the effective dose of Virusnip™ Aqua for horizontal transmission of White Spot Syndrome Virus in Pacific white shrimp *Litopenaeus vannamei*

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A study was conducted on an organic releasing chlorine based disinfectant (Virusnip™ Aqua), in order to assess its *in vivo* efficacy in protection of the Pacific white shrimp *Litopenaeus vannamei* from White Spot Syndrome Virus (WSSV). The efficiency for the prevention of virus transfer from one shrimp to another was investigated by co-habitation of WSSV infected shrimps and healthy virus-free in Virusnip™ Aqua-treated sea water for 2 weeks. To determine the effective concentration of Virusnip™ Aqua solutions ranging from 0.5, 1, 2 to 4 ppm were prepared either with one application at the start of the trial or two applications at the start and after 1 week. The negative control (no WSSV) showed 82% survival, whereas the positive control with untreated WSSV (without Virusnip™ Aqua) revealed 6% survival. Incubation of healthy and infected shrimps together with 0.5 ppm of Virusnip™ Aqua at two weekly applications improved the survival rate up to 60%. Only one application of Virusnip™ Aqua at the beginning of the trial or higher concentrations of Virusnip™ Aqua resulted in lower survival rates. Taken the results together, the application of low dose of Virusnip™ Aqua (i.e., 0.5 ppm) with a weekly application helps to prevent WSSV infection. Furthermore, the toxicity of Virusnip™ Aqua was studied by the determination of LC_{50} in pacific white shrimp *Litopenaeus vannamei*, which was 68.2 ± 5.5 ppm (more than 100 times of the recommended dose at 0.5 ppm of Virusnip™ Aqua).

ID255:

Determination of the effective concentration of Organic Releasing Chlorine (Virusnip™ Aqua) against White Spot Syndrome Virus in Pacific white shrimp *Litopenaeus vannamei*

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Pond preparation is a key procedure of the shrimp culture. The objective of this study was to determine the efficacy of Virusnip™ Aqua against White Spot Syndrome Virus (WSSV) and the disinfection procedure of the culture system prior to releasing the shrimp seeds into the grow-out pond. WSSV was treated with different concentrations of Virusnip™ Aqua for 30 minutes or 24 hours to determine the effective concentration (ranging from 0.25, 0.5, 1, 2 to 4 ppm). After incubating WSSV with Virusnip™ Aqua in various concentrations, the different virus solutions were injected into the Pacific white shrimp *Litopenaeus vannamei* to investigate the virus survival rate for 14 days. Control group (WSSV only) showed 100% mortality within 7 days after virus injection. The study revealed a promising protective effect in shrimps against WSSV infection with approximately 80% survival rate in both short (30 minutes) and long term (24 hours) exposure regardless of the concentration used (0.25 – 4 ppm). In conclusion, Virusnip™ Aqua was considered effective as disinfectant against WSSV in pond preparation prior to shrimp culture.

ID240:

Persistence of *Penaeus stylirostris* densovirus delays mortality caused by white spot syndrome virus infection in black tiger shrimp (*Penaeus monodon*)

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Persistent infection of *Penaeus stylirostris* densovirus (*Pst*DNV) or IHHNV and its non-infectious inserts into the black tiger shrimp, *Penaeus monodon* (*P. monodon*) genome are commonly found without apparent disease. Here, we introduced the method of multiplex PCR in order to differentiate shrimp with viral inserts from ones with the infectious virus. The method allowed us to study the effect of pre-infection of IHHNV, in comparison to IHHNV inserts, on WSSV resistance in *P. monodon*. A multiplex PCR system was developed to amplify the entire IHHNV genome, ensuring the accurate diagnosis. Field samples containing IHHNV DNA templates as low as 20 pg or equivalent 150 viral copies can be detected by this method. By challenging the two groups of diagnosed shrimp with WSSV, we found that shrimp IHHNV infection and those with viral inserts responded to WSSV differently. Considering cumulative mortality, average time to death of shrimp in IHHNV-infected group (day 14) was significantly delayed relative to that (day 10) of IHHNV-inserted group. Real-time PCR analysis of WSSV copy number indicated the lower amount of WSSV in the IHHNV-infected group than the virus-inserted group. The ratio of IHHNV:WSSV copy number in all determined IHHNV-infected samples ranged from approximately 4 to 300-fold. The multiplex PCR assay developed herein proved optimal for convenient differentiation of shrimp specimens with real IHHNV infection and those with insert types. Diagnosed shrimp were also found to exhibit different WSSV tolerance. After exposed to WSSV, the naturally pre-IHHNV infected *P.monodon* were less susceptible to WSSV and, consequently, survived longer than the IHHNV-inserted shrimp.

ID232:

Development of a simple and cost-effective WSSV diagnostic kit for the Philippine shrimp aquaculture industry

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Playing a major role in the Philippine economy, the shrimp aquaculture industry has provided livelihood and revenue for Filipinos across the country. Considered as one of the deadliest pathogen in the country's shrimp aquaculture history, the White Spot Syndrome Virus (WSSV) has a fatal mechanism of inducing 100% mortality 2-10 days post-infection resulting in millions of revenue losses. Early diagnosis is viewed as one of the options of controlling disease outbreaks; However measures for diagnosis have proven to be inaccessible and complicated for shrimp farmers. Utilizing affordable and readily available reagents, a DNA extraction protocol suitable for non-technicalpersonnel was developed producing quality templates from shrimp tissues and possibly from other invertebrates as well. As a DNA-based virus, the first step to diagnosis of WSSV is the isolation of high- quality DNA suitable for Polymerase chain reaction (PCR) or Loop-mediated isothermal amplification (LAMP). In addition to the protocol, the study utilized a locally fabricated heat block offering a more practical, convenient, and efficient way of detecting WSSV with the benefit of preservation shrimp life by using only pleopods. Through the application of the developed protocol, DNA from shrimp pleopods was successfully extracted. The DNA yield and purity was comparable with two commercially available extraction kits. The developed extraction protocol offers a costing per sample that is 3 to 5 times cheaper than the two commercial kits. LAMP was optimized for WSSV detection at 63°C and was proven to exhibit ten folds sensitivity in comparison with conventional PCR. The methodology and technology developed in this research has been optimized for screening of shrimp farms without the need for technical personnel thereby reducing risks of WSSV outbreaks and aiding local farmers.

ID333:

Bioinformatic prediction of WSSV-host protein-protein interaction

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White spot syndrome virus (WSSV) is one of the most dangerous pathogens in shrimp aquaculture. However, the molecular mechanism of how WSSV interacts with shrimp is still not very clear. In the present study, bioinformatic approaches were used to predict interactions between proteins from WSSV and shrimp. The genome data of WSSV (NC 003225.1) and the constructed transcriptome data of *F. chinensis* were used to screen potentially interacting proteins by searching in protein interaction databases, including STRING, Reactome, and DIP. Forty-four pairs of proteins were suggested to have interactions between WSSV and the shrimp. Gene ontology analysis revealed that 6 pairs of these interacting proteins were classified into "extracellular region" or "receptor complex" GO-terms. KEGG pathway analysis showed that they were involved in the "ECM-receptor interaction pathway." In the 6 pairs of interacting proteins, an envelope protein called "collagen-like protein" (WSSV-CLP) encoded by an early virus gene "*wsv001*" in WSSV interacted with 6 deduced proteins from the shrimp, including three integrin alpha (ITGA), two integrin beta (ITGB), and one syndecan (SDC). Sequence analysis on WSSV-CLP, ITGA, ITGB, and SDC revealed that they possessed the sequence features for protein-protein interactions. The data provided new insights into the interaction mechanisms between WSSV and shrimp. In order to confirm the predicted results, *SDC* gene was cloned and its function during WSSV infection was studied. *SDC* gene was ubiquitously expressed in all tested tissues, with the highest level in Oka, moderate level in haemocytes, intestine, gill, heart, nerve and stomach, and the lowest level in muscle and hepatopancreas. The transcription level of *SDC* in haemocytes was apparently up-regulated after WSSV challenge. After silencing of *SDC* gene with siRNA, the WSSV copy number in WSSV infected shrimp was much lower than that in non-silenced shrimp. These results indicated that *SDC* gene played important role during WSSV infection.

FISH IMMUNOLOGY

ORAL PRESENTATIONS

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

ID73:

A successful case of phage therapy in aquaculture practice

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Bacteriophage has stimulated various basic and applied areas of scientific studies since its discovery in the mid-1910s. Studies on "phage therapy" in aquaculture became popular since the late 1990s, with an increasing interest in recent years.However, available information is limited compared with that in agricultural and medical fields. We have studied phage therapy against *Pseudomonas plecoglossicida* infection in pond-cultured ayu (*Plecoglossus altivelis*), an amphidromous fish with a 1-year life cycle. In general, serial research steps are required to establish procedures in phage therapy: (i) isolation and characterization of lytic phages against the target bacterium, (ii) examination of phage infectivity against various prevailing strains of the bacterium, (iii) selection of therapeutic phages, (iv) evaluation of therapeutic efficacy of the phages under experimental and natural conditions, including the effective administration method (route, dose, and frequency), (v) examination for known virulence or toxin genes in the candidate phages, and (vi) consideration to phage-mediated environmental perturbation. We selected two phages (PPpW-3 and PPpW-4) as therapeutic agents, which belong to the Myoviridae (dwarf myovirus) and the Podoviridae, respectively. Full genome analysis found no deleterious genes in these phages. In a laboratory setting, oral administration of a cocktail of these phages incorporated into feed (10^8 pfu/fish) was highly protective against oral challenge of the pathogen. The administered phages quickly appeared in the circulatory system and were detected in the organs for at least 6 h. Through field testing, we found that consecutive bid administration of phages for greater than 10 days were efficacious in reducing fish mortality and completely eliminating the disease from the pond. Interestingly, no phage-resistant *P. plecoglossicida* emerged throughout the phage treatments. These results support commercial use of phages in aquaculture in the future.

ID160:

Immune responses of yellowtail kingfish (*Seriola lalandi*) to *Photobacterium damselae* subsp. *damselae*, *Vibrio anguillarum*, and *Vibrio harveyi*

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The yellowtail kingfish (*Seriola lalandi*) is a marine pelagic fish present globally in subtropical and temperate waters of the Pacific and Indian Oceans. This species can be grown in sea cages and land-based recirculating systems, and is a good candidate for aquaculture development in Western Australia because of its high growth rate and high market value. A combination of factors including the bacteria *Photobacterium damsela*, diet and environmental factors were identified as responsible for fish mortalities in several trial sea cages carried out a few years agoFew studies have investigated *S. lalandi* immunity in response to bacterial infections. Using flow-cytometry and light microscopy, the present study characterized the different cells present in the head kidney, the spleen and the blood including lymphocytes, monocytes, neutrophils, and macrophages. The impact of different bacteria strains and species was investigated by flow-cytometry to study phagocytosis, intracellular oxidative activity, cell mortality, and presence of lysosome in different cell subpopulations. This study revealed differences in cell sub-populations in terms of functions and morphology, and responses to the different bacteria species used.

ID121:

Immunogenicity of Toll-like receptor 9 inactivated vaccine against CpG oligonucleotides and alum in cobia (*Rachycentron canadum*)

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In the present study, adjuvant effects of unmethylated cytosine phosphorothioate guanine oligonucleotides (CpG-ODN) 2006, 2395, 1668 and control ODN 2137 in combination with inactivated formalin-killed bacterial vaccine and alum were investigated. We analyzed immune gene expressions (RCTLR9, MyD88 & IL-1β), humoral response in serum, and histological changes in spleen and liver, in CpG-ODN or ODN treated and vaccinated cobia challenged with live *Photobacterium damselae* subsp. *piscicida*. CpG-ODN 1668 treatment resulted in significant RCTLR9 expression in spleen (~11 folds), liver (~20 folds) and in interleukin-1β in liver (~4 folds). The highest lysozyme and peroxide content was found in CpG-ODN 1668 treated fish. Bactericidal activity as measured by bacterial percentage survival was significantly less in CpG-ODN 1668 (25%) compared to other treatments. The liver cells of treated fish showed high lipid content at 6 and 10 dpi. CpG-ODN 1668 and 2395 treated groups have an increased white pulp area in spleen at 10 dpi. Cobia immunized with CpG-ODN 1668 and 2395 treated cobia had survival rates by 3 weeks post-challenge of 90 & 70%, respectively, after live *Photobacterium damselae* subsp. *piscicida* challenge. This study demonstrated that different CpG-ODNs have different abilities to augment protection against Photobacterium, and that CpG-ODN 1668 can be a potential adjuvant candidate for cobia vaccination.

ID138:

Characterization of specific innate immune genes IκBa, Rac2 and Rab21 during persistent Cyprinid herpesvirus 2 infection in crucian carp *(Carassius auratus gibelio)*

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NF-κB inhibitor IκBa, small GTP binding protein Rac2 and Rab GTPases Rab21 play important roles in innate immunity. Suppression subtractive hybridization (SSH) assays were used to identify differentially expressed immune-related genes in crucian carp (*Carassius auratus gibelio*) infected with Cyprinid herpesvirus2 (CyHV-2). IκBa, Rac2 and Rab21 were previously identified as significantly up-regulated genes in fish that survived Cyprinid herpesvirus 2 (CyHV-2) infections. The complete open reading frames (ORF) of IκBa, Rac2 and Rab21 genes were cloned and sequenced in this study. The full-length cDNAs of the three genes contained an ORF of 933, 540 and 648bp, encoding polypeptides of 311, 170 and 216 aminoacids, respectively. Phylogenetic analysis indicated that these three genes shared high homology to other bony fish species including zebrafish. A real-time RT-PCR assay was developed to quantitatively examine their tissue distribution. IκBa, Rac2 and Rab21 were found expressed at low levels in all of the tissues examined. The expression level of IκBa, Rac2 and Rab21 were determined at different time points in kidney after infection, to monitor the response of these genes to CyHV-2 or *Aeromonas hydrophila* infections.Significant up-regulation of IκBa, Rac2 and Rab21 were observed 6h, 12h and 24h post-CyHV-2 injection ($P<0.01$), whereas significant up-regulation was observed from 6h to 72h after infection with *A. hydrophila* ($P<0.01$). This study demonstrated IκBa, Rac2 and Rab21 genes were markedly and acutely involved in host innate immune response to CyHV-2, but at sustained low levels in response to *A.hydrophila*. This suggested that CyHV-2 might induce host innate response including neutrophil function and NF-κB activation, but attenuates EGF-mediated MAPK signaling during persistent infections.

ID317:

Protective immunity against *Streptococcus agalactiae* in Tilapia (*Oreochromis niloticus niloticus* L.), using recombinant FbsA and Alpha-enolase

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Streptococcus agalactiae, a gram-positive bacterium, can infect a variety of fish species reared in both seawater and fresh water, and cause serious economic losses in aquaculture. In this study, we identified, expressed and purified two putative cell surface proteins FbsA and α-enolase from *Streptococcus agalactiae* THN strain, and determined their effects on blood leukocyte respiratory burst activity, serum lysozyme activity and specific antibody titers weekly post immunization in tilapia. The protective immunity of both proteins against *Streptococcus agalactiae* infections was studied. Our findings showed that both putative proteins shared similar structural characteristics with that of other bacterium species. Recombinant FbsA (rFbsA) and α-enolase (rEnolase) were expressed using *E. coli* BL21 system, and purified. Post immunization with rFbsA or rEnolase in tilapia, the respiratory burst activity and lysozyme activity were significantly enhanced when compared with PBS control groups. The serum specific antibody titers against rFbsA or rEnolase was increased, with rFbsA inducing higher antibody titers. Both recombinant proteins were found to confer protection against *Streptococcus agalactiae*, with protection rates of 47.2% and 66.7% for rFbsA or rEnolase, respectively.*Streptococcus agalactiae* vaccine, adjuvant and PBS control groups produced protection rates of 94.4%, 25.0% and 11.1%,respectively. This study showed that FbsA and α-enolase regulate the host innate immune responses as well as specific antibody responses, and give protective immunity against *Streptococcus agalactiae*, although protection rates conferred by each protein was different. It also supports FbsA and α-enolase as potential vaccine candidates. to

ID282:

Effects of high-concentration ascorbic acid supplementation on disease resistance and innate immune responses in rainbow trout

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The rainbow trout (*Oncorhynchus mykiss*) is a cold water fish native to North America from Alaska to Mexico, and is one of the most suitable fish for cultivation in cold waters worldwide. It was introduced to Japan from the USA in 1877, and now accounts for 20% of freshwater aquaculture products in Japan. However, since some viral and bacterial diseases are causing serious problems in trout cultures, many producers are searching for optimal disease control methods to minimize the potential risks of disease. The use of high-concentration ascorbic acid (AsA) as a dietary immunostimulant may be one approach for fish culturists to reduce losses due to disease in their facilities. AsA is an essential vitamin for the normal growth and physiological function of fish, and some studies indicate that a diet supplemented with high-concentration AsA of at least 1000 mg/kg diet protects rainbow trout from several stresses. We demonstrated that administration of AsA at 5,000 mg/kg diet significantly improved survival of juvenile rainbow trout from infectious hematopoietic necrosis (IHN). The present study was therefore designed to investigate the efficacy of high-concentration AsA supplementation against viral or bacterial pathogens that cause serious disease in rainbow trout farms in Japan. Non-specific immune parameters, including lysozyme activity, phagocytic activity, and mitogen response, were examined. Fish were fed commercial diets supplemented with 5,000 mg/kg of AsA per kg of diet for 7 days, and then challenged by bath exposure with either hematopoietic necrosis virus (IHNV), Oncorhynchus masou virus (OMV), *Streptococcus iniae*, or *Vibrio ordalii*.

Mortality decreased significantly in IHNV, *S. iniae* and *V. ordalii* challenged fish. All non-specific immune parameters were significantly higher in fish fed high-concentration AsA compared to those fed a diet without AsA. In contrast, AsA supplementation in the diet did not significantly reduce the mortality of rainbow trout as a result of infection with OMV. These results indicate that although high-concentration AsA supplementation may be ineffective against some salmonid pathogens, this administration enhances resistance against many diseases in rainbow trout and is effective in reducing losses in trout culture due to disease.

ID163:

Alpha Ject Panga 1-The first vaccine against *Edwardsiella ictaluri* in striped catfish (*Pangasianodon hypophthalmus*) in Vietnam

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Five pairs of earthen ponds were included in a large scale study conducted on four different commercial fish farming locations in Dong Thap and Ben Tre provinces in the Mekong Delta. 1,800,000 striped catfish, (*Pangasianodon hypophthalmus*) juveniles weighing 10 to 30g, were vaccinated intraperitoneally with ALPHA JECT® Panga 1 (0.05 ml/fish) and stocked in five ponds. For reference, comparable ponds with non-vaccinated fish were included in the study. The size of the ponds ranged from 0.3 - 1.5 ha and depths from 3.5 - 5 m, with stocking densities ranging from 20 - 80 fish/m^2. In vaccinated and unvaccinated ponds, mortality, disease diagnosed, weight gain, FCR and any treatment during the entire production cycle were recorded and analyzed. These results will be presented and discussed.

ALPHA JECT® Panga 1 is the first fish vaccine licensed for aquaculture in Vietnam. The vaccine is an effective tool in reducing losses due to *E. ictaluri* and will contribute to improved fish health and environment, as well as sustainability of the industry in the future.

ID 417:

Functional recombinant type I interferon of medaka (*Oryzias latipes*), produced using mammalian cell line

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Type I interferon (IFN1) is one of most important cytokines for responsible for antiviral responses in fish innate immunity, after the induction pathway following pattern recognition.

The purpose of this study is to identify the biological properties of recombinant IFN1 (rIFN1) in medaka (*Oryzias latipes*). The mature peptide sequence of medaka IFN1 cDNA was cloned into the pFUSE-mIgG2a vector, modified with mouse IL-2 signal sequence (coding secretion signal) and C-terminal histidine-tag (His-tag). To optimally produce post-translational modified (glycosylation and disulfide bond) recombinant proteins, the construct was transfected into HEK293T cells. The recombinant IFN1 fusion His-tag was purified by two column chromatographic steps of nickel-chelating sepharose and gel filtration. To demonstrate the biological activity, hematopoietic cells derived from whole kidney were cultured with recombinant medaka IFN1.The expression of IFN-stimulated genes in cultured cells was analyzed by quantitative real-time PCR.

Approximately 20 μg of the purified protein was recovered from 10 ml of culture supernatant. The molecular size of recombinant medaka IFN1 was determined as approximately 26 kDa by Western blot analysis, and larger than predicted molecular size. It is possible that the higher molecular size was caused by two consensus sequences for the *N*-glycosylation sites, based on the medaka IFN1 amino acid sequence. We report the expression level of IFN-stimulated genes in renal hematopoietic cells after rIFN1 treatment.

ID 328

Immune response in carps to nanoparticle based delivery of recombinant outer membrane protein of *Aeromonas hydrophila*

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Aeromonas hydrophila is primarily responsible for causing diseases in fresh water fish that often hamper the aquaculture industry causing huge economic losses. Disease prevention by the use of bacterial outer membrane proteins (OMPs) reported to be very effective to control the disease problems and considered as potential vaccine candidates. In this study, the efficacy of Poly D, L-lactide-co-glycolic acid (PLGA) as a carrier of recombinant outer membrane protein W (rOmpW) of *A. hydrophila* in freshwater fish *Labeo rohita* was tested. The expression of rOmpW was carried out in *E. coli* M15 clone was estimated to be approximately 22 kDa by 15% SDS-PAGE. Purity of recombinant protein was about 90% and protein concentration of rOmpW was around 1.2 mg/ml. These antigens were efficiently loaded in the PLGA nanoparticles, reaching encapsulation efficiency of 53.56%. Particle size analyser revealed that the formulated nanoparticles had an average diameter of 370-375 nm. The charges of antigen loaded nanoparticle were -19.3 mV. *In-vitro* release test showed high initial release during the first eight hours was 15%. The total release of antigen rOmpW was 35% in 48 hrs. Feed was prepared mixing with PLGA-rOmpW protein using an extruder and fishes were vaccinated orally. The vaccine carrying feed was fed to fish for 21 days. After 30 days of post vaccination, fish were challenged with *A. hydrophila* and relative percentage survival (RPS) was calculated as 53%. Challenge studies revealed that PLGA nanoparticle loaded with rOmpW was highly effective in eliciting immune response affording protection. Thus, the approach could be followed for vaccination to protect fish against *A. hydrophila* in aquaculture sector.

FISH IMMUNOLOGY POSTER PRESENTATIONS

ID400:

Generation and evaluation of virulence attenuated mutants of *Edwardsiella tarda* as vaccine candidates to combat edwardsiellosis in flounder *(Paralichthys olivaceus)*

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Edwardsiella tarda is an intracellular pathogen that causes edwardsiellosis in fish. The development of a live attenuated vaccine may be an effective approach for preventing this disease in fish. In this study, we introduced deletions of *esrB, esaC, evpH, rpoS, and purA into the E. tarda LSE40ΔaroA strain, thereby generating five double-gene mutants (ΔaroAΔesrB, ΔaroAΔesaC, ΔaroAΔrpoS, ΔaroAΔevpH, and ΔaroAΔpurA,)* and two triple-gene mutants *(ΔaroAΔesrBΔevpH and ΔaroAΔesaCΔevpH)*. When blue gourami *(Trichogaster trichopterus)* was used as a fish model for the primary screening and evaluation of the vaccine candidates, all mutants were attenuated significantly by more than 2 to 3 logs at 50% lethal dose (LD50). Five double-gene mutants yielded relative percentage survival (RPS) rates of 26.1–82.6% after challenge with wild-type *E. tarda*. The *ΔaroAΔesrB* mutant that conferred the highest RPS (82.6%) in blue gourami was evaluated in flounder *(Paralichthys olivaceus)*. After vaccination via intramuscular (i.m.) injection or immersion, this mutant persisted in the flounder for 14–35 days and induced higher serum antibody titers than in the control fish ($P < 0.01$). Flounder vaccinated via i.m. injection at doses of 103–107 CFU/fish had RPS rates of 14.3–66.7% after i.m. challenge with 104 CFU/fish, using wild-type *E. tarda*. Flounder vaccinated via immersion at a dose of 107 CFU/mL exhibited 100% RPS against immersion challenge with 107 CFU/mL using wild-type *E. tarda*. These results indicate that the *ΔaroAΔesrB* mutant could be used as an effective live vaccine to combat edwardsiellosis in flounder.

ID71:

Vaccine development for atypical *Edwardsiella tarda* infections in Red Sea bream (*Pagrus major*)

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Atypical *Edwardsiella tarda,* a non-motile phenotype, is the causative agent of edwardsiellosis in cultured Red Sea bream (*Pagrus major*). The disease predominantly occurs in summer and fall seasons in Japan, causing severe losses in aquaculture. We investigated the efficacy of a formalin-inactivated atypical *E. tarda* vaccine in Red Sea bream, as a means to control the disease. LD_{50} of the *E. tarda* strain used for vaccine preparation in Red Sea bream juveniles by intraperitoneal (IP) injection was approximately 10^6 cfu/fish. A single IP-injection of the vaccine (10^8 cells/fish) to Red Sea bream juveniles induced high antibody production at 3 weeks post-vaccination. High levels of protection was achieved by the vaccination, with RPS values greater than 60% when fish were IP challenged with the homologous strain. Reproducibility of the results was confirmed by 3 independent experiments. The protection conferred by the vaccine lasted at least up to 4 months post vaccination. This vaccine induced no apparent abnormalities in fish, with good growth recorded at 10-times dose rates. During challenge with atypical *E. tarda*, the numbers of the bacteria in blood, spleen and liver were lower in vaccinated fishas compared to control fish. Studies using normal and immune sera of Red Sea bream revealed that atypical *E. tarda* strains were highly resistant to the serum complement-killing involving both classical and alternative pathways. Macrophage phagocytic and killing activities against atypical *E. tarda* were not significantly different between vaccinated and unvaccinated fish. This study demonstrates the effectiveness of an inactivated vaccine against Red Sea bream edwardsiellosis, although clearance mechanism for *E. tarda* in vaccinated fish remains unknown.

ID175:

Investigations into the role of serine protease substrate in cell-mediated immunity in fish

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Due to crowding in aquaculture settings, infectious diseases are causing serious economic losses in the aquaculture industry. There is an urgent need for development of effective vaccines to control diseases. In order to evaluate vaccine potency, more effective methods other than challenge tests are requiredElevation of antibody in immunized fish is not observed for most fish vaccines, and challenge tests need many fish and involve considerable labor. We have demonstrated that cytotoxicity of CD8a+ T cells correlates well with the Granzyme B-like activity in a model fish, ginbuna crucian carp. Therefore, serine protease activity assay is useful to assess cytotoxic activity or cell-mediated immunity of aquacultured fish in which genetic information on granzymes, T cell markers or antibodies against T cell subsets are not available. In this study, we report the candidate substrate of serine protease involved in the cytotoxicity induced by allo-sensitization.

To find the candidate substrates, enzyme activity of serine protease in lymphocyte lysate from allo-sensitized fish was measured using six types of substrates. Protease activity was enhanced by allo-sensitization when Ac-IETD-MCA, Bz-R-MCA, Z-GPR-MCA, K-MCA, and Suc-AAPF-MCA were used as substrates, while the activity was not increased in the case of M-MCA. To classify the group of protease involved in cytotoxicity, a protease inhibitor was added to enzyme assays together with the substrates, which have been shown to be enhanced by allo-sensitization. The inhibitor of serine protease "AEBSF" significantly inhibited enzyme activity only when Z-GPR-MCA was used as a substrate, and there was no inhibition with cysteine protease inhibitor "E-64".

These results suggest that serine protease which cleaves Z-GPR-MCA is involved in cytotoxicity. We are in the process of establishing a method for assessment of vaccine efficacy using Z-GPR-MCA substrate for enzyme assay of lymphocyte.

ID242:

Identification of immune-related genes in leukocyte subpopulations in ayu (*Plecoglossus altivelis altivelis*)

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Ayu (*Plecoglossus altivelis*) is one of the most economically important fish in freshwater aquaculture in Japan. However, there is little genetic information available on this fish species. This has hampered understanding of immune system of ayu. In the present study, expressed sequence tag analyses of three leukocyte subpopulations, B cells, neutrophils and thrombocytes of ayu were performed using a next generation sequencer. The B cells, neutrophils and thrombocytes were isolated from peripheral blood leukocytes by use of monoclonal antibody conjugated to magnetic microspheres, and the purity evaluated by flow cytometry. The cDNA libraries were normalized with duplex-specific nuclease and sequenced by Ion Torrent PGM sequencer. The sequencing of B cell, neutrophil and thrombocyte cDNA library produced 5,657,811, 5,720,737 and 6,452,590 reads, respectively. De novo Assembly yielded 16,505, 22,733 and 22,494 contigs from B cell, neutrophil and thrombocyte cDNA library, respectively. Blast analysis directed to non-redundant protein database annotated 41.0 %, 39.9 % and 45.0 % of the sequences in the B cell, neutrophil and thrombocyte cDNA library, respectively. GO analysis revealed that 291, 342 and 328 sequences from B cell, neutrophil and thrombocyte cDNA library were assigned to immune system process (GO:0002376) at level 2 in the biological process. Pathways related to phagosome (ko04145), endocytosis (ko04144) and lysosome (ko04142) were found in all of the cDNA libraries by KEGG Automatic Annotated Server analysis. The B cell library owned 1,590 unique sequences including CC chemokine 19, CC chemokine with stalk CK2 and B cell linker protein. The neutrophil cDNA library owned 3,056 unique sequences including interleukin (IL)-1β, IL-1β receptor, IL-8 and IL-8 receptor A. The thrombocyte cDNA library owned 2,894 unique sequences including CC chemokine CK3, IL-31 receptor A and Toll-like receptor 8-1. In this way, a great number of immune related genes were identified from the three leukocyte cDNA libraries. This study provides valuable sequence resources for future studies on the immune system of ayu.

ID280:

Host responses to *Aphanomyces invadans*: a comparison between resistant and susceptible fish

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The oomycete *Aphanomyces invadans*, causative agent of epizootic ulcerative syndrome (EUS) is a pathogen of international significance. To date more than 100 species of fish have been reported to be naturally infected with *A. invadans* and the host range is expanding. In spite of the current and potential impacts of *A. invadans* infection on fisheries and aquaculture sectors of the world, very little is known about host- *A. invadans* interactions. In the present study, following experimental infection with *A. invadans* in one EUS resistant fish, common carp (*Cyprinus carpio*), sequential changes in various innate immune parameters were monitored and compared with one EUS susceptible fish, Indian major carp (*Labeo rohita*). The results indicated that in infected common carps, the innate immune parameters i.e. respiratory burst, myeloperoxidase, antiprotase, and α-2 macroglobulin activities were not significantly different from that in the control; but the lysozyme and alternate complement activities were higher. On the other hand, in the susceptible *L. rohita*, particularly at advanced stages of infections, the lysozyme and anti-protease activities were significantly lower in infected *L. rohita*. Expression analysis of the immune-regulatory genes indicated significant up-regulation in of some genes like complement C3, and CxCa in susceptible fish. The results obtained in this study may help strengthen the understanding of the mechanisms of host immune responses to *A. invadans*.

ID316:

Identification of vaccine candidate proteins from *Cryptocaryon irritans* by proteomics analysis

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Cryptocaryoniasis is a severe disease of cultured marine fish caused by a parasitic ciliate *Cryptocaryon irritans,* with considerable economic losses. Immunoprophylaxis is considered a promising control method for this disease.However, suitable protein antigens for vaccine development have not been identified. This study aims to screen for candidate proteins from *C. irritans* for vaccine development, by differential proteomics and immunoproteomics. A total of 53 protein spots in differential abundance based on pair-wise comparison among theronts, trophonts and tomonts were studied, and identified as 12 distinct proteins. In addition, 33 and 27 protein spots were determined to comprise of 9 and 11 distinct proteins, using rabbit and grouper anti-sera, respectively. Among these identified proteins, actin, α-tubulin, β-tubulin, polypyrimidine tract-binding protein, NADH-ubiquinone oxidoreductase 75 kDa subunit, glutamine synthetase, enolase, protein kinase domain containing protein, malate dehydrogenase, TNFR/NGFR cysteine-rich region family protein and vacuolar ATP synthase catalytic subunit α in infective theronts were immunoreactive with rabbit and grouper anti-sera. Parasitic trophonts, actin, heat shock protein 70, mitochondrial-type hsp70 and vacuolar ATP synthase catalytic subunit α were immunoreactive only with rabbit anti-sera. In addition to finding increasing abundance of these proteins in theronts and trophonts, actin, α-tubulin, β-tubulin, enolase and vacuolar ATP synthase catalytic subunit α were detected in infective theronts and parasitic trophont, using rabbit and grouper anti-sera. Using rabbit and grouper anti-sera, β-tubulin appeared to occur in all three developmental stages. Therefore, five proteins were identified in this study (actin, α-tubulin, β-tubulin, enolase and vacuolar ATP synthase catalytic subunit α) as promising candidate proteins from *C. irritans* for vaccine development, based on their presence in multiple stages of this parasite.

ID171:

Functional vaccine against streptococcosis in Nile tilapia (*Oreochromis niloticus Linn.*) based on the serotypes of *Streptococcus agalactiae* in Thailand

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The culture of Nile tilapia (*Oreochromis niloticus*) has reached the highest ranking in freshwater aquaculture in Thailand in the past decade. Annual production reached 300,000 metric tons, with a steady increase due to domestic demand and export. Most farmers employ intensive culture systems, which opens the possibilities for many etiological agents to cause diseases. Thus, it is imperative for fish health researchers to develop effective and practical approaches for disease prevention. Streptococcosis is the most common bacterial disease of tilapia culture in Thailand. Our laboratory has identified 120 isolates of *Streptococcus agalactiae*using 16S rRNA PCR, from tilapia grow-out farms in the central, north, south and northeastern parts of Thailand. Multiplex-PCR has been developed to identify 14 virulence genes and *cps* cluster genes. We have successfully established the main serotypes of *S. agalactiae* as serotype Ia and III, the first reports in Thailand. A study on pathogenicity of these two serotypes showed that serotype III is more virulent than serotype Ia. These two serotypes are used as candidate antigens for the development of functional vaccines.

This project on *S. agalactiae* vaccine development for tilapia culture in Thailand has been supported by SATREPS program, National Research Council of Thailand and Kasetsart University. Studies on vaccine preparation indicated that formalin-killed (FK) vaccine yielded the highest antibody titers when compared with FK + Extracellular products (ECP) and lysed-cell vaccine. However, the protection against virulent *S. agalactiae* by these three vaccines were not significantly different ($P > 0.05$) but significantly different from unvaccinated fish ($P < 0.05$). Response of tilapia to vaccination by injection prepared from these two serotypes indicated good primary and secondary immune response. Vaccinated fish developed significant protection against the respective antigen, and certain extent of cross protection was detected. The efficacy of the single and combined antigen-vaccine and bath vaccine in tilapia fry are being investigated. Development of subunit vaccine is also under investigated. The goal of this project is to establish the vaccination program at two stages of tilapia culture: grow-out stage from fingerling to marketable size and if applicable, the nursery stage from fry to fingerling.

ID117:

Molecular characterization and antigenicity of outer membrane protein (OMP) of *Vibrio alginolyticus,* isolated from diseased tiger grouper (*Epinephelus fuscoguttatus)*

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Vibriosis, caused by bacteria belonging to the genus *Vibrio* has been recognized as one of the most destructive diseases in the aquaculture industry. *Vibrio alginolyticus*, a Gram-negative bacterium, is considered an important pathogen in humans and marine animals such as shrimp, shellfish and marine fish, which leads to serious economic losses. The outer membrane proteins (OMPs) of bacteria play an important role in interaction between bacterium and host, during infection and induction of host immune response. Based on previous studies, conserved OmpK and OmpW regions are good vaccine candidates against *V. alginolyticus*. In this study, a total of eight bacterial strains isolated from diseased tiger grouper were collected from the National Fish Health Research Centre (NaFisH) in Penang, Malaysia. These bacterial strains were identified by polymerase chain reaction (PCR) using four different genes: 16S rRNA, internal transcribed spacer (ITS), OmpK and OmpW. OmpK and OmpW genes PCR targeted conserved regions of *Vibrio* species. PCR using these four distinct genes enabled these eight bacterial strains to be successfully differentiated as *Vibrio* species. Protein profiling of OMPs using SDS-PAGE revealed major and minor protein bands in the strain. Western blot analysis showed ~37 kDa protein in the strain is antigenic, and able to elicit an immune response towards *V. alginolyticus*. This antigenic protein will be further studied as a vaccine candidate for tiger grouper.

ID323:

Identification and expression analysis of two important molecules in TLR signal pathway: IRAK4 and TRAF6

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TLR signal pathway plays a crucial role in host innate immune responses. *Cryptocaryon irritans* is a pathogenic ectoparasite ciliate that infects salt-water fish causing heavy economic losses. However, the role of TLR signal pathway in host defense against C. irritans infection is poorly understood. In this paper, we firstly cloned two important signal transduction molecules of IRAK4 (EcIRAK4) and TRAF6 (EcTRAF6) from grouper (Epinephelus coioides). Full-length of IRAK4 was 1859 bp containing an ORF of 1395 bp encoding a putative polypeptide of 464 amino acid residues. EcIRAK4 has a conserved death domain (DD) and a serine/threonine/ tyrosine protein kinase domain (STYKc). TRAF6 has an ORF of 1713 bp encoding 570 amino acids. The putative amino acids possess the characteristic motifs of other TRAF6, including a ring domain, two zinc fingers, a coiled-coil region, and a MATH domain. Phylogenetic analysis and multiple alignments indicated that EcIRAK4 and EcTRAF6 was respectively clustered into fish group and shared a 62-83% and 65-86% sequence identity with that of other fish species. Both genes were constitutively expressed in all tested tissues, with the highest expression level in the head kidney and blood, respectively. Post infection with C. irritans, we detected both genes' expression in the local infection sites and system immune organs. Results demonstrated the both genes were significantly up-regulated in the skin, but slightly fluctuated in other three tissues at most time points, implying that parasite infection could regulate TLR signal pathway responses in local attack site.

ID315:

Resistance genes to antimicrobial drugs in tilapia pathogen, *Streptococcus agalactiae*

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Information on antimicrobial resistance genes and horizontal gene transfer in pathogenic fish bacteria is limited in Thailand. In this study, 70 isolates of Gram-positive bacteria *Streptococcus agalactiae* were isolated from diseased tilapia (*Oreochromis niloticus*). Bacterial isolates were tested for antimicrobial drug sensitivity against tetracycline, enrofloxacin and sulfametrozaxole-trimethoprim, which are currently licensed for use in fish culture in Thailand. 43 Bacteria isolates which exhibited resistant characteristics to these 3 drugs were employed for DNA extraction. Resistance genes to antimicrobial drugs were examined using PCR technique. PCR showed that tetracycline resistance genes namely tet (M), int and xis occurred at percentages of 13.95, 11.62 and 9.30, respectively. Gyr A and Par C which are enrofloxacin resistance genes were found in all 43 isolates (100%). Resistance gene for sulfametrozaxole-trimethoprim was not detected in this study. PCR products from five antimicrobial resistance genes were purified and used for DNA sequencing. Nucleotide sequencing and BLASTX results confirmed these genes as genuine resistance genes. The authors also investigated the transfer of resistance genes from *S. agalactiae* to *Escherichia coli* and *Aeromonas hydrophila*. The results from our study showed the occurrence of horizontal gene transfers between *S. agalactiae* and other bacteria. This is a warning for introduction and spread of antimicrobial resistance genes in aquaculture. Responsible use of antimicrobial drugs in aquaculture will act as a safeguard to minimize the spread of antimicrobial resistance genes .

ID135:

Effects of *Astragalus membranaceus* and Rooibos (Aspalathus *linearis*) herbal extracts on non-specific immune response and disease resistance in barramundi (*Lates calcarifer*)

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Barramundi or Asian sea bass (*Lates calcarifer*) is a promising new fish species for intensive aquaculture worldwide. However, fish reared in intensive conditions are more susceptible to infectious diseases. Detrimental effects of intensive rearing conditions can be reduced by using immunostimulants, compounds that can enhance the innate (non-specific) immune response. Recently, herbal extracts have been investigated by many researchers as potential immunostimulants. To test the effects of herbal immunostimulants on innate immune response of barramundi, an eight-week feeding trial was carried out using three different herbal extracts. Fish (average weight 84g) were allocated into three experimental groups, each with 60 fish. Fish were fed diets containing 0.5% *Astragalus membranaceus* extract or 0.5% Rooibos (*Aspalathus linearis*) green (non-fermented) extract. Control group was fed basal diets without herb supplements. Blood samples were taken from five fish from each group, on the first, second, third and eighth week of trial to determine non-specific immune response parameters . Following eight weeks of feeding trial, fish were challenged with *Aeromonas hydrophila* and cumulative mortalities registered. Both herbal extracts enhanced the innate immune response and disease resistance. Rooibos extract was more effective, reduced mortality after challenge by 40% and significantly enhanced most non-specific immune parameters examined. *Astragalus* extract reduced mortality by 15% and significantly enhanced the phagocytic activity of leukocytes. Our results showed that both herbal extracts enhanced the innate immune response and disease resistance, and can be used as immunostimulants for intensive rearing of barramundi.

ID294:

Effects of *Moringa oleifera* on non-specific immune response and disease resistance against *Streptococcus agalactiae* Biotype 2 in Nile tilapia (*Oreochromis niloticus*) Chitralada strain

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The aim of this study is to determine the dietary effects of *Moringa oleifera* on non-specific immune response and disease resistance against *Streptococcus agalactiae* Biotype 2 in Nile tilapia (*Oreochromis niloticus*) Chitralada strain. In this experiment, fifteen aquaria (150 L) were stocked with 30 fish (32g) each and fed (5% body weight twice a day) with five diets containing 0%, 1.5%, 2%, 2.5%, and 3% of *Moringa* leaf extract (MLE) for 48 days. To evaluate the immune response and resistance to *S. agalactiae* Biotype 2 infections, immunological parameters were investigated at 0, 16 and 32 days of feeding MLE, and again after 14 days post-challenge. The highest serum bactericidal activity (SBA) count before challenge experiments (after 32 days of feeding MLE) was observed in fish fed 2.5 % MLE, at 8.21±0.35 cfu/ml (log). SBA count increased in all groups except 2.5 % MLE after 48 days of feeding (14 days post challenge). Serum lysozyme activity (SLA) count in fish fed 3% MLE was significantly different from all the other groups after 16 & 32 days of feeding. SLA counts of fish fed 2.5 & 3% MLE significantly differed from the other groups after 48 days of feeding. Typical clinical signs such as hemorrhage (on pectoral fin and the body), distended abdomen due to ascites, corneal opacity and bilateral exophthalmia were observed in this study. MLE treatment increased the relative percentage survival (RPS) in tilapia challenged with *S. agalactiae,* to 80% at 2.5% MLE in diet (1 x 10^7cfu/ml), 86.66 % at 2 % (1 x 10^8cfu/ml) MLE and 100% in fish fed 1.5% & 3 % MLE , as compared with untreated control groups. In conclusion, a dietary MLE of 1% provides the best survival rate for tilapia (*O. niloticus*) when challenged with *S. agalactiae* Biotype 2.

ID211:

Hyperosmotic infiltration in vaccination of Atlantic salmon (*Salmo salar*) against *Yersinia ruckeri*

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Yersinia ruckeri is a pathogen which causes yersiniosis and significant losses in farmed Atlantic salmon (*Salmo salar*) in the Southern Hemisphere. Immersion vaccination employs a natural route of antigen entry and is an effective method of vaccine administration in fish. Most common ways of immersion vaccination used are direct immersion, hyperosmotic infiltration and spray. Hyperosmotic infiltration that immerses the fish in a hypertonic solution such as sodium chloride or urea for a short period of time followed by immersion in the vaccine, has been shown to increase the vaccine uptake and enhance the efficacy of vaccines. This study has been undertaken to compare hyperosmotic infiltration (4.5% NaCl) with direct immersion in Atlantic salmon (*Salmo salar*) by using an ammonium sulphate inactivated whole-cell vaccine *Y. ruckeri* to vaccinate Atlantic salmon. The present study clearly demonstrates that hyperosmotic infiltration can improve protection afforded by an immersion vaccine against *Y. ruckeri* for Atlantic salmon.

ID100:

Immunostimulatory effects of herbal bioconditioners in tiger grouper (*Epinephelus fuscoguttatus*) against *Vibrio parahaemolyticus* infections

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In the present study, protective effects of herbal-based conditioners as immunostimulants were trialed in juvenile tiger grouper (*Epinephelus fuscoguttatus*) at various stages of their culture period, and their resistance against bacterial infections tested. The trial used a single formulation of herbal-based bioconditioners with scheduled water changes during the treatment. Three fish groups subjected to herbal-based bioconditioner exposure for 6 h, 12 h and 24 h, respectively and a control group were trialled using a randomized experimental design. All groups were subjected to a challenge test using a pathogenic bacteria, *Vibrio parahaemolyticus* at concentration of 10^5 cells ml^{-1}. Percentage survival and host-pathogen interactions were determined at the end of exposure and challenge test. Our challenge tests showed that herbal-based bioconditioners (AquaHerb) significantly increased the percentage survival ($P<0.05$) of tiger grouper and improved their resistance against *V. parahaemolyticus* infections. Statistically, percentage of leukocytes, monocytes, lymphocyte and neutrophils treated with 24 h of AquaHerb immersion were higher than 6 h or 12 h immersions, and control group ($P<0.05$). In addition, tiger grouper immune functions in treated groups was found to be better than in the control group. From the positives impact of herbal-based bioconditioners observed in this study, this prophylactic approach can be an effective alternative to the use of antibiotics and other chemicals.

ID130:

The influence of dietary fungal-derived β-glucan on immune function and immune gene expression in *Pangasianodon hypophthalmus* challenged with *Edwardsiella ictaluri*

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Striped catfish (*Pangasianodon hypophthalmus*) is an important fish species cultured in Southeast Asia, including Vietnam. Rapid expansion and intensification in the culture of this species has resulted in increased disease problems, especially with *Edwardsiella ictaluri*. Immunostimulants are food additives used by the aquaculture industry to enhance the immune response of fish; β-glucans are now commonly used for this purpose. The aim of this study is to determine the effects of dietary fungal derived β-glucan on immune function, immune gene expression and ultimately, resistance to *E. ictaluri* in *P. hypophthalmus*.

Two trials were performed. In the first trial *P. hypophthalmus* were fed for 28 days with 0.0%, 0.05%, 0.1% or 0.2% fungal-derived or 0.1% commercial yeast- derived β-glucan. The fish were then challenged with *E. ictaluri* by immersion for 1 h (8 x10^4CFU/mL), and the percentage cumulative mortalities (PCM) recorded at 14 days post infection (d.p.i.). Immune function was measured prior to, and 14 days post- infection (dpi). A number of statistically significant differences were found in the blood parameters (haematocrit and white blood cell counts) and immune responses (complement, head kidney phagocytic function and respiratory burst, total plasma protein, total IgM) as a result of immunostimulation and/or the *E. ictaluri* infection.

In the second trial fish were fed 0% or 0.1% fungal derived β-glucan or 0.1% commercial yeast derived β-glucan for 14 days before challenge with *E. ictaluri* by immersion (1 x10^6 CFU/mL) for 30 min. At 1 d.p.i, the expression of immune genes in liver, kidney and spleen were analysed by qPCR. Significant differences were found in the level of expression of precerebellin-like protein, transferrin and C-reactive protein in liver, 2a MHC class II in liver and spleen, complement factor B and interleukin-1β in kidney between infected and uninfected fish. Transferrin was the only gene affected by diet, being reduced by β-glucan feeding. Significant differences were also found in the PCM between fish fed the basal control diet (30% ±12%) and the two immunostimulated groups [0.1% fungal-derived β-glucan (17% ±8%) and commercial yeast-derived β-glucan (16% ±5%)] at 14 d.p.i.

ID134:

Effects of different feeds in common carp (*Cyprinus carpio*) broodstocks on fatty acid content of larvae and stress resistance in fry

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Vegetable oil is a promising alternative source of essential poly-unsaturated fatty acids for fish feeds, and a possible replacement for fish meal and fish oil. Within the framework of the EU-funded ARRAINA project, the effects of vegetable oil was investigated on the fatty acid content of common carp larvae and stress response of fry, through feeding of broodstock.

Pond reared male and female common carp broodstock were placed into a flow-through fish rearing system. Segregated experimental groups were fed on feeds supplemented with fish oil (FO group), vegetable oil (VO group) or wheat (control group).Fish were spawned after five months of feeding these supplements. Larvae were stocked into earthen pondsand kept on natural feeds. Poly-unsaturated fatty acid levels (ARA, EPA, DHA) were measured in larvae 30d after stocking into ponds. ARA levels were significantly higher ($p<0.05$) in progeny of FO and VO groups compared to the progeny of control group.

Fry from each treatment group in triplicates of 10 fry each, were placed into water with low oxygen content (0.3 mg/l), and the number of distressed fish recorded for two hours. Progeny of VO and FO groups tolerated hypoxia much better than control group. Mucus samples were taken from fish after 30, 60 and 120 minutes into hypoxia trial, to measure stress response. Lysozyme, total protein and imunoglobulin levels were not significantly different among the groups. Cortisol levels in progeny of VO group were significantly decreased.

Feeding broodstock with vegetable oil increased the level of ARA in progeny in our study. Tolerance of fry against hypoxia significantly increased in the progeny of FO and VO groups. Moreover, characteristic stress response could not be detected in progeny of VO group.

ID205:

Investigation of in-shore hagfish adaptive immune response, focused on immune related gene and variable lymphocyte receptors (VLRS)

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Inshore hagfish (*Eptatretus burger*), a jawless fish, is known to have variable lymphocyte receptors (VLRs) namely VLR-A, -B and -C. Among these receptors, VLR-B shares common characteristics with mammalian B cell receptors or immunoglobulins. In mammals, immune stimulation triggers changes in B cells to plasma cells and hence induce the secretion of immunoglobulins. Several genes are known to regulate lymphoblast transformation, proliferation and differentiation into plasmacytes. Some of these regulator genes are Ikaros, CD45 and Spi. In this study, we investigated the expression of the regulator genes (Ikaros, CD45 and Spi) in both short and long term periods after immunization with low-pathogenic avian influenza virus (H9N2) hemagglutinin (HA). We also examined the gene expression levels of VLR-A, -B and –C, and evaluated the VLR-B expression level using ELISA. Results showed that the gene expression level of Ikaros was up-regulated in the short term period but was down-regulated in the long term period. In the case of CD45, there was an up-regulation of the gene only in the long term period. In the case of Spi, there was no significant gene upregulation observed in both short and long term periods. The gene expression level of all three VLRs showed up-regulation patterns in short term period. In long term period, VLR-A and VLR-C showed similar gene expression levels with day 0, while VLR-B showed a consistent up-regulation pattern in the long term period. In ELISA test, high-titer anti-HA VLR-B responses were observed in plasma from the immunized hagfishes, which provide evidence of adaptive immune responses of hagfish against soluble proteins. Hence, these data suggest that VLR-A and VLR-C, which are T-lymphocyte-like cells in mammals, are involved in the initial stage of the immune response in hagfish. On the other hand, VLR-B, which is known as a B-lymphocyte-like cell in mammals, is involved in the latter stage of immune response. These observations bears a high similarity with the adaptive immune response observed in the mammalian immune system.

ID186:

Identification of cytokine homologues in giant grouper (*Epinephelus lanceolatus*) and tiger grouper (*Mycteroperca tigris*) using next generation sequencing

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Groupers are among the most attractive aquaculture species in East and South-East Asian countries because of their high economic value. In Thailand, much research have been conducted to improve the aquaculture techniques of the giant grouper (*Epinephelus lanceolatus*) and the tiger grouper (*Mycteroperca tigris*). The control of infectious diseases is important in aquaculture development, and vaccinations are promising measures for controlling diseases. Vaccination relies on the immune systems of the host animal, and cytokines which are key regulators of immune systems of vertebrates play important roles in vaccine efficacy. Recent advances in fish immunology revealed that fish cytokines are useful not only as markers to check immunological conditions after vaccination, but also as an adjuvant for vaccines. We have performed transcriptome analyses using NGS, and identified a number of cytokine homologues in these two grouper species,. These include some chemokines, and inflammatory cytokines such as IL-1β, IL-6, IL-10, TNF and IFNγ. In addition, some cytokines involved in T-cell development such as IL-12 were identified. We are now comparing their structures with those previously identified in other fish species, and characterizing their features. This research was supported by JST/JICA, SATREPS.

ID 141

Phosphoglycerate kinase enhanced immunity of the whole cell of *Streptococcus agalactiae* in tilapia, *Oreochromis niloticus*

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Streptococcus agalactiae is a Gram-positive bacterium and a severe aquaculture pathogen that can infect a wide range of warmwater fish species. The outer surface proteins in bacterial pathogens play an important role in pathogenesis. We evaluated the immunogenicity of two of the identified surface proteins namely phosphoglycerate kinase (PGK) and ornithine carbamoyl-transferase (OCT). PGK and OCT were over-expressed and purified from *Escherichia coli* and used as the subunit vaccines in tilapia. Tilipia immunized with the *S. agalactiae* modified bacteria vaccine (whole cell preparations with recombinant PGK and OCT proteins) individually were tested for the efficacy. OCT and PGK combined with WC had a higher survival rate. A high-level protection and significant specific antibody responses against *S. agalactiae* challenge was observed upon the vaccinated tilapia with the purified PGK protein and *S. agalactiae* whole cells. The specific antibody titer against *S. agalactiae* antigen suggested that increased antibody titers were correlated with post-challenge survival rate. Il-1β expression profile was higher in PGK+WC-treated group. Tnf-α expression in the PGK+WC group was significantly increased. Taken together, our results suggested the combinations of recombinant protein and whole cell may elicit immune responses that reach greater protection than that of individual *S. agalactiae* components.

ID140:

Molecular cloning of orange-spotted grouper (*Epinephelus coioides*) CXC chemokine ligand 12 gene, and characterization of its expression in response to nodavirus infections

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CXC chemokine ligand 12 (CXCL12) is an inflammatory chemokine of the immune system. The chemokine CXCL12 and its receptor, CXCR4, are believed to be a specific ligand-receptor pair which is involved in several physiological and pathophysiological processes. In this study, we cloned and characterized the CXCL12 gene from *Epinephelus coioides* (*osgCXCL12*). The open reading frame of *gCXCL12* consisted of 98 amino acid, with an estimated molecular mass of 11.27 kDa. Reverse transcription polymerase chain reaction (RT-PCR) and real- time PCR were utilized to examine *osgCXCL12* expression levels in different tissues. Our results suggest that *osg*CXCL12 was constitutively expressed in several tissues (the fin, gill, liver, and spleen; $p < 0.01$) with especially high expression levels in the head kidney ($p < 0.01$). Furthermore, time- course analysis of *osgCXCL12* and *osgCXCR4* (CXCR4 from *E. coioides*) expression levels in nervous necrosis virus (NNV) infected groupers revealed a significant increase after 12 and 24 h of challenge with NNV ($p < 0.05$), respectively. The *osgCXCR4* gene expression level also increased with NNV in a time-dependent manner until post-infection ($p < 0.05$). Immune related genes expression level at different development stages of *E. coioides* (0-28 day) was evaluated by real time PCR.These data provide valuable information for further study of grouper chemokine signaling pathways and their roles in immune responses to virus infections.

ID107:

Transcriptional regulation of orange-spotted grouper (*Epinephelus coioides*) myostatin gene promoter correlated with nodavirus infection

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Myostatin is a negative regulator of skeletal muscle development and growth in many animals. Earlier studies showed that loss of myostatin function was associated with the double-muscle growth. However, the regulation and mechanism of myostatin at the transcriptional level are unclear. A few articles report that myostatin expression levels are affected by virus infection in mammals. To better understand the effects of nodavirus on myostatin promoter function in fish, we amplified and sequenced the orange-spotted grouper myostatin promoter, with a total length of 1936 bp. Sequence analysis of 1.9 kb of the grouper myostatin gene upstream region showed that it contains 10 E-box (CANNTG) motifs, which are the binding sites for the basic helix-loop-helix transcription factors such as myogenic regulatory factors that might influence the expression of myostatin gene. Different length myostatin promoter fragments were amplified and cloned into pGL3-Basic luciferase reporter vector. Vectors were transfected into grouper fin cells (GF-1) and muscle cells of juvenile grouper, to analyze the transcriptional activity of promoter through dual-luciferase reporter assays. The mutation of either a specific individual E box or a combination of some E box motifs was performed by systematic 5'-deletion and site-directed mutagenesis. Mutated fragments were sub-cloned into the pGL3-Basic vector to find the specific binding-site of transcriptional activator and suppressor for myostatin gene under poly (I:C) treatment. The results indicated that the E-box regions, E5 and E6, may be critical for the transcriptional activity of the grouper myostatin gene in nodavirus-infected groupers, particularly the HNF-3b site on E6 region, as well as E6 E-box site and c-Ets site on E5 region.

ID98:

Functional analysis of interferons in orange-spotted grouper (*Epinephelus coioides*)

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The importance of the interferon (IFN) system as a host defense against viral infections is illustrated by the finding that a number of IFN-induced antiviral proteins, the RNA-dependent protein kinase (PKR) and the GTPases Mx protein antagonize nodavirus. Immunohistochemical (IHC) analysis indicated that IFN expression is predominantly membrane-localized in healthy grouper, but has a zonal distribution in nodavirus-infected grouper. Recombinant IFN activates grouper Mx and PKR, leading to upregulated antiviral activity. It has been previously found that an overexpression of grouper Mx act as a negative regulator of nodavirus activity through direct interaction, such that grouper Mx induction expression might bind and perturb intracellular localization of coat protein. The presence of grouper Mx in dsRNA poly[I:C] interferon system inhibited nodavirus infection, which showed that overexpressing grouper Mx had an inhibitory effect on both coat protein and RdRp of nodavirus antigens, resulting in reducing levels of virus yields. The grouper Mx promoter was highly induced after treatment with recombinant IFN. The present study showed that the expression of grouper IFN may participate in the immunologic barrier function of nodavirus.

ID90:

Molecular cloning and characterization of heat shock transcription factor 1 isoform genes from orange-spotted grouper (*Epinephelus coioides*) exposed to temperature changes and nodavirus infection

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Heat shock transcription factor 1 (HSF1) mainly regulates the production of heat shock proteins (HSPs), that assist protein folding and inhibit protein denaturation in organisms in response to high temperature. Two orange-spotted grouper HSF1 isoform genes were cloned and named *osgHSF1α* and *osgHSF1β*, with an open reading frame of 2100 and 2028 nucleotides, encoding a polypeptide of 544 and 520 amino acids, respectively. In this study, quantitative PCR systems were designed to evaluate their expression levels in response to temperature changes and nodavirus infection. Under normal physiological conditions, the two isoform genes were differentially expressed in all tissues, with the highest *osgHSF1α* and *osgHSF1β* expression levels in brain and liver, respectively. During virus infection, *osgHSF1α* and *osgHSF1β* also showed differences in expression patterns compared to normal conditions, with their highest expression were in brain and heart, respectively. These two isoform genes were induced differentially in grouper larval stages during heat and cold shock responses. The *osgHSF1α* showed an increased expression in both temperature changes while the *osgHSF1β* increased at low temperature and decreased at high temperature. *osgHSF1α* showed a higher gene expression level than *osgHSF1β* in virus-infected grouper larval stages while the downstream HSPs genes like *osgHSP90AB* and *osgHSC70* were both increased. *osgHSF1α* expression in grouper larvae after challenge with virus was elevated throughout the experimental periods. These results suggest that *osgHSF1* isoform genes play important roles at different temperatures and *osgHSF1α* is important in nodavirus infection.

ID408:

Protective immunity of recombinant fbsa and α-enolase in tilapia (*Oreochromis niloticus* niloticus l.) against S*treptococcus agalactiae*

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Streptococcus agalactiae, a gram-positive bacterium, can infect a variety of fish species reared in seawater and fresh water and cause serious economic losses in aquaculture. In this paper, we identified, expressed and purified two putative cell surface proteins FbsA and α-enolase from Streptococcus agalactiae THN strain, and determined their effects on the blood leukocyte respiratory burst activity, serum lysozyme activity and specific antibody titer weekly post immunization tilapia. Also, the protective immunity of both proteins against *Streptococcus agalactiae* infection was detected. Results indicated that both putative proteins shared similar structure characteristics with that of other bacterium species. Using E. coli BL21 system, we expressed recombinant FbsA (rFbsA) and α-enolase (rEnolase) and purified. Post immunization with rFbsA or rEnolase in tilapia, the respiratory burst activity and lysozyme activity were significantly enhanced when compared with the PBS control. The serum specific antibody titer against rFbsA or rEnolase was also increased at different degree, and rFbsA induced higher antibody titer. Both recombinant proteins can confer fish protection against *Streptococcus agalactiae*, with the protection rate of 47.2% and 66.7%, respectively, and *Streptococcus agalactiae*, adjuvant and PBS control groups respectively produced protection rate of 94.4%, 25.0% and 11.1%. These results indicated that FbsA and α-enolase can function as candidate vaccine antigens to regulate the host innate immune responses and specific antibody responses, and give host protective immunity against *Streptococcus agalactiae*, while protection rate offered by each protein was different.

ID409:

Identification of vaccine candidate proteins from *Cryptocaryon irritans* by proteomics analysis

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Cryptocaryoniasis is a severe marine cultural fish disease with considerable economic loss caused by a parasitic ciliate *Cryptocaryon irritans*. Immunoprophylaxis is considered as a promising treatment for this disease, however, antigenic proteins used for vaccine development remain largely unknown. This study was aim to screen candidate proteins of *C.irritans* for vaccine development by differential proteomics and immunoproteomics. 53 protein spots in differential abundance based on pair-wise comparison among theronts, trophonts and tomonts, were identified and characterized as 12 proteins. Meanwhile, 33 and 27 protein spots were recognized with rabbit and grouper anti-sera respectively, which were identified as 9 and 11 proteins correspondingly. Among these identified proteins, actin, α-tubulin, β-tubulin, polypyrimidine tract-binding protein, NADH-ubiquinone oxidoreductase 75 kDa subunit, glutamine synthetase, enolase, protein kinase domain containing protein, malate dehydrogenase, TNFR/NGFR cysteine-rich region family protein and vacuolar ATP synthase catalytic subunit α in infective theronts showed immunogenicity with rabbit and grouper anti-sera. While in parasitic trophonts, actin, heat shock protein 70, mitochondrial-type hsp70 and vacuolar ATP synthase catalytic subunit α were immunoreactive with rabbit anti-sera. Combined with the results of proteins increasing in theronts and trophonts, actin, α-tubulin, β-tubulin, enolase and vacuolar ATP synthase catalytic subunit α were detected in infective theronts and parasitic trophont, and recognized by rabbit and grouper anti-sera. Furthermore, β-tubulin appeared in all three developmental stages and commonly recognized by rabbit and grouper anti-sera. Therefore, five proteins selected in this study (actin, α-tubulin, β-tubulin, enolase and vacuolar ATP synthase catalytic subunit α) might be considered as promising vaccine candidate proteins of *C.irritans* for vaccine development.

ID 269:

In vitro assessment of cellular immunomodulatory effects of *Centella asiatica* (Linn.) and *Portulaca oleracea* (Linn.) extracts in Nile tilapia (*Oreochromis niloticus* Linn.)

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Centella asiatica and *Portulaca oleracea* are indigenous plants from the Philippines that have been reported to possess various bioactivities, including immunomodulatory effects. Interests in the use of natural products to enhance the health and disease resistance of aquaculture species have intensified in recent years. In the present study, the immunomodulatory potentials of natural products from the two plant species were assessed in *Oreochromis niloticus in vitro*. The immunomodulatory actions of the ethanolic, ethyl acetate and hexane extracts of both plants were evaluated using cell-based assays such as lymphocyte proliferation, production of reactive oxygen species (ROS) and phagocytic activity assay. The results showed that 10 ug/ml of *C. asiatica* ethanolic extract induced the highest lymphocyte proliferative activity of 46.77% while *P. oleracea* ethanolic extract at 5 ug/ml concentration resulted in a 55.6% lymphocyte proliferation, which was comparable to the action of the mitogens concanavalin (ConA) and lipopolysaccharide (LPS). The *C. asiatica* ethyl acetate extract at 50 ug/ml induced the highest proliferative activity (94.95%) while *P. oleracea* hexane extract at 100 ug/ml resulted in 101.49% lymphocyte proliferation. For ROS production assays, 10 and 100 ug/ml of *C. asiatica* crude ethanolic extract and 50 and 100 ug/ml of *P. oleracea* ethanolic extracts were evaluated. Both concentrations of *C. asiatica* and the 50 ug/ml *P. oleracea* were found to induce significantly higher ROS levels than in unstimulated head kidney macrophages. Only the 50 ug/ml *P. oleracea* induced significantly higher ROS levels than in the PMA-stimulated group. However, the ethyl acetate and hexane fractions of both plants produced lower ROS levels than the unstimulated and PMA-stimulated macrophages. The phagocytic activity of macrophages treated with crude ethanol extract (10 ug/ml) of *C. asiatica* showed a significantly higher activity compared to negative control cells. The results demonstrated the capacity of the extracts from these two plant species to improve the immune responses of Nile tilapia, even at low concentrations. Further studies are required to fully assess the effects of these natural products on other aspects of fish immunity and their possible use in aquaculture.

TILAPIA AND CATFISH DISEASES

ORAL PRESENTATIONS

ID267:

Major Diseases and health management in most commonly cultured species in the Mekong River Delta, Vietnam

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Aquaculture has developed very rapidly in Vietnam and had a great contribution to global aquaculture production. However, diseases have been one of significant problems for sustainability and profitability of the aquatic farming sector in the country, especially in the Mekong River Delta where large areas for aquaculture is located. Emergence and spread of diseases has greatly increased over the past few years along with rapid expansion and intensification of the sector. Although parasites and bacteria have been documented as the most common pathogens, bacterial agents have been identified for the major epizootics in farms where most important aquatic species are cultured. Recently, some new diseases have been reported in cultured aquatic species but the causative agents which are responsible for clinical signs of those diseases have not yet been identified. In this article, major diseases in most commonly cultured species in the Mekong River Delta including catfish, snakehead, mudskipper and tilapia will be described. Economic losses, rapid diagnositcs and health management in farms of these species will be presented and discussed.

ID412

Disease outbreaks in Pangasius catfish farming: past, present and future

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In the last decade, production of the freshwater catfish species *Pangasianodon hypophthalmus* (Sauvage) has superseded all expectations and now has an established place on the international table market. It is produced in many Asian countries but Vietnam has by far, the largest market share. This whitefish species is popular globally as more than 90% of the farmed pangasius is exported into over 100 countries. The development of this aquaculture industry has been one of the fastest recorded for a single aquatic species. However, this emerging aquaculture sector has suffered from mass fish losses due to infectious and non-infectious diseases, some of which have had a significant socio-economic impact on the production chain. As early as 1999, infectious disease outbreaks were reported due to parasitic and bacterial diseases, some of which continue to affect the supply chain in 2014. The aim of this presentation is to provide a comprehensive review of the lessons learned over the last 10 years by reviewing the historical, current and emerging disease threats affecting the health and welfare of farmed *P. hypophthalmus*.

ID145:

Dermocystidium, Streptococcus parauberis, and liposarcoma: new disorders in ornamental fish in Israel

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Two unusual infections and a rare condition are reported in ornamental freshwater fish reared in intensive aquaculture systems in Israel.

Cardinal tetra (*Paracheirodon axelrodi* (imported from Singapore and kept in quarantine facilities presented whitish, non-motile, worm-like structures of up to 1 cm long, located sub-dermally mostly at the base of fins and around the eyes. Histological analysis revealed that these were elongated cysts containing a large number of spherical spores with a large central vacuole, the morphology of which was consistent with *Dermocystidium* sp. Mortality in the affected fish was limited. Removal of affected fish resulted in clearance of the parasite from the system.

High mortality in Ram cichlid (*Mikrogeophagus ramirezi*) from an Israeli intensive aquaculture farm was reported. Affected fish presented significant redness on their skin. Histopathologically, focal inflammation was observed in the muscle and internal organs. A Gram-positive coccus was isolated from internal organs of symptomatic fish and the bacteria were identified by rRNA sequencing as *Streptococcus parauberis*. The bacterium was sensitive to Florfenicol and resistant to Oxyteracycline.

Clownfish (*Amphiprion ocellaris)* from an intensive recirculating system exhibited abnormally distended abdomen and laborious swimming. Gross pathology showed a whitish mass occupying the abdominal cavity, and severely pressing against internal organs and musculature. Histopathology revealed that the whitish mass was composed of adipose cells. The condition was characterised as a well-differentiated liposarcoma. Aggregates of adipose cells were present in different organs, appearing as distinct masses from the main abdominal tumor. The condition occurred in the summer of 2011 and affected approximately 0.3% of the fish stock. The individuals exhibiting clinical signs were removed and the condition did not reoccur.

ID209:

Identification and pathogenicity investigation of *Chryseobacterium indologenes* in red tilapia (*Oreochromis* sp.)

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Chryseobacterium indologenes is an opportunistic pathogen associated with infection in human, and is widely distributed in the aquatic environment. Previous study showed high mortality rate of yellow perch infected with *C. indologenes. C. indologenes* has been isolated from red tilapia (*Oreochromis* sp.) and their culture environments in our pilot research. *C. indologenes* infection in red tilapia has never been documented previously. The aims of this study were to isolate, identify, and study the pathogenicity of *C. indologenes* in red tilapia in Thailand. Twenty isolates of yellow pigmented colonies suspected to be *C. indologenes were* isolated from liver, spleen, and kidney of 100 diseased red tilapia which showed clinical signs of septicemia. Subsequently, all isolates were sub-cultured and identified by phenotypic and biochemical characteristics. However, *Chryseobacterium* species identification is not possible using biochemical chracteriztion only, thus, the twenty isolates were identified as *Chryseobacterium sp.* Additionally, five isolates which showed biochemical characteristics close to *C. indologenes were* selected for genotypic identification with polymerase chain reaction (PCR) amplification of partial 16s rRNA and sequencing. The result showed that four isolates were *C. indologenes* with 99 % homology to *C. indologenes* accession no EU2211399 and JN831444, while one remindes were 99% homologous to *C. massilliae* accession no AF531766 and FJ812379. The four isolates of *C. indologenes* were selected for pathogenicity by *in vitro* haemolytic and enzymatic activities. One isolate with highest pathogenicity was selected for virulence determination by LD_{50} test Thirty healthy red tilapia were intra-peritoneal injected with three different bacterial concentrations per fish ($2.9x10^5$, $2.4x10^6$ and $1.4x10^7$ CFU/fish). Mortality rate was observed ten days after infection and LD_{50} value determined ($2.78x10^6$ CFU/fish). During ten days of observation, moribund fish expressed clinical signs of anorexia, loss of balance, fin erosion and abnormal swimming. The results suggested that *C. indologenes* can be a causative agent of the disease observed in red tilapia.

ID208:

Francisellanoatunensis subsp. *orientalis* as the causative agent of visceral granulomas disease in cultured red tilapia (*Oreochromis* sp.) in Thailand

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Since 2012, several outbreaks of an emerging disease characterized by multiple white granulomas in internal organs have occurred in cultured red tilapia (*Oreochromis* sp.) in some provinces in Thailand. The etiological agent of this disease has not yet been determined. The objective of the present study was to identify the causative agent of this infectious disease by fulfilling Koch's postulates. Ten diseased fish were collected from two affected farms for bacterial isolation, histological studies, and polymerase chain reaction (PCR) diagnosis for suspected pathogen, *Francisella* sp.. All fish samples were positive with *Francisella* genus-specific PCR. One bacterial strain, designated VMCU-FNO131, was successfully recovered from the kidney of diseased fish, using cysteine heart agar supplemented with 10% sheep blood and Polymixin B 100 units mL^{-1}. Species identification of this strain was established by amplification and nucleotides sequencing of 16S rRNA gene. BLAST analysis revealed 100% identity to *Francisella noatunensis* subsp. *orientalis* strains available in the Genbank database. Phylogenetic analysis exhibited that Thai strain formed in the same cluster with others recovered from warm water fish. Subsequently, two groups of apparently healthy red tilapia fingerlings were challenged with 1.08 x 10^4 CFU mL^{-1} and 1.08 x 10^6 CFU mL^{-1} by intra-peritoneal injection. The same ccumulative mortality of 86.7 ± 23% was observed within 16 days and 5 days post-injection respectively. The same clinical signs and histopathological manifestation of typical granulomas were found in multiple organs of the both naturally and experimentally infected fish. The present study had fulfilled the Koch's postulates to confirm that *F. noatunensis* subsp. *orientalis* is the causative agent of visceral granulomas disease in red tilapia in Thailand.

ID103:

Outbreak of polycystic liver in red hybrid tilapia, *Oreochromis niloticus* (L.) x *Oreochromis mossambicus* (Peters)

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Polycystic liver disease is a rare disease in fish but has been reported in many different fish species in extensive marine and aquaculture environment around the world. A group of 700 red hybrid tilapia (*Oreochromis sp.*) juveniles weighing approximately 80 g was obtained from a local hatchery in Negeri Sembilan, Malaysia. After 2 months, several fish were noticed to have distended abdomen and showed swimming difficulties. Post-mortem examinations on 30 randomly selected fish weighing between 130 g and 250 g revealed pale liver with numerous cysts of variable size that contained clear fluid, involving approximately 33% of the fish. No other anomalies were observed, while no parasite and bacteria were isolated from the affected fish. Histological examinations revealed typical cysts that were lined a single layer of epithelial cells. The cysts were surrounded by thick connective tissue that might have originated from the bile duct. The hepatocytes surrounding the cysts were compressed and some were necrotic. The most likely explanation for this disease is genetic predisposition. This is the first reported case of polycystic livers in red hybrid tilapia.

ID333:

Francisellosis in Tilapia

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Francisella noatunensis (Fn), the causative agent of "Piscine francisellosis" affects several marine and fresh water fish species worldwide, including farmed salmon, cod and tilapia. The species is subdivided into two subspecies *orientalis* (*Fno*) and *noatunensis* (*Fnn*). Among the different hosts of *Fno*, tilapia is one of the most severely affected species. Francisellosis has been associated with high mortalities and severe economic losses in tilapia fish farms. Mortalities due to *Fno* have been reported in fish ranging from 5 to 90 g in weight during periods of cold stress, mainly in Latin America and Asia. Its facultative intracellular lifestyle and fastidious nature can make its isolation difficult. A new selective "cysteine blood-tilapia" agar was developed at IoA, which was successfully used to isolate a novel *Fno* isolate (Stir-Gus-F2f7) from moribund farmed tilapia in Europe and this represented the first documented report of *Fno* isolation from tilapia in Europe.

A polyphasic approach based on ecological, phenotypical, serological, chemotaxonomical, genetic, and genomic analyses was used to characterise this isolate. The use of this approach for comparative analyses with *Fnn* suggests that this and other *Fno* isolates represent a separate *taxon*, advocating the creation of the new bacterial species *Francisella orientalis* (*Fo*). An experimental challenge infection model with STIR-GUS-F2F7 was established at IoA in naïve Mozambique and Nile (red and wild type) tilapia. The disease model resulted in the death of fingerlings with granuloma formation and widespread multifocal white nodules, and allowed the LD_{50} of the isolate to be determined in each genetic group and a comparison of cumulative mortalities between the species.

A whole cell inactivated autogenous vaccine was tested in the red Nile tilapia *Oreochromis niloticus using isolate* Stir-Gus-F2f7 emulsified with an mineral oil based adjuvant. The tilapia were intraperitoneally immunised (i.p.) with either the vaccine, adjuvant mixed with phosphate buffered saline (PBS) or PBS alone. Fish were then challenged i.p. with 4.0 x 10^3 CFU/ml 30 days post-vaccination. The relative percent survival for the vaccine and adjuvant groups was 100% and 46.6%, respectively at 27 days post-infection. The correlation between survival and specific antibody production indicates the effectiveness of this vaccine in protecting tilapia against francisellosis.

ID218:

Putative virulence gene profiles and pathogenicity of *Flavobacterium columnare* isolated from red tilapia (*Oreochromis* sp.)

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Flavobacterium columnare, the causative agent of columnaris disease, is a threat to many fish species worldwide including red tilapia (*Oreochromis* sp.). However, information on virulence factors and pathogenicity of *F. columnare* are still limited. The objective of this study was to investigate existence of putative virulence genes among *F. columnare* isolates recovered from red tilapia and its pathogenicity. Specific primers targeting five putative virulence genes including *cls, m50, sprA, sprB* and *gldF* which encode chondroitin AC, membrane associated zinc matalloprotease, surface proteins, and gliding protein, respectively, were designed for virulence gene profiling. The results revealed that ten out of 51 isolates exhibited positive results with five tested genes (P1), whereas the rest of the isolates were positive to three genes *cls, gldF,* and *m50* (P2). These results indicated coexistence of two distinct putative virulence gene profiles among isolates of *F. columnare* isolated from red tilapia in Thailand (P1, P2). Five isolates in each virulence gene pattern were randomly selected for immersion challenge at a dose of 10^7cfu/ml. The consequences of pathogenicity revealed that the mean mortality rate of fish challenged with *F. columnare* isolates in pattern P1 varies from 0 to 100%, while in P2, fish mortality fluctuated from 20 to 80%. Our results suggested that virulence of *F. columnare* is not dependent on the five types of virulence genes that were studied nor on their virulence gene profiling patterns. Other virulence genes or expression of virulence genes maybe associated with virulence of isolated *F. columnare*.

ID264:

Bacterial disease and management of Nile Tilapia (*Oreochromis niloticus*) cage cultured in Chi River in Northeastern Thailand

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Tilapia (*Oreochromis niloticus*) is the most economically important aquaculture species in northeastern Thailand. Tilapia farming ranges from rural subsistence farming to large-scale commercial operations depending on the intensity management employed. Cage culture of tilapia in Chi River are normally intensive with high stocking density, thus high risks for infection if the farmers fail to provide the fish with optimal water conditions. This can cause stressful condition and compromise the immune system which will eventually result to disease outbreak. A survey on bacterial diseases of tilapia in Chi River was carried out at Khon Kean, Maha Sarakham, Roit, Yasothon and Ubon Ratchathani provinces. Fish samples were collected and diagnosed during March 2012 to February 2013. Bacterial infections observed were identified to be caused by *Streptoccocus*, *Aeromonas*, *Pseudomonas*, *Staphylococcus*, *Flavobacterium* and *Enterobacter*. These bacteria infect all stages of culture. *Streptococcus* is the most important pathogen affecting tilapia culture. However, severe mortality was observed with co-infection with digenean, *Trichodina*, *Ichthyophthirius* and some zoosporic fungi. During disease outbreak, farmers apply preventive measures including transfer of cages to better sites with enough water currents, cage cleaning, provision of good water quality, optimum density, and good feeding management. Antibiotics and chemicals are commonly used for prevention and treatment, while vaccination is also of interest. On the other hand, avoidance of other risk factors such as wild pathogen contamination, authropogenic transfer of carrier stocks, and climate change among others, can make the tilapia more prone to diseases.

ID234:

Load and composition of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines

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The quantity and composition of the bacterial microbiota in the rearing water, sediment, gills and intestines of tilapia *Oreochromis niloticus* collected fortnightly from Day 30 to Day 120 after stocking for grow-out culture in 6 earthen brackish water ponds in the Philippines were examined. The mean viable counts obtained in the water, sediment, gills and intestine of tilapia ranged from $1.3 \pm 1.9 \times 10^4$ – $4.6 \pm 1.8 \times 10^4$ c.f.u. ml^{-1}, $2.9 \pm 4.1 \times 10^4$ – $2.3 \pm 1.3 \times 10^5$ c.f.u. g^{-1}, $2.5 \pm 4.7 \times 10^6$ – $1.2 \pm 3.3 \times 10^7$ c.f.u. g^{-1}, and $2.2 \pm 4.1 \times 10^5$ – $3.8 \pm 3.3 \times 10^6$ c.f.u. g^{-1}, respectively. In terms of composition, a total of 20 bacterial genera and 31 species were identified with the preponderance of gram-negative bacteria constituting 84% of the overall bacterial isolates examined. *Aeromonas hydrophila*, *Bacillus* spp., *Plesiomonas shigelloides*, *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Staphylococcus* spp. and *Vibrio cholerae* were the dominant bacteria identified in the gills and intestine of tilapia. These bacteria also dominated in the pond sediment and rearing water, except for the nil isolation of *S. putrefaciens* and *V. cholerae* in the water samples examined, indicating that resident bacteria in the pond water and sediment congruently typify the composition of bacterial microbiota in the gills and intestine of tilapia which under stressful conditions may propel the ascendance of disease epizootics.

ID162:

Genome sequencing and comparative analysis of four strains of *Edwardsiella ictaluri* from the Mekong Delta.

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Edwardsiella ictaluri, the causative agent of enteric septicemia of catfish (ESC), is a well known bacterial pathogen with significant economical consequences in both Vietnamese and American aquaculture. Antibiotics are used for treatment and there has been identified resistance towards a large range of antibiotics, which causes concern to the industry, the environment and human health. To better understand if there are important variations among the different isolates of *E. ictaluri* from the Mekong Delta, we have analyzed several isolates from diseased farmed striped catfish (*Pangasianodon hypophthalmus*). Although these strains display some variation in terms of virulence and plasmid content, they are indistinguishable by phylogenetic analysis of the 16S, *gyrB*and *glnA* genes. Therefore, to fully uncover any differences, we have performed full genome sequencing using Illumina technology on four different isolates. Preliminary results reveal that isolates from the Mekong Delta are significantly different from the American *Edwardsiella ictaluri* 93-146strain. Furthermore, our comparative analyses show that the four strains are strikingly similar to each other, being almost identical at the genomic level. However, we have identified genetic differences which might be significant at the phenotypic level and findings that shed light upon the concerns of antibiotics in the context of human and environmental safety.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

TILAPIA AND CATFISH DISEASES

POSTER RESENTATIONS

ID224:

Study on Edwardsiellosis in clown knifefish (*Chitala chitala*) in the Mekong Delta, Vietnam

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This paper describes the fisrt report of *Edwardsiella tarda* isolated from hemorrhaged clown knifefish in the Mekong Delta, Vietnam. The study investigated 178 diseased clown knifefish collected from commercial farms in Mekong Delta provinces including Haugiang, Dongthap, and Cantho. Diseased fish showed gross signs of abnormal swimming, exophthamia with hemorrhages, skin ulcerations, and petechial hemorrhages in fins and body. Internally, ascites with heamoperitoneum, hemorrhaged kidney, light-colored nodules on the liver and splenomegaly were also observed. There were 43 *E. tarda* isolates obtained from fish samples. Conventional and rapid identification systems, and further phylogenetical (16S rDNA) characterization were used to identify the pathogen. The experimental challenge was performed in healthy clown knifefish fingerlings (mean weight 15±4g). The experiement was randomly designed with triplicated intraperitoneal injection with 0.1 ml of *E. tarda* at concentrations of 10^4, 10^6, 10^8 CFU/ml. Control groups were injected with 0.1 ml sterile saline solution. The results fulfilled Koch's postulates and showed clinical signs similar to those observed in the natural infection, while no mortality was observed in the control groups. The 12h LD50 value was $4.89x10^5$ CFU/mL. The susceptibility of all isolates to 16 selected antibiotics using disk diffusion method is described in the study.

ID341:

Induction of disease susceptibility in *Streptococcus agalactiae* infected Nile tilapia (*Oreochromis niloticus*) by elevation of water temperature

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The relationship between severity of streptococcosis associated with *Streptococcus agalactiae* (Group B streptococcus; GBS) infection in Nile tilapia (*Oreochromis niloticus*) and the increase of water temperature was investigated in this study. At high temperature, GBS showed the enhancing of in vitro pathogenicity since bacteria grown at 35°C required only 4 hours to reach the log-phase, while it was 7 hours at 28°C growing condition. The viability of bacteria in tilapia whole blood increased from 2% to 97% and the hemolysis activity drastically increased 5 times in the high temperature condition. Strong up-regulation of virulence genes of GBS, comprised of cylE (β-hemolysin/cytolysin), cfb (CAMP factor) and PI-2b (pili-backbone), occurred at 35°C grown GBS. For in vivo pathogenicity, Nile tilapia reared at 35 and 28°C were challenged with GBS and the accumulated mortality was found to be 85% and 45%, respectively. At 35°C, infected tilapia exhibited tremendous up-regulation (30 to 40 folds) of inflammatory-related genes (cyclooxygenase-2, IL-1β and TNF-α) between 6 and 96 hours-post infection. These results suggest that the increase of GBS pathogenicity due to the water temperature is related to massive inflammatory responses which may lead to acute mortality in Nile tilapia.

ID325:

The presence of virulence genes in *Aeromonas hydrophila* isolated from striped catfish (*Pangasianodon hypophthalmus*)

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During three-year period from 2012 to 2014, we collected 260 isolates of *Aeromonas hydrophila* from striped catfish from six provinces in the Mekong Delta of Vietnam including Vinh Long, An Giang, Dong Thap, Can Tho, Ben Tre and Tien Giang. The presence of seven virulence genes of *A. hydrophila* isolates were detected by PCR method. It was observed that *ahsA*, a gene encoding S-layer protein, was absent in all the isolates collected. The gene encoding heat-stable cytotonic enterotoxin, *ast*, was only present at 14.6% frequency, which was statistically lower ($p < 0.01$) compared to other five genes. The gene encoding heat-labile cytotonic enterotoxin, *alt*, was the most common gene among all *A. hydrophila* isolates (92% frequency). The four genes, *aerA*, *lip*, *ahpA* and *ahh*1 has 75% to 84% frequency. The distributions of these genes were combined into 14 genotypes. The genotype $aerA^+ahh1^+alt^+ahpA^+lip^+ast^-$ was the most common (57.3%) among all the isolates and was distributed in the representative from all six provinces. Thid genotype appeared in all the isolates of *A. hydrophila* collected from fingerlings with haemorrhage in Tien Giang province in June and July 2014. Interestingly, this genotype also existed in bacteria isolated from healthy striped catfish and in the water samples collected from five provinces in 2012 and 2013. These results showed that the hemorrhage disease caused by *A. hydrophila* occurred in striped catfish to a large extent in time and location especially during rainy season, and caused huge loss for the farmers in the Mekong Delta.

ID261:

Isolation and characterization of *Streptococcus disgalactiae* from mudskipper (*Pseudapocryptes elongatus*) cultured in the Mekong Delta, Vietnam

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Hemorrhagic disease caused by bacteria has been one of significant problems in cultured mudskipper (*Pseudapocryptes elongatus*) in the Mekong Delta of Vietnam. Usual clinical signs of disease include haemorrhage in skin, fin and anus with high mortality. Although these clinical signs are easy to spot for the layman, it is insufficient to determine the species of bacteria responsible for the disease. These pieces of information are crucial in order to anticipate prevention and treatment measures. Therefore, diseased specimens were collected from intensive farms of mudskipper in Bac Lieu and Soc Trang provinces. Microscopic observation of fresh smear of blood, liver, kidney and spleen from these specimens revealed small cocci, gram positive bacterial cells. Bacterial isolates from brain and head kidney were recovered on brain heart agar and were analyzed as Gram positive, non-motile and oxidase negative, and they were identified as *Streptococus disgalactiae* using a combination of conventional biochemical test, API 20 strep system, PCR and gene sequencing. Histopathological examination of diseased specimens showed a typical sign of bacterial necrosis in kidney, spleen and liver. Challenge experiments using injection method showed that they can cause the observed disease signs with the LD_{50} value of about 4.25×10^4 CFU/m. It is the first report of *Streptococcus disgalactiae* outbreak in mudskipper in Vietnam.

ID220:

Status of Antibiotic resistance in *Edwardsiella ictaluri* and *Aeromonas hydrophila* from striped catfish farming in the Mekong Delta, Vietnam

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The purpose of this study is to assess the *in vitro* antibiotic resistance of 150 *Edwardsiella ictaluri* and 91 A*eromonas hydrophila* isolated from diseased striped catfish in the Mekong Delta during January 2010 to March 2014. The isolates were screened against 15 antibiotics by disk diffusion method. The results showed that *E. ictaluri* isolates were highly resistant to tetracycline, enrofloxacine, streptomycine, florfenicol and chloramfenicol, completely resistant to trimethoprim/ sulfamethoxazole, and sensitive to ampicillin and amoxicillin. On the other hand, *A. hydrophila* isolates were highly resistant to tetracycline, florfenicol, completely resistant to trimethoprim/sulfamethoxazole, cefalexine, and sensitive to doxycycline, cefotaxime and ciprofloxacine. Most of isolates displayed multi-drug resistance with MAR (Multi-antibiotic resistant index) recorded a >0.2. The conjugation experiments showed that *E. ictaluri* isolates can transfer antimicrobial resistant genes to *E. coli* RC85. Likely, transconjugants (*Ei-R*) can transfer these genes to *A. hydrophila* isolates. Results of this study showed that *E. ictaluri* isolates pathogenic to freshwater catfish have high multi-resistance and can transfer antimicrobial resistant genes, which have relation with plasmid of *E. coli* pathogenic to human.

ID291:

Identification and characterization of *Flavobacterium columnare* isolated from Nile Tilapia (*Oreochromis* sp.) and striped catfish (*Pangasionodon hypophthalmus*) in Thailand

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Flavobacterium columnare has been recognized as an important bacterial pathogen of various freshwater fish species worldwide since 1922. However, characterization of this pathogen originated from economically valuable fish is still poorly explored in Thailand. The purposes of this study were to identify, investigate phenotypic characterization, genetic diversity and assign genotype for isolates of *F. columnare* isolated from Nile tilapia (*Oreochromis* sp.) and striped catfish (*Pangasionodon hypophthalmus*) in different geographical locations in Thailand. Thirty putative isolates of *F. columnare* isolated from diseased Nile tilapia and six isolates from diseased striped catfish were identified as *F. columnare* based on biochemical characteristics and species-specific PCR method. All thirty- six isolates were assigned to genomovar II based on the restriction fragment length polymorphism of the 16S rRNA gene (16S-RFLP). It was noticeable that 16S-23S rRNA intergenic spacer region (ISR) of these isolates represented an exception as sharing one amplified band with approximate size of 500 bp in size and two distinct bands with approximate size of 400 bp and 600 bp in tilapia and striped catfish originated isolates, respectively. Interestingly, one isolate (NK13) revealed a novel ISR containing up to three amplified bands with the approximate size of 400 bp, 500 bp and 600 bp, respectively. This study firstly reported and assigned genomovar for isolates of *F. columnare* isolated from Nile tilapia and striped catfish in Thailand. Furthermore, genetic diversity of *F. columnare* based on 16S rRNA and 16S-23S rRNA ISR was phylogenetically investigated in the present study.

ID154:

Identification of *Edwardsiella ictaluri* isolates recovered from natural infections in *Pangasianodon hypophthalmus* in Vietnam

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This study describes the biochemical, biophysical characteristics, plasmid profiles, and antibiotics resistance as well as the relatedness of *Edwardsiella ictaluri* isolates recovered in farmed striped catfish over last ten years from 2002 to 2011. The isolates were collected both years at the same 40 farm sites consisting of 30 BNP outbreak farms and 10 recovered farms in 4 provinces of Vietnam. The isolates were first identified using both traditional primary and biochemical bacterial identification tests and after that their genetic relatedness was assessed using pulsed-field gel electrophoresis (PFGE). From these data, representative isolates from each genetic cluster were then selected for additional biophysical and biochemical investigation. The minimum inhibitory concentration (MIC) values were also investigated for these isolates. The PFGE results showed 6 main groups with a similarity of 82% and the corresponding genotypes of the prevalent isolates illustrated geographic and annual differences but no differences in biochemical characteristics were observed. The results of biophysical tests showed that a pH value between 5.5 to 6.5 and salt concentration between 0-0.5% was optimal for the growth of *E. ictaluri*. Plasmid profiles identified 3 distinct groups. The results of MICs showed that all Vietnamese isolates were resistant to Oxolinic acid, Sulfadimethoxine/ Ormetoprim (Romet), Oxytetracycline and Amoxicillin.

ID244:

Novel genetic characterization of *Flavobacterium columnare* isolated from diseased red tilapia (*Oreochromis* sp.) in Thailand

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Flavobacterium columnare is the causative agent of columnaris disease and severely affects various freshwater aquaculture fish species worldwide. The purposes of this study was to determine the genetic variability among *F. columnare* isolated from red tilapia in Thailand. Fifty isolates from diseased fish in different geographic locations were used in this study. These isolates showed homologous phenotypic characteristics but exhibited genetic diversity. Based on 16S-RFLP, one isolate (CUVET1215) was assigned to genomovar I while the rest (*n*=49) were assigned to genomovar II indicating the coexistence of two genomovars but predominance of genomovar II. Phylogenetic analysis of the 16S-23S ISR sequences revealed that a subset of the Thai isolates (*n*=25) contained a smaller ISR (523-537 bp) and formed a novel ISR phylogenetic group. Phylogenetic analysis of the 16S rRNA gene supported the unique cluster of Thai isolates. Interestingly, present study also addressed that failure of amplification of 16S rRNA gene using forward primer R1500 proposed in the standard method 16S-RFLP occurred as a result of nucleotides variability in 16S rRNA sequences of Thai-originated *F. columnare* isolates. This is the first description of the molecular characteristics of *F. columnare* isolated from red tilapia in Thailand as well as five isolates of *F. columnare* derived from other fish species including Nile tilapia, koi carp and Vietnamese striped catfish. Additionally, pathogenicity of two representatives and adhesion dynamic of a virulent isolate (CUVET1201) and an avirulent isolate (CUVET1214) were investigated in fry red tilapia model in the present study.

ID295:

Histopathology of *fusarium* sp. infected with striped catfish (*Pangasianodon hypophthalmus*)

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Fungi are considered as one of the pathogen infections in fish. The study was carried out to observe the histopathological changes in fish infected with *Fusarium* sp. A total of 54 fish samples were collected for fungal identification and histological examination. Most fungal colonies are round, light red backside and frontside, and the hyphae grow closely on PDA. Other colonies are round, yellow in backside and cotton-wool growth on PDA. Results of histology showed that tissues of gill, muscle, liver, kidneys do not harbor spores or hyphae of *Fusarium* sp., but changes in the cell structures were observed. Presence of dense spores and hyphae in sinus of swim bladder was observed.

ID226:

Diversity of tetracycline resistance genes in *Aeromonas hydrophila* and *Edwardsiella ictaluri* from the striped catfish farmed in the Mekong Delta, Vietnam

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The aim of this study was to determine the genetic determinants responsible for tetracycline resistance in tetracycline resistant bacteria from Tra catfish (*Pangasianodon hypophthalmus*) farmed in the Mekong Delta, Vietnam. Thirty-six isolates (including *A. hydrophila* and *E. ictaluri*) resistant to tetracyclines group antibiotics (tetracycline and doxycycline) were carried out by disk diffusion method. Polymerase chain reaction (PCR) was used to detect tetracycline resistance genes. The results revealed that *tetA*, *tetB* and *tetS* were found in both bacteria, in which *tetA* (70%) was the most common determinant, followed by *tetS* (55%) and *tetB* (30%). The high prevalence of a variety of tetracycline resistance genes in *A. hydrophila* and *E. ictaluri* isolates from Tra catfish culture in the Mekong Delta may pose potential public health risk due to possible transfer of resistance to other bacteria in the environment.

ID223:

The presence of class 1 integrons in *Aeromonas hydrophila* causes disease on the striped catfish (*Pangasianodon hypophthalmus*) farmed in the Mekong Delta, Vietnam

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The objective of this study was to detect the presence of class 1 integrons in *A. hydrophila* that causes haemorrhagic disease on the striped catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta, Vietnam. A total of 40 *A. hydrophila* isolates were collected from 2013 to 2014. These isolates were screened against 15 antimicrobial agents by disk diffusion method. The results showed that most of *A. hydrophila* isolates displayed multiple resistance phenotypes. Thirty-two *A. hydrophila* isolates showed the presence of class 1 integrons by using PCR technique (polymerase chain reaction). The results demonstrated that the percentage of class 1 integrons in *A. hydrophila* isolates were 21.9%. The presence of such mobile genetic elements in *A. hydrophila* in this research revealed the ability of bacteria to transfer antimicrobial resistance genes to other bacteria in the natural environment. Therefore, using antibiotics for disease prevention in aquaculture should be managed more strictly. This is the first report on class 1 integrons detected in pathogenic *A. hydrophila* causing disease in striped catfish.

ID212:

Characterization of a mutant of *Edwardsiella ictaluri* lacking the gene coding a component of type VI secretion system

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Edwardsiella ictaluri is originally known as a Gram-negative pathogen causing enteric septicemia of catfish, and recently infection by the bacterium has occurred in ayu *Plecoglossus altivelis* in Japan. Secretion systems are the important virulence factor in most of Gram-negative bacteria, and type III secretion system of *E. ictaluri* has been reported to be involved in the virulence in fish. However, the role of type VI secretion system (T6SS) of the bacterium in the pathogenicity is not clear. In this study, we focused on a core membrane-associated protein of T6SS, designated as EivpO, which is a homologue of EvpO of *E. tarda* and IcmF of *Legionella pneumophila*, and constructed an *eivpO* in-frame deletion mutant of a virulent *E.ictaluri* strain (PH0744) isolated from diseased ayu through double crossover allelic exchange using a suicide vector pRE112. Δ*eivpO* was not different from the wild type in cell morphology. It was found that *in vitro* growth rate of Δ*eivpO* at 28°C was higher than that of wild type. This result suggested that *eivpO* may involve in quorum sensing regulation of *E. ictauri*. In addition, Δ*eivpO* reduced ability of adherence to and invasion in fish cell lines, EPC, BB (brown bullhead) and ayu fin. Together with results of experiments for detection of virulence in fish, we discuss the role of EivpO and T6SS of *E. ictaluri* in the pathogenicity.

ID340:

Molecular characterization of *Streptococcus agalactiae* strains isolated from diseased fish.

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Streptococcus agalactiae (Lancefiels group B) is a Gram positive, chain –forming coccus that can affect human and many other animal species. It has been recognized as one of the major causes of meningitis in neonates, bovine mastitis as well as meningoencephalitis in aquatic species such as tilapia. From diseases identified in farmed tilapia streptococcosis is considered to be the most devastating and responsible for heavy economic loses *e.g.* large-scale streptococcal outbreaks which took place in China from 2009-2011, occurred in approximately 95% farms and cased up to 80% mortality. This research aims to provide an insight into population structure of aquatic *Streptococcus agalactiae* which is essential for further vaccine development research.

A total of 90 *Streptococcus agalactiae* strains were isolated from cultured tilapia *Oreochromis niloticus* L. in USA, Costa Rica, Canada, Honduras and China. Bacteria were identified using classic bacterial identification methods and then investigated further using biotyping, serological typing and multilocus sequence typing (MLST) using established typing methods (Jones N. et al 2003, Kong et al. 2005 and Poyard et al. 2007). The characterisations show that of the 90 strains 75 belongs to biotype 1 with β-haemolysis, fast growing characteristic, and serotype Ia typically associated with *S. agalactiae* with human origin. Of those 75 strains just one was collected outside China. Remaining 15 isolates were characterized as non-haemolytic, and belongs to Ib serotype. Strains are been analysed also by MLST typing. Until now the Streptococus strains were grouped into two clonal complexes (CC): CC 552 and CC 7. However, the MLST typing process is still on-going and the final results will be presented.

ID330:

Epidemic of Bacterial Diseases in Nile tilapia (*Oreochromis niloticus*)

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Nile tilapia (*Oreochromis niloticus*) is one of the most commonly cultured fishes in Thailand. Aquacultures of Nile tilapia are mostly traditional and some are more developed intensive farm. However, the overall production is significantly dependent on occurrence of bacterial diseases. The objective of our research is to study the epidemic of bacterial diseases in tilapia. Two hundred and six diseased Nile tilapia were sampled from 37 farms in Northern provinces: Chiang Mai(4 farms), Lamphun (3 farms), Kamphaengphet (7 farms); and Central provinces: Nakhonsawan (11 farms), Suphanburi (8 farms), Nakhonpathom (4 farms). The common clinical signs of infected fish were haemorrhagic lesions on the skin, rotten gill with brownish gray patches and swollen abdomen. Bacteria were isolated from liver, spleen, kidney, eyes and lesions using TSA media. Species identification was performed by biochemical method. Antibiotic sensitivity test was also determined. Our results showed majority of causative bacteria were *Aeromonas sobria*, *Aeromonas hydrophila* and *Streptococcus agalactiae*. Either *A. sobria* or *A. hydrophila*, or both species were causative agent for bacterial diseases in all studied provinces. The combination of these two bacteria with *S. agalactiae* was detected in Suphanburi and Nakhonsawan. The complexity of bacterial diseases due to *A. sobria*, *A. hydrophila*, Epitheliocystis and Mycobacterium were found only in Kamphaeng phet province. Antibiotic sensitivity testing showed sensitivity of bacteria to both tetracycline and sulfatrimethoprim.

ID93:

Genome analysis of *Streptococcus dysgalactiae* 12-06 isolated from amberjack, *Seriola dumerili*

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Streptococcus dysgalactiae (GCSD) of Lancefield group C has been isolated from farmed amberjack (*Seriola dumerili*) and yellowtail (*Seriola quinqueradiata*) in the western part of Japan since 2002. Recently, GCSD infections have been detected in farmed fish. Whole-genome sequencing of *S. dysgalacticae* subsp. *equisimilis* isolated from humans had been previously performed, and the sequence was deposited in a GenBank. However, GCSD isolated from fish had not been analyzed, and comparative genomic analysis of strains isolated from fish and from mammals had not been performed. This study determined the draft genome sequence of GCSD 12-06 isolated from amberjack, and compared its sequence to that of *S. dysgalactiae* subsp. *equisimilis,* isolated from humans. The whole-genome shot-gun sequencing of the GCSD 12-06 strain was performed using a sequencer (454 GS-FLX; Roche) and sequences were assembled into contigs. The gaps were closed by sequencing of polymerase chain reaction products. Blast2GO and GeneMarks were used to predict open reading frames (ORFs). Virulence-related genes were identified and the virulence of GCSD 12-06 was compared to that of other *Streptococcus* species, including *S. dysgalacticae* subsp. *equisimilis.*The draft genome sequence of GCSD 12-06 consisted of a single circular chromosome with a guanine-cytosine content of approximately 39.6%. A total of 2,055 ORFs, 57 tRNA genes, and 5 operons of 5S-16S-23S rRNA were identified. Most of the gap sequences were found to contain different types of insertion sequences (ISs). The frequency of IS1161 in GCSD 12-06 was more than three times of that found in *S. dysgalactiae* subsp. *equisimilis* strains. IS981 was found only in GCSD 12-06. Major virulence-related genes were found in both GCSD 12-06 and *S. dysgalactiae* subsp. *equisimilis.*

SHRIMP IMMUNOLOGY

ORAL PRESENTATIONS

ID405:

Crustacean immunity; hemocytes are most important for immunity

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In this overview I will talk about the most important immune player in immunity and that is the hemocyte (blood cell). When an infection occurs the hemocyte number goes down dramatically and more than 90% of the circulating hemocytes are lost and thus there is a need for new hemocyte production. We have been studying hemocyte synthesis and their proliferation for the past 15 years and we have in detail deciphered the ways in which hemocytes are synthetized and how they proliferate. Thus we have now a good knowledge how to avoid that animals get problems with production of new blood cells and which will lead to stress and more susceptibility to disease and pathogens.

ID257:

Functional elucidation and characterization of *Mr*FH in *Macrobrachium rosenbergii* using RNA interference

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Shrimp aquaculture is a major source of income in the intertropical countries of the Southeast Asian region. However, a great decline in production was observed due to a major viral pathogen, the White Spot Syndrome Virus (WSSV) which continues to prevail despite many preventive measures applied to deter the virus. RNA interference (RNAi) technology has been employed to reveal functions of specific genes in the virus and its host with the aim of controlling WSSV by elucidating complex host-virus interactions. This study determined the involvement of *Mr*C20, a DNA fragment that is part of the genome of *M. japonicus* and was shown to be present in *M. rosenbergii*. Phylogenetic analysis revealed that *Mr*C20 is highly homologous to homotetrameric enzyme fumarate hydratase (*Mr*FH) which functions in the tricarboxylic acid cycle during aerobic respiratory metabolism. Moreover, *Mr*FH is ubiquitously expressed in vital organs suggesting that it is essential to metabolic functions of the shrimp and may also play a role in its innate immune system as highlighted in its expression in the hemocytes. Rapid cloning of 5' and 3'-cDNA ends PCR (RACE-PCR) was employed to determine the unknown 5' and 3'-cDNA termini of *Mr*FH. Four set-ups were prepared to analyze the sequence specific silencing of *Mr*FH namely: *Mr*FH-dsRNA treated, GFP-dsRNA treated, PBS treated, and naive control. One Way Analysis of Variance (ANOVA) of the mortality assay indicates that *Mr*FH-dsRNA treatment has a significant protective effect against WSSV compared to GFP-dsRNA and PBS treated shrimps.

ID118:

Application Frequency of Dietary *Vibrio harveyi* Lipopolysaccharide on Growth and White Spot Syndrome Virus Resistance of *Penaeus monodon* Post Larvae

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The effects of dietary *Vibrio harveyi* lipopolysaccharide (LPS) as immunostimulant given at different frequencies in improving growth performance, immune responses, and disease resistance of post larvae *Penaeus monodon* against white spot syndrome virus (WSSV) infection were evaluated. Shrimp were fed with a treatment diet containing *Vibrio harveyi* LPS at a predetermined dose of 50 mg kg^{-1} diet given daily, every 2 days, every 5 days, and every 7 days. A basal diet was given to the treatment groups on the period that the shrimp should not receive dietary immunostimulant. A feeding trial was conducted for 8 weeks and resulted to 90-96% survival of the test animals. Weight gain (WG) and specific growth rate (SGR) were significantly enhanced in shrimp receiving LPS every 2 days. However, no significant difference was observed in the feed conversion ratio (FCR) of all test groups. Following WSSV challenge, the groups receiving LPS-supplemented diet exhibited increased disease resistance than the control group. Shrimp fed with LPS every 2 days exhibited statistically enhanced survival (53%) than the rest of the treatments. The same group consistently showed significantly higher values in all immune indices measured including total haemocyte count (THC), phenoloxidase (PO) activity, and respiratory burst activity (RBA). Collectively, the present work suggests that dietary administration of *Vibrio harveyi* LPS at 50 mg kg^{-1} concentration fed every 2 days is optimum to enhance growth, immune responses, and protection against WSSV in post larval *P. monodon*.

ID270:

Interplay between white spot syndrome virus and shrimp*Penaeus onodon* melanization immune response

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Melanization is one of the important immune response in invertebrates which is performed by a key enzyme phenoloxidase (PO) and tightly regulated by the prophenoloxidase (proPO) activating cascade. In insects, it has been clearly demonstrated that melanization plays role against bacterial and viral pathogens. Recently, our research group also showed the antimicrobial activities of melanization reaction products to counteract both bacteria and fungi in the shrimp, *Penaeus monodon*. Furthermore, the effect of melanization reaction on viral infection was examined. It was observed that shrimp injected with melanization reaction-treated white spot syndrome virus (WSSV) resulted in high survival rate and reduction of viral replication. In the present study, the potential role of shrimp melanization against WSSV was investigated emphasized on host-viral interaction.Protein-protein interaction analysis between *Pm*proPOs (*Pm*proPO1 and *Pm*proPO2) and WSSV was carried out using yeast two-hybrid approach. The interaction screening result was successfully obtained from *Pm*proPO2 but not *Pm*proPO1. With respect to confirmation test in yeast, four candidate WSSV proteins were found to specifically bind to *Pm*proPO2 but not *Pm*proPO1. One of the interested viral proteins, WSSV164, previously identified as an immediate-early gene, was subsequently selected for further characterization. Co-immunoprecipitation assay in insect Sf9 cells indicated that WSSV164 interacts directly with *Pm*proPO2 but not *Pm*proPO1. An *in vitro* PO activity was also examined in sf9 cell co-expressing WSSV164 and *Pm*proPO2. Gene silencing of WSSV164 in WSSV-infected shrimp was revealed that knockdown of WSSV targeted by*Pm*proPO2 could significantly alter shrimp PO activity. Taken together, it was suggested that WSSV might interfere shrimp immune response by protein targeting in proPO system and melanization also plays essential role during WSSV infection.

SHRIMP IMMUNOLOGY POSTER PRESENTATIONS

ID199:

Development of white spot syndrome virus (WSSV) vaccine using shrimp glucose transporter 1 (Glut1)

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White spot syndrome virus (WSSV) is a large enveloped DNA virus, and in Taiwan and many other countries it is the causative agent of a disease that has led to serious mortality and huge economic losses in cultured shrimps industry. To reveal the mechanism of the virus entry and to establish the antiviral strategy, the cell receptor for virus entry and receptor binding protein should be identified. In our pervious study by using yeast-two-hybrid system, we found that a surface protein, glucose transporter1 (Glut1), can interact with WSSV envelope proteins VP53A. In order to identify Glut1's localization, *Litopenaeus vannamei* hemocytes were applied to confirm that Glut1 was localized on the cell surface by using confocal microscopy. Our previous research demonstrated that Glut1 could interact with *Penaeus monodon* chitin-binding protein (PmCBP), which is involved in WSSV infectome, therefore we can assume that Glut1 is being involved in WSSV infectome. Furthermore, we prove that Glut1 also can interact with other WSSV envelope proteins by western blotting analysis and co-immunoprecipitation (co-IP). As our result of *in vivo* and *in vitro* neutralization, we confirm that Glut1 plays an important role in WSSV infectome that can inhibit WSSV infection. To conclude, all of these results had important implications for our understanding of WSSV entry.

ID278:

Antiviral from YHV infected shrimp can reduce YHV infection

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Antiviral defense mechanisms in shrimp are relatively poorly understood and are generally believed to depend mainly on the innate immune system. In previous work, we discovered anti-viral peptides in insect cells persistently infected with viruses, and we hypothesized that similar peptides might exist in shrimp since they are also arthropods. To test this hypothesis, we prepared ultrafiltered (5 kDa pore size) hemolymph extracts from shrimp persistently infected with yellow head virus (YHV) and tested them for ability to protect naïve shrimp against YHV infection. Normal shrimp were injected with a small volume (25 µl) of the extract while control shrimp were injected with an equal volume of buffer. Both were held for 60- 72 hours before challenge with YHV. A negative control group was injected with buffer only. At 24 h after YHV injection, hemolymph was collected from the test and positive control shrimp and hemocytes were examined for YHV infection by immuno-staining. The results showed that YHV immunopositive hemocytes were reduced by 80% in shrimp injected with the 5 kDa filtrate when compared to the buffer-injected control. Mortality in the test group proceeded more slowly and morality at 5 days post challenge was 80% compared to zero in the control group. There was no mortality in the negative control group. In a second, similar experiment, the test shrimp were injected simultaneously with YHV and the 5 kDa filtrate. At 5 days post-challenge mortality was 40% in the test group compared to 70% in the positive control group. There was no mortality in the negative control. These preliminary experiments suggest that very small antiviral peptides similar to those described from insects may also be present in shrimp.

ID334:

Characterization of anti-lipopolysaccharide factor isoforms in penaeid shrimp and their potential applications

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Anti-lipopolysaccharide factor (ALF) is a kind of antimicrobial peptides (AMPs) with broad-spectrum antimicrobial and antiviral activities. In crustacean, multiple ALF isoforms always exist in one organism. Based on our transcriptome data, seven ALF isoforms were isolated from *F. chinensis*. Tissue distribution analysis revealed that different *FcALF* isoforms exhibited distinctive expression profiles. The mainly expressed tissues were stomach for *FcALF1*, lymphoid organ (Oka)/nerve/eyestalk for *FcALF2*, Oka for *FcALF3*, eyestalk for *FcALF4* and *FcALF5*, Oka/nerve/haemocyte for *FcALF6*, and Oka/haemocyte for *FcALF7*. In addition, the expression levels of *FcALF* isoforms could be apparently regulated after challenging with *Micrococcus lysodeikticus*, *Vibrio anguillarum* and white spot syndrome virus (WSSV). In order to study the function of *FcALF*s, we designed and synthesized peptides corresponding to the LPS-binding domain (LBD) of *FcALF*s and their structure-modified isoforms. The antibacterial and anti-WSSV activities of these LBD peptides were tested and the relationship between the sequence features of LBD and its activities were investigated. Different LBD peptides showed distinctive activities against Gram-positive and Gram-negative bacteria. Among them, the LBD peptide of FcALF2 showed apparently inhibitory effects on Gram-positive bacteria, while *FcALF7* exhibited apparently inhibitory activities against both Gram-negative and Gram-positive bacteria, especially against *M. luteus* and *V. anguillarum* with MIC ranges of 1-2 µM and 2-4 µM, respectively. Moreover, *in vitro* incubation of WSSV with *FcALF1*, *FcALF2*, *FcALF5*, or *FcALF7* could reduce the *in vivo* propagation of WSSV. Comparison on the antibacterial and antiviral activities among LBD peptides and their structure-modified peptides revealed that the disulfide loop and the basic amino acids in LBD peptides played key roles in its antibacterial activities. After increasing the amount of basic amino acids in the synthetic LBD peptides, their antibacterial activity spectrums were broaden and the antibacterial effect was significantly enhanced. Adding lysine residue into the LBD peptide could enhance its anti-WSSV activity which suggested that lysine was very important for its antivirus activity. These data comprehensively characterized the features and function of ALF in penaeid shrimp, and also provided a promising possibility for developing peptide drugs.

ID303:

Inside a shrimp's gut: its bacterial community, pathogenesis and immunity

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In addition to the digestion of food and the absorption of nutrients, the intestine establishes a strong defense against aggressions from the external environment. This important defensive task is composed of three essential parts: the microflora, the mucosal barriers and the local immune system. As a first entry point for many pathogens, the digestive tract and its defense mechanism are central to the health of the animal. Many diseases in shrimp, including the recently emerging EMS/AHPNS, originate or show symptoms in the gastrointestinal (GI) tract, and the cause of this disease it has been concluded is *Vibrio parahaemolyticus*. To understand pathogenesis of shrimp under natural conditions, the interactions between shrimp and a pathogenic bacterium in the GI tract was investigated. Using scanning electron microscopy (SEM) and histopathological studies, shrimp were infected via ingestion with pathogenic *Vibrio harveyi* (*Vh*) or *V. parahaemolyticus* (*Vp*) contained in *Artemia* as a feed and the results showed that shrimp had numerous bacteria attached randomly across the surface of the stomach, both as single and in large biofilm-like clusters at 6 h post ingestion. The persistence of highly virulent *Vh* or *Vp* resulted in the development of severe infections in the hepatopancreas and upper part of the midgut, but not in the posterior midgut and hindgut or even in the circulatory system. Oral infection is a better method for mimicking a natural infection and host-bacteria interactions occurring in the shrimp stomach may provide new insights into how to avoid and prevent infections.

ID308:

Characterization of cytokine homologue gene, IL-17, in Kuruma shrimp *Marsupenaeus japonicus*: gene expression and genomic analysis

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The interleukin-17 (IL-17) is known as inflammatory cytokine and promotes the inflammatory response, cell migration and granulopoiesis in vertebrates. Regarding the study of IL-17, reports are very few in invertebrates. This is the first report in crustacean. We report the identification and characterization of genes of IL-17, from kuruma shrimp, *Marsupenaeus japonicus* in this study. *Mj*IL-17 encodes a protein of 244 amino acids with an estimated molecular mass of 26.8 kDa.*Mj*IL-17 had no intron in genomic analysis. *Mj*IL-17 was conserved the IL-17 domain which is known as characteristic domain of IL-17 family. In gene expression analysis, *Mj*IL-17 did not show the significant gene expression in various organs of healthy shrimp and after white spot syndrome virus (WSSV) or *Vibrio penaeicida* injection. *Mj*IL-17 did not show the significant gene expression. It suggested that *Mj*IL-17 may be pseudo-gene in kuruma shrimp.

ID307:

Molecular cloning and expression analyses of multifunctional cytokine, TGF-β family, in Kuruma shrimp *Marsupenaeus japonicus*

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In vertebrates, Myostatin (MSTN) is included in the transforming growth factor beta (TGF-β) family and is known as growth differentiation factor (GDF)-8. It shows more than 90% identity withGDF-11. Regarding physiological function, TGF-β family have various functions such as regulation of proliferation, differentiation of cell. MSTN is known to involve in muscle growth. We report the Identification and characterization of genes of MSTN, from kuruma shrimp, *Marsupenaeus japonicus* in this study. *M. japonicus* Myostatin (*Mj*MSTN) gene comprises 1,391 bp and encodes a protein of 428 amino acids with an estimated molecular mass of 49.0 kDa. The *in silico* analyses such as domain, homology and phylogenetic analyses were performed. In analyses using amino acids sequences,*Mj*MSTN was found to be closely related to insect MSTN. In simulation of 3D structure,*Mj*MSTN formed dimer as with human MSTN. In the gene expression analysis, *Mj*MSTN gene showed high level expression in muscle and heart. On the other hand, *Mj*MSTN did not show the significant gene expression in hemocytes. In pathogen infection test, *Mj*MSTN decreased at 108 hours after white spot syndrome virus (WSSV)-injection. This data suggested that *Mj*MSTN may be involved in virus infection in kuruma shrimp.

ID306:

Cytokine homologue genes, VEGF, MIF and astakine, in Kuruma shrimp *Marsupenaeus japonicus*: simulation of 3D structure, gene expression analysis during WSSV infection and gene knockdown

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Cytokines are known as signaling protein molecules for intercellular communication. In vertebrates, the vascular endothelial growth factor (VEGF) family have various function such as promotion of angiogenesis, chemotaxis for macrophages and granulocytes, and lymphangiogenesis. The macrophage migration inhibitory factor (MIF) is known as an inflammatory multi-functional cytokine. In invertebrate, Astakine is invertebrate cytokine and can induce the hematopoietic stem cell differentiation in freshwater crayfish, *Pacifastacus leniusculus*. We report the characterization of genes of VEGF, MIF and Astakine from kuruma shrimp, *Marsupenaeus japonicus* in this study. The full-length cDNA sequence of the *Mj*VEGF1, *Mj*MIF and *Mj*Astakine genes were 845 bp, 894 bp and 1,589 bp. *Mj*VEGF1 formed dimer in simulation of 3D structure. The gene expression of *Mj*VEGF1 and *Mj*Astakine increased after white spot syndrome virus (WSSV) injection. In gene knockdown experiment, the gene expression of *Mj*MIF decreased over the course of 2.5 to 7.5 days in the *Mj*MIF-dsRNA-injected group. Additionally, in *Mj*MIF-dsRNA injected group, survival rate decreased after injection compared with control groups. These data suggested that cytokine may be involved with the biological defense mechanism and homeostasis in shrimp.

ID266:

Production and application of dsRNA in probiotics bacteria for antiviral defense in shrimp

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RNA interference (RNAi) technology through the trigger of double-stranded RNA (dsRNA) has been applied to inhibit viral replication in penaeid shrimp. In this study, we developed probiotics bacteria to produce dsRNA instead of *Escherichia coli* (*E. coli*), the bacteria could pose significant risk to human health and environmental contamination. To explore the possibility of using probiotics bacteria (*Lactobacillus spp.*) for production of dsRNA, we constructed plasmid containing RNA dependent RNA polymerase (RdRp) gene of Yellow Head Virus (YHV) cassette in shuttle vector, pWH1520, that was transformed into *Lactobacillus plantarum* (*L. plantarum*) and *Lactobacillus casei* (*L. casei*) by electroporation. After transformation, more than 100 colonies were obtained and the transformant colony containing recombinant plasmid was selected and confirmed by PCR. We will next examine the dsRNA-YHV expression level in *Lactobacillus spp.* and antiviral efficacy of dsRNA-YHV will be evaluated.

ID238

Melanization reaction products of shrimp display antimicrobial properties against their major pathogens

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Phenoloxidase (PO)-mediated melanization plays a key role in the production of cytotoxic intermediates and melanin products for microbial sequestration in invertebrates. Here, we show that the melanization reaction products of shrimp exhibit antimicrobial properties towards the major shrimp pathogens. The substrate specificity of POs was investigated in the gene-knockdown shrimp. Two *Pm*POs (*Pm*proPO1 and *Pm*proPO2) were found to display a substrate specificity towards monophenols and diphenols, and exhibit relatively weak activity against 5,6-dihydroxyindole (DHI). Systemic infection of the *Pm*proPO1/2 co-silenced shrimp with the fungus, *Fusarium solani*, led to a significantly increased mortality, suggesting an important role of *Pm*proPOs in shrimp's defense against fungal infection. Using L-DOPA, dopamine or DHI as a substrate, the melanization reaction products exhibited *in vitro* antimicrobial activities towards Gram-negative bacteria (*Vibrio harveyi* and *V. parahaemolyticus*), Gram-positive bacteria (*Bacillus subtilis*), and the fungus (*F. solani*). SEM analysis revealed the morphological changes and damage of cell membranes of *V. harveyi* and *F. solani* after treatment with shrimp melanization reaction products. Together, these findings demonstrate the crucial functions of the proPO system and the importance of melanization reaction products in the shrimp's immune defense.

ID202:

Localization of vp28 protein on baculovirus envelope and its oral vaccination displayed enhanced protection against white spot syndrome virus in *Penaeus monodon*

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White spot syndrome virus (WSSV) is an enveloped dsDNA virus responsible for white spot disease in shrimp and other crustaceans. VP28 is one of the major envelope proteins of WSSV and plays a crucial role in viral infection. In an effort to develop a vaccine against WSSV, we have constructed a recombinant baculovirus with WSSV-immediate early 1 (ie1) promoter which expresses VP28 at an early stage of infection in insect cells. Baculovirus expressed VP28 (Bac-VP28) was able to maintain its structural and antigenic conformity as indicated by immunofluorescence assay and western blot analysis. Interestingly, confocal microscopy and transmission electron microscopy results revealed that VP28 protein was localized on the plasma membrane of insect cells and it was successfully acquired by budding baculovirus from the insect cell membrane respectively. In our earlier observations with Bac-VP28 as a vaccine against WSSV via injection route, shrimp showed a significantly higher survival rate compared with wild-type baculovirus (Bac-wt). In order to administrate the vaccine in a feasible way, we fed the shrimp, *Penaeus monodon* orally with Bac-VP28 or wild-type baculovirus (Bac-wt) continuously for 7 days and challenged with WSSV after 3 and 15 days. Bac-VP28 vaccinated shrimp showed significantly higher survival rates 81.7% and 76.7% than Bac-wt or non-treated shrimp. To verify the protective effects of Bac-VP28, we examined in vivo expression of VP28 by immunohistochemistry and quantified the WSSV copy number by quantitative real-time RT-PCR. Our findings indicate that oral vaccination of shrimp with Bac-VP28 is an attractive preventative measure against WSSV infection that can be used in the field.

ID197:

Characterization of copper zinc superoxide dismutase isoform 5 (MjCu/Zn SOD-5) gene, a lineage-hemocyte of kuruma shrimp *Marsupenaeus japonicus*

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The innate defense system of crustacean, including shrimp consist of humoral responses and cellular responses. Humoral responses comprise antimicrobial peptides, reactive intermediate nitrogen or oxygen and the cascade system while cellular responses relate to phagocytosis, nodule formations and encapsulations. Humoral responses and cellular responses are mutual interaction through hemocytes because humoral factors affect hemocyte activities and hemocytes play as a source of many humoral factors. Moreover, different types of hemocytes play different immune functions. Therefore, hemocyte classification study is very important to understand activities of hemocyte. In decapod crustacean, three types of hemocyte namely hyaline cell (HC), semigranular cell (SGC) and granular cell (GC) which can be distinguished based on their morphologies. Unfortunately, this classification method cannot be applied on hemocytes of kuruma shrimp due to the morphological similarity of hemocytes in this species. This study was conducted to find molecular marker that can be used to distinguish hemocyte types of kuruma shrimp. In this study, the coding sequence of MjCu/Zn SOD-5 in kuruma shrimp was identified. The expression pattern of MjCu/Zn SOD-5 was also checked. The immune challenge trials and *in situ* hybridization using MjCu/Zn SOD-5 probes were carried out. The results showed that the coding sequence of MjCu/Zn SOD-5 was composed of 585 bp and encoding 194 amino acids. Expression pattern showed that MjCu/Zn SOD-5 was predominantly expressed in hemocytes. White spot syndrome virus (WSSV) and *Vibrio penaeicida* infections up regulated the mRNA expression of MjCu/Zn SOD-5, suggesting that this isoform has role in the innate immune system of kuruma shrimp. *In situ* hybridization results showed that MjCu/Zn SOD-5 expression was present in certain hemocyte indicating that MjCu/Zn SOD-5 can be a hemocyte marker to distinguish hemocyte types of kuruma shrimp.

ID195:

Identification of genes induced by non-specific double-strand RNA in kuruma shrimp *Marsupenaeus japonicus*

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It has been claimed that double stranded RNA (dsRNA) induces non-specific biodefence against various pathogenic organisms in shrimp. We also showed that dsRNA encoding green fluorescence protein (dsRNA-GFP) influence on the susceptibility of kuruma shrimp against white spot syndrome virus (WSSV). To identify the genes involved in the non-specific biodefence induced by dsRNA-GFP, comprehensive gene expression profiling using microarray was performed and a number of genes were likely to be differently regulated by the treatment. We further characterized of the genes that were significantly up-regulated by the treatments. From microarray result, 3 candidate genes significantly up-regulated by dsRNA-GFP treatment were selected and primers were designed. Shrimp were injected with 30μg of dsRNA-GFP or PBS, and the hemocytes were collected at 0, 3, 6, 24 and 48 hours post injection (hpi). The tissue expressions were investigated in different tissues of healthy shrimp. Changes of mRNA levels in hemocytes after the treatments evaluated by real-time quantitative (RT-qPCR). The genes named CUST1937, CUST7659 and CUST7677 were choosen. The deduced amino acid sequences of these genes were showed 99%, 42% and 26% identities to C-type lectin2 of *Marsupenaeus japonicus* (*Mj*CTL2), Williams-Beuren syndrome chromosomal region 27 protein (*Mj*WBS27) and DNA binding-protein RFX2 of *Heterocephalus glaber* (*Mj*RFX2), respectively. The mRNA levels of *Mj*CTL2 (5-fold) and *Mj*WBS27 (11-fold) were highest at 24phi but that of *Mj*RFX2 (6-fold) was highest at 6hpi. All of those 3 genes could be detected in the heart, hepatopancreas and lymphoid organ for healthy shrimp, but none in the hemocytes.

ID188:

In vivo study of potential bacterial probionts against pathogenic effect of Vibriosis in artemia culture

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Probiotics are bacteria which can act as health promoter of other organism by modifying the ambient microbial community, enhancing feed nutritional value, enhancing the host response towards diseases, or improving the quality of its ambient environment. Potential probiotics identified as *Micrococcus* spp. (JAQ07) and *Bacillus* spp. (JAQ04) were used as potential probiotics in this study. Both probiotics were identified as Gram-positive bacteria with different morphology. *Micrococcus* spp. was a rod-shaped bacterium whereas *Bacillus* spp. was cocci-shaped bacterium. In *in-vivo* assay, Artemia was used as a host and treated with both probionts at different concentrations (10^2, 10^4 and 10^6 CFU ml^{-1}) and challenged with *Vibrio alginolyticus* at concentration of 10^5 CFU ml^{-1}. *Bacillus* spp. able to enhance the survival of Artemia better compared with *Micrococcus* spp. when challenged with *Vibrio alginolyticus*. Artemia treated with *Bacillus* spp. at concentration of 10^6 CFU/ml and challenged with *Vibrio alginolyticus* showed 70% of survival compared with the survival of challenged Artemia with *Vibrio alginolyticus* only (20% survival rate) after 7 days. Meanwhile Artemia pre-incubation with *Micrococuss* spp. and challenged with *Vibrio alginolyticus* showed 68% survival. Both probionts are not harmful because no significant of survival was found compared to the control. *Micrococcus* spp. was able to slightly reduce the vibrios load in Artemia and culture water. However, *Bacillus* spp. was not able to reduce the vibrios load in water culture and Artemia. Nevertheless, both probionts demonstrated good characteristics as probiotic candidates for aquaculture industry.

ID336:

Protection against white spot syndrome virus infection in *Penaeus monodon* by oral administration of VP28-*Bacillus subtilis*

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White spot syndrome virus (WSSV) is currently the most serious viral pathogen of shrimp worldwide. It causes up to 100% mortality within 7 to 10 days in shrimp farms, resulting in huge economic losses. Recently, the effectiveness of oral vaccination with WSSV recombinant proteins in giant freshwater prawn (*Macrobrachium rosenbergii*), whiteleg shrimp (*Penaeus vannamei*), kuruma shrimp (*Marsupenaeus japonicus*), and crayfish has been documented. In this study, we investigated the effectiveness of oral delivery of surface-displayed VP28 on *Bacillus subtilis* spores (VP28-*Bacillus subtilis*) in *Penaeus monodon* upon immersion challenge with WSSV. The immune parameters, including haemocyte count, phenoloxidase activity, and superoxide dismutase activity were evaluated for different feeding regimes. Through the value of relative percent survival, the type and feeding regime were determined in order to achieve the highest survival rate of *Penaeus monodon* after WSSV immersion challenge.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

MARINE AND OTHER AQUATIC DISEASES

ORAL PRESENTATIONS

ID351:

Tail-rot and scale-drop disease in Asian seabass (*Lates calcarifer*): A review

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Tail-rot and scale-drop disease is a serious disease affecting primarily Asian seabass. The tail-rot disease was first noted in 1982/83, and tail-rot and scale-drop disease in 1985/86 in Penang after large numbers of hatchery-produced seabass fries were imported from Songkhla, Thailand. Smaller size seabass were more susceptible to tail-rot than larger size fish. The scale-drop disease was observed during the raining season at the end of each year (November to February). By 1990/91, the disease was seriously affecting up to 90% of all batches of farmed seabass, and affecting all fish sizes.

Danayadol *et al.* (1983) reported that the tail-rot disease in seabass fingerlings in Songkhla was caused by long rod myxobacterium, *Flexibacter columnaris*. Perngmark (1992) reported that the outbreak of tail-rot disease in seabass fingerlings in Penang was caused by two microorganisms, a ciliated protozoan, *Cryptocaryon irritans* and myxobacterium *Flexibacter maritimus*. The bacterium enters through the damaged caudal fin caused by the protozoan and spreads to the muscle fibers. The epidermis of affected fish loosens from the dermis, has numerous inflammatory cells between them, and bacteria spreads along the collagen fibers, cartilage, and between muscle fibers in large numbers (Leong, Perngmark & Wong 1992).

Win, Leong & Wong (1998) reported the destruction of spleen and kidney tissues, reduction of circulating erythrocytes and increased in size and number of melanomacrophage centers in scale-drop disease in seabass in Penang. The severity and destruction of various organs depends on the progressive stages of the disease.

The pathogenic bacterium causing the scale-drop disease in seabass was confirmed to be *Tenacibaculum* (=*Flexibacter*) *maritimum*, (reference strain NCIMB 2154 (ATT 43398) University of Tokyo) from tropical marine environment (Win 2000). Labrie *et al.* (2005, 2007) reported that diseased seabass were also found infected with *Streptococcus iniae*.

Gibson-Kueh *et al.*, (2012) reported wide spread pathological damages associated with scale-drop disease in Asian seabass. Single and double enveloped hexagonal virions were observed in transmission electron microscopy that resembles iridovirus or herpesvirus. They suggested that the pathological changes are possibly of viral aetiology. In this review, the two causative pathogens associated with cultured Asian seabass tail-rot and scale-drop disease are discussed.

ID287:

Diseases of barramundi or Asian seabass (*Lates calcarifer* Bloch) – current knowledge and management strategies.

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In the last two decades, the aquaculture of barramundi or Asian seabass (*Lates calcarifer* Bloch) has grown from small scale operations producing table-sized fish to capital intensive industrial farms supplying the fish and chips industry and gourmet restaurants. A wide range of diseases have been recognised as the cause of significant losses at different stages in the aquaculture of *L. calcarifer*. Farms routinely vaccinate against Streptococcosis, and other vaccines against *Tenacibaculum maritimum* and iridovirus are now available. Vaccinations do not replace good fish health management. Successful disease prevention and vaccination programs require farm staff with a good understanding of fish health. Disease is a manifestation of the combined dynamic effects of husbandry, weather conditions, pathogens present and the general health status of fish. Recently, many farms have become wary of 'scale drop syndrome', a disease that is poorly understood and can be devastating as it affects larger more valuable fish. 'Big belly' is a significant disease of juvenile fish, and its persistence in grow-out fish and associated adverse effects on growth may be largely unrecognised. As the industry intensify and increase stocking density at each site, it will become necessary to adopt site specific and national disease control programs.

ID178:

Description and quantification of mortality in marine finfish aquaculture in Northern Vietnam

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Although Vietnam is one of the most productive aquaculture countries in the world, marine aquaculture represents only about 1.7% of the overall farmed food fish production in Vietnam (FAO 2014). The sector produces fish primarily for local consumption, and is a target for further development by the Vietnamese government. Mortality of fish is considered one of the main constraints for the industry. Since quantitative insight into mortality patterns is not available, the objective of this study is to investigate mortality related production variables in marine finfish aquaculture in Northern Vietnam. The study consisted of two parts. Firstly, in April 2014, a questionnaire-based survey was conducted in 120 randomly selected small-scale marine farms in the Cat Ba area in Northern Vietnam. The questionnaire focussed on general farming practices, network structure, mortality events and health determinants. Secondly, based on geographic location and participation in the questionnaire, 10 farms were randomly selected and requested to record daily cage-level mortality over a 7 month period. Results of the questionnaire show that farms averaged 9 years at their current location and 24 cages per farm. The farms cultured multi-species, mainly red drum (*Sciaenops ocellatus*; 88% of farms), grouper (*Epinephelus spp.;* 68%), snapper (*Lutjanus spp*; 67%), Asian sea bass (*Lates calcarifer*; 54%), cobia (*Rachycentron canadum*; 42%) and pompano (*Trachinotus blochii*; 23%). Average expected mortality between stocking and harvesting was between 50 and 75% in all these species, but was more than 75% in up to 30 % of cages (species dependent). Of all farmers, only 3% documented records of mortalities. Preliminary results on daily mortality show high variation between farms and between cages within farms, indicating that mortality is an important consideration for productivity outcomes. Detailed examination of mortality patterns will facilitate epidemiology studies of possible disease transmission and related production and environmental factors.

ID281:

Epidemiological survey of pathogenic bacteria in ayu and other feral fishes in a river

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Ayu (*Plecoglossus altivelis*) is an osmerid fish with a one year life cycle, and is one of the most important freshwater fish species in Japan. The fish matures in rivers in autumn and then descends to the lower reaches of the rivers for spawning. The offspring migrate upstream from coastal areas in the spring and grow by feeding on periphyton (algae attached to the riverbed) in summer. In Japan, a large number of ayu are produced in hatcheries or collected from Lake Biwa, local rivers and sea coasts, and annually released into local rivers for freshwater fisheries and spot fishing. However, it has been reported that wild- and cultured-ayu are suffering from serious disease due to the effects of various pathogenic bacteria such as *Listonella anguillarum, Aeromonas hydrophila, Streptococcus iniae, Pseudomonas plecoglossicida, Flavobacterium branchiophilum, F. psychrophilum,* and *Edwardsiella ictaluri*.The disease problems in wild-ayu have become more serious in recent years. Outbreaks related to bacterial cold-water disease (BCWD) by *F. psychrophilum* among wild-ayu have been found in many rivers since the mid-1990s. Mass mortality due to *E. ictaluri* infection in riverine ayu was recorded in some rivers of Japan in the summer of 2007. Furthermore, it has been suggested that these pathogenic bacteria that affect ayu are also pathogenic toward other freshwater fish species. However, there are few reports concerning the prevalence of pathogenic bacteria in ayu and other feral fishes for the entire length of rivers, throughout all seasons. In this symposium, we report the prevalence of pathogenic bacteria among ayu and other feral fishes in a class river of the Tokyo metropolis, Japan during 2011 and 2012.

ID 213

Relationship between the protozoan parasite *Perkinsus olseni* and a decrease in Manila clam resources in Japan

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The Manila clam (*Ruditapes philippinarum*) is one of the most common bivalves in the coastal waters of Japan. However, since the mid-1980s, the annual catch of Manila clam has decreased dramatically due to a decrease in the bivalve's population. In the late 1990s, an infection in the Manila clam population with the protozoan parasite *Perkinsus olseni*, which is an OIE-listed disease, was identified in Japan for the first time. At the time, the infection had spread nationwide. Although the infection was suspected as a cause of the decrease of Manila clam resources, most researchers working on resource recovery were still suspicious of the involvement of the parasite to the resource depletion because of a lack of information pertaining to its virulence. Thus, we evaluated the virulence of a *P. olseni* infection on Manila clam using challenge experiments and estimated its impact on wild clam populations through field surveys in three tidal flats in Japan. In the challenge experiments, the survival rate in the challenged groups was remarkably lower than those of the negative controls. Wet tissue weight (WTW) of ~10^6 cells/g was suggested to be lethal to the host clams, and the infection intensities and mortality rates increased more rapidly in juveniles than in adults, and at higher temperatures. Additionally, the infection had a negative effect on the growth, condition index, filtration activity, and burrowing activity of the host under experimental conditions. In the field survey at Ariake Bay, where the Manila clam catch declined dramatically since late 1970s, the density of each year's cohort appearing during the survey began to decrease when the infection intensities reached ~10^6 cells/g WTW in summer; suggesting that this infection intensity was close to the lethal level. At the other two study areas, where clam resources were relatively abundant, the infection intensities in the populations were much lower than those at Ariake Bay. These results indicate that *P. olseni* is lethal to Manila clams and is one of the major causes for the decrease in Manila clam resources in Japan.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

MARINE AND OTHER AQUATIC DISEASES

POSTER PRESENTATIONS

ID338:

Vibriosis resistance and wound healing in onion- and ginger-fed brown-marbled grouper (*Epinephelus fuscoguttatus*) exposed to acute stress and infectious challenge

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The effects of stressors (hypoxia and hyposalinity) were examined in *Epinephelus fuscoguttatus* fed 4 diets supplemented with onion (ON), ginger (GNR), vitamin C (VC), and β-glucan (BC) at 2%, 2%, 3%, and 1% of the diet, respectively, and a non-supplemented control diet (CON). Cortisol level after acute D.O. stress was signicantly higher in CON compared to those in ON, GNR, BC, and VC. Cortisol was significantly lower in ON than the rest of the groups. When the fish were subjected to acute salinity stress, cortisol level was significantly higher in the control group than in any of the treatments. Cortisol levels did not differ among the supplemented groups. Following acute D.O. stress, cumulative mortality (CM) 15 days post-infection with *Vibrio harveyi* was highest in CON (90%) followed by BC (60%), VC (45%), GNR (35%), and lowest in ON (30%). However, no significant differences were found among the supplemented groups. When fish were subjected to acute salinity stress followed by *V. harveyi* injection challenge, CM was again highest in the CON group (80%), followed by BC (55%), VC (32%), GNR (32%), and lowest in ON (28%). CM in all the supplemented groups was significantly lower than the control. However, no significant differences were found among VC, GNR and ON, although they were significantly lower than BC. When fish exhibiting ulcerative lesions were monitored until they fully recovered, mean lesion resolution time (MLRT, in days) was 12 for GNR, 14 for ON and VC, and 16 for BC and CON. Only GNR had significantly shorter MLRT than the control. In summary, all the supplemented groups performed better than the control when all the parameters were considered. However, the best supplements were onion and ginger which compared favorably with vitamin C.

ID169:

An unidentified 'skin ulcer disease' of rainbow trout reared in seawater in Korea

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Rainbow trout (*Oncorhynchus mykiss*) is commercially important in fresh water aquaculture industry in Korea. Recently, rainbow trout farming in seawater in Jeju and southern coast of Korea has become popular, because of better growth performance in seawater than freshwater. However, an unindentified disease in fish during seawater adapting phase in Jeju has caused serious economic losses.

In February 2013, a batch of 20,000 rainbow trout (1 ~ 1.2kg bodyweight) cultured in seawater fish farm on landexperienced daily mortality of approximately forty fish for several weeks. Most of the diseased fish had severe ulcers on the skin - hence it is called 'skin ulcer disease'. No parasites or fungi were observed in diseased fish. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed to test for IPNV, IHNV and VHSV, which are important viral diseases of salmonidae. No bands specific for these pathogens were detected by PCR. In No CPE were observed on CHSE-214 and FHM cell inoculated with 0.45 um filtered lysate of tissues from diseased fish.

Based on literature analysis and our findings, 'skin ulcer disease' is similar to 'strawberry disease' caused by rickettsia-like organisms (RLO) of rainbow trout. Further study is necessary to elucidate cause of rainbow trout skin ulcer disease.

ID142:

Characterization of the outer membrane protein (OMP) of *Vibrio alginolyticus* and *Vibrio vulnificus* isolated from diseased grouper (*Ephinephelus* sp.)

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Vibrio alginolyticus and *Vibrio vulnificus* have been identified as the causative agents for vibriosis in groupers resulting in high mortality. For this reason, a study was conducted to characterize the outer membrane proteins (OMPs) and to determine the most antigenic protein of both Vibrio species for potential vaccine candidate. OMP characterization and identification were determined using Sodium Dedocyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) while antigenicity was determined using Western immunoblot technique. The results revealed that the OMP of *V. alginolyticus* and *V. vulnificus* were located at 33kDa and 50kDa, and at 33kDa, 40kDa, 48kDa and 75kDa, respectively. Further study by western immunodetection showed that the most antigenic protein of V.alginolyticus is the 33kDa while for V. vulnificus, they are the 33kDa and 75kDa proteins. The 33kDa proteins of both *V. alginolyticus* and *V. vulnificus* showed cross-reactions. The antigenic 33kDa protein band can be a potential vaccine candidate against both Vibrio species.

ID204:

Isolation of *Vibrio vulnificus* biotype I from disease outbreaks in cultured tiger grouper (*Epinephelus fuscoguttatus* Forsskal, 1775)

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The first isolation of *Vibrio vulnificus* biotype I from disease outbreaks in cultured tiger grouper (*Epinephelus fuscoguttatus* Forsskal, 1775) in southern Thailand has been described. Gross signs of diseased fish included dark coloration of body, anorexia, petechial hemorrhages on skin of tail and fins and ulceration of skin. Haemorrhage was observed in intestine, body cavity and spleen. Sequence data from 16S ribosomal RNA gene confirmed that these isolates were *V. vulnificus*. Biochemical characteristics indicated that the isolates belong to the biotype I, the human clinical isolate, based on indole production, ornithine decarboxylation, growth at 42°C and acid production from mannitol. To determine the human virulence potential of those *V. vulnificus* isolates, genotyping analysis using hemolysin gene (*vvhA*) and virulence-correlated gene (*vcg*) were conducted. The results indicated that eight of *V. vulnificus* isolates were the clinical-type isolates and one isolate was both clinical-type and environmental-type isolates. The susceptibility of groupers to *V. vulnificus* isolate was investigated by intraperitoneal injection (i.p.) with 10^5 CFU/ml bacterial suspensions. Only tiger grouper showed mortalities within 5 days post-injection, and developed clinical signs of hemorrhagic septicemia, with lesions on skin and internal organs similar to those found in disease outbreak. In a vaccination trial, tiger grouper vaccinated with formalin inactivated whole-cell vaccine exhibited relative percent survival (RPS) of 68% following homologous isolate challenge.

ID111:

Isolation of pathogenic bacteria from diseased marine fishes

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This study was conducted to isolate and identify pathogenic bacteria from diseased marine fish prior to use of alternative herbal treatment. Twenty diseased sea bass and orange-spotted grouper were sampled for bacterial culture. Ten species of bacteria were isolated. The most frequent bacteria isolated were *Vibrio alginolyticus* and *Vibrio vulnificus*. Pathogenicity tests revealed that *Vibrio alginolyticus* was more pathogenic than *Vibrio vulnificus*. The LD50 of *V. alginolyticus* in sea bass was 10^7 and 10^9 in grouper compared to 10^8 and 10^9 for Sea bass and grouper, respectively for *V. vulnificus*.

Piper betle in-vitro sensitivity study produced inhibition zones of 21mm for *V. alginolyticus* and 20mm for *V. vulnificus*, suggesting its potential for use as an alternative treatment for fish bacterial disease. The in-vivo use of piper betle extract in infected fish showed evidence of recovery as early as day-3 post treatment.

ID139:

Isolation and molecular confirmation of *Flavobacterium columnare* from natural outbreaks of columnaris disease in farmed catla (*Catla catla* Hamilton), and comparative sequence analysis of 16S-23S rDNA intergenic spacer region among its genomovars

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Flavobacterium columnare is currently an important bacterial pathogen hampering the productivity of fish farming worldwide. A presumptive columnaris disease outbreak in farmed catla (*Catla catla* Hamilton), was investigated with the aim of isolating and identifying the causative pathogen. *F. columnare* (strain RDC-1) was isolated from gills of infected fish and identified by conventional biochemical methods, species specific polymerase chain reaction (PCR) and sequencing of the 16S rDNA for molecular identification. Strain RDC-1 belonged to genomovar II with ≥99% similarity to available 16S rDNA sequences of *F. columnare*, and also shared ≥70% DNA-DNA relatedness with known strain of *F. columnare*. This is the first study on molecular confirmation of *F. columnare* in farmed catla in India. Bath immersion studies of RDC-1 showed development of columnaris disease in catla fingerlings within 7 days, with a cumulative mortality of 83.3%. Comparative sequence analysis of 16S-23S rDNA intergenic spacer region (ISR) of RDC-1 with other known strains of *F. columnare* validate the existence of two groups (A & B) in the genomovar I and II, with RDC-1 belonging to IIA. This heterogeneity within the genomovars is due to the presence of three hyper variable regions (V1, V2 & V3) in the ISR.

ID326:

Incidence of *Vibrio cholerae* non-O1 from plankton and seafood samples in Tamil Nadu, INDIA.

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The aim of the present study was to investigate the prevalence of *V. cholerae* from plankton (copepods), water and different kind of sea foods. Sampling was done at different intervals from coastal area, landing centre and fish market along the south east coast of Tamil Nadu, India. All the samples were primarily enriched with alkaline peptone water (APW) and plated on selective medium such as thiosulphate citrate bile salt sucrose (TCBS). Suspected colonies that resembled *V.cholerae* were subjected for biochemical reactions followed by serotyping and molecular confirmation. A total of 1112 samples were examined for the presence of *V. cholerae* and other vibrios, only 379 (34.0%) samples showed positive for *V. cholerae* and other vibrios. Copepods found to be associated with maximum number of vibrios 25 (80.6%), followed by coastal water 51 (63.7%) and shrimp 27 (54.0%) from fish market. *V. cholerae* isolated from copepods showed positive for haemolysin gene A (*hlyA*), but negative for cholera toxin (*ctxAB*) and toxin co-regulated pilus (*tcpA*). Molecular analysis revealed that only 5 strains having the gene for Type III Secretion system (TTSS) and they belonged to O5, O185, O8 and O14 serogroups of *V. cholerae*. Genotypic profiles of *V. cholerae* from copepod showed 93% similarity with clinical strain of *V. cholerae*. Antimicrobial susceptibility pattern were tested by the standard disk diffusion technique. All isolates showed antibiotic susceptibility to tetracycline and quinolone group (ciprofloxacin and ofloxacin) followed by ampicillin and chloramphenicol. Some exhibited multi-drug resistance against nalidixic acid, erythromycin, streptomycin and tetracycline. Although the virulence genes and antibiotic resistance pattern of *V. cholerae* varies from source to source, presence of virulence marker was evident through molecular analysis. The isolation of these pathogenic *Vibrio* from plankton (copepods) and seafood is a serious concern in aquaculture production. Because copepods are being used as a live feed in many aquaculture practices round the globe thereby affecting aquaculture production. Thus, there is a possibility of high risk to reduce aquaculture production and thereby chances are transmitting disease to the human through aquaculture system.

ID285:

The development of a grading tool for 'Big Belly' histopathology

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Lates calcarifer (Asian seabass or Barramundi) is an increasingly important commercial food fish species in Australia and South East Asia. Losses from both infectious and non-infectious cases pose a threat to economic returns in the aquaculture of *L. calcarifer*. 'Big Belly' is a significant bacterial disease of barramundi fry in Southeast Asian hatcheries, which can cause mortalities of 80 to 100% in juvenile seabass. The 'Big Belly' pathogen causes severe abdominal distension and adhesions in juveniles (<5g). Intracellular coccobacilli have been observed in gastrointestinal tracts of barramundi in grow-out sea cages, suggesting that the disease may persist as a chronic infection in older fish.

To aid future assessment for the severity of 'Big Belly' disease during various husbandry stages and culture conditions, a novel histopathological scoring system for Big Belly lesions was developed, highlighting the key features in organs most commonly affected by the disease, the gastrointestinal tract, liver and kidney. This will help the the assessment of the severity of the disease in a batch of fish. Risk factors associated with the disease will be highlighted.

ID274:

Disease outbreak in cage cultured cobia (*Rachycentron Canadum*) associated with *Streptococcus dysgalactiae*

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Disease outbreaks in cage cultured cobia *Rachycentron canadum* (L.) in Taiwan in 2007 to 2013, with mortality of 2.5-10% , were characterized by the presence of polyserositis, pericarditis and peritonitis. The microorganisms isolated from internal organs were Gram-positive cocci. The isolates were confirmed as *Streptococcus dysgalactiae* by a polymerase chain reaction assay that yielded the expected specific 259 bp amplicon. Additionally, partial sequence of the 16S-23S rDNA intergenic spacer region of the GCS strain isolates from fish was compared and produced 100% sequence identity with *S. dysgalactiae* (GenBank accession number AB252398). The genetic characterization was then determined by pulsed-field gel electrophoresis (PFGE) analysis. Based on PFGE, the *Apa* I or *Sma* I digestion patterns of chromosomal DNA of these isolates were grouped into three main clusters. Taiwanese strains were grouped into two clusters, and the *tet*(M) gene was detected in cluster 1 (pulsotypes: A1-A2 and S1-S3), but not in cluster 2 strains (pulsotypes: A3-A4 and S4-S5). Three Japanese strains from amberjack, *Seriola dumerili* (Risso) were grouped into cluster 3 (pulsotypes: A5-A7 and S6-S8) and displayed no mortality to cobia in achallenge experiment. In contrast, Taiwanese strains from cobia and snubnose pompano, (*Trachinotus blochii* L.) displayed a mortality rate of 50-87.5% in cobia.

ID243:

Prevalence and antimicrobial susceptibility of ESBL-producing *Escherichia coli* isolated from fish in the Mekong Delta

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A number of studies have identified that *Escherichia coli* are resistant to beta - lactam antibiotics group (ESBL producing bacteria), due to bacteria producing enzymes (TEM, SHV and CTX - M). Moreover, ESBL producing bacteria are capable of performing plasmid transfer between different bacterial species, causing failure in the treatment of human disease. Up to now, there have been few studies to determine the level of antibiotic resistant *E. coli* which can potentially spread to aquatic animals through the waterborne route in fish pond environment. This study aimed to investigate the prevalence and antimicrobial resistance of ESBL-producing *E. coli* in cultured and non-cultured fish in the Mekong Delta, Vietnam. We isolated ESBL-producing *E. coli* from striped catfish (*Pangasianodon hypophthalmus*) and red tilapia (*Oreochromis* sp.), using ChromAgar ECC. Confirmatory tests for ESBL production in *E. coli* were performed using the combination disk method based on the inhibitory effect of clavulanic acid (CVA). Antibiotic susceptibility tests for ESBL-producing *E. coli* were performed using the disk diffusion method with 15 antibiotics (Ampicillin, Cefotaxime, Cefotaxime/ Clavulanic Acid, Ceftazidime, Ceftazidime/ Clavualanic Acid, Ciprofloxacin, Chloramphenicol, Gentamicin, Kanamycin, Meropenem, Nalidixic Acid, Streptomycin, Tetracycline, Trimethoprim-Sulfamethoxazol, Fosfomycin). Of these antibiotics tested, E. coli were highly resistant to Ampicillin, Cefotaxime**,** Trimethoprim-Sulfamethoxazol(>95%). The results obtained showed that fish were contaminated with ESBL-producing *E. coli* species, and that fish could potentially be a vehicle for the transfer across species of antimicrobial resistance genes.

ID216:

Detection of a partial homology pathogen of abalone herpesvirus associated with abalone chronic mortality

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Abalone herpesvirus (AbHV) infection of cultured abalone (*Haliotis diversicolor supertexta*) induced acute high mortality rates. After the outbreak of acute abalone herpesvirus infection in Taiwan, a low-rate and persistent mortality of cultured abalone was noted, and cumulative mortality was high. Viral particles were not observed in moribund individuals using electronic microscopy. In these chronic infection, DNA samples were tested by PCR using 20 primer sets from direct sequencing of AbHV Taiwan isolate, and no significant fragment was amplified. In the histopathology of chronic infection, hemocytes infiltration in the connective tissue of digestive tracts and hemocytes of various stages were evident. A primer set from DNA polymerase of herpesviridae has amplified a 489bp product from DNA sample from abalone with chronic infections. This sequences have 35 percent (7/20) homology to DNA polymerase processivity subunit of Gallid herpesvirus (NCBI access no.YP_182371.1), and 35 percent (18/51) homology to orf29 of Alcelaphine herpesvirus 2 (NCBI access no.YP_009044411.1). These data suggest that these sequences can be from an emergent viral genotype. Another 1406 bp sequences was subsequently amplified from DNA sample from chronic infection cases, and these sequences have 92 percent (553/602) similarity to target gene of AbHV Taiwan isolate (NCBI serial no. KF537536.1). PCR using primer sets derived from these sequences revealed 58% (32/56) archived chronic mortality samples tested produced significant signals. The study of abalone chronic infection is still on-going, and further studies of DNA sequence associated with chronic mortality will be done.

ID215:

Comparison of the susceptibilities of the Manila clam (*Ruditapes philippinarum*), and the hard clam (*Mercenaria mercenaria*) to *Perkinsus olseni*

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Perkinsus olseni, a protozoan parasite of marine mollusks, is a major cause for the depletion of Manila clam populations in Japan. The parasite has been detected worldwide in many species of bivalves and gastropods. However, its pathogenicity has not been confirmed in all reported hosts. In addition, there are few studies on the differences in the susceptibilities of different mollusk species to *P. olseni*. In this study, we compared the susceptibilities of the Manila clam and hard clam to *P. olseni* (the latter is an introduced species from northern America to Japan), in order to elucidate the mechanisms underlying the pathogenicity and host specificity of the parasite.

Adult clams of both species were challenged by injection of trophozoites of *P. olseni*, and juvenile clams were challenged by exposure to zoospore suspensions. The infection intensity remained high in adult Manila clams over three weeks, but it decreased rapidly with time in adult hard clams. In the juveniles, the infection intensity increased rapidly in Manila clams and much more slowly in hard clams.

In order to identify the factors involved in the difference in in-vivo trophozoite propagation in Manila and hard clams, proliferation of the trophozoites and their phagocytosis were compared. Trophozoite proliferation was examined using a WST-8 assay for one week at two different concentrations of clam plasma, and the rates of phagocytizing clam hemocytes were measured at 0.5, 1, and 3 h after exposing the trophozoites to hemocytes. No significant differences were observed in the proliferation of trophozoites and the phagocytosis rates between the two species.

This study showed that the different susceptibilities of the Manila and hard clams to *P. olseni*. However, the factors for the difference are still unclear. We are currently investigating the intracellular survival of *P. olseni* in clam hemocytes as a possible factor related to the different susceptibilities of the host species.

ID210:

Bacteriophage as a prophylactic agent for Vibrio infection caused by *Vibrio parahaemolyticus* in oysters

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Vibrio parahaemolyticus is a major cause of foodborne illness, related to the consumption of raw contaminated seafood, especially oysters. To evaluate the effectiveness of various applications of a bacteriophage (phage), pVp-1, against a multiple-antibiotic-resistant *V. parahaemolyticus* pandemic strain (CRS 09-17), we designed artificial contamination models that are most likely to be encountered during the culture of oysters. When live oysters were treated with bath immersion with pVp-1 after CRS 09-17 challenge, the growth of bacterial strain was significantly reduced. After 72 h of phage application with bath immersion, bacterial growth reduction was observed to be 8.9 × 10^6 CFU/ml (control group) to 1.4 × 10 CFU/ml (treatment group). Phage preservation tests were conducted as described elsewhere with modifications. When pVp-1 were stored under the variable temperatures at -80 °C, -20 °C, 4 °C, or 20 °C for 36 months respectively, pVp-1 showed very little loss of its PFU at 4 °C. Our successful phage application emphasizes the potential use of the phage as a prophylactic agent to avoid Vibrio infection caused by *V. parahaemolyticus* from aquaculture to consumption.

ID170:

Parasitic and shell diseases of abalone (*Haliotis asinina*) in the Philippines

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The donkey's ear abalone *Haliotis asinina* is a very promising commercial product in the Philippines and elsewhere. However, its grow-out is being plagued by parasitic and shell diseases irrespective of fry source, whether wild or hatchery-bred. This study describes the first screening of *H. asinina* for the presence of parasites and shell diseases in the Philippines, and the continued monthly screening of different life stages of abalone reared at the SEAFDEC/AQD hatchery in 2013. Shells were grossly examined for the presence of parasites and shell-boring polychaetes. Polychaetes were extracted from shell burrows or the surface of the abalone shells using chemical vermifuge. Gross examination revealed shells fouled with the presence of burrowing polychaetes, belonging to the family *Dorveillidae* (prevalence, 41%). Nematodes were found with prevalence of 12%. Histological results showed that large oval ciliates (prevalence, 38%) were observed in the gills and digestive gland. Metacestodes or plerocercid (prevalence, 5%) were seen within the foot surfaces. These parasites did not elicit any inflammation or other host response, and appeared to have no ill effects on their host. Values of the condition index (CI) calculated monthly show significant decline in abalone infested with shell-boring polychaetes. These results, arising from gross examination and standard histological techniques, will be used to formulate practical control/prevention.

ID94:

Atypical *Lactococcus garvieae* isolated from previously vaccinated yellowtail (*Seriola quinqueradiata*)

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Lactococcus garvieae is a pathogen of fish of the genus *Seriola,* including *Seriola quinqueradiata*, *S. dumerili*, and *S. lalandi*, farmed in Japan. This pathogen has been isolated not only from marine fish species but also from dairy products, mammals, and rainbow trout (*Oncorhynchus mykiss*). The diversity of *L. garvieae* strains isolated from various host animals has been investigated.

The number of *L. garvieae* infections in Japan has decreased owing to the development of injectable and oral vaccines. However, in 2012 and 2013, atypical strains were isolated from yellowtail (*S. quinqueradiata)*, raised at different fish farms. The atypical *L. garvieae* strains could not be agglutinated with either KG+ or KG- phenotype *L. garvieae* antiserum.

The atypical strains were compared to typical *L. garvieae* strains by analyzing their 16S rDNA sequences, testing their bacteriological characteristics (API 20 STREP), and performing *L. garvieae* species-specific PCR assays (16S rRNA, 16S-23S internal transcribed spacer region, and phospho-β-galactosidase dihydropteroate synthase). For further comparison, biased sinusoidal field gel electrophoretic (BSFGE) analysis, DNA-DNA hybridization, and antigenicity analysis (gel diffusion method) were also performed.

Analysis of bacteriological characteristics and 16SrDNA sequences revealed that the atypical strains could be identified as *L. garvieae*. In species-specific PCR assays, except the dihydropteroate synthase assay, the atypical strains showed positive reactions identifying them as *L. garvieae*. BSFGE analysis revealed that the electrophoretic patterns of the atypical strains differed from those of the typical strains. The DNA-DNA hybridization analysis indicated that the atypical strains were closely related to the typical strains.

ID92:

Induction and characterization of a lysogenic bacteriophage of *Lactococcus garvieae* isolated from marine fish species

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Lytic bacteriophages (virulent bacteriophages) of *Lactococcus garvieae* have been studied to determine whether they can be used to prevent *L. garvieae* infection in host fish by oral treatment or injection. However, no information is available for lysogenic bacteriophages of *L. garvieae*. This study investigated the induction and characterization of a lysogenic bacteriophage of *L. garvieae,* isolated from marine fish species.

The genomes of more than 400 *L. garvieae* strains isolated from several marine fish species between 1974 and 2012 were analyzed using biased sinusoidal field gel electrophoresis (BSFGE). The *L. garvieae* isolates were determined to belong to 16 genotypes (S1–S16). A representative of each genotype was used to induce a lysogenic bacteriophage (phage) by treatment with 0.5 μg mL^{-1} freshly prepared mitomycin C. Subsequently, a cross-spotting assay was used to confirm lysogenic and indicator genotypes. The lysogenic genotypes were selected for phage isolation and concentration, and then phage DNA was digested for BSFGE analysis. A portion of the lysogenic phage DNA was sequenced to design primers for polymerase chain reaction (PCR) detection of lysogenic phages.

Ten of the 16 genotypes were found to contain a prophage (lysogenic), and indicator *L. garvieae* genotypes were identified. BSFGE analysis of lysogenic phage genomic DNA showed that the lysogenic phages were homogeneous. PCR analysis of the prophages revealed that all S1 isolates were lysogenic (30/30), but no S16 isolates were lysogenic (0/30).

ID 182

Comparative genomic analysis of *Candidatus* Xenohaliotis californiensis detected in abalone species *Haliotis* spp. in Japan

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Candidatus Xenohaliotis californiensis, a *Rickettsiales*-like organism (RLO) is the causative agent of withering syndrome (WS) in abalone as listed in the Office International des Epizooties (O.I.E.). The first WS-RLO infection was reported in North American black abalone (*Haliotis cracherodii*) in 1986. Since the 1980s, this bacterial infection has been suspected to be broad worldwide. WS-RLO has been detected in abalone, the genus *Haliotis*, by PCR in North and South America, Europe and Asia. In 2011, WS-RLO has first been found in cultured seedling of Japanese black abalone (*H. discus discus*) in Japan. Although the WS-RLO found in Japan is considered to be originated from imported abalone from U.S.A., the genetic analysis of the WS-RLO that is detected in Japanese abalone has not yet been well conducted. The aim of this study is to investigate whether genetic variation exists in the WS-RLO in Japan. The cultured and wild abalone inhabiting the costal area around Japan were surveyed for potential infection with WS-RLO by PCR using OIE primers amplifying 158bp, and then the 16S rDNA segment (1200 bp) of WS-RLO was amplified from the PCR-positive samples to determine the nucleotide sequences. PCR-positive results were obtained in 4, 3, 3, 2 and 1 animals of Japanese black abalone, Ezo abalone (*H. discus hannai*), giant abalone (*H. gigantean*), small abalone (*H. diversicolor supertexta*) and subspecies of small abalone *H. diversicolor diversicolor*, respectively. By alignment of 1200 bp sequence, all the 16 nucleotide sequences of WS-RLO gained form 3 species including Japanse black, Ezo and giant abalone were identical to those of North American black abalone (*Candidatus* Xenohaliotis californiensis, GenBank Accession number AF069062 and AF133090). While 3 sequences of 2 small abalone species were shared and 9-bp or 15-bp of 1200-bp were different from *Candidatus* Xenohaliotis californiensis (AF069062 or AF133090). These results suggest that in Japan there are *Candidatus* Xenohaliotis californiensis and one variant, detectable in 3 species of Japanese black, Ezo and giant abalone, and in small abalone, respectively.

ID323:

Overview of aquatic disease impacts throughout the ASEAN Community

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In 2012, collectively the ASEAN Community's aquaculture industries, representing at least 96 different classes of aquatic product, accounted for 18,376,575 tons of production, *i.e.* 20.32% of global aquaculture production (see Table 1). Within the Community there has been phenomenal growth, demonstrated by a 63.65% increase in output across the period 2008-2012, with an average year-on-year increase of 13.11 ± 1.06% (range 12.0-14.46%). And yet, this growth has been achieved in the face of the ongoing challenges from both predictable and unpredictable disease events that can result in significant economic losses to both stock and profitability. Here, in a wallchart, we summarise some of the most notable aquatic diseases impacting on aquaculture enterprises throughout the ASEAN Community.

Table 1. Aquaculture production across the ASEAN Community† in freshwater, brackish and marine environments in 2012 presented in tons for each broad class of aquatic species. Figures are calculated from the FAO FishStatJ databases using the latest figures available.

Class	No. spp.	Tonnage			Total Prod.	% of ASEAN
		Fresh	Brackish	Marine		
Algae	5	0	776,166	8,056,249	8,832,415	48.063
Amphibia	2+	31,843	0	6	31,849	0.173
Aquatic inverts	1+	0	0	1,173	1,173	0.009
Crustacea	14	42,898	1,613,392	56,706	1,712,996	9.322
Holothuroidea	1	0	0	475	475	0.003
Mollusca	9	10,000	5	703,326	713,331	3.882
Pisces	62+	6,026,815	917,813	136,550	7,081,178	38.534
Sauropsida	2+	2,600	0	0	2,600	0.014

†National productions were: Brunei Darussalam 550 t; Cambodia 74,000 t; Indonesia 9,599,765 t; Lao PDR 101,895%; Malaysia 615,270 t; Myanmar 885,569 t; Philippines 2,541,965 t; Singapore 3,584 t; Thailand 1,233,877 t; Vietnam 3,3320,100 t.

ID 414:

Establishment of MALDI Biotyper system for rapid identification of two main causative agents of streptococcosis, *S. parauberis* and *S. iniae*, in olive flounder (*Paralichthys olivaceus*)

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Streptococcosis of cultured olive flounder contributes to major economic losses in the aquaculture industries in South Korea and Japan. The major etiological species include *Streptococcus parauberis* and *Streptococcus iniae*. In order to develop a novel diagnostic tool, we established mass spectrometry-based protein profiling of two causative agents using MALDI Biotyper matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) system that enables a rapid and simple microbial identification with great accuracy. Spectral profiling of respective bacterial colony was obtained from a total 249 isolates that have been preliminary examined using various phenotypic and biochemical assays. Microorganisms were identified by spectral pattern-based matching with the libraries in the Biotyper 2.0 software. With respect to *S. parauberis*, 94 (89.5%) isolates were correctly identified to the species and genus levels, whereas most of *S. iniae* isolates showed probable or no reliable identity, which is mainly due to a lack of relevant reference spectra included in the database. Overall, this study shows the high potential of using MALDI Biotyper for fast and relatively non-laborious identification of *S. parauberis* and further fills the gaps in the protein-based identification databases, particularly for *S. iniae*.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

DIAGNOSTICS
ORAL PRESENTATIONS

ID332:

Evolution of rapid diagnostic technologies – what does the future hold for aquaculture?

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Disease is considered a major constraint to aquaculture production globally. Control of disease is complex and relies heavily on a combination of pathogen detection, disease diagnosis, treatment, prevention and general health management. Clearly speed in pathogen detection is crucial to prevent the spread of disease. Rapid diagnostic technologies have evolved quickly for use in clinical and veterinary medicine and many of these methods have been adapted for use in aquaculture. Advances in modern biotechnology, nanotechnology and information technology have hastened the development of faster, more sensitive methods. Automated sequencing equipment is also available for proteins and whole genomes. Many new methods are under development and commercial products/pond side tests are now available, assisting with standardisation of protocols and availability of tests. Pathogen detection methods for use in aquaculture need to be robust yet sensitive, as well as affordable, and requirements will depend whether methods are to be performed in the laboratory or in the field. This presentation will provide a review of the evolution of rapid diagnostic technologies taking into consideration the limitations of both existing and novel methods, how applicable some of the new methods are to aquaculture, and a look into future prospects.

ID305:

The problem of emerging diseases – the role of microscopy and histopathology as an integrating component in disease diagnosis

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Investigating an emerging disease problem requires a multidisciplinary approach and integration of inspection, diagnostic and epidemiological expertise. It is important at the outset to 'invest' the time in taking comprehensive samples and field data to avoid the expense associated with repeat sampling. It is also crucial to ensure that mechanisms are in place to collate data from each of these disciplines in order to achieve a robust assessment of the problem. Histopathology is the oldest medical laboratory discipline but remains a font line approach for understanding the impact of disease at the individual and population level. High levels of technical expertise are required to produce the quality required for examination. However, histopathology (combined with electron microscopy) is the only technique that can link the presence of pathological change with the presence of pathogen and in addition, can identify toxicologic pathology and mixed infections. It also provides prognostic capability. As such, its application in elucidating the nature of an emerging condition is crucial, particularly where infectious agent have not been detected or isolated by other methods; red mark syndrome (RMS) in salmonids being an example. In our laboratory we strive to routinely take matched individual specimens for histology, EM and molecular techniques and this has proved valuable, particularly for viral and over dispersed parasitic infections in invertebrates. Recently, the advent of new approaches for molecular detection of pathogens has raised issues regarding significance of detection of pathogen DNA in environmental samples. Histological screening of resident populations provides a means to identify significant disease presence and has the potential to link this with the environmental signal; thereby contributing to assessment of significance of the latter. This presentation uses a series of examples showing the utility of histopathology for determining the nature of emerging disease and the necessity to combine pathological data with pathogen detection (molecular identification and sequencing), pathogen ecology, culture and epidemiological information to understand the nature of the disease, its impact and potential risk as well as informing decision making for control and additional investigation. The importance of networking and training for histopathologists is also highlighted.

ID158:

A novel immunomagnetic reduction assay for dection of fish iridovirus in groupers

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A rapid, reliable, sensitive, automatic detection method is an important issue for aquaculture. With an efficient detection method, we can monitor the pathogen spreading and enhance the economic benefits. In last decade, grouper iridovirus (GIV) cause serious economic loss, and it is an important aquatic virus which infects variable species of fish and frog. The technology to measure the magnetic signals in this work is called immunomagnetic reduction. By antibody-functionalized magnetic nanoparticles, specific viruses can be magnetically labeled, and furthermore it can be quantitatively detected by measuring the related magnetic signals. In this study, the detected GIV concentration using immunomagnetic reduction was 91% related to that of using real-time polymerase chain reaction. The low-detection limit in assaying iridovirus was found to be 10 $TCID_{50}$/ml. There was no significant interference from other grouper viruses, such as nervous necrosis virus (NNV). These results showed the feasibilities of screening iridovirus in grouper using immunomagnetic reduction.

ID329:

Development of monoclonal antibody based farmer level diagnostics for aquatic pathogens in India

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In the fast growing aquaculture in India, four pathogens – white spot virus (WSV) and *Vibrio* of shrimp, *Aeromonas hydrophila* and *Aphanomyces invadans* of fish – are causing annual estimated economic loss of more than Rs 1000 crores (165 million USD). Although, several DNA based and biochemical diagnostics are available for detecting these pathogens, their overall impact in health management is not significant. Against this background, monoclonal antibody (MAb) based flowthrough immuno diagnostics have been developed for simple, sensitive and low cost farmer level detection. Panel of MAbs against these four pathogens were raised, cloned and characterised by Westren blot and Isotyping. These MAbs used for antigen characterisation, epitope analysis and serotyping of the pathogens. Later from the panel selected MAbs were used for developing an enzyme based flowthrough immunoassay (FTA) and their sensitivity and cost compared with 1 step PCR. FTA is an improved version of immunodot which uses a nitrocellulose membrane baked onto adsorbent pads enclosed in a plastic cassette, favouring closer interaction between reagents (Ag & Ab and Antibody enzyme conjugates) resulting in rapid focused reaction with dots. Sharp purple dots developed with an antigen against the white background of the nitrocellulose membrane considered positive. The enzyme based FTA was later improvised by replacing with MAb linked with nanogold for better resolution and minimum background reaction. The FTA has been developed to a kit called 'RapiDot' for commercialization by industry. The RapiDot is much sensitive than the conventional immonodot. Sensitivity of the RapiDot in general for the white spot virus and *A. hydrophila* ranged from 100-1000 times higher than 1 PCR where as for *A. invadans* and *vibrio* it was equal to 1 PCR. RapiDot could also detect pathogen in higher number of samples compared to 1 PCR. The RapiDot was 15 to 20 % cheeper than 1 PCR each costing Rs 40/ sample (< a USD) hence cost effective. The test time including sample preparation is max 4 -5 min. The test is very simple to farmer level, ideal for use in farm, landing centre and hatcheries without need of any equipment and gadget. The test reagents have shelf life of 4 – 6 m at fridge temperature. The RapiDot for WSV has been evaluated in field since 2006, with nearly 350 kits (35,000 samples) sold by the university in India and abroad. White spot RapiDot is being transferred to a multinational company for commercialisation. The other 3 kits *A.hydrophila, A. invadans* and *Vibrio* are undergoing field trials.

ID310:

Evaluation of diagnostic tests for detection and identification of spring viraemia of carp virus in imported ornamental fish.

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Spring viraemia of carp virus (SVCV) causes an acute, severe haemorrhagic and contagious disease that predominantly affects cyprinid fish species, with the most susceptible being common carp (Cyprinus carpio carpio). The disease, spring viraemia of carp (SVC), is an OIE notifiable disease that was first identified in Europe but has since been identified in other regions around the world. SVCV is currently classified within the genus Vesiculovirus in the Rhabdoviridae family and is grouped with other related fish vesiculoviruses, including pike fry rhabdovirus. The fish vesiculoviruses comprise 4 genogroups, of which all SVCV isolates are found in Genogroup 1. Within Genogroup 1, four subgroups of SVCV exist and these can be differentiated based on genotype and geographic origins. Three are regarded as European subgroups and the fourth as an Asian subgroup (Taylor, 2013). The global movement of fish through the ornamental fish trade has led to the Asian SVCV subgroup being found in the UK and North America. The first outbreak of SVC in the U.S.A. was found to originate from fish imported from Asia. Australia imports up to 18 million ornamental fish annually with a large number of these being SVCV-susceptible species from Asia. The OIE prescribes the use of virus isolation for the detection of SVCV in sub-clinically infected fish with nested PCR being recommended for confirmation. This methodology is time-consuming, labour-intensive and expensive, and is not practical for large scale screening of samples. In order to assess the feasibility of using real-time PCR as part of an alternative testing strategy for testing imports, we evaluated and compared the relative analytical sensitivity and specificity of 3 real-time PCR assays with the currently recommended OIE procedures of virus isolation and nested PCR. We found that real-time PCR was specific for the SVCV genogroup 1 viruses tested and two of the real-time PCR assays had similar limits of detection to virus isolation. This suggests that real-time PCR could provide a rapid, cost effective method for the screening and detection of SVCV in imported ornamental fish.

ID80:

Bacteriophages as an indicator for prediction of bacterial disease occurrence in aquaculture

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It is generally difficult to isolate/detect fish-pathogenic bacteria from fish culture environments by conventional culture techniques or even molecular ones, mainly due to their few numbers if present. Bacteriophages exhibit strong specificity to host bacteria and appear abundantly in natural environments after bacterial cell destruction, and thus phages are expected to be a good indicator for the presence of specific pathogen in fish culture environments that will lead to the prediction of disease occurrence. In order to search this possibility, we examined a relationship between appearance of specific phages in fish-rearing seawater and progress of the disease in cultured fish. The present study targeted atypical *Edwardsiella tarda* infection (edwardsiellosis) in red seabream *Pagrus major*. The study was conducted at Ehime Prefectural Fisheries Research Center (EPFRC) and two aquaculture grounds (A, B) in Ehime Prefecture, Japan, in 2012-2013. *E. tarda* phages were isolated from seawater using an enrichment culture method followed by a double-agar-layer method. In a net pen culture of EPFRC, *E. tarda* phages were first isolated (10^1 pfu/L) in August and increased in the number (10^{2-3} pfu/L) in September and October when fish mortality due to edwardsiellosis was recorded, and decreased in the number (10^1 pfu/L) with cessation of the disease. In both aquaculture grounds, edwardsiellosis prevailed intensively from August to September, and the phages were first detected in April (A) and June (B) at 10^2 pfu/L and 10^3 pfu/L, respectively. The highest concentration of phage reached 10^4 pfu/L in August and then slowly decreased. Most of the *E. tarda* isolates from diseased fish during the survey were susceptible to the phage isolates, suggesting that the phages were originated from disease causing *E. tarda* cells. The present results suggest that *E. tarda* specific phages are useful as an indicator for prediction of the initial occurrence, peak, or cessation of edwardsiellosis in red seabream culture.

ID184:

Detection of concatemeric DNA as an indicator of koi herpesvirus infection

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Herpesvirus forms head-to-tail concatemer (long continuous DNA molecule containing multiple copies of the viral genome) as a replicating intermediate in the host. In this study, we developed a concatemeric DNA specific PCR assay for detecting the infection stage of koi herpesvirus (KHV). The 295 kbp dsDNA KHV genome consists of a 251 kbp unique long (UL) region and two 22 kbp direct repeats (DR_L and DR_R) at each genome terminus. We designed a new primer set (DR primer set) on the DR region spanning the presumptive concatemeric junction. Using the DR primer set, a PCR product was obtained from KHV-infected CCB cells, but not from the cell culture supernatant. Sequencing analysis of the PCR amplicon revealed that the concatemeric junction consisted of right terminus of DR_R and left terminus of DR_L. The synthesis of concatemeric genome in virus-infected CCB cells was examined in a time course experiment using the DR primer set together with viral mRNA of terminase gene, copy numbers of viral genome, and infectious viral titer. The mRNA was first detected in the CCB cells at 6 hours post-viral inoculation (hpi), and the copy number of viral genome in the cells increased at 12 hpi. Progeny virus was detected in the cell culture supernatant at 24 hpi. The concatemeric DNA was detected in the CCB cells at 18 hpi, just before detecting of the progeny virus. These results indicate that the developed PCR assay can be an indicator of intracellular viral infection, and it possibly has an ability to evaluate the host range of KHV.

ID312:

ShrimpGPAT: a gene and protein annotation tool for knowledge sharing and gene discovery in shrimp

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Although captured and cultivated marine shrimp constitute highly important seafood in terms of both economic value and production quantity, biologists have little knowledge of the shrimp genome and this partly hinders their ability to improve shrimp aquaculture. To help improve this situation, the Shrimp Gene and Protein Annotation Tool (ShrimpGPAT) was conceived as a community-based annotation platform for the acquisition and updating of full-length complementary DNAs, Expressed Sequence Tags, transcript contigs and protein sequences of penaeid shrimp and their decapod relatives and for *in-silico* functional annotation and sequence analysis. ShrimpGPAT currently holds quality-filtered, molecular sequences of 14 decapod species (~500,000 records for six penaeid shrimp and eight other decapods). The database predominantly comprises transcript sequences derived by both traditional EST Sanger sequencing and more recently by massive-parallel sequencing technologies. The analysis pipeline provides putative functions in terms of sequence homologs, gene ontologies and protein-protein interactions. Data retrieval can be conducted easily either by a keyword text search or by a sequence query via BLAST, and users can save records of interest for later investigation using tools such as multiple sequence alignment and BLAST searches against pre-defined databases. In addition, ShrimpGPAT provides space for community insights by allowing functional annotation with tags and comments on sequences. Community-contributed information will allow for continuous database enrichment, for improvement of functions and for other aspects of sequence analysis. Regularly updated and expanded with data on more decapods, ShrimpGPAT is publicly available at http://shrimpgpat.sc.mahidol.ac.th/ for the research community to contribute knowledge and insights about the properties of molecular sequences for better, shared, functional characterization of shrimp genes.

ID245:

The genome and occlusion bodies of marine *Penaeus monodon*nudivirus (PmNV, also known as MBV and PemoNPV) suggest that it should be assigned to a new nudivirus genus that is distinct from the terrestrial nudiviruses

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Penaeus monodon nudivirus (PmNV) is the causative agent of spherical baculovirosis in shrimp (*Penaeus monodon*). This disease causes significant mortalities at the larval stage and early postlarval (PL) stage and may suppress growth and reduce survival and production in aquaculture. The nomenclature and classification status of PmNV has been changed several times due to morphological observation and phylogenetic analysis of its partial genome sequence. In this study, we therefore completed the genome sequence and constructed phylogenetic trees to clarify PmNV's taxonomic position. To better understand the characteristics of the occlusion bodies formed by this marine occluded virus, we also compared the chemical properties of the polyhedrin produced by PmNV and the baculovirus AcMNPV (*Autographa californica* nucleopolyhedrovirus). We used next generation sequencing and traditional PCR methods to obtain the complete PmNV genome sequence of 119,638 bp encoding 115 putative ORFs. Phylogenetic tree analysis showed that several PmNV genes and sequences clustered with the non-occluded nudiviruses and not with the baculoviruses. We also investigated the characteristics of PmNV polyhedrin, which is a functionally important protein and the major component of the viral OBs (occlusion bodies). We found that both recombinant PmNV polyhedrin and wild-typePmNV OBs were sensitive to acid conditions, but unlike the baculoviral OBs, they were not susceptible to alkali treatment. From the viral genome features and phylogenetic analysis we conclude that PmNV is not a baculovirus, and that it should be assigned to the proposed*Nudiviridae* family with the other nudiviruses, but into a distinct new genus (*Gammanudivirus*).

ID164:

In vivo and *in silico* comparison of fish-associated and non fish-associated subtypes of *Streptococcus agalactiae*

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Streptococcus agalactiae causes morbidity and mortality in farmed, wild and ornamental fish. Based on epidemiological studies and challenge experiments, disease in fish is predominantly caused by beta-haemolytic strains belonging to clonal complex (CC) CC7, such as sequence type (ST) 7, or by non-haemolytic strains belonging to clonal complex (CC) 552, including ST260. The most common strain in aquatic mammals, ST23, has a wide homeothermic and poikilothermic host range but it has never been reported in fish. The aim of this study was to determine whether ST23 is non-virulent in fish and to identify potential genomic markers of fish-adaptation of *S. agalactiae*. Intraperitoneal challenge of tilapia with *S. agalactiae* showed that ST260 is lethal at doses from 10^7 down to 10^2 cfu per fish, whereas ST23 does not cause disease at a dose of 10^7 cfu per fish, confirming that ST23 is non-virulent in this host species. Comparison of the genome sequence of ST260 and ST23 with genomes of strains derived from fish, cattle and humans revealed the presence of a number of genomic elements that are either unique to CC552 or shared by strains of CC552 and CC7 but not by ST23 or strains belonging to other STs or CCs. Many of these genes occurred in clusters exhibiting typical signatures of mobile genetic elements, suggesting acquisition through horizontal gene transfer. PCR-based screening of a collection of *S. agalactiae* isolates from fish, aquatic and terrestrial mammals confirmed the association of selected genes with fish-derived strains. Some fish-associated genes encoded proteins that potentially provide fitness in the aquatic environment.

DIAGNOSTICS
POSTER PRESENTATIONS

ID 422:

An integrated workflow solution for shrimp pathogen detection

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An increasing number of new and emerging diseases are posing challenges for aquaculture, more specifically in the detection and management of these diseases. Diseases such as white spot syndrome, yellow head disease, and Taura syndrome have inflicted great economic and food production losses worldwide.

Quantitative, real-time polymerase chain reaction (PCR) is a powerful tool for fast, accurate, and sensitive detection. As a leader in real-time PCR reagents and instruments, we have leveraged this technology to develop a complete workflow consisting of reliable reagents for detection of shrimp pathogens. We offer a portfolio of products for singleplex (single-template PCR reaction) TaqMan assays to top 5 OIE listed shrimp pathogens. In this article, we describe some results of validation by external laboratories who achieved reproducible PCR efficiency with high specificity to the shrimp pathogen analyte. To address the need to save time and effort in shrimp molecular screening and analysis, we've also developed two shrimp multiplex (multiple template PCR reaction) assays. The multiplex assays, one for DNA viruses (IHHNV and WSSV with internal IPC) and one for RNA viruses (YHV, IMNV and TSV with internal IPC), are developed to work on the 7500 Fast Real-Time PCR system. Here, we also describe some success in preliminary testing conducted in an external laboratory as well as in ring test participation.

ID176:

Development of Co-agglutination Kit for the Diagnosis of *Edwardsiella tarda* Infection in Fish

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Immunodiagnostic test using staphylococcal co-agglutination has been recognized for its ability to detect pathogen in fish. The method is simple, fast, and does not required sophisticated laboratory equipment, but its value has been overshadowed by more expensive tools such as PCR or FAT. This study envisioned to develop a rapid test kit for detecting Edwardsiella tarda infection in fish. It was found that this method is sensitive and specific to detect of E. tarda directly from fish tissue with positive reaction characterized by granular appearance (clumps) of reagent-sample suspension visible to naked eye. The best result obtained with application of 50µl reagent with 50µl extracted organ, within sixty minutes of observation and 1:1 organ/tissue dilution (w/v). Kidney and ulcer provide best agglutination reaction within the test of different samples. The result indicated that co-agglutination can reduced an enormous time (from 4-5 days into a few hours after samples submission) and complicated preparation in conventional biochemical test, but also inexpensive compared to more advance test such as FAT or PCR. This method can cut through the idea of the most suitable method regarding the lack of equipment experienced by many laboratory or fisheries institution in South East Asia, especially in the upcoming AFTA 2015 where a more massive fish distribution and development of aquaculture is expected.

ID124:

Biotyping of *Streptococcus agalactiae* isolated from Nile tilapia farm in Thailand based on virulence genes categorization

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Streptococcus agalactiae is a pathogenic bacteria commonly found in Nile tilapia (*Oreochromis niloticus* Linn.) culture of Thailand and all around the world. Fish farmers treat this infection by applying antibiotic in the diet, but the results are not acceptable. Thus, it is imperative for fish scientists to find the measures that can effectively prevent the outbreak of this disease. Vaccination should be the practical method that can be applied with tilapia culture in Thailand. To develop the effective vaccine against this disease, biotypes or serotypes of the bacteria should be identified. This study investigated the biotype variation of *S. agalactiae* categorized from virulence genes. *S. agalactiae* were isolated from Nile tilapia, cultured in ponds and cages from the central, northern, northeastern and southern part of Thailand. Total of 390 fish samples were collected and 120 isolates were identified as *S. agalactiae* by 16S rRNA PCR. Three categories of 14 virulence genes (adhesins, invasins and immune evasions): *fbsA* (fibrinogen-binding protein FbsA), *fbsB* (fibrinogen-binding protein FbsB), *pavA* (fibrinogen-binding protein), *scpB* (C5a peptidase), *lmb* (laminin-binding protein), *cyl* (β-hemolysin/cytolysin), *cfb* (CAMP factor), *spb1* (hemolysin III), *hylB* (hyaluronate lyase), *rib* (surface protein rib), *bca* (C-α protein), *bac* (C-β protein), *cspA* (serine protease cspA) and *pbp1A/ponA* (penicillin-binding protein 1A) were identified by Multiplex PCR. The identified virulence genes can be categorized into 2 biotypes of *S. agalactiae*: Biotype I (*bac* gene) and Biotype II (*lmb*, *scpB* and *spb1* gene). The two biotypes shared *fbsA*, *fbsB*, *pavA*, *cyl*, *cfb*, *hylB*, *rib*, *bca*, *cspA* and *pbp1A/ponA* gene. The distribution of these biotypes was geographically located in tilapia culturing area of Thailand. It is interesting to find that the number of isolates identified under each biotype were quite similar. Pathogenicity of the biotypes was compared by intraperitoneal injection into juvenile tilapia. The challenge indicated the higher virulent of Biotype II than Biotype I. We hope to establish the serotype of *S. agalactiae* isolates categorized by one or two more virulence genes such as capsular polysaccharide gene (*cps* cluster). Eventually, the selection of candidate antigens will be finalized for the development of practical and effective vaccine for tilapia culture in Thailand.

ID79:

Quantitative detection of piscine nodavirus in fish-rearing seawater

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Viral nervous necrosis (VNN) caused by piscine nodavirus (betanodaviruses) has caused high mortality in a variety of cultured marine fish species worldwide for these two decades. Although vertical transmission of the virus from broodstock to offspring has been well understood in VNN of some fish species, virus transmission mode for fish at grow-out stages in the open sea still remains unclear. In the present study, we established a high sensitive method to detect betanodavirus (RGNNV genotype) from seawater by combination of virus concentration and real-time PCR method. Prior to real-time PCR detection of the virus, an iron-based flocculation method was used for concentration of the virus in seawater. This method was so effective that almost 100% of RGNNV experimentally spiked into seawater was recovered and the detection limit was 1.2×10^3 copies/L of seawater. In the first trial of virus detection from seawater, juvenile sevenband grouper *Epinephelus septemfasciatus* (donor) injected with RGNNV were placed in a tank, the drainage from which was introduced to a tank where virus-free sevenband grouper (recipient) were kept. There was a correlation between disease progression in the donor and RGNNV concentration in the drainage, and the highest virus concentration was 10^8 copies/L at 10 days post-infection when mortality of donor stopped with cumulative mortality of 44%. One week after the last death in the donor, mortality started in the recipient, resulting in 8% cumulative mortality. In the second trial, we succeeded in detecting RGNNV from seawater nearby floating net pens where VNN was prevailing in cultured juvenile sevenband grouper (n=115,000). The highest concentration of the virus was 10^6 copies/L. In addition, we successfully isolated RGNNV using E-11 cells from seawater. The present iron-based flocculation and real-time PCR method to detect RGNNV in seawater will be useful to investigate infection mechanism in VNN encountered during net-pen culture.

ID187:

Development of qPCR of *Nocardia seriolae* and its application to environmental samples

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Nocardia seriolae is a Gram-positive, acid fast bacterium with branched hyphae which causes nocardiosis in cultured marine and freshwater fish in Asia and Europe. Understanding of the dynamics of pathogenic microorganisms in aquaculture environment would be of great importance for disease control. However the ecology of *N. seriolae* in aquaculture environment is poorly understood. This study aims to develop a quantitative PCR (qPCR) to detect *N. seriolae* and quantify *N. seriolae* genome in seawater of artificially infected fish and those collected in fish farm. A primer set and a probe for qPCR was designed in *gyr*B region of *N. seriolae*. The detection limit was $5x10^1$copies/reaction. Yellowtails, *Seriola quinqueradiata* (average weight 49g) were divided into 3 groups (n=10 each) and immersed in *N. seriolae* suspension of $1.2x10^1$cfu/mL (low dose), $1.2x10^3$cfu/mL (high dose) or in seawater (control) for 1 hour. After the challenge, fish were reared in 50L tanks with flow-through seawater at 25°C and 1 liter of seawater was collected at 3 to 4 days intervals for 31 days. Each sample was filtered through 0.45μm pore size cellulose nitrate filter, and then continuously filtered through 0.22μm pore size filter. DNA was extracted directly from the 2 filters (0.45 μm and 0.22 μm) with QIAamp DNA Mini Kit (Qiagen), and subjected to qPCR. The copy numbers of *N. seriolae* in seawater of low dose group, which no mortality was recorded, was lower than the detection limit. On the other hand, the numbers of *N. seriolae* was $9.3x10^3$ to $4.4x10^5$copies/20mL seawater in high dose group, which 30% mortality was recorded. Seawater surrounding the fish farm was also collected at surface and 8 meters below the surface near net cages of yellowtail and amberjack, *Seriola dumerili* from May 2012 to January 2013 in Kagoshima prefecture. Samples collected in early to late summer, when outbreaks of nocardiosis occurred in the fish farm, were positive for *N. seriolae* ($1.1x10^1$ to $9.6x10^2$copies/20mL seawater). The qPCR and concentration method of seawater samples would be of benefit for understanding the ecology of *N. seriolae*.

ID299:

An *in-vitro* assay to determine susceptible host species to cyprinid herpesvirus 3 (KHV) using tissue culture of the scales

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At present, susceptible host species in viral infection are confirmed by the mortality in an experimental infection. However, this *in-vivo* test requires a large scale of equipment. Only carp *Cyprinus carpio* is generally regarded as susceptible species to cyprinid herpesvirus 3 (KHV). However, some researchers suspected other cyprinid fish may potentially be susceptible to KHV. In this presentation, we developed an *in-vitro* assay to determine susceptible host species to KHV using tissue culture of the scales.

The scales of 4 fishes, koi carp *Cyprinus carpio*, goldfish *Carassius auratus*, Dark chub *Nipponocypris temminckii* and rainbow trout *Oncorhynchus mykiss* (n=5 each) were peeled from their bodies, treated with 10x antibiotic-antimycotic liquid and exposed to KHV solution ($10^{3.3}$ $TCID_{50}$/mL). After 1 h exposure to virus, the scales were transferred in new MEM with 2% bovine serum and 1x antibiotic-antimycotic liquid in 6 well culture plates, and incubated at 20°C for 2 days. Approximately 20 mg of the scales were sampled at 0, 1 and 2 days post virus-exposure (dpe) to extract DNA. The number of DNA copies was quantified using TaqMan real time PCR to estimate whether the number increases or not during 2 days (*in-vitro* experiment). In another experiment, the 4 fishes (n=5 each) were exposed to the KHV solution for 1 h, transferred in 10 L aquarium and kept for 3 days at 23°C. A part of the pectoral fin (approximately 20 mg) was dissected at 0, 1, 2, and 3 dpe and examined for the quantification of KHV DNA (*in-vivo* experiment).

In the *in-vitro* experiment, the number of KHV DNA copies increased in the scales peeled from koi carp during 2 days incubation, but not in the scales from other 3 fishes. In the *in-vivo* experiment, the number of copies also increased in the fin of koi carp exposed to KHV, but not in the fin of other 3 fishes. These results suggest that the *in-vitro* assay in this presentation can determine or presume susceptible host species to KHV. Further, the results in both *in-vitro* and *in-vivo* experiments indicate that only carp *Cyprinid carpio* is susceptible species to KHV.

ID191:

Evaluation of loop-mediated isothermal amplification method for detection of *Kudoa septempunctata* (Myxozoa:Multivalvulida) in olive flounder (*Paralichthys olivaceus*)

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Kudoa septempunctata is a myxosporean parasite infecting muscle tissue of olive flounder (*Paralichthys olivaceus*). Several authors reported that the ingestion of raw olive flounder muscles containing a high concentration of *K. septempunctata* spores induces transient diarrhea and emesis. Thus, it is necessary to develop reliable detection method of *K. septempunctata*, to prevent outbreaks and ensure food safety. Molecular methods (PCR and real time PCR) were developed but have several disadvantages for routine use in field with a huge number of samples. Loop-mediated isothermal amplification (LAMP) assay was developed for the simple, rapid, sensitive, and inexpensive detection of *K. septempunctata* in olive flounder. A set of six specific primers was designed to target the 28S rDNA encoding gene. The reaction condition was optimized as 63°C, 45 min. LAMP successfully amplified *K. septempunctata* DNA in serially diluted purified spores ($1.32x10^6$ ~ $1.32x10^1$ spores/ml) and spiked muscle samples with serially diluted purified spores ($1.32x10^6$ ~ $1.32x10^1$ spores/g muscle). The detection limit was $1.32x10^3$ spores/ml and $1.32x10^3$ spore/g muscle, respectively. In conventional PCR, it was $1.32x10^4$ spore/ml and $1.32x10^4$ spores/g muscle, respectively. Thus, LAMP assay was 10 times more sensitive than PCR assay in this study. Using LAMP assay, field samples were analyzed and the results were compared with conventional PCR assay. Thirteen out of 94 (13.8%) samples were *K. septempunctata*-positive by conventional PCR, whereas 51 of 94 (54.3%) samples were *K. septempunctata*-positive by LAMP. The results indicate that LAMP can be a useful tool for monitoring and early field diagnosis of *K. septempunctata*.

ID181:

Production of monoclonal antibody against *Kudoa septempunctata*

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Kudoa septempunctata is a myxosporean parasite of *Paralichthys olivaceus* (olive flounder) that was identified as a causative agent of food-borne illness. The consumption of raw fish meat containing a high concentration of *K. septempunctata* spores induces transient vomiting and diarrhea. In the present study, the production of a monoclonal antibody was examined for developing a simple immunodiagnostic assay for detecting *K. septempunctata*. Four BALB/c mice were anesthetized and injected intramuscularly at the right and left tail base with an emulsion containing purified 10^5 spores and TiterMax® Gold adjuvant. Mice were sacrificed 14 days after injection. Iliac lymph nodes were collected and lymph node lymphocytes from 4 mice were pooled and used for cell fusion attempt. The lymphocytes were fused with SP2/0-Ag14 (ECACC) non-secreting myeloma cells using ClonaCell™-HY Hybridoma Kit. The antigens were immunoprecipitated overnight at 4°C by the addition of cell culture supernatant containing monoclonal antibodies to lysate of infected muscle tissue. The fusion of lymphocytes with myeloma cells produced 48 hybridoma lines, of which 2 cell lines were selected on the basis of their reactivities with the *K. septempunctata* pseudocyst shown by immunohistochemistry on frozen muscle sections of infected fish. One of two monoclonal antibodies immunoprecipitated a 59.5 kDa protein from *K. septempunctata* infected tissue lysate.

ID150:

www.aquatic.animalhealth.org: Digital Disease Detection for Aquatic Animal Health

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This poster presentation describes an Australian-based international research project to develop the International Biosecurity Intelligence System (IBIS; www.biointel.org). IBIS is automated intelligence software that gathers near real-time open-source strategic intelligence on aquatic animal pests and diseases from the World Wide Web. The sub-domain for aquatic animal health is found at www.aquatic.animalhealth.org. Emerging issues are identified automatically by IBIS or by the user community and are analyzed using both human and computer together. IBIS is open for anyone to join, and has attracted an international network of users to promote cross-sector intelligence analysis, crowd-sourced analysis and information sharing.

IBIS is able to track and forecast aquatic animal health issues from around the world and plays the central role in providing early warning, better planning and rapid response for its diverse global user community. Efficient and automated intelligence gathering and analysis on emerging and re-emerging pest and disease threats is critical to ensure that these risks are managed effectively, whether you are providing biosecurity for government, aquaculture, or information for researchers and private businesses.

Early warning, better planning and rapid response for emerging biosecurity threats actually works at the country level. For example: IBIS assisted in the early warning process by detecting and tracking early mortality syndrome (EMS) as it emerged in the international shrimp farming industry and widely communicated the information and associated risks. The Governments of India and Indonesia rapidly responded to the emerging information and implemented strict border controls on the entry of shrimp for aquaculture purposes. As a result, their shrimp farming industry was not affected by EMS during the time of the first major outbreak and spread of the disease, and producers were able to capitalize on the increased global demand for product.

ID129:

Characterization of a rapidly growing nontuberculous mycobacteria isolated from farmed thread-sail filefish *Stephanolepis cirrhifer* in Japan.

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In 2009 and 2010, high mortality episodes were recorded among cultured population of thread-sail filefish *Stephanolepis cirrhifer* reared at Ehime Prefectural Fisheries Research Center. Diseased fish had significant abdominal distention and numerous white nodules scattered on the surface of the serosae of internal organs and mesentery. The isolated mycobacteria indicated growth on 2% Ogawa egg slant and positive with Ziehl-Neelsen staining. As a result of BLAST search, the sequences of 16S rRNA and partial 16S and 23S rRNA genes (ITS) gene of the isolates showed high homology to those of *Mycobacterium chalonae* (JCM6388) and *Mycobacterium salmoniphilum* (ATCC13758) in Genbank database, respectively. In this study, we tried to identify the isolates using additional molecular analyses and biochemical characterization.

DNA was extracted from the isolates and subjected to PCR reaction and sequencing, targeting *rpoB*, *hsp65*, *recA* and *sodA* genes. Sequences were compared with type strains of *M.chelonae* (JCM6388, ATCC35752) and *M. salmoniphilum* (ATCC13758) in GenBank database. Biochemical analyses of the isolates were performed in comparison with type strains of *M. chelonae* (JCM6388), *M. salmoniphilum* (ATCC13758) and *M. marinum* (JCM17638).

The sequences of the isolates of *rpoB*, *hsp65*, *recA* and *sodA* genes showed high homology to *M.chelonae* (JCM 6388, ATCC35752), while the biochemical characteristics of them corresponded to *M. salmoniphilum* (ATCC13758). These results suggest the possibility that they could be classified into a novel mycobacterial species.

ID89:

Development of simple and sensitive detection for bacteria pathogens in Nile and red tilapia using colorogenic Loop-mediated isothermal amplification

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Loop-mediated isothermal amplification (LAMP) assay is a recently-invented method of detecting only few copies of target nucleic acid under a constant temperature utilizing self-recurringstand-displacement DNA synthesis initiated by specially designed primer sets. Although the LAMP amplicons were initially visualized by agarose gel electrophoresis stained with ethidium bromide, they may also be assessed indirectly by the amount of white magnesium pyrophosphate (P2O7□2Mg) precipitate formed as by-product of the LAMP reaction. However, the turbidity observation by naked eye gave some bias results in the low amount of LAMP products. In this study, an accelerated colorimetric LAMP assay with pre-addition of calcein was developed and achieved the result through the naked eyes. This assay relies on the assessment of magnesium ion reduction during LAMP reaction, being due to the formation of P2O7□2Mg as by-product, resulting to the change of orange color (negative) to green color (positive). Therefore, colorimetric LAMP yielded results immediately without end point detection by opening the reaction tube. This technique has applied to detect the bacteria in cultured tilapia; *Streptococcus agalactiae*, *S.iniae,* and *Flavobacterium columnare*. The sensitivity of newly method has more sensitive than the conventional PCR. Among broodstock, fertilized eggs and fry samples, *S. agalactiae* revealed the high prevalence at 72.1% (272/377), followed by *S. iniae* at 16.2% (61/377) and *F. columnare* at 11.3% (44/390). It is noteworthy that these bacteria contained in reproductive organs of broodstock before passing to their offspring. The water samples taken from the farm were also found these bacteria. This suggest the likelihood that transmission mode could be both vertically and horizontally transmitted. The colorimetric LAMP would be employed simple, inexpensive equipment and involved simple steps making it applicable for small field laboratories. Wider application of the method to screen broodstock before use in a hatchery, to test fry before transaction movement and monitor fish in rearing earthen-ponds would help to assess and reduce the negative impact of these pathogens in fish farming.

ID76:

Rapid screening of pathogens in aquaculture through the use of the loop-mediated isothermal amplification (LAMP) technology

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The aquaculture industry is one of the biggest contributors in the global food production. Over the years, rapid advancements in productions systems and increasing number of aquatic species are being utilized for aquaculture to meet the growing food demand of the growing population. However, continued expansion of this industry is hampered by several farming bottlenecks. One of them is the occurrence of disease outbreaks caused by pathogenic organisms. In fact, disease is recognized as one of the most serious threats to the success of commercial aquaculture. The nature of diseases affecting aquatic organisms in aquaculture systems is complex. There are more than 20 cutaneous and systemic bacterial diseases, and more than 30 viral diseases of commercially important finfishes and crustaceans. Early diagnosis of the disease has a crucial role in the effective management of any aquaculture facility. Several biochemical and serological tests have been developed for the detection of the different pathogens of fish and crustaceans, but there are inherent constraints in logistics that make these diagnostic tools difficult to adopt during the production cycle. Molecular-based techniques particularly the conventional polymerase chain reaction (PCR) and quantitative real-time PCR (Q-PCR) have become increasingly popular in diagnostics; however, their application is limited by the availability of financial resources in the procurement of expensive equipment and reagents. Recently, a technique called loop-mediated isothermal amplification (LAMP) has been developed to detect a number of pathogens. This method is a sensitive strand displacement technique, which amplifies target DNA from a few copies to millions of copies in less than an hour under isothermal conditions. This technique is rapid and sensitive; thus, it has been used for the detection of bacterial, viral, fungal and parasitic diseases in aquaculture. Moreover, this assay does not require the use of sophisticated and expensive equipment, making it to have a good potential for on-site diagnosis of pathogenic diseases. The application of LAMP technology in the detection of commercially-important bacterial and viral pathogens in shrimp and in various farmed fish in tropical and temperate environments is presented.

HUSBANDRY AND MANAGEMENT

ORAL PRESENTATIONS

ID239:

Calcium is important for survival of prawn infected with *Macrobrachium rosenbergii* nodavirus

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White tailed disease (WTD) is a viral disease that affects the commercially important fresh water prawns, *Macrobrachium rosenbergii*. A major causative agent of WTD has been identified as *M. rosenbergii* nodavirus (*Mr*NV). It causes serious economic losses to the prawn farming industry worldwide. Little information is available in terms of the molecular mechanisms of the host-virus interaction. The requirement of calcium during *Mr*NV infection has been investigated. In the primary hemocyte culture model, supplementation of $CaCl_2$ (10 mM) in the culture medium could significantly increase the replication rate of *Mr*NV, compared to those without $CaCl_2$ supplementation. Determination of gene(s) involved in shrimp survived from *Mr*NV infection using the suppressive subtraction hybridization technique revealed the most abundant transcripts (22%) is in the gene category of calcium homeostasis such as sarcoplasmic calcium binding, and calcification-associated peptide. The functions of these genes in molecular details on prawn-*Mr*NV interaction are on-going. It is hope that the study will be help in design a novel strategy to control WTD in prawns.

ID236:

Expression of virulence genes in Harveyi clade vibrios in relation to their virulence towards gnotobiotic brine shrimp

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Vibrios belonging to the Harveyi clade are pathogenic marine bacteria affecting both vertebrates and invertebrates, thereby causing a severe threat to the aquaculture industry. Hemolysin, metalloprotease, serine protease, the quorum sensing master regulator *luxR* and the regulator *toxR* have been associated with virulence of these bacteria. This study aimed at investigating the relation between the expression levels of these virulence genes and their virulence towards gnotobiotic brine shrimp. Virulence gene expression in ten different pathogenic Harveyi clade isolates was measured with reverse transcriptase real- time PCR with specific primers. Further, gnotobiotic brine shrimp larvae were challenged with the ten isolates to check the virulence of these organisms. There was relatively low variation in the expression levels of the quorum sensing master regulator *luxR* (7-fold), whereas for the other genes, the difference in expression between the isolates showing lowest and highest expression levels was over 25-fold among isolates. The expression of the *luxR* gene positively correlated with *toxR*, metalloprotease and serine protease gene expression, but there was no significant correlation with the expression of the hemolysin gene. The expression of the transcriptional regulatory gene *toxR*, showed a statistically significant positive correlation with the expression of *luxR*, metalloprotease and serine protease. Hemolysin gene expression showed a significant positive correlation with the expression of the serine protease gene. Finally, the expression levels of *luxR*, *toxR* and hemolysin in the pathogenic isolates were correlated with the survival of brine shrimp larvae challenged with the isolates, indicating that Harveyi clade vibrios that have a higher production capacity of the proteins encoded/regulated by these genes tend to be more virulent. This shows that the strains with higher production capacity of LuxR, ToxR and hemolysins tend to be more virulent. Serine protease and metalloprotease expression were positively correlated with virulence towards challenged brine shrimp, but the correlations were not significant. This study concludes that there is variation in the expression level of virulence regulators and virulence factors in pathogenic *Vibrio* isolates belonging to the Harveyi clade and this could be the reason for the variation observed in their virulence.

ID300:

Dietary effects of various antioxidant supplements on growth, survival, antioxidant capacity, immune response, metabolic response and oxidative stress status of Pacific white 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.

ID288:

Heat Shock Protein 70 (Hsp70): a crucial molecular chaperone for thermotolerance and pathogenic *Vibrio* resistance in the brine shrimp *Artemia*

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RNA interference (RNAi) is a highly specific and efficient strategy to determine protein function in cells. In this study, Hsp70 knockdown *Artemia* were produced to determine the function of Hsp70 in thermotolerance and protection against pathogenic bacteria. As revealed by RT-PCR and Western immunoblotting, injection of Hsp70 double stranded RNA (dsRNA) into the berried females of *Artemia* decreased the amounts of Hsp70 mRNA and Hsp70 in cysts and nauplii. Hsp70 knockdown nauplii were less resistant to heat perturbation and pathogenic *Vibrio campbellii*, with survival percentage reduced approximately 31% and 28%, respectively in challenge assays. Interestingly, a combined hypothermic/hyperthermic shock followed by recovery at ambient temperature induced Hsp70 synthesis in *Artemia* larvae. Thermotolerance was also increased as was protection against infection by *Vibrio campbellii*, the latter indicated by reduced mortality and lower bacterial load in challenge tests. Resistance to *Vibrio* improved in the face of declining body mass as demonstrated by measurement of ash-free dry weight. The resulting two-fold increase in survival of larvae in concert with stress protein synthesis suggested that endogenous Hsp70 functions in protection. This study provides new insights into the role of Hsp70 in the response to abiotic and biotic stress of the brine shrimp *Artemia*, an important species used in aquaculture.

ID260:

Involvement of Laminin receptor protein in shrimp hemocyte homeostasis

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Laminin receptor (Lamr) is a multifunctional protein that binds to capsid or envelope proteins of some RNA viruses and plays essential role in shrimp viability. Here, we further investigated the potential function of Lamr in regulation of hemocyte homeostasis in the whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei*. Experimental silencing of *Pv*Lamr transcripts leads to a significant decrease in the numbers of total hemocyte and hyaline cell counts. However, no sign of apoptosis was detected in the hemocytes and the hematopoietic cells of the *Pv*Lamr-depleted shrimp. To further examine the possible causes of hemocyte loss, the crustacean hematopoietic factor-like protein (*Pv*CHF-like) and hemocyte homeostasis-associated protein (*Pv*HHAP) genes were identified and cloned from *P. vannamei* hemocytes. Subsequent RT-PCR analysis revealed that suppression of *Pv*Lamr expression down-regulated both *Pv*CHF-like and *Pv*HHAP. Protein interaction analysis by yeast two-hybrid screening confirmed that *Pv*Lamr could bind with *Pv*CHF-like and *Pv*HHAP. These finding implied that *Pv*Lamr is involved together with *Pv*CHF-like and *Pv*HHAP in regulation of the hemocyte homeostasis in shrimp.

ID286:

Efficacy of herbs against *Streptococcus* infection in different Tilapia sp: Review

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The most common strategy to fight aquaculture disease is the use of antibiotics; however, such usage has been reported to have adverse effects. Natural herbs have gained prominence in research as a means of treatment and control of *Streptococcus* infections in tilapia. Immunostimulants from plants have become increasingly important as research focuses on environmental sustainability. This paper reviews the use of immunostimulants for *S. iniae* (*Thymus vulgaris, Rosemarinus officinalis, Trigonelia foenum-graecum, Cuminum cyminum, Pimenta dioica), S. agalactiae* (*Andrographis paniculata, Helichrysum plicatum, Murraya, koenigii),* and *Streptococcus* sp (*Syzygium aromaticum, Vinca minor, Nuphar lutea,, Cantella asiatica, Citru microcarpa, Morinda citrifolia*). Detailed antimicrobial test for the use of herbs against *Streptococcus* has been reported for only two herbs: *S. aromaticum and A. paniculata* with the latter producing the highest efficacy (95%) in *O. niloticus* as well as overall efficacy. This paper has reviewed various herbs as effective treatment or preventive agent against *Streptococcus* infection in tilapia.

ID279:

Genetic characterisation of multidrug resistant *Citrobacter* spp. isolated from septicaemic fresh water ornamental fish

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Citrobacter freundii, a Gram negative bacteria within the family Enterobacteriaceae, has an established role as a fish pathogen causing systemic infections. However, its isolation from diseased fish is infrequent and hence rarely reported. During an investigation to identify septicaemia causing bacterial pathogens of freshwater ornamental fish in Sri Lanka, we have isolated *Citrobacter* spp. as the sole pathogen from 12.3% (10/81) of the total septicaemic cases investigated. As an aetiological agent causing septicaemia, its occurrence was only second to motile *Aeromonas* spp. With the growing interest in aquarium fish trade, a thorough understanding of the pathogens associated with the commercial ornamental fish culture is invaluable for accurate diagnosis and effective control. In this context, we genetically characterised 10 isolates of *Citrobacter* spp. that were isolated from moribund ornamental fish showing signs of generalized septicaemia. The isolates were first identified phenotypically by conventional biochemical tests and thereafter by sequence analysis of 16S rDNA and dnaG genes. The antimicrobial susceptibility of the isolates to eight antimicrobial agents that are commonly used in aquaculture was also determined. The genetic diversity was assessed by the enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). The genome of a selected strain, *C. freundii* CF04 isolated from moribund, septicaemic giant gourami (*Osphronemus goramy*) was sequenced using Ion Torrent PGM.

All the isolates were identified as *C. freundii* based on the phenotypic and phylogenetic identification. Strain typing through ERIC-PCR fingerprinting showed that three strains belong to a single clone even though they were isolated from different host species in different geographical locations. Rest of the isolates were genetically distinct. 90% of the isolates were resistant to tetracycline, erythromycin and amoxicillin while antimicrobial multi-resistance (resistant to ≥3) was observed in 80%. All isolates were sensitive to enrofloxacin. The draft genome of *C. freundii* CF04 assembled to a total length of 5,148,798 bp (216 contigs, 51.5 GC% with 5390 coding sequences) encodes for an array of genes related to virulence (adherence, Type 6 secretion system, siderophores, sialic acid metabolism, quorum sensing), multidrug resistance and metabolic versatility. To our knowledge, this is the first genome sequence of a fish borne C. freundii clinical strain and would provide valuable insights into this fish pathogen. Challenging experiments to test the pathogenic potential of C. freundii CF04 in adult zebra fish are ongoing. Given the important role of this bacterium as a zoonotic pathogen its existence in aquaculture could also pose a public health risk.

ID147:

Selection of probiotic bacteria and study of their inhibitory activity against pathogenic *Vibrio* spp. for Larval Asian Seabass, *Lates calcarifer* Bloch culture

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The Asian Seabass, *Lates calcarifer,* is the leading marine finfish species which is being cultured in Malaysia due to high value in local market, tasty flesh and suitability for comercial aquaculture. However, rapid intensification of aquaculture production exposed the industry to diseases pathway caused by bacteria such as *Vibrio* spp. The use of antibiotic as preventive measures has been limited in most country including Malaysia because of their negative effect to environment, human health and causing antimicrobial ressistance. Alternatively, the use of probiotic is one of the best option to control diseases in aquatic environment. Thus, the aim of this study was to screen for potential bacteria that can act as probiont to protect sea bass against vibriosis. A total of 255 bacteria were successfully isolated from 15 healthy juveniles *L. calcarifer*. Out of total, 89 isolates were collected from intestines and 20 isolates were from livers. The target pathogens used in this study consist of *Vibrio anguillarum, V. alginolyticus* and *V. harveyi*. This study demonstrated that nine isolates showed capability to inhibit the growth of selected pathogens using spot lawn assay. The inhibition zones were recorded ~2.0±0.2 cm in diameter. Meanwhile, in cross-streak assay, the inhibition lines were observed from only two potential isolates namely SG111 and SL21. The inhibition lines were measured around ~ 0.7 cm after 24 hour pre-incubation with the respective pathogens. Furthermore, potential probionts able to reduce numbers of Vibrios in a co-culture assay. The probionts were then underwent molecular characterization for identification. Based on the results of three different *in vitro* assay, at least nine isolates obtained from healthy *Lates calcarifer* showed good potential as probionts for fish health management due to their ability to inhibit the growth of different pathogenic *Vibrio* spp. However, *in vivo* investigation is needed in order to confirm their potential in protecting the host from vibriosis.

ID128:

Antibacterial activity of rampe, *Pandanus amaryllifolius* against a pathogenic strain of *Aeromonas hydrophila* and efficacy of rampe root extract in controlling *A. hydrophila* infections in guppy, *Poecilia reticulata*

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Use of broad spectrum antibiotics for prophylactic and therapeutic treatments could increase antibiotic resistance among bacteria in exposed systems and application of phytomedicine is becoming popular as an environmentally friendly way of controlling fish diseases. Rampe, *Pandanus amaryllifolius* is a tropical plant with recorded medicinal properties for some human health issues. Previous studies have revealed that, among recorded bacterial pathogens, the most common pathogenic species in Sri Lankan ornamental fish industry is *Aeromonas hydrophila*.

The present study was carried out to investigate the antibacterial effect of rampe on a pathogenic strain of *A. hydrophila* isolated from commercial, temporary holding facilities of guppy, *Poecilia reticulata*. Two concentration series were prepared separately with methanol extracts of rampe roots and leaves respectively to test the antibacterial effect using disc diffusion method on lawns of *A. hydrophila*. Three groups of apparently healthy guppy with four replicate tanks for each group were challenged with *A. hydrophila* by immersion technique and then the first group was treated with root extract of rampe at 5 gL^{-1}, second group was treated with oxytetracycline at 80 mgL^{-1}(positive control) and third group was maintained without any treatment (negative control). Disease development and mortality of guppy was recorded for each replicate tank over the two weeks of post challenge. Significantly larger inhibitory zones were produced by the methanol extract of rampe roots indicating significantly higher antibacterial effect ($P<0.05$) compared to leaf extract. Mean percentage cumulative mortalities recorded for guppy challenged with *A. hydrophila* and then treated with rampe root extract (10.5%) and oxytetracycline (7.5%) were significantly lower ($P<0.05$) than that was recorded for the guppy in control group (70.5%). Methanol root extract of rampe could be used as an antibacterial phytomedicine to control A*eromonas hydrophila* infections in guppy.

ID119:

Characterization of the potential probiotics, *Bacillus* spp. isolated from striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) in Vietnam

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Culture of striped catfish (*Pangasianodon hypophthalmus*) has been the most important freshwater aquaculture in Vietnam for the past decades. With intensive culture system, the production can reach 200-300 metric tons per hectare. Unfortunately, many diseases have been found in the culture system, especially by *Edwardsiella ictaluri*. Since chemical and drug treatments can cause many negative consequences, prevention approach should be highly encouraged. Application of probiotics is an alternative not only for growth enhancement but also for disease prevention. The objectives of this study were to investigate the distribution and *in vitro* characterization of potential probiotics, *Bacillus* spp. collected from the intestine of striped catfish in Vietnam. Specific primers were designed to amplify 16S rRNA for the identification of 6 species of *Bacillus* (*B. subtilis, B. amyloliquefaciens, B. cereus, B. megaterium, B. licheniformis* and *B. pumilus*). Multiplex PCR was developed to identify 120 isolates collected from striped catfish farms in 3 provinces in the southern part of Vietnam: An Giang, Dong Thap, and Can Tho. The *in vitro* analysis included antagonistic activity against pathogenic bacteria *Edwardsiella ictaluri*, protease activity, stomach acidic, bile tolerance test and antibiotic susceptibility. The result showed that 27% of the isolates were identified as *B. megaterium* which was the highest number of *Bacillus* population collected from the three provinces and there was no isolates identified as *B. licheniformes*. The two *Bacillus* spp. chosen for *in vivo* experiments were 54A-*B. amyloliquefaciens* with high protease activity and 47B-*B. pumilus* which showed high inhibition activity against *E. ictaluri*. These two strains were also resistant to stomach acid and low bile salt condition after 3 h and 24 h of exposure. Evaluation of antibiotic susceptibility showed that all six probiotics candidates were susceptible to amoxicillin, ampicillin, erythromycin, florfenicol, neomycin, trimethoprim, tetracycline and ciprofloxacin while spectinomycin, oxytetracycline, and sulfamethoxazole showed inconsistent results.

ID271:

AHL-Lactonase from *Bacillus licheniformis* DAHB1 inhibits Vibrio biofilm formation in vitro and reduced zebrafish mortality against *Vibrio parahaemolyticus* infection

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Diseases caused by biofilm formation of *Vibrio* spp. can be problematic and lead to economic loss in fish farming cultures. Pathogenic vibrios in fish farming were controlled by chemotherapeutics, probiotics, plant based compounds, antibiotics and vaccines. However, interfering with the biofilm formation of *Vibrio* spp. is one way to disrupt quorum sensing and serves as an alternative to the use of antibiotics in aquaculture practices. *Vibrio* virulence is associated with biofilm formation and is regulated by N-acylated homoserine lactone (AHL)-mediated quorum sensing. AHL plays a critical role in hydrolyzing the lactone bond within the acyl homoserine lactone moiety, thus changing the relative conformational structure of the signalling molecule; this prevents binding to the LuxR transcriptional regulator, resulting in quorum sensing inhibitory activity against pathogens. In an attempt to reduce vibrio colonisation of fish and mortality, in the present study the *Bacillus licheniformis* DAHB1 native intestinal bacilli was used which showed biofilm-inhibitory activity (quorum quenching) against the pathogen *V. parahaemolyticus* VpDAHV2. The strain also inhibited biofilm-forming exopolysaccharide and altered cell surface hydrophobicity of *Vibrio parahaemolyticus* VpDAHV2. Oral administration of *Bacillus licheniformis* DAHB1, significantly reduced zebrafish mortality caused by VpDAHV2 challenge, and inhibited colonization of VpDAHV2 in the gills and intestine of zebrafish as evidence by confocal laser scanning microscope and selective plating. Furthermore, zebrafish receiving DAHB1-containing feed had increased phagocytic activity of its leucocytes, increased serum activities of superoxide dismutase. The results suggest that *Bacillus licheniformis* DAHB1 could protect zebrafish from *V. parahaemolyticus* infection by inhibiting biofilm formation and enhancing defence mechanisms of the fish. These findings suggest that the *B. licheniformis* DAHB1 quorum- quenching AHL might be developed for use as a prophylactic treatment to inhibit or reduce vibrio colonisation and mortality of fish in aquaculture.

BOOK OF ABSTRACTS
The 9th Symposium on Diseases in Asian Aquaculture (DAA9)

HUSBANDRY AND MANAGEMENT

POSTER PRESENTATIONS

ID122:

The effect of Aqui-S® (Isoeugenol) on the post-transport survival rate and post-harvest fillet quality of Pangasius in Vietnam

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The current practice of handling non-sedated fish for transport and harvest causes high mortality, reduced immune system and reduced fillet quality due to stress. Aqui-S® is sedative for aquatic animals in various countries with a zero-withholding time. The purpose of this study was to determine the effect of Aqui-S® (10-15ppm) sedation during fish handling. Two fingerling transportation and two harvesting trials were conducted similarly and under field condition. The trial fish were sedated/ non-sedated in different steps including (a) pond crowd, (b) transfer from pond to boat, (c) boat transport to the new sites, and (d) crowded again before being unloading from boat and transferred to pond (fingerling)/ processing factory (harvesting).

The first trial simulated the use of Aqui-S® during four handling steps of Pangasius fingerling (40-60g bw, total 7 treatments and control, 55 head/treatment with two replicates) transport. The result showed higher survival rate (abcd-98%, ab-89%, b-87%, bd-79%, d-67%, bdc-67%) and healthier fish in the sedated compared with the control (51%). The second trial analyzed the effects of Aqui-S® sedation in two handling steps of the fingerling (37g bw; total 3 groups of treatments and control, 10,000 head/ group and 2 replicates) transport under field conditions that also showed higher survival rate (bd-99%, b-92%) of the sedated fish compared with the control (89%). Under both simulation and field condition, Aqui-S® significantly reduced the negative impact of handling stress on the fingerling.

The third trial investigated the benefit of sedation with Aqui-S® during two harvest related handling processes. Total 24 fishes at harvest size (0,7-0,8 kg bw) were sedated in steps bd compared with non-sedated. The results showed the sedated fish were significantly less damaged rigor onset and lighter fillet color and higher fillet pH after one hour, all indicating better fillet quality. The fourth trial analyzed the effects of Aqui-S® sedation on the harvest size pangasius during a commercial harvest and transport to the processing site. Two replicates of fish (5 to 112 mt) were sedated in steps bd compared with two non-sedated replicates. The results showed that sedated fish had higher survival rate (+2.07%), improved fillet conditioning (higher monophosphate uptake of +2,65%) and higher fillet color score and fillet ratio (+2,1%). Overall Aqui-S® helped to improve the fillet quality during a commercial sized harvest.

In conclusion, Aqui-S® shows promise as a zero with holding sedative to reduce the impact from handling stress to reduce mortality and to improve fillet quality for Pangasius. The application is cost efficient and complies with animal welfare standards in Pangasius aquaculture.

ID207:

Mutations in *gyrA* and *parC* associated with quinolone resistance in *Flavobacterium columnare* isolated from freshwater fish in Thailand

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Flavobacterium columnare is the causative agent of columnaris disease in many fish species both cultured- and wild- freshwater fish worldwide. In Thailand, several kinds of antibiotics have been widely used for the treatment of columnaris disease, especially tetracycline group and quinolone group. Major mechanisms of quinolone resistance in most Gram-negative bacteria are mutations in specific regions called quinolone resistance- determining regions (QRDRs) of *gyrA* or both *gyrA* and *parC*. The mutations in quinolone target genes associated with their resistant results of members in Genus *Flavobacterium* have limited report. Therefore, the purpose of this study was to investigate the association between quinolone susceptibility and mutations in *gyrA* and *parC* of *F. columnare*. In this study, totally 50 isolates of *F. columnare* originated from Red tilapia, Nile tilapia, and Koi carp in Thailand were examined. The minimum inhibitory concentration (MIC) value of oxolinic acid (OA) was evaluated by broth microdilution method. Out of 50 isolates, 16 were resistant (MIC≥4 μg ml^{-1}), and 9 were intermediate (MIC=2 μg ml^{-1}) to OA. MIC_{50} and MIC_{90} of OA of all isolates were 1 and 8 μg ml^{-1}, respectively. The QRDRs of *gyrA* and *parC* were amplified by designed specific primers and sequenced. The results of sequence analysis revealed the mutations responsible for amino acid substitutions in QRDRs of *gyrA* and *parC* of all OA-resistant isolates related to their MIC values, in *gyrA* at position 83 according to *Escherichia coli* numbering: Ser → Phe, Ser → Tyr (MIC=4 μg ml^{-1}), and Ser → Ala (MIC=8,16 μg ml^{-1}) whilst in *parC* at position 87: His → Tyr (MIC≥4 μg ml^{-1}). This study is the first investigation of the evidence of quinolone resistance mechanism in *F. columnare*. These results suggest that *gyrA* and *parC* are the primary targets of OA in *F. columnare*. However, other quinolone target genes and mechanisms should require further study.

ID173:

Antimicrobial susceptibility of *Streptococcus agalactiae* isolated from tilapia (*Oreochromis* sp.) in Thailand

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Streptococcus agalactiae is Gram-positive bacterial pathogen that has been frequently isolated from fish in many countries. 32 isolates were recovered from tilapia (*Oreochromis* sp.) in Thailand, and identified by both biochemical tests and PCR with species-specific primers. Susceptibility of these isolates to 12 antimicrobial agents and minimal inhibitory concentration (MIC) of enrofloxacin were determined by disk diffusion and micro-broth dilution method respectively, in compliance with Clinical and Laboratory Standards Institute guidelines. The results showed that all isolates were susceptible to sulphamethoxazole/trimethoprim, chloramphenicol, florfenicol, cephalexin, ceftiofur, amoxicillin, and doxycycline. While only 3% of isolates showed resistance to either ampicillin or erythromycin, intermediate resistance and resistance to fluoroquinolones (norfloxacin, ciprofloxacin, and enrofloxacin) were 3% and 9%, respectively. None of the isolates showed multiple drugs resistance. MICs of enrofloxacin to resistant isolates were higher than 64µg/ml. Investigation on gyrA gene, one of the targets of fluoroquinolones in bacteria, showed no difference in nucleotide sequences of gyrA gene among susceptible, intermediately resistant, and resistant isolates. Interestingly, high MIC of fluoroquinolones, an important class of antimicrobial also used for treatment of bacterial infection in human signified the need for more careful consideration and prudent use of antimicrobials in aquaculture.

ID157:

Motile Aeromonas Septicemia Infection in *Pangasius sp.*, Its Pathogenecity And Acceptability Of Plant Extract As Potential Treatment

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Motile Aeromonas Septicemia (MAS) has been identified as a problem in *Pangasius sp.* culture in Malaysia. Continuous reports of high mortality (30-40%) were obtained from the Temerloh district of Pahang since 2008, especially during the dry season in farms along Sg. Pahang. The critical culture period was found to be between 1 to 3 months. The clinical signs of infected fish were severe, particularly Inflammation around both mandibles, basal region of all fins, while internally pale liver with occasionally white nodules, congested kidney, and enlarged spleen with tiny nodules. Pathogenecity study conducted showed the LD50 of *Aeromonas hydrophila* to be $5.18x10^8$ cfu/mL. The LD50 result was high since *A. hydrophila* is an opportunistic bacteria, only causing disease whenever there is stress. MAS can be controlled either by antibiotics or herbal treatment as alternative medication. The plant extract of study has earlier shown better antimicrobial properties than antibiotics. Hence, the acceptability test was conducted in laboratory to determine the optimal dose of the plant extract that could be incorporated in feed for alternative treatment. Duplicates of five different doses of plant extract incorporated into feed in ascending order of 20 ppm, 40ppm, 60ppm, 80ppm and 100ppm were fed to the fish including control. A total of 20 fish were used per group. Percentage of pellet consumption were recorded for two consecutive weeks. Results showed that *Pangasius sp.* accepted all the doses studied including 100ppm which was found earlier to be effective in treating other bacteria species infection. The result thus indicated that feed incorporated with the plant extract can be taken up without any difficulty. This study hence revealed the potential of plant extract to be used as oral treatment in *Pangasius sp.* for Motile Aeromonas Septicemia.

ID132:

Efficacy of *Piper betle* extract as an anesthetic for Koi (*Cyprinus carpio*)

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Introduction: The present study investigated the use of Thai medicinal plants as an anesthetic for Koi (*Cyprinus carpio*). The ethanolic extract from leaves of *P. betle* showed a potential to induce anesthesia in indicated species. Consequently, the efficacy of *P. betle* as anesthetic for *C. carpio* was investigated.

Objectives: To verify the efficiency of crude extract of *P. betle* extract to induce anesthesia in *Cyprinus carpio*.

Method: The induction time and recovery time of anesthesia induced by extract were evaluated. The mean 50% effective concentrations (EC_{50}) values of *P. betle* extract to produce anesthesia was determined.

Results: The increasing concentrations of *P. betle* extract proportionally decreased the time required for sedation and anesthesia induction. On the contrary, recovery times were directly proportional to the concentrations of the extract. The lowest effective concentration of *P. betle* extract for induction of anesthesia in stage 1, stage 2 and stage 3 was 125 µg/ml. In the stage 4 of anesthesia showing different behavior changes, the lowest concentration of *the* extract to induce total loss of equilibrium was 250 µg/ml, while to inhibit pain reflex was 300 µg/ml. The EC_{50} values of the extract to induce stage 3, 4, 5 and 6 of anesthesia were calculated as 320, 350, 450 and 600 µg/ml, respectively. Interestingly, the efficacy of the extract (400 µg/ml) to induce anesthesia showed similar efficacy as positive control, 50 ppm of eugenol.

Conclusion: The use of *P. betle* is as an anesthetic for carp fish is an alternative as it can be safely utilized on them and recovery times are not significantly difference with eugenol. *C. carpio* were sufficiently sadated for normal sampling in crude extract at concentration 125 mg/ml. However, for surgical procedures, the fish were required to induce deep anesthesia (stage 4) as well as total loss of equilibrium was 250 mg/ml, while to inhibit pain reflex was 350 mg/ml or higher. Therefore, *P. betle* ethanolic extract showed a potential as an anesthetic in *C. carpio*.

ID116:

Disinfection of rotifer against bacterial flora using chemotherapeutant

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Rotifers have been used as live feed for fish larvae. They carry bacterial load that can be transferred into tanks and fish, leading to infection. Therefore, low bacterial content in rotifer is needed to reduce the possibilities of bacterial infection on fish larvae. Vibriosis is one of the major threats in marine fish culture that comes from live prey such as rotifer. Thus, this study was carried out to determine the effectiveness of two chemotherapeutants against bacterial flora, particularly on *Vibrio* sp. Rotifers were disinfected in sodium nifustyrenate and oxytetracycline at different concentrations for 24 hours before the bacterial flora within the rotifer was determined. Bacterial flora, including Vibrio groups in rotifer were found to significantly decreased from $1x10^5$ to $1x10^2$ CFU following sodium nifustyrenate treatment. The rotifer, however, showed low survival rate at high concentrations. Reductions of bacterial flora following oxytetracycline treatments were not significant. In conclusion, sodium nifustyrenate at 3 ppm for 24 hours treatment was the best concentration for significant removal of bacterial flora in rotifer without affecting the survival rate.

ID109:

Dietary effects of garlic (*Allium sativum*)on mucus parameters, growth performance and survival in Caspian roach,(*Rutilus caspicus*)

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The purpose of this study was to determine the response of Caspian roach (*Rutilus caspicus*) fingerling to garlic (*Allium sativum*)-added dietary doses with regard to immune response, growth and disease resistance. During this study, fish samples with average weight 1±0.07 g after 10 days of adaptation were distributed in 12 tanks with 20 liters water and fed twice daily (3% BW) with commercial diets containing 0, 5, 10 and 15 g garlic per kg. After two months feeding, mucus and blood sampling was performed. Results indicated a significant increase in growth performance and hematocrit in garlic added diet treatments than the control. Also, alkaline phosphatase enzyme and soluble mucus protein were significantly increased compared to the control. Lyzozyme activity was negative in all treatments. *Escherchia coli*, *Serratia marcescens*, *Micrococcus luteus* and *Sterptococcus faecium* showed the highest sensitivity into the mucus antibacterial activity. No mortality was observed in treatments during 13 ppt salinity shock.

ID411:

An outlook of diseases in commericial fishes in punjab, Pakistan

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The present paper is a brief account of diseases of culturable carps and ornamental fishes observed in Punjab, Pakistan. Six species of culturable carps and several ornamental fish species: *Carassius auratus* (goldfish comet, shubunkin, oranda, black moor, fantail); *Cyprinus carpio* (koi carp); *Poecilia reticulata* (guppy); *Poecilia sphenops* (molly): *Xiphophorus maculatus* (platy) and *Xiphophorus helleri* (sward tail) were examined and found with mild to serious parasitic, fungal and bacterial infections. Parasitic infections were caused by protozoan (*Ichthyophthirius multifilis*; *Trichodina* sp; *Chilodonella* sp, *Piscinoodinium pillulare*; *Tetrahymena* sp, *Epistylis* sp.); monogenean (*Dactylogyrus vastator, D. extensus, Dactylogyrus* sp. *Costia* sp; *Gyrodactylus turnbulli, Gyrodactylus* sp.); digenean (*Posthodiplostomum cuticula*; *Cryptocotyle* sp.), nematode (*Camallanus* sp. *Capillaria* sp.); crustacean (*Lernaea cyprinacea, L. polymorpha, L. oryzophila, L. ctenopharyngodonis*; *Argulus foliaceus, Argulus* sp.) and fungal infections by *Saprolegnia* sp. *Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, Penicillium oxalicum, Alternaria* sp, *Mucor* sp. *Rhizopus* sp, *Blastomyces* sp. Bacterial diseases like motile aeromonad septicaemia, fin rot, haemorragic ulcers on skin were also reported from some species of carps and ornamental fishes. Lernaeasis is most prevalent disease in carps; whereas protozoan and monogenean infections are high in ornamental fishes. Due to gradual progress in fish disease diagnosis, our attention must be focused on diseases of wild commercial fishes. This may be achieved through the use of advance diagnostic techniques. The understanding of biology, epidemiology and control measures of pathogens may help us to have more fish for human consumption. On the other hand, strict regulations on import of live ornamental fish may also be imposed to control the transmission of serious pathogens into Pakistan.

ID251:

The use of furazolidone in the treatment of white spot disease in gold fish (*Carrassius auratus*)

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White spot disease, also known as Ich, is a parasite disease caused by ciliated protozoan, *Ichthyophthirius multifilis*. There are several chemicals can be used for treatment of disease such as formalin, sodium chloride, hydrogen peroxide. This study was performed in order to evaluate the effect of furazolidone on *Ichthyophthirius multifililis*. Furazolidone is a nitrofuran derivatives that interferes with parasitic enzyme. Although its exact mechanism of action is unknown, it is effective against *Giardi* sp., Coccidia, Trichomonas and some entric bacteria. 120 Gold fish were divided in four groups in duplicate. Groups 1 to 3 were experimentally infected with *Ichthyophthirius multifilis* in water temperature18°C. Group 4 was not infected and considered as control. 72 hours post - infection mortality was started in challenged groups. Presence of parasite was detected on the skin and fins of dead fish under microscopic examinations. Three days after mortality initiation, group 1 was treated by bath with 50 mg/L furazolidone for one hour and group 2 was treated with formalin at concentration of 250 ppm for one hour. Fish in group 3 was not treated. Treatments were repeated after three days. The fish were monitored for 2 weeks. Dead fish were collected every day and monitored for Ich disease. After 2 weeks mortality in experiment groups were compared statistically.

The result of this study shows the difference between mortality in groups were significant ($p \leq 0.05$). Mortality in furazolidone exposed group was lower than other groups while higher death rate was in non-treated group and formalin group was in between. Mortality rate in furazolidone group (1), formalin group (2), non-treated group (3) and control were 17%, 40%, 67%, 0 %, respectively. However the results showed that furazolidone is more effective than formalin against Ich disease but excess studies are necessary to understand different concentration and durations of application of furazolidone, the number of treatment and the time intervals between treatment .

ID290:

Acute Effects of combined herbicide, 2,4-D + MCPA liver enzymes, ALT and AST, in Rainbow trout (*Onchrhynchus mykiss*)

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In the recent years, we confronted with harmful effects of toxins such as herbicides on aquatic species due to unregulated use of these compounds in agricultural operations and their disposal to water ecosystems. In the present study, the effects of combined herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid) + MCPA (2-methyl-4-chlorophenoxyacetic acid), "the frequently used herbicide in Kurdistan province" on the liver enzymes in rainbow trout as the main aquatic species farmed in this area was assessed. After determination of LC50 using Probit model, 60 healthy trout fish with an average weight of 97g were divided into two groups. The first group was considered as control and second group exposed to1 cc/L herbicide (equivalent to 2,4-D 360 mg/L + 315 mg/L MCPA) for one hour. After 72 hours, liver enzymes including ALT and AST were measured. The levels of liver enzymes, ALT and AST, in the treatment group increased significantly when compared with the control group ($P<0.05$).The increase in liver enzymes level probably associated with pathological effects of the herbicide on liver tissue.

ID253:

Growth Performance of Sea Bass Larvae in Different Concentration of Newly Isolated *Enterobacter ludwigii* from Sea Bass Intestine

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The rearing condition of fish in aquaculture industry is highly influenced on their growth performance and success. In this regard, the observation of suitable dosage of the *Enterobacter ludwigii* was observed by mixing with the larval feed of Asian sea bass. The growth performance, water quality and the ability to protect the sea bass larvae against the fish pathogen through *in vivo* test were also observed and tested. Five Randomized Complete Block Design (RCBD) treatments for different concentrations of *E. ludwigii* were mixed with sea bass feed, i.e., T1 (control; without *E. ludwigii*), T2 (1×10^1 cfu/g of *E. ludwigii*), T3 (1×10^3 cfu/g of *E. ludwigii*), T4 (1×10^6 cfu/g of *E. ludwigii*), T5 (1×10^9 cfu/g of *E. ludwigii*) and fed to the sea bass larvae for 28 days. *In vitro* test showed that *E. ludwigii* formed a clear inhibition zone against 3 fish pathogens (*Aeromonas hydrophila* ATCC7966, *Vibrio alginolyticus* ATCC33839 and *V. parahaemolyticus* ATCC43996) at concentration level of 1×10^9 cfu/ml via well and disc diffusion method. A concentration of 1×10^9 cfu/g of *E. ludwigii* in feed was safe to be used to the sea bass larvae as high survival rate (83.3%) was found in T5 compare to control (T1; 78%). The number of *E. ludwigii* in the gastrointestinal tract and water sample were also not significantly ($P>0.05$) different, whereas, the trend of increasing in *E. ludwigii* counting in gastrointestinal tract and water sample were observed with the increasing of its concentration in the feed. Significant ($P<0.05$) variation in nitrate, ammonium and phosphate concentrations of water were observed within the treatments used to rear the sea bass larvae. The concentration of nitrate was lower (0.001mg/l) in T5 followed by phosphate concentration of 0.03 mg/l. Ammonium concentrations was found to be lower in T4 with the value 0.10 mg/l. The improvement of water quality in the rearing tank of sea bass larvae using high concentration of *E. ludwigii* was remarkable. Observation after 7 days of challenge test against *V. parahaemolyticus* (2×10^8 cfu/ml) showed that the highest survival (100%) rate was found for T5 compared to T1 (79%). The findings of the study revealed that *E. ludwigii* could probably be a potential probiotics for rearing of fish larvae specially sea bass.

ID177:

Description and quantification of basic production and mortality variables of red tilapia (*Oreochromis niloticus*) cultured in the Mekong Delta, Vietnam.

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Vietnam is one of the most productive aquaculture countries in the world with 80% originating from the Mekong Delta. The most cultured fish, pangasius (striped catfish; *Pangasianodon hypophthalmus*), is mainly exported, while red-tilapia (*Oreochromis niloticus*), often cultured in close proximity to pangasius, is important for local consumption. Little is known about the frequency of mortality events, or their contributing factors, in red-tilapia in the Mekong Delta. Objectives of this pilot study were to describe and quantify basic production variables (e.g. duration of production cycle, fallow period, biosecurity measures, record keeping) and mortality related variables. In July 2014, approximately 50 farmers of red-tilapia aquaculture cages floating in rivers in An Giang (AG), Vinh Long (VL), Dong Thap (DT) and Ben Tre (BT) provinces (total 201) were randomly selected and interviewed. If a farmer was not able to participate, another farmer in close proximity was selected. Results represent percentages of interviewed farmers per province. More than 66% of interviewees reported 2 cycles of red tilapia per year. Sixty percent reported application of a fallow period (time between harvesting and stocking a new crop) of 1 – 7 days, while the longest fallow period was more than 10 weeks (2% of farmers in DT). Most farmers (12 – 42%) reported "usual mortality between stocking and harvesting" to be 31 – 40%, the total range was 0 - 70%. Cause of mortality was usually not confirmed; more than 90% of farmers in AG, DT and BT and 51% in VL reported never sending fish to a diagnostic laboratory for testing. More than 75% of farmers reported removal of dead fish once per day and 64 - 88% of farmers reported selling mortalities as food for catfish or other fish farms. More than 86% of farmers reported never keeping records of mortalities. These results provide evidence-based knowledge on determinants of health in tilapia cage culture that can be used in further studies to examine associations between basic production variables and mortality.

ID77:

Probiotics research for sustainable aquaculture: utilization of host-derived probiotic candidates

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Research in probiotics for aquaculture has evolved through the years and its diversification is an adaptation to the growing number of farmed species that utilize these beneficial bacteria. The application of probiotics in aquaculture is perceived to be a sustainable and promising strategy not only in the context of disease control but also in ensuring good nutrition, better growth and increased disease resistance. Though recently used in aquaculture, most of the probiotics that fish farmers use are from terrestrial origins. Because of the differences in the environment of the fish and the probionts, the beneficial effects of the terrestrial probiotics may be affected; thus, it is important to use host-derived probionts to ensure better colonization of the gut. Using a cold-water fish, Atlantic cod (*Gadus morhua*) as a model, host-derived probiotic candidates were isolated from the gut and further characterized *in vitro and in vivo* for their potential to be developed as probiotics in aquaculture. The isolates exhibited differences in their antagonism to the bacterial pathogens under the varying conditions. The probionts were able to adhere on gut epithelial cells and the adhesion was segment-dependent. Transcriptional responses of gut epithelial cells to the candidate probionts showed differential regulation of some immune-associated genes. There was no mortality of fish during the pathogenicity experiment, confirming the safety of the candidates for further applications. A feeding trial was conducted by incorporating the candidate probionts in feed. Generally, there were no significant differences in some of the metabolic enzymes in the blood and in selected cellular immune parameters between the fed fish and the control. However, the fed fish had better serum-mediated bacterial reduction capacity against pathogenic bacteria than the control. Oral administration of the probiotic candidates through feeding modulated the expression of immune-associated genes in the gut and the level of transcription was shown to be segment- and sampling point-dependent. These series of studies clearly indicate that host-derived probionts can be developed from fish and has the potential for further development as probiotics to ensure a sustainable aquaculture.

ID321:

Impact of snails in goldfish farm

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Snails, commonly known as an intermediate host for digenea trematode, have been observed with an increasing number in Nakorn Pathom and Ratchaburi provinces, Central part of Thailand. Fourteen goldfish (*Carassius auratus*) farms from these two provinces have been selected to investigate the relationship of prevalence between snails and metacercariae in goldfish. Goldfish and snail samples were collected from March to June, 2014 at 5-10% prevalence. There were two types of goldfish culture in this study: earthen and concrete pond. Both goldfish and snails were collected from six earthen pond farms. Among these, one farm showed parasitic infestation both in snail and fish, two farms had infested fish only, the rest digenean parasite was not found neither in fish nor snail. The other eight farms, only goldfish were sampling from their concrete ponds. The results showed that infested fish were found in three farms while five farms were free from the digenean parasite. 1-184 metacercarial cysts, which were identified as *Centrocestus formosanus* by morphological and PCR technique, were found in gill tissue of infested fish. High variation of the mean intensity of infestation was detected among fish farms (3.07-88.34 cysts per fish). Three species of snails from the earthen ponds of six farms were morphologically identified as *Melanoides* sp., *Camptoceras* sp. and *Filopaludinia* sp. However, only *Melanoides* sp. was found to be infested with redia of this parasite. These results suggest that the distribution of *C. formosanus* in goldfish farms is related to either a presence of intermediate host, *Melanoides* sp., in their culture system or the use of water contaminated with the cercaria, including the careless movement of infested fish among fish farms.

ID 421:

The efficacy of dietary short-chain fatty acids in enhancing growth performance, resistance to *Vibrio* bacterial infections and hepatopancreatic structural integrity of the giant freshwater prawn, *Macrobrachium rosenbergii*

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The use of short-chain fatty acids (SCFA) or organic acids in the diets of aquacultured animals as a growth promoter and prophylactic to bacterial disease is receiving increasing attention, but so far the productivity implications to decapod crustaceans and their subsequent resistance to disease are lacking. An 8-week feeding trial was conducted to measure the growth performance, feed conversion ratio (FCR) as well as hepatopancreatic total viable colony forming units (CFU) and histology of the giant freshwater prawn, *Macrobrachium rosenbergii,* when fed diets supplemented with formic (FA), citric (CA), lactic (LA), propionic acid (PA), an organic acid blend (OAB) or a control diet (no additives). A second experiment was performed to examine the resistance of the prawns and associated hepatopancreatic histopathological changes after a two-week challenge to *Vibrio harveyi* when fed these SCFA supplemented diets. While all the SCFA supplemented diets tended to improve prawn growth, those fed the CA diet had a significantly higher ($P<0.05$) growth performance and slightly better ($P>0.05$) FCR than the control treatment. Prawns fed the SCFA supplemented diets tended to have lower hepatopancreatic CFU counts than those fed the control diet, but only those fed the LA or FA diets were significantly lower than the control treatment. Survival of prawns to *V. harveyi* challenge was significantly higher when fed the LA diet, followed by the CA diet, than the other treatments, likely due to less bacterial induced hepatopancreatic damage and higher energy reserves compared to those fed the other diets. Results demonstrated that dietary CA and LA supplementations can be an effective growth promoter and prophylactic against *V. harveyi*, respectively, which may help reduce the reliance of antibiotics in the prawn farming industry. This is the first report on the efficacy of SCFA in enhancing growth and imparting hepatopancreatic protective properties to freshwater prawns when challenged with a bacterial infection.

ID 416:

Herbal Therapy in small scale aquaculture: an Ethnobotanic approach in North Vietnam and Central Java, Indonesia"

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Several researches mainly developed in Asia, demonstrating that plant biodiversity can be a valuable and a sustainable alternative to allopathic treatment. However, knowledge about plants and their use in health practices carried out by fishfarmers are not very thorough knew. Ethnobotany and Ethnopharmacology allow identifying plants and their use by humans for healing and health, but these approaches are rarely used in aquaculture. As part of the regional project ESTAFS, ethnobotanical researches were conducted in two regions of northern Vietnam and in Central Java (Indonesia). Knowledge Attitude Practices (KAP) surveys were conducted through semi-structured questionnaires involving more than 650 farmers from about 130 villages in each country. Fish farmers that use plants represent 79.5% ± 4.1% (n = 379) in central Java and 66% ± 5.5 (n = 280) in Vietnam. The number of identified plants is higher in Java than in Vietnam, (56 and 24 plants respectively), but the median number of plants used by fish farmers is the same (2) in the two countries. Despite the different ecological conditions, some plants are common between the two study areas. Plants are mainly used for the treatment of diseases; but their use to prevent disease is also widespread. Various factors specific to the two countries, determine the use or not of plants and these factors probably reflect societal and environmental differences among the farmers of both countries. The ethnobotanical approach to study the identification of new natural substances and to improve the sustainability of aquaculture is discussed.

CRUSTACEAN DISEASES GENERAL POSTER PRESENTATIONS

ID258:

Antimicrobial resistance in *Vibrio parahaemolyticus* isolated from shrimp farms in the Mekong Delta, Vietnam

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Outbreak of acute hepatopancreatic necrosis syndrome (AHPNS) appeared in large region and seriously affected to shrimp farming in Vietnam. With intensification in shrimp production, the pressure from AHPNS has greatly increased, some farmers have dealt empirically with AHPNS by using chemicals and antibiotics without laboratory diagnostic support and veterinary supervision. The aim of this study was to determine the sensitivity of 67 *Vibrio parahaemolyticus* bacterial isolates to 8 common antibiotics used in shrimp farming. These isolates were recovered from shrimp displayed typical pathology of AHPNS such as hepatopancreatic atrophy, empty gut and showed hepatopancreaic changes including dysfunction of hepatopancreatic cells, hemocytic infiltration and bacterial infection. Results of antibiotic susceptibility testing by the disk diffusion method showed that single resistance to tested antibiotics was relatively rare. The majority of isolates tested exhibited multi-resistance to more than one tested antibiotics. The minimum inhibitory concentrations (MICs) for oxytetracyline and florfenicol was also determined for the sensitive isolates using a broth macrodilution method. MIC value for oxytetracyline of tested isolates was 64 ppm, whereas MIC value for florfenicol was 16-32 ppm. The current study suggests caution in the use of antibiotics for prevention and treatment of AHPNS in shrimp farming and there is an urgent need to apply alternative strategies for prevention and treatment of AHPNS in shrimp farms.

ID156:

Policy issues in shrimp health management in Sri Lanka: Recent trends and future directions.

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Sustainability of the shrimp farming industry in Sri Lanka is challenged by frequent outbreaks of white spot virus (WSV). Lack of appropriate policy and related issues have been identified as major contributory factors for these outbreaks. The main objective of the present communication is to review recent policy intervention and to highlight future directions identified to reduce the risk of WSV outbreaks.Continuous consultation programs were conducted with the appropriate stakeholders under the IDRC/CRDI project during 2012 / 2013. Main live Ministry of Fisheries and Aquatic Resources (MOFAR), Provincial Ministry of Fisheries, 23 shrimp farmer organizations, Sri Lanka Aquaculture Alliance (SLADA) which is the main umbrella organization covering farmers, service providers, processors were involved in the consultations. Outcome of these consultation were transform to programs, procedures, regulations or practices to address the challenges and issues related to frequent disease outbreaks in the industry. Introduction of a crop calendar facilitates staggered stocking among 23 shrimp farming subzones, reducing the pressure on water source and prevents contamination of adjoining zones in case of disease outbreaks. Mandatory requirement of screening of all brood stock, post larvae through PCR, farmers to become members of farmer association, adoption of stipulated pond preparatory procedures and bio security procedures are some of the existing policy interventions.Introduction of concessionary rates for PCR tests, perusal of shrimps through PCR after one month of stocking, establishment of a steering committee under the chairmanship of Provincial Ministry of Fisheries, establishment of dedicated website and facilitations of knowledge through SMS are some of the recent policy decisions. Identified future directions in policy includes incorporation of mandatory BMP's to the constitutions of farmer societies, strengthening of farmer societies for regular water quality management, establishment of a monitoring group, accreditation of PCR laboratories, formulation of standards for farm effluents and establishment of Sri Lankan standards for shrimp feeds. These policy interventions will help to improve the sustainability of the shrimp farming industry in Sri Lanka reducing the risk of disease outbreaks.

ID155:

Impact of cluster approach on health management in small scale shrimp farming in Sri Lanka

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Shrimp culture in Sri Lanka has now got transformed to an industry dominated by small scale farmers from an industry which was controlled by multinational companies and large scale shrimp farmers. The present communication assess and compares the present status, farm performances, and disease situation small scale farms in North Western Province(NWP) and Eastern Province (EP) which are the main shrimp farming zones of Sri Lanka Regular consultations and interviews with farmer societies, questionnaire based interviews and regular farm visits were used to collect information with the assistance from IDRC/CRDI project during 2012 / 2013.Most of the small scale shrimp farms in NWP are located in environmentally sensitive intertidal areas and are scattered around The Dutch canal and the Mundal lagoon. These areas are identified as high risk areas for WSV disease. Lack of water treatment facility, direct water exchange, poor aeration, weaknesses in biosecurity measures, high stocking densities, lack of water quality monitoring facility, less organized farmer societies and poor infrastructure are identified as main contributory factors for continuous crop failure. Well organized cluster of small scale farms have been established in the village of Wattawan in EP bordering the Uppar lagoon. A common reservoir to treat water, well established farmer society organized to form a company, good infrastructure facility, moderate stocking density, availability of technical support and water quality monitoring facility are the salient features that have contribute for good farm performance. No serious disease outbreaks and only incidences of bacterial infections are reported.Organized cluster approach developed in the EP can be used as a model to improve the sustainability of shrimp farming industry in other areas of Sri Lanka to manage health and improve the sustainability of the industry.

ID127:

Virulency of five species of *Vibrio* isolated from *Penaeus monodon* (black tiger shrimp) hatcheries in Sri Lanka and the sensitivity of them to antibiotics

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Though Sri Lankan shrimp hatcheries use UV sterilized sea water, significant mortalities occur during the rearing process of different larval stages due to vibriosis. In one of our previous studies, five species of*Vibrio* were isolated and identified as most common ones in Sri Lankan shrimp hatcheries. Present study investigated the virulency of those five species of *Vibrio,* freshly isolated from culture facilities of shrimp hatcheries, on mysis larvae and on twelve days old post larvae (PI12); antibiotic sensitivity test was performed for each species of *Vibrio*. Apparently healthy mysis larvae and PI12 of *Penaeus monodon* were challenged separately with each species of *Vibrio* at 4 concentrations (1× 102, 1× 103, 1×104 and 1×105 CFUmL-1) with four replicates for each concentration and for the control. At 48 hours post challenge with 1×105 CFUmL-1,the highest mean percentage cumulative morality of mysis was recorded for *Vibrio parahaemolyticus*followed by *Vibrio alginolyticus* and *Vibrio harvaeyi* (respective values were 97.5%, 96.25% and 93%); mean percentage cumulative mortality recorded for *Vibrio fluvialis* and *Vibrio vulnificus*(78.25% and 56% respectively) were significantly lower than those were recorded for former three species (P□ 0.05). At 48 hours post challenge, mean percentage cumulative mortality observed for PI12was significantly higher (P□ 0.05) when they were challenged with *Vibrio* followed by *Vibrio harveyi* (60%) *alginolyticus* (77.75%) and *Vibrio parahaemolyticus* (43.75%) challenge with *Vibrio* at 1×105CFUmL-1 *vulnificus* and *Vibrio fluvialis* resulted mean percentage cumulative mortality of 21.75% and 16.25% respectively in P112. The most virulent *Vibrio* species isolated from Sri Lankan *Penaeus monodon* hatcheries were *Vibrio parahaemolyticus, Vibrio harveyi* and *Vibrio alginolyticus*; virulancy of them seemed to vary when mysis larvae are grown up to PI12. *V. alginolyticus, V. vulnificus, V. fluvails* and *V. harveyi* were sensitive to Chloramphenicol. *Vibrio parahaemolyticus* showed resistance to Chloramphenicol, Ampicillin, Oxytetracycline and Erythromycin but were sensitive to Gentamycin. All five species were resistant to Ampicillin while only *V. vulnificus* was sensitive to Erythromycin.

ID104:

Lagenidium thermophilum isolated from eggs and larvae of mud crab *Scylla tranquebarica* in Sabah, Malaysia

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Seed production of mud crab *Scylla tranquebarica* is attempted at a hatchery in Sabah, Malaysia. A fungal infection was found in eggs and larvae of the mud crab. Fungi were isolated from the eggs and larvae with fungal infection using PYGS agar, and tried to identify the fungi from the morphological characteristics and molecular analysis. First the isolated fungus was cultured in PYGS broth at 28□ for 3 days, and then transferred to sterilized seawater. Discharge tubes developed from the mycelia, and a vesicle for zoospore formation was produced at the top of each discharge tube. After that zoospores swam away in seawater from vesicle. The isolate (IPMB1401) was identified as *Lagenidium* sp. from the morphological characteristic of an asexual reproduction of the fungus. Second ITS1 sequence of the nuclear rRNA gene of the isolate IPMB1401 was compared with that of pathogenic *Lagenidium* strain in crustaceans. As a result, the similarity between *L.* sp IPMB1401 and *L. thermophilum* was 99-100%; therefore the isolate IPMB 1401 was identified as *Lagenidium thermophilum*. This is the first record of *Lagenidium* infection in Malaysia

ID322:

Two-month mortality syndrome and other problems encountered by shrimp farmers in the Philippines

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The Philippines is an archipelago composed of more than 7000 islands. This makes aquaculture and fisheries a major source of livelihood for the Filipinos. One of the major specie scultured in the Philippines is the tige shrimp, *Penaeus monodon*. Tiger shrimp production was a luxurious business venture until the early 1990's when the industry wasd evastated by luminous bacteria, and by the white spot sydrome virus (WSSV) in the late 1990's. This study describes other problems encountered by *P. monodon* farmers in the Philippines and how they may relate to globally reported disease. Data were gathered using a structured questionnaire. Respondents were shrimp farmers (n=174) from 8 provinces in the Philippines. Luminous bacteria, though easily controlled by farmers into semi-intensive monoculture is still a problem to some farmers applying extensive culture. WSSV continuos to be a problem to farmers both in semi-intensive monoculture and extensive polyculture with milkfish and/ or crab. Aside from WSSV and luminous bacteria, one of the major problems of farmers into extensive polyculture is the two month mortality syndrome (TMMS). Farmers call it the TMMS because the shrimp die before they reach DOC 60, some as early as 2 weeks. Mortality is up to 100%. TMMS maybe similar to the reported early mortality syndrome (EMS), but this can not be confirmed due to the absence of proper diagnosis such as histopathology and bacterial isolation. Other minor disease problems are protozoan infestation in the gills and cooked-like appearance even when alive. Other problems are environmentals uch as pollution due to reddish culture water released by adjacent farms, siltation which do not allow water to drain into the sea and decayed bamboo poles used for mussel/oyster culture. Possible risk factors of the TMMS and other problems will be presented during the presentation. Some preventive measures against TMMS and other disease problems might be through the proper implementation of the greenwater culture system and organic farming.

ID314:

Probiotics as Antimicrobial Agent Can Control Diseases in Shrimp Hatchery: Bangladesh Perspective

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Recently, diseases mostly caused by bacteria and viruses, decline shrimp PL (post larvae) production in Bangladesh. The leading bacterial flora (*Vibrio* sp.) related with shrimp larvae showing symptoms of zoea 2 syndrome, mysis mold syndrome, swollen hindgut syndrome, luminescent vibriosis, white body disease, and 'slime' has been determined. Antibiotics, which have been widely used to control bacterial infections, are now showing their ineffectiveness resulting more pathogens that are antibiotic resistant in the system. Among the 32 operating hatcheries of Bangladesh, 24 (75%) were surveyed in which eight different commercial probiotics namely Epicin, Top 10, Top 25, ABS, Pro 4000X, VC-7, Bio-guard, and Novobiotic-Plus have been used for preventing those diseases. About 50% of the surveyed hatcheries have been used Epicin and 40% using ABS in different dose rate before the nauplii stocking and continued to PL_{15}. Rest of the probiotics has been used in different stages i.e. zoea, mysis and PL at various concentration. In Cox's Bazar, a shrimp hatchery that had been devastated by zoea 2 syndrome, luminous *Vibrio* disease and 'slime' problem while using heavy doses of antibiotics, attained survival of 60-80% of shrimp PL, treated with Novobiotic-Plus. This probiotic contains *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Sacharomyces cerevisiae*.

ID313:

Experimental culture of *Rickettsia*-like bacteria causing spiny lobster's Milky Haemolymph Syndrome on Grouper Embryonic cell line

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Milky Haemolymph Syndrome (MHS) is an infectious disease which can be found in many different spiny lobsters species (*Panulirus* spp.) cultured in the Central Region of Vietnam. With incredible fast infection speed, the disease has heavily damaged lobster culture regions in Vietnam particularly during2006-2008 with no proper method for prevention or treatment until now. Pathogen analysis led to the revelation of one small Gram negative, curved-rod shape bacterium, thriving with high populationintra-cellularly and in the disease lobsters' haemolymph. In addition, this bacterium could not be cultured in formulated media thus was suggested to belong to *Rickettsia* genus. Our study proposed a full procedure of culturing this bacterium from isolating it from infected spiny lobster's haemolymph to culturing it in grouper embryonic cell line (GE). After 3 days of infecting GE cell culture with the isolated MHS bacterium, we observed cytopathological effects on GE cells. The cell staining results showed a populated growth of intracellular bacteria. This suggested that MHS-*Rickettsial*-like bacterium was able to be cultured in GE cell line. This success would serve as a critical step for further research on this kind of pathogen.

ID237:

The application of bactericidal and HSPi compounds to improve PL quality in terms of disease & stress resistance

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Loss of production due to bacterial and viral infections has been a recurring problem in shrimp aquaculture for decades now. The fact that shrimp mortality is often perceived as uncontrollable, is mainly due to a lack of a holistic approach in developing control strategies. Only focusing on the pathogens does not suffice, and both the environment and the host's defense have to be measurably improved. The recent case of *Vibrio parahaemolyticus* epizootic clearly illustrates this. By employing broad-spectrum disinfectants or antibiotics in ponds, it is possible to suppress temporarily the growth & bloom of bacteria. However, the root of the problem and the cause why some opportunistic r-strategist suddenly can rise to power in a shrimp pond is not addressed by this action. Hence, we advocate for the application of targeted, selective bactericides which specifically inhibit pathogenic *Vibrio*, immediately followed by the re-colonization of the empty niche with probiotic bacteria. Simultaneously, the shrimp's defense can be augmented by stimulating its endogenous protective pathways, such as increased heat shock protein synthesis. Heat shock proteins (HSP) function as chaperones for other proteins, protecting them from denaturation, and enabling survival during stressful conditions. Increased HSP levels allow the shrimp to cope with lethal levels of radicals, toxins and abiotic stress. In practice, HSP induction (HSPi) results in a spectacularly better performance of shrimp in abiotic stress tests and bacterial challenges.In a joint research project with Ghent University, we selected and tested a variety of compounds from plant origin which combine selective bactericidal and HSP inducing properties. The antibacterial action of the compounds was first studied in vitro in 96-well plates, analyzing the bacterial growth of specific bacterial strains in the presence of different components and concentrations. A gnotobiotic *Artemia* model was used to proof the concept that the selected compounds induced an increased production of HSP, as detected and quantified by Western blot. In subsequent *Vibrio* challenges, prophylactic HSPi compounds significantly increased the survival of *Artemia* from 30±5% to 60±7%. Experimental studies performed under normal culture conditions showed that the application of these HSPi compounds resulted in significantly better survival of *P. vannamei* PLs in stress tests from 45±8% to 75±10%. Continuous application of HSPi compounds during the hatchery cycle from mysis to PL10 also consistently resulted in significantly more and stronger PLs. Overall survival and total biomass in treated tanks were 16-25% higher than in untreated controls, while significantly reducing TCBS counts in culture water. In conclusion, through extensive scientific research and development, a synergistic mix of HSPi compounds was formulated which manages the microbial community by selectively suppressing pathogenic bacteria and at the same time enhances the intrinsic disease and stress resistance of shrimp. These documented additive effects proved to significantly improve disease management both under laboratory conditions and in commercial shrimp culture operations.

ID248:

Histopathology of Whiteleg shrimp (*Penaeus vannamei*) influenced by *Vibrio harveyi* and spo oil

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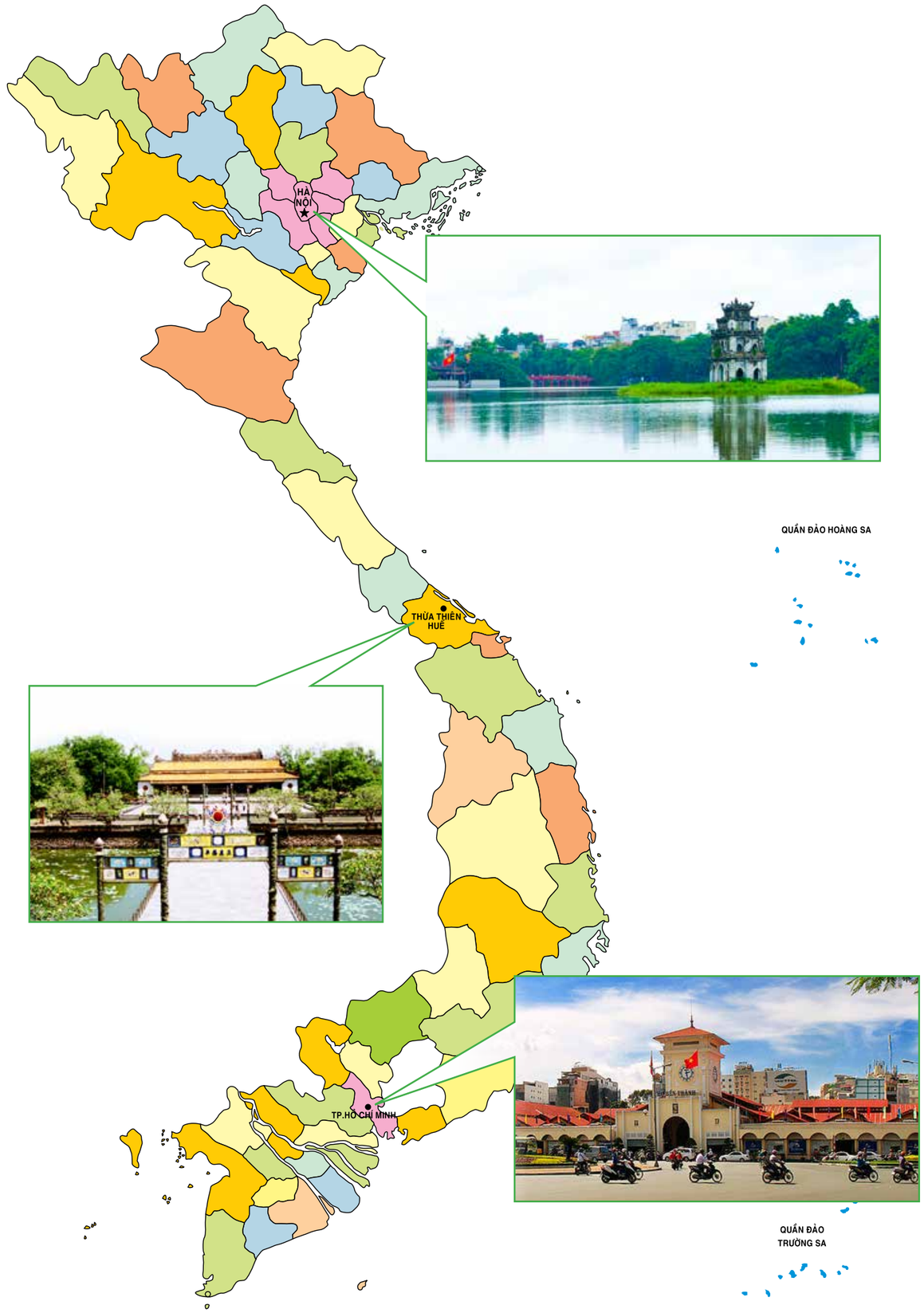
Vibrio harveyi was used for challenge experiment in shrimp which were collected from indoor and outdoor experiments at the injection doses of 10^7 CFU/ml. Experimental shrimp were fed the food supplemented SPO oil with the rate of 0%, 0,1%, 0,2%, 0,4% for 30 days. A study was carried out to determine the histopathology of whiteleg shrimp (*Penaeus vannamei*) influenced by SPO oil and *Vibrio harveyi*. Histopathplogical observation revealed most of the challenged shrimp had lesions in skeletal muscle, including hemocytic infiltration and multifocal necrosis muscle. The foci of rod bacteria cells presented in the muscle of shrimp cultured outdoor. The hepatopancreas (HP) tubule epithelial cells separated and lose connection with tubule membrane. There are lack B, E and F cells in the HP tubules. Massive necrosis in HP tubules caused multufoci bacteria.



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