Eighth Symposium on Diseases in Asian Aquaculture
21-25 November, 2011

BOOK OF ABSTRACTS

Organised by

Fish Health Section, Asian Fisheries Society
and
College of Fisheries
Karnataka Veterinary, Animal and Fisheries Sciences University
Mangalore, India

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EIGHTH SYMPOSIUM ON DISEASES IN ASIAN AQUACULTURE

Welcome to DAA VIII

On behalf of the College of Fisheries, Mangalore, we extend our warm welcome to all the delegates to the DAA8 being held in Mangalore, India from November 21-25, 2011. India is hosting this event for the first time and the theme "Fish for Food security" fits well in the present context of addressing the world's hungry population. The world community, on providing food to the one billion hungry mouths, needs to deliberate on "greater production of food by reduction in losses due to diseases". Like all food producing sectors, the aquaculture sector, which is claimed to be the fastest growing one is plagued with disease problems and losses. The coming together of over 200 delegates from several countries of the world and India is a reflection of the growing interest in Asian aquaculture through various collaborations between the east and the west, north and south. Never has the "one health" concept and the synergies therein been more relevant than the present time.

Thank you all for your whole hearted support and scientific contributions to this conference. Coming together is a beginning and staying together is progress. Many of us have built our co-operation through this forum by drawing up collaborative projects to address problems in aquaculture in the Asia-Pacific region. We hope the conference will forge new partnerships and help in moving towards producing more 'Fish for Food security'.

Distance has never been a reason for shirking from responsibilities. The local organisers express their deep appreciation for the continued support by Dr. C.V. Mohan, our former colleague, presently with NACA, Thailand.

The local hosts and members of the College of Fisheries wish all of you a useful and enjoyable time in this shore city of India enriched by her natural beauty of swaying coconut and arecanut palms, temples, churches and mosques that showcase the confluence of religions, the dense forests, the irresistible cashews and cuisines…etc etc. We also hope you enjoy the social and cultural evenings as you get a glimpse of the variety in India.

Welcome again to Incredible India and Magical Mangalore

The hosts of DAA8, Mangalore……

K.M. Shankar
M.N. Venugopal
Indrani Karunasagar
Malathi Shekar

Greetings from FHS

It is with a sense of pride and fulfillment that the FHS looks back at the over two decades gone by since the floating of the focused group working on improving aquaculture production, taking stock of the problems of emerging and reemerging diseases afflicting the sector and looking at methods to overcome them through diagnostic, preventive and therapeutic approaches.

As we forge ahead with dreams to address "Fish for food security" it is necessary that global concerns are deliberated by global experts. The goal of bringing together health professionals, researchers and students from several countries is once again accomplished as is evident from the >70 oral, ~200 poster presentations that will span through five days. The vision and tempo of those who started this in a small way in Bali, Indonesia in 1990 and moved through Phuket, Thailand (1993), Bangkok, Thailand (1996), Cebu, The Philippines (1999), Gold Coast, Australia (2002), Colombo, Srilanka (2005), Taipei, Taiwan (2008) and now Mangalore, India (2011) needs to be carried forward to other countries in the Asia-Pacific region by the younger, involved, enthusiastic members of the organisation.

Basic studies are essential to answer the vagaries that come about in the ever dynamic aquatic environment and the animals therein. This will enable us to answer the innumerable questions that arise during the varying experiences encountered during aquatic animal culture. This conference presents many such studies having immense practical applications for the future of the industry to help produce more "Fish for Food security".

The field of fish health has emerged as an important biological interface between the host, the pathogen and the environment. The information collected in the last two decades have been useful in explaining how the growth and development of the aquatic animals has been impacted by infectious and non-infectious agents. Application of molecular biology, rDNA technology, advanced immunological techniques along with bioinformatics tools have revealed the complexity of this field.

In the ecological niche of aquatic animals, multifaceted regulations of physiological and biochemical transformation control a wide array of functional attributes. The awareness of these challenges is required to be disseminated globally among researchers and this is an ideal platform for meeting and discussing. We are certain the conference will be intellectually rewarding, socially satisfying and culturally enriching.

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SCIENTIFIC PROGRAM SCHEDULE - ORAL

Scientific Program Schedule - Oral

Monday, 21 November

08.30 -10.00 : Registration
10.00 -11.00 : Opening Ceremony
11.00 -11.30 : Tea Break

Session 01 : **Global Aquaculture and Emerging Aquatic Animal Health Issues -Thematic Presentations (TP)**
Chairpersons : Tarun Shridhar and Timothy W Flegel

11.30 - 11.55 : TP-1: ***Implementation of good aquaculture practices to prevent animal diseases and ensure consumer protection***
Lahsen Ababouch (Italy)

11.55 - 12.20 : TP-2: ***Disease Control in Aquaculture: the Future for Food Security***
Rohana Subasinghe (Italy)

12.20 - 12.45 : TP-3: ***New and emerging OIE standards for aquatic animal health***
Barry Hill (United Kingdom)

12.45 - 13.05 : TP-4: ***Zoonotic potential of pathogens of aquatic animals and public health issues associated with aquaculture***
Iddya Karunasagar (Italy)

13.05 - 14.00 : Lunch Break

Oral Presentations (OP)

Session 02 : **Epidemiology of Shrimp Disease**
Chairpersons : Rohana Subasinghe and Chu Fang Lo

14.00 - 14.25 : OP-A1: ***Epidemiology and evolution of white spot syndrome virus: implications for shrimp farming***
Keynote - 01
Just Vlak (The Netherlands)

14.25 - 14.50 : OP-A2: ***Longitudinal disease studies in small-holder black tiger shrimp (Penaeus monodon) ponds in Andhra Pradesh, India***
Keynote - 02
Peter J Walker (Australia)

14.50 - 15.05 : OP-A3: ***Effect of different temperatures on the mortality and viral load of experimentally infected Penaeus monodon with White spot syndrome virus (WSSV)***
Eleonor A Tendencia (Philippines)

15.05 - 15.20 : OP-A4: ***Variable tandem repeat structure of white spot syndrome virus genome populations correlates with shrimp disease status***
Tran Thi Tuyet Hoa (Vietnam)

15.20 - 15.35 : OP-A5: ***Effects of fluctuating temperature and the lower salinity on the disease transmission of WSSV in Penaeus monodon***
Tuyen Ngo Xuan (Vietnam)

15.35 - 15.50 : OP-A6: ***Cost-benefit analysis of removable rain protection for shrimp ponds***
Roel H Bosma (The Netherlands)

15.50 - 16.30 : Tea Break

16.30 - 19.00 : Local sight seeing (Pilikula /Kudroli Temple /Aloysius Chapel

19.30 onwards : Welcome Dinner and Cultural show

Tuesday, 22 November

Session 03 : **Application of Diagnostics**
Chairpersons : Andrew Goodwin and Alexandra Adams

9.00 - 9.25 : OP-B1: ***Loop mediated isothermal amplification (LAMP): Application for fish and shellfish diseases***
Keynote - 03
Mashahiro Sakai (Japan)

9.25 - 9.45 OP-B2: ***Research development in Crustacean viral diseases***
Keynote - 04
Jean Roberts Bonami (France)

9.45 - 10.00 : OP-B3: ***Gyrase B gene sequencing and molecular detection of Vibrio harveyi***
Linda M Nunan (USA)

10.00 - 10.15 : OP-B4: ***False rumours of disease outbreaks caused by infectious myonecrosis virus (IMNV) in the whiteleg shrimp in Asia***
Saengchan Senapin (Thailand)

10.15 - 10.30 : OP-B5: ***Evaluation of several detection methods for Koi herpesvirus (KHV)***
Kei Yuasa (Japan)

10.30 - 10.45 : OP-B6: ***White tail disease (WTD) of Macrobrachium rosenbergii***
A S Sahul Hameed (India)

10.45 - 11.00 : OP-B7: ***In vitro propagation of shrimp Taura syndrome virus (TSV) in a C6/36 mosquito cell line***
Jurairatana Phromjai (Thailand)

11.00 -11.15 : OP-B8: ***Development of a multiplex PCR for the identification of pathogenic Edwardsiella tarda and application to edwardsiellosis diagnostics***
Zhao-Lan Mo (China)

11.15 -11.30 : Tea Break

Session 04 : **Crustacean Viruses and Immune Response**
Chairpersons : Peter Walker and I.S. Bright Singh

11.30 - 11.55 : OP-C1: ***The role of viral inserts in the shrimp response to viral pathogens***
Keynote - 05 :
Tim W Flegel (Thailand)

11.55 - 12.20 : OP-C2: ***Interfering RNA for control of viruses in crustacea***
Keynote - 06
Leigh Owens (Australia)

12.20 - 12.35 : OP-C3: ***Random, natural inserts of parvovirus genome fragments occur in the shrimp genome and have implications for viral disease diagnosis and control***
Vanvimon Saksmerprome (Thailand)

12.35 - 12.50 : OP-C4: ***Development and demonstration of 'Shrimpvac-I' for the management of white spot syndrome virus in shrimp grow-out system***
I S Bright Singh (India)

12.50 - 13.05 OP-C5: ***Uptake of white spot syndrome virus and WSSV-vaccines in the gut of black tiger shrimp, Penaeus monodon***
Amod Kulkarni (The Netherlands)

13.05 - 14.00 : Lunch Break

14.00 - 15.00 : **Poster Session**

15.00 - 15.30 : Tea break

Session 05 : **Fish Disease and their Management**
Chairpersons : Aoki T. and Barry Hill

15.30 - 15.55 : OP-D1: ***Managing viral infections endemic in farmed cyprinids: Goldfish herpesviral hematopoietic necrosis virus (CyHV-2) and the aquareoviruses***
Keynote - 07
Andrew Goodwin (USA)

15.55 - 16.20 : OP-D2: ***Nodavirus in Norway: lessons learned and disease management strategies***
Keynote - 08
Sonal Patel (Norway)

16.20 - 16.35 : OP-D3: ***Diseases of Asian seabass (or barramundi) Lates calcarifer Bloch***
Gibson-Kueh Susan (Australia)

16.35 - 16.50 : OP-D4: ***Difference in virulence of marine and freshwater isolates of viral hemorrhagic septicemia virus in vivo correlates with in vitro ability to infect gill epithelial cells and macrophages of rainbow trout (Oncorhynchus mykiss)***
Bjørn E. Brudeseth (Norway)

16.50 - 17.05 : OP-D5: ***Temperature dependent virus replication and anti-viral apoptotic response in viral haemorrhagic septicaemia virus infected olive flounder (Paralichthys olivaceus)***
Satheesha Avunje (Korea)

Wednesday, 23 November

Session 06 : **Immunology and Fish Vaccines**
Chairpersons : Jean Roberts Bonami and Dinakaran Michael

9.00 - 9.25 OP-E1: ***Molecular immunology and genomic science of shrimp***
Keynote - 09 Ikuo Hirono (Japan)

9.25 - 9.50 : OP-E2: ***Micro- and nanoparticles for vaccine delivery***
Keynote - 10 Roy Dalmo (Norway)

9.50 - 10.05 : OP-E3: ***Chelated mineral zinc for growth and immune response of Pangasius***
Ei Lin Ooi (Vietnam)

10.05 - 10.20 : OP-E4: ***Ontogeny of the thymus in barramundi (Lates calcarifer): a light microscope study***
Nguyen Huu Dung (Vietnam)

10.20 - 10.35 : OP-E5: ***Marine macroalgal immunostimulants - An alternative to finfish vaccines ?***
R Dinakaran Michael (India)

10.35 - 10.50 : OP-E6: ***Efficacy data of the first vaccine for pangasius in Vietnam***
Vo Thanh Tung (Vietnam)

10.50 - 11.05 : OP-E7: ***Development and characterization of monoclonal antibodies against putative T-cell of Catla catla***
Neeraj Sood (India)

11.05 -11.30 : Tea Break

Session 07 : **Immunology and Fish Vaccines (contd.)**
Chairpersons : Mashahiro Sakai and Leigh Owens

11.30 - 11.55 : OP-E8: ***Vaccine development for viral nervous necrosis of groupers***
Keynote - 11 Toshihiro Nakai (Japan)

11.55 - 12.20 : OP-E9: ***From discovery to characterization of cytokines: Applications in vaccine development and diseases***
Keynote - 12 Ram Savan (USA)

12.20 - 12.35 : OP-E10: ***Immune responses in Atlantic cod following immunisation with different bacterial antigens in oil*** adjuvant
Helene Mikkelsen (Norway)

12.35 - 12.50 OP-E11: ***The immune response and disease resistance of clonal lines of Nile tilapia Oreochromis niloticus***
Kim Thompson (UK)

12.50 - 13.05 OP-E12: ***Immune markers for disease resistance to aeromoniasis using rohu carp as a model species***
P.K.Sahoo (India)

13.05 - 14.00 : Lunch

14.00 - 15.00 : **Poster Session**

15.00 - 15.30 : Tea break

Session 08 : **Bacterial Pathogens and their Management**
Chairpersons : Toshihiro Nakai and Roy Dalmo

15.30 - 15.55 : OP-F1: ***Managing microbial activity in larviculture: the quorum sensing case***
Keynote - 13
Peter Bossier (Belgium)

15.55 - 16.20 : OP-F2: ***Bacterial interaction in crustacean guts: pathogenesis and gene expression***
Keynote - 14
Pikul Jiravanichpaisal (Thailand)

16.20 - 16.35 : OP-F3: ***Phage therapy in aquaculture -Lysozyme helps overcome phage resistance***
Indrani Karunasagar (India)

16.35 - 16.50 : OP-F4: ***Establishing Caenorhabditis elegans model for screening anti-infectives against aquaculture pathogens***
K Balamurugan (India)

16.50 - 17.05 : OP-F5: ***Major Diseases and health management in striped catfish (Pangasianodon hypophthalmus) farming in the Mekong River Delta, Vietnam***
Dang Thi Hoang Oanh (Vietnam)

17.05 - 17.20 : OP-F6: ***Antibiofilm compounds from marine bacteria - A novel strategy to conquer antibiotic resistance in aquaculture industries***
S Karutha Pandian (India)

17.20 - 17.35 : OP-F7: ***Sustaining Shrimp Culture in the Philippines Using Molecular Applications***
Benedict A. Maralit (The Philippines)

18.00 - 19.30 **9th TGM of FHS (AFS)**

Thursday, 24 November

Session 09 : **Genomics, Bioinformatics and Novel Approaches**
Chairpersons : Just Vlak and Ingo Ernst

9.00 - 9.25 : OP-G1: ***Using systems biology approaches to understand shrimp cellular responses during viral infection***
Keynote - 15
Chu Fang Lo (Taiwan)

9.25 - 9.50 : OP-G2: ***Development of SPF and SPR stocks for shrimp aquaculture***
Keynote - 16
Carlos Pantoja (USA)

9.50 - 10.05 : OP-G3: ***Mining a comprehensive collection of shrimp expressed sequence tags for viral responsive genes***
Anuphap Prachumwat (Thailand)

10.05 - 10.20 : OP-G4: ***Molecular characterization, down-stream signaling analysis, and prediction of ligand binding key domains in toll-like receptor 2 (TLR2) of the Indian Major Carp, rohu (Labeo rohita)***
Mrinal Samanta (India)

10.20 - 10.35 : OP-G5: ***White spot syndrome virus induces a Warburg-like effect in shrimp hemocytes in the early stage of infection***
KC Han-Ching Wang (Taiwan)

10.35 - 10.50 : OP-G6: ***Reverse genetics of fish viruses***
K Riji John (India)

10.50 - 11.05 : OP-G7: ***Semiconductor based sequencing technology: Applications and tools***
Anupama Gaur (India)

11.05 - 11.30 : Tea Break

Session 10 : **Immune Mechanisms and Pathogenesis**
Chairpersons : Nobuaki Okamoto and Carlos Pantoja

11.30 - 11.55 : OP-H1: ***Induction of type I interferon gene expression mediated by pattern recognition receptors in Japanese flounder***
Keynote - 17
Takashi Aoki (Japan)

11.55 - 12.20 : OP-H2: ***Anti viral and anti-bacterial defence in a crustacean: role of Dscam and gC1qR***
Keynote - 18
Kenneth Söderhäll (Sweden)

12.20 - 12.35 : OP-H3: ***Cloning, expression analysis, and silencing study of three inhibitor of apoptosis protein genes (IAP) from Litopenaeus vannamei shrimp***
Jiann-Horng Leu (Taiwan)

12.35 - 12.50 : OP-H4: ***Pathogen recognition receptors (PRRs) in teleost fish: An overview***
Rajendran K V (India)

12.50 - 13.05 : OP-H5: ***Profiling expressed genes in the lymphoid organ of Australian banana prawn (Penaeus merguiensis) using suppression subtractive hybridization***
Rusaini (Australia)

13.05 - 14.00 : Lunch

14.00 - 15.00 : **Poster Session**

15.00 - 15.30 : Tea break

Session 11 : **Parasitic and other Diseases of Fish**
Chairpersons : Øystein Evensen and Pikul Jiravanichpaisal

15.30 - 15.55 : OP-I1: I***n vitro and in vivo efficacies of ionophores against Cryptocaryon irritans***
Keynote - 19
Tomoyoshi Yoshinaga (Japan)

15.55 - 16.10 : OP-I2: ***Nested PCR assay, a quick and sensitive tool for the diagnosis of the Apicomplexan parasite, Perkinsus spp. in commercially important marine molluscs***
Vijayan K. K (India)

16.10 - 16.25 : OP-I3: ***Use of Bio-Surfactant as a prophylactic agent against fish protozoan parasite, Cryptocaryon irritans in Asian seabass, Lates calcarifer***
Beng Chu Kua (Malaysia)

16.25 - 16.40 : OP-I4: ***Caligus Müller, 1785 parasitizing cultured Lates calcarifer Bloch, 1970 in Malaysia***
Muhd. Faizul (Malaysia)

16.40 - 16.55 : OP-I5: ***Prevalence, mean intensity and site preference of Caligus rotundigenitalis Yü, 1933 1 (Copepoda: Caligidae) on cage cultured crimson snapper (Lutjanus erythropterus Bloch, 2 1790) from Bukit Tambun, Penang, Malaysia***
Leaw Yoon Yau (Malaysia)

16.55 - 17.10 : OP-I6: ***First molluscan theta class Glutathione S-Transferase: Identification, cloning, characterization and transcriptional analysis post immune challenges***
Kasthuri Saranya Revathy (Korea)

17.10 - 17.25 : OP-I7: ***Resurgence of Epizootic Ulcerative Syndrome (EUS) in India***
Pravata Kumar Pradhan (India)

17.30 - **Group Photo**

Friday, 25 November

Session 12 : **Translating Academic Research to Field Application**
Chairpersons : Kenneth Söderhäll and Peter Bossier

9.00 - 9.25 : OP-J1: ***Identification of fish vaccine antigen candidates - finding needles in a haystack***
Keynote - 20 Alexandra Adams (UK)

9.25 - 9.50 : OP-J2: ***Novel vaccine technologies - also for farmed fish (?)***
Keynote - 21 Øystein Evensen (Norway)

9.50 - 10.05 : OP-J3: ***Field trial study on vaccine ALPHA JECT® Panga 1 against Edwardsiella ictaluri, in pangasius catfish (Pangasianodon hypophthalmus)***
Tu Thanh Dung (Vietnam)

10.05 - 10.20 : OP-J4: ***Streptococcosis in fish: implications for vaccine development***
Saravanane Poobalane (Singapore)

10.20 - 10.35 : OP-J5: ***Establishment of the technological platform for the development of rapid detection kits of aquatic animal pathogens***
Qing-Li Zhang (China)

10.35 - 10.50 : OP-J6: ***Detecting WSSV and IMNV by using insulated isothermal polymerase chain reaction (iiPCR)***
Chen Su (Taiwan)

10.50 - 11.05 : OP-J7: ***A comparison of the effect of live and dead Aeromonas hydrophila on the immune response of Pangasius hypophthalmus***
Wanna Sirimanapong (Thailand)

11.05 - 11.30 : Tea Break

Session 13 : **Disease Preparedness and National Plans**
Chairpersons : Iddya Karunasagar and Eduardo Leano

11.30 - 11.55 : OP-H1: ***Current state of fish diseases in Japan***
Keynote - 22 Nobuaki Okamoto (Japan)

11.55 - 12.20 : OP-H2: ***Arrangements for effective emergency aquatic animal disease response***
Keynote - 23
Ingo Ernst (Australia)

12.20 - 12.35 : OP-H3: ***Building capacity in aquatic animal health - Challenges, Strategies & Opportunities: Role and activities of the ASEM Aquaculture Platform in Aquatic Animal Health***
John Bostock (UK)

12.35 - 12.50 : OP-H4: ***WSSV disease and socio- economic behavior of shrimp aquaculture industry in Sri Lanka***
Aruna C.D.A.M. Dissanayake (Sri Lanka)

12.50 - 13.05 : OP-H5: ***Mycobacteriosis in ornamental fish tank in Iran, what mycobacterium sbsp. can be accused?***
Nader Mosavari (Iran)

13.05 - 13.20 : OP-H6: ***Current status and problems of infectious myonecrosis virus (IMNV) disease in Penaeus vannamei farming in Indonesia***
Yuri Sunanto (Indonesia)

13.20 - 14.30 : Lunch

Session 14 : **Plenary Session**
Panel Coordinators : Tim Flegel, Barry Hill and Lahsen Ababouch

14.30 - 15.30 : ● Wrap up of oral and poster presentations
● Facilitated open discussions
● Recommendations and way forward

15.30 - 16.00 : Formal closing

16.00 - 16.30 : Tea

19.00 - : Cultural show, Award ceremony and Banquet

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SCIENTIFIC PROGRAMME SCHEDULE - POSTER (PP)

Scientific Programme Schedule -Poster (PP)

Tuesday, 22 November, 2011

A: Aquatic Animal Diseases	(A1 -A19)
B: Health Management, Probiotics, Immunostimulants, Phages	(B1-B23)
C: Diagnostics, Treatment, Cell Lines	(C1-C23)

Wednesday, **23 November, 2011**

D: Crustacean Viruses and Immunity	(D1-D32)
E: Parasitic Diseases	(E1 -E20)

Thursday, 24 November, 2011

F: Fish Vaccination and Immunity	(F1 - F23)
G: Molecular Biology, Genomics and Bioinformatics	(G1 - G29)

A: Aquatic Animal Diseases

Sl.No	*Poster Title/ Presenting Author*	*Poster Code*
1.	***First identification of Flavobacterium columnare infection in farmed striped catfish Pangasianodon hypophthalmus*** Tu Thanh Dung (Vietnam)	PP-A1
2.	***The study fungi contamination in rainbow trout farm*** Monireh Faeed (Iran)	PP-A2
3.	***Prevalence, epidemiology and histopathology of tumour (Odontoma) in Sphyraena obtusata, South East coast of India*** Gopalakrishnan A. (India)	PP-A3
4.	***Virulence and susceptibility studies of Vibrio species isolated from cultured P. monodon affected by Monodon Slow Growth Syndrome(MSGS) and Loose Shell Syndrome (LSS)*** Jayasree Loka (India)	PP-A4
5.	***Outbreak of Betanodavirus in cage-cultured Asian seabass (Lates calcarifer Bloch): A case study*** K.P.Jithendran (India)	PP-A5
6.	***MS marker distribution pattern in iridovirus infected rock bream, Oplegnathus fasciatus*** Myung-Hwa Jung (Korea)	PP-A6

B. Health Management, Probiotics, Immunostimulants, Phages

C: Diagnostics Treatment Issues, Cell Lines

E: Parasitic Diseases

F: Fish Vaccination and Immunity

G: Molecular Biology, Genomics and Bioinformatics

ORAL PRESENTATION

TP - 1
Implementation of good aquaculture practices to prevent animal diseases and ensure consumer protection

Lahsen Ababouch

Food and Agriculture Organisation, Rome, Italy
Email: Lahsen.Ababouch@fao.org

As much as diseases in aquaculture are highlighted as a major concern to sustainable production and profitability, ensuring consumer safety through reducing human health hazards from cultured aquatic products has become an equally important challenge. The volume of internationally traded aquaculture products is rapidly increasing globally and there is a risk of increasing health hazards to consumers. As a result, major importing countries have been implementing more and more stringent food safety standards for aquaculture products, particularly on the levels of residues of antimicrobials and harmful microorganisms. While harmful microorganisms in rearing waters can originate from off-farm sources; i.e. water containing faecal bacteria, animal and poultry manure containing harmful microorganisms, etc., most antibacterial residues result from their direct usage in the farm. Any effort to reduce contamination by harmful organisms should consider both aspects; maintaining animal health and improving food safety. In this respect, the role of the fish farmer in managing health should change from merely reducing mortalities and improving production at farm level to being an indispensable part of a chain for the production and delivery of safe, high quality products to the consumer. This is most important because, like any other food chain, the aquaculture one is only as strong as its weakest link. Rearing water quality and the misuse of antimicrobials are important links. This paper addresses the significant synergies that can be developed when both aquatic animal health and food safety are addressed concurrently to make aquaculture economically profitable while ensuring maximal consumer protection".

TP - 2
Disease control in aquaculture: The future for food security

Rohana P. Subasinghe

Senior Aquaculture Officer, Fisheries and Aquaculture Management Division, Fisheries and Aquaculture Department, Food and Agriculture Organization of the UNViale delle Terme di Caracalla 00153, Rome, ITALY
Email: Rohana.Subasinghe@fao.org

Aquaculture remains a growing, vibrant and important food production sector and contributed 52.5 million tones of aquatic animals accounting for 45.7 percent of the world's fish food production for human consumption in 2008. Considering the growing world population and the need for more food and accepting the fact that the production from capture fisheries will not be increased significantly, at least to maintain the global per capita fish consumption at the current level, we will have to produce an additional 30 million tones of aquatic animal products by the year 2030. This is a significant challenge and if not achieved, not only will it affect the global food security, but also will impinge on the nutrition and health of millions of people living in the planet.

Disease control and health management in aquaculture is increasingly considered as one of the most, if not the most, important constraint in meeting the global demand for food fish in the future. Recent outbreaks of fish and shellfish diseases in aquaculture and in the wild and the losses incurred remind us of the importance of addressing the health issues in aquaculture, developing strategies for reducing the risks of disease in aquaculture production systems, and need for conducive policies, strategies and plans for maintaining good biosecurity at all levels. Recent FAO and World Bank estimates indicate that, annually, aquaculture sector suffers losses over 6.5 billion US Dollars due to diseases.

The presentation discusses the experiences and trends in aquatic animal disease in global aquaculture and the opportunities and constraints for reducing the risks for ensuring contribution of aquaculture for meeting the global food fish demand in the coming decades.

TP - 3
New and emerging OIE standards for aquatic animal health

Barry Hill

President, OIE Aquatic Animal Health Standards Commission, c/o Cefas, Weymouth DT4 8UB, United Kingdom
Email: b.j.hill@cefas.co.uk

OIE, as the World Organisation for Animal Health, is continuously developing standards to ensure the sanitary safety of international trade in live aquatic animals and their products. This is achieved by providing guidelines on the measures to be used by the veterinary authorities and/or aquatic animal health services of importing and exporting countries to prevent the transfer of agents pathogenic for aquatic animals, while avoiding unjustified trade barriers. The OIE also provides detailed guidance on the best methods for detection and identification of important pathogens of aquatic animals (amphibians, crustaceans, fish and molluscs) to increase knowledge and awareness of their occurrence and distribution.. The standards and methods are published in the OIE Aquatic Animal Health Code ("Aquatic Code") and the OIE Manual of Diagnostic Tests for Aquatic Animals ("Aquatic Manual"), both of which are updated annually to take account of advances in scientific knowledge. On-going development of the Aquatic Code and Aquatic Manual is the responsibility of one of the OIE's Specialist Commissions, the Aquatic Animal Health Standards Commission (AAHSC), which is scientifically assisted by several ad hoc groups of experts in specific topics, internationally renowned independent experts and the expertise at the many OIE Reference Laboratories for aquatic animal diseases.

Recently revised or new standards adopted include:

- amendments to the listed aquatic animal diseases
- chapters for the new listed diseases
- criteria to assess the safety of aquatic animal commodities
- listing of safe aquatic animal commodities for international trade
- handling, disposal and treatment of aquatic animal waste
- principles for responsible and prudent use of antimicrobial agents in aquatic animals
- welfare of farmed fish during transport
- welfare aspects of stunning and killing of farmed fish for human consumption
- control of hazards in aquatic animal feeds.

New standards currently in development include:

- guidance and criteria for listing aquatic animal species as susceptible to a disease
- pathogen differentiation for some listed diseases
- killing of farmed fish for disease control purposes
- monitoring of the quantities and usage patterns of antimicrobial agents used in aquatic animals
- national antimicrobial resistance surveillance and monitoring programmes for aquatic animals
- disease-specific surveillance model chapters
- definition of 'aquatic animal health professional.

Details of these revised and new standards, and an outline of the standards in development, will be presented and discussed.

TP- 4

Zoonotic potential of pathogens of aquatic animals and public health issues associated with aquaculture

Iddya Karunasagar

Products, Trade and Marketing Service, Fisheries and Aquaculture Department, Food and Agriculture Organisation, Rome, Italy
Email: Iddya.Karunasagar@fao.org

Aquaculture is contributing to about half of the global food fish production and as the contribution of products from this sector in international fish trade is increasing, there are also concerns about consumer safety of these products. However, current epidemiological records show that aquaculture products are rarely involved in outbreaks of foodborne illnesses. There are few pathogens of aquatic animals that have a zoonotic potential. *Vibrio vulnificus* has been associated with eel infections and shrimp mortalities. This organism has been isolated both from wound infections and in septicaemia in humans. *Edwardsiella tarda*, a common pathogen of warm water aquaculture is associated with both intestinal and extra-intestinal infections in humans. *Streptococcus iniae*, another important pathogen in warm water aquaculture has been occasionally isolated from human infections. Though *Aeromonas hydrophila* has been considered a potential human pathogen, its involvement in gastrointestinal illnesses has been questioned. Though *Salmonella spp* are not considered pathogens of aquatic animals, their associations with aquaculture environments and aquaria have led to public health concerns. Some parasites like fishborne trematodes have adapted to survive in fish without inducing any pathology or immune reactions. Residues of antimicrobials and antimicrobial resistance being selected due to antimicrobial use in aquaculture are other public health issues related to fish health management.

OP - A1

Epidemiology and evolution of white spot syndrome virus: implications for shrimp farming

Just M. Vlak, *Tran Thi Tuyet Hoa, Bui Thi Minh Dieu, Ngo Xuan, Tuyen Mark P. Zwart Mart and C.M. de Jong*

Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
Email: just.vlak@wur.nl

White spot syndrome virus (WSSV) is the most important disease agent in cultured penaeid shrimp in the last two decades. Since its discovery in the early 1990s the virus has quickly spread from its origin in Southeast Asia to almost all shrimp growing areas around the world. The disease has not only caused huge economic losses, but has also led to environmental problems, habitat destruction and endangered livelihoods in shrimp growing areas. WSS is a notifiable disease (OIE) and has been reported in most if not all shrimp growing countries. The virus is highly infectious and is easily transmitted both horizontally, through contact and cannibalism, and vertically, via eggs and broodstock. Why the virus is so infectious and which factors contribute to outbreaks of the disease is not clear. A further problem is the wide host range among crustaceans, where the infection can be asymptomatic but which could serve as a reservoir for WSSV.

WSSV is about the largest animal DNA virus, with a genome of about 300,000 base pairs. The function of the 180 open reading frames is being investigated but largely enigmatic. WSSV isolates from around the world are highly homologous (> 99% on the basis of nucleotide sequence), but there are five well defined areas with high variation: two regions with insertion / deletions ('indels') and three regions with a highly variable number of tandem repeats (VNTRs). These regions have been used to reconstruct the evolution of WSSV over time and space and have led to a model explaining the evolutionary path of this virus.

Recently, the structure of these variable regions has been correlated with virulence and more recently with outbreak status and type of shrimp production system. Furthermore, WSSV isolates appeared to be mixtures of genotypes and the genetic population structure of WSSV in ponds to be a predictor of the health status of the shrimp production system. This information renders functional significance to these variable regions in the WSSV genome and may lead to novel early warning, mitigation or control strategies to combat the disease

OP - A2

Longitudinal disease studies in small-holder black tiger shrimp *(Penaeus monodon)* ponds in Andhra Pradesh, India

***PJ Walker[1]**, N Gudkovs[1], CV Mohan[2], V Stalin Raj[3], B Pradeep[4], E Sergeant[5], AB Chandra Mohan[6], G Ravibabu[6], TC Santiago[3], I Karunasagar[4], KK Vijayan[7], NR Umesh[6] and PA Padiyar[2]*

[1]CSIRO Livestock Industries, Australian Animal Health Laboratory, AUSTRALIA
[2] Network of Aquaculture Centers Asia-Pacific, Bangkok, 10900, THAILAND
[3]Central Institute for Brackishwater Aquaculture, INDIA
[4]College of Fisheries, Karnataka Veterinary, Mangalore, INDIA
[5]AusVet Animal Health Services, Brisbane, AUSTRALIA
[6]National Centre for Sustainable Aquaculture, Kakinada INDIA
[7]Central Marine Fisheries Research Institute, Kochi, INDIA.
Email: Peter.Walker@csiro.au.

Longitudinal disease studies were conducted between January 2005 and August 2007 in small-holder black tiger shrimp ponds in Andhra Pradesh. The studies applied PCR testing and genotype analysis using the ORF94 variable number tandem repeat (VNTR) marker to detect evidence of WSSV infection in shrimp, wild crustacean and plankton samples at various times during grow-out. The first study, involving 457 BMP ponds in West Godavari, indicated a high risk of WSSV infection during grow-out stage but a relatively low incidence of disease, despite a high prevalence of heavy WSSV infection in non-outbreak ponds. The second study, conducted in a cluster of 61 non-BMP ponds in Krishna, confirmed a high risk of exposure to WSSV infection during grow-out but a much higher risk of disease outbreaks, and demonstrated that multiple WSSV genotypes were circulating simultaneously in the farming area. The spatial and temporal pattern of WSSV genotype distribution suggested transmission of infection within two clusters of ponds. The third study, conducted in a cluster for 12 ponds in the same location, involved sampling farmed shrimp, wild shrimp, crabs and plankton at intervals of 10 days. Of the 375 samples collected, 216 (57.6%) were WSSV-positive by Taqman PCR. The overall prevalence of WSSV infection varied significantly amongst sample types and was higher in farmed shrimp (76.0%) than for any other sample category (56% for crabs and plankton, 47% for wild shrimp). A wave of WSSV infection in plankton and wild crustaceans occurred across the study site, commencing at day 10, intensifying at day 20, subsiding at days 30 and 40, and passing off by day 50. The pattern of WSSV infection in farmed shrimp appeared to follow the wave of infection in plankton and wild crustaceans, with heavy viral genetic loads detected in most samples collected from days 30 to 80. Genotype analysis identified a wide range of concurrently circulating WSSV genotypes with multiple genotypes commonly detected in individual samples. Although there were several examples of simultaneous heavy infections with the same genotype in several ponds, there was no clear pattern of association of a single genotype or constellation of genotypes with disease outbreaks.

OP - A3

Effect of different temperatures on the mortality and viral load of White spot syndrome virus (WSSV) experimentally infected *Penaeus monodon*

Eleonor A. Tendencia [1,2], *Roel H. Bosma*[2] *and Johan A.J. Verreth*[2]

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan 5021 Iloilo, Philippines

[2]Aquaculture and Fisheries Group, Wageningen University, The Netherlands

Email: gigi@seafdec.org.ph; eleonor.tendencia@wur.nl

White spot syndrome virus (WSSV) has plagued the shrimp industry for more than two decades. Low temperature is reported to be a risk factor while high temperature a protective factor. A temperature of 29°C is reported to be optimum for shrimp culture, while others found that WSSV virus does not replicate at this temperature. To validate the effect of different temperatures on WSSV infection, tank experiments with three replicates per treatment were done. Healthy shrimp were injected with WSSV at high (90 x SID50)(SID50 = shrimp infectious dose with 50% endpoint) and low (10x SID50) viral dose (SID50= 105.5) and cultured at different temperatures (20°, 29°, 36°C). Control groups were injected with sterile phosphate buffer solution. Mortality was recorded daily. Shrimp were analyzed for WSSV by qPCR. Another experiment was done to determine the effect of the 3 temperatures on viral multiplication in shrimp injected with low viral dose.

Preliminary statistical analysis showed higher mortalities in shrimp, injected with both low and high viral dose, cultured at 22°C than those kept at 29°C and 36°C. Higher WSSV load was also observed in 22°C treatments at 72 hours post injection (hpi). Based on the results of the univariate analysis, the injected virus (P=0.005) and the viral load (P=0.002) accounted for the observed mortalities; temperature (P=0.064) weakly accounted for the viral load of shrimp. In the experiment wherein shrimp were injected with low viral dose and cultured at 22°C, 29°C and 36°C, significantly higher viral load in shrimp exposed to 29°C at 24 hpi was observed. This was accounted for by the luminous bacteria present (P=0.001), injected virus (P=0), temperature (P=0), and the interaction between last two (P=0). At 48 and 72 hpi, significantly higher viral load was observed in injected shrimp cultured at 22o and 29°C. This was accounted for by the percentage of green Vibrios (P= 0.049) in addition to temperature, injected virus and interaction of the last 2 factors. A 4% mortality was observed in injected shrimp cultured at 36°C at 24 hpi. At 48 hpi 8% mortality was observed in injected shrimp cultured at 29°C.

Results validated that low temperature (22°C) is a WSSV risk factor and high temperature (36°C) a protective factor; although, high temperature could have overwhelmed the shrimp immune system after 24 hpi resulting in mortality and higher viral load (1.0 x 105 WSSV/mg). The drop in viral load (to 5.75 x 102 WSSV/mg) at 36°C at 48 hpi suggested that high temperature could be a protective factor against WSSV which is in accordance with earlier findings. Contrary to previous reports, WSSV virus multiplied at 29°C resulting in mortality at 48 hpi in this study.

OP - A4

Variable tandem repeat structure of white spot syndrome virus genome populations correlates with shrimp disease status

Tran Thi Tuyet Hoa,[1,2,3,] *Mark P. Zwart*[3,4]*, Nguyen T Phuong*[2]*, Mart C.M. de Jong*[3] *and Just M. Vlak*[1]

[1]Laboratory of Virology, Wageningen University, Radix building (# 107) Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
[2]College of Aquaculture and Fisheries, Can Tho University, Campus II, 3/2 street, Can Tho City, Vietnam
[3]Quantitative Veterinary Epidemiology, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
[4]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-UPV, Valencia, Spain
Email: ttthoa@ctu.edu.vn

Investigating the genetic variability among natural WSSV populations is a novel approach to understand the evolution and pathogenesis of this virus. We characterized the number of repeat units located in the variable number of tandem repeat regions (VNTR) of ORF75, ORF94 and ORF125 (WSSV-TH strain; Van Hulten et al., 2001) from 662 WSSV-DNA samples collected from 104 shrimp ponds. The results showed that: (i) a high genetic diversity of WSSV isolates in the small scale shrimp farming operations in the Mekong delta, Viet Nam, occurred; (ii) the pattern of repeat regions in ORF94, ORF125 was positively correlated with disease status (disease outbreak or not); (iii) low repeat unit numbers have a significantly higher prevalence in outbreak ponds compared to non-outbreak ponds, especially for ORF94, suggesting that this VNTRs region is useful for monitoring WSSV populations for virus outbreak status. There is no significant correlation of VNTR repeat unit structure and shrimp cultivation practices. These findings add to our understanding of the role of WSSV population structure and disease outbreaks.

OP – A5

Effects of fluctuating temperature and lower salinity on the disease transmission of WSSV in *Penaeus monodon*

***Tuyen Ngo Xuan*[1,2], *Johan Verreth*[3], *Just M. Vlak*[4] *and Mart C.M. de Jong*[1]**

[1]Quantitative Veterinary Epidemiology Group, WU Animal sciences, Wageningen University, 6700AH Wageningen, the Netherlands
[2]Research Institute for Aquaculture No. 2, 116 Nguyen Dinh Chieu St., HoChiMinh City, Vietnam,
[3]Aquaculture and Fisheries Group, WU Animal sciences, Wageningen University, 6700AH Wageningen, the Netherlands
[4]Laboratory of Virology, Wageningen University, Wageningen, the Netherlands
Email: ngoxuan.tuyen@wur.nl

White spot syndrome virus (WSSV) is an infectious disease ravaging farmed penaeid shrimp worldwide. A comparison was made of the disease transmission and survival rates of WSSV in *Penaeus monodon* juvenile between optimal conditions and more adverse conditions being low salinity condition or fluctuating temperatures.

The experiments, done with group cohabitation, were conducted in glass tanks where the experimental conditions were controlled. In the experiment dead shrimp were either sampled and left in the tank (keep) or were removed immediately when found during inspection (remove). A PCR method was used to test for virus infection. The Generalized Linear Model and Kaplan-Meier regression model were used to estimate respectively the transmission parameters of WSSV and survival of shrimp.

The transmission rate parameter of WSSV in shrimp was not significanly different between optimal conditions and low salinity condition or fluctuating temperature condition independent of the keep or remove protocol. The mortality rate parameter of WSSV infected shrimp was significantly different between optimal and low salinity. In contrast, there was only a difference between optimal temperature and fluctuating temperature when the keep protocol was used. In these experiments low salinity and fluctuating temperature increases the mortality rates in infected shrimp, but not the transmission rates of WSSV. The consequence is that the WSSV is expected to transmit best under optimal conditions but more clinical symptoms will be seen under the adverse conditions.

OP – A6

Cost-benefit analysis of removable rain protection for shrimp ponds.

Roel H. Bosma[1], *Pim Mostert[2] and Eleonor Tendencia[1,3].*

[1] Aquaculture and Fisheries, Animal Sciences Dep., Wageningen University, Wageningen, Netherlands

[2] Farm Technology (BSc student), Wageningen University, Netherlands

[3] Aquaculture Dep., Southeast Asian Fisheries Development Center, Tigbauan 5021 Iloilo, Philippines

E-mail: roel.bosma@wur.nl

Shrimp farmers experience outbreaks of white spot syndrome virus (WSSV) among others because of heavy rain causing changes in salinity and temperature of the water. Therefore in the Philippines many farmers stock only once a year, while 1.5 times on average can be reached, considering time needed for pond preparation. Protection against rainwater falling in the ponds might enable this increased harvest frequency. In this study we investigated the profitability of investing in a removable rain screen for shrimp ponds.

Economic indicators were collected from 9 farms in the Philippines in 2009. Average shrimp yield was 6,650 kg ha-1 with an average market price of about 6.4 US$ kg-1 and a profit of close to 0.4 US$ kg-1. The benefit-cost analyses (CBA) of the rain screen was computed for a baseline scenario considering a lifespan of 15 years, a one hectare pond, 1.5 harvest per year, and the 2009 shrimp market. We also calculated benefits for a 10% higher price. We considered an annual interest rate of 5% and a discount factor of 3.5.

Total investment cost were estimated at 63,000 US$ ha-1. The pay-off period for the 2009 market price of shrimp was 14 years. Net Present Value (NPV) of total benefit of investing in a rain screen was positive for the 2009 shrimp market price but doubles if shrimp price increases with 10% (Table). Considering the given parameters, the maximum affordable costs of the rain screen varied from 6.9 to 10.6 US$ m-2. Investing in a removable rain screen is cost effective for the considered values, but will depend on the shrimp market price, on the realized increase in the number of harvests, and on the realized decrease in the mortality due to the disease. The interest of enterprises to invest in a rain screen to increase harvest frequency will also depend on the cost of land.

Undiscounted and discounted cost and benefits, NPV, Benefit Cost Ratio (BCR) and Internal Rate of Return (IRR) of investing in a removable rainscreen for a one hectare pond considering 15 year pay-off for two shrimp prices (US$).

Shrimp	Undiscounted		Discounted				
price kg-1	costs	benefits	costs	benefits	NPV	BCR	IRR
6.4	86,881	130,764	69,175	93,451	24,276	1.35	25
7.1	80,711	169,980	67,892	120,791	52,899	1.78	37

OP – B1
Loop mediated isothermal amplification (LAMP): Application for fish and shellfish diseases

Masahiro Sakai, *Toshiaki Itami and Tomoya Kono*

Faculty of Agriculture, University of Miyazaki, Gakuen kibanadai nishi 1-1,Miyazaki, 889-2192, Japan
Email: m.sakai@cc.miyazaki-u.ac.jp

The LAMP (loop mediated isothermal amplification technique) has been developed which can amplify nucleic acids with high specificity, sensitivity and rapidity under isothermal conditions in 2000. The LAMP reaction employs a DNA polymerase and a set of four specific primers that recognizes a total of six distinct sequences on the target DNA making the amplification of the target sequence highly selective. Various bacterial, viral, fungal and parasitic pathogens in both animal and plants have been detected using LAMP. In aquaculture, LAMP technique has also been reported for detection of many fish and shrimp pathogens such as KHV, SVCV, IHNV, WSSV, *Edwardsiella tarda, Vibrio nigripulchritudo,* YHV, *Nucleospora salmonis* etc. According to these reports, the sensitivity of LAMP is almost the same as PCR and it suggest the possibility for use of this technique for diagnosis of these diseases. Furthermore, the real-time LAMP method has been recently reported for quantitative detection of pathogens. In this review, the advantages and the potential application of LAMP in the detection of pathogens associated with aquaculture are discussed.

OP – B2
Research development in crustacean viral diseases

Jean-Robert Bonami

C.N.R.S. Pathogens and Environment, Université Montpellier 2, 34095 Montpellier Cedex 5, France

Email:bonami.jean-robert@neuf.fr

Since the report in 1966 of a virus infecting crab, the number of viral diseases reported in aquatic crustacean has dramatically increased in parallel with aquaculture development. These diseases were described in marine and freshwater crabs, in shrimp and freshwater prawn, in crayfish species and lobsters. To date, the listed viruses in crabs (more than 30 viruses) are at least as numerous as shrimp and prawn viruses. A total of more than 70 viruses were reported in crustacean literature from both freshwater and marine environment. Of course, shrimp/prawn viruses were the most investigated due to their negative impact on economy with losses estimated in billions of US $.

As invertebrate immunity is not so well developed as in vertebrates, disease control based on vaccination is not yet available in farms and limited to date to experimental treatment. Consequently selection of healthy broodstock or specific pathogen free animals and health checking of pond populations were developed to prevent and limit disease impact. These methods are principally based on nucleic acid detection by PCR or RT-PCR.

Underlining the importance of viral diseases in crustacean, the OIE includes six viral diseases on the eight listed: Infectious hypodermal and haematopoietic necrosis (IHHN), Infectious myonecrosis (IMN), Taura syndrome (TS), White spot syndrome (WSS), White tail disease (WTD) and Yellow head disease (YHD).

Some of these viruses are able to develop in other groups of crustacean. A good example is the WSSV (White spot syndrome virus), which can infect quite all crustacean, crabs, shrimp and prawn, crayfish and lobsters. Some exhibit strong morphological similarities with viral particles previously reported in other groups; unfortunately, in these cases, old data are only limited to histo- and cyto-pathological results, with no possibilities of genomic comparison.

All the six viral diseases listed above were deeply investigated, etiological agents were isolated, characterized and their genomes fully sequenced. These results and the very high number of scientific papers published on these subjects underline the impressive progress made during the last four decades in the field of crustacean virology.

OP - B3

Gyrase B gene sequencing and molecular detection of *Vibrio harveyi*

Linda M. Nunan, *Leone Mohney and Donald V. Lightner*

The University of Arizona, Department of Veterinary Science and Microbiology, 1117 E. Lowell St., Tucson, Arizona, USA

Email: lmn@email.arizona.edu

Vibriosis can be caused by many different species or strains of *Vibrio*. Two of the most common *Vibrio spp.* which are responsible for economic loss in the shrimp industry include *V. harveyi* and *V. parahaemolyticus*, and are considered primary pathogens. Microbiological methods require isolation of the organism. A molecular method for detection of *V. harveyi* from ethanol preserved samples was the endpoint for this research.

Literature review revealed a PCR method developed by Fikui and Sawabe (Microbes Environ 2007, 22:1-10) that targets pathogenic *V. harveyi*. This method was assessed for specificity and sensitivity. To determine the specificity of the PCR assay, the 16S rRNA and gyrase B genes from various *Vibrio spp*. isolates in the University of Arizona Aquaculture Pathology Laboratory bacterial collection were sequenced. Sequencing the 16S rRNA gene proved complicated due to the genomic structure of *Vibrio spp.*, in that they possess two chromosomes. For many of the isolates, the 16S PCR amplification jumped chromosomes and the resultant data was corrupt. This was not the case when sequencing the gyrase B gene.

From the gyraseB sequencing data, 5 bacterial isolates clustered with *V. harveyi.* These isolates were tested using the PCR protocol outlined by Fikui and Sawabe. The results from the PCR assay revealed that the Fikui and Sawabe protocol amplified three *V. harveyi* isolates, but not the remaining 2 isolates. This result showed the lack of specificity in the published protocol.

Following the evaluation of the published protocol, UAZ developed a PCR assay for *V. harveyi*, using primers designed from the gyrase B gene. The primers designated as 1018gyr2F/R detected the 5 V. harveyi isolates. Initial testing revealed cross-reactivity with *V. fluvalis.* To be able to distinguish between *V. harveyi* and *V. fluvalis*, an additional primer pair was used in a duplex PCR reaction with the 1018gyr2F/R primers.

This duplex PCR reaction differentiates between *V. harveyi* and *V. fluvalis* dependent on visualized fragment size. *V. harveyi* generates a 447 bp fragment, contrasted to *V. fluvalis* which generates multiple amplicons that are all derived from the gyrase B gene and range in size from 356 to 997 bp. All other *Vibrio spp.* were not detected.

OP - B4

False rumours of disease outbreaks caused by infectious myonecrosis virus (IMNV) in the whiteleg shrimp in Asia

***Saengchan Senapin*[1,2]*, Kornsunee Phiwsaiya*[1,2]*, Warachin Gangnonngiw*[1,2] *and Timothy W. Flegel*[1,2,3]

[1]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, 12120, Thailand
[2]Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Rama VI Rd., Bangkok, 10400, Thailand
[3]Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok, 10400, Thailand
Email: saengchan@biotec.or.th

Infectious myonecrosis virus (IMNV) disease outbreaks in cultivated whiteleg shrimp *Penaeus (Litopenaeus) vannamei* are characterized by gross signs of whitened abdominal muscles and by slow mortality reaching up to 70%. In 2006 the first disease outbreaks caused by IMNV in Asia occurred in Indonesia. Since then rumours have periodically circulated about IMNV disease outbreaks in other Asian countries. Our findings indicate that these are false rumours. Our continual testing by nested RT-PCR of shrimp samples suspected of IMNV infection from various Asian countries since 2006 has yielded negative results, except for samples from Indonesia. Our results are supported by the lack of official reports of IMNV outbreaks since January 2007 in the Quarterly Report on Aquatic Animal Diseases (QAAD) from the Network of Aquaculture Centers in Asia Pacific (NACA). In most cases, our shrimp samples for which tissue sections were possible showed signs of muscle cramp syndrome that also commonly causes muscle whitening in stressed whiteleg shrimp. Thus, we suspect that most of the false rumors in Asia about IMNV outside of Indonesia have resulted because of muscle cramp syndrome.

OP - B5

Evaluation of several detection methods for koi herpesvirus (KHV)

Kei Yuasa[1], *Satishi Miwa[2], Morihiko Kawana[3], Takafumi Ito[2], Ikunari Kiryu[1], Motohiko Sano [4] and Norihisa Ohseko [1]*

[1]National Research Institute of Aquaculture, Fisheries Research Agency, Minami-Ise, Mie 516-0193, Japan

[2]National Research Institute of Aquaculture, Fisheries Research Agency, Tamaki, Mie 519-04, Japan

[3]Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Sapporo 062-0922, Japan

[4]National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama 236-8648, Japan

Email: yuasa@fra.affrc.go.jp

The abilities of several detection methods for KHV were tested using experimentally infected koi, from the early stage of infection to the time when the virus became latent. Three specimens of koi carp exposed to KHV and reared in running well water at 23°C were randomly sampled at 1, 2, 3, 7, 14, 21, 28, 35, 49, 70 and 210 days post-exposure to the virus (dpe). Sampled fish were kept in 1 L well water for 1 h and the water was quantitatively analyzed for KHV with TaqMan real-time PCR. To detect infectious virus, two naïve common carp were cohabited with the sampled fish for 1 h. The serum antibody titer against KHV in the sampled fish was measured with ELISA. DNA and RNA were extracted from the gills, skin, kidney, intestine and brain using commercial extraction kits. DNA in each organ was quantitatively analyzed with TaqMan real-time PCR for KHV genome. RT-PCR was conducted to detect the mRNA for KHV terminase.

When the experimentally infected koi sampled at 2-28 dpe were kept in 1L well water, the water was positive for KHV. Common carp cohabited with infected koi carp sampled at 2-21 dpe died of KHV infection. High antibody titers against KHV were detected from 14 to 49 dpe at the termination of the ELISA test. KHV genome was detected from the skin and gills of all fish at 2-35 dpe and from the intestine and brain at 3-210 dpe. The mRNA from the gills and skin was detected in samples at 2-21 dpe. In conclusion, PCR with the gill or skin was useful to diagnose the disease, but additional PCR with the brain and intestine or ELISA is essential to detect latent KHV. In addition, PCR detection from the water, cohabitation and RT-PCR using the gills or skin were similarly effective to detect fish shedding the virus.

OP - B6
White Tail Disease (WTD) of *Macrobrachium rosenbergii*

A.S. Sahul Hameed

OIE Reference Laboratory for WTD, C.Abdul Hakeem College, Melvisharam - 632 509, Vellore Dt., TN., India

E-mail: cah_sahul@hotmail.com

Macrobrachium rosenbergii is an economically important crustacean and being cultured in large-scale in different parts of the world. One of the major constraints limiting the prawn production all over the world is diseases. Generally, *M. rosenbergii* is considered to be a moderately disease-resistant species when compared to penaeid shrimp. A new viral disease, white tail disease (WTD) has been observed in freshwater prawn hatcheries and nursery ponds in different parts of world, causing high mortalities and huge economic losses. This disease was first reported in the French West Indies (Arcier et al., 1999), later in China (Qian et al., 2003), India (Sahul Hameed et al., 2004), recently in Thailand (Yoganandhan et al., 2006) and very recently in Taiwan (Wang et al., 2008) and Australia (Owens et al., 2009). Clinical signs observed in the infected animals include lethargy, opaqueness of the abdominal muscle, degeneration of the telson and uropods, and 100% mortality within 4 days. The causative organisms have been purified and identified as *Macrobrachium rosenbergii nodavirus* (*Mr*NV) and extra small virus-like particle (XSV). *Mr*NV is a small icosahedral non-enveloped particle, 26-27 nm in diameter, identified in the cytoplasm of connective cells. XSV is also an icosahedral virus and 15 nm in diameter. These viral pathogens have been detected by nucleic acid and protein based diagnostic methods such as RT-PCR, dot-blot hybridization, in situ hybridization and ELISA. In experimental infection, these viruses caused 100% mortality in post-larvae but failed to cause mortality in adult prawns. Our research work indicates the possibility of marine shrimp acting as reservoir for *Mr*NV and XSV and maintaining their virulence in tissue system of marine shrimp. Experiments were carried out to determine the possibility of vertical transmission of *Mr*NV and XSV in *M. rosenbergii.* The results indicate that WTD may be transferred from infected brooders to their offspring during spawning. Artemia is a live feed organism to larvae and post-larvae of prawn and experiments were carried out to investigate the possibility that it might transmit these viruses to PL of freshwater prawns. The results showed that Artemia act as a carrier for these viruses. Recently, WTD has been observed in hatchery reared post-larvae of marine shrimp (*Penaeus monodon* and *P. indicus*). Clinical signs observed in these animals were found to be similar to those found in the post-larvae of *M. rosenbergii.* Producing the seeds of marine shrimp and freshwater prawn in close proximity invites the possibility of transmitting pathologically significant organisms from native to non-native hosts as observed in our study.

OP - B7

In vitro propagation of shrimp Taura syndrome virus (TSV) in a C6/36 mosquito cell line

Jurairatana Phromjai, *Narong Arunrut[1,2], Warachin Gangnonngiw[1,2],Siriporn Sriurairatana[1] and Wansika Kiatpathomchai[1,2]*

1Centex Shrimp, Faculty of Science, Mahidol University, Rama 6 Road , Bangkok 10400, Thailand
2National Center for Genetic Engineering and Biotechnology (BIOTEC)
National Science and Technology Development Agency Klong 1, Klong Luang, Pratum Thani 12120, Thailand
Email: jiabju@yahoo.com

Taura syndrome virus (TSV) is the causative agent of Taura syndrome. It initially caused serious shrimp mortality in reared *Penaeus vannamei (P. vannamei)* or whiteleg shrimp in the Americas but it subsequently spread to Asia via international transfer of shrimp stocks for aquaculture. Here we describe the challenge of C6/36 cells (derived from the mosquito *Aedes albopictus*) followed by serial split passage to produce cultures with the cells persistently positive for TSV by RT-PCR and by immunohistochemistry for TSV capsid antigen using confocal microscopy. Supernatant solution from homogenates of these TSV-positive insect cells from passage 5 were used to successfully infect the *P. vannamei* with TSV as confirmed at 8 days post challenge by immunohistochemical analysis of hemocytes. This work revealed that TSV could be propagated successfully in C6/36 cells that showed no gross signs of infection. Although the virus was not released into the culture supernatant solution in detectable quantities, supernatant solutions from homogenates of whole cells were capable of producing very light TSV infections in whiteleg shrimp. Since the TSV-infected insect cells could be stored at -80^{o}C and revived at will, this method will be useful for preservation and propagation of TSV.

OP - B8

Development of a multiplex PCR for the identification of pathogenic *Edwardsiella tarda* and application to edwardsiellosis diagnostics

Zhao Lan Mo, *Gui Yang Li and Peng Xiao*

Key Lab of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
E-mail: zhlmo@qdio.ac.cn

Edwardsiella tarda is a common fish pathogen that causes the disease edwardsiellosis. The present study was to develop a multiplex PCR (mPCR) for the identification of pathogenic *Edwardsiella tarda* and application to edwardsiellosis diagnostics. *E. tarda* rpoS, esaV, katB and hlyA and an *E. ictaluri* specific DNA fragment were used for primers design. The conserved 16S rRNA gene was as an internal positive control. The established mPCR can specifically identify *E. tarda* and *E. ictaluri,* also allowing differentiation of the pathogenic *E. tarda* from various bacterial species in a single reaction. The mPCR assay was adapted to edwardsiellosis diagnostics and monitoring the presence of *E. tarda* in turbot fish farm. The assay could be used successfully in monitoring the presence of potential pathogens to assess edwardsiellosis risk in turbot culture environments.

OP - C1

The role of viral inserts in the shrimp response to viral pathogens

Timothy W. Flegel

Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok 10400, Thailand and National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathum Thani 12120, Thailand.
E-mail: sctwf@mahidol.ac.th

Shrimp tolerate viral pathogens as persistent, active infections with no visible signs of disease. A viral accommodation concept to account for this phenomenon proposed that shrimp have an active (adaptive) mechanism to accommodate viral pathogens in a manner that leads to persistent infections without signs of disease, specifically blocks viral-triggered, massive apoptosis called kakoapotosis and also provides some protection against mortality upon subsequent superinfection with the same virus. An essential element of the concept was a memory mechanism that allowed specific recognition of each viral pathogen by an initially unknown process later proposed to consist of the viruses themselves in persistent infections. However, it has more recently been hypothesized that the memory consists of viral genome fragments randomly and autonomously endogenized from "foreign" mRNA of both RNA and DNA viruses via host reverse transcriptases (RTs) and integrases (INs), and that some of these sequences, by chance, result in the production of antisense, immunospecific RNA (imRNA) capable of stimulating RNAi that suppresses viral propagation. A test of the hypothesis revealed many random genomic insertions of a densovirus (IHHNV) in the genome of the giant tiger shrimp (*Penaeus monodon*) and the whiteleg shrimp *(Penaeus vannamei*). Genome walking in one *P. monodon* specimen revealed two viral inserts linked to host microsatellite-like fragments. Use of chimeric shrimp/virus primer pairs revealed similar insertions in many shrimp specimens, including some infected with IHHNV but showing no signs of disease. At the same time, work with several generations of breeding stocks of *P. monodon* free of white spot disease and continuously negative for white spot syndrome virus by PCR tests for several viral genes did give positive PCR results for other WSSV gene fragments. For one of these fragments, RT using sense and antisense primers with 4 shrimp specimens revealed that two produced antisense RNA fragments, one produced ambisense RNA fragments and one produced no RNA from the WSSV target sequences. These results constitute the first experimental support for the hypothesis-based prediction that random numbers and lengths of sequence fragments from a single virus genome commonly occur in the shrimp genome and may produce antisense RNA fragments.

OP - C2

Review: Interfering RNA for control of viruses in crustacea

Leigh Owens, *Kathy La Fauce, Esti Harpeni, Dewi Sayhidah and Orachun Hayakijkosol*

Microbiology and Immunology, School of Veterinary and Biomedical Sciences, James Cook University, Townsville, Australia 4814

Email: leigh.owens@jcu.edu.au

The interfering RNA (iRNA) pathway is a eukaryotic cell's method of recycling misfolded mRNA and regulating genes by either silencing genes, or silencing gene repressors, thus up-regulating genes. Examples of the effect of iRNA in crustacea have been published. IRNA is even being used to target and experimentally knock out genes to observe the function of a particular gene. As the IRNA pathway is clearly operational in crustacea, it opens the door for manipulation against viruses.

Against crustacean viruses, iRNA has been used to effectively knock down yellowhead virus (YHV), white spot syndrome virus (WSSV), *Macrobrachium rosenbergii nodavirus* (MrNV) and by inference from research in crickets, *Penaeus merguiensis Densovirus* (PmDV) also known as hepatopancreatic parvovirus. The capsid gene was targeted for knockout in WSSV (VP19 & VP26) and PmDV infections whilst a combination of viral encoded protease gene and a host Rab7 GTPase were targeted for efficient knockdown of YHV. Interestingly when a second gene, NS2 in PmDV was targeted by iRNA, the viral titre was knocked down by 1 log10 but mortality was a third higher suggesting that NS2 may be analogous to a vital house-keeping gene in crickets.

Nodaviruses produce a novel protein, B2 which binds to dsRNA intermediates produced during transcription of the nodavirus which prevents nucleic acid degradation via the iRNA system's enzyme Dicer. Targeting the viral anti-iRNA was a successful strategy in circumventing the viruses' ability to successfully protect itself from the cells' iRNA. Mortality in *Cherax quadricarinatus* exposed to MrNV decreased from 60% to 10% when treated with iRNA antagonistic to the gene expressing protein B2.

One observation that has occurred a couple of times in the literature is the fact that non-target, non-specific dsRNA used as negative controls in the experiments has had a protective effect against some viruses. In other animals this would suggest that an interferon pathway (INF) via Toll-like receptors was operating in crustacea. However, other published work suggests that the decapod INF pathway has regressed to be non-functioning (Nehyba et al 2009). Indeed experimental exposure of crayfish to doses of α-INF from 100 to 210 IU/g body weight (BW) showed increasing mortality in a dose-dependent manner. Perhaps the therapeutic dose of INF is below 100 IU/g BW as the INF effect is clearly present in the literature.

IRNA offers a novel method of combating viruses in crustacea if it can be delivered in a cost efficient manner.

OP - C3

Random, natural inserts of parvovirus genome fragments occur in the shrimp genome and have implications for viral disease diagnosis and control

Vanvimon Saksmerprome[1,2], *Sarocha Jitrakorn*[1,2], *Kanokporn Chayaburakul*[3], *Seansook Laiphrom*[4], *Khanittha Boonsua*[4] *and Timothy W. Flegel* [1,2,5]

[1]Centex Shrimp, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

[2]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathum Thani 12120, Thailand.

[3]Department Anatomy, Faculty of Science, Rangsit University, Pathumthani 12000, Thailand

[4]Shrimp Genetics Improvement Center, Surat Thani, Thailand

[5]Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Email: vanvimon.sak@biotec.or.th

Scattered reports of viral inserts in shrimp and insect genomes led to the hypothesis that random, autonomous insertion of such sequences occurs in these organisms and leads to specific, hertitable immunity. To test the prediction regarding random insertion of viral sequences into the shrimp genome, we examined the giant tiger shrimp for random genomic insertions of a shrimp parvovirus (densovirus) called IHHNV. By PCR analysis of giant tiger shrimp specimens using a set of 7 overlapping primer pairs to cover the whole IHHNV genome (4 kb), PCR failure with some pairs indicated sequence gaps that revealed a random pattern of putative viral inserts in the shrimp genome. Targeting a putative insert from one arbitrarily selected specimen, we used genome walking to reveal a viral insert linked to a host microsatellite-like fragment. In one specimen, 2 slightly different inserts were revealed, probably on paired chromosomes. By design and use of chimeric shrimp/virus primer pairs we proved that similar insertions occurred in many shrimp specimens, including those infected with IHHNV, but showing no signs of disease. These inserts gave false positive PCR test results for IHHNV viral infection using currently recommended PCR protocols. This is the first experimental support for the hypothesis-based prediction that a random number and length of sequence fragments from a single virus genome commonly occur in the shrimp genome. These inserts can give false positive results for infectious IHHNV with official detection methods used to regulate international seafood trade. In addition, discard of domesticated shrimp breeding stocks based on such false positive results might have negative consequences, if such inserts are related to shrimp viral disease tolerance, as also hypothesized.

OP - C4

Development and demonstration of 'Shrimpvac-I' for the management of white spot syndrome virus in shrimp grow-out system

M.Manjusha[1], N.S. Sudheer, S.Ranjit[1], S.Somnath Pai[1], Anitha Thankachan[1], A. Anas[1] Rosamma Philip[2] and ***I. S. Bright Singh[1]***

[1]National Centre for Aquatic Animal Health,
[2]Department of Marine Biology, Microbiology and Biochemistry Cochin University of Science and Technology, Lakeside Campus, Fine Arts Avenue, Kochi - 682016, Kerala, India.
Email: bsingh@md3.vsnl.net.in

A cocktail vaccine 'Shrimpvac-I' consisting of inactivated White Spot Syndrome Virus (WSSV), bacterins (inactivated pathogenic isolates of Vibrio harveyi MCCB 151 & *Vibrio alginolyticus* MCCB 142) and an immunostimulant, Peptidoglycan (PG Aqua), was developed for oral 'vaccination' of shrimp and validated under field conditions to protect the crop from WSSV. Vaccination commenced from Post larvae (PL-10) and repeated 7 times continuously for 3 consecutive days providing an interval of 10 days in grow out system. The culture could be completed in the grow out with in 90 days with an over all survival of 56.5% at the time of harvest. A total biomass of 687.7kg could be harvested from 0.80 hectare pond. Animals sampled during harvest survived 4 consecutive challenges with WSSV at an interval of 15 days under laboratory conditions. Three days after the 4th WSSV challenge the hematological profile showed high level of reactive oxygen intermediates (ROI) and low prophenol oxidase activity. The histological observation revealed enlarged eosinophilic granulated nuclei. The results showed that successful prawn culture would possible by a periodic 'vaccination' using the cocktail vaccine - Shrimpvac-I. A cost benefit analysis demonstrated economic feasibility of shrimp vaccination against WSSV in both hatchery and grows out systems.

OP - C5

Uptake of white spot syndrome virus and WSSV-vaccines in the gut of black tiger shrimp, *Penaeus monodon*

***Amod Kulkarni*[1]*, Jan HWM Rombout*[1, 2]*, I S. Bright Singh*[3]*, N.S. Sudheer*[3]*, Christopher MA Caipang*[1]*, Monica F Brinchmann 1and Viswanath Kiron*[1]

[1]University of Nordland, Bodø, Norway,
[2] Wageningen University, Wageningen, The Netherlands.
[3] National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Kochi, India.
Email: amod.kulkarni@uin.no

White spot syndrome virus (WSSV) incurs significant economic losses to the shrimp farming industry. Hence several attempts to evoke resistance in shrimp to WSSV through 'vaccination' have been made. Although WSSV envelope protein VP28 and formalin-inactivated virus (IVP) are currently employed as candidate vaccine components, their uptake mechanisms upon oral delivery are not clearly understood. Therefore after these components were orally intubated, their fate was investigated by immunohistochemistry, employing antibodies against VP28 and haemocytes. In addition, the same techniques were also applied following oral infection with WSSV. From our observations it is evident that midgut is the main site where WSSV-immunoreaction is predominant. We confirmed that the WSSV-antigen from the lumen first adheres to the apical surfaces of the epithelial cells. They are then transported to the cytoplasm of the epithelial cells, from where it moves to the adjacent connective tissue and gets accumulated. Finally it makes its way out to the outer surfaces of the midgut. Moreover, some WSSV-immunoreactivity appeared in the supranuclear vacuoles of the epithelial cells, which are supposed to be the endolysosomal compartments. Interestingly, the number of haemocytes was found to increase in the connective tissue and they seem to be activated (degranulation) by the transported antigens, especially in the case of live WSSV. Further, through gene expression studies on immune-competent molecules, we have examined the immunomodulation following the uptake of vaccine.

OP - D1

Managing viral infections endemic in farmed cyprinids: Goldfish herpesviral hematopoietic necrosis virus (CyHV-2) and the aquareoviruses

Andrew E. Goodwin

University of Arkansas at Pine Bluff, Aquaculture/Fisheries Center, 1200 N. University Dr, Pine Bluff, AR 71601 USA.

Email: agoodwin@uaex.edu

Several of the viruses affecting cyprinid fishes are very widespread and may infect most farmed populations. The wide distribution and possible vertical transmission of these pathogens means that most farms must me managed to reduce disease rather than to avoid infection. An example is Herpesviral Hematopoietic Necrosis, a disease of goldfish, *Carrasius auratus,* caused by Cyprinid Herpes virurus-2 (CyHV-2) infection. In an epidemiological study, we did quantitative PCR on tissue homogenates from healthy goldfish fingerlings, broodfish, eggs and fry directly sampled from commercial farms, from moribund fish submitted to our laboratory for disease diagnosis, and on naturally-infected CyHV-2 carriers subjected to experimental stress treatments. We found that the virus is present in almost all farmed populations, that the virus appears to be vertically transmitted in an egg-associated way, and that subjecting healthy CyHV-2 carriers to cold shock (from 22 to 10 ºC) but not heat, ammonia, or high pH, increased viral copy numbers. The CyHV-2 is widespread on commercial goldfish farms and outbreaks apparently occur when healthy carriers are subjected to a sharp temperature drop followed by holding at the permissive temperature for the disease. Prevention of CyHV-2 losses on commercial farms can be achieved by careful attention to temperature changes known to trigger viral replication, and by manipulating temperatures to promote the recovery of diseased fish.

Aquareoviruses are another important group of cyprinid fish pathogens. Our work with the golden shiner virus (GSV, Aquareovirus C) and the American grass carp reovirus (AGCRV, Aquareovirus G) has shown that these viruses are common and widespread in farmed cyprinids in the USA. Using quantitative rt-RTPCR we have confirmed that GSV replication increases in stressed fish, but we have been unable to discover any association between infection by these aquareoviruses and clinical disease. Surprisingly, the GSV is nearly identical in RNA sequence to the Chinese grass carp reovirus (CGCRV) and GSV is likely to actually be CGCRV that was brought to the USA in infected grass carp *(Ctenopharyngodon idella)* during the early 1970's. Given that CGCRV is a very important pathogen of grass carp cultured in China, it is very surprising that GSV has failed to produce losses in GSV-infected grass carp cultured in the USA. We need to establish an international collaboration to determine if the differences in apparent pathogenicity are due to minor mutations in the virus, or due to differences in fish culture methods between the US and China. If the differences are due to mutations, the US strain might provide protective immunity for fish grown in China. If the differences are due to fish husbandry methods, then discovery of the relevant differences would benefit grass carp farmers in both the USA and in Asia.

OP - D2

Nodavirus in Norway: lessons learned and disease management strategies

Sonal Patel

Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway.
E mail: sonal.patel@imr.no

Nodavirus, causing the disease viral nervous necrosis (VNN) affects farmed species like halibut, cod and turbot in Norway. Following an outbreak of VNN in Atlantic cod in western Norway, both laboratory and field studies were carried out to study susceptibility to other healthy cod, and transmission modes. Additional studies on tissue tropism and susceptibility to other important species like Atlantic salmon carried out at the Institute of Marine Research could identify some of the measures that might be useful to follow by the aquaculture industry. The main conclusions from long term studies can be summarized as: 1) Nodavirus is infectious between species, though resulting in varying degree of infection. 2) Tissue tropism analysis suggests that biopsy and analysis of anterior kidney is a good candidate to screen and select for virus-free broodstock. 3) Salmon are potentially susceptible host, and co-location with known susceptible hosts such as halibut and cod should be avoided. 4) Although both horizontal and vertical transmission is known for nodavirus, both laboratory and field studies on Atlantic cod showed little or no notable transmission during a period of 2 yrs after an outbreak. 5) Genetics of fish may play an important role in susceptibility to nodavirus and subsequent development of pathology and disease.

Based on current knowledge of nodavirus along with some other diseases and their outbreak and spread in Norwegian aquaculture and along the coast, several control measures can be recommended. Especially during the planning for new locations, expansion and considering breeding of new species in aquaculture, such control measures are important, to prevent and reduce the risk of transmission of nodavirus both vertically and horizontally, and probably boost the aquaculture against Nodavirus.

OP - D3

Diseases of Asian seabass (or barramundi) *Lates calcarifer* Bloch

Gibson-Kueh S[1], *B. Jones[2] and P. Nicholls[1]*

[1]School of Veterinary and Biomedical Sciences, Murdoch University, South St., Murdoch, Western Australia 6150, Australia and
[2]Fish Health Laboratory, Department of Fisheries, 3 Baron-Hay Court, South Perth, Western Australia 6151, Australia.
Email: Susan.Kueh@murdoch.edu.au

This is a histopathological study of the major disease agents observed in Asian seabass (or barramundi), *Lates calcarifer* Bloch at various stages of the culture cycle. The impacts of viral nervous necrosis (VNN), pot belly disease (PBD), systemic iridoviral disease, streptococcosis and tenacibaculosis are discussed. Systemic iridoviral disease was usually observed in 10 to 20 g *L. calcarifer* 2 to 3 weeks post-transfer into sea cages. The presence of inclusion bodies in clinically healthy nursery-stage *L. calcarifer* less than 1g bodyweight suggested that the iridovirus infection occurred well before stocking at sea. PBD previously reported in less than 1g fish in hatcheries was observed to persist in 80 to120 g fish in sea cages. These diseases also often occurred concurrently, complicating their diagnosis and control. 'Scale drop syndrome' (SDS) was previously observed in 100 to 300g *L. calcarifer* in sea-cages, and recently in 3 to 5 kg fish with no prior history of disease. Onset of SDS often coincided with periods of stressful events. SDS which affected larger more valuable fish is expected to have greater economic impacts.

Successful management of disease requires better understanding of the disease process rather than sole dependence on detection of disease agents. Blood-borne septic emboli associated with streptococcosis can have serious effects on blood and tissue oxygen levels. The handling stress associated with antimicrobial bath treatment in sea cages is impossible without exacerbating mortality due to streptococcosis. The ability of *Streptococcus* and the PBD bacterium to persist intracellularly will affect the efficacy of antimicrobial therapy. Farms have reported good results with vaccination against Streptococcosis. However, the occurrence of VNN, PBD and systemic iridoviral infections in less than 1g *L. calcarifer* excludes vaccination as a potential control measure. The way forward may lie in the vaccination of juvenile fish from 'clean' broodstocks. The high prevalence at which disease is encountered in aquaculture is likely an indicator of stressed fish rather than the emergence of serious pathogens. Stress factors in husbandry practices including nutrition should be addressed in tandem with the management of specific pathogens and improved biosecurity.

OP - D4

Difference in virulence of marine and freshwater isolates of viral hemorrhagic septicemia virus *in vivo* correlates with *in vitro* ability to infect gill epithelial cells and macrophages of rainbow trout (*Oncorhynchus mykiss*)

***Bjørn E. Brudeseth*[1], *Helle F. Skall*[2] *and Øystein Evensen*[3]**

[1]PHARMAQ AS, PO Box 267, N-0213 Oslo, Norway
[2]Technical University of Denmark, National Veterinary Institute, Hangøvej 2, DK-8200 Aarhus N, Denmark
[3]Norwegian School of Veterinary Science, PO Box 8146 Dep, N-0033 Oslo Norway
Email: bjorn.brudeseth@pharmaq.no

Two strains of viral hemorrhagic septicemia (VHS) virus with known differences in virulence characteristics *in vivo* were studied (time-course) for their ability to infect and translocate across a primary culture of gill epithelial cells (GEC) of rainbow trout (RBT; *Oncorhynchus mykiss*). The strains included one low-virulent marine strain (ma-VHSV; ma-1p8) and a highly pathogenic freshwater strain (fw-VHSV; fw-DK-3592B). Infectivity to trout head kidney macrophages was also studied (time-course) and difference in in vivo virulence was reconfirmed, the aim being to determine any correlation between in vivo virulence with in vitro infectivity. The in vitro studies showed that the fw-VHSV isolate infected and caused cytotoxic effect in monolayers of GEC (virulence) at early time (2 h) and the same virus strain translocated over a confluent, polarized GEC-layer by 2 h post inoculation. The marine isolate did not infect monolayers of GEC and a delayed translocation across polarized GEC was seen by 48 h post inoculation. Primary cultures of head kidney macrophages were also infected with fw-VHSV, with a maximum of 9.5% virus positive cells by 3 days post infection, while for the ma-VHSV strain only 0.5% of the macrophages were positive by 3 days of culture. *In vivo* studies showed that the fw-VHSV strain was highly virulent for RBT fry and caused high mortality, with classical features of VHS. The ma-VHSV showed very low virulence (only one pool of the sampled dead fish was VHSV positive). This study has shown that the difference in virulence between ma- and fw-strains of VHSV following in vivo infection in rainbow trout correlates with in vitro ability to infect primary cultures of gill epithelial cells and head kidney macrophages of the same species.

OP - D5

Temperature dependent virus replication and anti-viral apoptotic response in viral haemorrhagic septicaemia virus infected olive flounder (*Paralichthys olivaceus*)

Satheesha Avunje, *Myung-Joo Oh and Sung-Ju Jung*

Department of Aqualife Medicine, Chonnam National University, Chonnam 550-749, Korea.
Email: s.avunje@gmail.com

Olive flounder (*Paralichthys olivaceus*) succumb to viral haemorrhagic septicaemia virus (VHSV) infection in winter season but are found to be resistant in summer. Apoptosis plays an important role in effective control of the growth and spread of virus in the host. We studied real-time PCR based relative immune expression of host apoptotic genes (granzyme, perforin, FasL, p53 and caspase 3) comparing with mature viral copies and its transcription rate. For the experiment, a less lethal viral infection was developed in olive flounder, maintained at 15^0C and 20^0C. Sampling interval was set to get information on early infectious period of 3 hours post infection (hpi) to 1 day post infection (dpi) as well as recovery period of 2 to 7 dpi. The viral growth was noted to be rapid at 20^0C than 15^0C fish, but an effective apoptotic response at 20^0C by the fish contained viral replication by 2 dpi. On the other hand, viral replication in 15^0C fish was slower at initial infection stage (3 hpi to 1 dpi) but increased rapidly at 2 dpi. Fish of 20^0C group, steadily expressed apoptosis inducing genes throughout the experiment. Fish of 15^0C, though expressed a higher level of apoptotic genes at early stage of infection (3 to 6 hpi), as the viral load increased, transcription of these genes was under-expressed. In late recovery stage, some fish of this group showed recovery of apoptotic mechanism. From 2 dpi, fish of 20^0C group expressed very high level of caspase 3 indicating an effective apoptosis and a drastic decline in viral copies. Compared to this, caspase 3 transcription in 15^0C remained under-expressed, but virus grew in large number. Interestingly, fish maintained at 15^0C expressing high caspase 3 carried very low or undetectable levels of viral transcripts. Thus, from the results of our experiment, we could conclude that the apoptotic immune system plays a pivotal role in resistance of VHSV-infected olive flounder in summer, but susceptibility in winter temperature.

OP - E1
Molecular immunology and genomic science of shrimp

Ikuo Hirono

Tokyo University of Marine Science and Technology, Tokyo, Japan
E-mail: hirono@kaiyodai.ac.jp

Because of the importance of penaeid shrimp in world aquaculture, there is much interest in understanding their immune system and genome science to improve their resistance to pathogenic microorganisms.

Unequivocal evidence points to the efficiency of RNA interference (RNAi) in studying gene function and their involvement in mounting antiviral responses in eukaryotes. The potency of double stranded RNA (dsRNA) to knock-down gene expression (gene silencing) has been successfully demonstrated in studying gene function in shrimp. Here, some of our recent research results of kuruma shrimp (*Marsupenaeus japonicus*) immune-responses against microbial pathogens after silencing *in vivo* of immune-related genes including 2 types of lysozymes, antimicrobial protein crustin and penaeidin, clotting protein and proPO are presented.

Genomic information of penaeid shrimp is essential for understanding further their immune mechanisms and other biological information of interest. To resolve this, we have constructed Bacterial Artificial Chromosome (BAC) library (MjBL2). The MjBL2 represents about 3.3 times coverage of 2,000 Mbp kuruma shrimp haploid genome. From this, we found that kuruma shrimp genome has several different homologous genes of white spot syndrome virus (WSSV), a virulent caused pathogen known to affect crustaceans. Some of these WSSV homologous genes in the kuruma shrimp genome were knocked down by using the RNAi technology. After knock down of some of them, one group (knock down of one specific gene) of shrimp were more sensitive to WSSV and other group of shrimp which showed more resistance to shrimp when compared to the control group.

OP - E2
Micro- and nanoparticles for vaccine delivery

Børge N. Fredriksen[1], Jostein Grip[1], Linn B. Hølvold[1], Hetron M Munang'andu[2], Jarl Bøgwald1, Øystein Evensen[2] and ***Roy A. Dalmo[1]***

[1]University of Tromsø, Faculty of Biosciences, Fisheries & Economics, 9037 Tromsø, Norway.
[2]Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, Section of Aquatic Medicine and Nutrition, P.O. Box 8146 Dep, N-0033 Oslo, Norway.
Email: roy.dalmo@uit.no

Polymers of lactic and glycolic acid (PLGA) have been shown to hold a great potential as vaccine drug delivery matrices as they are easily manipulated in terms of their physical- and chemical properties that make them biodegradable. In antigen delivery, particles within the phagocytic size range, normally considered to be less than 5 μm, may be employed as depot and/or antigen vehicles targeted to intracellular compartments in antigen presenting cells (APCs). It is known that vaccines by virtue of PLGA encapsulated antigens induce robust antibody responses, as well as CTL responses. Not only antigens can be encapsulated but also immune directing adjuvants such as TLR receptor ligands. We have compared antibody responses of Atlantic salmon using PLGA "nanovaccines" and "microvaccines", with or without adjuvants, to conventional oil-formulated vaccines. Furthermore, the antibody response in fish has been correlated to antigen retention and to tissue distribution of PLGA encapsulated vaccines. To use PLGA carried vaccines in fish it is pertinent to perform preclinical assessment in terms of early innate and adaptive responses. While antibody assessment is quite easy to perform, a functional analysis of resulting immune responses are rather difficult in. Thus, gene expression analysis has been done to assess responses up to one-week after immunization. The efficacy of vaccines encapsulated in PLGA particles has also been evaluated after experimental challenge of IPNV. Detailed results will be presented at the DAA8 conference.

OP - E3

Chelated mineral zinc for growth and immune response of Pangasius

***Ei Lin Ooi*[1], *Pham Minh Anh*[1], *Bui Chau Truc Dan*[1], *Vo Thi Minh Thu*[2], *Nguyen Thi Kieu Tuyen*[2], *Nguyen Tri*[2] *and Nguyen Huu Thinh*[2]**

[1]Novus Aqua Research Center, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
[2]Nong Lam University, Faculty of Fisheries, Linh Trung Ward, Thu Duc District, Ho Chi Minh, Vietnam

Email: eilin.ooi@novusint.com

Trace minerals, particularly zinc are required for proper development and function of the immune system in humans and animals. Zn deficiency reduces the activity of both T and B lymphocytes. Thus Zn deficiency can cause decreased antibody response to vaccination. The present experiment evaluated a new class of zinc mineral that is chelated with 2 molecules of methionine hydroxy analogue. Effects on performance, immune response and response to pathogen challenge were evaluated in Striped catfish (*Pangasianodon hypophthalmus*). An initial experiment was designed to better understand the bioavailability of different sources of zinc by comparing various doses of Zn-sulfate and the Zn-chelate. It was found that even at the highest supplementation of 120 mg/kg, animals on Zn-sulfate diets did not reach the growth potential of animals on only 30 mg/kg Zn-chelate diets. In a follow up trial, Pangasius (450/treatment) were fed a control practical diet containing 44 ppm Zn, or diets supplemented with Zn-sulfate or Zn-chelate to contain 107 ppm Zn. After 6 weeks on the diets, Pangasius from all 3 dietary treatments were either mock-vaccinated with PBS, or vaccinated with a formalin-killed *Edwardsiella ictaluri* vaccine. Titers were measured by agglutination assay prior to vaccination (day 0), and on days 14 and 21 post-vaccination. All treatments were negative on day 0 and the 3 mock-vaccinated treatments remained negative for titers on all days. On day 14, the vaccinated Zn-sulfate and vaccinated chelated-Zn treatments exhibited titers greater than the unvaccinated treatments ($P<0.05$), whereas the vaccinated control treatment did not. On day 21, all vaccinated treatments achieved titers greater than the unvaccinated treatments ($P<0.05$), but titers in the Zn-chelated group were higher ($P<0.05$) than all other treatments. At least 42 fish per treatment were challenged by immersion with *E. ictaluri*, and survival was monitored over a 14 day period. Vaccination significantly increased survival across dietary treatments ($P<0.0001$). Relative percent survival of the Zn-chelate group was highest at 66.5% while the Zn-sulfate and control groups were 60.9% and 49.5% respectively. In sum, these data indicate a more robust growth performance and antibody response to vaccination with chelated zinc diets.

OP - E4

Ontogeny of the thymus in barramundi (*Lates calcarifer*): a light microscope study

Dzung Nguyen Huu, *Hich Tran Vi and Dang Thi Sao Mai*

Department of aquaculture, Nha Trang University, Vietnam
Email: hdnguyen.ntu@gmail.com

In teleost fish, the thymus is regarded as an important immune organ. In this study, the ontogeny of this lymphoid organ was studied in barramundi (*Lates calcarifer*). The larvae of barramundi were sampled at 6 hours intervals from hatching to the size of 3 cm in total length and sectioned for examination of the developing thymus of fish. The data showed that the mucous cells of the thymus were observed on the epithelial layer in larvae from 11 days after hatching (DAH). From 14 DAH, two zones could be differentiated in the thymus and from 28 DAH, the thymus appeared fully formed with three zones clearly distinguishable: an apical zone (with pale-stained cubical epithelial cells and elongated epithelial cells), outer zone (with small lymphocytes) and inner zone (with loosely distributed lymphocytes and epithelioid-type cells).

OP - E5

Marine macroalgal immunostimulants - An alternative to finfish vaccines?

R. Dinakaran Michael

Centre for Fish Immunology, School of Life Sciences, Vels University, Chennai-600117, India.
E-mail: rdmichael2000@yahoo.co.in

Asian Aquaculture faces substantial loss in the finfish production due to infectious diseases and disease-outbreaks. To prevent and treat these diseases, the fish farmers depend mainly on the use of antibiotics. Though vaccines are the proven efficacious immunoprophylatics for higher vertebrates, they have not been introduced and used on large scale in Asian aquaculture due to various problems and issues.

In this context, the use of immunostimulants for fish is considered as an alternative immunoprophylactic measure. Immunostimulants by definition, are substances, which enhance especially, the non-specific defense mechanisms though they also stimulate specific antibody responses if the treatment is followed by infection or vaccination. Immunostimulants seem to be effective and important to fishes, since the fishes, unlike mammals, depend more on the non-specific immune mechanisms for their disease resistance. Plants have been traditionally used by many regions in the world to prevent and treat human and veterinary diseases. This presentation aims to focus on some of the major findings made in the author's laboratory on selected marine macroalgae and the immunostimulatory and disease protective properties of their extracts and fractions to draw attention of the stake holders to the potential of these macroalgal immunostimulants as immunoprophylactics in preventing or substantially reducing the disease loss in finfish production in Asian aquaculture and aquaculture elsewhere. Being plant products, these formulations are eco-friendly and easily biodegradable. If the raw materials are available, these immunostimulants can be relatively inexpensive. The impressive immunostimulating and disease-resisting performance of these macroalgal immunostimulants indicates the scope of using them as an attractive alternative to finfish vaccines.

OP - E6

Efficacy data of the first vaccine for Pangasius in Vietnam

Vo *Thanh Tung*[1] , *Pham Cong Thanh*[1], *Tu Thanh Dung*[2] *and Kjersti Gravningen*[1]

[1] PHARMAQ Vietnam Ltd,
[2] College of Aquaculture and Fisheries, Cantho University, Vietnam.
Email: Vo-thanh.tung@pharmaq.no

The efficacy of vaccine ALPHA JECT Panga 1 was demonstrated in a laboratory study on pangasius fingerling (*Pangasianodon hypophthalmus*) 14 ± 1 g. Total 420 fish was intraperitoneally (IP) injected with 0,05ml of formalin inactivated oil-based vaccine produced by PHARMAQ AS. Control group was injected with 0.05ml of sterile saline. The fish were marked by fin clipping and mixed in the same tanks during the trial. At 2, 5, 10 and 20 weeks post vaccination (WPV) blood was sampled from 5 fish per group and antibody measured by direct agglutination. Simultaneously, 50 fish from each group were challenge IP with Edwardsiella ictaluri isolated from diseased pangasius in the Mekong Delta, Vietnam. The challenge doses ranged from 4.2 x 103 to 5.6 x 104 cfu/fish in the different challenges.

The mortality after challenge was always significant lower in the vaccinated group than in the control group ($P<0.05$). The Relative Percentage Survival (RPS) for the vaccinated group was always more than 60. The antibody of vaccinated fish reached to level 8 (log2) at 2WPV, and was continuously increasing to10.8 at 5WPV and 11.8 at 10 WPV, while there were no antibodies detected before vaccination and in the control group during the study. After 20 WPV, the antibody in vaccinated fish still maintained at high level. The results show that injection with 1 dose (0.05ml/fish) of vaccine ALPHA JECT Panga 1 would give good protection against *Edwardsiella ictaluri* at least 20 weeks. The vaccine ALPHA JECT Panga 1 was a promising vaccine candidate for the field trial in Vietnam. The trial did also include higher and a lower dose of the vaccine, all results will be presented.

OP - E7

Development and characterization of monoclonal antibodies against putative T-cell of *Catla catla*

*Dharmender K. Chaudhary[1], **Neeraj Sood**[1], P.K. Pradhan[1], Peyush Punia[1], N.K. Agarwal[2] and Gaurav Rathore[1]*

1National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow- 226002, India
[2]Department of Zoology, H.N.B. Garhwal University (Campus), Badshaithaul, Tehri Garhwal, Uttarakhand
E-mail: sood_neeraj@rediffmail.com

*Catla catla i*s an important freshwater food fish in many Asian countries including India. Thymus mononuclear cells (MNCs) of *Catla catla* were isolated using Histopaque-1077 and passed through a nylon wool column. The non-adherent cells were fixed in paraformaldehyde and used to immunize BALB/c mice. The splenocytes from immunized mice were fused with myeloma cells and positive hybridomas were screened by enzyme-linked immunosorbent assay and fluorescent antibody test. One monoclonal antibody (MAb) (B8) showing strong reactivity in ELISA was selected and further characterized. Western blotting of reduced T-cell membrane protein showed that B8 MAb reacted with a 168.2 kDa peptide. In flow cytometric analysis, the percentage of gated lymphocytes detected by B8 MAb was 77%, 11%, 2% and 32% in thymus, kidney, spleen and blood MNCs, respectively. B8 MAb was also successfully employed to demonstrate T-cells in sections of thymus, kidney, spleen as well as blood smears. These MAbs can be useful reagent to study cell mediated immunity.

OP - E8
Vaccine development for viral nervous necrosis of groupers

Toshihiro Nakai[1], *Hirofumi Yamashita*[2], *Koh-ichiro Mori*[3], *Kazuhiro Hanyu*[4], *Shinji Tanaka*[4], *and Akashi Kuroda*[5]

[1]Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan
[2]Fisheries Research Center, Ehime Research Institute of Agriculture, Forestry and Fisheries, Ehime, Japan?
[3]Research Management Department, Fisheries Research Agency, Yokohama, Japan
[4] Mie Prefecture Fisheries Research Institute, Mie, Japan
[5]Nippon Institute for Biological Science, Tokyo, Japan
E-mail: nakaitt@hiroshima-u.ac.jp

Viral nervous necrosis (VNN) caused by betanodaviruses (Nodaviridae) has seriously damaged marine aquaculture worldwide since the first report in 1990. VNN generally prevails among hatchery-reared larvae and juveniles under the presence of broodstock as an infection source of the virus. This vertical transmission can be prevented by selection of virus-free broodstock and disinfection of fertilized eggs. However, some grouper species, such as sevenband grouper *Epinephelus septemfasciatus* and longtooth grouper *E. bruneus,* continue to be susceptible to red-spotted grouper nervous necrosis virus (RGNNV) even at the grow-out stages, resulting in heavy losses during net-pen culture. Both species are important for marine stock enhancement programs and aquaculture industries in Japan.

We recently developed the formalin-inactivated RGNNV vaccine to prevent VNN in the groupers. A single intraperitoneal injection of the vaccine was proved to be effective in protecting fish against RGNNV infection in both laboratory and field settings. There was also significant correlation between virus-neutralizing antibody titres (ND50) and relative percent survival (RPS) values. In case of sevenband grouper, the minimum effective inoculation dose of the vaccine was 107.0 TCID50 per fish and the minimum mean ND50 enabling significant protection was approximately 1:200. Large-scale vaccination trials in field setting demonstrated that the RGNNV injection vaccine is no harm to fish and effective to reduce mortality of fish due to naturally occurring VNN. The present vaccine is expected to be useful for any fish species susceptible to RGNNV. In addition, we revealed that combined inoculation with the vaccine and an aquavirnavirus (FBV) as an inducer of interferon-related substance(s) confers rapid onset of non-specific protection and long-lasting specific protection against VNN.

OP - E9

From discovery to characterization of cytokines: Applications in vaccine development and diseases

Ram Savan

Cancer and Inflammation Program, National Cancer Institute, Frederick, MD 21701 USA
Email:savanr@mail.nih.gov

As fish form a major link between invertebrates and mammals, they are a good model organism to study the evolution of immune processes. It is challenging to discover immune genes in fish as they share very low sequence identities with human counterparts. Comparative genomics exploits the knowledge that blocks of chromosomes are conserved in fish and human. Using this methodology, a number of new cytokine genes Th1, Th2, Th17 cytokines, TNF superfamily, CXC and CC chemokines have been identified in fish. There are teleost specific cytokines like IFN-γ-like gene (IFN-γ-rel) and a teleost specific IL-2 family gene. The identification of these cytokines establishes new and important veterinary applications, such as the use of cytokines as DNA vaccines or adjuvants. Additionally, researchers are now better able to establish lymphoid cell lines through the use of these cytokines and, foremost, to understand the immune system of fish and the regulation of these molecules during disease.

OP - E10

Immune responses in Atlantic cod following immunisation with different bacterial antigens in oil adjuvant

H. Mikkelsen[1], M. Seppola[1] and Bjørn Brudeseth[2]

[1] Nofima, Department of fish health, P.O. Box 6122, N-9291 Tromsø, Norway.
[2] PHARMAQ AS, P.O.Box, 267 Skøyen, N-0213 Oslo, Norway
Email: helene.mikkelsen@nofima.no

Atlantic cod, *Gadus morhua*, is a fairly new species in aquaculture in Norway. The most common bacterial diseases affecting cod is atypical furunculosis, francisellosis and vibriosis caused by facultative intracellulare bacteria atypical *Aeromonas salmonicida, Fransicella noatunensis* and the extracellular bacteria *Vibrio anguillarum* respectively. Atlantic cod has a unique immune system lacking both major histocompatibility complex class 11 (MHC-11) and the CD4, Invariant chain, IL-2, IL-4 and Toll like receptors 1,2,4,5,6, proteins that are important in production of specific immune response as specific antibodies and activation of macrophages.

In this context we found it interesting to study whether immunisation with either intracellular or extracellular bacteria had different ability to activate a specific immune response. We have measured the immune response by antibody production and expression of genes both in the innate and adaptive immune system. Groups of cod were injected intraperitoneally with oil adjuvanted bacterin. Head kidney and spleen were sampled on RNA-later at several time points while blood were sampled 49 days post immunisation.

The immune response was specific with regard to antibody production, all antibodies reacted only with homologous strain and not with heterologous strains. Both atypical *A. salmonicida* and *F. noatunensis* resulted in high antibody response (titer 4096) while *V. anguillarum* resulted in lower response (titer 512). The immune gene expression was highest in spleen and showed that *V.anguillarum* resulted in highest expression of inflammatory (IL-1_, IL-6, IL-8,) and anti-inflammatory (IL-10) genes, inducers of cellular immune response (IL-12p40, IFN_ and TNF_) and antibacterial genes (cathelcidin, hepcidin). None of the bacteria resulted in changed expression of immune globulin genes as secretory and membrane bound IgM and IgD, acute phase respone (pentraxin) or antiviral genes (interferon regulator factor 1: IRF1).

OP - E11

The immune response and disease resistance of clonal lines of Nile tilapia *Oreochromis niloticus*

Kim D. Thompson[1], M. Rafiq [1]. Sarder[2], Sayema Sayeed[3], José G.R. Paredes[1], Randolph H[1]. Richards, Alexandra Adams[1], Brendan J[1]. McAndrew[1] and David J. Penman[1].

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
[2]Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh
[3]University of Canberra, Abbott, ACT, Australia
Email: kdt1@stir.ac.uk

Intensification of farming systems for aquaculture makes fish more susceptible to stress and disease. Farmers currently treat with drugs, such as antibiotics, or use vaccines for controlling disease. Genetic selection as a means of improving disease resistance of stock is presently receiving a great deal of attention. Some strains of fish are naturally resistant to a particular pathogen, and as such are very useful for studying the underlying mechanisms involved in disease resistance. Inbred strains of fish, produced in the laboratory using conventional sibmating, are ideal for immunological studies, since phenotypes for different immune traits can be defined in the different strains and these in turn can be related to disease resistance. However, these strains can take between 15 and 20 generations to develop. Gynogenetic or androgenetic reproduction offers a rapid alternative for developing fully inbred homozygous clones of fish within two generations. Since the clones have identical genotypes, the genetic variability between clones can be monitored and clones with favourable or unfavourable genes, which are important in disease resistance, can be identified.

Studies have been carried out at the Institute of Aquaculture, University of Stirling in which the genetic variation of clonal lines of Nile tilapia (*Oreochromis niloticus* L.) was examined in relation to disease resistance and innate and adaptive immune responses. Mitotic gynogenesis was used to produce a first generation of completely homozygous female Nile tilapia, and inbred clones were then established from mitotic gynogenetic females by meiotic gynogenisis. Outbred clones were also produced by crossing the different inbred clones. A group produced by ordinary crossing was used as an unrelated control. The results of this work, together with current activities, are described in this paper, from which it is apparent that the clonal lines vary in their immune response parameters and a positive correlation exists between the level of infection with *A. hydrophila* and the parameters measured. Current work is aimed at trying to understand the genetic control of these immune mechanisms and their role in disease resistance in these fish.

OP - E12

Immune markers for disease resistance to aeromoniasis using rohu carp as a model species

P.K. Sahoo[1], *K.D. Mahapatra*[1], *J.N. Saha*[1], *S. Das1, M. Baranski*[2] *and N. Robinson*[2,3]

[1] Cental Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
[2]Nofima, PO Box 5010, 1432 Ås, Norway
[3]Flinders University, Sturt Road, Bedford Park, SA 5042, Australia
Email: pksahoo1@hotmail.com

Aeromonas hydrophila is frequently associated with diseases in rohu carp showing small surface lesions, local haemorrhages and septicaemia, dropsy, exophthalmia, and fin and tail rot. The advance made in developing a suitable commercially viable vaccine to prevent this infection is still in its infancy. The exploitation of intrinsic resistance factors, which will aid in the protection of fish against diseases throughout its life cycle may be harnessed via selective breeding. The basis for selection is genetic variation in disease resistance. Selection of fish disease resistance may be performed directly via survival and challenge data, or indirectly via the identification of the underlying resistance markers responsible for the differential survival. In a selection program that is going on at CIFA, Bhubaneswar, for obtaining higher growth in rohu, we have attempted to add another trait i.e., resistance to aeromoniasis. Based on a selective breeding approach, two divergent lines (susceptible and resistant) have been generated. To evaluate the possible role of developing indirect immune markers, an array of innate immune indicators were standardized for large scale analysis from around 50 families of rohu every year starting from 2003 till 2009. Based on our observations, substantial genetic variation between survival and immune parameters were obtained. For example, the serum ceruloplasmin level showed a positive correlation with survival to aeromoniasis (0.49) and a good heritability (0.5). For the first time, a novel approach has been used as a first-pass scan to identify genes associated with resistance to *A. hydrophila* in rohu. The first comprehensive database of sequences of transcribed genes in rohu (consisting of over 137 thousand contiguous sequences) has now been analysed and annotated. Seventeen percent of the 100 most highly differentially expressed transcripts in the resistant line rohu showed homology to genes with putative functions affecting innate immune response. Results suggest that innate immunity is enhanced in naïve resistant line fish because higher quantities of particular compounds are expressed, and because of the presence of particular polymorphisms, or linked genes.

OP - F1

Managing microbial activity in larviculture: the quorum sensing case

Tom Defoirdt[1,2], H.A.Darshanee Ruwandeepika[1,3], Indrani Karunasagar[3], Nico Boon[2] , Natrah Ikhsan[1], Li Xuan[1], Kristof. Dierckens[1], Patrick Sorgeloos[1] and ***Peter Bossier***[1]

[1]Lab. Aquaculture & Artemia Reference Center, Ghent University, Gent, Belgium
[2]Lab. Microbial Ecology and Technology, Ghent University, Gent, Belgium
[3] UNESCO MIRCEN for Marine Biotechnology, College of Fisheries, Mangalore India
Email: peter.bossier@ugent.be

Microorganisms play an important role in aquaculture and in larviculture in particular. Despite the poor background in fundamental knowledge, many researchers have tried to improve survival in larviculture through the application of probiotics. Typically, beneficial results have been observed without strong evidence for the mode of action *in vivo.*

This review will summarise the results that have been obtained in gnotobiotic experimental systems (avoiding largely stochastic and temporal interference) and where possible complement these observations with results obtained under non-gnotobiotic experiment conditions. Gnotobiotic experimental conditions have been developed for *Brachionus, Artemia,* and European seabass. Using gnotobiotic *Artemia* as experimental model, a Vibrio challenge test could be developed. The first results revealed that quorum sensing is indeed important *in vivo*. Using *Vibrio* strains carrying single or double mutations in its quorum-sensing systems, it could be demonstrated that a mutation in the AI2 system (either in the production or the sensor) considerably reduced the virulence towards Artemia. Encouraged by these results, we have developed systems to demonstrate that quorum-sensing molecules (mainly acyl homoserine lactone, AHL, also called AI1 molecules) are important in other systems. In turbot and *Macrobrachium* larviculture it could be shown that the daily addition of a mixture of AHL molecules (1mg/l) can have a strong negative effect on the larval survival. Microbial communities able to degrade AHL molecules *in vitro* and *in vivo* have been shown to mitigate the negative effect of added AHL molecules. This suggests that influencing the balance between AHL production and degradation is a possible option to manipulate the standing microbial community, reducing its virulence towards target organisms. In conclusion, gnotobiotic systems are useful experimental systems that allow to conceptually verify novel treatments and their mode of action. Such knowledge will definitely contribute to a knowledge-based application of novel concepts and products in larviculture.

OP - F2

Bacterial interaction in crustacean guts: pathogenesis and gene expression

Pikul Jiravanichpaisal

Aquatic Molecular Genetics and Biotechnology Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin, Thailand Science Park, Thailand.
Email: pikul.jir@biotec.or.th

The digestive tract of the crustacean is composed of three major sections: foregut, midgut, and hindgut. The length of each segment is different among species, and only the foregut and the hindgut are covered with cuticle. The midgut of crayfish is much shorter in comparison to the midgut of the penaeid shrimp. For this reason crayfish is highly tolerant to pathogenic *Aeromonas hydrophila* by oral administration since this bacterium appears to infect through the midgut.

To understand shrimp's pathogenesis and defense mechanism against infections in the most natural setting, the interactions between shrimp and a pathogenic bacterium in the intestinal tract was investigated. When the shrimp was infected via ingestion with highly pathogenic *Vibrio harveyi* (*Vh*), it normally dies within three days. By studying the effects of this *Vh* infection on the expression of the fifteen immune-related genes, we found that nearly all of these genes were constitutively expressed at high levels and only six of them were affected by the bacterial challenge. Oral administration of *Vh* in shrimp leads to the transfer of bacteria from the mouth to the posterior part of the alimentary tract. Unlike non-pathogenic bacteria, pathogens use sophisticated strategies to counteract such immune responses in the gut. The persistence of *Vh* can result in the colonization and multiplication of the pathogen in the shrimp gut. Subsequently, *Vh* and/or its toxins destroy the epithelium and try to evade the local immunity to gain entry into the body cavity. At this stage of infection, circulating hemocytes are recruited to the site of invasion to fight against the pathogens and also to heal the damaged tissues. If the pathogens can overcome this protection, they will subsequently proceed to initiate systemic infection and lead to death of the host. However, if the pathogens are eliminated from the gut, the host will prevail.

OP -F3

Phage therapy in aquaculture -Lysozyme helps overcome phage resistance

Indrani Karunasagar

Department of Fisheries Microbiology, UNESCO-MIRCEN for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575 002, India.

E.mail: karuna8sagar@yahoo.com

Phage therapy is an efficient and eco-friendly solution to manage bacterial diseases as opposed to the application of antibiotics that result in issues of resistance among microbes and residues in animals and environment. In any crustacean aquaculture, due to the presence of chitinous exoskeleton in the animals, diseases due to vibrios are a major problem. The most important agents of 'vibriosis' in shrimp aquaculture are members of the 'Harveyi' clade, especially *Vibrio harveyi*. Therefore, the use of phages to control 'luminous vibriosis' was taken up as a viable alternative. The study on phages and their application included the isolation, propagation, characterization and performance of several phages isolated from estuarine and marine environments and animals present in those niches. For application in hatcheries, studies were conducted on dose (volume), frequency, number of phage particle etc. Resistance of bacteria to phage was encountered sometimes after phage treatment and was presumed to be due to lysogenization of the phage. Subjecting such bacterial colonies to mitomycin treatment did not revert them to the lytic state (phage sensitive). We hypothesized a 'quasi resistance' phenomenon which could be due to failure of phage entry into the bacterial cell. The treatment of a mixture of phage and lysozyme to such resistant bacteria reverted them to phage sensitive state (lytic zones seen). We surmise that lysozyme promotes the entry of phage into the bacteria. Application of a combination of phage and lysozyme brought about greater reduction in *Vibrio harveyi* numbers in sea water than phage or lysozyme alone. Lysozyme possibly helps phage entry by making pores and the reversal to lytic state seen in "quasi resistant bacteria". The results on phage therapy will be discussed.

OP - F4

Establishing *Caenorhabditis elegans* model for screening anti-infectives against aquaculture pathogens

S. Durai and ***K. Balamurugan***

Department of Biotechnology, Alagappa University, Karaikudi-630003, Tamilnadu, India
Email: bsuryar@yahoo.com

Major aquaculture pathogenesis is contributed to *Vibrio* spp infection. *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are responsible for wide range of pathogenesis in marine environment. The transmission of *V. alginolyticus* from the aquatic environment, through fishes, shellfishes to humans has been well documented. Infection by *V. alginolyticus* is frequently associated with mass mortality of aquaculture larvae in the estuarine ponds. Biofilm formation on biotic and abiotic surface by vibrios is considered to be the major cause for antibacterial resistance. The attachment of bacteria and the formation of biofilm on biotic surfaces are extensively examined. We have established *C. elegans* as a model for *V. alginolyticus* and *V. parahaemolyticus* infection. Physiological status including intestinal colonization, pharyngeal pumping, egg count assay and chemotactic response was analyzed using *C. elegans* as a host. Molecular analysis of host immune responsible genes proved the regulation of lys-7, clec-60 and clec-87 over a period of 24 hours. In recent years *C. elegans* has been used as an amenable model for screening bioactive compounds in various fields. In the present study, using *C. elegans* as *in vivo* model system, we screened marine sponge associated bacteria (SAB) for their anti-infective activities against *V. alginolyticus* and *V. parahaemolyticus*. Survival assays, *in vivo* colonization reduction, pharyngeal pumping were analyzed in the presence of SAB extracts. The reduction in colonization was further analyzed with GFP-tagged pathogens using Confocal Laser Scanning Microscopy. Results showed that the SAB isolates significantly increase the survival of *C. elegans* infected with *Vibrio* spp. by reducing in vivo colonization. One of the positive isolate was characterized at the molecular level by 16S rRNA gene sequencing. In addition, the positive extracts were characterized using FTIR analysis. Owing to the increase in antibacterial resistance by aquaculture pathogens, the present *in vivo* screening approach would provide a molecule with potent characteristics for future drug. *This work was supported in part by DBT and UGC-Major grants from Govt of India to K.B.*

OP - F5

Major Diseases and health management in striped catfish (*Pangasianodon hypophthalmus*) farming in the Mekong River Delta, Vietnam

Dang Thi Hoang Oanh[1] *and Nguyen Thanh Phuong*[1]

[1]Department of Aquatic Biology and Pathology, College of Aquaculture and Fisheries, Cantho University, Cantho city, Viet Nam.
Email: dthoanh@ctu.edu.vn

Striped catfish (*Pangasianodon hypophthamus*) farming has been developed very rapidly in Vietnam and has made a tremendous contribution to global aquaculture production. However, diseases have been one of the significant problems for sustainability and profitability of the striped catfish farming sector in Vietnam. 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 farmed striped catfish. Recently, some new diseases have been reported in cultured striped catfish but causative agents which are responsible for clinical signs of those diseases have not yet been identified. In this article, major diseases in farmed striped catfish will be described and health management in striped catfish farming in the Mekong River Delta will be presented and discussed.

OP - F6

Antibiofilm compounds from marine bacteria - A novel strategy to conquer antibiotic resistance in aquaculture industries

Karutha Pandian S. *and C. Nithya*

Department of Biotechnology, Alagappa University, Karaikudi 630003, Tamil Nadu, India.
E-mail: sk_pandian@rediffmail.com

Vibrios are important bacterial pathogens for animals reared in aquaculture. *V. alginolyticus, V. salmonicida, V. parahaemolyticus* and *V. vulnificus* are among the main bacterial pathogens of several fish species. Much is known about the formation of biofilm by *Vibrios.* However, only very few studies have been carried out on the biofilm inhibition of *Vibrio* spp. A biofilm is a multicellular consortium that consists of more than one species and shows high resistance to antibiotics. Currently we are unable to treat these infections successfully because biofilms are resistant to the existing antimicrobials. Considering the emergence of increasing antibiotic resistant bacteria, the use of signal-molecule-based drugs to attenuate bacterial pathogenicity rather than bacterial growth is attractive. The goal of our study was to identify and characterize novel marine bacterial isolates for antibiofilm activities against aquaculture pathogens. Sediment associated bacteria isolated from Palk Bay coastal areas were screened for antibiofilm activity. Eight of them (S8-01, S8-05, S8-15, S6-01, S6-15, SS-03, SS-04 and SS-08) exhibited 60-94% antibiofilm activity against aquaculture pathogens (*Vibrio* spp.). Among the eight isolates, SS-03 (*Bacillus niabensis*), S6-01 (*B. indicus* - MTCC 5559) and S6-15 (*B. pumilus* - MTCC 5560) distrupted in the mature biofilm of *Vibrio* spp. The structure of the active compounds in S6-15 and SS-03 were elucidated using GC-MS, 1H NMR, 13C NMR spectra and identified as 4-phenyl butanoic acid and 5-hydroxy dodecanoic acid, delta lactone/5-dodecanolide respectively. This is the first report showing the production of phenyl butanoic acid, an aromatic short chain fatty acid and 5-hydroxy dodecanoic acid with the functions of molecule chaperon by marine bacterial isolates. The purified compounds showed promising broad spectrum antibiofilm activity (at BIC 5-20 μg/ml). Furthermore, they disrupted the biofilm architecture by reducing the hydrophobicity index and EPS production. Although different biological tests were applied, no antibiotic activity was detected. From the promising results obtained in the current study, it becomes obvious that, the novel antibiofilm compounds isolated from the marine bacterial isolates *B. pumilus* (S6-15) and *B. niabensis* (SS-03) with broad spectrum antibiofilm activity could very well serve as valuable tools for aquaculture applications.

OP - F7

Sustaining shrimp culture in the Philippines using molecular applications

Benedict A. Maralit, *Minerva Fatimae H. Ventolero and Mudjekeewis D. Santos*

Genetic Fingerprinting Laboratory, National Fisheries Research and Development Institute, Quezon Avenue, Quezon City, Philippines

Email: benedict_bdek@yahoo.com

In the Philippines, three key factors have been identified in order to successfully revive the country's shrimp industry including 1) the culture of specific-pathogen free (SPF) and specific-pathogen resistant (SPR) broodstock and "high health" fry ; 2) use of best management practices (BMPs) such as probiotics and biosecurity measures; and 3) marketing and compliance to food safety regulations (Mojica, 2008). Here, we started to address these issues using molecular biology and biotechnology-based applications. We specifically report 1) the generation and comparison of gene expression profiles of resistant and susceptible shrimp species that may be used for broodstock selection; 2) the development of new primers that enhances the efficiency and sensitivity of PCR- and LAMP-based diagnostic methods, and 3) the use of DNA barcoding for identification of mislabeled products, authenticity testing and species traceability. These studies highlight the importance of adopting molecular techniques as essential tools for reviving and sustaining the shrimp culture industry in the country.

OP - G1

Using systems biology approaches to understand shrimp cellular responses during viral infection

Chu-Fang Lo[1]*, Geen-Dong Chang*[2]*, Kay-Hooi Khoo*[3] *and KC Han-Ching Wang*[4]

[1]Institute of Zoology, College of Life Science, National Taiwan University, Taipei, Taiwan
[2]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan
[3]NRPGM Core Facilities for Proteomics & Glycomics, Academia Sinica, Taipei, Taiwan
[4]Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan
Email: gracelow@ntu.edu.tw

White spot syndrome virus (WSSV) is the causative agent of white spot disease (WSD), a disease which affects most aquatic crustaceans. WSSV is unique: over 90% of its ORFs show no significant similarity to other known proteins. Our work brings together expertise and technological platforms from biological, bioinformatic and computational research teams to address several challenging issues in host-pathogen interactions using a new perspective: holism instead of reductionism. We use systems biology approach to address the "big picture" question of virus host interaction under different global conditions, such as different infection states, different disease states, and different disease resistance states. For this presentation, the focus will be shrimp cellular changes during viral infection. Using systems biology approach, we found changes in several cellular metabolic pathways. A shift from glycolysis to the pentose phosphate pathway led to increased NADPH as well as increased levels of intermediates for the synthesis of amino acids and nucleotides. Proteomic analysis further showed that the energy-producing TCA cycle was incomplete but that this provided intermediates for the biosynthesis of lipids and amino acids. All of these changes are very likely to be of benefit to the virus in its replication cycle. We also have known previously that the WSSV-genome contains many IRES (Internal Ribosomal Entry Site). Meanwhile, our proteomics data suggested that cap-dependent translation is inhibited at the late stage of infection, suggesting that IRES and other cap-dependent translation mechanisms play an important role in viral protein production at the late stage of the viral replication cycle. To investigate whether the composition of the ribosomal machinery had been changed to favor translation of viral proteins, we purified ribosomes from infected cells. Our results show that viral protein[s] had been incorporated into the host cell ribosomes, although the biological meaning of these insertions has yet to be determined.

OP - G2
Development of SPF and SPR stocks for shrimp aquaculture

Carlos R. Pantoja

The University of Arizona, Aquaculture Pathology Laboratory, 1117 E. Lowell St., Bldg. 90, Rm 106, Tucson, Arizona 85721, USA
Email: cpantoja@u.arizona.edu

Infectious diseases are one of the most important factors that have contributed to shaping the international shrimp farming industry. The predominance of *Penaeus (Litopenaeus) vannamei*, the dominant shrimp species cultivated globally today, is a good example. *P. vannamei* came to replace *Penaeus (Litopenaeus) stylirostris* after showing better survival during the devastating outbreaks caused by infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the early 1970s and 1980s. Later, it became clear that IHHNV-free *P. vannamei* outperformed IHHNV-infected shrimp of the same species, and the term Specific Pathogen Free (SPF) was employed to refer to such IHHNV-free stocks. Advances in pathogen detection technology, mostly based on PCR, were applied along with strict quarantine protocols to breeding programs in the USA producing SPF stocks for the domestic shrimp farming industry. Then, the emergence of Taura syndrome virus (TSV), to which *P. vannamei* is highly susceptible, demonstrated that other disease control strategies were necessary. Fortunately, *P. vannamei* appears to have additive genetic variation for resistance to TSV and significant improvements in TSV resistance were quickly made, which increased the popularity of this species worldwide. Resistance to other viral pathogens, such as white spot syndrome virus (WSSV) is not as easily inherited in penaeid shrimp. For years since it first emerged, attempts to breed resistance to WSSV in *P. vannamei* have been disappointing. However, during a challenge experiment performed early this year at the University of Arizona's Aquaculture Pathology Laboratory (UAZ-APL), *P. vannamei* stocks developed in Panama by a private company showed survival rates that ranged from 23% to 57%. No WSSV was detected by real time PCR in survivor shrimp 17 days after *per os* exposure to WSSV. This is the first time since 1996, when UAZ-APL first began performing WSSV challenge studies, that a stock of *P. vannamei* has shown significant survival in laboratory challenge studies that are designed to mimic white spot disease outbreaks in typical shrimp farms.

OP - G3

Mining a comprehensive collection of shrimp expressed sequence tags for viral responsive genes

Anuphap Prachumwat [1,2], *Amornrat Tangprasittipap*[1], *Timothy W. Flege*[1,2] *and Kallaya, Sritunyalucksana*[1,2]

[1] Shrimp-Virus Interaction Laboratory, Agricultural Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumthani, Thailand
[2] Center of Excellence for Shrimp Molecular Biology and Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand
Email: anuphap.pra@biotec.or.th

Information on how shrimps respond to pathogens remains limited especially for viral responsive shrimp genes. To reveal putative viral responsive shrimp genes, a comprehensive collection of expressed sequence tags (ESTs) of *Penaeus monodon* and *P. (Litopenaeus) vannamei* challenged by yellow head virus (YHV) and white spot syndrome virus (WSSV) were *in silico* characterized in a comparison with unchallenged shrimp EST libraries. The total of 54,778 EST clones, after contig assembly, from gills, hemocytes, lymphoid or whole shrimp were searched for homologs in NCBI non-redundant protein database with E-value < 10-4 and at least 25 amino acid residues of the alignable region. ESTs were projected to KEGG's gene annotation by these homologs for identifying shrimp genes found exclusively in the viral challenged EST libraries (namely, putative viral responsive genes). 221 and 74 putative viral responsive genes were present in WSSV- and YHV-challenged libraries, respectively. Two out of these 221 putative WSSV-responsive genes are found in both *P. monodon* and *P. vannamei:* SMAD, Mothers Against DPP 2/3 and acyl-CoA dehydrogenase. Interestingly, none of WSSV responsive and YHV-responsive genes is shared in either *P. monodon* or *P. vannamei.* The experiments to confirm the correlation of the two WSSV-responsive genes (SMAD, Mothers Against DPP 2/3 and acyl-CoA dehydrogenase) and WSSV infection in shrimp is underway.

OP - G4

Molecular characterization, down-stream signaling analysis, and prediction of ligand binding key domains in toll-like receptor 2 (TLR2) of the Indian Major Carp, rohu *(Labeo rohita)*

Mrinal Samanta*, Banikalyan Swain , Madhubanti Basu, Padmaja Panda, Girish B. Mohapatra, Bikash R. Sahoo and Nikhil K. Maiti*

Fish Health Management Division, Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, Orissa 751002, India.
Email: msamanta1969@yahoo.com

Toll-like receptors (TLRs) are one of the key components of innate immunity. Among the TLRs, TLR2 is involved in recognizing specific structures on pathogens (PAMPs), and triggers MyD88-dependent signaling pathway to induce pro-inflammatory cytokines. We cloned and characterized TLR2 gene in rohu *(Labeo rohita)* which is highly important fish in the farming-industry of India and its neighboring countries. The full length rohu TLR2 (rTLR2) cDNA comprised of 2691 bp with a single open reading frame (ORF) of 2379 bp encoding a polypeptide of 792 amino acids (aa), with an estimated molecular mass of 90.74 kDa.. Phylogenetically, rohu TLR2 was closely related to common carp. Basal expression analysis of rTLR2 showed its constitutive expression in all the tissues examined; highest was in the spleen and the lowest was in the eye. Inductive expression of TLR2, and MyD-88 dependent signaling was analyzed in response to various TLR2 ligands and *Streptococcus uberis* and *Edwardsiella tarda* infections, and the results suggested the key role of TLR2 in inducing the immunoregulatory cytokine interleukin (IL)-8. The important sites of interaction between PAMPs (pathogen associated molecular patterns) and the extra-cellular domain (ECD) representing LRR-regions of rTLR2 (rTLR2-ECD) were identified by homology modeling and molecular docking. These key domains are expected to be involved in pathogen recognition, and the activation of TLR2 signaling resulting in the induction of innate immunity, and the protection of fish against diseases.

OP - G5

White spot syndrome virus induces a Warburg-like effect in shrimp hemocytes in the early stage of infection

KC Han-Ching Wang,[1] *Yun-Tzu Huang,*[1] *I-Tung Chen,*[2] *Suh-Yuen Liang,*[3] *Shu-Yu Lin,*[3] *Yen-Chen Chen,*[4] *Jan-Ming Ho,*[4] *Geen-Dong Chang,*[5] *Kay-Hooi Khoo,*[3,6] *and Chu-Fang Lo,*[2]

[1]Institute of Biotechnology, National Cheng Kung University, Tainan, Taiwan
[2]Institute of Zoology, College of Life Science, National Taiwan University, Taipei, Taiwan
[3]NRPGM Core Facilities for Proteomics & Glycomics, Academia Sinica, Taipei, Taiwan
[4]Institute of Information Science, Academia Sinica, Taipei, Taiwan
[5]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan
[6]Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan
Email: wanghc@mail.ncku.edu.tw

White spot syndrome virus (WSSV) is the causative pathogen of white spot disease (WSD), which continues to have a major economic impact on the cultured shrimp industry. In our previous study, we found that mitochondria may play an important role during WSSV infection through dsRNA silencing of the mitochondrial outer membrane protein, VDAC (voltage dependent anion channel). To elucidate the mitochondria-related pathogenesis of WSSV, several metabolic pathways were monitored in shrimp hemocytes after the injection of phosphate-buffered saline (PBS) and WSSV. At the WSSV genome replication stage (12 hpi), all of the observed results were characteristic of the Warburg effect, which is an abnormal glycolysis response associated with mammalian cancer cells. Subsequently, at the late stage of WSSV infection (24 hpi), these metabolic changes led to cell death. This is the first time that any Warburg-like effect has been shown for any invertebrate virus. Further, WSSV is unique in being a non-cancer inducing virus that can nevertheless induce a Warburg-like effect. To date, however, we have been unable to use dsRNA silencing of VDAC to investigate its rate in the WSSV-induced Warburg effect, so it is unsafe to draw any conclusion about VDAC's function in this effect. To explore in more detail the gene regulation networks that produced this WSSV-induced Warburg effect, we performed a comprehensive quantitative analysis of protein profiles using a gel-free mass spectrometry-based high throughput proteomics platform. The resulting overview of the protein alterations during WSSV infection, suggested that activation of the PI3K/AKT/mTOR signaling pathway is critical for inducing the Warburg effect during the WSSV genome replication stage. Contingent upon further validation, these new insights into WSSV's unique pathogenesis are likely to be useful in developing effective ways to combat WSSV infection.

OP - G6
Reverse genetics of fish viruses

K Riji John, *M. Rosalind George and Reyas Ahmed*

Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi 628008, India
Email: rijijohn@gmail.com

Reverse genetics is a process of generating a cloned complimentary DNA of RNA viral genome by *in vitro* reverse transcription and transfecting permissive cell lines with manipulated DNA or *in vitro* transcribed RNA to generate infectious virus particles. This technique has now become a powerful tool to generate vaccine strains of viruses and viral vectors capable of carrying multiple antigens, study virus biology, tissue tropism and virulence factors and for modifying host specificities and multiplication deficient viruses. Reverse genetics system for the positive sense RNA viruses was the first to develop as the *in vitro* synthesized RNA could be immediately translated by the host cell machinery upon transfection. Negative sense viral RNA genomes were less amenable to manipulation as it required conversion to positive sense by an RNA polymerase before translation, correct 5' and 3' untranslated regions (UTRs) for viral packaging and ribonucleoprotein (RNP) complexing of genomic and antigenomic RNAs. However many of these hurdles were overcome by developing minireplicons to produce individual viral RNAs of the genome. In aquaculture, potentially problematic RNA viruses belong to rhabdoviridae, birnaviridae, betanodavirdae, togavirdae and orthomyxoviridae. First reverse genetics system for a fish virus was established in 1998 for the birnavirus infectious pancreatic necrosis virus (IPNV). Stable live attenuated vaccines, a dream for many infectious diseases and developed by continuous passaging in cell culture systems, are generally more effective than inactivated viruses and sub unit vaccines. Establishment of reverse genetics systems for many of the fish RNA viruses from cDNA greatly widened the scope for developing stable attenuated vaccine strains of these viruses apart from generating multivalent live viral vaccines, which could reduce vaccine cost, improve protection efficiency and would couple ease of application. Generation of single-replication cycle virus-based vaccine vectors could further improve the reverse genetics technology to generate safe live vaccines.

OP - G7

Semiconductor based sequencing technology: Applications and tools

Anupama Gaur

Business Development Manager-NGS, South Asia, Invitrogen BioServices India Pvt. Ltd., 372, Udhyog Vihar Ph II, Gurgaon, Haryana - 1220126, India
Email: anupama.gaur@lifetech.com

DNA sequencing is an inherent tool to all Life science scientists for decades now. The golden era of slab gels followed by capillary sequencing has a paradigm shift with onset of Next generation Technologies. With the advent of next-generation sequencing, large amounts of sequence data can be generated very rapidly. Recently launched semiconductor based sequencing technology in which scalable, low-cost manufacturing techniques are used to make an integrated circuit able to directly perform non-optical DNA sequencing of genomes. Sequence data are obtained by directly sensing the ions produced by template-directed DNA polymerase synthesis using all-natural nucleotides on this massively parallel semiconductor-sensing device or ion chip. The ion chip contains ion-sensitive, field-effect transistor-based sensors in perfect register with 1.2-12 million wells, which provide confinement and allow parallel, simultaneous detection of independent sequencing reactions. Use of the most widely used technology for constructing integrated circuits, the complementary metal-oxide semiconductor (CMOS) process, allows for low-cost, large-scale production and scaling of the device to higher densities and larger array sizes.

For Ion Personal Genome Machine™ (PGM™) sequencer analysis, DNA is fragmented, and each discrete DNA construct is amplified on a single bead-a process termed "clonal amplification." This streamlined sample preparation of the PGM™ sequencing workflow is critical in serious public health outbreaks. Recently a *Escherichia coli* outbreak was contained and countered with this high speed technology. The technology can be used for de Novo sequencing, re-sequencing, target sequencing (Life Technologies also provide Target sequencing kits), Metagenomics, RNA sequencing, CNV, CHiP sequencing and many more applications being added in near future.

OP - H1

Induction of type I interferon gene expression mediated by pattern recognition receptors in Japanese flounder

Takashi Aoki[1,2], *Maki Ohtani*[1], *Jun-ichi Hikima*[1], *Seong Dong Hwang*[2], *Carmelo S. del Castillo*[1], *Takahiro Morita*[2], *Yoshiaki Suzuki*[2], *Goshi Kato*[2], *Hidehiro Kondo*[2], *Ikuo Hirono*[2]*and Tae Sung Jung*[1]

[1]Aquatic Biotechnology Center of WCU Project, College of Veterinary Medicine, Gyeongsang National University, 900 Gajwa-dong, Jinju 660-701, South Korea
[2]Laboratory of Genome Science, Tokyo University of Marine Science and Technology, 4-5-7 Minato-ku, Tokyo 108-8477, Japan
E-mail: aoki@kaiyodai.ac.jp

Type I interferon (IFN-I) is one of the most important cytokines to enhance antiviral activity. In mammals, IFN-I is induced by viral infection as mediated by pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs). In our previous studies, these PRR genes [such as TLR-3, LGP2 (laboratory of genetics and physiology 2) and MDA5 (melanoma differentiation-associated gene 5)] and IPS-1 (mitochondrial adaptor IFN-? promoter stimulator-1), which is an adaptor molecule in RIG-I signaling, have been identified from Japanese flounder (Paralichthys olivaceus), and their functions have been shown. In order to understand the details in transcriptional regulation of the IFN-I gene downstream of PRR signaling, the 5' up-stream region (1.36 kb) of Japanese flounder IFN-I gene, which contains numerous canonical motifs to bind transcription factors [such as NF?B and the IFN regulatory factors (IRFs) 1, 3, 4, and 7], was cloned and analyzed by transient reporter assay. The reporter assay results showed enhanced IFN-I transcriptional activity in the -634 to -179 bp region after both extracellular and intracellular poly I:C stimulation. Interestingly, overexpression of IRF3 strongly enhanced transcriptional activity in the presence of poly I:C. These results indicated that IRF-3 is a key transcriptional regulatory factor for IFN-I gene expression in Japanese flounder. To confirm whether PRRs such as TLR-3, LGP2 and MDA5 induce IFN-I gene expression, these PRRs were co-transfected with IFN-I promoter. The transcriptional activities of the IFN-I promoter region were enhanced by overexpression of these PRRs in the presence of poly I:C. These results suggest that IFN-I gene expression is probably triggered by PRR signaling pathways in a manner similar to mammalian systems.

OP - H2

Antibacterial and antiviral defence in a crustean; the role of Dscam,TEPs and gC1qR

Kenneth Söderhäll

Department of Comparative Physiology, Uppsal University
Email: Kenneth.Soderhall@ebc.uu.se

The role and function of TEPs, Dscam and gC1qR in antibacterial and antiviral defence will be discussed.

OP - H3

Cloning, expression analysis, and silencing study of three inhibitor of apoptosis protein genes (IAP) from *Litopenaeus vannamei* shrimp

Jiann-Horng Leu, *Kuan-Yu Chen and Huai-Ting Huang*

Institute of Marine Biology, National Taiwan Ocean University, No.2 Beining Road, Keelung City 20224, Taiwan, ROC

Email: jiholeu@ntou.edu.tw

The members of inhibitor of apoptosis protein (IAP) family are involved in the regulation of diverse cellular processes, including apoptosis, signal transduction and mitosis. Organisms with higher complexity have several different IAP genes in their genomes; for example, the human and the fruit fly have eight and four IAP genes, respectively. Only one IAP gene (*Penaeus monodon* IAP, PmIAP) had been discovered in penaeid shrimp, and this study aimed to identify and characterize more penaeid shrimp IAP genes. A homology search against NCBI EST database revealed that several *Litopenaeus vannamei* ESTs showed a certain degree of similarity to PmIAP gene. Based on these ESTs, we successfully cloned three IAP genes from *Litopenaeus vannamei* shrimp: LvIAP1, LvIAP2, and *LvSurvivin*. The full-length cDNAs of LvIAP1, LvIAP2, and *LvSurvivin* were 3166, 2160 and 864 bp, respectively. LvIAP1, the homolog of PmIAP, has 699 amino acid residues, consisting of three BIR domains and one RING domain. LvIAP2 is 226 residues long with two BIR domains. *LvSurvivin* has 139 residues with only one BIR domain. Their mRNA expression levels in various tissues were determined by absolute quantitative real-time RT-PCR. The results showed that of the three IAP genes, *LvIAP1* has highest expression levels in almost all examined tissues and *LvSurvivin* are lowest. Further, among the examined tissues, lymphoid organ highly expresses all three genes. The importance of the three genes to shrimp survival was investigated by gene silencing through dsRNA injection. All *LvIAP1* dsRNA-injected shrimp died by 48 hours post injection, whereas injection of the other two dsRNAs showed no harm to shrimp. In the *LvIAP1* dsRNA-injected shrimp, the number of circulating hemocytes was dramatically decreased, and the hemocytes underwent extensive apoptosis, as shown by nuclear morphology and increased caspase-3 activity. In conclusion, this study identified three IAP genes from *L. vannamei* shrimp, and one of them, the *LvIAP1*, is essential for the survival of hemocytes in *L. vannamei* shrimp.

OP - H4

Pathogen recognition receptors (PRRs) in teleost fish: An overview

Rajendran K.V.

Central Institute of Fisheries Education (CIFE), Versova, Andheri (W), Mumbai-400061, India
Email: rajendrankv@hotmail.com

Innate immune system plays a key role, in fishes and higher eukaryotes, to recognize and sense potentially harmful pathogens/dangers and to rapidly trigger appropriate defense mechanisms that prevent or minimize tissue damage. Primarily, the innate response is mediated by germ-line encoded pathogen-recognition receptors (PRRs) that recognize the conserved molecular signatures associated with pathogens termed pathogen-associated molecular patterns (PAMPs). After sensing the PAMPs, host innate immune cells initiate a broad spectrum of defense mechanisms that result in the development of inflammation and host resistance to infection. PRRs comprise an array of sensors and are found in the extracellular space, membrane-associated variant cell types or in the cytosol. Three major classes of PRRs have been identified: (1) The Toll-like receptors (TLRs) that recognize ligands on either extracellular surface or within the endosome, (2) the NOD-like receptors (NLRs) that are cytoplasmic receptors and (3) RIG.I-like receptors (RLRs), a virus recognizing intracellular receptors. TLRs represent type I transmembrane receptors characterized by the presence of leucine-rich repeats (LRRs) in their extracellular domain and by the presence of a Toll/interleukin-I receptor domains (TIR domain) in the C-terminal in the cytosol that initiates signal transduction Teleosts possess majority of tetrapod TLR orthologs in addition to fish-specific TLRs. The typical characteristics of the NLR family include the presence of three structural domains: a) An N-terminal protein-protein binding or effector domain b) A central nucleotide oligomerization (NACHT) domain and c) A C-terminal leucine-rich repeat domain (LRR) domain. However, the NLRs do not possess a signal peptide or transmembrane domain, which indicate their locations in the cytosol. RLRs consist of three members, retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). The structure of RIG-I and MDA5 are very similar, as the N-terminal region of RIG I and MDA5 are characterized by the presence of two tandem arranged caspase activation and recruitment domains (CARDs) involved in protein-protein interaction. This presentation will briefly discuss about the wide array of all the three PRRs present in fish, their phylogeny and expression patterns in different tissues.

OP - H5

Profiling expressed genes in the lymphoid organ of Australian banana prawn (*Penaeus merguiensis*) using suppression subtractive hybridization

Rusaini[1,2] *and L. Owens*[2]

[1]Aquaculture, Faculty of Agriculture, University of Tadulako, Central Sulawesi 94118, Indonesia.
[2]Microbiology and Immunology, School of Veterinary and Biomedical Sciences,
James Cook University, Townsville, Queensland 4811, Australia.
E-mail: rusaini.rusaini@my.jcu.edu.au; rmallullu@yahoo.com

Several viruses have been reported to cause diseases associated with lymphoid organ spheroid (LOS) cell development in wild and cultured banana prawn *Penaeus merguiensis*. Currently, in north Queensland, Australia approximately 75 - 100% of cultured P. merguiensis from different family lines have spheroids that occupy more than 40% of the lymphoid organ area. However, no candidate virus has been identified in causing these spheroid formations. Furthermore, differential gene transcripts in particular immune related genes in banana prawns are poorly understood. Therefore, this study was performed to determine viral genomes and differentially expressed genes in the lymphoid organ of *P. merguiensis.*

Suppression subtractive hybridization (SSH) was performed to generate a cDNA forward library between hatchery animals with spheroids (tester) and wild caught animals without spheroids (driver). A total of 316 sequences were clustered into 8 functional categories including immune-related genes (2.5%), proteases and inhibitors (7.0%), structural and cytoskeletal related molecules (10.1%), synthesis, processing and regulation-related proteins (9.8%), energy and metabolism factors (1.9%), ribosomal proteins (6.3%) and other sequences from various organisms (13.9%). Transcripts that had no significant amino acids/nucleotides similarity were grouped into unknown sequences (48.4%).

Firstly, the absence of viral genes from the SSH libraries, may indicate these two populations are infected with the same virus, with spheroids only evident in the hatchery population. Secondly, the concentration of viral genes in the cDNA from the hatchery population was too low to be expressed using SSH. Thirdly, the poly A tail on the viral mRNA may be too short for detection using SSH. Finally, the virus causing spheroid formation in the lymphoid organ of hatchery prawns may not have a poly A tail, therefore unable to be expressed in the SSH libraries. Nevertheless, the health status of these two populations of banana prawns are different; resulting in differentially expressed genes in the two populations, with some genes only being up-regulated in the hatchery population.

OP - I1

In vitro and in vivo efficacies of ionophores against *Cryptocaryon irritans*

Tomoyoshi Yoshinaga, *Hyun Jung Im, Sayako Nishida and Kazuo Ogawa*

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
Email:atyoshi@mail.ecc.u-tokyo.ac.jp

Aiming at the development of oral chemotherapy against *Cryptocaryon irritans,* the causative agent of 'white spot disease of marine fish', we assayed the effects of a variety of antiprotozoal compounds against trophonts of *in vitro* using the double layered media that we developed previously for the culture of the parasite. In the assay, ionophores, particularly sodium salinomycin, showed apparent killing and growth-suppression effects against the parasite. As there was no mortality in Japanese flounder *Paralichthys olivaceus* that were fed a diet containing sodium salinomycin (200 g/g) for two weeks, we evaluated the efficacy of 200 g/g sodium salinomycin against *C. irritans* in Japanese flounder. We fed Japanese flounders a medicated diet for 5 d prior to and 3 d after challenge with *C. irritans.* In the experimental group, the number of protomonts recovered from the fish and the size of tomonts that were transformed from the protomonts were significantly reduced, when compared to the control group. Furthermore, in a different experiment, the fish that were fed a diet medicated with sodium salinomycin survived longer than those fed an unmedicated diet after challenge. Sodium salinomycin can be a good candidate drug for chemotherapy and control of *C. irritans* infection.

OP - I2

Nested PCR assay, a quick and sensitive tool for the diagnosis of the Apicomplexan parasite, *Perkinsus* spp. in commercially important marine molluscs

Vijayan K.K., *Sanil N.K., Suja G., Lijo J. and Thomas P.C.*

Fish Health Section, Marine Biotechnology Division, Central marine Fisheries Research Institute, P.B No. 1603, Ernakulam North P.O., Cochin - 682018, India.
Email: vijayankk@gmail.com

Perkinsosis, an epidemic disease of marine molluscs caused by *Perkinsus* spp. often lead to mass mortalities. Infections with *P. olseni* and *P. beihaiensis* have been reported from the Indian subcontinent in the Pearl oyster, *Pinctada fucata* and edible oyster *Crassostrea madrasensis* respectively. At present, *Perkinsus* diagnosis is carried out using Ray's fluid Thioglycolate medium (RFTM) culture, histology and normal single step PCR techniques. RFTM and histology fails in sensitivity during very low and localized infections. Lack of specificity of RFTM has been reported earlier in many instances. Single step PCR though an effective means for specific and rapid detection, its sensitivity fails in one stage DNA amplification during low intensity infections. Template inhibition by host DNA might be one of the reasons behind the failure of amplification to a detectable level in low intensity infected samples.

Development of a diagnostic procedure with improved sensitivity and suitability is required for the effective and extensive use as a diagnostic tool. In this context we have developed a quick and sensitive nested PCR assay which could be used for rapid screening of samples for *Perkinsus* at genus level. Total DNA isolated form *Perkinsus* infected host tissue was used as the template. The first reaction was done using *Perkinsus* genus specific ITS primers followed by nested amplification using newly designed internal primers. The nested primers have been designed specifically from the conserved 5.8S ribosomal RNA gene and the internal transcribed spacer 2 (ITS 2) regions after aligning the available *Perkinsus* ITS sequence data to increase the specificity for the genus *Perkinsus.* Mass screening for *P. olseni* and *P. beihaiensis* have been carried out using the newly developed nested PCR assay. Results of nested PCR assay when compared with those obtained using classical methods of diagnosis of *Perkinsus* viz. histology, RFTM and single step PCR showed high sensitivity than the normal single step PCR, histopathology and RFTM. The assay being specific, sensitive, user friendly and cost effective, is appropriate for the rapid screening of large numbers of samples. This nested PCR is presently being developed as a PCR kit for the stake holders in bivalve mariculture.

OP - I3

Use of Bio-Surfactant as a prophylactic agent against fish protozoan parasite, *Cryptocaryon irritans* in Asian seabass, *Lates calcarifer*

Beng Chu Kua *and M. Thavalingam*

National Fish Health Research Centre (NaFisH), FRI NaFisH Batu Maung, Fisheries Research Institute, Department of Fisheries Malaysia, 11960 Batu Maung, Penang, Malaysia.
Email:kuaben01@fri.gov.my

Bio-Surfactant has been widely used in controlling plant diseases caused by insects and fungi. It is also extensively used in the medical industry in treating fungal and bacterial diseases as well as head lice in humans. Bio-Surfactant has never been applied to aquatic organisms. Bio-Surfactant is a polysaccharide-lipid consisting of an ionic and non-ionic surface, a liquid product derived through a biotechnological process from an oil palm waste.

Bio-Surfactant was experimentally applied to Asian seabass fry and fingerling infected with protozoan white spot, *Cryptocaryon irritans*. Results showed that seabass fry and fingerling can be treated with bio-Surfactant in three applications with a survival rate of 60% as compared to 0% in control fish without treatment. Bio-Surfactant was applied on day-3, 6 and 9 after infection. Typically, infection of *C. irritans* would lead to high mortality (80-100%) in the Asian seabass fry or fingerling in hatchery after 6 days of infection as a result of its characteristic life cycle. Thus, bio-surfactant could be used as a prophylaxis against fish protozoan parasite, *C. irritans* in Asian seabass, *Lates calcarifer* by terminating the life cycle of this parasite when it first multiplies after 6 -7 days, infecting the fish.

OP - I4

Caligus Müller, 1785 parasitizing cultured *Lates calcarifer* Bloch, 1970 in Malaysia

***Muhd. Faizul** H. A. H.[1], S. Bhassu[1] and Kua B. C[2]*

1 Institute of Biological Sciences, Faculty of Science, University of Malaysia, 50603 Kuala Lumpur, Malaysia
[2] National Fish Health Research Centre, NaFisH, Fisheries Research Institute, FRI, 11960 Batu Maung, Penang, Malaysia
Email: faizul_helmi@yahoo.com

Present study unveils the parasitism of three *Caligus* Müller (1785) species in cultured *Lates calcarifer* Bloch (1790) in Malaysia. The divergence in between the species was investigated through three perspectives; the presence, morphological and phylogenetic. Scanning electron microscopy conclusively reveals the species as *C. chiastos, C. epidemicus* and *C. rotundigenitalis.* Incessant infection by *C. epidemicus* was observed throughout the study; more than 90% of the total isolated specimens showed presence at all observed states. Followed by the infection by *C. rotundigenitalis,* it covered approximately 6% of the total specimens isolated from Johor and Penang. One individual of *C. chiastos* was isolated from Penang. Molecular phylogenetic analysis of partial 28S rRNA summarized the three species into monophyletic group of *Caligidae. C. chiastos* forming monophyletic relationship with *C. elongatus, C. rotundigenitalis* evolves from the group and *Caligus* epidemicus tend to be the most primitive. So far there is no report on the parasitic invasion by *C. chiastos* and *C. rotundigenitalis* onto *L. calcarifer.* This result would enhance the knowledge regarding on the parasitic infection in aquaculture activity in Malaysia.

OP - I5

Prevalence, mean intensity and site preference of *Caligus rotundigenitalis Yü,* 1933 1 (*Copepoda: Caligidae*) on cage cultured crimson snapper (*Lutjanus erythropterus* Bloch, 2 1790) from Bukit Tambun, Penang, Malaysia

Leaw Yoon Yau[1]*, Faizah Shaharom*[2]*, Anil Chatterji*[2] *and Beng Chu Kua*[3]

[1]Faculty of Agrotechnology and Food Science (FASM), Universiti Malaysia Terengganu 21030 5 Kuala Terengganu, Terengganu.
[2]Institute of Tropical Aquaculture (AQUATROP), Universiti Malaysia Terengganu 21030 Kuala 7 Terengganu, Terengganu.
[3]National Fish Health Research Center (NaFisH), Fisheries Research Institute, 11960 Batu 9 Maung, Penang, Malaysia.
E-mail: ivanisme86@hotmail.com

Snapper culture has been started in Malaysia since 30 years ago like any other marine species cultured. This study was carried out in order to determine the prevalence, mean intensity and site preference of *Caligus rotundigenitalis* (*Caligidae, Siphonostomatoida*) parasitic copepod on cage cultured crimson snapper, *Lutjanus erythropterus* from Bukit Tambun, Penang Malaysia). A total of 70 specimens of cultured snapper from floating cages were examined based on different infestation sites such as head, body as well as operculum and 3 different groups considering the size of the fish. *Caligus rotundigenitalis* was found to be the only species infesting *L. erythropterus* with prevalence 81.4% and a mean intensity of 5.6 ± 4.4. The significant difference between the prevalence of site infestation of the body and inner operculum sites was found when the data tested statistically. The prevalence of *C. rotundigenitalis* was highest on the operculum of the fish followed by the body and head. There was no significant difference in the distribution of *C. rotundigenitalis* over the different infestation sites derived from the 3 groups of different size. The present study is important for preventive measures to control the ectoparasitic copepod infection to benefit farmers to avoid outbreak in their cages.

OP - I6

First molluscan theta class glutathione S-transferase: identification, cloning, characterization and transcriptional analysis post immune challenges

Kasthuri Saranya Revathy[1], *Navaneethaiyer Umasuthan*[1] *and Jehee Lee*[1,2]

[1]Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Special Self-Governing Province 690-756, Republic of Korea
[2]Marine and Environmental Institute, Jeju National University, Jeju Special Self-Governing Province 690-814, Republic of Korea
Email: saranyarevathi@gmail.com

Glutathione S-Transferases (GSTs) are multifunctional cytosolic isoenzymes, distinctly known as phase II detoxification enzymes. GSTs play a significant role in cellular defense against toxicity and have been identified in nearly all organisms studied to date, from bacteria to mammals. In this study, we have identified a full-length cDNA of the theta class GST from *Ruditapes philippinarum* (RpGSTθ), an important commercial edible molluscan species. RpGSTθ was cloned and the recombinant protein expressed in order to study its biochemical characteristics and determine its physiological activities. The cDNA comprised an ORF of 693 bp, encoding 231 amino acids with a predicted molecular mass of 27 kDa and an isoelectric point of 8.2. Sequence analysis revealed that RpGST? possessed characteristic conserved domains of the GST_N family, Class Theta subfamily (PSSM: cd03050) and GST_C_family Super family (PSSM: cl02776). Phylogenetic analysis showed that RpGSTθ grouped with other theta class homologues. By quantitative PCR, RpGSTθ was found to be ubiquitously expressed in all tissues examined, with the highest levels occurring in gills, mantle, and hemocytes. Since GSTs may act as detoxification enzymes to mediate immune defense, the effects of lipopolysaccharide endotoxin and intact *Vibrio tapetis* bacterial challenge on RpGSTθ gene transcription was studied. Furthermore, the RpGSTθ expression changes induced by immune challenges were similar to those of the antioxidant defense enzyme manganese superoxide dismutase (RpMnSOD). To our knowledge, RpGSTθ is the first molluscan theta class GST reported, and its immune-related role in manila clam may provide insights into potential therapeutic targets for protecting this important aquaculture species.

OP - I7

Resurgence of epizootic ulcerative syndrome (EUS) in India

P.K. Pradhan, *Gaurav Rathore, Neeraj Sood, Manoj Yadav and Peyush Punia*

National Bureau of Fish Genetic Resources , Canal Ring Road, Lucknow, India
Email: pradhanpk1@gmail.com

In the year 2010-11, consequent to the report of heavy fish mortality, twenty fish farms in different parts of Uttar Pradesh were surveyed. During the survey, large scale mortality ranging from 20 to 50% of the total stock was observed in almost all the fish farms. Based on the information collected through discussions with the farmers, it was estimated that the direct economic losses due to epizootic ulcerative syndrome outbreaks was approximately rupees 10,000/- to 20,000/- per hectare. A total of 97 samples representing 12 different species were collected and through silver staining, fungal hyphae of *A. invadans* were observed in 95.8% of the samples (N=93). Using PCR technique, *A. invadans* was detected in 11 out of total 12 species. The sequence analysis of PCR product revealed 100% homology with the *A. invadans* sequences in GeneBank. *A. invadans* was successfully isolated from the ulcerated Mrigal and Puntius and the disease was successfully reproduced under laboratory conditions. Even with injection of 10 zoospores of *A. invadans,* 100% mortality was observed in the experimentally infected fishes. These observations are against the general perception that EUS incidence has declined and no longer a problem in aquaculture farms in the country.

OP - J1

Identification of fish vaccine antigen candidates - finding needles in a haystack

Alexandra Adams and *Kim D Thompson*

Institute of Aquaculture, School of Natural Sciences, University of Stirling, Scotland, UK
E-mail: alexandra.adams@stir.ac.uk

Vaccination has been proven to be effective in preventing fish disease and in reducing the use of antibiotics. The number of fish vaccines commercially available have grown in recent years but there are still numerous diseases for which no vaccines are available, or cases where existing vaccines do not perform well. The most crucial step in developing an effective vaccine is identification of the protective antigens. This is not an easy task, like 'finding a needle in a haystack', and the most effective approach taken depends on the type of pathogen (e.g. virus or bacteria, extracellular or intracellular bacteria) and the final end use envisaged for the vaccine (e.g. cost, fish species, immersion versus injection). Fish vaccines have in general become much more sophisticated in recent years. Technologies such as recombinant and DNA vaccines are powerful tools for vaccine development as these enable the separation of potential protective antigens from suppressive ones. These are being developed because the simpler approach of using inactivated whole cell vaccines has been unsuccessful for many important diseases, and attempts at attenuated vaccines in general have not been encouraged from a safety point of view. This paper will focus on the technologies that can be used to identify antigens for vaccine development, giving specific examples of *in vitro* methods used to identify isolates from a given serotype to include in whole cell vaccines and describing technologies for the identification of specific antigens for recombinant or peptide vaccines. These include *in vivo* expression technology (IVET) and immunoproteomics for bacterial vaccines, epitope mapping for viral vaccines and third generation vaccines using reverse genetics. Such methods are broadly applicable for the development of fish vaccines.

OP - J2
Novel vaccine technologies - also for farmed fish (?)

Øystein Evensen

Norwegian School of Veterinary Science, PO Box 8146 Dep, N-0033, Oslo, Norway
Email: oystein.evensen@nvh.no

Vaccination of farmed fish has become an increasingly important tool in disease control and has resulted in a reduced consumption of antibiotics in salmon production, as one example. The majority of the vaccines used are inactivated (non-replicating), whole cell preparations, usually formulated with some type of adjuvant that potentiates the immune responses and prolong the duration of protection through slow release of antigen from the injection site (depot-effect). Slow-release formulations are usually water-in-oil emulsion that will allow integration of water droplets into a continuous oil phase. Such formulations are dependent on the use of stabilizer and emulgators and will induce some degree of inflammatory reaction at the injection site, for salmon the peritoneal cavity, with secondary effects like growth retardation and unwanted immune responses (autoimmunity). The vaccines that have proven efficacious in the market are against bacterial diseases, while viral vaccines have not conferred the same level of immunity.

On this basis, there is need for new and less reactogenic vaccine preparations/ formulations that maintain the immunogenicity profile of the vaccines and at the same time confer long lasting immunity, particularly since prime-boost strategies are usually not applicable in aquaculture. There is also need for more efficacious viral vaccines, designed to induce T-cytotoxic responses. These issues pose challenge to the scientific community and to the vaccine industry, and aspects of reactogenicity, immunogenicity and duration of immunity would have to go hand-in-hand when new concepts and modalities of antigen delivery are developed. In this presentation I will draw up some possible trends that hold promise for vaccination of fish, including new formulations based on nano- or microparticle-based formulations for cytosol-based delivery, novel adjuvants with immunomodulating potentials that direct the immune responses towards T-cell polarity, use of reverse genetics methods for design of live, attenuated virus vaccines as well as molecular methods used to design live, attenuated bacterial vaccines. Last but not least, I will discuss the use of plasmid-based vaccines, and their potentials and limitations. Assessment of host immune responses will be briefly discussed towards the end of the talk.

OP - J3

Field trial study on vaccine ALPHA JECT® Panga 1 against *Edwardsiella ictaluri*, in pangasius catfish (*Pangasianodon hypophthalmus*)

***Tu Thanh Dung*[1],*Vo Thanh Tung*[2],*Pham Cong Thanh*[2], *Nguyen Thi Thu Hang*[1] *and Kjersti Gravningen*[2]**

[1] College of Aquaculture and Fisheries, Cantho University, Vietnam
[2] PHARMAQ Vietnam Ltd,
Email: ttdung@ctu.edu.vn

The purpose of the present study was to assess the safety and efficacy of vaccine ALPHA JECT Panga 1 against *Edwardsiella ictaluri*. Field trials were located on 3 commercial pangasius catfish (*Pangasianodon hyopophthalmus*) farms in three provinces in the Mekong Delta, Vietnam. The average fish body weight at vaccination ranged from 28 to 58g. The vaccine was injected intraperitoneally to fish with the dose 0.05ml/fish. Totally, there were 360,991 vaccinated fingerlings and 358,636 non injected fish (control). All groups were observed for 170 days post vaccination. Mortality, diagnosis, temperature and oxygen were recorded daily. Antibodies and weight were evaluated at day 10, 20, 30, 40, 50, 60, 80, 110, 140 and 170. Injection site reactions were evaluated at 60, 110 and 170 days. Vaccinated groups and control groups were compared.

During the study, natural outbreak of *E. ictaluri* was observed in two provinces. The *E. ictaluri* specific RPS during outbreak reached 65 in one province and 50 in the other. In all 3 farms, the vaccine induced antibodies against *E. ictaluri* reached high levels already at day 10 post vaccination, and remained significantly higher than control groups (at P<0,001) during the observation period of 170 days. After vaccination no abnormal mortality or behaviour was observed. There was no significant difference in growth rate between groups. When the fish were observed for any possible side-effects (day 60, 110 and 170), we did not observe any melanin in the fish at the injection site or any adhesions between the abdominal wall and the viscera.

In conclusion, the vaccine ALPHA JECT Panga 1 protects against *E. ictaluri* in *P. hypothalmus*, the onset of immunity based on antibodies is 10 days and the duration of protection is at least 170 days. The vaccine ALPHA JECT Panga 1 is safe and efficacious for use as an injection vaccine to *P. hypophthalmus.*

OP - J4

Streptococcosis in fish: implications for vaccine development

Saravanane Poobalane, *Lee Yeng Sheng, Shi Hui, Neil Wendover, Chang Siow Foong and Luc Grisez*

Intervet Norbio Singapore Pte Ltd, 1 Perahu Road, Singapore - 718847
Email: info.aquaINS@merck.com

Streptococcosis is a devastating disease which causes severe losses in the aquauclture industry. It has a complex aetiology as there are different types of *Streptococcus* spp. causing disease in different fish species in both marine and fresh water environment. Epidemiological surveys have further revealed that different species of *Streptococcus* occur in geographically distinct regions.

Streptococcosis caused by *Streptococcus iniae* and *S. agalactiae* commonly occur in seabass and Tilapia respectively. *S. iniae* is a globally relevant pathogen and most commonly associated with the marine environment, causing chronic mortality in grow-out stock. *S. agalactiae* is a fresh water pathogen which can be classified into two distinct biotypes (biotype I & II). *S. agalactiae* biotype I is limited to Asia and causes acute mortality peaks often associated with higher temperatures; whereas biotype II is considered a significant pathogen present in both Asian and Latin American countries.

The significance of these distinct streptococcal clusters are of fundamental importance during vaccine develpoment, as there is no cross protection between species and biotypes. Furthermore, extensive analysis of the bacteria revealed two serotypes within *S. agalactiae* biotype I which showed no cross protection against each other.

MSD Animal Health has developed effective vaccines which have been proven to confer protection against streptococosis. The application of these vaccines in this region shows that vaccination is a practical and cost effective disease control tool in Asian aquaculture.

OP - J5

Establishment of the technological platform for the development of rapid detection kits of aquatic animal pathogens

Qing-Li Zhang, *Jie Huang, Mei-Xia Li, Ying-Bin Du, Qing-Tao Wang, Bing Yang, and Xiao-Ling Song*

Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao, Shandong 266071, China
Email: zhangql@ysfri.ac.cn

Infectious aquatic animal diseases cause heavy losses in world aquaculture every year. Rapid detection kits for pathogens is regarded as one of the most efficient tool for the early-warning, diagnosis, and prevention of aquatic animal diseases. A technological platform for the development of rapid detection kits of aquatic animal pathogens was established in this study. The technological platform integrates the method of sample transportation under normal temperature, the method of field minute preparation of nucleic acid samples, contamination control measures by built-in nucleic acid dye, isothermal amplification methods for nucleic acid of aquatic animal pathogens and reagent preservation technology for transportation under normal temperature. Based on the technological platform, the detection process from sample preparation till finishing the detection can be finished in one hour in the field and requires only a simple heating device for incubation. The rapid detection kits for 16 pathogens, including White spot syndrome virus (WSSV), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Taura syndrome virus (TSV), Infectious myonecrosis virus (IMNV), Yellow head virus (YHV), Monodon-type baculovirus (MBV), *Baculovirus penis* (BP), *Hepatopancreatic parvovirus* (HPV), Necrotising hepatopancreatitis bacteria (NHPB), *Macrobrachium nodavirus* (MrNV), Penaeus vannamei nodavirus (PvNV), *Spiroplasma sp*., Turbot reddish body iridovirus (TRBIV), Spring viraemia of carp virus (SVCV), *Edwardsiella sp.*, Acute viral necrosis virus of scallop (AVNV), were developed using this technological platform. The technological platform has great potential for the development of rapid detection kits for more aquatic animal pathogens for early-warning of aquatic animal diseases.

OP - J6

Detecting WSSV and IMNV by using insulated isothermal polymerase chain reaction (iiPCR)

Chen Su, *Pin-Hsing Chou, Ping-Hua Teng and Te-Yu Chung*

GeneReach Biotechnology Corporation, No. 19, Keyuan 2nd Rd. Central Taichung Science Park, Taichung, Taiwan 407
Email: sales@genereach.com

Insulated isothermal polymerase chain reaction (iiPCR) is a new method for nucleic acid amplification. The target gene can be amplified in the specially designed reaction tube heated from the tube bottom by constant high temperature under the insulated environment without using traditional PCR thermal cycler. Instead of repetitive heating and cooling the reagent, a temperature gradient in the reaction tube drives thermal convection. According to the convection, the reaction liquid circulates between hot and cool regions of the tube. The temperature gradient covers the temperature that PCR needs and which is for denature, annealing and elongation. PCR is carried on as long as the temperature gradient is kept. Moreover, we also found that the temperature and convection of the liquid in the tube can be controlled by heating temperature and a reverse transcription (RT) can be introduced prior the PCR procedure. Based on our discovery, we have tested it for amplifying the target genes of WSSV and IMNV those are mostly concerned viruses in shrimp farming industry. The results of gel electrophoresis showed the detection sensitivity of WSSV and IMNV is 10 copies per reaction.

In order to reduce the detection time and avoid the chance of contamination during electrophoresis steps, we designed specific primer sets of virus and shrimp housekeeping gene (β-actin gene) and their specific TaqMan probes labeled by FAM and JOE respectively. The results can be detected and identified by a combination of different wave length filters. According to this, multiplex fluorescence detection can be integrated with iiPCR and as a closed qualitative detection system. The advantages of iiPCR include high sensitivity and low cost of the required device. It has high potential as an alternative for on-site PCR detection for shrimp virus diseases.

OP - J7

A comparison of the effect of live and dead *Aeromonas hydrophila* on the immune response of *Pangasius hypophthalmus*

Wanna Sirimanapong[1,2,] *Kan Kledmanee*[2], *Prawporn Thaijongrak*[2], *Kim D. Thompson*[1] , *Bertrand Collet*[3,] *Ei Lin Ooi*[4] *and Alexandra Adams*[1]

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom.
[2]Faculty of Veterinary Sciences, Mahidol University, Salaya campus, Nakornpathom, Thailand 73170.
[3]Marine Scotland-Science, Scottish Government, Marine Laboratory, Aberdeen, AB11 9DB, Scotland, United Kingdom.
[4]Novus Aqua Research Center, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
Email: ws7@stir.ac.uk

The immune system of pangasius catfish has not been fully described despite the recent growth in aquaculture for this species. Motile *Aeromonas septicaemia* (MAS) caused by *Aeromonas hydrophila* is reported to be one of the main diseases affecting *Pangasius hypopthalmus* the major species cultured in Vietnam. It is well known that in general live bacteria induce different immune responses than dead bacteria. Thus, samples collected from fish challenged with live or dead *A. hydrophila* were used help elucidate the immune system of *P. hypopthalmus* and to determine differential immune responses. Four treatment groups of 40 fish per group (40-50g) consisting of an untreated control group, a group injected intraperitoneally with adjuvant (Montanide ISA 760 VG) only, a group injected with heat-killed *A. hydrophila* $1x10^9$ cfu/ml mixed with adjuvant and a group injected with a subclinical dose of live *A. hydrophila* $2.7x10^5$ cfu/ml were used in the study. Samples were collected 0, 1, 3, 7, 14 and 21 days post injection (d.p.i.) to assess the immune response of fish, and included total red and white blood cell (WBC) counts, blood haematocrit values, plasma peroxidase activity, total plasma Immunoglobulin M (IgM) concentrations, serum lysozyme activity, serum complement activity, phagocytic and respiratory burst activity of head kidney macrophages and specific antibody titre against *A. hydrophila* (determined by ELISA). The results indicated that challenge with both live and dead bacteria stimulated the immune response in *P. hypopthalmus* significantly above control groups with respect to specific antibody titre, lysozyme activity, phagocytosis and plasma peroxidase at 7 or/and 14 d.p.i. Moreover, on 21 d.p.i. complement activity, total IgM, specific antibody titre and lysozyme activity from both of live and dead *A. hydophila* challenge groups were significantly different to the control groups. Differential immune responses between live and dead bacterial challenges were also observed as only live *A. hydrophila* significantly stimulated WBC counts, phagocytosis and plasma peroxidase at 3 d.p.i. with the greatest increase in WBC counts noted at 21 d.p.i. and in phagocytosis at 14 d.p.i. By 21 d.p.i only the macrophages from fish challenged with dead *A. hydrophila* showed significantly stimulated respiratory burst activity. This study provides useful basic information on the immune response in pangasius catfish that can be applied to the health control of this speices.

OP - H1
Current state of fish diseases in Japan

Nobuaki Okamoto[1] *and Motohiko Sano*[2]

[1]President of Japanese Society of Fish Pathology, Tokyo University of Marine Science and Technology, Tokyo, Japan.
[2] Secretary-in-Chief of Japanese Society of Fish Pathology, National Research Institute of Fisheries, Yokohama, Japan.
Email: nokamoto@kaiyodai.ac.jp

With the increasing number of aquaculture species in Japan, outbreaks of diseases have increased. Some of the major diseases of the important aquaculture species in Japan are: streptococcicosis caused by *Lactococcus garvieae* and nocardiosis of yellowtail (*Seriola quinqueradiata*) and amberjack (*S. dumerili*), edwardsiellosis of flounder (Paralichthys olivaceus) and iridoviral disease of red sea bream (*Pagrus major*). A long history of aquaculture has resulted in numerous diseases in salmonids. Of these, IHN in rainbow trout and erythrocytic inclusion body syndrome (EIBS) in coho salmon are the most significant. In ayu (*Plecoglossus altivelis*), bacterial coldwater disease (BCWD) from *Flavobacterium psychrophilum* infection is frequently found. Various diseases of larvae and small juveniles occur in hatcheries. Of these, viral nervous necrosis (VNN), caused by betanodavirus infection, is a major obstacle in the production of many fish species.

Previously, fish disease problems have been limited to aquaculture facilities, where fish are reared in high density conditions. However, in recent years, diseases such as koi herpes virus disease (KHVD) and BCWD have spread and caused damage to wild fish populations.

In recent years in Japan, estimated losses of cultured fish from diseases have halved (i.e. to around 4-5%) after previously accounting for 10% of total production. Current vaccine usage against streptococcicosis caused by *L. garvieae*, which causes major losses in yellowtail aquaculture, has contributed a high proportion of the reduced losses. The loss of yellowtail production from infection was previously around 90 million dollars per year, but decreased to around 9 million dollars in 2005. This is compelling evidence that vaccine control is one of the most effective measures against disease in aquaculture. Vaccines have also been developed and licensed to treat vibriosis of salmonid fish and yellowtail, streptococcicosis caused by *Streptococcus iniae*, pseudotuberculosis caused by *Photobacterium damselae* subsp. *piscicida*, and red sea bream iridoviral disease. Vaccines have been increasingly used to treat cultured yellowtail since the late 1990's, and almost all individuals of cultured yellowtail in Japan are now vaccinated; the measures are becoming the "prophylaxis" for fish disease.

OP - H2

Arrangements for effective emergency aquatic animal disease response

Ingo Ernst

Aquatic Animal Health Program, Animal Health Programs Branch, Australian Government Department of Agriculture, Fisheries and Forestry, GPO Box 858, Canberra ACT 2601, Australia
Email: ingo.ernst@daff.gov.au

Effective arrangements for managing aquatic emergency animal diseases (EADs) requires a comprehensive set of elements aimed at minimising impacts on aquatic animal production, fisheries resources, ecosystems and trade. The arrangements should be developed with consideration of the particular system of government and the legislative responsibilities of government agencies.

EAD preparedness and response arrangements include elements that apply across all phases of an aquatic EAD response, including: EAD freedom (risk mitigation activities to preserve disease status and market access), "alert" (suspicion of an EAD), "incident definition" (investigation to determine if the incident relates to an EAD), "emergency response" (to attempt eradication of the EAD), and "recovery" (surveillance to prove freedom, resumption of trade, disease containment or mitigation).

Key elements of the "alert" and "incident definition" phases include detection (relying on disease awareness for recognition of suspected aquatic EADs), reporting (including legal requirements to report notifiable diseases and unusual mortality) and diagnostic and field investigation capability to confirm the presence of an aquatic EAD. Key elements of the "emergency response" phase include response facilities and personnel, arrangements for decision-making and technical advice in accordance with agreed contingency plans, and communication mechanisms. Where possible, harmonisation of approaches between animal and aquatic animal sectors should be pursued. Key elements of the "recovery" phase include surveillance capability to prove freedom (if eradicated) and research and development capability to support disease management and control (if not eradicated).

Cooperation between governments and industries is an important element of emergency disease response and can be formalised by agreements that define government and industry responsibilities across all phases of an EAD incident-including arrangements for cost-sharing.

OP - H3

Building capacity in aquatic animal health - Challenges, strategies & opportunities: Role and activities of the ASEM Aquaculture Platform in aquatic animal health

John Bostock[1] *and Patrick Sorgeloos*[2]

[1] Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK.
[2] Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier, 44, 9000 Ghent, Belgium
E-mail: j.c.bostock@stir.ac.uk

Disease remains a major challenge to profitable and sustainable aquaculture production across most species and systems. The global trade in seafood adds additional risk with respect to disease transfer and the spread of zoonosis. New disease problems are also emerging with the intensification of production. Governments and consumers have high expectations of Aquatic Animal Health Professionals to deliver effective and timely answers with limited funding, whilst industry are often buying whatever remedy is offered on the market rather than investing in research for longer-term solutions.

The challenge facing the Aquatic Animal Health Community is to increase the efficiency, effectiveness and impact of our work. The way to achieve that is no secret, and involves greater interdisciplinary collaboration, improved access to knowledge and learning, and reduction in unnecessary duplication of effort. The barriers to this are however substantial, from language, culture (institutional as well as social), geography and technology. Overcoming these require the active engagement of the whole community and a willingness to build bridges through concrete collaborations and knowledge sharing.

The ASEM aquaculture Platform is an initiative funded by the European Union to help build those bridges and promote collaboration and knowledge sharing between Europe and Asia. With respect to Aquatic Animal Health, the expectation is that management will improve, losses reduce and problems will be solved more quickly. Mechanisms are therefore being put in place to enhance knowledge sharing and help facilitate new collaborative initiatives. These involve both high-level actions to link existing networks, and efforts to foster new connections across the Aquatic Animal Health community. The opportunities for further development are discussed.

OP - H4

WSSV disease and socio- economic behavior of shrimp aquaculture industry in Sri Lanka

***C.D.A.M.P.A.Dissanayake*[1] , *V.D.C.N. Vithana*[2] *and U. Edirisingha*[3]**

[1] Post Graduate Institute of Agriculture, University of Peradeniya, Sri Lanka
[2] Department of Planning, Sri Lanka
[3] Department of Animal Science University of Peradeniya , Sri Lanka
E-mail: dissanayake.aruna@gmail.com

In 1984 shrimp culture has been started in Sri Lanka. With the high profit industry expanded around lagoons and Dutch canal in North Western Province. Total land extent is recorded as 8000Ha in North Western Province.

In 1996 it was recorded that WSSV shrimp disease ruined the industry. Two years later in 1998 and 2000, we recorded their maximum production and after that it reduced up to 30% from the highest recorded production. The main reason was the WSSV disease outbreak to production decreases and the sector is unable to recover from the condition and or cannot reach its highest recorded value. To prevent this condition, independent mechanisms such as PL screening, effluent water treatment, recycling system (close system), crop calendar and best management practices has been practiced by authorities and farmers in North western Province. The production results are a proof that these independent approaches are not effective to control WSSV disease condition in Sri Lanka.

Long term WSSV persistency has led to an increase in the risk factor in the shrimp industry in North Western Province. In case of WSSV, the land use pattern for aquaculture is changing rapidly. With the frequent WSSV disease risk, 6.6% of the total shrimp culture lands have been converted to salt production areas and still is progressing in high salinity areas of the province. The study found that 18% of the land area from the total didn't operate even once in the last 10 years. Aquaculture farms conversion to coconut planting also progressing and its percentage is now 3.5% of the total aquaculture land area. Aquaculture farms conversion to residential areas establishment of industries and wind power plants are the other shrimp farm land utilization in the area.

Multiple disease prevention system together with best aquaculture policy planning will be effective to minimize WSSV disease threat and increase shrimp aquaculture production in Sri Lanka.

OP - H5

Mycobacteriosis in ornamental fish tank in Iran, what mycobacterium sbsp. can be accused?

Shirin Akbari[1&4], Majid Nojavan[1], Amir Tuckmachi[2], Hooman Rahmati-holasoo[3], Keyvan Tadayon[4], Nafiseh Shakibamehr[4], Mohammad Mohammadtaheri[4] and ***Nader Mosavari[4]***

[1] Urmiah Azad University, Biology Department
[2] Urmiah Azad University, Pathobiology & Biotechnology Department, Artemia & Aquatic animal Institute
[3] Tehran University, Veterinary Medicine, Aquatic Animal Health Department
[4] Razi Vaccine & Serum Research Institute, Mycobacteriology (Tuberculosis) Department
Email: nmosavari@gmail.com; n.mosavari@rvsri.ir

Here we are reporting isolation of *Mycobacterium* sub sp. from clown loach fish and common carp in two fish tanks in Iran.

Head and tail from two newly dead clown fish and one common carp were individually ground, decontaminated with a cocktail of NaOH and N-acetyl L-cystein followed by culture on mycobacterium-specific culture media including glycerinated Lowenstein-Jenson, pyruvated Lowenstein-Jenson, MGIT®, mycobactin-supplemented Herold-egg and plain Herold-egg media. The same method was employed for bacterial culture of viscera from the three animals with the exception that NaOH was the exclusive decontaminant used. Cultured tubes were incubated at 25ºC for two weeks when microscopic slides were prepared for acid-fast and fluorochrome staining of the bacterial growth.

Characteristic mycobacterial bacilli were identified in light and fluorescent microscopy of all the three specimens. When genomic DNA from isolates was PCR-amplified against a genus-specific locus of mycobacteria located on the 16S rRNA gene, all isolates produced the expected 543 bp amplicon. While further work is ongoing to fully characterize and also genotype the isolates, presumptive diagnosis based on necropsy backed by microscopy and bacterial culture observations, confirm these fish have suffered from mycobacteriosis.

OP - H6

Current status and problems of Infectious Myonecrosis Virus (IMNV) disease in *Penaeus vannamei* Farming in Indonesia

Agus Sunarto[1] *and* ***Yuri Sutanto***[2]

[1] Fish Health Research Laboratory, Central Research Institute of Aquaculture, Ministry of Marine Affairs and Fisheries Jakarta 12540, Indonesia.
[2] Aquatic Animal Health Center, PT. Central Proteinaprima, Jakarta-Indonesia
Email: yuri.sutanto@cpp.co.id

Pacific white shrimp (*Penaeus vannamei*) was officially introduced into Indonesia in 2000. Since then *P. vannamei* has dominated Indonesian shrimp aquaculture and contributed about 60% of its production. Infectious myonecrosis virus (IMNV) was first reported in East Java in May 2006. Subsequent analysis of complete genome sequences revealed that the Indonesian IMNV shares 99.6% identity to that of Brazilian IMNV. The high identity of the genome sequences of IMNV from Brazil and Indonesia indicates that Indonesian IMNV may have originated from Brazil. This is another classic example of trans-boundary disease which occurred due to irresponsible movement of contaminated stocks for aquaculture.

Since 2006 the disease has spread to several neighboring provinces where *P. vannamei* is being cultured. The affected shrimp showed clinical signs of whitish necrotic muscle and reddened tail fan, similar to those reported for infectious myonecrosis (IMN) in Brazil. Diseased shrimps show histopathological changes including coagulative necrosis of striated muscle (myonecrosis), haemocytic infiltration and fibrosis. Mortality rate range from 40 to 60%. It is estimated that IMNV has caused direct economic losses of US$0.2-1 billion.

Since the end of 2008, IMNV spread had accelerated and now been confirmed in other major farming regions in Indonesia, and has caused major production loss in some of those regions.

Analysis of the RdRp sequence of IMNV isolates from the various regions showed the presence of single nucleotide insertion at nucleotide 7431 that distinguish the Brazillian and Indonesian isolate, as previously reported (Senapin et al, 2007). Therefore, the data indicates that the recent IMNV outbreak in Indonesia was originated from East Java. The RdRp sequence of all isolates show >99% homology to the RdRP sequence of Brazilian and 2007-East Java isolates.

POSTER PRESENTATION

PP - A1

First identification of *Flavobacterium columnare* infection in farmed striped catfish *Pangasianodon hypophthalmus*

***Tu Thanh Dung*[1], *Nguyen Thi Tien*[1], *Nguyen Anh Tuan*[1] *and Margaret Crumlish*[2]**

[1] College of Aquaculture and Fisheries, Cantho University, Vietnam
[2] Institute of Aquaculture, Stirling University, Stirling, FK9 4LA, Scotland. U.K.,
Email: ttdung@ctu.edu.vn

The bacterium *Flavobacterium columnare* was recovered and identified as the aetiological agent causing freshwater columnaris infection in farmed catfish *Pangasianodon hypophthalmus* fingerlings, suffering from high mortality rates within commercial hatchery ponds in Vietnam. The gross clinical signs were typical of columnare infected fish and included eroded tail fins, depigmented, necrotic areas on the body, yellow discolouration on the fins and necrotic gills. Histological examination found numerous Gram negative, filamentous, long bacteria present on the skin, muscle and gill tissues of affected fish. Bacterial recovery was performed from the skin, gill, kidney and spleen using selective PYES agar. The yellow pigmented bacteria isolated were identified as *F. columnare* using primary, secondary and molecular PCR methods. An experimental immersion challenge study was performed and fulfilled Kochs postulates. Furthermore the immersion challenge study provided LD50 values of 1.7×10^5 cfu per ml and 3.2×10^6 cfu per ml for strains FC-HN and FC-CT, respectively. To the best of our knowledge this is the first report of freshwater columnaris infection in *P. hypophthalmus*.

PP - A2

A study on the mortality resulting of bacterial contamination in Rainbow trout from Guilan Province

Faeed M, *Ghasemi, M Amini, M Ramezani B and Asgharnia M*

Inland water Aquaculture Research Institute ,P.O.BOX 66 . Anzali ,Iran
Email: m_faeed@yahoo.com

In 2010, we studied 40 specimen of infected fish and water in three fish farms in Guilan Province. In this research, different bacteria were isolated and identified. The isolated bacteria belonged to the families *Entrobacteriacea, Vibrionacea, Psudomonacea* and *Sterptococcacea.* The maximum and minimum rate of bacterial contamination were from family *Enterobacteriacea* (53.5%) and *Psudomonacea* (12%) respectively, and the highest and the lowest bacterial contamination of rainbow trout observed in family *Vibrionacea* (42.5 %) and *Psudomonacea* (2.5%). Rate of contamination of some bacteria in rainbow trout and water was significant ($P< 0.05$). *Aeromonas salmonicida* and *Streptococcus iniae* bacteria were dominant in diseased rainbow trout.

PP- A3

Prevalence, epidemiology and histopathology of tumour (Odontoma) in *Sphyraena obtusata*, south east coast of India

A. Gopalakrishnan

Assistant Professor, Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai 608 502, Tamil Nadu
E-mail: aquagopal@gmail.com

The barracuda were examined during January to December 2010 at three landing centers along the Bay of Bengal, south-east coast of India. The Barracuda is abundantly distributed along the East coasts of India. It is one of the major food fish species for humans in India and is marketed as fresh, dried, dried-salted, smoked and canned. Barracuda occupies a pelagic-neritic niche living at a depth range of 20-200 m.The fishes were examined at the three landing centers. The tumour bearing fishes were examined for lesions. Lesions in the barracuda were never found on the body surface. Instead, they were present only in and around the mouth specifically, around the teeth. Grossly, they were mostly red and extremely firm, varying in diameter from 33 to 80 mm. They usually extended into the mouth, often deforming the jaw to such an extent that the fish was unable to close its mouth properly. A single mouth neoplasm examined was comprised histopathologically of dense fibrous tissue that extended into the bony trabecular tissue of the jaw. Aside from local expansion of the tumours, there was no indication in either species of metastasis to internal organs, nor any other significant lesions. Radiography in the barracuda revealed that the tumour contained moderately dense bone that merged with the mandibular bone. The transmission electron microscopic (TEM) study showed the viral particle in the tissue section.

PP - A4

Virulence and susceptibility studies of *Vibrio* species isolated from cultured *P. monodon* affected by Monodon Slow Growth Syndrome(MSGS) and Loose Shell Syndrome (LSS)

***Jayasree L*[1], *Janakiram P*[2], *Geetha G.K*[2], *Sivaprasad B*[2] *and Veerendrakuma, M*[2]

[1] Central Marine Fisheries Research Institute, Karwar Research Centre, Karwar, Karnataka -581301
[2] Dept. of Marine Living Resources, Andhra University, Visakhapatnam - 530 003, A.P
E mail: lokasree@gmail.com

Vibrio spp. isolated from shrimp infected with Monodon Slow Growth Syndrome (MSGS) and Loose Shell Syndrome (LSS) were subjected to experimental infectivity studies. Out of eight isolates of *V. harveyi* identified from MSGS and LSS affected shrimps, *Vibrio harveyi* isolated from LSS affected shrimp was found to be the most virulent with LD_{50} value of 1 x 10^4 cfu/g when experimentally challenged to healthy juvenile shrimp. Whereas, the LD_{50} value of *V. alginolyticus* isolated from MSGS was 1 x 10^5 cfu/g. Bath challenge infections were given to the healthy post larvae revealed LD_{50} value of *V. harveyi* was 1 x 10^7 cfu/ml and 1 x 10^9 cfu/ml for *V. alginolyticus*. Antibiotic susceptibility studies for all the *Vibrio* isolated from MSGS and LSS affected shrimps exhibited sensitivity towards ciprofloxacin. The susceptibility of other species *Vibrio* varied with species as well as antibiotics.

PP - A5

Outbreak of betanodavirus in cage-cultured Asian seabass (*Lates calcarifer* Bloch): A case study

K.P. Jithendran[1]*, P.K. Sahoo*[2]*, C.P. Binesh*[1] *and B.R. Mohanty*[2]

[1]Central Institute of Brackishwater Aquaculture, Santhome High Road, R. A. Puram, Chennai - 600 028, India
[2]Central Institute of Freshwater Aquaculture , Kausalyaganga, Bhubaneswar, Orissa - 751 002, India
Email:kpjithendran@hotmail.com

Viral encephalopathy and retinopathy (VER), otherwise known as viral nervous necrosis (VNN) caused by betanodavirus, is a neuropathological condition affecting more than 40 fish species. Although VER affects mainly marine fish, the disease has also been detected in certain species reared in low-saline and freshwater environments. A betanodavirus associated massive mortality was investigated in juvenile seabass *Lates calcarifer*, maintained in cage culture facilities in brackishwater pond in Orissa (India). Histopathology revealed vacuolation of the nervous system, suggesting an infection by a betanodavirus. The presence of virus was detected by nested RT-PCR assay and confirmation by sequencing and analysis of PCR products. Sequencing of the T4 region of the coat protein gene indicated a phylogenetic clustering of this isolate within the red-spotted grouper nervous necrosis virus type. However, the seabass betanodavirus isolate from the study formed a unique branch distinct from other betanodavirus isolates. The reservoir of virus at the origin of the outbreak remains unidentified. This is the first report of mortality associated with natural infection of betanodavirus infection in seabass culture facilities in India.

PP -A6

MS marker distribution pattern in iridovirus infected rock bream, *Oplegnathus fasciatus*

Myung-Hwa Jung[1], *Jong-Oh Kim*[1], *Jehee Lee*[2], *Myung-Joo Oh*[1] *and Sung-Ju Jung*[1]

[1]Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Republic of Korea.

[2]Department of Aquatic Biomedical Sciences, Jeju National University, Jeju 690-756, Republic of Korea.

Email: gloomy8983@hanmail.net

Rock bream (*Oplegnathus fasciatus*) is a highly valued aquaculture species in Asia-Pacific region including Korea. However, due to the iridovirus infection in summer, aquaculture industry suffers a huge economic loss (Jung and Oh, 2000). Excessive hypertrophy of spleen and kidney of the virus infected rock bream leads to mass mortality. In this experiment, 90 fish (70-150g of 14-20cm) were injected with 500µl of iridovirus (10^4 $TCID_{50}$/ml) and maintained at 26-28^{0}C for 30 days. Fins from all dead fishes were preserved in 100% ethanol. The sampled fish, two groups were selected; first group which died early and the second, which died later. Genomic DNA was extracted from fins of 20 fish died earlier in the experiment and from 20 fish died later. The fish DNA was used for genotyping using MS marker (CA-03, CA-10, CA3-05, CA3-06, and CA3-36); among these CA3-05, CA3-06, and CA3-36 showed very good allele distribution for early and late died groups. In CA-03 and CA-10 marker, the allele distribution was not significantly different. For CA3-05 marker the allele distribution for the fishes succumbed early ranged from 156-167 base, with maximum allele distribution at 166 base and the range for the fish that died later was 154-165 base. For CA3-06 marker allele distribution range was from 154-171 base, with maximum distribution at 169 base in early died fishes, while the range was 150-171 base, with maximum allele distribution at 154 base later died fishes. For CA3-36 marker the allele distribution ranged from 158-174 base, with maximum allele distribution at 194 base, in the first group while was 158-194 base, with maximum allele distribution at 159 base in the second group. In-depth studies will provide valuable information in selection of stronger or resistant variety of the fish. Thus, from this study, using MS marker and genotyping, we could detect differences in two groups of rock bream, which are susceptible to iridovirus at varied infectious period stressing these markers will facilitate in selection of iridovirus resistant rock bream.

PP -A7

Korean and Japanese isolates of viral hemorrhagic septicemia virus from olive flounder are pathogenic to rainbow trout

Wi-Sik Kim[1], *Toyohiko Nishizawa*[2], *Jeong-Ho Kim*[3], *Rungkarn Suebsing*[3], *Sung-Ju Jung*[2], *and Myung-Joo Oh*[2]

[1]The Fisheries Science Institute, Chonnam National University, Yeosu 556-901, Korea
[2]Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Korea
[3]Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, Korea
Email: slaidcats@hanmail.net

Viral haemorrhagic septicaemia virus (VHSV) is an epidemic virus in olive flounder (*Paralichthys olivaceus*) farms in Korea and Japan, although VHSV was never detected in salmonids including rainbow trout (*Oncorhynchus mykiss*) until now. In the present study, the pathogenicities of Korean and Japanese isolates of VHSV from olive flounder were investigated with rainbow trout. The cumulative mortalities of fish challenged with FYeosu05 (Korean) and Obama25 (Japanese) isolates at $10^{6.5}$ $TCID_{50}$/fish were 64% and 48%, respectively. No mortality was observed in fish challenged with either of the isolates at $10^{5.5}$ $TCID_{50}$/fish, or in mock-challenged fish. The affected fish showed darkening of the body, expanded abdomen, pale gills, enlarged spleen and diffuse necrosis in splenic and interstitial hematopoietic tissues. From all the dead fish, VHSV was re-isolated by cell culture and detected by reverse transcription loop-mediated isothermal amplification (RT-LAMP). It was thus confirmed that Asian VHSV isolates from olive flounder are pathogenic to rainbow trout, although with low virulence.

PP-A8
Prevalence of vibrios in wild caught and cultured marine finfish of South West coast of India

***Muliya Sankappa Nithin**, M. N. Venugopal and Indrani Karunasagar*

Department of Fisheries Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore- 575 002, India
Email: ms.nithin@gmail.com

Vibrios are autochthonous flora of the aquatic environment and hence commonly associated with aquatic animals. Many species are capable of causing diseases in fish and shrimps while some also cause intestinal and extraintestinal infections in humans. The present work was aimed at studying the prevalence of vibrios in wild caught and cultured marine finfish of South West coast of India.

In this study, 102 samples were analysed of which 87 (85.29%) samples were positive for vibrios which included 65 of 74 (87.83%) finfish samples, 20 of 21 (95.23%) water samples and 2 of 7 (28.5%) sediment samples. The isolates were speciated as *Vibrio parahaemolyticus* which was the dominant species in finfish and water, followed by *V. cholerae, V. alginolyticus, V. fluvialis, V. damsela* and *V. mimicus. V. harveyi* and other environmental luminescent *vibrios of Harveyi clade* were also isolated. Molecular characterization by PCR was carried out for some of the commonly recognized pathogenic vibrios.

Though several *Vibrio* species are distributed in South West coast of India, *V. parahaemolyticus, V. harveyi, V. alginolyticus, V. damsela* and *harveyi clade* have been recorded as finfish pathogens. They can cause vibriosis in aquaculture settings. Hence, it is important to assess the risk associated with these *vibrios* in aquaculture. The need for preventing/reducing problems due to vibriosis in commercial aquaculture can be achieved by following good practices such as water quality management, vaccine/immunostimulant administration, probiotic/ bioremediator application etc.

PP -A9

Experimental challenge immersion studies of *Edwardsiella ictaluri* in striped catfish *Pangasianodon hypophthalmus* (Sauvage)

Nguyen Ngoc Phuoc, *Randolph Richards and Margaret Crumlish*

Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK.
Email: phuoc.nguyenngoc@stir.ac.uk

The bacterium *Edwardsiella ictaluri*, described as the causative agent of bacillary necrosis of *Pangasianodon* (BNP), continues to be a significant health threat to the sustainable development of the Vietnamese striped catfish industry. Previous experimental challenge studies on the Vietnamese striped catfish provided different doses and immersion time of *E.ictaluri.* In this study, an initial challenge or LD_{60} study was conducted, which provided information concerning doses and immersion times to be used in the challenge. All experiments were conducted using a bacterial strain of *E.ictaluri* (E136) that had been recovered from a natural outbreak of BNP in Vietnamese *Pangasianodon hypophthalmus*. The challenge study was performed using four treatments groups. All fish of the three first treatment groups received 1 x 10^7 cfu per ml with immersion times of 30 seconds, 1 minute, and 2 minutes for the treatment group 1, 2, and 3 respectively. Fish from the fourth group (sham-immersion control) were exposed to the same amount of saline solution with the longest time of immersion (2 minutes) which did not contain bacteria. Within 3 or 4 days post challenge, all fish from the first three treatment groups showed clinical signs commonly associated with *E.ictaluri* infection. The cumulative percentage of mortalities was the highest in the treatment of the longest time for immersion (2 minutes) (96.67%), while 80% mortality was presented in the treatment of immersion for 1 minute, and the lowest (63.33%) was observed in the treatment of immersed *E.ictaluri* for 30 seconds. The cumulative mortality percentage rose dramatically from day 5 to day 7 and gradually increased from day 8 to day 11 in all bacterial treatments. Fish were healthy, and no mortality occurred in the control group. The bacteria that were isolated from moribund and fresh dead fish with clinical signs of BNP were identified as *E. ictaluri.* This study provides a reliable challenge model for use in subsequent challenge studies.

PP -A10

Current status of Lancefield group *C Streptococcus dysgalactiae* infection in farmed fish

Issei Nishiki[1]*, Yasushi Horikiri*[2]*, Toshiaki Itami*[3] *and Terutoyo Yoshida*[3]

[1] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.
[2] Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.
[3] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.
Email: nb11004@student.miyazaki-u.ac.jp

Lancefield group *C Streptococcus dysgalactiae* subsp. *dysgalactiae* (GCSD) has been reported as a causative agent of mastitis in cattle, endocarditis in domestic animals, and cardiopulmonary diseases in humans. Since 2002, there has been a rise in the GCSD infection in the farmed fish *Seriola dumerili* and *S. quinqueradiata* in Japan. Several years have passed since the emergence of GCSD infection in fish farms. In this study, we report the molecular epidemiological information for GCSD isolates from fish. In addition, GCSD isolates obtained from fish were characterized by comparing fish isolates with mammalian isolates.

A total of 284 bacterial strains were collected from various fish farms between 2002 and 2008. Clinical isolates of GCSD were obtained from pigs diagnosed with endocarditis. Molecular epidemiological analyses of GCSD strains were performed using biased sinusoidal field gel electrophoresis (BSFGE). DNA-DNA hybridization and sequence analysis of three housekeeping genes (16S rDNA, 23S rDNA, and *hsp60*) were also performed to compare fish isolates with mammalian isolates. To discriminate between the fish and mammalian isolates, a primer set for PCR targeting *sof* gene was determined.

A total of 284 fish isolates collected in Japan were classified into 16 electrophoretic profiles. The dendrogram created in this study revealed that all 284 fish isolates obtained in Japan were closely related. On the other hand, the restriction patterns of mammalian isolates were different from those of fish isolates. The genetic analysis revealed that the fish isolates were genetically very similar to each other with high DNA-DNA relatedness (>95.4%) and sequence homology. Meanwhile, the DNA relatedness between mammalian isolates and the fish isolates was 73.4%-82.6%. These finding suggested that fish isolates were genetically close to each other and a clonal expansion occurred. The designed oligonucleotides targeting *sof* gene could discriminate the fish GCSD from mammalian isolates.

PP - A11
Histopathological manifestations of vibriosis in Asian seabass (*Lates calcarifer* Bloch)

S.R.Krupesha Sharma *and K.K.Philipose*

Central Marine Fisheries Research Institute (Indian Council of Agricultural Research), Karwar Research Centre, Karwar -581 301, Uttara Kannada, Karnataka, India
E mail: krupeshsharma@gmail.com

Asian seabass (*Lates calcarifer*) has been designated as a candidate species for open sea cage culture in India. Histopathological manifestations of a case of vibriosis in Asian seabass reared in open sea floating cages are reported. Haemorrhage and ulcer were observed grossly in the diseased fish. The consistent histological lesion seen in all the organs in both natural and experimental infection was haemorrhagic in nature. Congestion, haemorrhage and necrosis were observed in vital organs in naturally infected fish. Extensive vacuolation of hepatocytes and increased expression of melanomacrophage centres in kidney were consistent features in experimental infection.

PP -A12
Experimental *Streptococcus iniae* infection in barramundi (*Lates calcarifer*)

Hich Tran Vi *and Dzung Nguyen Huu*

Department of aquaculture, Nha Trang University, Vietnam
Email: tranhich@gmail.com

Streptococcus iniae is one of causative agents of streptococcosis in many fish. In Vietnam, it was only reported as the disease agent from an outbreak in tilapia. In this study, we describe the first isolation of *Streptococcus iniae* in barramundi cultured in Vietnam and confirmed the capability of it to cause disease in barramundi by intraperitoneal injection. The pathogen was found to have a LD_{50} of $10^{4.8}$ colony form units. Haemorrhages in or around the eye, base of the fin, anus or elsewhere on the body were observed in moribund fish in this experiment. The moribund fish also exhibit erratic swimming or pop-eye. In some cases, the fish may show haemorrhages in the liver. It is suspected that *Streptococcus iniae* was highly pathogenic for barramundi.

PP -A13
Emerging disease problems in Indian bivalve mariculture

***Sanil N.K**, Suja G, Lijo J, Asokan P.K and Vijayan K.K*

Fish Health Section, Marine Biotechnology Division, Central marine Fisheries Research Institute, P.B No. 1603, Ernakulam North P.O., Cochin - 682018, India.
Email: nksanil@gmail.com

The Indian sub continent has a very rich bivalve fauna including many commercially important species. Though bivalve aquaculture is gaining importance in India, information on the pathogens and diseases affecting them are lacking. During the past decade, culture of green mussel (*Perna viridis*) and edible oyster (*Crassostrea madrasensis*) showed a phenomenal increase along the south west coast, raising the production of 20,000 tons in 2010. Usually, the risks involved increase with the intensification of farming activities and in aquaculture systems, containing a disease outbreak is a difficult proposition, leaving preventive strategies as the only option.

World over, there have been many reports of mass mortalities in natural and farmed bivalves. The first report of *Perkinsus olseni,* an OIE listed pathogen from the pearl oyster, *Pinctada fucata* from the southeast coast of India came in 2010. Subsequent studies have revealed that 12 species of bivalves from the Indian subcontinent harbor *Perkinsus spp.* In the Indian waters, *Perkinsus* is represented by 2 species, *P. olseni* in *P. fucata* and *P. beihaiensis* in *C. madrasensis*. Considering the pathogenic potential of *Perkinsus sp.* and its ability to destroy bivalve populations, it has to be monitored on a regular basis. Though no mass mortalities have been reported so far, the presence of *Perkinsus* and its broad host range in the subcontinent is a disturbing factor for Indian bivalve mariculture. Similarly, preliminary studies have also indicated the presence of two other important molluscan pathogens, *Bonamia* and *Marteilia* in bivalves from the subcontinent which again is a cause of concern.

Specific diagnosis of most of the OIE listed diseases is difficult mainly due to sensitivity issues associated with subclinical/low level infections. Use of novel molecular biological tools in tandem with conventional diagnostics needs to be employed for the surveillance of these pathogens. As bivalve culture in the country is in its initial phase of expansion, there is an opportunity to put a scientific health management strategy in place, before any disease outbreaks happen.

PP -A14

Screening of wild caught and cultured marine finfish from west coast of India for presence of nodavirus and iridovirus

***Santhosh S**, Mahesh Shetty and Indrani Karunasagar*

Department of Fisheries Microbiology, UNESCO-MIRCEN for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore - 575 002, India.

Email: sanofish009@gmail.com

Production of marine fish in India has been initiated in recent years because of their economic value and to address food and nutritional security. Rapid expansion and intensification of aquaculture, however, has been accompanied by increased incidence of disease outbreaks. The morbidity and mortality caused by viruses in cultured fish population is very high with nodavirus and iridovirus infection occurring frequently. Hence, the present study was carried out to understand the prevalence of nodavirus and iridovirus in cultured and wild caught marine finfish from west coast of India.

Of 43 wild caught and cultured fish samples analyzed for nodavirus by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers, 17 were positive. All the 3 organs viz. brain, retina and kidney samples taken from the individual fish were positive for the virus. The RT-PCR amplification of the T4 region of the coat protein gene (by T4NV-F/R primers) gave a PCR product of 426 bp. The RT-PCR analysis of another set of primer (LCNV-F/R) gave an amplicon of 294 bp in all 17 individual fish samples. The risk associated with nodavirus outbreaks are seen from the results of the study.

The study also included 44 samples of both cultured and wild caught fish for iridovirus. DNA extracted from the sampled tissues was subjected to single step PCR using Major capsid protein and ATPase gene primers. None of the 44 samples was positive for lymphocystis iridovirus. The present study indicates the absence of lymphocystis disease in samples from west coast of India when PCR amplification of MCP and ATPase genes were carried out.

PP - A15

Aeromonas spp. as aetiological agents of diseases in freshwater ornamental fishes: characterization, pathogenic potential and antibiotic susceptibility pattern

K.Sreedharan[1]*, Rosamma Philip*[2] *and I.S.Bright Singh*[1]

[1]National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Fine Arts Avenue, Cochin-682 016, Kerala, India

[2]Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin-682 016, Kerala, India

E-mail: shreedin77@gmail.com

Aeromonas spp. are ubiquitous aquatic organisms, and has recently been considered emergent human pathogens, causing multitude of diseases in many species of animals, including fishes and humans. As part of disease surveillance programme across the state of Kerala, we met with several instances of disease outbreaks in ornamental fishes. Majority of these fishes exhibited clinical signs such as haemorrhagic septicemia, dropsy and tail rot and fin rot, from which pure cultures of *Aeromonas spp*. could be recovered. All the isolates under study were phenotypically characterized, and identified as *A. aquariourum* (from gold fish and carp), *A. jandaei* (from *Puntius denisonii*) and *A.veronii* (from Oscar, gouramy and goldfish) by sequencing 16S rRNA gene and gyrB. They produced potential hydrolytic enzymes, haemolytic activity and cytotoxicity in varying proportions. The virulence potential of the isolates were also assessed by PCR screening of virulent genes such as enterotoxins (act, alt and ast), haemolytic toxins (hlyA and aerA), genes involved in type 3 secretion system (ascV, aexT, aopP, aopO, ascF-ascG, and aopH), and glycerophospholipid-cholesterol acyltransferase (gcat). The challenge study and LD_{50} values further support the virulence of the isolates, and the organism could be subsequently recovered from the lesion as well as from the internal organs. In addition to the clinical isolates, we have also isolated and characterized the *Aeromonas spp*. from source water. In general, the pathogenic potential of clinical isolates was substantially higher than the environmental isolates. On screening the isolates against commercially available antibiotics, multiple antibiotic resistances could be seen widely distributed.

The data generated suggested that the ornamental fresh water fishes are always under the threat of an *Aeromonas* infection because, they live in an environment with a normal flora of *Aeromonas* equipped with at least a couple of virulent genes having the capability of their expression in moments of stress. Moreover, the increase in antimicrobial resistance poses a growing challenge in the treatment of *Aeromonas* infections. If such antibiotic resistant aeromonads, which are true human and aquatic pathogens, are able to multiply within fresh water ornamental fish culture systems, they obviously may cause problems to public health.

*Presenting Author

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PP - A16

Disease Problems Causing Mortality in Floating Net-Cages of Striped Catfish (*Pangasius* spp.) in Sg. Pahang, Temerloh

Siti-Zahrah A [1], *Zulkafli A.R*[2], *Zamri-Saad M* [3], *Amal A.M.N* [3], *Nur-Nazifah M* [3], *Firdaus-Nawi M* [3], *Ramley B*[2], *Fahmi S* [1], *Aziel S* [1], *Iftikar A.R* [1], *Oo M.G* [1] *and Shahidan H* [1]

[1]National Fish Health Research Center (NaFisH), Fisheries Research Institute (FRI), 11960 Batu Maung, Penang, Malaysia.
[2]Freshwater Fisheries Research Center (FFRC), Fisheries Research Institute (FRI), 71650 Jelebu, Negeri Sembilan, Malaysia.
[3]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, UPM, Serdang, Selangor, Malaysia.
Email: siti.zahrah.abd@gmail.com

A study was conducted in Sg. Pahang after receiving reports of fish mortality. Five sites were chosen before fish with clinical signs and water were monthly sampled for a period of 25 months to determine the pathogens and prominent tissue changes. Examinations of the affected fish revealed reddish skin inflammation throughout body especially the basal areas of dorsal, anal and ventral fins, occasionally with fin rots. Inflammations were also observed on skin of both mandibles and pectoral regions. Bacterial isolation revealed presence of both Gram positive and Gram negative bacteria, particularly *Staphylococcus spp*(28.7%), *Micrococcus* spp (23.4%), *Aeromonas hydrophila* (22.8%) and *Plesiomonas shigelloides* (18.6%). *Aeromonas hydrophila* was isolated from all sites. Each sampling site showed different correlations between bacterial presence and water quality parameters indicating their variability in a flowing water body. Histological examinations revealed necrosis and hemorrhages of the kidneys and liver, while the spleen was congested. There was focal encephalitis with infiltration of mononuclear cells, particularly around the ventricle and blood vessels.Polymerase chain reaction (PCR) revealed the presence of channel catfish virus (CCV). Since CCV does not usually exhibit specific clinical signs like *Aeromonas*, its presence could be secondary.

PP-A17

Diseases of cultured mulloway (*Argyrosomus japonicus*) in Western Australia

Dina Zilberg[1]*, B. Jones*[2]*, P. Nichols*[3]*, Gibson-Kueh S*[3] *and F. Stephens*[2]

[1] French Associates Institute for Agriculture and Biotechnology of Drylands, Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel.
[2] Animal Health Laboratory, Department of Fisheries, Government of Western Australia, South Perth, Australia.
[3] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Australia.
Email: dzilberg@bgu.ac.il

Mulloway (*Argyrosomus japonicus*) is an estuarine and costal water fish distributed through the Indian and Western Pacific Oceans and is an emerging aquaculture species. Mulloway culture history in Australia is of about nine years. In Western Australia (WA), mulloway culture had started in 2008, in a seacage farm offshore Geraldton. Fish health was closely monitored at the Department of Fisheries, WA during early production stages at Chellenger TAFE (Fremantle, WA) and subsequently routine diagnosis of monthly submitted fish by the farm.

Juvenile mulloway from TAFE were susceptible to ectoparasitic protozoans and to *Uronema sp.,* systemic and external infection. Broodstock in recirculating tanks were diagnosed with fluke infections. The main findings in farm-reared fish included systemic bacterial infections by *Vibrio harveyi.* and *Photobacterium damselae* monogenean parasites were repeatedly diagnosed, most commonly gill flukes, including the blood-feeding *polypistocotelean*, morphologically similar to *Sciaenacotyle sciaenicola* and monopisthocotylean resembling *Calceostoma glandulosom*; but also the capsalid skin fluke *Benedinia scianiae* (monopisthocotylean).

Shortly after stocking in the seacages, an encysted parasite of unknown nature was identified, located in the kidneys of all fish. Histologically, granolomatous lesions were present mainly in the kidney. Inside the granuloma was an aggregate of round shaped organisms, with a diameter of 4.2-5.4 μm. The parasite did not have the pronounced staining with Giemsa that is characteristic of myxosoan parasites but did positively stain with Grocott (a silver stain). Electron microscopy analysis revealed that the enclosed organism was composed of 2 cells, a peripheral cell surrounding a central cell and contained a primitive mitochondria.

Apart from the localized kidney pathology, the fish did not appear to be affected by the parasite, although reduced growth rate and elevated susceptibility to other infections is likely to have occurred. Treatments were applied to control skin flukes. The gill fluke did not require treatment.

PP - A18
Diseases of Marine Cultured Fish in Kuwait

Ahmed Al-Marzouk, *Azad Saheb and Hashem Al-Gharabally*

Mariculture and Fisheries Department, Kuwait Institute for Scientific Research, P.O. Box 1638, Salmiya, 22017, Kuwait.
Email: ABOHASSN58@GMAIL.COM

The Department of Mariculture and Fisheries (MFD), at the Kuwait Institute for Scientific Research (KISR) is undertaking research into larvae rearing technologies for a range of marine finfish. They are the blue-fin bream *Sparidentex hasta* (sobaity), orange-spotted grouper *Epinephelus coioides* (hamoor), yellow-fin bream *Acanthopagrus latus* (sheam) and silver pomfret *Pampus argenteus* (zobaidy). Between 2005 and 2011, they encountered different parasitic, bacterial and viral diseases. This paper presents a synopsis of diseases seen in these species.

Disease outbreaks due to two ciliated protozoan *Cryptocaryon irritants* and *Uronema sp.*, and one flagellated protozoan *Amyloodinium ocellatum* were recorded. *Uronema sp.* was found infesting only cultured zobaidy adults and sub-adults, whereas the *C. irritans* and *A. ocellatum* was found infesting the other cultured species. The incidences of *Uronema sp.* was associated with a raise in the water temperature, increased bacterial load and the sensitivity of the fish species. The skin monogeneans detected were the capsalid monogenean *Tarrenia acanthopagri* that infest sobaity and sheam, while the *Megalocotyloides epinepheli* was found specifically infesting the grouper. Gill monogeneans were the microcotylid *Polylabris angifer* that infests only the breams, while *Lamellodiscus acanthopagri* infests specifically the yellow bream and the *Diplectanum microphallus* infests specifically the grouper. Crustacean parasite *Caligus anntenatus* was found infecting sheam causing high mortality among the grow-out fish.

Bacterial diseases caused mainly by Vibrio species (*V. anguillarum, V. algionlyticus, V.harveyi* and *Proteus vulgaris*) and *Streptococcus agalactiae.* The virus detected was the viral nervous necrosis (VNN) that causes severe mortalities among the grouper of different stages.

PP-A19

Study on natural reservoirs of shrimp pathogenic viruses

***S. A. Mohan.**, M. N. Venugopal and Indrani Karunasagar*

Department of Fisheries Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575 002, India.

E mail I D: saminu@gmail.com

Among infectious diseases of cultured shrimp, certain viral diseases are highly significant due to the loss they cause to the shrimp industry. The study was carried out to understand the incidence and prevalence of DNA viruses such as White spot syndrome virus, Hepatopancreatic parvovirus, Monodon baculovirus and Infectious hypodermal and hematopoietic necrosis virus in cultured as well as in wild caught crustaceans. First step and nested PCR was performed to study the presence of viruses in several samples associated with shrimp culture and hatchery environment. The role of bivalves that are sometimes used as feed in ponds and crabs that are used in hatcheries as feed for brooders were also analyzed. Among cultured shrimps, *Penaeus monodon* (adults and postlarvae) and *P. vannamei* were found to be infected with multiple viruses. Multiple viral infection in *P. vannamei* is being reported for the first time from India. Wild caught shrimps such as *Seranopsis longiceps*, *Solenocera choprai, Metapenaeus brevicornis & P. canaliculatus* were found positive for multiple infection and other wild shrimps such as *Aristeus alcocki* and *P. fissuroides* were negative for all the viruses. Crabs such as *Charybdis hoplites* and *Portunus pelagicus* were found to be infected with multiple viruses. *C. riversandersoni* was found positive for WSSV and this is the first report of the presence of the virus in this species. Wild *Macrobrachium rosenbergii* harboured three viruses as seen from the positive PCR results. Bivalaves such as clam (*Meretrix casta*) and mussel (*Perna viridis*) were also found to be passive carriers of shrimp viruses. Aquatic insects such as Gerries, Nepa, Notonecta, Ranatra and Dragon nymphfly were found to be positive for WSSV. The presence of viruses in bivalves as passive carriers and insects of euphridae family studied is being reported for the first time.

PP- A20

Mixed infection of Wild Mullet Mugil cephalus with two type of Myxosporean

Min-Seok Jang, *Wi-Sik Kim, Toyohiko Nishizawa, Myung-Joo Oh**

Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Republic of Korea.

Email: ohmj@chonnam.ac.kr

Mullet *Mugil cephalus* is a commercially important species common in coastal waters and estuaries in the temperate zone, subtropics and tropics. It is mainly consumed as sliced raw fish in Korea, and as dried salted fish roe in several other countries, including Taiwan, Japan and the southeastern United States. Mullet is known to be infected with several *Myxobolus.* These *Myxobolus* species mainly cause systemic infection, gill and skin lesions in their hosts, resulting in debilitation and commercial rejection. In 2009, many wild mullet with white cystic masses on their scales were found in Yeosu on the south coast of Korea. Infected fish have no commercial value, though they do not differ in behavior from normal fish. In the present study, we investigated the morphological and molecular characteristics of the myxosporean and histopathology of the wild mullet. Cyst-like plasmodia consisted of a large number of mature myxosporean spores and numerous sporogonic stages. Spores were oval-shaped in their front view, tapering anteriorly to a blunt apex, and lenticular in their lateral view, bearing several distinct triangular markings on the sutural edge. Cysts consisted of a large number of myxosporean trophozoites with mature spores and spoloblasts. Spores from scale and intestine were measured 7.0 μm (6.2-7.6) and 11.1 μm (10.0-11.9) in length and 5.2 μm (4.0-6.2) and 8.7 μm (7.3-10.1) in width, with polar capsules of 3.5 μm (2.5-4.5) and 3.7 μm (2.5-4.5) in length and 2.0 μm (1.6-2.3) and in 2.2 μm (1.8-2.9) in width, respectively. In histopathological examination, spores were observed not only in the plasmodia on the scales, but also in intestine, pancreas, heart, kidney, stomach, gill, skin, spleen and liver. Nucleotide sequences of the 18S rRNA gene of the myxosporean parasites from scale and intestine showed 99.8% homology with *Myxobolus episquamalis* and 94.5% homology with *Myxobolus spinacurvatura* from mullet, respectively. These results indicate that wild mullets were least infected with *M. episquamalis* and *Myxobolus spinacurvatura* as a mixed infection.

PP- B1

Study on the effect of neeem oil *(Azdirachta indica)* on growth parameters, immune response and sexual maturity of red tilapia; *(Oreochromis niloticus x Oreochromis mossambicus)*

Azri, B.Y[1] *and* ***Abdelhadi, Y.M.***[1,2]

[1]Aquaculture Dept., Faculty of Agriculture, University Putra Malaysia (UPM) 43400, Serdang, Selangor, Malaysia.
[2]The Central Laboratory for Aquaculture Research (CLAR), Abbassa 44662, Abou-Hammad, Sharkia, Egypt.
E.mail address: ymhadi@yahoo.com

Neem oil was used as a feed additive in the rates of 0.5, 1 and 1.5% with 300 tilapia (average total body weight and length of 2 g and 7 cm respectively) for 3 months to study the effect of neem oil on growth parameters, survival rate, immune response and sexual maturity of tilapia. Three replicates were assigned for each treatment, with 25 fish per each and were kept in glass aquaria of 160 liters capacity. The fish in all treatments were fed twice daily in the rate of 3% of total biomass. LD50 and LC50 were detected. Total body weights and length were measured every 2 weeks. Two fish from each replicate were sacrificed at the end of experiment for blood sampling and dissecting to get livers, spleens and gonads. Blood parameters (total erythrocytic & leucocytic counts, haemoglobin, PCV, total protein, albumin and globulin), hepato/somatic, spleno/somatic and G/S indices were calculated. Ten fish were collected from each treatment and were challenged (I/P) with a hot strain of *Aeromonas hydrophila.*

Results revealed that neem oil has a toxic or lethal effect on fish and showed LD50 of 5% after 1 week and 20% after 4 days. On the other hand, LC50 was 5% after 2 days and 3% after 3 days. There was no significant difference in estimates of growth parameters and survival rate between fish treated with 0.5% neem and fish in the control group. However, the fish in both groups had significantly higher growth and survival rates than fish treated with 1 & 1.5% neem oil with feed. Challenge test results showed no significance difference between treated fish and those of the control group regarding relative percent survival (RPS). Blood parameters showed that Neem oil had a negative effect on most of blood parameters, where red & white blood cell counts, hemoglobin, PCV, and MCV decreased significantly in fish treated with neem oil than in fish of the control group. However, there was no significant difference in total protein, albumin and globulin between treated fish and fish of the control group.

PP- B2

Hexane soluble fraction of *Tinospora cordifolia* leaves as a feed supplement enhanced the non-specific immune mechanisms and disease resistance in *Oreochromis mossambicus*

Catherine P.Alexander [1], *C. John Wesly Kirubakaran*[2] *and R. Dinakaran Michael*[3]

[1]P.G. Department of Zoology, Jeyaraj Annapackiam College, Periyakulam-625601, India
[2] SGS Life Sciences, Taramani, Chennai-600113, India
[3]Centre for Fish Immunology, School of Life Sciences, Vels University, Chennai-600117, India
Email: catherine_ruskin@yahoo.co.in

Due to intensive culture practices, disease management continues to pose a serious threat to the aquaculture industry. In intensive aquaculture, use of antibiotics and chemotherapeutics for prophylaxis and treatment has been widely criticized for their negative impacts like bioaccumulation of drugs, development of microbial drug resistance and immunosuppression in fish. Therefore, development of an effective vaccine may be the best way to check disease outbreak. The wide range of pathogens in fish farm also limits the feasibility and effectiveness of vaccines. Hence, there is an urgent need to look for eco-friendly disease preventative measures to promote sustainable aquaculture. One of the most promising methods of controlling diseases in aquaculture is strengthening the defense mechanisms through prophylactic administration of immunostimulants. Immunostimulants are known to enhance innate immunity in fish. Natural immunostimulants are biocompatible, biodegradable, cost effective and safe for the environment. There has been growing interest in the immune-stimulating functions of some herbs and other plants in aquaculture.

This study was undertaken to examine the immune response of tilapia and its resistance to *Aeromonas hydrophila* after receiving hexane soluble fraction of *Tinospora cordifolia* (TC) leaves, a traditional Indian medicinal plant. Hexane soluble fraction of TC was administered orally as a feed supplement at doses of 0.01%, 0.1% or 1% for 3 weeks. Non-specific immune parameters such as serum lysozyme, antiprotease and alternate complement haemolytic (ACH50) activities, the production of cellular reactive oxygen species (ROS), reactive nitrogen intermediate (RNI) and myeloperoxidase and the disease resistance against live virulent *A. hydrophila* were investigated after 1, 2 or 3 weeks of feeding with hexane soluble fraction supplemented diet.

The results of this study indicated that feeding tilapia for 1, 2 or 3 weeks with selected doses of hexane extract of TC leaves significantly enhanced serum lysozyme, alternate complement activities and cellular ROS, RNI and MPO production. It was evident from the disease resistance test that feed supplemented with TC leaves extract at 0.1% or1% level significantly reduced the mortality of *O. mossambicus* and a 3-week feeding with 1% extract-supplemented diet appears to be the optimal regimen for maximal disease resistance. Thus, the study indicates the scope of using the TC leaves extract as an immunoprophylactic in fin fish aquaculture.

PP- B3

Isolation, screening and probiotic properties of autochthonous bacteria from marine fish of Kuwait

I.S. Azad, *A.Al-Marzouk, K.A. Al-Elah, T. Abu-Rezq and H. Al-Gharabally*

Mariculture and Fisheries Department, Kuwait Institute for Scientific Research, PO Box-1638, Salmiya 22017, Kuwait
Email: azadis@hotmail.com

Mariculture activities in the Arabian countries have been on the rise during recent times. Primitiveness of immune competence at the younger ages of the marine fish make the application of probiotics an effective alternative to antibiotics thus, obliterating the possible antibiotic contamination of the environment and development of multiple antibiotic resistant bacterial strains. However, the science of probiotics in aquaculture is in its infancy.

Gut bacteria from different marine fish (cultured and wild population) of Kuwait were isolated and screened yielding three potential bacterial species, Sh, Sq and L8, respectively from yellowtail bream (*Acanthopagrus latus*), silver pomfret (*Pampus argenteus*) and brown spotted grouper (*Epinephilus coioides*). These isolates were compared with a standard probiotic species (*Lactobacillus divergens*, ATCC).

PP- B4

Growth and diseases resistance in fingerlings of *Labeo rohita* fed with dietary omega-3 fatty acids

***Prathibha Bharathi C**, Sumanth Kumar Kunda[2] and D. Madhu Prasad[3]*

Department of Zoology and Aquaculture, Acharya Nagarjuna University Nagarjuna Nagar, Guntur, INDIA

Email: prathibach9@gmail.com

To assess the role of omega-3 fatty acid diets on growth and immunomodulation in rohu fingerlings a 60 days feeding trial was conducted. 300±20 fingerlings (average body weight 1.793 ± 0.022g) were randomly distributed into five treatment groups with each of three replicates. Five isonitrogenous (protein 19.60g) and isocaloric (337 k cal/ 100 g) test diets were prepared with different levels of omega-3 fatty acids viz. Control (basal diet), T1 (basal + 1% ω-3 fatty acid), T2 (basal + 3% ω-3 fatty acid), T3 (basal + 5% ω-3 fatty acid) and T4 (basal + 7% ω-3 fatty acid). The results indicated that the fishes which received the feed T1 (127.4±0.1) consisting of 1% of ω-3 fatty acid showed significantly increase of average body weight and specific growth rates (SGR) compared to control and other treatments T2 (120.1±0.3), T3 (112.6±0.3) and T4 (109.0±0.3). No significant differences were observed in feed utilization amongst the treatment groups. The present study indicated that T1 and T2 groups showed increase of length, weight and specific growth rate compared to T3, T4 and control. These growth studies revealed that increase of percentage of feed composition of ω-3 fatty acids beyond 3 percentage of ?-3 fatty acid are insignificant. The survivability studies showed that 100 percent survival of rohu fingerlings throughout the experimental period. The changes in level of omega-3 fatty acid percentages had not shown any effect on survivability of the fishes. The challenge study with *Aeromonas hydrophila* showed that the relative survival percent was also highest in T1 group followed by T2 and T3 and the lowest in T4 and control groups. It is concluded that, under the experimental conditions, the increase of dietary omega-3 fatty acid level beyond 1% had no beneficial effects on growth and supplementation of ω-3 fatty acid at 1% registered higher immunological responses and increase of supplementation of ω-3 fatty acid (7%) in the diet caused immunosuppression in *Labeo rohita* fingerlings. Supplementation of omega-3 fatty acid in the aquaculture diets would also reduce the usage of antibiotics and chemicals.

Key words

ω-3 fatty acid, *Labeo rohita, Aeromonas hydrophila*, Specific Growth Rate, Feed efficiency.

PP-B5

Application of lytic phages for controlling of *Vibrio parahaemolyticus* in aquaculture systems

***M.M. Dechamma**, S.A. Mohan and I. Karunasagar*

Department of Fisheries Microbiology, UNESCO-MIRCEN for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore- 575002, India.

Email:dechamma.mm@gmail.com

Members of the family *Vibrionaceae* occurring in marine environment are a serious problem in aquaculture settings such as hatchery and farms. *Vibrio parahaemolyticus* are found closely related to *V. campbellii, V. natriegens, V. alginolyticus* and *V. harveyi*. Consequently, these species have been grouped together as a clade, referred to as the Harveyi Clade. The present study assessed the applicability of phages to control *V. parahaemolyticus* numbers in aquaculture environments. Eight phages (A to H) with a broad host range were isolated. One of the phage (H) showing higher activity and maximum host range, with a titer of $2x10^{10}$ PFU/ml was used for further studies. For quantification of phage application, MIC was estimated in broth. 200 µl of phage ($2X10^{8}$ PFU/ml, final concentration) was adequate in reducing V. parahaemolyticus numbers by 2.5 log10 units in 16 h. Lytic activity was found to be maximum within 4 h of phage addition. Application of two doses at 4 h intervals was found effective in controlling *V. parahaemolyticus* effectively. DIG labeled probe targeting tlh gene was designed for enumeration of *V. parahaemolyticus*. The sensitive detection of *V. parahaemolyticus* by the DIG labeled probe was found to be specific at a hybridization temperature of 42.50C. This probe was used in experiment for the enumeration of *V. parahaemolyticus* to see the effectivity of the specific phage in lowering the viable counts. Phages isolated could have practical application in controlling pathogenic *V. parahaemolyticus*. The results of the study will be presented.

PP-B6

Enhancement of immune responses and disease resistance by *Nyctanthes arbortristis* leaves in *Oreochromis mossambicus*

L.D.Devasree[1] *and R. Dinakaran Michael*[2]

[1]Department of Zoology, The Madura College, Madurai-625011, Tamil Nadu, India.
[2]Centre for Fish Immunology,School of Life Sciences,Vels University,Pallavaram, Chennai-600117, Tamil Nadu, India.
Email: ganeshkumar.devasree@gmail.com

Use of immunostimulants as a prophylactic measure in preventing the diseases of fish is considered to be an attractive and promising area. Medicinal plant extracts as immunostimulants have recently received more attention as they cost less, can be easily incorporated into diet and have very low impact on the environment. Hence, the present study aims to determine the effect of hexane soluble fraction of the Indian medicinal plant, *Nyctanthes arbortristis* on the immune responses and disease resistance in *Oreochromis mossambicus* (Peters). Fishes were injected intraperitoneally with 0, 3.2, 16, 80 or 400mgKg-1 body weight of hexane soluble fraction. The specific immunity (antibody response), non-specific immunity (lysozyme , reactive oxygen species production) were tested. The functional immunity in terms of percentage mortality and Relative Percent Survival (RPS) on a challenge with live *Aeromonas hydrophila* was assessed. The antibody response was significantly enhanced by hexane soluble fraction irrespective of the dose used. The serum lysozyme level and the intracellular reactive oxygen species production were also enhanced by the fraction. Similarly all the doses of hexane soluble fraction administered as a single or double dose gave impressive protection against *A. hydrophila* in terms of reduced percent mortality which is reflected in the increased Relative Percent Survival (RPS).Thus, the study indicates the scope of using hexane soluble fraction of NAT leaves as an prophylactic to prevent infectious diseases in finfish aquaculture systems.

PP-B7

Immunostimulatory effect of alginic acid extracted from brown seaweed *Sargassum wightii* on *Penaeus monodon* against *Vibrio parahemolyticus*

Grasian Immanuel, *Madasamy Sivagnanavelmurugan, Kudus Kadharsha and Arunachalam Palavesam*

Department of Marine Biotechnology, Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam - 629502, Kanyakumari District, Tamilnadu
Email: gimmas@gmail.com

The present investigation was carried out to study the immunostimulatory effect of alginic acid extracted from the brown seaweed *Sargassum wightii* on *Penaeus monodon* against *Vibrio parahaemolyticus*. For this, the alginic acid was extracted from *S. wightii* and supplemented with pellet feed at three different concentrations (0.1, 0.2 and 0.3%). Then the alginic acid supplemented diets were fed to *P. monodon* for 45 days and challenged with *V. parahaemolyticus* and the mortality percentage was recorded daily upto 21 days. During challenge test, the control group displayed 78 % mortality within 21 days, whereas, experimental groups fed on 0.1 - 0.3% alginic acid supplemented diets displayed 39 to 59 % mortality within 21 days of challenge experiment. The cumulative mortality index (CMI) of control group showed high mortality rate and the reduction in mortality percentage of experimental groups over control group ranged from 26.05 to 54.46%. During challenge experiment, the immunological parameters such as THC, prophenoloxidase activity, Respiratory burst activity, superoxide dismutase activity; phagocytic activity, bactericidal activity and bacterial clearance ability were analyzed on 0, 10th and 21st days of the challenge test. All the immunological parameters of experimental groups challenged with *V. parahemolyticus* were significantly ($P< 0.05$) increased when compared to control group. During challenge study, *V. parahaemolyticus* load was also enumerated from the infected shrimp at 0, 10th and 21st days of challenge experiment. In control group, the *Vibrio* load was high in hepatopancreas and muscle tissues on 10th and 21st day of challenge test. But in the experimental groups, the *Vibrio* load in hepatopancreas and muscle tissues declined well from 10th day on wards in all the tested concentrations of alginic acid.

PP-B8

Effect of Levamisole on growth, survival and haemato-biochemical parameters in the catfish, *Pangasius sutchi* (Fowler, 1937) fingerlings

Latha C. *and Munuswamy, N.*

Unit of Aquaculture & Cryobiology, Department of Zoology,University of Madras, Guindy Campus, Chennai - 600 025, Tamilnadu, India
Email: kv.krishnakumar@hotmail.com

The present study documents the possible effects of Levamisole supplemented diets on growth, survival and haemato-biochemical profiles in the catfish, *Pangasius sutchi* fingerlings. Fingerlings of 10.89 ± 0.52 cm in length and 22 ± 1.23 g in weight were used. Experimental animals were fed formulated diets supplemented with varying concentration of Levamisole (viz. 100, 200, 400 and 800 mg/kg of feed), at the rate of 3 % of the body weight for a period of six weeks. Control animals were fed with formulated diets without Levamisole. The results indicated that Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and survival were high in the fish fingerlings fed Levamisole supplemented diets, irrespective of dosage, compared to control diet. Haemato-biochemical profiles such as Total serum protein, albumin, globulin, albumin globulin ratio and serum glucose were also significantly higher in the experimental groups compared to control group. Among the four concentrations of Levamisole used, 200 mg concentration increased the growth, survival and enhanced health status of *P. sutchi* fingerlings.

PP-B9

Phage therapy against Pseudomonas infection of cultured ayu on site

Yasuhiko Kawato[1]*, Kentaro Takegami*[2]*, Takahiro Nagai*[3] *and Toshihiro Nakai*[1]

[1]Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan
[2]Shiga Prefectural Fisheries Experiment Station, Shiga, Japan
[3]Fisheries and Marine Technology Center, Hiroshima Prefectural Technology Research Institute, Hiroshima, Japan
E-mail: nakaitt@hiroshima-u.ac.jp

Phage can be a promising agent to control bacterial infections in aquaculture. Our previous phage therapy study demonstrated that oral administration of a cocktail of two lytic phages, PPpW-3 (myovirus) and PPpW-4 (podovirus), was effective in reducing the mortality of a freshwater fish, ayu *Plecoglossus altivelis,* caused by *Pseudomonas plecoglossicida* infection in the field setting. In the present study of in vivo kinetics of phages orally administered with feed in ayu, it was identified that an effective phage dose in feed was 10^9 pfu/g or higher and that phage titres in the kidney of fish reached their peak ($10^{4.9}$ pfu/g) in 6 h and disappeared within 24 h after administration. Following this result, phage therapy experiments were conducted in ayu culture facilities where the disease prevailed in the rearing ponds (average body weight of fish: 3-5 g, fish number: 60,000-150,000). All three trials resulted in rapid fall of fish mortality as seen in the previous study. However, a couple of administration of phage was not enough to completely eliminate the disease from cultured fish populations. Instead, a continuous multiple administration of phage, i.e. twice a day for approximate 10 days, was required for the eradication. We discuss how optimum the phage behaves in a pond of fish population comprising various infectious stages for treatment and prevention of bacterial infection by using a simple simulation model.

PP-B10

Evaluation of probiotic viz-a-viz antibiotics based seed production in penaeid shrimp larviculture

***A. Panigrahi**, S. K. Otta, C. Gopal, P. K. Patil, S. Sivagnanam, P. Ravichandran and A.G. Ponniah*

Central Institute of Brackishwater Aquaculture, 75, Santhom High Road, R.A. Puram, Chennai-600 028

Email: apanigrahi2k@gmail.com

Probiotics is drawing more and more attention as alternative health management tool in aquaculture though there is no clarity over the mechanisms involved in such host-probiotic interactions. The present study evaluates the performance evaluation of tiger shrimp *P. monodon* seeds produced following probiotics (commercial-Prob1 & Prob2 and known strain-Prob3) vis-a-vis antibiotics in inducing better larval performance and protective response upon pathogenic challenge in shrimp hatchery. The experimental larvae of this shrimp at mysis stage were exposed to antibiotics (1-2 ppm) and three probiotics (two commercial- as per manufacturer's instructions and one farm-grown probiotic strain *Lactobacillus rhamnosus* @ 10^6 CFU/ml of the rearing media, while the control group of larvae were reared without any such exposure. The experiment was conducted for nearly 5 weeks in triplicate tanks (500 l), started with a 50,000 protozoea/tank each, and subsequently challenging with virulent pathogenic strain *Vibrio anguillarum*. The performance of the larvae in terms of survivability, growth and metamorphosis pattern were observed following antibiotics and probiotics based rearing and post challenge.

The survivability was significantly ($p<0.05$) higher in antibiotics (82.21 ± 1.92 %) and two out of three probiotic treated groups (80.29 ± 5.65 and 76.38 ± 5.63 %) of shrimp larvae compared to the control group without any treatments (70.55 ± 3.99 %) while rearing from protozoea to PL-25 stage. The weight gain was significantly higher ($p<0.01$) in these groups of probiotic supplementation compared to that of the control and antibiotic treated groups. Upon challenge with pathogen, there was a significant difference in the cumulative mortality rate between the probiotic fed and control group. The percentage survival significantly vary ($p<0.01$) between the treatments so also between the time points. All the three probiotic groups showed significantly higher ($p< 0.005$) survival rate compared to that of the control and antibiotic treated groups, however, there is no significant difference ($p> 0.05$) between the antibiotic and control groups with regard to the survival of the challenged larvae. This study establishes the probiotic superiority in shrimp hatchery larval production systems compared to that of the use of antibiotics and depending on the strain certain probiotics are good in giving better larval performance. Probiotics may modulate the functioning of immune system both at systemic and mucosal levels, thus, giving protective response to the animal whereas antibiotics are known to bring on immunosuppression.

PP-B11

Effective manipulation of CN ratio alters the bacterial population structure in marine microcosms

Jemila Thangarani A, ***Satya Prakash****, Balakrishnan S, Rosalind George M, K and Riji John*

Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi
Email: satya.p1988@gmail.com

Differences in the levels of nutrients and composition of major elements have been found to alter the population structure of bacterial communities in a dynamic system like shrimp farming systems. We investigated the efficacy of nitrifying and other heterotrophic bacteria in controlling growth of shrimp pathogenic bacteria and maintenance of water quality under simulated farm conditions. We isolated 85 hetrotrophs (*Bacillus sp*. 23%) and 119 nitrifiers (2/3rd ammonia oxidisers and 1/3rd nitrite oxidisers) from shrimp farming systems. *Bacillus* strains capable of controlling vibrios through bacteriocins were identified by cross streaking and double layer method.

Microcosms were set up in a series of poly jars of 5 l capacity with sediments collected from shrimp farms up to a depth of 2 cm and filled with 4 l of seawater of 25 ppt. Jars were supplied with shrimp feed supplemented with starch to adjust the input CN ratio (CNr) to 4:1 and 5:1. Nitrifiers showed higher survival in treatments with lower CNr than in treatments with higher CNr. Significant reduction was observed ($P<0.05$) up to 6 days in the microcosm receiving sterile soil and water with only nitrifiers (CNr 4:1) and up to 9 days in microcosm with nitrifiers and *Bacillus* sp. (CNr 5:1). The ammonia oxidisers and nitrite oxidisers showed a significant reduction in water ($P< 0.05$) over the duration of the experiment falling from an initial count of 2.9×10^6 to 1.10×10^5 CFU/ml and 2.40×10^6 to 1.53×10^5 CFU/ml respectively, while the sediment population did not have significant changes. In treatment with non sterile soil and water with *Vibrio harveyi* and nitrifiers at CNr 4:1 no significant reduction of vibrios was observed in water or sediment indicating the inability of inoculated indigenous nitrifiers to control the vibrio population even at low CNr. However, the above treatment showed significant reduction in NH_3-N and NO_2-N in the system. The results derived from study indicate that in addition to water quality control, enhanced CNr supports higher number of heterotrophic / probiotic strains, which could help in controlling *vibrio* population in shrimp farming systems, which is the most common pathogenic bacteria for shrimps.

PP-B12

Effects of tricaine methanesulfonate (MS222), clove oil and electro anesthesia in narcosis stage on respiratory burst activity of rainbow trout (*Oncorhynchus mykiss*)

Mirzargar Seyed Saeed[1], *Gholipour kanani Hosna*[1], *Soltani Mehdi*[1], *Abrishamifar Adib*[2] *and Ebrahimzadeh Mousavi Hosseinali*[1]

[1]Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran P.O. Box 14155-6453, Tehran, Iran
[2]School of Electrical Engineering, Iran University of Science and Technology, P.O. Box 16846, Tehran, Iran
Email:zargarm@ut.ac.ir

There is few available information on suppressive effect of anesthesia on immune response in fish specially electroanesthesia. In the present study, two anesthetics, MS222 (50 ppm), clove oil (25 ppm), and electroanesthesia were tested in rainbow trout (*Oncorhynchus mykiss*) in narcosis stage in order to observe their effects on innate immune system. The results showed that electroanesthesia reduces light emission in chemiluminscense assay both 1 and 24 h post anesthesia. Clove oil and MS222 decreased light emission 24 h post anesthesia. In addition clove oil, MS222 and electroanesthesia had no effect on nitroblue tetrasolium assay.

From aquaculture practice point of view, these data showed that the effect of anesthesia should be taken into account to avoid possible immunodepression in rainbow trout.

PP-B13
Building fish health professionals for sustained aquaculture: Students perspective

Saloni Shivam[1] *and Satya Prakash*[2]

[1]Aquautic Environment and health Management division, Central Institute of Fisheries Education, Mumbai
[2] Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi
Email: salonishivamfcri@gmail.com

Indian Aquaculture passed its aquarium phase (phase in which maintenance of crystal clean water with no assemblage of various fish food organisms and left over feed was emphasised) and entered into meat production phase some 20 years back. This transformation led to a burst in fish production with the current aquaculture production reaching to 3.47 million tonnes (FAO,2008). While this increase in production is attracting more number of farmers and entrepreneurs towards aquaculture but till date what ails them is lack of experts who can suggest proper protection and remedial measures in the event of outbreak of a disease. Indian fisheries education system which should be credited with this much desired increase in fish production over the period of time should also hold the accountability of failing to provide the specialised fish health professionals who can shoulder the above mentioned responsibility. Production and protection must go hand in hand, alienation between these two can provide success in short run but in long run the very existence of sector will be jeopardised. At present Fisheries graduates are neither equipped nor permitted through the law of land to prescribe a drug for a disease unlike that of veterinarians and agriculture graduates. Present curriculum although seeks to address the issue of Fish reproduction and fish culture in greater detail but fails to recognise the need of training students for disease diagnosis, treatment and recommendation of pharmacological compounds if a disease is identified. This issue apart from having a professional and ethical dimension (Fish disease treatment by veterinarians with very limited or no knowledge of fish physiology) has a social dimension too in form of increasing the cost of production (Farms seek the guidelines from a veterinarian although having employed a fisheries graduate on permanent basis) thereby minimising the profit margin. This triennial symposium should serve as a platform to find solutions for demanding issues as this in addition to discussing research. This poster intends to represent students' perspective and find hope in their present and power in future with help from experts gathered.

PP-B14

Oral administration of seeds of *Achyranthes aspera* (Amarantheceae) enhances the growth, immune response and survival rate of Indian major carp *Labeo rohita* larvae challenged with *Aeromonas hydrophila*

Praveen Kumar Srivastava *and Rina Chakrabarti*

Aqua Research Lab, Department of Zoology, University of Delhi, Delhi 110 007, India
Email: aquaresearchlab@yahoo.co.in

Immunostimulatory and disease resistance properties of indigenous medicinal plant *Achyranthes aspera* was tested with Indian major carp *Labeo rohita* (rohu) larvae. Two experiments were conducted. In first experiment, larvae were fed with four different diets containing 0.1, 0.5 and 1.0% *Achyranthes aspera* seeds and control diet. Fish were immunized with c-RBC after 30 days and blood samples were collected on days-7, 14 and 21 after immunization. Significantly ($P<0.05$) higher average weight (2.565±0.02 g) and SGR were found in 1.0% seeds incorporated diet fed fish compared to others. The average weight of fish showed a direct relationship with the increasing dose of seeds in diets. FCR was significantly ($P < 0.05$) lower in 1.0% seeds incorporated diet fed group. Total serum protein, albumin and globulin levels were always higher in treated groups compared to the control one, whereas the antigen-specific antibody titre level was lower in the latter group. SGOT, SGPT and ALP levels were always significantly ($P<0.05$) higher in the control group. Significantly ($P<0.05$) higher level of myeloperoxidase was recorded in 1.0% seeds incorporated diet fed rohu (1.561-2.558, 450 nm) compared to others. In other experiment, rohu larvae were fed with four different diets, containing 0.1, 0.25 and 0.5% seeds and control diet. Fish were challenged with live *Aeromonas hydrophila* by giving intraperitonial injection after 70 days of feeding. In control group, first mortality was recorded within 12 h of exposure and 50% fish died within 72 h, whereas in all treated groups, first mortality was recorded after 24 h. After 7 days post challenge, the total accumulated percentages of mortalities were 50, 40, 35 and 15% in control, 0.1, 0.25 and 0.5% plant incorporated diet fed groups, respectively. Lysozyme and NOS levels were significantly ($P<0.05$) higher in 0.5% seeds incorporated diet fed group. The present study documented the immunostimulatory properties of seeds. This indicated that the feeding of carp larvae with *Achyranthes aspera* seed improved the non-specific immunity at early developmental stage and it enhanced the resistance of fish against the pathogen. Moreover, this enhanced the growth of larvae.

PP-B15

Anti White Spot Syndrome Virus activity of a mangrove plant *Ceriops tagal*

***N.S Sudheer*[1], *Rosamma Philip*[2] *and I.S Bright Singh*[1]**

[1] National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Lake Side Campus, Cochin-16, Kerala, India.
[2] Department of Marine Biology Microbiology and Biochemistry, Cochin University of Science and Technology, Lake Side Campus, Cochin-16, Kerala, India.
Email: cusatmarine@gmail.com

White spot syndrome virus, a novel genus under the family Nimaviride still continues to be a serious threat to shrimp aquaculture. As on today no effective remedial measure could be evolved other than a few managerial protocols to control the WSSV out break. Majority of shrimp cultivating areas are found in proximity to the mangrove ecosystem. Presently mangroves have been extensively destroyed for construction of aqua farms. Considering the requirement and also on the basis of the fact that several antiviral molecules have been reported, seven mangrove plants were chosen for screening anti WSSV activity in *Penaeus monodon.* Plants were screened through feeding as well as injection routs. Through this investigation anti WSSV property could be confirmed in the aqueous extracts of *Ceriops tagal* under experimental conditions. A Preliminary phytochemical analysis and an HPLC finger print of the aqueous extract were generated. The results revealed the presence of alkaloids, flavonoids, polyphenolics, cardiac glycosides, saponins and sterols. The animal bioassay results showed that the plant extract was less toxic to shrimps and could inactivate WSSV at a concentration of 30-50 mg/ml. The concentration of the extract required in the feed to protect shrimps from WSSV on oral challenge was 500mg/kg animal body. The possible mode of action of the aqueous extract from *Ceriops tagal* has been noticed, through temporal gene expression analysis of immune genes and viral genes involved in various stages of virus multiplication cycle, as virucidal rather than immunostimulatory. The absence of virus particle in medicated animals which survived WSSV challenge was confirmed through histopathology and indirect imunohistochemistry using WSSV VP28 specific monoclonal antibody. In an attempt to purify the active principles involved in the anti WSSV activity an active virucidal saponin rich fraction could be separated. The present study also strengthens the need for protecting mangrove plants and adopting mangrove friendly aquaculture rather than destroying mangrove plants for construction of aqua farms.

PP-B16
Characteristics and history of aquatic animal disease introduction to Japan

Nanae Takagishi *and Tomoyoshi Yoshinaga*

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
Email: aa107055@mail.ecc.u-tokyo.ac.jp

About 20 significant, non-indigenous aquatic animal diseases have been introduced to Japan in the past 50 years. Although Act on the Protection of Fishery Resources to manage risk of invasion was amended in 1995, introduction of pathogens non-indigenous to Japan persists and poses a serious threat to economic returns in aquaculture and fisheries industries. To prevent further introduction of diseases, we investigated the characteristics and history of aquatic animal disease introduction to Japan through a review of published literature and prefectural reports, and interviews of prefectural officers who were involved in the response to introduced diseases.

The characteristics and history of aquatic animal disease introduction investigated by this study are: (1) Some introduced diseases spread to wild animals. (2) Introduction of previously unknown diseases has increased since the 1990s. (3) Almost all introduced diseases were not listed as important diseases, and these invasions should be prevented. (4) The origin of the majority of pathogens shifted from North America to East Asia around 1990. (5) Between the 1960s and 1980s, the most prevalent introduced diseases affected salmonid fish and eel, but recently, diseases that affect various aquatic animals including marine fishes, mollusks and crustaceans have become more prevalent. These characteristics and history offer important insights for improving systems to prevent invasion and spread of non-indigenous diseases in Japan.

PP-B17

Immunostimulant potential of selected plant extracts to control *Aeromonas hydrophila* infection in *Channa striatus*

R.Thaya *and B.Vaseeharan*

Department of Animal Health and Management, Alagappa University, Karaikudi.
Email: thayarajgopal@gmail.com

Aquaculture operations strive to produce large numbers of healthy fish by means that are biologically and economically efficient. *Channa striatus* is indigenous, commonly seen in the rural streams, canals, drains, ponds and reservoirs. It is valued for its supposed medicinal value, when eaten it is reported to be good for healing bodily wounds and thus recommended for post-operation patients. *C. striatus* was affected by many microbial pathogens and cause heavy losses due to mortality, reduced growth as well as unmarketable appearance in fish. In fishes *Aeromonas hydrophila* Gram negative bacteria cause dermal ulceration, tail, fin rot, exophthalmia, erythrodermatitis, haemorrhagic septiceamia, red sore disease, red rot disease and scale protrusion disease. A wide range of antibiotics has been used to treat this disease. Eventhough antibiotics and several other chemical give positive effects they cannot be recommended, due to their residual effect in fish tissue and other side effects. Problems arise due to the antibiotics, chemicals in the environment and this led to the emergence of multiple resistant strains. To overcome these problems immunostimulation is one element in a strategy to achieve microbial control. In the present study *Channa striatus* of average weight 500gms were used as test animals, separated into two groups one as control and another as experimental. The experimental animals were immunized with selected plant extracts through intra-peritoneal injection and the fishes used as control had received same amount of saline. Following challenge with *A. hydrophila,* there was a reduction in mortality compared with the controls. Furthermore, the fish, which were immunized, recorded enhanced haematological and immunological parameters including phagocytic, respiratory burst, lysozyme, bactericidal, complement, myloperoxidase and antiprotease activities, and total protein, compared to the controls. The active compounds present in the plant extract play an important role in the stimulation of nonspecific immunity and stimulation of specific defence mechanism and seem to be the most promising methods for preventing microbial diseases. The alternative plant extracts prove to be very effective in aquacultural operations to treat the fish diseases.

PP-B18

Specific pathogen free Red Tilapia broodstock development: establishing a list of current significant pathogens for screening

Sakranmanee Krajangwong[1], *Kim D. Thompson*[1], *Boonsirm Withyachumnarnkul*[2], *Matthijs Metselaar*[1], *Tharangani Herath*[1], *David J. Penman*[1], *Warren Turner*[3], *Dave Little*[1] *and Alexandra Adams*[1]

[1]Institute of Aquaculture, University of Stirling, Scotland, UK
[2]Centex Shrimp, Faculty of Science, Mahidol University Bangkok, 10400, Thailand
[3]Nam Sai Farms Co.Ltd., Amphur Ban Sang, Prachinburi 25150 Thailand
Email: kdt1@stir.ac.uk

Tilapia (*Oreochromis spp.*) is one of the major fish species cultured in global aquaculture. The incidence of disease in tilapia farming systems has increased, and problematical pathogens include protozoa, *Streptococcus* spp, rickettsia-like organisms (RLOs), *Chlamydia* and *Francisella spp*. The concept of Specific Pathogen Free (SPF) stock is to exclude pathogens from the farm facility by selectively breeding disease-free juveniles. Broodstock that test positive for pathogens during disease screening are destroyed to reduce risk of horizontal and vertical transmission of disease. This allows further selective breeding to proceed based on healthy offspring with improved performance. However, no up-to-date list is available for the pathogens currently affecting tilapia production systems; especially in Thailand where this study was set. A monosex tilapia farm in Prachinburi Province, Thailand was screened to establish which pathogens were present in the broodstock and offspring. Both clinically healthy and moribund fish (broodstock and fry) were examined by histology, bacteriology, and molecular methods for *Streptococcus iniae, S. agalactiae, Chlamydia spp.* and *Francisella spp.* and also by immunohistochemistry to confirm the presence of *Francisella sp.* in fish tissues.

Francisella spp. and *S. agalactiae* were identified by polymerase chain reaction (PCR) in healthy broodstock at levels of 87 % and 8.3 %, respectively. Simultaneously, epitheliocystis caused by *Chlamydia spp.* was found in 15 % of the fish by histology. Statistical analysis showed that co-infection by streptococcosis with a cryptic infection of *Francisella sp.* caused an expression of clinical signs 2.6 times higher than in fish infected with only a single pathogen. *Francisella sp.* was the most important pathogen contributing to deteriorating fish health and high morbidity as a result of opportunistic infections.

This study was the initial step for developing SPF broodstock in red tilapia. The current pathogens for broodstock screening proposed include *Francisella sp, S. agalactiae* and *Chlamydia spp.*

PP-B19

Immunomulatory effect of supplementation of *Ficus baghensis* prop root & *Leucaena leucocephala* pod seed powder in the artificial feed in Indian freshwater murrel, *Channa punctatus* against *Aeromonas hydrophila*

Vipin Kumar Verma *and Om Prakash*

Sri Venkateswara College, University of Delhi, Benito Jaurez Road, Delhi-110021.
Email: vipinkumarverma1@yahoo.com

Immuno-stimulatory effect of *Ficus baghensis* (aerial root) or *Leucaena leucocephala* (pod seeds) 5% powder as supplementary ingredients in the artificial fish feed of Indian freshwater murrel, *Channa punctatus* was compared with control group of fishes which were fed with artificial feed alone. The fishes were fed with control and experimental feed for 15 days prior to start of experimentation. Fishes were challenged with *Aeromonas hydrophila,* a major disease causing bacteria in freshwater fishes on weekly intervals for four weeks. Serum samples were collected on 7th, 14th, 21stand 28th day after challenge. The specific immunity (antibody response) and non-specific immunity (lysozyme activity & phagocytic response) of the fish were evaluated.

The increase levels of SGOT & SGPT in liver is a marker of damage of most targeting organs by *A. hyrophila* in fishes fed with simple artificial feed, however the levels did not changed significantly in fishes fed with experimental feed. Nitric oxide, SOD, ALP indicates the lower stress level in experimental fed fishes compared to control group of fishes. In fished fed with supplementary feed showed increased total serum protein level, lysozyme activity and phagocytic index. Antibody was detected in the serum by ouchterlony double immuno-diffusion & dot blot. The levels of immonoglobulins in the serum analyzed by sandwich ELISA showed higher antibody production in fishes fed with supplementary feed.

The present study indicated the immuno-stimulatory response of powder of *Ficus baghensis* and *Leucaena leucocephala* when supplemented in the feed of in the Indian freshwater murrel, *Channa punctatus.*

PP-B20

Piper Betle (Sireh) extract in feed as potential cure for nocardiosis in red snapper, *Lutjanus erythropterus*

Siti-Zahrah A[1], *Nik-Haiha N.Y*[2], *Afiqah M.J*[3], *Zamri-Saad M* [4] and *Jaafar S*[3]

[1] National Fish Health Centre (NaFisH), Batu Maung, 11960 Penang
[2]FRI Tg. Demong, 22200 Besut, Terengganu
[3] Myikan Sdn Bhd, Lot 6656 Mukim Bebar Badong, Pekan 26680 Pahang,
[4]Faculty of Doctor Veterinary Medicine, Universiti Putra Malaysia(UPM), Serdang, Selangor, Malaysia.
Email: siti.zahrah.abd@gmail.com

Earlier study revealed that *Piper betle* extract had profound antimicrobial properties, on both Gram positive and negative bacteria. Therefore, it was used to treat a case of nocardiosis outbreak involving fingerlings of Red Snapper, *Lutjanus erythropterus* cultured in re-circulating tank systems after more than three months. The infected fish were lethargic, gulping near water surface, inappetance with skin discoloration and emanciated. There was occasional bulging of muscle at caudal peduncle and surrounding the eyes. Post-mortem examinations on the affected fish showed whitish nodules in almost all organs including the digestive tracts. Histological examinations of the kidneys and spleen stained with Grocotts revealed hyphae-like organisms, suggesting *Nocardia sp.* infection. The infected fish were separated into 2 grow-out tanks each containing 9000 and 7000 fish (5-6") respectively prior to antibiotic and chemical treatment for a month. Total mortality was observed in the tank that was treated with potassium permanganate at an overdose level of 20 ppm given by the farmer, while the latter tank of 7000 fish treated with oxytetracycline followed by acriflavine the mortality was 47%. Thus, the remaining affected fish were again isolated before they were treated with *Piper betle* extract in feed at 100ppm at alternate days for a period of 4 weeks. The treatment eventually reduced the mortality to 15% while the surviving fish showed full recovery and was harvested at 7 months at an average weight of 700g. This preliminary study concluded that *Piper betle* extract could be an effective alternative herbal treatment for *Nocardia sp*. infection in fish.

PP -B21

The effect of *Alieae sativum, zataria multiflora* and *Echinacea angustifolia* on white blood cells of rainbow trout (*Oncorhynchus mykiss*)

Maryam Ghodratnama[1], *Shafigh Shafeie*[1], *Mehdi Soltani*[1], *Ali Taheri Mirghaed*[1], *Abbas Mokhtari*[2]

[1]Aquatic Animal Health and Diseases, Faculty of Veterinary Medicine, University of Tehran,Tehran, Iran

[2] Faculty of Veterinary Medicine, Islamic Azad University of Shahrekord,shahrekord, Iran
Email: ghodratnama@ut.ac.ir

In recent years, some medicinal herbs have been shown to modulate fish immune system and are used as an alternative to antibiotics in aquaculture industry. The aim of this study was to investigate the stimulatory effect of *Alieae sativum* (garlic), *Zataria multiflora* and *Echinacea angustifolia* on WBCs of rainbow trout. Forty trout weighing 50 ± 5g were kept in four small tanks under similar condition (12-14?C, hardness 200mg/l, O2 8-9mg/l and ammonia <0.01). The study was conducted in 3 treatments and a control group. 60 ml (30ml in the morning and 30 ml in the afternoon) of herbal extracts were sprayed on pellets per 100 kg fish, daily. After 7 days blood samples were collected from peduncle. According to the results, mean lymphocyte for control, *A. satvium, Z. multiflora* and *E. angustifolia* were 48.66, 50, 61.33, 74%, mean monocyte for mentioned groups was 23, 46.33, 37.66, 61.33%, mean neutrophil was 0, 2.33, 0.66, 2.66% and mean eosinophil 1.33, 1.33, 0.33 and 0.33%, respectively. Mean lymphocyte, monocyte and neutrophil were significantly higher in *E. angustifolia* treatment than control and other groups. No significant difference in mean eosinophil was seen between groups. It can be concluded that the *E. angustifolia* can be used as an immunomodulator in rainbow trout culture.

PP- B22

Acute and sub-lethal toxicity of Nuvan®, an organophosphate to fresh water fish *Labeo rohita* and its effect on selected haematological parameters

G. Dash[1], Perisetti Bhargvi[1] and ***Santosh Kumar Udgata***[2]

[1]Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria Campus, Kolkata-700 094, West Bengal, India.
[2]College of Fisheries, OUAT, Rangailunda, Berahampur-760 007, Odisha,India
Email: udgatask2002@gmail.com

Acute and sub-lethal toxicity of an organophosphate insecticide, Nuvan®, on the fingerlings of *Labeo rohita* was evaluated under static conditions for 24, 48, 72 and 96 h. The median lethal concentration (LC50) values for 24, 48, 72 and 96h were 31.25, 28.10, 24.33, 16.67 ppm respectively. Fishes were exposed to a sub-lethal dose of Nuvan i.e. 1.11 ppm (1/15th of LC50 96h for a period of 15 days). The alterations in haematological parameters were studied at the end of 24, 48, 72 and 96h in lethal concentrations and were studied at the end of 15 days in sub-lethal concentrations. Haematological parameters like total erythrocyte count (TEC), haemoglobin concentration (Hb) and haematocrit values (Hk%) were decreased while, total leukocyte count (TLC) and plasma glucose level increased in exposed fishes compared to control. The drop in TEC in pesticide exposed fishes may be due to erthropoisis, haemosynthesis and osmoregulatory dysfunction. Decrease in haemoglobin concentration may be due to release of oxygen radical brought about by toxic stress of Nuvan. Increase in the TLC and glucose could be due to stimulated lymphopoiesis and hyperglycaemia in pesticide exposed fishes. The observed haematological parameters may be used as non-specific biomarkers in the field of environmental toxicology.

PP- B23

Histopathological changes in major organs of *Labeo rohita* upon exposure to acute and sub-lethal toxicity of Nuvan®, an organophosphate

Perisetti Bhargvi[1], ***Santosh Kumar Udgata***[2] *and G. Dash*[1]

[1]Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria Campus, Kolkata-700 094, West Bengal, India.
[2]College of Fisheries, OUAT, Rangailunda, Berahampur-760 007, Odisha,India
Email: udgatask2002@gmail.com

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PP - B24

Efficacy of three herbal extracts in enhancing immune response of *Labeo rohita* against experimental infection with Flavobacterium branchiophilum

Gayatri Devi, *Y and Shameem, U*

Dept. of Zoology, Andhra University, Visakhapatnam - 530 003
Email: ummey.shameem@gmail.com

Investigations were undertaken to study the efficacy of three herbal extracts *Asparagus recemosus* (satavari), *Bhoerhavia diffusa* (punarnava) and *Sida cordifolia* (bala) in enhancing immune response in *Labeo rohita* challenged with a lethal dose of *Flavobacterium branchiophilum*, the causative agent of Bacterial Gill Disease in carps. Dried powder of whole plant extracts was used in the present experimental study. A set of 60 fish were used for treatment with each variety of herbal extract, and each set was divided into three batches with 20 fish in each batch. Batches 1&2 were regarded as positive and negative controls, where as the third batch was regarded as experimental group and fed with feed mixed with herbal extract at the rate of 7.5gm/kg. Except negative controls all fishes were challenged with a lethal dose of *F.branchiophilum* (1x103.27 cfu/µl) on 20th, 40th and 60th day of the experiment.

Both cell mediated and humoral mediated immune responses were analyzed by employing trypan blue dye exclusion test for cell viability, NBT test for activated macrophages and neutrophils and agglutination for antibody titers. Besides the activity of myeloperoxidase, lysozyme and antiprotease enzymes was also analyzed.

The test results indicated an increase in the number of activated neutophils, macrophages and antibody titre values in fish fed with herbal extracts when compared to fish fed with normal diet.

PP -C1

Development of monoclonal antibody based flowthrough immunoassay for rapid detection of *Aphanomyces invadans* of Epizootic ulcerative syndrome (EUS)

***Adil B**, Shankar K.M, Ramesh K.S, Naveen Kumar B.T, Rajreddy Patil, Omkar V. Byadgi, Prabhugouda Siriyappagouder and Bharath Kumar*

Fish Pathology and Biotechnology Laboratory, Department of Aquaculture, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore 575002, Karnataka, India
E- mail: adil.b04@gmail.com

Epizootic ulcerative syndrome (EUS) is a serious fish disease caused by *A. invadans*, and has caused large scale mortality of fresh and brackish water fishes in several parts of south -East Asia, Africa and North America. A monoclonal antibody (MAb) based flow through immunoassay (FTA) was developed, with nitrocellulose membrane laid on the top of adsorbent pads enclosed in a plastic cassette with a test hole at the centre. Muscle homogenate of EUS suspected fish dotted with appropriate positive and negative control. The dotted membrane and treated with MAb-C5 recognising *A. invadans* followed by rabbit anti mouse IgG - HRP and substrate chromogen. FTA could be completed within 10 min with clear purple dots developed with *A. invadans* against white background of nitrocellulose membrane. The limit of detection of FTA and Immunodot was 7 and 56µg/ml of homogenate prepared from *A.invadans* culture respectively. In serially diluted EUS infected fish sample homogenate, FTA and PCR could detect the fungus up to 10-11 dilution, where as immunodot only up to 108 dilution. FTA and PCR assay could detect fungus in field samples 100%, where as Immunodot only 89.04%. FTA reagents were stable and giving expected results for 4 months when stored at 40 - 80C. FTA has potential to be developed to a field level kit.

PP- C2

Development and evaluation of PCR primers for the detection of monodon baculovirus in marine shrimp, *Penaeus monodon*

D.S. Akhila, *P .P. Bhowmick, M.N. Venugopal and I. Karunasagar*

Department of Fisheries Microbiology, UNESCO-MIRCEN for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575 002, India.

Email: akhilads87@gmail.com

Monodon baculovirus (MBV) was the first viral agent studied that was responsible for causing disease in cultured marine shrimp *Penaeus monodon*. A heavy infection of hatchery-reared larvae and postlarvae of *P. monodon* with MBV results in stunted growth and mortalities causing substantial economic loss to the shrimp farmers. Its presence and isolation is also an indication of poor hygie in the hatchery. The risk of carriers in the spread of this disease is of paramount importance to control its devastating effects. Though a number of diagnostic methods are in place, the need for developing a highly sensitive and specific detection was the reason for this study. Samples of marine crustaceans consisting of shrimps, crabs, prawns and squilla were analyzed for the presence of MBV by using the highly sensitive and specific DNA based molecular technique polymerase chain reaction (PCR). Three sets of published primers (1.4F/R, P1/P2 and 261F/R) as well as primers designed in this study (PmNP 189F/R and NPV 210F/R) for a conserved region of polyhedrin gene were used. Of the 415 samples examined, 8 shrimp samples (3.4%) were positive for MBV with primer (1.4F/R) in first step reaction generating an amplicon of 533 bp while nested primer (1.4NF/NR) gave positive reaction in 31 samples (11.10%) with an amplicon of 361 bp. The published primer set 261F/R used in one step reaction yielding an amplicon of 261bp enabled the detection of MBV in 26 samples (10.6%). None of the samples were positive for MBV with primer pair P1/P2 or the primer pair PA1/PA2 for nested reaction. Primers designed in this study for a conserved region of polyhedrin gene (PmNP 189F/R and NPV 210F/R) detected MBV in 27 (10.6%) samples by first step PCR. The results clearly demonstrate the superiority of the designed primers in the sensitive and specific detection of virus in shrimps and other carriers by a single step reaction.

PP- C3

Media optimization for the development of primary cell cultures from different tissues and hemocytes of mud crab, *Scylla serrata*

***Deepika Anand**, M. Makesh and K.V. Rajendran*

Central Institute of Fisheries Education, Panch Marg, Versova, Mumbai-400061, India
Email: deepikaanand12@gmail.com

Crustaceans have received special attention as donors of cells and tissues for *in vitro* cultures, as they are easy to obtain and handle in the laboratory. Using the methods developed for mammalian and insect cell culture, tissues from shrimps and crabs have been tried for developing primary cell cultures for different research purposes, but very few attempts have been successful, the media used for culturing cells being one of the reasons for limiting the success of such approaches. The present study attempted to establish primary cell cultures from different tissues and hemocytes of mud crab, *Scylla Serrata*. The organs used to develop primary cell cultures included hepatopancreas, heart, testis and hemocytes. The optimum conditions for primary culture in vitro were obtained in double-strength L-15 medium with osmolality of approximately 894-1047 mOsmol/kg, adjusted using crab saline and incubation at 28°C. The supplements tried included crab hemolymph serum, crab muscle extract, vitamin mix, non- essential amino acid mix, lipid concentrate and fetal bovine serum (5-10%). The cells from each organ were cultured in different growth media. Hemocytes showed better attachment and spreading than other tissues. After 24 to 48 hours of incubation, cell migration was observed in hemocytes cultured in 2X L-15 supplemented with 5% FBS and 2X L-15 supplemented with 5% FBS as well as 5% crab hemolymph serum. Cells from heart cultured in 2X L-15 prepared in crab saline+10% FBS showed adherence and cell migration within three days. Cells from hepatopancreas could be separated into two fractions, by centrifugation, showing different morphology - the upper fraction consisting of small cells and the lower fraction of larger cells. Larger cells were viable as suspension cultures for 15-30 days, but cell viability decreased after subculture. Cells from testis showed adherence and were also viable as suspension cultures when cultured in 3X L-15+1X non-essential amino acid mixture and 1% lipid concentrate. Cell proliferation was observed as indicated by the increase in the number of cells. The cells from other tissues remained viable for 20 to 30 days, but cell division was not seen. Cell viability decreased after subsequent subcultures. The primary cell cultures developed using these methods can be used for the isolation and identification of viruses infecting crustaceans.

PP-C4

Investigation of the propagation efficiency of fish cell lines to fish nodavirus

***Sarath Babu**, Nathiga Nambi, Abdul Majeed, Taju Gani, Ravi Mani, Vimal Sugumar, Sundarraj and Sahul Hameed*

Department of Zoology, C. Abdul Hakeem College, Melvisharam-632509, Tamil Nadu, India.
Email: sarathbabuv@yahoo.com

Viral encephalopathy and retinopathy or viral nervous necrosis is an OIE significant disease occurring with high mortalities among larvae and juveniles of 40 fish species reaching pandemic status. Although several fish cell lines have focused on the susceptibility of nodavirus, it is important to study the replication efficiency of different fish cell lines to increase the sensitivity of nodavirus isolation and enhance the number of viable viral particles using suitable cell lines.

The susceptibility/propagation efficiency of 8 readily available fish cell lines were tested to study the replication efficiency of fish nodavirus. The cell lines developed from grouper (*Epinephelus sp.*), seabass (*Lates calcarifer*), Indian walking catfish (*Clarius batrachus*) and pearlspot (*Etroplus suratensis*) fishes along with the control cell lines SSN1 and E11 (*Ophicephalus striatus*) were used. The susceptibility of the cell lines to nodavirus were carried out by infecting the cell lines, confirmed by cytopathic effects and further by RT-PCR assay using four published and one pair of nodavirus specific primers designed in our lab. The nodaviral load in these cell lines was tested by infecting a batch of cell culture flasks and collected from all the infected cells at day 1,3,5,7 and 9. The viral replication efficiency was determined by TCID50, real-time RT-PCR assay and ELISA assays.

Our results showed that all the infected cells formed CPE and complete degeneration of monolayer on 7 day post-infection and further confirmed by RT-PCR. The steady increase in nodavirus propagation was observed in TCID50, real-time RT-PCR at different time intervals and standard increase in the absorbance signal was witnessed, correlating the proliferation of nodavirus titre by ELISA using the anti-nodavirus antibody. All the mock infected control cultures remained negative for CPE, RT-PCR and ELISA in the infected cells. The results indicate that all these cell lines were permissible to nodavirus and the seabass (spleen and kidney), grouper eye and pearl spot (eye and gill) cells gave the best results for propagation as observed in SSN-1 and E-11 cell lines, whereas smallest variability was seen in the other fish cell lines.

PP- C5
Development of primary cell culture from *Penaeus monodon*

Vinayak Biradar *and Deepti Deobagkar*

Molecular Biology Research Laboratory, Department of Zoology, Center for Advanced Studies, University of Pune. Pune - 411 007, India
E-mail: v2biradar@gmail.com

WSSV infects shrimp aquaculture and it has caused great economic loss. There is no *"in vitro"* model system to effectively support multiplication of WSSV. There is a need to develop various strategies for understanding the host-parasite relationship in case of WSSV. We have made an attempt to establish primary shrimp cell culture from different organs of *Penaeus monodon.* Shrimps brought from different sources were first checked for infection using WSSV specific primers. The WSSV free animals were dissected and different organs such as lymphoid organ, ovary, and hemocytes and tissue were used to set up primary cell cultures. The basic culture medium was varied for osmolarity, serum concentration and pH. It was found that 2X L-15 medium pH 7.0 -7.2 supplemented with various growth factors along with 10% FBS, 100 IU/ml penicillin and 100 µg/ml streptomycin with final osmolarity at 680 to 720 mmol/kg was suitable for lymphoid organ, hemocytes and ovaries. The results of these explant cultures and hemocyte will be presented. Potential applications of this culture system in understanding virus host interactions will also be discussed.

PP- C6

In vitro minimum inhibitory concentration of gut environment modifiers against field isolates of aquatic pathogens

Hoang Thi Dieu Thuy, *Bui Chau Truc Dan and Ooi Ei Lin*

Novus Aqua Research Center, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
Email: chautrucdan.bui@novusint.com

Disease management is a priority for successful aquaculture. The use of antibiotics in aquatic diets may help control disease outbreaks but public concerns on the potential development of antibiotic resistance have led to reduction and even elimination of the use of such substances worldwide.

Gut environment modifiers (GEMs), such as organic acids and essential oils, have attracted interest for their antibacterial properties, but without the potential negative impacts associated with the use of antibiotics. ACIDOMIX® AFL premix preservative, ACTIVATE® DA and MERA™ Cid nutritional feed acids are Novus proprietary blends of organic acids, while NEXT Enhance®150 is a patented 1:1 blend of thymol and carvacrol, essential oil extracts from oregano (*Oreganum vulgare*). These products were tested using an *in vitro* minimum inhibitory concentration (MIC) assay against five important aquatic bacteria pathogens, including *Aeromonas hydrophila, Edwardsiella ictaluri, Edwardsiella tarda*, *Streptococcus agalactiae, Vibrio anguillarium* and *Vibrio harveyi.* All the GEMs tested demonstrated some antibacterial properties. As anticipated based on the mode of action, the organic acid blends were generally less effective at neutral pH, while the essential oil blends were roughly equally effective under both conditions tested, and the NEXT Enhance®150 essential oil blend stands out as being the most potent with consistently the lowest MIC values against all tested bacterial strains. The MIC assay has utility in the screening of product efficacies under controlled conditions.

PP- C7

Three continuous cell lines developed from the fin and caudal peduncle tissues of the three spot damsel, *Dascyllus trimaculatus* (Rüppell, 1829)

*K. S. Sobhana, **Githa Ann George**, Sheethal Mary Sunny, S. Sreedevi, K. K. Surendran, Boby Ignatius and P. U. Zacharia*

Central Marine Fisheries Research Institute, Ernakulam North P. O., Kerala - 682 018, India
E-mail: githageorge@gmail.com

The global marine ornamental fish trade has been expanding in the recent years and India has vast potential in this trade. *Dascyllus trimaculatus* is one among the most widely traded pomacentrids in the international market. Successful hatchery production of commercially important marine ornamental fish species is a pre-requisite for the development of a long term sustainable trade. The Central Marine Fisheries Research Institute (CMFRI), Kochi has developed hatchery production methods for several species of marine ornamentals including the three spot damsel, *D. trimaculatus*. Establishment of sensitive, species specific fish cell lines are important in the much required national and international quarantine and certification programme for producing virus free fish stock. Fish cell lines are also powerful tools for studying toxicology, immunology, transgenics and functional genomics in fish.

Three continuous cell lines have been developed from the caudal peduncle and fin tissues of *D. trimaculatus*. The cell line derived from fin explants viz., DT1F4Ex has crossed 120 passages over a period of 3 years. Two cell lines have been developed from caudal peduncle tissue viz., DT1CpEx and DT1CpTr by explant and trypsinization methods respectively. DT1CpEx and DT1CpTr have crossed 115 and 105 passages respectively over 3 years. All the cell lines grow at an optimum temperature of 28 + 2ºC in Leibovitz L-15 medium supplemented with 2 to 5% foetal bovine serum. The cell lines have also been characterised by karyotyping and the modal chromosome number is estimated at 48. All the 3 lines have been successfully cryo-preserved, exhibiting >90 % survival rate even after an year of storage in liquid nitrogen.

PP- C8

Development of RT-PCR assay for the detection of White Muscle Disease in *Macrobrachium rosenbergii*

***Naveen Kumar S.**, Nithin M. S., Venugopal M. N. and Indrani Karunasagar*

UNESCO-MIRCEN for Marine Biotechnology, Department of Fisheries Microbiology, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore 575002, India

E-mail: aquas510@gmail.com

The giant fresh water prawn *Macrobrachium rosenbergii* is widely cultured species in most of the South-East Asian countries and in Caribbean Islands. Global prawn culture suffered severe losses in recent years due to the occurrence of White muscle disease (WMD) that affects post larvae and juveniles of *M. rosenbergii* causing 100% mortality within few days of infection. *Macrobrachium rosenbergii Nodavirus* (MrNV), an RNA virus with two ss RNA genome and Extra Small Virus (XSV) which is also an RNA virus with a ss RNA as its genome are held responsible for the outbreak of WMD. Development of specific and sensitive RT-PCR is essential for diagnosis of white muscle disease and can find application for routine health monitoring in prawn hatcheries and farms.

Total RNA from WMD infected tissue sample was extracted and cDNA of RNA1 and RNA2 of MrNV and RNA of XSV synthesized using reverse transcriptase enzyme. cDNA's were PCR amplified and sequenced. Comparative sensitivity of different primers designed for the sequenced RNA strands of MrNV and XSV was established by carrying out RT-PCR using combinations of primers at different annealing temperatures. β-actin of *M. rosenbergii* was used as a internal control to monitor the RNA decay. A 98% homology was observed in RNA1 and RNA2 when blasted with the sequence available in GenBank confirming the sequence to be indeed of MrNV genome. Sequence alignment of RNA of XSV showed 99, 97 and 96% homology with RNA of XSV from Taiwan, China and France respectively. Among different combination of primers, those generating an amplicon of 564 bp and 525 bp for MrNV and XSV respectively showed specific band even at very low RNA concentration. Amplified products yielding specific bands were cloned and sequenced for confirmation. RT-PCR developed in this study for the diagnosis of WMD can be extended to regular health monitoring as the technique has been universally accepted as the most sensitive diagnostic tool.

PP- C9

Replication of white spot syndrome virus in primary hemocyte culture from mud crab, *Scylla serrata*

*A. Deepika, **M. Makesh** and K.V. Rajendran*

Central Institute of Fisheries Education, Panch Marg, Versova, Mumbai-400061, India
Email: mmakesh@yahoo.com

White spot syndrome virus causes high mortality in many species of penaeid shrimp leading to severe damages to shrimp culture industry. Although considerable progress has been made in understanding the viral structural proteins, little is known about the mechanism of infection and pathogenesis. The major obstacles include the lack of appropriate cell lines for WSSV infection and proliferation. Though WSSV infects a wide range of crustaceans, crabs are easy to obtain and maintain in laboratory for obtaining hemolymph for the culture of hemocytes. The present study was carried out to establish primary cell culture of hemocytes from the mud crab *Scylla serrata* as an *in-vitro* model for infectivity studies with white spot syndrome virus. Hemolymph was withdrawn from the animal in sodium citrate buffer; hemocytes were separated by centrifugation and cultured in double-strength L-15 prepared in crab saline, for adjusting the osmolality, supplemented with 5% fetal bovine serum and antibiotics (penicillin 100 IU/ml streptomycin 100 µg/ml and amphotericin B 0.25 µg/ml). The cells adhered to substrate within four hours after seeding and showed proliferation up to 72 hours. Different dilutions of WSSV inoculum prepared from infected *Penaeus monodon* were inoculated into the cultured hemocytes and cytopathic effects like detachment, rounding of cells and clear areas of depleted cells were observed after 48 hours. Cells exposed to WSSV inoculum were harvested at 0, 24, 48 and 72 hours post-infection, and the DNA was isolated. WSSV infection was confirmed by PCR and real-time PCR using WSSV-specific primers which revealed that WSSV could infect hemocytes of *S. serrata.* This preliminary study showed that mud crab hemocyte culture can support WSSV replication and it can be used as an *in vitro* tool for WSSV replication.

PP- C10

Early detection of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) in post-larvae of *Macrobrachium rosenbergii* by RT-PCR and immunological methods

Ravi Mani *and A.S. Sahul Hameed*

OIE Reference Laboratory for WTD, Department of Zoology, C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India.
E.mail: ravimicro_2004@yahoo.com

White tail disease (WTD) causes a high mortality rate in the freshwater prawn *Macrobrachium rosenbergii*. The pathogenic agent is a small virus, *Macrobrachium rosenbergii* nodavirus (*Mr*NV), 25 nm in diameter, associated with extra small virus (XSV), a virus-like particle, 15 nm in diameter. RT-PCR and immunological techniques such as Western blot and ELISA were used for early detection of MrNV and XSV in post-larval samples obtained from time-course experiments at different time intervals. Two viruses were purified from diseased post-larvae of *M. rosenbergii* by a combination of low and high speed centrifugation using sucrose and CsCl gradients to raise the antisera separately. One structural protein with molecular weight of 43 kDa (CP-43) was identified from the purified preparation of *Mr*NV, and two overlapping polypeptides of about 17 kDa (CP-17) and 16 kDa (CP- 16) were found in XSV particles by SDS-PAGE. The antisera raised against CP-43 of *Mr*NV, CP-16 and CP17 of XSV in mice were used to detect *Mr*NV and XSV by Western blot and ELISA. Published primers specific to *Mr*NV and XSV were used for the early detection of these viruses by RT-PCR and nested RT-PCR. The post larval samples collected at 3 h post infection (h p.i.) showed positive for both viruses by nested RT-PCR and negative by RT-PCR, Western blot and ELISA techniques. The samples collected at 24 h p.i. and thereafter were found to be positive for *Mr*NV and XSV by RT-PCR, ELISA and Western blot analyses.

PP- C11

Anti-quorum sensing potential of malonic acid in preventing *Vibrio harveyi* infection

Khadar Syed Musthafa*, Issac Sybiya Vasantha Packiavathy, Angusamy Annapoorani, Shunmugiah Karutha Pandian and Arumugam Veera Ravi*

Department of Biotechnology, Alagappa University, Karaikudi - 630 003, Tamil Nadu, INDIA.
Email: syed_musthafa@rediffmail.com

Vibrio harveyi is a gram-negative, luminescent, marine bacterium recognized as important bacterial pathogen in aquaculture industry. The indiscriminate and large scale usage of antibiotics leads to the development of resistance in *V. harveyi* to available antibiotics. The acquired resistance makes the antibiotic treatment become ineffective and necessitates an alternative treatment measures. One of the alternative strategies that have recently been developed to control infections caused by antibiotic-resistant *V. harveyi* is the disruption of quorum sensing (QS) system. QS mediated by auto-inducer molecules such as N-acyl homoserine lactones (AHL) and Furanosyl borate diester (AI-2) play a vital role in the regulation of biofilm formation and virulence factors production in *V. harveyi*. Thus, given the importance of warfare against emerging antibiotic resistant *V. harveyi* infection, the present study was made to search for potential anti-quorum sensing (anti-QS) compounds in preventing such infection among aquaculture organisms. The secondary metabolite malonic acid identified from the marine bacteria Bacillus spp. SS4 (GU471751) effectively inhibited the QS dependent violacein production in QS marker strain *Chromobacterium violaceum* CV026. Further, malonic acid was assessed for its anti-QS potential in reducing QS dependent factors production in *V. harveyi*. The QS dependent bioluminescence production and biofilm formation was significantly reduced when treated with malonic acid. In *in-vivo* challenging analysis also the test compound enhanced the survival of Artemia nauplii to the level of 80% against *V. harveyi* infection. Furthermore, the test compound showed no antibacterial activity against *V. harveyi*. Hence, the findings of the study evidence the anti-QS potential of secondary metabolite malonic acid and establishes an alternative treatment measure to antibiotic usage in preventing the *V. harveyi* infections among aquaculture organisms.

PP- C12

Efficacy of ivermectin against natural infections of ecto-paarasite *Caligus* sp. in Pearlspot fish, *Etroplus suratensis*

***M. Natarajan**, P.Rajesh, K.P.Jitendran, G.Thiagarajan and S.M.Pillai*

Central Institute of Brackishwater Aquaculture (ICAR), 75 Santhome High Road, Chennai 600 028
E-mail: natarajanm@ciba.res.in

The efficacy of orally administered ivermectin through feed against natural infestations of the copepod ecto-parasite *Caligus* sp. in the Pearlspot fish, *Etroplus suratensis,* was studied. Oral administration of ivermectin at 0, 0.845, 1.69 and 3.38 mg per kg fish b.wt. did not cause any noticeable adverse reactions or mortality. Feed containing 5 mg ivermectin per kg given for six days or 10 mg ivermectin per kg given for three days, did not affect the growth and survival of Pearlspot fish fingerlings. Treated fishes grew as well as the control groups when reared for 45 days in indoor FRP tanks. Ivermectin dosage of 1.4 mg/kg fish b.wt. provided adequate protection against natural infections of *Caligus sp* for at least a month in fishes reared in FRP tanks and outdoor net cages. Repeat medications may be required for protection for longer periods. After 30 days, the level of infestation was higher in the untreated control groups (0.8 to 8 nos. / fish) than in treated fishes (0 - 0.3 nos. / fish). Similarly, in outdoor net cages, natural infection of *Caligus* sp. occurred very quickly in control group compared to treated groups of Pearlspot fish. Under poor water quality conditions, the infections were severe. Providing sufficient water exchange in the rearing tanks/cages delayed the onset of parasitic infections. A dose of 1.4-1.5 mg ivermectin/kg fish b.wt. was not effective in protecting Pearlspot fish against *Caligus* sp. under poor water quality conditions. In outdoor net cages, Pearlspot fish treated with 2.38 mg ivermectin/kg fish b.wt. was adequately protected from *Caligus* infestations for 30 days and thereafter the level of infection was comparatively lower than untreated control fish (17 nos./fish versus 43 nos./fish). The lower levels of infection resulted in faster growth (113%) compared to control. Ivermectin treatment through feed at a dosage of 1.4-1.5 mg/kg fish b.wt. could be used to control ecto-parasitic copepod *Caligus sp.* infection in indoor nursery rearing of Pearlspot fish, while higher doses could be adopted for outdoor rearing. In all cases the effectiveness of ivermectin lasted for about a month.

PP-C13

Antibacterial effect of feeding artemia enriched with fucoidan of *Sargassum wightii* on *Vibrio parahemolyticus* resistance in *Penaeus monodon* postlarvae

***Arunachalam Palavesam**, Madasamy Sivagnanavelmurugan, Thangapandi Marudhupandi and Grasian Immanuel*

Department of Marine Biotechnology, Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam - 629502, Kanyakumari District, Tamilnadu
Email: plavesh06@gmail.com

A study was carried out to determine the effect of fucoidan from brown seaweed *Sargassum wightii* on *Penaeus monodon* postlarvae (PL) against *Vibrio parahaemolyticus*. Initially fucoidan was extracted from brown seaweed *S. wightii* and the yield observed was 2.832 ± 0.204%. The antibacterial activity of fucoidan against *V. parahemolyticus* was screened by agar well diffusion method and the maximum zone of inhibition observed was 15.66 ± 0.942mm at 20mg/ml concentration. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined by double dilution method and the MIC and MBC of fucoidan observed was 12 mg/ml. Then the fucoidan was enriched with artemia nauplii at four different concentrations (100, 200, 300 and 400mg/l) for 12h. These enriched artemia nauplii were fed to *P. monodon* PL for 20 days. After feeding experiment, the PL were challenged with *V. parahaemolyticus* and the mortality percentage was recorded daily untill 30 days. During challenge test, the control group displayed 64% mortality within 30 days. But in the experimental groups fed on 100 - 400mg/l fucoidan displayed only 8.0 to 40 % mortality, within 30 days of challenge experiment. The cumulative mortality index (CMI) of control group showed high mortality rate. The reduction in mortality percentage of experimental groups fed with 100 - 400mg/l fucoidan enriched Artemia nauplii over control group was ranged from 36.97 to 89.86%. The *V. parahaemolyticus* load was enumerated from the infected shrimp at an interval of 10 days during challenge experiment. In control group, the *Vibrio* load in hepatopancreas and muscle tissues increased from 10 to 30th day of challenge test. But in the experimental groups, the *Vibrio* load in hepatopancreas and muscle tissues decreased positively from10 to 30th day in all the tested concentrations (100 to 400mg/l) of fucoidan.

PP- C14

ESSA1 embryonic stem like cells from gilthead seabream: a new tool for fish bone cell biology

***Parameswaran V**, Laizé V, Gavaia PJ and Cancela ML*

Centre of Marine Sciences (CCMAR), University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
Email: paramesh@ualg.pt

Sparids are a large group of marine teleost fish, exploited and farmed for human consumption worldwide. The high levels of skeletal abnormalities in farmed fish is a major concern for aquaculture industries worldwide because of decreased survival rate of abnormal fish and increased production costs. In an effort to understand skeletal development and mechanisms of skeletal mineralization in Sparidae, we developed a pluripotent embryonic stem like cell line designated as ESSA1 derived from blastula stage embryos of gilthead seabream *Sparus aurata.* ESSA1 cells have a polygonal morphology, grow exponentially in culture (Leibovitz's L-15 medium supplemented with 5% fetal bovine serum without a feeder layer) and form dense colonies. ESSA1 cells also exhibit intense alkaline phosphatase activity, normal karyotype and are positive for stage-specific embryonic antigen-1 (SSEA1) and octamer-binding transcription factor 4 (OCT4) markers, even after 30 passages. Upon treatment with all-trans retinoic acid, these cells differentiate into neuron-like, oligodendritic, muscle and melanocyte cells, indicating their pluripotency, which was further confirmed by embryoid body formation upon seeding in bacteriological plates. For the first time, fish-derived embryonic stem like cell line could be induced to differentiate into osteogenic, chondrogenic and osteoclastic cell lineages and to produce a mineralized extracellular matrix *in vitro*. Osteogenic and chondrogenic differentiation was assessed by histological analysis including von Kossa's, alizarin red and alcian blue staining, while osteoclastic differentiation was assessed by TRAP (tartrate-resistant acid phosphatase) enzymatic activity and further confirmed by immunocytochemistry using lineage specific markers. ESSA1 cells represent a promising model for investigating mechanisms of cell differentiation towards bone and cartilage cell lineages in fish and also highlight the potential of piscine stem cell research. ESSA1 cells also represent a suitable host to study viral diseases prevalent in sparid species (e.g. viral encephalopathy and retinopathy and lymphocystis disease).

PP-C15

Development of monoclonal antibody based diagnostic ELISA for specific detection of Infectious hypodermal and hematopoietic necrosis virus (*Penaeus stylirostris densovirus*)

***Praveen Rai**, Muhammed P. Safeena, Devananda Devegowda, Deekshit V. Kumar, and Indrani Karunasagar*

Department of Fishery Microbiology, UNESCO Microbial Resource Centre (MIRCEN) for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore - 575 002, India
Email: raiprav@gmail.com

Infectious hypodermal and hematopoietic necrosis virus (IHHNV) of shrimp, recently classified as *Penaeus stylirostris densovirus* (*Pst*DNV), is one of the major viral pathogens of penaeid shrimps worldwide. Molecular detection of IHHNV in shrimp is complicated by the fact that certain virus-related sequences are integrated into the genome of *Penaeus monodon.* This would result in false positive results for infectious *Pst*DNV during the PCR based screening of the shrimp stocks. In this study, monoclonal antibodies (mAbs) were raised against recombinant structural protein (54 kDa) of PstDNV for the specific detection of infectious *Pst*DNV. The structural protein of the virus was cloned into a pET32a (+) expression vector for the production of the recombinant fusion protein with His tag. After induction, the protein was purified by Ni-NTA column chromatography and used for immunization of BALB/c mice. The SP2/0 myeloma cells were used for fusion with spleen cells of immunized mice. After single cell cloning, seven stable antibody producing clones were obtained using indirect-ELISA. The specific immunoreactivity of the mAb produced to the viral antigen was studied by dot-ELISA and further confirmed by western blotting. Since this antibody could detect *Pst*DNV infections in field samples of *Penaeus monodon* and *Penaeus vannamei* it has potential diagnostic application in detection and differentiation of infectious *Pst*DNV.

PP- C16

Farnesol - A potential anti-quorum sensing compound to prevent aquatic bacterial diseases

Arumugam Veera Ravi, *Issac Sybiya Vasantha Packiavathy, Khadar Syed Musthafa, Angusamy Annapoorani and Shunmugiah Karutha Pandian*

Department of Biotechnology, Alagappa University, Karaikudi - 630 003, Tamil Nadu, INDIA.
Email: aveeraravi@rediffmail.com

Quorum sensing (QS) - bacterial cell to cell communication system is mediated by signaling molecules called autoinducers. Many aquatic bacterial pathogens utilize these QS autoinducer molecules for the expression of various phenotypic characters including the biofilm formation, virulence factors production and bioluminescence production. Since, virulence factors production and biofilm formation are under the control of QS, targeting bacterial QS could possibly reduce the pathogenicity of aquatic bacterial pathogens to host. In view of this fact, in this present work, the test compound farnesol was assessed for its anti-Quorum Sensing (anti-QS) potential in reducing QS dependent factors production in aquatic bacterial pathogens. Farnesol exhibited 90% reduction in the QS dependent violacein production in CV026. In biofilm quantification assay, in the presence of farnesol, the target aquatic bacterial pathogens showed reduction in the formation of biofilm biomass to the level of 70%. Light microscopic and confocal laser scanning microscopic analysis revealed the visible reduction in the biofilm formation of aquatic bacterial pathogens when treated with the test compound. Further, the QS dependent bioluminescence production in *Vibrio harveyi* was also reduced to 99% when treated with the test compound. Moreover, in the presence of farnesol, Artemia nauplii showed enhanced survival rate to the level of 86% against *V. harveyi* infection. Thus, the present study evidenced the antipathogenic potential of farnesol in preventing the aquatic bacterial infection by interfering with bacterial QS system.

PP -C17

Comparative evaluation of latency-associated genes of white spot syndrome virus (WSSV) for developing PCR test to detect latent infection

***Reshma K. J**, ReenaYadav, M. Makesh, C. S. Purushothaman and K. V. Rajendran*

Central Institute of Fisheries Education (CIFE), Indian Council of Agricultural Research, Off-Yari Road, Versova, Mumbai - 400 061, India

Email: reshjanardhan@gmail.com

White spot syndrome virus is a prevalent and virulent pathogen affecting both wild and cultured penaeid shrimps worldwide. PCR is considered as an important diagnostic tool used for the detection and elimination of this pathogen in cultured systems.Conventional single step PCR, which uses the primers targeting structural proteins, is widely used for the detection of WSSV. However, one of the common problems associated with available conventional PCR tests is their inability to detect very early and latent infection. Studies showed the latent infection of WSSV in shrimps, which were verified as WSSV negative by the conventional PCR detection method, with the possibility of reactivation. Moreover, studies also showed the possibility of incorporating latency-associated gene transcripts in diagnostic tools to increase the sensitivity in order to detect even the latent infection. In the present study, seven sets of primers targeting latency-associated genes of WSSV and two sets which target structural genes of the virus and that are being widely used in PCR tests, were evaluated for their sensitivity in detecting early infection. Healthy, WSSV-free *Penaeus monodon* were injected with a known WSSV inoculum and tissues like gill, pleopod and lymphoid organ were collected from the injected shrimps at 6, 12, 24, 48 and 72 h post-injection. DNA extracted from these tissues, collected from 10 individual animals, were subjected to PCR analysis using primers targeting latency-associated genes (*wssv*151, *wssv*366, *wssv*403, *wssv*407, *wssv*427, *wssv*285 and *wssv*332) and primers used in the routine diagnosis of WSSV (*vp*28, *wssv*115). Except for primers *wssv*285, *wssv*332 and *vp*28, all other primers were able to detect 60 to 100% of the samples collected at the earliest stage of infection (6 h p.i.). Among these primers, *wssv*366 was found to detect WSSV infection in all tissues tested for all 10 samples as early as 6 h post-injection (h p.i). The other primer which could detect maximum number of samples at all time points was wssv407. Among the tissues tested, pleopod samples showed the highest rate of detection at early as well as late stage of infection.Considering consistency in the detection, the present study revealed that the primer targeting latency-associated gene, wssv366 could be an ideal candidate for developing sensitive PCR tests that could detect early and latent infection of WSSV. Further, the study showed that pleopod would be the tissue of choice for early WSSV diagnosis and since the sampling of this tissue can be done in a non-sacrificial way, this method could be used in screening broodstock more effectively.

PP -C18

Antibiotic resistant *Salmonella spp* isolated from fresh water loach, *Lepidocephalicthys guntea* (Hamilton Buchanan)

Rudra Prasad Roy[1]*, Ruksa Nur*[1]*, Min Bahadur*[2] *and Sudip Barat**[1]

[1] Aquaculture & Limnology Research Unit, Department of Zoology, University of North Bengal, District Darjeeling, 734013, West Bengal, India.
[2] Genetics & Molecular biology Laboratory, Department of Zoology, University of North Bengal, District Darjeeling, 734013, West Bengal, India.
E.mail: r.rudra85@rediffmail.com

An investigation was conducted on the antibiotic resistant *Salmonella spp* that was isolated from skin, gill and gut of a fresh water loach, *Lepidocephalichthys guntea*,. This loach has both ornamental and edible value and also very common in the rivers and streams of Darjeeling District, West Bengal, India. A total of 14 antibiotic resistant *Salmonella spp* were isolated. Confirmed identification of *Salmonella spp* was done by different biochemical procedures and polymerase chain reaction (PCR) amplification using *Salmonella spp* specific 16S rDNA primers for increasing reliability of identification. Antibiotic resistance pattern was determined by the disc diffusion method. Maximum resistance was exhibited for Penicillin-G (100%), Erythromycin (100%) and Cephalothin (100%). Moxifloxacin (20%) and Tetracycline (0%) showed minimum resistance in fish. Two isolates found in gut were resistant to seven antibiotics among the ten antibiotics studied. Three strains isolated from gill and one from skin were resistant against five antibiotics. Most of the resistant *Salmonella spp* were observed in the gut of the fish. Presence of *Salmonella spp* and its resistance against multiple antibiotics indicates that the fish was contaminated with multi antibiotic resistant enteric pathogenic bacteria. This study, therefore, provides valuable information for making policy decisions aimed at reducing microbial contamination of fish and the indiscriminate use of antibiotics. There is need for research on antibiotic susceptibility surveillance in the aquatic environments where fresh fish and water are obtained for human consumption.

PP-C19

Development of monoclonal antibody specific to recombinant capsid protein of Hepatopancreatic parvovirus (*Penaeus monodon densovirus*)

Muhammed P. Safeena, *Praveen Rai, Krishna K. Ballemoole, Devananda Devegowda, and Indrani Karunasagar*

Department of Fishery Microbiology, UNESCO Microbial Resource Centre (MIRCEN) for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore - 575 002, India

Email: safeena.mp@gmail.com

Hepatopancreatic parvovirus (HPV) more recently classified as *Penaeus monodon densovirus* (*Pm*DNV) of penaeid shrimp is an emerging shrimp virus that causes considerable economic loss to shrimp farmers and is known to infect several penaeid shrimp species. In this study, an 857 bp sequence encoding the capsid protein gene of *Pm*DNV was ligated into pET-32 a (+) expression vector and transformed to BL21 (pSBET A) E. coli competent cells. After induction, the recombinant capsid protein (48 kDa) was purified by Ni-NTA and used to immunize mice for monoclonal antibody (mAb) production. Specific mAb was raised against the recombinant capsid protein of *Pm*DNV and characterised. The SP2/0 myeloma cells were used for fusion with spleen cells of immunized BALB/c mice. After single cell cloning, five stable antibody producing clones were obtained using indirect-ELISA. The production of antibody was studied by dot-ELISA and further confirmed by western blotting. The mAbs could detect capsid protein in extracts of shrimp naturally infected with *Pm*DNV both by western blotting and dot-ELISA. Additionally, these mAbs did not exhibit cross-reactivity to extracts from uninfected shrimp or shrimp infected with other viruses which infect shrimp thus, indicating the specificity of the mAb developed. The mAb developed in this study would be useful for the diagnosis of *Pm*DNV infection in shrimp.

PP-C20

Morphological characterisation of blood cells from *Pangasius hypophthalmus*

Wanna Sirimanapong[1,2], *Kim D. Thompson*[1] , *Kan Kledmanee*[2], *Prawporn Thaijongrak*[2], *Bertrand Collet*[3], *Ei Lin Ooi*[4] *and Alexandra Adams*[1]

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
[2]Faculty of Veterinary Sciences, Mahidol University, Salaya campus, Nakornpathom, Thailand 73170.
[3]Marine Scotland-Science, Scottish Government, Marine Laboratory, Aberdeen, AB11 9DB, Scotland, UK.
[4]Novus Aqua Research Center, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
Email: ws7@stir.ac.uk

Pangasius hypophthalmus is a common cultured species in Asia, however hematologic data for this species has not yet been been established. The aim of this study was to provide basic information on the morphological characteristics of red and white blood cells from *P. hypophthalmus* for further study on its imune system. Blood samples were collected from 25 fish (150-200g). Hematologic data were presented by mean ±SD of 25 fish. The normal haematocrit value (%) for *P. hypophthalmus* blood was 39.1 ±5.7 %, the white blood cell (WBC) count was 3.0 ±0.9 ($x10^7$ cell/ml) and the total red blood cell (RBC) count was 1.7 ±0.3 ($x10^9$ cell/ml). Erythrocyte volume was 3,259.2 ±0.785 (μm^3) and volume of nucleus was 547.11 ±0.123 (µm3).

The blood smears were stained with Romanowsky stain (using a commercial staining kit) and differential blood cell counts performed. The percentage of lymphocytes in the blood was 34.4 ±8.6 (%), granulocytes 20.6 ±9.4 (%), thrombocytes 40.5 ±7.2 (%) and monocytes 4.5 ±2.1 (%). The size of *P. hypophthalmus* RBCs and WBCs were then determined. RBCs were 21.5 ±0.4 µm in length and 17.0 ±0.5 µm in wide, neutrophils ranged from between 20.7 ±1.0 - 18.4 ±1.3 µm in length, basophils between 19.7 ±2.7 - 17.8 ±1.0 µm, monocytes between 19.6 ±2.8 - 17.2 ±3.0 µm, lymphocytes between 12.1 ±1.0 - 10.2 ±0.8 µm, thrombocytes between 15.1 ±1.7, 8.2 ±1.1 and eosinophils between 20.0 ±1.2 - 18.5 ±1.5 µm. The morphologic and staining features of neutrophil, monocyte and lymphocyte of *P. hypophthalmus* are similar to channel catfish *Ictalurus punctatus*. However, the size of leukocyte was different. These data provides reliable information for use in subsequent health monitoring studies in this species.

PP-C21

Establishment of five new cell lines from the cobia, *Rachycentron canadum* (Linnaeus, 1766)

K. S. Sobhana, Githa Ann George, ***Sheethal Mary Sunny****, S. Sreedevi, K. K. Surendran, M. K. Anil1, B. Santhosh[1], C. Kalidas[2] and P. U. Zacharia*

Central Marine Fisheries Research Institute, Ernakulam North P. O., Kerala - 682 018, India
[1]Vizhinjam Research Centre of Central Marine Fisheries Research Institute, Vizhinjam, Thiruvananthapuram, Kerala - 695 521, India
[2]Mandapam Regional Centre of Central Marine Fisheries Research Institute, Mandapam Camp, Tamil Nadu - 623 520, India
E-mail: shee.sunny@gmail.com

Cobia, *Rachycentron canadum* is a highly priced, commercially important food fish distributed worldwide in tropical and warm-temperate waters. Cobia has the qualities that define an excellent candidate species for aquaculture and has an emerging global potential for mariculture. Central Marine Fisheries Research Institute has been successful in broodstock development of the species and has successfully bred Cobia for the first time in India. One of the major limiting factors in hatchery rearing and culture of marine fish is the occurrence of diseases, specifically those caused by pathogenic viruses which causes heavy economic losses. Iridovirus and nervous necrosis virus (nodavirus), the two emerging viral pathogens have been identified as the most important pathogens infecting marine fish. The establishment of healthy and sensitive fish cell lines is essential for isolation, identification and characterization of infectious viruses from fish. For development of precise diagnostics and prophylactics of the viral pathogens, establishment of cell lines is very important. Fish cell lines also have widespread application in the fields of toxicology, cytogenetics, transgenics etc.

The present paper discusses the development of 5 successful cell lines from the brain, fin, heart and caudal peduncle tissues of *R. canadum*. Tissues from various organs such as fin, gill, caudal peduncle, heart, liver, spleen, kidney and brain were evaluated for developing cell lines by trypsinisation method. The culture medium used was Leibovitz L-15 supplemented with 20% foetal bovine serum (FBS). Among the different organs, cells from brain, fin, heart and caudal peduncle gave rise to confluent monolayers which were subcultured successfully and have crossed >40 passages. The cell lines derived from the above tissues viz., RC3BrTr, RC4F1Tr, RC4F2Tr, RC4H1Tr and RC4CPTr have reached passages upto 58, 65, 74, 46 and 51 respectively. Chromosome counts of metaphase spreads from the cobia cell lines revealed that the chromosome numbers varied from 42 to 55. All the five cell lines derived have been successfully cryopreserved and the viability of cells were found to be >90% when revived after 6 months of storage in liquid nitrogen.

PP -C22

A SYBR Green-based quantitative real-time PCR for hepatopancreatic parvovirus (HPV) infecting *Penaeus monodon*

***Reena Yadav**, Makesh M, Aparna Chaudhari and Rajendran K.V.*

Central Institute of Fisheries Education, Panch Marg, Versova, Andheri (W), Mumbai- 400061, India

Email: ren1902@gmail.com

Hepatopancreatic parvovirus (HPV) infects *Penaeus monodon* and causes mortality in the larval stages. Further, it has been implicated in the growth retardation in cultured *P. monodon*. Here, we developed a SYBR Green-based real-time PCR for the absolute quantification of the virus. A 441 bp PCR amplicon was generated and cloned in pTZ57R/T vector and the plasmid copy number was estimated based on the concentration and molecular mass. A 10-fold serial dilution of this plasmid DNA from 2 x 10^9 copies to 2 copies was prepared and used as a standard. The primers were tested initially using the standard on a conventional PCR format to determine the linearity of detection. Real-time PCR primers were designed using the cloned sequence of HPV. The standards were further tested on real-time PCR format using SYBR Green chemistry and a standard curve was generated based on the Ct values from three well replicates for each dilution. The assay was found to be sensitive, specific and reproducible with wide dynamic range (2 x 10^9 down to 2 copies with coefficients of regression (R^2) > 0.99, calculated average slope -3.196). The intra-assay and inter-assay coefficients of variation of the Ct values ranged from 0.26% to 0.94% and 0.43% to 2.49%, respectively. This assay was further tested to detect and quantify HPV in field samples of *P. monodon* post-larvae.

PP- C23

Development of DNA based diagnostic PCR for simultaneous detection of multiple viral infection of crustaceans

***S. A. Mohan.**, M. N. Venugopal and Indrani Karunasagar*

Department of Fisheries Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575 002, India.

E mail I D: saminu@gmail.com

The greatest risk in shrimp aquaculture is the presence of viruses in larval stocks. The presence of multiple viruses in precipitating disease outbreaks in shrimp hatcheries and farms has been a concern worldwide. Early detection of viruses can help in adopting best management practices. To achieve this objective, DNA based test PCR and qPCR was developed for the rapid, sensitive and specific detection of more than one virus simultaneously. In this regard, eleven different primer combinations for multiplex PCR for detecting the DNA viruses White spot syndrome virus, Hepatopancreatic parvovirus, Monodon baculovirus and Infectious hypodermal and hematopoietic necrosis virus have been developed. They could detect two, three or four viruses simultaneously. The method was designed for screening all life stages of shrimp. The developed multiplex PCR was as sensitive as single step PCR and could detect upto picogram level of viral DNA. Uniplex real-time PCR for detection and quantification of viral DNA was developed that could detect as low as 10 copies of DNA of WSSV (0.2 fg), 10^5 of HPV (0.28 fg), 10^4 of MBV (0.31 fg) and IHHNV (0.41 fg). A standard curve was created for all the four viruses. Duplex real-time PCR was developed for the shrimp virus combination HPV-MBV, HPV-IHHNV and MBV-IHHNV. The method was shown to be specific, sensitive, less-time consuming and successful in detecting shrimp viruses in cultured as well as wild caught shrimps.

PP-D1

Functional characterization of crustin and phosphatase, defense proteins of black tiger shrimp *Penaeus monodon*

***Debashis Banerjee**, Biswajit Maiti, M.N. Venugopal and Indrani Karunasagar*

UNESCO-MIRCEN for Marine Biotechnology, Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575002, India.

Email: debashis.banerjee@gmail.com

Antimicrobial proteins and peptides are among the first line of nonspecific defense against invading pathogens in crustaceans. The objective of this study was to clone and express the molecules and characterize the antimicrobial property of recombinant crustin and phosphatase, isolated from *Penaeus monodon*. The genes encoding the molecules were PCR amplified, cloned and expressed in *Escherichia coli*. The expression was studied in 15% SDS-PAGE and the protein/peptide purified by Ni-NTA affinity chromatography. The SDS-PAGE showed distinct bands of ~22 and ~ 25 kDa for crustin and phosphatase respectively. The antimicrobial effect of the recombinant protein/peptide was checked by solid phase agar diffusion assay against twelve different bacteria, viz. *Bacillus coagulans, B. cereus, Staphylococcus aureus, S. epidermidis, Aeromonas hydrophila, Salmonella weltevreden, S. paratyphi, Vibrio parahaemolyticus, V. harveyi, V. campbellii, V. anguillarum* and *V. alginolyticus*. The agar diffusion assay showed clear zones of inhibition by both the recombinant molecules against all the bacterial cultures used in the study. There was inhibition of both gram positive and gram negative pathogens including the potent shrimp pathogens, *V. harveyi* and *V. campbellii*. The minimum inhibitory concentration (MIC) of the protein/peptide against all the twelve bacterial isolates was determined by microtitre plate method and was in the range of 5-160 µg/ml for phosphatase while for crustin it was 2.5-160 µg/ml. The antimicrobial activity of recombinant crustin and phosphatase seen in this study is a pointer to their potential application in control of bacterial pathogens in aquaculture and some human pathogens.

PP-D2
ProPO-system mediated innate response in shrimp immunity

Walaiporn Charoensapsri,[1] *Piti Amparyup,*[1, 2] *Ikuo Hirono,*[3] *Takashi Aoki*[3] *and Anchalee Tassanakajon*[1]

[1]Center of Excellence for Molecular Biology and Genomics of Shrimp, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.
[2]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Road, Klong1, Klong Luang, Pathumthani 12120, Thailand.
[3]Laboratory of Genome Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan.
Email: walaiporn_lai@hotmail.com

The prophenoloxidase activating system (proPO-system) has long been considered as one of the major innate responses that plays an essential role in the defense against bacterial and fungal infections in many invertebrate species. So far, the proPO-system mediated response in shrimp immunity was obscure and not yet well understood. Using the recent advance of RNA interference (RNAi) technologies, we have succeeded in investigation of the potential function of two *Pm*PPAEs (*Pm*PPAE1 and *Pm*PPAE2) and two *Pm*proPOs (*Pm*proPO1 and *Pm*proPO2) genes in the black tiger shrimp, *Penaeus monodon*, proPO-system. Moreover, a significant increase in the cumulative mortality and an increase in the bacterial numbers in the knockdown shrimp hemolymph were observed after challenge with the highly pathogenic bacterium, *Vibrio harveyi*. Taken together, these data provide the first evidence on clarification of the important functions of the proPO-system in shrimp immune defense against *V. harveyi* infection.

PP-D3

Using liquid chromatography-electrospray ionization-mass spectrometry as a system-wide metabolomic platform to monitor the perturbation in shrimp hemocyte metabolites induced by white spot syndrome virus infection

I-Tung Chen,[1] *Der-Yen Lee,*[2] *Geen-Dong Chang,*[2] *KC Han-Ching Wang,*[3] *and Chu-Fang Lo*[1]

[1]Institute of Zoology, College of Life Science, National Taiwan University, Taipei, Taiwan.
[2]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan.
[3]Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan.
Email: d99b41004@ntu.edu.tw

Shrimp white spot disease (WSD) results in high mortality and great economic losses to the world's shrimp industry. The causative pathogen is the white spot syndrome virus (WSSV), and while WSSV's pathogenesis is still poorly understood, like all other viruses, it is an obligate parasite that reprograms the host metabolism to biosynthesize all the macromolecules needed for viral replication. To elucidate the pathogenesis of WSSV, in this study, we established a shrimp metabolomics platform to study metabolic activity within the virally infected shrimp hemocytes. This platform used liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) to measure the levels of different intracellular metabolites in a system-wide model with multiple biological pathways, including glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway (PPP). In this study, all observations were made in shrimp hemocytes with nano molar sensitivities at the different WSSV infection stages. These metabolomic observations allow us to understand the actual physiological phenomena that occur during virus-host interactions *in vivo*. We found that WSSV reprogrammed glucose metabolism and the pentose phosphate pathway, both of which are related to energy production. WSSV also induced the biosynthesis of macromaterials such as nucleic acids, which are essential to support the viral life cycle. The next step will be to reconfirm these observations using other *in vivo* biochemical assays. Meanwhile, this is the first time that a systems level metabolomic platform has been established for shrimp, and in the future it should continue to be very useful for understanding the biological networks induced by WSSV infection.

PP-D4

Assessment of infectivity potential of Indian white spot syndrome virus isolates

***Rosalind George M**, Riji John K, Mohamed Mansoor and M J Prince Jeyaseelan*

Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi 628008.
Email: rosalindmg@yahoo.com

An assessment of the infectivity potential of white spot syndrome virus (WSSV) was carried out using five isolates obtained from different parts of India. The WSSV infection is one of the most severe problems in shrimp farms world over. The current study was formulated to test whether there is any difference in the ability of the virus in inducing the infection leading to the typical 100% mortality with or without the clinical signs when administered parenterally. The samples used for the source virus was tested for the presence of WSSV by single step PCR using OIE primers. The individual virus isolate preparations were injected intramuscularly to juvenile tiger shrimps (*Penaeus monodon*) with equal quantified doses at different dilutions and maintained in individual tanks. Mortality pattern of the experimental animals were monitored at regular intervals. The isolates were genotyped with reference to VNTR, deletion and variable regions and select functional ORFs. Results of the study indicated that while four of the five isolates killed all the experimental animals in 23-136 h, one isolate which was also positive by single step PCR, killed only 45.83 % of the animals at the end of experimental duration of 20 days. VNTR analysis of ORF 94 of the five isolates indicated that 100 % mortality was caused by isolates with 12, 7 and 5 TRs, while the low virulent isolate (Q13) showed multiple bands revealing the presence of 17, 10 and 6 TRs. Analysis of polymerase gene showed an additional band for Q13 isolate indicating difference from the other four isolates. While four highly virulent strains tend to group into distinct genotypes, the low virulent strain could not be classified as a single genotype due to the presence of multiple bands in two of the nine genomic regions analysed.

PP-D5

Cannibalism, the only transmission route for Appendages Deformities Syndrome (ADS) in monoculture ponds of *Macrobrachium rosenbergii* in Andhra Pradesh

Haribabu P,[1] *Sahul Hameed A.S,*[1] *Pani Prasad K,*[3] *Reddy A. K,*[3] *Pal A.K,*[3] *Nazeer Basha*[2] *and Mukherjee S.C* [3]

[1]College of Fishery Science, SVVU, Muthukur 524 344 SPSR Nellore Dist, AP, India.
[2]Aquaculture Biotechnology Division, C. Abdul Hakeem College, Melvisharam, Vellore, TN, India.
[3]Central Institute of Fisheries Education, ICAR, Versova, Andhri (W), Mumbai 400 061, India.
Email: hbabu208@gmail.com

During the period 1999-2005, the giant freshwater prawn, *Macrobrachium rosenbergii*, a native species became the choicest candidate for its aquaculture potential to the low saline shrimp (*P. monodon*) farming areas of the coastal Andhra Pradesh, India. Freshwater prawn production from aquaculture was 7,140 MT in the year 2000 and 35,870 MT in 2004. During this period 71 freshwater prawn hatcheries have registered with MPEDA, GOI with a combined installed capacity of 1.83 billion post larvae. This facility was in addition to the 237 shrimp (*P. monodon*) hatcheries out of which few have catered to the needs of the freshwater prawn seed demand as their off season business.

However, by the end of year 2005, the freshwater prawn farmers felt that they were left in the lurch by *M. rosenbergii* because of the novel and endemic status of Appendages Deformities Syndrome (ADS) which has not been reported from any other part of the world where *M. rosenbergii* is under aquaculture use till date. The economic loss experienced by the prawn farmers on account of ADS was estimated approximately at US $ 30 million per annum during 2003, 2004 and 2005 (US $ 1= INR 45 approx for that period).

The present report has been a part of the research being carried out from 2002 and the observations have been continued since then. The ADS affected prawn population was free from any of the known dominant shrimp/prawn viruses. The ADS was observed in the sexually matured prawn only so, this was not a problem at hatchery or early nursery ponds. The affected prawn population did not grow further and continued to survive but for cannibalism by the intermolts. Experimental evidence supported to state that the endemic status of ADS is non contagious by cohabitation but through cannibalism only. The details are discussed with an attempt on case definition.

PP-D6

Comparative proteomic profile of shrimp hemocytes during WSSV infection

Yun-Tzu Huang,[1] *I-Tung Chen,[2] Suh-Yuen Liang,[3] Shu-Yu Lin,[3] Yen-Chen Chen,[4] Jan-Ming Ho,[4] Geen-Dong Chang,[5] Kay-Hooi Khoo,[3, 6] Chu-Fang Lo[2] and KC Han-Ching Wang [1]*

[1]Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan.
[2]Institute of Zoology, College of Life Science, National Taiwan University, Taipei, Taiwan.
[3]NRPGM Core Facilities for Proteomics & Glycomics, Academia Sinica, Taipei, Taiwan.
[4]Institute of Information Science, Academia Sinica, Taipei, Taiwan.
[5]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan.
[6]Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.
Email: L66994013@mail.ncku.edu.tw

Since 1990, outbreaks of shrimp white spot disease have caused huge economic losses. The causative pathogen is the white spot syndrome virus (WSSV), the pathogenesis of which is still unclear. In a previous study, we monitored the concentrations of glucose and lactate in shrimp hemolymph in the early stage of WSSV infection, and found that glucose was consumed while lactate was accumulated. The same phenomena are among those seen in the Warburg effect, which is a classical metabolic feature in most cancer cells. To explore the possibility that WSSV might also induce a Warburg-like effect, we performed a comprehensive study using a label-free LC-MS/MS quantitative proteomics platform. The advantages of this quantitative proteomic approach include high mass accuracy, sensitivity and high-throughput. In shrimp hemocytes collected at the WSSV genome replication stage, the expression of over 600 identified host proteins was significantly changed compared to control hemocytes. From these data, we identified several biological networks that appear to be involved in the early stage of WSSV pathogenesis. The altered biological networks include glucose metabolism, the pentose phosphate pathway, HIF-1α, and the PI3K/AKT/mTOR signaling pathway. These results not only provide a "big picture" overview of the interrelated metabolic effects caused by WSSV infection, they also support the hypothesis that WSSV is in fact inducing a Warburg-like effect in infected cells. These results are a very useful starting point for subsequent studies that can look more closely at the details of the virus-host interactions.

PP-D7

Penaeus monodon thioredoxin restores the DNA binding activity of oxidized WSSV IE1

Jiun-Yan Huang,[1] *Wang-Jing Liu,*[1] *KC Han-Ching Wang,*[2] *Geen-Dong Chang* [3] *and Chu-Fang Lo* [1]

[1]Institute of Zoology, College of Life Science, National Taiwan University, Taipei, Taiwan, ROC.
[2]Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan, ROC.
[3]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan, ROC.
Email: d94b41003@ntu.edu.tw

Thioredoxin (Trx) is a multi-function redox protein that controls the function[s] of its target proteins via oxidation and reduction (redox reaction). A previous study reported an increase in the number of thioredoxin EST clones when shrimps (*Penaeus monodon*) were infected with white spot syndrome virus (WSSV). In the present study, we used a shrimp cDNA microarray to analyze the expression profiles of Trx and Trx-related genes in *P. monodon* gills following WSSV infection. Our results showed that the Trx-related genes were not significantly affected and that *P. monodon* Trx (PmTrx) was the only gene to be up-regulated. The transcriptional and translational expression levels of PmTrx were further confirmed by real time PCR and immunoblotting. The functional motif of Trx is Cys-X-X-Cys (CXXC) and interestingly, several Trx target proteins are known to contain this same motif. A search for the CXXC motif in the WSSV genome produced over 70 matches, one of which was located in the WSSV immediate early gene #1 (IE1). IE1 is one of the most important non-structural genes of WSSV, and since it also has DNA binding activity, which is a function that is often under Trx redox control, the interactions between PmTrx and WSSV IE1 were selected for further study. A GST pull-down assay and a co-immunoprecipitation assay both showed that PmTrx can interact with the WSSV IE1. An electrophoretic mobility shift assay (EMSA) further showed that the DNA binding activity of WSSV IE1 was controlled by the redox reaction, and that PmTrx restored the DNA binding activity of the inactivated oxidized WSSV IE1. By using an LC/ESI/MS system, we also found that the oxidative stress of WSSV-infected shrimps changes significantly at different hpi. Taken together, these results suggest that the host defense molecular Trx might be hijacked to rescue WSSV IE1's functional activity, which in turn would benefit the virus.

PP-D8

Dscam expression was evaluated after bacterial and viral challenges in *Litopenaeus vannamei*

Hsin-Yi Hung, *Chung-Wei Lee, Pin-Hsiang Cho and KC Han-Ching Wang*

Institute of Biotechnology, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan 701, Taiwan.
Email: L66991031@mail.ncku.edu.tw

In arthropods, the Down syndrome cell adhesion molecule (Dscam) is believed to be involved in "specific immune priming". The extracellular region of arthropod Dscam can generate a great number of isoforms via mutually exclusive alternative splicing. This variability has been shown to function as a neural wiring adaptor in neural wiring and an antigen-specific receptor in arthropod special immune responses. In shrimp Dscam, in addition to the extracellular region, cytoplasmic tail variants arise through the alternative splicing of 8 elements. These variants are potentially able to trigger different subsequent signal transductions. This study is the first to investigate the responses of shrimp Dscam during shrimp pathogenic invasion by *Vibrio harveyi* and white spot syndrome virus (WSSV). Expressions of shrimp Dscam and variable cytoplasmic tail variants were stimulated by the injections of *V. harveyi* and WSSV. The *Vibrio*-induced shrimp Dscam isoforms were subsequently collected and grouped. A phylogenetic analysis showed that half of the major *Vibrio*-induced shrimp Dscam isoforms were grouped into one clade that was clearly separated from the normal shrimp Dscam isoforms. These results suggested that shrimp Dscam may be involved in responses against bacterial and viral invasions. Our next step will be to investigate whether the specific *Vibrio*-induced Dscams show specific inhibition activity against *V. harveyi*.

PP-D9

cDNA cloning and expression analysis of astakine gene, *MjAstakine*, in Kuruma Shrimp *Marsupenaeus japonicus* and change of hemocyte count on *MjAstakine* knockdown

Mari Inada,[1] *Junichi Nishi*,[2] *Maki Yoshimine*,[2] *Keisuke Kihara*,[2] *Tomoya Kono*,[3] *Raja Sdhakaran*,[4,5] *Terutoyo Yoshida*,[4] *Masahiro Sakai*[4] *and Toshiaki Itami*[4]

[1] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[2] Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[3] Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuenkibanadai-nishi 889-2192, Miyazaki, Japan.

[4] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[5] Department of Biotechnology, Thiruvalluvar University, Serkadu - 632 106, Vellore, Tamilnadu, India.

Email: mari_k_p_sky_melody@yahoo.co.jp

In vertebrates, cytokines have been known to regulate hematopoiesis and immune responses. In invertebrates, however, information about cytokines or cytokine-like factors concerning the hematopoiesis is very few, although the importance of the hemocytes has been well known in crustacean immune responses. Astakine, the first cytokine-like factor, found in crustacean can induce the shrimp hematopoietic stem cell differentiation. We present here the entire cDNA sequence (1,589 bp) of the kuruma shrimp *Marsupenaeus japonicus* astakine (*MjAstakine*). The open reading frame of *MjAstakine* encoded a protein of 124 amino acids with an estimated mass of 13.3 kDa. Sequence homology of *MjAstakine* was 79.8% and 52.4% to these of the tiger shrimp *Penaeus monodon* and freshwater crayfish *Pacifastacus leniusculus*. Amino acid sequence and 11 cysteines were highly conserved in prokineticin domain of crustaceans. In healthy shrimp, *MjAstakine* mRNA was highly expressed in the brain and hemocytes. On the other hand, *MjAstakine* mRNA showed low level expression in hepatopancreas and ovary. When white spot syndrome virus (WSSV) was injected into the kuruma shrimp, *MjAstakin*e expression in the hemocytes reached its peak one day and decreased to its normal level 5 days after the injection. On re-collection of hemolymph, hemocyte count increased in control group. On the other hand in *MjAstakine* knockdown group, hemocyte count decreased. This study was supported, in-part, by research fellowships of the Japan Society for the promotion of science for young scientists, the research and development program for new bio-industry initiatives and the University of Miyazaki's program for the support of women in sciences.

PP-D10

Prevalence of White Spot Syndrome Virus, Monodon Baculovirus and Hepatopancreatic Parvo - like viral infections in shrimp hatcheries of Visakhapatnam Coast

Janakiram P,[1] *Jayasree L* ,[2] *Sivaprasad B* ,[1] *Geetha G.K* ,[1]*and Veerendrakumar M* [1]

[1]Dept. of Marine Living Resources, Andhra University, Visakhapatnam - 530 003, A.P.
[2]Central Marine Fisheries Research Institute, Karwar Research Centre, Karwar, Karnataka-581301.
Email: pasupuleti9999@gmail.com

A survey was undertaken on the prevalence of viral infections viz., White Spot Syndrome Virus and Hepatopancreatic Parvo-like Virus in the brood stock and larvae of *Penaeus monodon* during 2009 for two cycles. The brood stock and post larvae were collected from ten different hatcheries along Visakhapatnam Coast and screened for the presence of WSSV and HPV infections. Samples were subjected to Flegel's rapid test, histopathological and PCR studies and found 30% of the brood stock was infected with WSSV and 15% with HPV infections. Prevalence of infection with WSSV in post larvae 25% and 50-60% of post larvae were infected with HPV infection. Double infections of MBV and HPV were most common in both brood stock and postlarvae of shrimp.

PP-D11

*Pm*MasSPH1, a masquerade-like serine proteinase homologue plays a role in the proPO system and immunity of shrimp

Miti Jearaphunt,[1] *Piti Amparyup,*[1,2] *Walaiporn Charoensapsri,*[1] *and Anchalee Tassanakajon* [1]

[1] Center of Excellence for Molecular Biology and Genomics of Shrimp, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.
[2] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Paholyothin Road, Klong1, Klong Luang, Pathumthani, 12120, Thailand.
Email: miti.jea@gmail.com

Clip domain serine proteinase homologues (clip-SPHs) play critical roles in various biological functions including prophenoloxidase (proPO) system. A *Pm*MasSPH1 from the shrimp *Penaeus monodon* is a multifunctional pattern recognition molecule in shrimp defense. In this study, RNAi-mediated silencing of the *Pm*MasSPH1 gene, by injection of double-stranded RNA (dsRNA) corresponding to the *Pm*MasSPH1 gene in shrimp, significantly reduced *Pm*MasSPH1 transcript levels. The total PO activity in shrimp hemocytes from *Pm*MasSPH1 knocked down shrimp was reduced to 30% compared to the levels seen in the GFP dsRNA control, suggesting that *Pm*MasSPH1 is involved in the proPO system. The RNAi-mediated silencing of the *Pm*MasSPH1 gene also affects the transcript expression levels of some other genes in proPO system (*Pm*MasSPH2 and PmPPAE2, a proPO-activating enzyme) and antimicrobial peptides (penaeidins and crustins). The results suggest that *Pm*MasSPH1 plays a role in shrimp immunity.

PP-D12

Identification and Expression Analysis of Toll interacting protein gene, *MjTollip,* in kuruma shrimp *Marsupenaeus japonicus*

Keisuke Kihara,[1] *Mari Inada,*[2] *Junichi Nishi,*[1] *Maki Yoshimine,*[1] *Tomoya Kono,*[3] *Raja Sdhakaran,*[4, 5] *Terutoyo Yoshida,*[4] *Masahiro Sakai*[4] *and Toshiaki Itami*[4]

[1] Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.
[2] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki Pref. 889-2192, Japan.
[3] Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuenkibanadai-nishi 889-2192, Miyazaki, Japan.
[4] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.
[5] Department of Biotechnology, Thiruvalluvar University, Serkadu - 632 106, Vellore, Tamilnadu, India.
Email: ad11002@student.miyazaki-u.ac.jp

Toll receptor signaling pathway in invertebrates is activated by infections of Gram-positive bacteria and fungus, and subsequently induces the antimicrobial peptides. In vertebrates, Toll-like receptor (TLR) signaling pathway prevents the infection and is known as an important factor in the immune system. Excessive activation of the immune system by the TLR signaling pathway is suppressed by Toll interacting protein (*Tollip)* in vertebrates. However, there is no information of Tollip in crustaceans such as shrimp. In this study, we have cloned *Tollip* from kuruma shrimp *Marsupenaeus japonicus* (*MjTollip*). Degenerate primers were designed in the conserved region of the C2 domain from insects *Tollip* gene. PCR was performed using the cDNA prepared above to obtain the initial predicted sequence. Based on the partial nucleotide sequence of the *Tollip* obtained by PCR, we performed rapid amplification of cDNA ends (RACE) and got full length cDNA of *MjTollip*. The full length cDNA of *MjTollip* consists of 1,062 bp with 816 bp open reading frame (ORF) encoding 272 amino acids. The deduced amino acid sequence of *MjTollip* showed 49.6 % and 48.4 % identity with sequences of European honey bee (*Apis mellifera*) Tollip and flour beetle (*Tribolium castaneum*) Tollip. *MjTollip* has C2 domain which has the ability to bind to phospholipid calcium-dependently and CUE domain which has a function of the ubiquitination to *Tollip*. Phylogenetic analysis revealed that *MjTollip* adjoin a group of insects. By the expression analysis of *Tollip* in the healthy kuruma shrimp, gene expression was confirmed with gill, brain, nerves, blood lymph, lymphoid organ, stomach, midgut gland, heart and intestine. The highest expression was found in blood lymph, whose expression was 57 hold higher than that of the gill.

PP-D13

A new avenue for development of prophylactic vaccine for *Penaeus monodon* by N-terminal bacterial lipid modification of WSSV viral targets

Subramani Kumar, *Vidhyapriya Murugan, Anand Kumar Pemula, Chandru Kamalakkannan and Krishnan Sankaran*

Centre for Biotechnology, Anna University, Chennai-600 025, India.
Email: karthicskumar@gmail.com

Aquaculture, especially shrimp farming, is a major economic activity of our country supporting a large coastal population. Owing to the enormous commercial potential there are intensive and highly competitive culture practices to increase the number of harvests and the number of animals per harvest. However, the yields have been severely affected by the frequent outbreak of infectious diseases especially White spot disease in shrimps. The global annual economic loss has been estimated to be about $3 billion and it is rising without effective strategies for prophylaxis and control. However several new strategies are evolving worldwide for prophylactic vaccine development with emphasis on inducing long-lasting protective immunity. Adjuvant categories like mineral salts, microorganism-derived adjuvant, emulsions, cytokines, polysaccharides, lipids and oils have been well known, but limiting in use due to side effects. Based on the proven superior adjuvant qualities, we are using bacterial N-terminal lipid-modification, N-acyl S-diacyl glceryl Cysteine, as a new approach. We have chosen three WSSV viral targets VP28, VP281 & ICP11 for developing vaccine. All the three WSSV viral targets were attempted for bacterial lipid modification by Sec-dependent and TAT-dependent strategies. We achieved the lipid modification by both the ways only for WSSV-ICP11; the other two WSSV viral targets were achieved by TAT-dependent expression. The localisation of the lipo-form of WSSV viral targets in the *E.coli* membrane has been studied. The lipid-modified proteins are expected to increase the stability and trigger more antigencity of these native protein targets in shrimps. If proven, lipid modification by bacterial recombinant method could play a crucial role for the development of prophylactic vaccines for infectious diseases in aquaculture.

PP-D14

Prevalence of certain viral pathogens in the brood stock of Black Tiger Shrimp, *Penaeus monodon* along the coast of Andhra Pradesh, India

P. Ram Mohan Rao[1] *and K. SreeRamulu*[2]

[1] Asst.Director of Fisheries, State Institute of Fisheries Technology, Kakinada-533002, Andhra Pradesh, India.
[2] Head, Department of Zoology, Andhra University, Visakhapatnam-530 003, Andhra Pradesh, India.
Email: mohanponnapalli@yahoo.co.in

India is the second largest aquaculture production country in the world with a production of 2.47 million tones next to China. At present, 300 shrimp hatcheries are in operation in India and 178 of them are spread along the coast of Andhra Pradesh viz., 31 in North Andhra, 72 in Central Andhra and 75 in South Andhra. Andhra Pradesh being the leading aquaculture state in India also suffers from serious aquatic animal diseases. Three important viruses viz, White Spot Syndrome Virus (WSSV), Monodon Baculo Virus (MBV) and Hepatopancreatic Parvo-like Virus (HPV) were analysed in the present study to assess the prevalence in shrimp brood stock. A total of 428 samples are tested by PCR throughout the coast of Andhra Pradesh. Out of these, 58.6% tested positive for WSSV, 25%for MBV and 16.8% for HPV. In North Andhra Region- the percentage of positivity is 53.3% for WSSV, 31.1% for MBV and 19.3% for HPV. In Central Andhra Region, 60.6% are positive for WSSV, 21.3% for MBV and 15% positive for HPV. In South Andhra Region, the percentage of positivity for WSSV is 61.6%, 23.3% for MBV and 16.5 % for HPV. Among the samples, 160 samples are exclusively positive for WSSV (37.4%), 35 are exclusively positive for MBV (8.2%) and 26 (6.1%) are positive exclusively for HPV. Only 108 samples are free from all viruses (Negative) (25.2%) and 11 samples are positive for all the three viruses of WSSV, MBV and HPV (2.6%). In the remaining samples, 53 are positive for WSSV and MBV (12.4%), 26 are positive for WSSV and HPV (6.1%) where as 9 samples are positive for MBV and HPV (2.1%).

PP-D15

Presence of multiple viruses in newly introduced White leg shrimp, *Penaeus vannamei* and assessment of their risk to aquaculture in India

***Rajeish Moger**, S. A. Mohan, M. N. Venugopal and Indrani Karunasagar*

UNESCO Centre for Biotechnology-MIRCEN, Department of Fisheries Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575 002, India.

Email: rajbelke@gmail.com

The culture of tiger shrimp, *Penaeus monodon* is facing disease problems mainly due to viruses that have resulted in severe economic loss to the shrimp farmers not only in other countries of the world, but also in India. To overcome this situation, diversification of species has been an approach that the Indian aquaculture industry has adopted by bringing in *Penaeus vannamei,* an exotic, specific pathogen free marine shrimp, a native of South America. This was recently introduced and its culture is fast expanding. Knowledge on the viral pathogens associated with this shrimp during culture operation is essential for the sustainability of its farming.

A total of 86 shrimp samples, collected from both East and West coast of India, were analyzed for DNA viruses like White spot syndrome virus (WSSV), Monodon baculovirus (MBV), Hepatopancreatic parvovirus (HPV) and Infectious hypodermal and hematopoietic necrosis virus (IHHNV) using both non-nested and nested PCR. Reverse transcription-polymerase chain reaction (RT-PCR) was carried out to detect RNA viruses like Taura syndrome virus (TSV), Yellow head virus (YHV), Infectious myonecrosis virus (IMNV) and Laem-Singh virus (LSNV). Out of 86 shrimp samples screened, 62 were positive for WSSV, 18 each for HPV and MBV and 28 for IHHNV. RNA viruses were absent in all the samples. Infection with more than one virus was observed in 44 samples screened. However, no signs of disease due to these viruses were observed in any of the shrimp samples tested. Any environmental stress to the animals is likely to result in outbreak of full-blown disease. It is important to recognize the risks and threats associated with the introduction and plan appropriate management strategies.

The study clearly proves that the introduction of *P. vannamei* as an alternative to *P. monodon* will not be a panacea to the disease problems faced by farmers involved in shrimp aquaculture as multiple viruses have been found associated with *P. vannamei.*

PP-D16

Molecular expression of potential immunogenic molecules for the treatment of White Muscle Disease in *Macrobrachium rosenbergii*

***Naveen Kumar S**, Dilip Kumar and Indrani Karunasagar*

UNESCO-MIRCEN for Marine Biotechnology, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore 575002, India.
Email: aquas510@gmail.com

Macrobrachium rosenbergii is a widely cultured species in many parts of the world and recently, the global production suffered severe losses due to the occurrence of White Muscle Disease (WMD). WMD affect larvae and post larvae of *M. rosenbergii* causing 100% mortality within 2 to 3 days post infection. *Macrobrachium rosenbergii Nodavirus* (*Mr*NV), an RNA virus with two ss RNA positive-sense genome and Extra Small Virus (XSV) which is also an RNA virus with a positive-sense ss RNA as its genome are responsible for WMD. Invertebrates are reported to lack true adaptive immunity; however, recent reports of existence of high degree of memory and specificity in some invertebrates have opened new vistas for study of the immunogenic potency of recombinant proteins for the treatment of invertebrate diseases.

Total RNA was extracted from sample showing clinical signs of WMD that tested positive by RT-PCR. cDNA of RNA1 and RNA2 of *Mr*NV and RNA of XSV was synthesized using reverse transcriptase enzyme. cDNA's were PCR amplified and sequenced. Antigenicity of coding regions was predicted using a computer program generated Kyte Doolittle hydropathy profile. Genes encoding the N-terminal region of *Mr*NV capsid protein, B2 protein and XSV capsid proteins were expressed using prokaryotic expression system. 3126 bp of sequenced RNA1 contains two Open reading frames (ORF's) encoding two non-structural proteins, an RNA-dependent RNA polymerase (RdRp) and B2 protein with an estimated size of 15 kDa. Sequenced RNA2 has 1175 bp consisting of one ORF encoding capsid protein of *Mr*NV with an estimated size of 43 kDa. XSV genome contains overlapping coding sequence of 16 and 17 kDa capsid proteins. SDS-PAGE analysis of the overexpressed genes of N- terminal region of *Mr*NV capsid, B2 and XSV capsid had bands corresponding to 36, 30 and 21 kDa size and proteins had an estimated pI of 11.05, 5.36 and 9.89 respectively. Expressed genes were confirmed by sequencing. Studies on *in vivo* immunogenic potential of the expressed proteins are in progress.

PP-D17

Studies on viability of monoclonal antibodies to White spot syndrome virus in hepatopancreas and haemolymph of the *Penaeus monodon* for prophylaxis

Omkar V. Byadgi, K.M.Shankar, ***Naveen Kumar B.T,*** *Abhiman, Rajreddy Patil and Iqlas Ahmed*

Fish pathology and biotechnology Laboratory, Department of Aquaculture, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore 575002, Karnataka, India.

Email: naveenkumar504@gmail.com

A study was conducted to detect viability of monoclonal antibody (mAb) in the gut and haemolymph of *Penaeus monodon* and its potential for protection against white spot syndrome virus (WSSV) upon challenge. mAb C-5 raised against WSSV was purified and coated on to a commercial shrimp feed at dosages of 5, 10 and 15 mg/kg feed. The feed was fed to *P. monodon* and viability of the mAb in hepatopancreas and hemolymph was determined by Immunodot and Western blot. Immunodot results indicated the presence of mAb for 2 h post feeding in hepatopancreas and hemolymph which was dose dependent. mAb was also detected in hemolymph by Western blot up to 1 h post feeding. Shrimp fed with mAb were challenged with WSSV by oral and injection. In shrimp fed at 15 mg antibody/ kg feed (0.45 μg mAb/ g shrimp/ day) WSSV infection delayed in oral and injection challenge with a high survival of 65 and 70 % respectively up to 15 days post challenge.mAb was viable in shrimp for passive immunization against WSSV and could be a potential tool for prophylaxis against the virus.

PP-D18

Susceptibility of juvenile European lobster (*Homarus gammarus*) to White Spot Syndrome Virus (WSSV) from naturally infected commodity shrimp products

James L. Munro,[1, 2] *Kelly S. Bateman* [2]*, Bryony Uglow*[2] *and Grant D. Stentiford*[2]

[1] School of Animal and Veterinary Sciences, The University of Adelaide, Australia, 5005.
[2] European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Weymouth, Dorset, DT4 8UB, United Kingdom.
Email: James.Munro@adelaide.edu.au

The European Union is a major importer of penaeid shrimp which are likely to originate from areas where white spot disease, caused by white spot syndrome virus (WSSV) is known to be endemic. Juxtaposing this, crustacean capture fisheries in Europe, and particularly the UK, are one of the most important and valuable fishery resources, often out-competing finfish resources in economic value within the Union. The aim of the project was to determine if commodity shrimp imported into the UK contain viable WSSV that is capable of infecting local species.

Polymerase chain reaction (PCR) was used to screen for the presence of WSSV in commodity shrimp sourced from local markets. WSSV positive tissue from commodity shrimp was fed to juvenile European lobster (*Homarus gammarus*). To ensure the viability of WSSV obtained from the commodity shrimp and if the European lobster was an adequate host, WSSV positive homogenate from commodity shrimp was injected into specific pathogen free Pacific white shrimp (*Litopenaeus vannamei*) and the susceptibility of the European lobster towards WSSV was determined by feeding them high dose WSSV-infected shrimp tissue.

Prevalence of WSSV in commodity shrimp ranged from 0% to 100%. Shrimp injected with WSSV positive tissue from commodity shrimp had up to 100% mortality and were PCR positive for the virus. Lobsters fed high dose WSSV infected tissue displayed a cumulative mortality of 55%. Viral infection was determined by histology and confirmed by transmission electron microscope. Lobsters fed commodity shrimp displayed up to 70% prevalence of WSSV, determined by PCR. DNA sequencing confirmed at least 99% homology to WSSV. However, histological examination of commodity fed lobsters failed to reveal any obvious characteristic signs of WSSV as seen in penaeid shrimp.

This work demonstrates that commodity shrimp pose a risk of importing viable WSSV into the EU and that WSSV is capable of infecting local species. Further work is needed to determine whether the infected hosts from commodity shrimp would display signs of disease if they were exposed to a stressful event such as moulting.

PP-D19

Computational genome analyses in White Spot Syndrome Virus by the approach subtractive genomics for drug target identification

S. Jeevan Amos, N. Subhalaxmi and ***K. Umadevi***

DBT - Bioinformatics Programme, Department of Marine Living Resources, Andhra University, Visakhapatnam -530 003, A. P., India.
Email: andhrauniv.btisnet@nic.in

White spot syndrome (WSS) is the devastating diseases in shrimp aquaculture industry responsible for enormous economic losses all over the world. Disease outbreaks can reach a cumulative mortality of up to 100% within 3 to 7 days of infection. White spot syndrome virus (WSSV), the causative agent of WSS is a virulent, contagious, and widespread dsDNA eukaryotic virus with high pathogenicity. Prevention and inhibition of infection by this virus at the molecular level can be difficult due to the ability of WSSV to survive for a long time in the environment. There are no approved antiviral therapies for the treatment of WSS in shrimp. Large genomic sequencing projects of pathogens as well as shrimp genome leads to immense genomic and proteomic data which would be very beneficial for the novel target identification in pathogens.

The objective was to identify and locate those essential proteins of WSSV that are unique i.e. absent in host and performing normal function within the host and to shortlist them in vaccine development. Subtractive genomics approach is one of the recently adopted strategies helpful in identification of potential targets. The approach works by subtracting the genes or proteins between the host and pathogen proteome provides information for a set of proteins that are likely to be essential to the pathogen but absent in the host. This approach was employed to identify novel drug targets in WSSV.

The complete WSSV genome has been assembled into a circular sequence of 305,107 nt which encodes 531 putative open reading frames.Our analysis had revealed that out of 531 coding sequences of the pathogen, 156 represented non-homologous protein to pathogen. On further comparison of these 156 non-homologous proteins, the list of minimal set of 56 unique essential genes was predicted as putative drug targets. Identification of different protein functions facilitates a mechanistic understanding of WSSV infection and opens novel means for drug development. Functional assignment of essential non-homologous WSSV proteome was done through support vector machine. The identified potential drug targets form a platform for further investigation in discovery of novel therapeutic compounds against WSS.

PP-D20

Characterization of Argonaute2 gene in kuruma shrimp (*Marsupenaeus japonicus*)

Junichi Nishi,[1] *Mari Inada,*[2] *Maki Yoshimine,*[1] *Keisuke Kihara,*[1] *Terutoyo Yoshida,*[3] *Tomoya Kono,*[4] *Raja Sudhakaran,*[3,5] *Masahiro Sakai*[3] *and Toshiaki Itami*[3]

[1] Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[2] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[3] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[4] Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuenkibanadai-nishi 889-2192, Miyazaki, Japan.

[5] Department of Biotechnology, Thiruvalluvar University, Serkadu - 632 106, Vellore, Tamilnadu, India.

Email: ad10005@student.miyazaki-u.ac.jp

RNA silencing (RNAi) is a common antiviral defense mechanism in eukaryotes from plants to animals. RNAi is functioning through the sequence-specific small RNA by RNA degradation or translational repression. As RNAi-related factors, RNA-induced silencing complex (RISC) recognizes the target mRNA and causes degradation and translational repression. Argonaute (AGO), a major component of RISC in the RNAi pathway, has been reported and five types of AGO genes in *Drosophila* were found. However, only one type of AGO gene has been reported in kuruma shrimp. In the present study, we report another type of AGO gene (*MjArgonaute*2) in kuruma shrimp, *Marsupenaeus japonicus*. We have designed degenerate primers on the basis of nucleotide sequences conserved between the European honey bee (*Apis mellifera*) Argonaute2 and the expressed sequence tag (EST) database of Argonaute2 in invertebrates. PCR was performed using the cDNA prepared above to obtain the initial predicted sequence. Based on the partial nucleotide sequence of *Argonaute2* obtained by PCR, we performed RACE (rapid amplification of cDNA ends) and got full length cDNA of MjArgonaute2. The full length cDNA of *MjArgonaute2* consists of 2,633 bp with a 39 bp 5'-untranslated region (UTR), a 38 bp 3'-UTR and a 2,556 bp open reading frame (ORF) encoding 852 amino acids. The molecular mass of the mature peptide is about 97.2 kDa. Analysis of the deduced amino acid sequence indicated that the mature peptide showed the highest similarity to whiteleg shrimp Argonaute2 (*Lv*Argonaute2) with a maximum identity of 76.4% and second highest similarity to black tiger shrimp Argonaute1 (*Pm*Argonaute1). Argonaute gene contains two functional recognition domains: one is PAZ domain, the other is Piwi domain. Quantitative expression analyses of *MjArgonaute2* in different organs of healthy kuruma shrimp revealed high level expression in muscle, heart, hematopoietic organ and lymphoid organ.

PP-D21

Occurence of Infectious Hypodermal and Haematopoietic Necrosis virus (IHHNV) infections in wild-caught freshwater prawn *Macrobrachium rosenbergii* in Sungai Rubana, Perak and Sungai Timun, Negeri Sembilan, Malaysia

Hazreen Nita M.K,[1] *Kua B.C,*[2] *Bhassu, S* [1] *and R.Y. Othman*[1]

[1]Genetics and Molecular Biology, Institute of Biological Sciences, 50603, Centre of Biotechnology for Agriculture (CEBAR), University Malaya, Kuala Lumpur, Malaysia.
[2]National Fish Health Research Centre (NaFisH),11960 Batu Maung, Pulau Pinang, Malaysia.
Email: subhabhassu@um.edu.my

The purpose of this study was to determine the prevalence of IHHNV in wild-caught giant freshwater prawn, *Macrobrachium rosenbergii* broodstocks in Malaysia by nested polymerase chain reaction (PCR). Over a period of one year monitoring in 2009, *M. rosenbergii* were collected from two selected rivers in Malaysia. A total of 50 wild-caught giant freshwater prawns were collected monthly from each location; Sungai Rubana (Perak), and Sungai Timun (Negeri Sembilan). The detection of IHHNV was based on the conventional PCR using primers 389bp as suggested by OIE (OIE, 2003) while for the nested step, newly designed primers with 247 bp were used. Through the year 2009, the prawns captured from Sungai Timun and Sungai Rubana showed positive results by nested PCR detection with the prevalence of the infected prawns ranged from 48.7% to 64.4 % respectively. There are significant differences found in IHHNV prevalence ($p<0.05$) among the infected prawns with respect to the body weight of *M. rosenbergii.* Our results showed that IHHNV infection were common in adult prawn. The economic impact caused by IHHNV in wild-caught *M. rosenbergii* was unknown as all the infected samples did not show any mortality. However, the gross sign such as deformity of rostrum and pleopods were seen in the infected prawn.

PP-D22

Effect of yeast expressed white spot virus proteins on protection of *Penaeus monodon* larvae against the virus

E. Praveena,[1] T. Bhuvaneswari,[1] A.K. Panigrahi [2] and ***S.K. Otta*** *[1]*

[1]Aquatic Animal Health and Environmental Division, Central Institute of Brackish water Aquaculture, 75 Santhome high Road, RA Puram, Chennai 600028.
[2]Crustacean Culture Division, Central Institute of Brackish water Aquaculture, 75 Santhome high Road, RA Puram, Chennai 600028.
Email: subhendu@ciba.res.in

In an attempt to find out the interacting proteins of two white spot virus (WSSV) proteins, VP28 and VP15, by yeast two-hybrid technique, baits were constructed for these two. The same bait constructs were transformed to yeast, *Saccharomyces cerevisiae*, and used for this experiment to see their protective effect against the virus when fed to *Penaeus monodon* shrimp larvae. The DNA binding domain vector, pGBKT7 transformed to yeast served as vector control. The post-larvae (PL8) were either fed directly or the yeast cells were initially fed to artemia and then supplied to shrimp larvae. Following 5 days of continuous feeding, the larvae were subjected to stress test and challenged with WSSV (through oral route). Shrimp larvae fed with yeast cells, either with the bait or the empty vector, showed better survival and stress tolerant than the control larvae. Mortality of larvae started after 72 hours of virus challenge and the survivability was better in yeast fed larvae (44.37 to 74.99%) compared to the control (31.35%). Control larvae recorded 100% mortality at 84 hours of post challenge. The larvae fed directly with yeast cells recorded survivability of 8.84% (Vector control), 20.83% (VP15 bait) and 24.24% (VP28 bait) after 96 hours of post challenge. Similarly the survivability of yeast fed shrimp larvae through artemia were 10.71% (Vector control), 23.17% (VP15 bait) and 25.8% (VP28) respectively. The mortality of larvae due to WSSV infection was confirmed by PCR.

PP-D23

Expression profiling of WSSV ORF 249 and shrimp ubiquitin conjugating enzyme in WSSV infected *Penaeus monodon*

*Jeena S, **K. Pani Prasad,** Gireesh Babu and Mujahid Pathan*

Central Institute of Fisheries Education, Off Yari Road, Panch Marg, Versova, Mumbai - 400 061.
Email: pprasad35@gmail.com

White spot syndrome virus (WSSV) is one of the major pathogens in shrimp aquaculture. WSSV249 and WSSV199 are predicted to encode a RING H2 domain, which in presence of ubiquitin conjugating enzyme (E2) in shrimp can function as viral E3 ligase and modulate the host ubiquitin proteasome pathway. Modulation of host ubiquitin proteasome pathway by viral proteins is implicated in viral pathogenesis. In the present study, expression profile of PmUbC was studied at both transcript and protein level while it was at transcript level for WSSV249 and WSSV199 genes in WSSV challenged shrimp. A time expression analysis was carried out at 0, 3, 6, 12, 24, 48 and 72 h post WSSV challenge by semi- quantitative RT-PCR as well as Real Time PCR. EF1α was used as a reference control to normalize the expression levels. A significant increase in PmUbC expression at 24 hours post infection (h.p.i) was observed followed by a decline till 72 h. p. i. The WSSV249 and WSSV 199 were shown to express at 24 h. p. i in WSSV challenged *P. monodon*. The PmUbC expression pattern studied at protein level by ELISA with PmUbC antibodies confirmed the results obtained in RT-PCR analysis. Since the up-regulation of PmUbC was observed at 24 h. p. i where WSSV 249 and WSSV 199 expression was detected, it can be speculated that these proteins might interact with host ubiquitination pathway for viral pathogenesis. This is the first report on the expression profiling of WSSV 199, a RING finger domain protein. However, further studies need to be done to unfold the molecular mechanism of interaction between host and virus to devise efficient control strategies for this chaos in shrimp culture industry.

PP-D24

Non-specific effect of DNA construct (pCMV-GFP-LH) on the expression of prophenoloxidase (proPO) and ferritin genes in *Penaeus monodon*

*Greeshma S. S., **Anutosh Paria**, Aparna Chaudhari, M. Makesh, C. S. Purushothaman and K. V. Rajendran*

Central Institute of Fisheries Education (CIFE), Indian Council of Agricultural Research, Off-Yari Road, Versova, Mumbai - 400 061, India.

Email: anu.cife@gmail.com

RNA interference (RNAi) has emerged as a stable, cost-effective and efficient therapeutic tool in crustacean viral disease management in recent years. However, the major constraint of the methodology is in relation to the specificity with dsRNA and siRNA can silence target genes in sequence-specific manner and can also deregulate certain endogenous genes. The present study explored the non-specific effect of DNA construct (pCMV-GFP-LH) on the expression of prophenoloxidase (proPO) and ferritin genes, important components of shrimp immune system. Healthy *Penaeus monodon* was injected with a DNA construct (pCMV-GFP-LH) designed against green fluorescent protein (GFP) which was capable of expressing lhRNA *in vivo* and an empty vector (pcDNA). To understand the tissue distribution of DNA construct (pCMV-GFP-LH), DNA extracted from gill, hepatopancreas and lymphoid organ was subjected to PCR using *gfp* and CMV promoter-specific primers. The DNA construct was detected in all these tissues at 24 h post-injection. mRNA expression levels of proPO and ferritin genes in haemocytes were determined by semi-quantitative RT-PCR and quantitative real-time PCR (qRT-PCR) with EF1α as internal control. Phosphate buffered saline (PBS) injected animals were used as experimental control. In DNA construct-injected shrimp a high level of proPO2 expression was noticed at 24 and 48 h post-injection (h p.i.) with the highest level noticed at 48 h p.i. This was followed by a significant decline at 72 h p.i. ($p<0.05$) but the expression was well above the normal level. On the other hand, ferritin expression was found to be down regulated at all time points except 48 hpi In the case of empty vector-injected shrimp, proPO expression showed a drastic decline at 6 h p.i. followed by a steady increase. However, the expression levels were found to be lower than that of the control at all time-points. On the contrary, ferritin mRNA expression in this group showed a steady down regulation. The study showed that the DNA construct (pCMV-GFP-LH) can enhance the immune response through proPO activation at early hours of introduction in shrimp. Therefore, the protection offered by the construct against white spot syndrome virus, as reported earlier, might be partly due to the non-specific immune response elicited by the DNA construct.

PP-D25

Production of monoclonal antibodies for detection of *Macrobrachium rosenbergii* Nodavirus (*Mr*NV) in Giant freshwater prawn in India

A. K. Sahoo, [1,2] *K. M. Shankar,* [1] *Raj Reddy Patil,* [1] *Abhiman,*[1] *Nabeen B. T.* [1]*and Iqlas Ahmed* [1]

[1] Fish Pathology and Biotechnology Laboratory, Department of Aquaculture, College of Fisheries, KVAFSU, Mangalore, India 500 007.
[2] Central Inland Fisheries Research Institute, Barrackpore, Kolkata, India 700120.
Email: amiya7@gmail.com

Macrobrachium rosenbergii Nodavirus (*Mr*NV) has been a major concern of disease outbreak both in hatchery and nursery farms of giant freshwater prawn, and known as the causative agent of White tail disease (WTD). Clinical signs of the disease include development of white spots in muscles or milky muscles throughout the body. Histopathology shows darkly basophilic, reticulated cytoplasmic inclusions in the connective tissue cells. Eventhough several molecular based diagnostics have been developed, there is a need for developing simple, specific and sensitive monoclonal antibody (mAbs) based field level assay for detection of the virus, *Mr*NV. A panel of three mAbs, (A1, C1 and D11) specific against *Mr*NV were developed and characterized. Two mabs (A1, D11) reacted strongly with the *Mr*NV 42 kDa coat protein in Western blot, while the third mAb (C1) showed little immunoreactivity. The mAbs showed no cross reaction with the White spot virus (WSV), *Aeromonas hydrophila* and fungus *Aphanomyces invadans*. The mAb based immuno-histochemistry showed viral inclusions bodies in the muscle necrotized area, which were highly conspicuous from the tissue sections stained with hematoxylin and eosin. The mAbs produced against *Mr*NV were used for development of an immunodot assay for simple and specific *Mr*NV detection. Virus isolates collected from the East and West coast of India found to be similar by mAb based serotyping immunodot, indicating single *Mr*NV isolate in India.

PP-D26

Cloning and characterisation of a antimicrobial peptide gene Penaeidin-3 (Fi-Pen3) from the haemocytes of Indian white shrimp *Fenneropenaeus indicus*

***Shanthi S**, B.Vaseeharan, R.M. Manikandan and N.M.Prabhu*

Department of Animal Health and Management, Alagappa University, Karaikudi.
Email: shanthisathappan@gmail.com

Aquaculture is the fastest growing food sector globally. During the past decades, commercial shrimp farming has been severely hit by epidemics associated with viruses and vibriosis, resulting in severe economic losses. Hence, the immunology can be considered as a prophylactic target to establish strategies dealing with the management and control of disease in aquaculture. Antimicrobial peptides (AMPs) are a major component of the innate immune defence system in marine invertebrates. Penaeidins, are a family of AMPs that are synthesized and stored in the shrimp granular haemocytes. In the present study, we investigate the AMP Pen-3 gene family cloned from the haemocytes of Indian shrimp *F. indicus* using degenerate primers with PCR amplification. The full length cDNA consisted of open reading frame with 243 bp encoding 80 amino acids. The mature peptide had a predicted molecular weight of 84.9 KDa. Fi-Pen3 was found to be expressed in haemocytes, heart, hepatopancreas, muscles, gills, intestine and eyestalk .However higher expression was observed in haemocytes. In addition, to confirm the expression RT-PCR analysis was carried out after injection of *Vibrio parahaemolyticus* to *F. indicus.* The hemocyte shows mRNA up-regulation which indicates the expression of Fi-pen3 and confirms the innate immune defence mechanism in shrimps.

PP-D27
Background, invasion, spread, and consequences of the Akoya oyster disease

Nanae Takagishi *and Tomoyoshi Yoshinaga*

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.
Email: aa107055@mail.ecc.u-tokyo.ac.jp

Since 1994, mass mortality of the Akoya oyster (*Pinctada fucata martensii*) has been reported in a large number of Japanese Akoya culture fields. Mortality was due to a disease known as Akoya oyster disease, which discolors the adductor muscle. The disease is currently believed to be infectious and introduced from China by way of Chinese Akoya oysters used for pearl culture. The case of Akoya oyster disease provides valuable lessons for future improvement of the quarantine system to prevent introduction of non-indigenous diseases of aquatic animals. We investigated the background, invasion, spread and consequences of Akoya oyster disease through a review of published literature and prefectural reports, and interviews with prefectural officers who were involved in the response to the disease.

We found that prior to the introduction of the disease, pearl oyster producers believed that excessive selective breeding had weakened the tolerance of Akoya oyster against environmental change and deterioration. To tackle this perceived problem, Chinese Akoya oysters were imported for breeding. It took almost 4 years before researchers proved that the disease was infectious but the pathogen remains unidentified to this date. Previously, formalin used as parasiticide for fish aquaculture, and starvation due to low phytoplankton density were suspected to be the causes of the disease. During the 4 years of uncertainty over the cause, no control measures were implemented, and Chinese Akoya oysters continued to be imported because of their higher resistance to the disease. The disease spread rapidly throughout the country because the region where the outbreak first occurred was the source of more than 70% of the Akoya oysters in Japan.

Improvements by cross-breeding between Japanese and Chinese oysters and by rearing oysters in low temperature areas in winter have saved pearl oyster production from complete decimation by the disease. However, the total production value of pearls remains low, at a quarter of the industry's peak value, indicating that there has been a far from complete recovery.

PP-D28

Autonomous shrimp genetic modification for production of viral antisense RNA

Heny Budi Utari, [1, 4] *Chumporn Soowannayan,* [1, 2] *Timothy William Flegel,* [1, 3] *Maleeya Kruatrachue* [4] *and Boonsirm Withyachumnarnkul* [5]

[1]Center of Excellence for Shrimp Molecular Biology and Biotechnology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, 10400.
[2]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumtani.
[3]Department of Biotechnology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, 10400.
[4]Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, 10400.
[5]Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, 10400.
Email: heny.budi@gmail.com

Recent studies have revealed the presence of non-retroviral virus sequences inserted into the shrimp genome. It has been hypothesized that these insertions arise from both RNA and DNA viruses via endogenous, host reverse transcriptases and integrases that recognize viral mRNA and randomly generated DNA insertions, some of which produce viral antisense RNA fragments. To test this hypothesis, giant tiger shrimp (*Penaeus monodon*) free of white spot disease for 7 generations was analyzed for the presence of white spot syndrome virus (WSSV) inserts and antisense RNA fragments. They were first confirmed negative for WSSV using standard nested-PCR screening methods for several WSSV genes. They were subsequently screened using WSSV targets for open reading frames (ORF) 151, 366 and 427 previously reported from their ancestors by microarray assay. From 97 specimens tested, 26 were positive for the target sequences by nested PCR. Using 20 specimens positive for ORF 366, reverse transcriptase reactions were carried out to amplify sense or antisense RNA fragments from DNase-treated RNA extracts. Nested-PCR with the resulting cDNA preparations revealed two specimens that produced only antisense RNA fragments, one that produced both sense and antisense fragments and one that produced no fragment. This supported the hypothesis for autonomous, random insertion of non-retrovirus sequences into the shrimp genome in a manner that can lead to individuals with the capacity to produce viral antisense RNA. Using nested PCR with spermatophore (germ cells) from 5 arbitrarily chosen specimens, it was found that all were positive for ORF 366 and 151. The size of the PCR products for ORF 366 was matched with expected target band, but for ORF151 was larger than expected. All 5 were negative for ORF 427. If such inserts are protective, crossing of such individuals in shrimp breeding programs may lead to the development of disease resistant stocks.

PP-D29

In silico modeling and motif prediction of Lipopolysaccharide and β-1,3-glucan binding protein (LGBP) from Indian white shrimp *Fenneropenaeus indicus*

J. Sivakama Valli *and B.Vaseeharan*

Department of Animal Health and Management, Alagappa University, Karaikudi -630 003, Tamil Nadu, India.

Email:

Aquaculture is the fastest growing food production zone in the world. Peanaeid shrimps especially, *Fenneropenaeus indicus* is one of the economically important species cultured along coastal areas of India. Farming of the species faces lot of problem due to the occurrence of opportunistic pathogens including bacteria and viruses. To increase the aquaculture production, research on disease management through biotechnological approaches is the need of the hour. Invertebrate immune mechanism has the ability to recognize the invading organisms as foreign substances, with the help of pattern recogition proteins. The present study focuses on the isolation and identification of lipopolysaccharide and β-1,3-glucan binding protein gene from Indian white shrimp *F. indicus.* Lipopolysaccharide and β-1,3-glucan binding protein (LGBP) are the genes involved in the pattern recognition mechanism in invertebrates, and induce cell mediated and humoral mediated immune reponse like encapsulation, phagocytosis, nodule formation, clotting, synthesis of antimicrobial peptides, and activation of the Prophenoloxidase (proPO) system. LGBP gene was isolated and sequenced from the Indian white shrimp *F. indicus* (Fein). Multiple alignment of *Fein*-LGBP sequence showed the Glucanase domain, catalytic triads, two conserved putative integrin-binding motif (cell adhesion sites), bacterial glucanase motif (GM) and two polysaccharide recognition motifs for the polysaccharide binding motif (PsBM) and β-glucan recognition motif (β-GRM). We report the theoretical model of novel immune related gene LGBP by *in silico* homology modeling and its motif prediction. Physico-chemical characterization of Fein LGBP provides the information of the protein stability. Prediction of motifs, patterns, disulfide bridges and secondary structure was performed for functional characterization of the Fein LGBP. The modeling of the three dimensional structure of the proteins showed that models generated by Modeller9V8 were more acceptable in comparison to that by Swiss Model and validated by NIH server. Until complete biochemical and structural data of LGBP are determined by experimental means, this model can serve as a valuable reference for characterizing the protein and could be explored for drug targeting by design of suitable agonists. This study will facilitate the exploration of other life sciences like pharmacokinetics, toxicology, drug designing and chemo informatics.la of LGBP

PP-D30

Cloning and expression of glutathione peroxidase, superoxide dismutase in the shrimp by *Vibrio parahaemolyticus* infection

G.Vinoj *and B.Vaseeharan*

Department of Animal health and Management, Alagappa University, Karaikudi, Tamil nadu, India.
Email: vinojvino@gmail.com

In shrimp both reactive oxygen species (ROS) and the corresponding scavenging system components especially glutathione peroxidase (GPx) and superoxide dismutase (SOD) are indispensable for normal development of the organism. In the present study, three bacterials isolates were isolated biochemically and by 16S rRNA sequence analysis they were identified as *Vibrio paraheamolyticus* (Vp). Vp cells extracted and washed twice with PBS saline solution and resuspended in 50 µl of the sterile saline solution were used for infection and gene expression assay in shrimp. Accordingly, the goal of this study was to validate maximum expression of SOD and GPX transcripts in the haemocytes, gills, the hepatopancreas, intestines, and muscles during different time intervals like 6,12,18,24 post infection on Vp injection. In the present observation Vp has the capacity to induce both immune-related genes like SOD, GPX in shrimp. To investigate the function of SOD and GPX in crustaceans, we cloned and characterized a full length GPx and SOD transcript in the shrimp after challenge with Vp. Sequence comparison showed that SOD and GPX deduced amino acid of our experimental shrimp had an overall similarity of 62% and 64% respectively that of *P. leniusculus, P. monodon,* and *L. vannamei.* Moreover, quantitative real-time PCR analysis showed that in hemocytes and hepatopancreas there is high level expression of GPX and SOD after 24 h of the Vp post infection. In the present study the maximum expression of the antioxidant gene transcript was found in the hemocytes followed by heptopancreas and muscles. In conclusion, result show Vp cells stimulating high immune response in hemocytes and hepatopancreas of shrimp.

PP-D31

Kazal-type serine proteinase inhibitor in disk abalone (*Haliotis discus discus*): Transcriptional response upon bacterial and viral immune challenges

Wickramaarachchi W.D.N.,[1] Mahanama De Zoysa[2] and Jehee Lee[1]

[1]Department of Marine Life science, school of Biomedical sciences, Jeju National University, Jeju Special Self-Governing Province,690-756, Republic of Korea.
[2]Laboratory of Aquatic Animal Diseases, College of Veterinary Medicine, Chungnam National University, Yuseong-gu, Daejeon, 305-764, Republic of Korea.
Email: niroshanwdn@yahoo.com

Proteinase inhibitors play a key role in many biological processes such as blood coagulation, activation of complement system, inflammation, immune response and development. The proteinase inhibitors involve in regulating the activity of the respective proteinases. Presence of single or multiple kazal domains are the specific feature of the KPIs and perform the specified activity irrespective of the number of domain within the KPI. All invertebrate Kazal domains share a common structure which is dictated by six conserved cysteine residues forming three intra-domain disulfide cross-links despite the variability of amino acid sequences between the half-cystines.

A kazal-type serine proteinase inhibitor was cloned from the disk abalone *Haliotis discus discus* which is presumably involved in innate immune response. The full-length cDNA sequence of Ab-KPI consists of 600 nucleotides with a poly A tail, and an open reading frame (ORF) encoding a polypeptide of 143 amino acids with a putative signal peptide of 17 amino acids. The deduced amino acid sequence of Ab-KPI contained 2 tandem Kazal domains with high similarity to other Kazal-type SPIs. Each domain consist of reactive site (P1) residue as leucine (L) and threonine (T) which is located in second amino acid after the second cysteine of each domain. Temporal expression of Ab-KPI in hemocytes, gills and mantle was assessed by quantitative real time PCR after bacterial, viral hemorrhagic septicemia virus (VHSV) challenge and tissue injury. After the bacterial and VHSV challenge, hemocytes showed the nearly 14-fold and 4-fold increase in relative expression compared to control at 6th hour respectively. In the case of tissue injury, up-regulation was started at 6th hour and highest was observed in 9th hour after the injury in hemocytes. The up-regulated mRNA expression of Ab-KPI in the abalone following injury or immune challenge indicates that the Ab-KPI gene is inducible and involved in wound healing and the immune response.

PP-D32

Characterization of *cytochrome c* in kuruma shrimp (*Marsupenaeus japonicus*)

Maki Yoshimine,[1] *Mari Inada,*[2] *Junichi Nishi,*[1] *Keisuke Kihara,*[1] *Ayaka Shiki,*[1] *Raja Sdhakaran,*[3,5] *Tomoya Kono,*[4] *Terutoyo Yoshida,*[3] *Masahiro Sakai*[3] *and Toshiaki Itami*[3]

[1]Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[2] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki Pref. 889-2192, Japan mari_k_p_sky_melody@yahoo.co.jp.

[3] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan.

[4] Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuenkibanadai-nishi 889-2192, Miyazaki, Japan.

[5] Department of Biotechnology, Thiruvalluvar University, Serkadu - 632 106, Vellore, Tamilnadu, India.

Email: ad10012@student.miyazaki-u.ac.jp

Apoptosis, programmed cell death, is known as a defense mechanism against virus infection in animals. However, the apoptosis mechanisms of shrimp are still far from well understood. In mammals, *cytochrome c* are released from mitochondria and form a complex with apoptotic protease-activating factor 1 (Apaf-1) and caspase-9. Caspase-9 is activated in this complex. The activated caspase-9 activates effector caspase, which induces apoptosis. In this study, we have cloned *cytochrome c* from kuruma shrimp *Marsupenaeus japonicus* (*Mj cytochrome c*). Primers designed on the basis of nucleotide sequences conserved between the desert locust (*Schistocerca gregaria*) *cytochrome c* and the expressed sequence tag (EST) database of *cytochrome c* in invertebrates. PCR was performed using the cDNA prepared above to obtain the initial predicted sequence. Based on the partial nucleotide sequence of the *cytochrome c* obtained by PCR, we performed RACE (rapid amplification of cDNA ends) and got full length cDNA of *Mj cytochrome c*. The full length cDNA of *Mj cytochrome c* consists of 1,107 bp with a 95 bp 5'-untranslated region (UTR), a 598 bp 3'-UTR and a 414 bp open reading frame (ORF) encoding 104 amino acids. The deduced amino acid sequence of *Mj cytochrome c* showed 80 % and 76 % identity with sequences of fruit fly (*Drosophila melanogaster*) *cytochrome c* and human (*Homo sapiens*) *cytochrome c*. Both showed a high homology with *Mj cytochrome c*. *Mj cytochrome* c has cytochrome c domain, which acts as electron carrier activity, iron ion binding activity and heme binding activity. Phylogenetic analysis revealed *Mj cytochrome c* and monsoon river prawn (*Macrobrachium malcolmsonii*) cytochrome c are the most closely related, and formed a single cluster by shrimp. By the expression analysis of *cytochrome c* in the healthy kuruma shrimp, expression was confirmed with brain, lymphoid organ, hematopoietic organ, intestine and nerve. The highest expression was found in intestine, with an expression 28 fold higher than that of the muscle.

PP- E1
Parasite infestation in portunid crabs

T. Anand, *P. Soundarapandian and D.Varadharajan*

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India.
Email: thaanand31@gmail.com

Portunid or swimming crabs are common in tropical and subtropical estuarine and nearshore habitats. There are 14 commercially important portunid crabs were recorded from Portonovo coast. These crabs are not only commercially important, but also ecologically important. A variety of parasites have strong impact on portunid population elsware in the world, that have impact upon by influencing the growth, reproduction, egg survival, longevity and marketability. Recent survey suggesting that a decrease in portunid populations, revealing the need for frequent monitoring of natural stocks. Hence in the present study some of the parasites were identified from the portunid crabs along Portonova coast. Symbiotic nemerten worm's C*arcinonemertean* sp. was identified from the berried crabs of *P. pelagicus.* These worms are egg predators and cause high egg mortality. The berried female of *P. pelagicus* stripped out her entire egg mass from the pleapods of the abdominal flab using her walking legs while infested by nemertean egg predators. *Sacculina sp.* was recorded in portunid crabs of *P. sanguinolentus*, *C. ferrieta, C. hellerii* and *C. hoplites*. Among these four species, *P. sanguinolentus* was infested more. Further four species of *Octolasmis* barnacles were collected from five different species of portunid crabs.

PP-E2

Effect of water volume and bottom surface on fish-borne zoonotic trematodes infection to common carp

Annette S. Boerlage,[1] *Elisabeth A.M. Graat,*[1] *Johan A. Verreth*[2] *and Mart C.M. de Jong*[1]

[1] Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands.

[2] Aquaculture and Fisheries Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands.

Email: annette.boerlage@wur.nl

Fish-borne zoonotic trematodes (FZT's) cause major human health problems. Little is known about factors influencing trematode transmission to fish, e.g. whether density of FZTs in ponds have an effect. Aim was to quantify effects of water volume and bottom surface on prevalence and attack rates of parapleurolophocercous-cercariae to common carps in two experiments.

First, effect of cercariae dose and water volume on prevalence and attack rate (i.e. number of metacercariae per fish per cercariae exposed) were tested in a 3x4 factorial design (n=48). Carps (1.12±0.28 g) were individually exposed to 0, 10, 50 or 250 parapleurolophocercous-cercariae in 100, 500 or 1000 ml water with equal bottom surface (121cm^2). Metacercariae were counted 21 days post exposure. Prevalence of fish with metacercariae was resp. 64, 100, and 100% at doses of 10, 50 and 250 cercariae, and different from control fish (dose=0) (0%, P<0.01). Prevalences did not differ between volumes, being resp. 61, 67, and 73% (P=0.76). Attack rates for exposed fish were not different for exposure doses, resp. 0.09, 0.13, and 0.11 (P=0.44) or water volumes, resp. 0.11, 0.12, and 0.10 (P=0.95).

Second, effect of bottom surface and water volume on attack rates was tested. Carps (0.34±0.17 g) were individually exposed to 0 (n=91) or 250 parapleurolophocercous-cercariae in 25, 50, 100, 250, and 500 ml water at a bottom surface of 21 cm2 (n=241) or with bottom surface of 4, 12, 21, 30 and 49 cm2 with volumes of 100 ml (n=246). Metacercariae were counted 7 days post exposure. Control fish and 2% of exposed fish were not infected. Attack rates for exposed fish were not different between bottom surfaces, resp. 0.063, 0.068, 0.070, 0.065, and 0.061 (P=0.57) and water volumes, resp. 0.070, 0.072, 0.069, 0.062, and 0.065 (P=0.67).

To conclude, different exposure doses to cercariae results in very high (up to 100%) prevalences, however, attack rates were not different. Furthermore, attack rates did not differ between tested bottom surfaces and water volumes. Therefore, transmission of cercariae is considered independent on cercariae density, implying that pond dimensions in aquaculture are less important with regard to FZT transmission to fish.

PP-E3
Zoonotic trematode infection in fish; does fish size matter?

Annette S. Boerlage,[1] *Elisabeth A.M. Graat,*[1] *Johan A. Verreth*[2] *and Mart C.M. de Jong* [1]

[1] Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands.
[2] Aquaculture and Fisheries Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands.
Email: annette.boerlage@wur.nl

Fish-borne zoonotic trematodes (FZTs) affect the health of more than 40 million people worldwide. Observational studies have reported contradicting effects of fish size on prevalences in fish. Aim of our study was to quantify the association between fish size and attack rates of cercariae to common carps. Two experiments were performed.

In experiment 1, effects of fish size and cercariae dose were tested in a 3x4 factorial design with 5 fish per combination of treatments (n=60). Individually kept small (1 g), medium (25 g) and large (45 g) carps were exposed to 0, 10, 50 or 250 parapleurolophocercous-cercariae for 48 hours. Metacercariae were counted and identified 21 days post exposure. Percentages of infected fish and attack rates of cercariae to fish were higher for carps of 1g (63%, 0.08 fish infected per cercariae) than for 25 g (20%, 0.004) and 45 g (5%, 0.0007), but never 0 (P=0.0002). Fish size is considered an important risk factor for transmission of FZTs. Decrease in prevalence and attack rate between 1 g and 25 g fish was not measured. Therefore, a second experiment was performed to test whether this decrease is abrupt or more gradual, which is interesting with respect to intervention possibilities.

In experiment 2, carps varying between 0.2 and 22 g were used as controls (n=66) or exposed to 250 parapleurolophocercous-cercariae (n=254) for 24 hours. Seven days post exposure metacercariae were identified and counted. Risk to be infected decreased when fish weight increased (Odds Ratio=0.95 per gram; P=0.02). Percentages of infected fish and attack rates of cercariae to carps of 1 g (88%, 0.0087 fish infected per cercariae) were not different from 4 g (84%, 0.0073; P=0.1156), but different from 8 g (63%, 0.0040) and 14 g (61%, 0.0033) fish (both P<0.01). It was concluded that prevalence and attack rates gradually decrease with fish size.

We demonstrated the importance of fish size as risk factor for transmission of FZTs. Control measurements aiming at reducing transmission to small fish may reduce the absolute amount of FZTs in the environment, which indirectly might reduce incidence of human infection with FZTs.

PP-E4

Distribution of zoonotic trematodes in snails in ponds at integrated agriculture-aquaculture farms

Annette S. Boerlage,[1] *Elisabeth A.M. Graat,*[1] *Johan A. Verreth*[2] *and Mart C.M. de Jong*[1]

[1] Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands.

[2] Aquaculture and Fisheries Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen University, Wageningen, The Netherlands.

Email: annette.boerlage@wur.nl

Fish-borne zoonotic trematodes (FZTs) are among the most neglected tropical diseases. Life-cycle involves aquatic snails, fish, and humans and other animals as final hosts. In integrated agriculture-aquaculture (IAA) farming, animal and human excreta are used as fish feed and pond fertilizer, thereby enhancing transmission of FZTs from final hosts to snail. Spatial distribution of final hosts and habitat has been reported to effect prevalence of FZTs in snails. Areas bordering ponds might cause patchiness in snail infections within ponds, which might provide indications for control measures. Objective was to estimate the effect of bordering areas on prevalence and parasite burden of FZTs in snails in different areas within IAA ponds.

Nine sample areas within a pond were assigned in six ponds in Nam Dinh province, Vietnam. Per site, 120 *Melanoides tuberculata* snails were collected and type of bordering area was recorded as: high-risk area (pond access for humans, livestock sty, water connection to canal), and low-risk area (road, rice planted in pond, agriculture, middle of pond). Snail size was recorded and cercariae were counted after 24 hours shedding.

In total, 5,392 snails were collected. Non-FZT *Transversotrema* positive snails were found in 2 ponds with overall prevalence of 0.6%. Parapleurolophocercous-cercariae were present in all ponds and varied between 6% positive snails in low-risk areas and areas with livestock sty only to 14.5% in areas with both human access point and livestock sty. Only the latter was significantly different from low-risk areas ($P<0.01$). Percentage of snails with xiphidio-cercariae varied between 5.4% and 9.8%, and was not significantly different between risk areas.

Mean snail size was 15.2 mm (SD=2.6). Risk of snails to be infected increased with increasing snail size ($P<0.01$); resp. 1.29 and 1.18 increased risk per mm increase in shell length. Median number of parapleurolophocercous- or xiphidio-cercariae per snail was resp. 69 and 126 with no significant effects of snail size ($P=0.26$ and $P=0.06$ resp.).

To conclude, risk areas surrounding ponds were not associated with variation in FZT infection in snails within ponds. Therefore snail control targeting specific areas within ponds is not considered a potential intervention measure.

PP-E5

Unusual occurrence of parasitic sacculina infestation in brachyuran crab, *Portunus sanguinolentus* of Tamilnadu coastal waters

***V. Elumalai**, C. Viswanathan, T.V. Suresh, M. Pravinkumar, A.R. Logesh, K. Jaganathan and S.M. Raffi*

CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamilnadu, India.

Email: raffi_cas@yahoo.co.in

Sacculina are Rhizocephalan barnacles that parasitize brachyuran crabs. Sacculina infestation in crabs induces severe modifications in morphology, physiology and reproduction of their host. A survey on the prevalence of sacculina infestation in a commercially important crab, *Portunus sanguinolentus* was conducted for a period of six months (May 2010 - October 2010) at two landing centers of Tamilnadu, namely, Annankoil and Mudasalodai. The average size group of infested crabs of *P. sanguinolentus* was between 25mm and 88mm in carapace width. The average ratio of infested to noninfested crabs were 1:6.28 and 1:7.22 for Annankoil and Mudasalodai stations respectively. The sexwise data among infestated for male and female crabs were in the ratio of 1: 0.74 and 1: 0.65 for Annankoil and Mudasalodai stations respectively.

PP-E6
Estimation of loss due to argulosis in carp culture ponds in India

*P. K. Sahoo,[1] J. Mohanty,[1] **S. K. Garnayak**,[1] B. R. Mohanty,[1] Banya Kar,[1] Hemaprasanth[2] and J.K. Jena[3]*

[1]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar,Orissa, India.
[2]Regional research centre, Central Institute of Freshwater Aquaculture, Bangalore, Karnataka, India.
[3]National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkhusa, Telibagh, Lucknow 226 002, India.
Email: skgarnayak@gmail.com

Parasitic diseases pose a serious threat to aquaculture industry in terms of growth reduction, secondary infections and mortality. Argulosis caused by crustacean ectoparasite, *Argulus sp.* is one of the parasitic diseases that pose a major loss to aquaculture industry around the globe. In recent years occurrences of argulosis appear to be more frequent due to extensive aquaculture activities and non-availability of suitable long-term control measures. The actual cost that is incurred due to this disease has gone mostly unnoticed. In the present study the economic burden imposed by *Argulus sp.* in carp culture ponds was estimated based on the data obtained from survey in major aquaculture zones in India during 2008-10 covering 1067.22 ha of water area in 8 states of India. During this period, an interview-based study was conducted using a standardized questionnaire based on the management and outbreak status of argulosis by repeated quarterly visit to individual farms. The loss due to argulosis was estimated by taking into consideration the mortality, loss in growth by reduction in weight, and expenditure towards drugs/chemicals applied for this disease. The expenditure towards labour charges was not included due to lack of sufficient authentic data. It was estimated that total loss due to argulosis was ~ 29494.18 INR (~US$614)/ha/year Few farmers also undertook regular use of chemicals such as cypermethrin, deltamethrin, dichlorovos and sanitizers as preventives for argulosis in broodstock farms in the states of West Bengal and Andhra Pradesh leading to an additional expenditure of about Rs. 2600/ha/year. Hence, management of this disease should be given top priority to save the aquaculture industry from the colossal loss.

PP-E7

Gill sphaerosporosis in swordtail, *Xiphophorus helleri* - first report from Sri Lanka

M. Hettiarachchi,[1] *D. C. Hettiarachchi*[2] *and C. K. Hemachandra*[1]

[1]Department of Zoology, University of Kelaniya, Kelaniya, Sri Lanka.
[2]Maxies & Co. (Pvt.) Ltd. Chilaw Road, Wennappuwa, Sri Lanka.
Email: manga@kln.ac.lk

Heavy mortalities in swordtail, *Xiphophorous helleri* were reported by some farmers in grow-out ponds/tanks, specially, in temporary holding tanks just before sale causing significant economic losses. To investigate the cause/causative agent, swordtails were maintained in glass aquaria under conditions simulated to temporary holding facilities of farmers. Water quality parameters were recorded, fish were observed for behavioural changes and external symptoms while recording mortality. Wet mounts and histological preparations of different tissues from affected, moribund, fish samples collected from farmers' holding tanks and from simulated tanks were observed under the microscope.

In early stages, gill filaments were pale, swollen and covered with excess mucous. In later stages gill filaments were covered with thick mucous and swelling was intense with necrotic areas. Trophozoites and pseudoplasmodial sporogenic stages of a *Sphaerospora* sp. were observed in excessive numbers in wet mounts of gill filaments. Histologically, there were severe epithelial hyperplasia, lamellar fusion, intense inflammatory response with granulocytic infiltration surrounding the parasite; cartilage necrosis also was observed. Intracellular uninucleate trophozoites (10-20 µm), oval to round extrasporogenic stages (18.21 ± 2.14 µm) and pseudoplasmodial sporogenic stages (21.09 ± 2.03µm) of a Sphaerospora sp. were present in gill tissues. Fish exhibited severe respiratory distress before death and mortality varied between 15% - 85%. Parasitic stages were not observed in liver, spleen, kidney or brain tissues.

Gill sphaerosporosis was the cause of mortality in swordtail. This is the first record of *Sphaerospora* sp. in an ornamental fish species reared in Sri Lanka.

PP-E8

Increasing of temperature for Oodinium disease treatment in marine fishes

Sahandi Javad [1], *Shohreh Poulin* [2] *Jafariyan Hojatollah*[3] *and Rezaei Hamid*[3]

[1] Young Researcher Club, Islamic Azad University, Islamshahr branch, I.R.Iran.
[2] Graduated of Veterinary of Ferdosi University Mashhad I.R. Iran.
[3] Gonbad Higher Education Center, Gonbad,I.R. Iran.
Email: sahandijavad@gmail.com

Now-a-days with development of marine fish keeping technology there are many people that like to keep marine fish as pet. This fishes have variety of colors and shapes. Oodinium is the most important parasite in this species. Use of chemical material in marine aquarium is really expensive. The best treatment for this disease is improving of temperature that could be a little hard because high temperature can kill some organism of water. The best temperature for marine tank is between 24-26oC. But with increasing of temperature of water Oodinium parasites leave the surface of fish body and migrate between gravels. With this migration we can remove them through water exchange. We have to change water up to 10 cm of low level of water and use siphon for washing gravels and emit Oodinium parasites.

PP-E9

A host specific parasitic copepod *Pseudocycnus armatus* infesting spotted seer fish distributed along the Malabar coast

[1]***Sudha Kappalli,*** *[1]Aneesh P.T, [1] Helna A. K, [1]Arshad K* and *[2]G. Anilkumar*

[1]Post Graduate Department of Zoology, Sree Narayana College, Kannur 670 007 India.
[2]School of Biosciences and Technology, VIT University, Vellore 632014 India.
Email: ksudha50@rediffmail.com

The spotted seer fish, *Scomberomorus guttatus* is of great demand globally and is largely available along the Malabar coast (Kerala, India). The present study reveals that this fish variety is heavily infested by the parasitic copepod *Pseudocycnus armatus.* A total of 424 fish samples (*S. guttatus*) were surveyed during the period from January 2010- August 2011; approximately 4173 of the parasitic copepod *P. armatus* were recovered from the gills of 421 fish signifying the existence of heavy parasitization. The number of copepods recovered from a single fish sample was found to range between 3 and 52. *P. armatus* firmly clings the host's gill filament using the heavily sclerotized claws of the second antennae and maxilliped. No seasonal fluctuation in the rate of infestation could be observed; our survey reveals a uniform degree of infestation of this parasite throughout the year. Significantly, all the 4173 specimens of parasitic copepods *P. armatus* collected round the year from the fish population *S. guttatus* were females bearing a pair of egg sacs, each containing 180-200 eggs. In order to assess the host specificity, if any, fifty seven different species of fish samples belonging to 22 families, collected from the Malabar coast were subjected to thorough observation throughout the year. Interestingly, *P. armatus* could not be recovered from any of the fishes other than *S. guttatus*, suggesting that the parasite is highly host-specific. The gill damage caused by the parasitic copepod is also discussed.

PP-E10

Susceptibility studies of carps to fish ectoparasite, *Argulus siamensis*

*P. K. Sahoo,[1] Hema Prasanth[2], J. Mohanty,[1] J.K. Jena[3], **B. Kar**,[1] S.K. Garnayak[1] and B.R. Mohanty[1]*

1Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India.
2Regional Research Centre of CIFA, Hessarghhata, Bangalore 560 089, India.
3National Bureau of Fish Genetic Resources, Canal Ring Road, Dilkhusa, Telibagh, Lucknow 226 002, India.
Email:

Most parasite species show high levels of host specificity. The fish louse *Argulus siamensis* shows non-host specific trait and parasitizes a wide range of freshwater fish species causing heavy economic loss. Though *A. siamensis* is a generalist parasite, it demonstrates some apparent host preferences. This study aims at use of field and experimental observations to record the susceptibility pattern of some of the widely-cultured carp species to *A. siamensis.*

In a nationwide survey initiated by CIFA under NFBSRA project, argulosis outbreak and prevalence were surveyed in major aquaculture zones of eight states of India comprising of 1067 hectare of water area. In most of the zones in general, *Labeo rohita* was found to be most susceptible species followed by *Cirhinnus mrigala, Catla catla* and *Cyprinus carpio* except the berries of West Bengal where *C. catla* was found to be most susceptible followed by *C. mrigala.* In order to confirm this susceptibility pattern of the carp species used in polyculture, *L. rohita, C. mrigala, C. catla* and *L. fimbriatus* were challenged with 200 metanaupliar larvae of *A. siamensis* each in aquaria. *L. rohita* was found to be the first affected and most preferred species. *C. mrigala* was also heavily affected, *C. catla* being moderately affected and *L. fimbriatus* the least affected species. To further understand the susceptibility pattern of few widely cultured carp species, a field experiment was conducted in six ponds each of 0.04 hectare area stocked with *L. rohita, C. catla, C. mrigala* and *Hypophthalmichthys molitrix* (n = 130, 20, 20, and 20, respectively). The experimental infection was initiated by co-habitational challenge in four of the ponds. The ponds were sampled monthly to evaluate the susceptibility. *L. rohita* appeared to be the most susceptible species harboring maximum quantum of parasites in all samplings followed by *C. mrigala* and *C. catla. H. molitrix* was the least affected species and appeared to be resistant to *Argulus* infestation. It is probable that the susceptibility of fish species to argulosis depends on species-specific behavioral and ecological traits which make these species easy targets for the parasite.

PP-E11

Effectiveness of freshwater bath on marine leech *Zeylanicobdella argumensis*, a parasite of marine cultured fish

***Beng Chu Kua**, Muhd. Naim Abd. Malek and Horng Chung Kok*

National Fish Health Research Centre (NaFisH), FRI NaFisH Batu Maung, Fisheries Research Institute,Department of Fisheries Malaysia,11960 Batu Maung, Penang, Malaysia.
Email:kuaben01@fri.gov.my

Freshwater bath had been practised by local marine fish culturists in order to dislodge the ectoparasite. It is a prophylatic measure in fish health management in marine floating cages. However, the effectiveness of this freshwater bath treatment against the marine leech infectation is yet to be proven. The present study was carried out to determine the survival percentage of adult and juvenile marine leech exposed to freshwater bath. The hatching percentage of juvenile marine leech from cocoon laid in different time of day and exposed to freshwater was also studied.

Results showed that 38 -100% of adult and 60 - 100% of juvenile marine leech were able to recover in seawater. Results of the hatching percentage showed a lower hatching rate (27.5%) on the Day 1 cocoon treated with freshwater as against 81.6, 77.0, 74.7 and 61.7% in control (no freshwater bath), Day 3, 5 and 7 respectively. There was a significant difference in hatching percentage between the cocoon laid time of day as compared to the freshwater bath. However, there was no significant difference between the exposure time (5, 30 and 60 minutes) with hatching percentage of cocoon. This study showed that a 5 to10-minute freshwater bath could reduce the adult and juvenile marine leech. However, reinfection can occur as 25 to 33% of adult and 17 to 50% of juvenile marine leech are able to infect the fish after freshwater bath. A delay of freshwater treatment on the laid cocoon after day 1 onward would increase the hatching rate of juvenile marine leech, hence increasing its population in the cultured fish.

PP-E12

Spread of *Caligus sclerotinosus* (Copepoda: Caligidae), a Pest of Cultured Red Seabream *Pagrus major* (Sparidae) to Korea

B. A. Venmathi Maran, *Sung-Yong Oh, Hee Jung Choi and Jung-Goo Myoung*

Marine Biology and Living Resources Research Department, Korea Ocean Research & Development Institute, Ansan, P. O. Box 29, Seoul 425-600, Korea.
Email: bavmaran@gmail.com

Caligid copepods (Crustacea) called as sea lice are known to be pests of cultured fish, since they cause serious diseases and economic losses in fish aquaculture worldwide. However, the study on sea lice in Korean aquaculture is scanty. Caligus sclerotinosus Roubal, Armitage & Rhode, 1983 (Caligidae), a parasitic copepod primarily infecting the highly prized cultured red seabream *Pagrus major* (Temminck & Schlegel, 1843) (Sparidae) in Japan, has been considered as a serious pest. Recently, in neighbouring Korea, red seabream culture has been carried out extensively and replaced with the yellow tail culture. However, until now, there have been no reports on caligid infection from the cultured red seabream in Korea. Our survey conducted in 2011 of 100 cultured individuals (range from 16 to 36 cm in total length) from Tongyeong floating net-cage fish farms, Gyeongsangnam-do, Korea revealed severe infection of the sea louse *C. sclerotinosus* on the body surface of cultured red seabream. Prevalence was recorded as 100% and the maximum number of individuals per host was 22. Adult ovigerous females, males and few developmental stages were observed. The adults of C. sclerotinosus seem to undergo ontogenetic host switching after the final moult. It has a high host-specificity for cultured red seabream, but surprisingly is not found on wild host collected from Tongyeong and Yeosu Fish Markets located at the southern coast of Korea. Severe infection of this sea louse can cause secondary infection. Route of infection of this caligid is focused either on natural dispersal, during migrations of other fish hosts across the narrow and shallow Tsushima and Korea Straits or import of fish from Japan. Regardless of route of entry, we expect this pest will have an impact on Korean red seabream fisheries equally serious to that being experienced in Japan. It can be considered as an alien parasitic copepod in Korea.

PP-E13

Morphology and SEM studies of the trematode, *Dinurus barbatus* infecting the dolphin fish *Coryphaena hippurus Linnaeus,* from Visakhapatnam coast, Bay of Bengal

***Indira Ashalatha Peter**, K. Padma Dorothy and G.Gnana Mani*

Dept. of Zoology, Andhra University Visakhapatnam-530003.

The dolphin fish, *Coryphaena hippurus* is a pelagic fish, cosmopolitan in tropical and sub-tropical waters. Besides being a food resource, it is an important game fish in many parts of the world. An investigation was undertaken from December, 2009 to November, 2010, with a view to examine the helminth parasites of *C. hippurus* off Visakhapatnam coast, Bay of Bengal. Specimens of *Dirurus barbatus* (Digenea: Hemiuridae) have been collected from the stomach of the dolphin 140 of 241 fish examined were found harbouring this trematode parasite. The morphology of the parasite as seen with light microscope and the structural features as observed by scanning electron microscope are being furnished. The possible consequences of infection are considered.

PP-E14

First report of nematode (*Camallanus* sp.) infection in *Puntius denisonii*, an indigenous ornamental fish endemic to the western ghats of India

*C K Manoj, **Devika Pillai**, C Mohanakumaran Nair and K R Salin*

Department of Aquaculture, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi - 682 506, Kerala, India.
Email: devikamanoj.pillai@gmail.com

We report the infection of nematode parasite, *Camallanus* sp., for the first time in *Puntius denisonii*, a highly valued and globally traded ornamental fish endemic to the Western Ghats, India. The infected fishes were collected prior to their breeding season from the upper stretches of Chalakkudy River, Kerala. Worms with characteristic red colour were seen protruding from the anus, of the weak fishes. Other clinical signs observed were bloating and reddish hemorrhages on the lateral and ventral sides of the fish and also at the anal region. The vent was reddish and swollen. More number of parasites was seen anchored towards the posterior intestine of the fish using hooks present in their cephalic region. They were viviparous with large number of young ones present inside the body cavity. *Camallanus* infection is highly contagious in aquarium systems as they can adopt a direct life cycle, in the absence of intermediate host. Treatment using metronidazole @ 5 ppm has yielded partial success in controlling the parasite. Considering the importance of *P. denisonii* in global ornamental fish trade, effective quarantine and treatment measures are to be standardized to prevent mortality and further spread of this dreadful parasite. Recently classified as vulnerable by the IUCN, we recommend that further studies should be carried out to find whether these parasites affect the breeding and recruitment of *P. denisonii* in the wild.

PP-E15

Co-infection of isopod and copepod parasites in halfbeak fish, *Hemiramphus marginatus* from the Coramandel coast of India

S.N. Sethi,[1] *K.P. Jithendran*[2] *and S.K anappan*[2]

[1]MRC OF CMFRI, 75, Santhom High Road, R.A.Puram, Chennai-28, India.
[2]CIBA, 75, Santhom High Road, R.A.Puram, Chennai-28, India.
Email: sethisatyanarayana@yahoo.co.in

The study was carried out to establish the extent of double parasitation of the isopod, cymothoidae and copepod pennellidae on halfbeak fish, *Hemiramphus marginatus*, from Coramandel coastal waters during September 2011. This is the first report from this region and the infection is discussed in relation to environmental and biological parameters. The double parasitation on *Hemiramphus marginatus* could also be related to the availability of numerous parasitic larvae particularly cymothoid larvae, which in turn depend upon the season and environmental temperature. The female parasite specimen (23-28 mm) and the male (13-18 mm) were attached on the same side or other side. Total number of eggs per adult parasites varies from 450-600 with an average of 526 ± 55.09. Total number of larvae per adult parasite varied from 391-420 with an average of 402.2 ± 36.93. Total number of Juveniles (Mancae/ pullus-stage-II) per adult parasites varied from 350-450 with average 414.2 ± 37.85. Percentage of occurrence of parasites on halfbeak fish was 60 %. The number of parasites per fish ranged from 1 to 3 with average number of 1.73 ± 0.59. Cymothoids harm the fish in several species of fish. Mancae (juvenile parasitic stages of cymothoid) feed voraciously and easily kill fry and fingerlings through tissue damage. Permanently attached adult parasite stunt the growth of fish and retard or inhibit reproduction. Parasites in the gill chamber are usually associated with stunted gills, partly from pressure atrophy and partly from damage associated with feeding and attachment that leads to anaemic gill condition. This may lead to severe economic loss in the commercial species of the marine fishes of India.

PP-E16

Diversity of parasite fauna of feral and cultured Indonesian shortfin eel (*Anguilla bicolor bicolor* McClelland) in Kerala, India

Sheeba, S *and Bijukumar, A.*

Department of Aquatic Biology & Fisheries, University of Kerala, Thiruvananthapuram 695 581, Kerala, India.

E mail: sheebaaqb@gmail.com

Fish parasites are an integral part of biodiversity of aquatic ecosystems and are recognised as a critical limiting factor in realising the full potential of aquaculture operations. Indonesian shortfin eel *Anguilla bicolor bicolor,* common in the freshwater systems of Kerala state, India, has now been cultivated in brackish water ponds in Kerala, and practically nothing is known on the parasites and diseases of these eels in India. In countries where eels are cultured, the swim bladder nematode *Anguillicola crassus* has been considered as a serious menace. The lack of information about the parasite and diseases of *A. bicolor bicolor* inhabiting feral water bodies and culture systems prompted the present work. This species, exhibiting catadromous migration, was collected from different freshwater and estuarine waterbodies of Kerala and from the culture ponds at Cherthala, Alappuzha district, Kerala. While 13 species (1 Protozoa, 2 Digenea, 3Cestoda, 2 Nematoda, 1 Acanthocephala, 2 Copepoda and 2 Isopoda) were collected from fishes inhabiting natural environments, six species (1 Protozoa, 1 Digenea, 1 Cestoda, 1 Nematoda, 1 Acanthocephala and 1 Copepoda) were collected from culture systems. The parasites collected included *Myxobolus sp.* (Protozoa), *Opegaster anguilli* and *Phyllodistomum magnificum* (Digenea), *Nybelinia* sp., pseudophyllidean larva and *Pleuronectis* sp. (Cestoda), *Procamallanus anguillae* and *Heliconema n.* sp. (Nematoda), *Arhythomorhynchus sp.* (Acanthocephala) *Ergasilus* sp. and *Caligus sp.* (Copepoda) and praniza larva and *Alitropus typus* (Isopoda). Parasites such as *Phyllodistomum magnificum, Nybelinia* sp., pseudophyllidean larva, *Heliconema n.* sp., *Caligus* sp., praniza larva and *Alitropus typus* were absent in cultured fishes. While prevalence and mean intensity of infection in wild fishes was 62.27% and 25.53%, respectively, in cultured systems, the values recorded were 100% and 86%, respectively. The higher prevalence and intensity of parasites in cultured fishes may be due to the abundance of intermediate hosts in the culture system, demanding better pond management practices while culturing eels. Though the parasite diversity was less in eels in culture systems, presence of harmful parasites such as *Ergasilus* sp., *Arhythomorhynchus* sp. in the culture systems suggests the need for precautionary principle while initiating the culture operations in India.

PP-E17

Investigations on *Perkinsus* spp. infections in bivalve populations along the Indian coast

Suja G, *Sanil N.K, Asokan P.K and Vijayan K.K*

Fish Health Section, Marine Biotechnology Division, Central Marine Fisheries Research Institute, P.B No. 1603, Ernakulam North P.O., Cochin - 682018, India.
Email: sujagangadhar@gmail.com

Parasites of genus *Perkinsus* are considered as one of the most important pathogens causing mass mortalities in bivalves across the world. In many cases, live transfer of asymptomatic carriers for farming or ornamental purposes has resulted in the introduction of these protozoan parasites into new geographical regions. As therapeutical options are not available, management of the stocks through early detection and avoidance is the only way out. In India, farming of mussel and oysters is a fast growing mariculture activity producing about 20,000 tonnes in 2010, but information on the pathogen profile and diseases affecting them is almost lacking. The first and only report on a Perkinsus infection from India is that of *P. olseni* in the pearl oyster, *Pinctada fucata.* In this backdrop an inventory of the OIE listed protozoan parasites in bivalves is required.

Bivalve samples were collected from the Southeast and Southwest coasts of India during the period from 2008 to 2011. Commercially important, cultured species like *P. fucata, Crassostrea madrasensis* and *Perna virdis* and other bivalves sharing the same habitat were screened. The OIE recommended screening methods, RFTM incubation, histology and PCR were followed for screening the samples. Since RFTM and histology techniques were unable to diagnose low level infections, highly sensitive nested PCR techniques were also used. *Perkinsus* infections were observed in the following oyster species - *C. madrasensis, Saccostrea cuculata, P. fucata, P. margaritifera* and *Isognomon sp.* In mussels, *P. indica, P.virdis* and *Pinna bicolor* were infected while the clams *Meretrix casta, Gelonia gelonia, Paphia malabarica* and *Donax sp.* were infected. Sequencing results showed *P. fucata, P. virdis, M. casta* and *P. malabarica* were infected with P. *olseni,* while *C. madrasensis, S. cuculata* and *G. gelonia* were infected with *P. beihaiensis*. The present study revealed the presence, geographical distribution and host range of *Perkinsus spp.* from the southeast and southwest coasts of the Indian subcontinent. The presence of various *Perkinsus* spp. and their broad host range poses a serious concern for the growing bivalve culture in India and this highlights the need for regular surveillance of wild and farmed bivalve stocks in the Indian waters.

PP-E18

Development of a real-time PCR assay for discrimination and quantification of two *Perkinsus spp.* in the Manila Clam *Ruditapes philippinarum*

Kousuke Umeda and ***Tomoyoshi Yoshinaga***

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
Email: atyoshi@mail.ecc.u-tokyo.ac.jp

The Manila clam *Ruditapes philippinarum* is infected with two *Perkinsus species, Perkinsus olseni* and *P. honshuensis*, in Japan. The latter was described as a new species in Mie Prefecture, Japan in 2006. *Perkinsus* infection is suspected as a major cause of the nation-wide decline of the stock size and catch of Manila clam in Japan since the mid-1980s. Ray's Fluid Thioglycollate Medium (RFTM) assay has been most commonly used to quantify *Perkinsus* infection. However, this assay cannot discriminate between species that resemble one another morphologically. We developed a real-time PCR assay for the specific quantification of *P. olseni* and *P. honshuensis.* DNA was extracted using Chelex resin. Cultured *P. olseni a*nd *P. honshuensis* cells were counted and spiked into uninfected clam gill tissue prior to DNA extraction to generate a standard curve, which allowed quantification based on the PCR cycle threshold values. We compared the RFTM assay with real-time PCR by quantifying *Perkinsus* spp. in gill tissue samples from the same individual clams. Infection intensities estimated by both assays were significantly correlated (r2=0.63). Our results suggest that the prevalence and infection intensity of *P. honshuensis* is much lower than for *P. olseni* in Manila clams.

PP-E19

Infection of guppies by the *Tetrahymena protozoan:* Study of the immune response against the parasite and potential immunization strategies

Dina Zilberg,[1] *Galit Sharon,*[1] *Pulak Ranjan Nath*[2] *and Noah Isakov*[2]

[1]The French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Sede Boqer.

[2]Department of Microbiology and Immunology, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Email: dzilberg@bgu.ac.il

Guppies (*Poecilia reticulata*) are among the most popular ornamental fish species, widely traded across the world. Infection of guppies by *Tetrahyma sp*., a cosmopolitan protozoan parasite, constitutes a serious problem in guppy production. The infection starts at the skin, through which the parasite penetrates the organism, and becomes a systemic disease. *Tetrahyma sp.* can infect all internal organs, as well as brain and eyes. Conventional treatments are only effective against external infection.

Guppies severely infected with *Tetrahymena sp.,* were imported by a commercial ornamental fish farm and brought to our laboratory. High levels of *Tetrahymena sp.* were identified in external and internal organs. The parasite was aseptically isolated from internal organs and cultured in RM-9 medium, at 25°C. Immunization by intra-peritoneal injection, along with adjuvant, protected the fish from infection. Homogenate of immunized fish was able to immobilize *Tetrahymena sp.* in vitro, suggesting the existence of anti-*Tetrahymena* antibody.

Our aim is to further study the immune response of guppies against *Tetrahymena sp.*-derived antigens, and compare the efficiency of different immunization strategies, which can be applicable in commercial aquaculture.

Here, controlled infection with *Tetrahymena* was calibrated by IP injection and immersion. An LD50 value of 946 *Tetrahymena*/fish was established for infection by IP injection. Infection by immersion was achieved, with mortality reaching a maximum of 23%. To analyze acquired immunity in these fish, an ELISA for quantification of guppy antibody is currently being developed. Guppy Sera was separated by SDS-PAGE, clearly demonstrating the existence of heavy (~75kD) and light chain (~25kD) immunoglobulins (Ig). Ig heavy chain was used for mouse immunization, antisera was produced and will be used for ELISA development. Future studies are aimed at determining the nature of the *Tetrahymena sp.*, immunogens, and characterization of their localization on the parasite, using different parts of the parasite, such as cilia, for immunization. Different vaccination routes are being considered, with emphasis on the oral route. In a preliminary study, fish were successfully intubated, and *Tetrahymena sp.* lysate was administered via the oral or anal routes. The consideration of carriers for induction of immunity will be discussed.

PP- E20

Discovery of parasites on floating cage cultured marine fishes in Korea

B. A. Venmathi Maran, *Sung-Yong Oh, Hee Jung Choi and Jung-Goo Myoung*

Marine Biology and Living Resources Research Department, Korea Ocean Research & Development Institute, Ansan, P. O. Box 29, Seoul 425-600, Korea.
Email:bavmaran@gmail.com

Parasites on cultured fish cause serious diseases and economic losses in marine aquaculture worldwide. In Korea, some of the major cultured fishes are found with severe infection of parasites which are reported here. Our survey conducted in 2011 at Tongyeong floating net-cage fish farm, Gyeongsangnam-do, Korea resulted in the severe infection of parasitic copepods (Caligidae, Lernaeopodidae, Pennelidae) and monogeneans such as: *Caligus sclerotinosus* Roubal, Armitage & Rhode, 1983, *Clavellotis sargi* (Kurtz, 1877), *Clavella sp.* 1 and an unidentified monogenean from red seabream *Pagrus major; Caligus latigenitalis* Shiino, 1954, *Cl. sargi* and an unidentified monogenean from blackhead seabream *Acanthopagrus schlegelii schlegelii*; *Lepeophtheirus elegans* Gusev, 1951, *Clavella parva* Wilson, 1912 and *Microcotyle sebastis* Goto, 1894 from Korean rock fish *Sebastes schlegelii;* an unidentified monogenean from black rock fish *Sebastes inermis*; *Caligus hoplognathi* Yamaguti & Yamasu, 1951 and an unidentified monogenean from barred knifejaw *Oplegnathus fasciatus, Peniculus minuticaudae* Shiino, 1956 from file fish *Thamnaconus modestus.* Prevalence and mean intensity has been recorded. Attachment sites of the parasites were the body surface and the gill filaments. *Caligus sclerotinosus* and *P. minuticaudae* are considered as a new record to Korea. Some of the findings constitute a new host record.

PP- F1

Innate immune responses of the orange-spotted grouper *(Epinephelus coioides)* to a fish pathogenic strain of *Vibrio harveyi*

***Edgar C. Amar**, Joseph.P. Faisan, Jr, and Rolando V. Pakingking, Jr*

Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC/AQD)Tigbauan, Iloilo 5021, Philippines
Email: eamar@seafdec.org.ph

Ex-vivo assays and changes in epinecidin mRNA transcripts were employed to characterize the non-specific immune response of *E. coioides* to a fish pathogenic strain of *Vibrio harveyi* isolated from affected cage-cultured fish.

Naïve hatchery-bred *E. coioides* obtained from the AQD marine fish hatchery were reared to the experimental size of 80 g in 250 L tanks in the department's biosecure facility. Various bacterial concentrations (colony forming units (CFU) ml^{-1}) were intramuscularly (IM) injected to the fish to determine the 50% lethal dose (LD_{50}) and the challenge dose that gave about 30% mortality. The fish were IM-injected with the bacteria and plasma and tissue samples were obtained 3, 6, 12, 24, 48, 72, 96, 120, and 240 h post-injection. Head kidney (HK) was excised to isolate the HK cells. Total RNA extracted from skin, liver, spleen and kidney was subjected to semi-quantitative RT-PCR. HK cells respiratory burst (RB), plasma lysozyme activity, and plasma total immunoglobulin (Ig) was measured. Cumulative mortality and total bacterial load in liver was monitored at different time points and correlated with the immune responses.

The RB (OD_{620}) of HK cells increased from basal (0.8-0.9) at 0-72 h, to peak levels (1.2-1.3) at 96-120 h, and returned to a basal value (0.9) at 240 h post-challenge. Conversely, liver total bacterial load (TBC; CFU ml^{-1}) increased (0 -10^3) at 0-12 h, peaked (10^7) at 24h, started to decline (10^5-10^6) at 48-72 h and was undetectable at 120-240 h post-challenge. Total *Vibrio* count (TVC) likewise peaked at 24 h ($10^{6.5}$) but was undetectable before 6 h and after 96 h. An inverse relationship was found between RB and TBC (R^2=-0.34). Lysozyme activity (µg ml^{-1}) increased from 0 (before 6 h) to 12-13 (24-48 h) and back to 0 at 120 h post-challenge. Lysozyme activity and TBC were positively correlated (R^2=0.59). No significant changes were seen in plasma total Ig. Upregulated epinecidin gene expression in skin, liver, spleen, gill, and kidney tissues were observed upon bacterial challenge, which persisted up to 10 days post-infection. Respiratory burst, lysozyme activity, and epinecidin gene expression are critical parameters to describe the innate immunity of *E. coioides* to *Vibrio harveyi.*

PP-F2

Nonspecific immune responses in Nile tilapia (*Oreochromis niloticus*) infected with Aeromonas hydrophila: the effects of orally-administered mangosteen- (*Garcinia mangostana*) and corn silk- (*Zea mays*) coated feeds

***Elena S. Catap**, Xiomarah Gina F. Dominguez, Ma. Rexie R. Jimenez and Leonora P. Nudo*

Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, 1101, Philippines
Email: elenacatap@yahoo.com

In this research, the immunomodulatory properties of *Garcinia mangostana* and *Zea mays* corn silk were evaluated in Nile tilapia infected with *Aeromonas hydrophila,* one of the opportunistic pathogens of cultured tilapia in the Philippines. In the first trial, differential white blood cell counts, phagocytosis and lysozyme levels were determined in tilapia that were fed with powdered rind of *Garcinia mangostana,* which was coated on feed pellets. In the second trial, corn silk (*Zea mays*) was coated on feed pellets administered to tilapia, and subsequently, phagocytosis, lysozyme levels and production of reactive oxygen species (ROS) were measured. In both trials, three experimental groups were employed: (1) negative control fish that were fed with uncoated feeds; (2) positive control fish that were fed with uncoated feeds (30 days) and then injected with 10^6 cfu/ml of *A. hydrophila;* and (3) supplemented fish, which were fed with coated feeds (either with *G. mangostana* or *Zea mays*) for 30 days and subsequently injected with 10^6 cfu/ml of *A. hydrophila.*

Results showed that mangosteen-treated fish exhibited significantly higher phagocytic activity compared with the positive control fish (*A. hydrophila* infected only). Differential counts of leukocytes showed that mangosteen-treated fish had the highest percentage of lymphocytes among the three treatment groups. The thrombocytes of mangosteen-treated fish were also higher than the two other groups, but the difference was not significant. The positive control fish had the highest lysozyme activity among the three groups; however there was no significant difference detected. In fish treated with corn silk, phagocytosis was likewise significantly higher compared with the positive control. Lysozyme levels were also higher in fish treated with corn silk compared with the positive control, but the difference was not significant. Production of reactive oxygen species (with or without phorbol myristate stimulation) was lower in corn silk treated fish compared with the negative control fish, which suggests the capacity of corn silk as an anti-oxidant. These studies are the first report on the use of *G. mangostana* and *Zea mays* silk as immunomodulants in fish. The preliminary results indicate the potential of both feed supplements; however, further testing and evaluation of both natural products are required.

PP -F3

Cloning, sequence analysis, ontogeny and expression study of hepcidin gene of *Puntius sarana* in response to *Aeromonas hydrophila* infection

Abhilipsa Das *and P.K. Sahoo*

Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
E-mail: abhilipsadas@gmail.com

Hepcidin is an antimicrobial peptide (AMP) produced by liver in response to inflammation and plays an important role in iron homeostasis. This cysteine rich AMP is responsible for the first line defence against various microbes and a key mediator of innate immunity. In the present study hepcidin (hamp) gene was amplified, cloned, sequenced from an endangered medium carp *Puntius sarana* and the phylogenetic analysis was also performed. To detect the appearance of *hamp* gene in early time periods (unfertilised egg, 0, 1, 3, 6, 9, 12, 18, 24, 48 h and 4, 7, 14 and 21 days post-fertilisation), RNA collected from the above sampling times were subjected to RT-PCR analysis. Further, an artificial infection experiment was performed with *Aeromonas hydrophila* after injecting juveniles of *P. sarana* i.p with 2.24×10^7 CFU/fish to investigate the change in expression level of the *hamp* gene in infected liver tissues at 0, 1, 3, 6, 12, 24, 48 h and 4, 7 and 14 days post-challenge (pc). The obtained *hamp* amplicon produced a predicted ORF of 279 bases having 92 amino acids and the nucleotide sequence showed 99% identity with zebrafish hepcidin. The amino acid sequence analysis revealed a signal peptide of 24 amino acids in length with a propeptide cleavage site located between Lys^{66} and Arg^{67}. In the ontogeny study, *hamp* gene expression was detected from 6 hpf onwards. Upon challenge with *A. hydrophila*, the gene showed its significant ($p < 0.05$) peak expression level at 3 and 6 hpc, whereas down-regulation of the transcript was evident after 24 hpc onwards till day 14 post-challenge in liver tissues of survivors as compared to control fish liver, thus indicating the potential role of hamp in bacterial disease modulation. Hence, further work is needed to check the spectrum of antimicrobial activity of the gene by its expression in *in-vitro* system.

PP-F4

Immune response analysis of outer membrane protein OmpC of *Aeromonas hydrophila* in *Labeo rohita*

P. Dash[1], *Sunita Kumaari Yadav*[2], *L.C. Garg*[3], *A. Dixit*[2] *and P.K. Sahoo*[1]

[1]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
[2]School of Biotechnology, Jawaharlal Nehru University, New Delhi-110067, India
[3]National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi-110067, India
Email: pujarini24@gmail.com

Aeromonas hydrophila is responsible for mass mortality in various fish species causing motile aeromonad septicaemia (MAS), haemorrhagic septicaemia, tail and fin rot and ulcers at different parts of the body. Development of a suitable vaccine is one of the most important steps towards effective management and control of mortality and related losses. A wide range of vaccine candidates have been explored in experimental conditions with varied levels of protection in fish. Out of different outer membrane proteins, OmpC is reported to be a porin of *A. hydrophila* that is regulated by the two component regulatory system and is essential for bacterial survival. The present work has been carried out to evaluate the modulation of innate immune response by the OmpC of *A. hydrophila* in *Labeo rohita*. Various immune parameters *viz.*, total serum antiprotease and ceruloplasmin, myeloperoxidase and lysozyme activities, bacterial agglutination and haemolysin titres were measured from the serum samples collected at different time periods, i.e., 0, 10, 28, 42 and 140 days post-administration in control fish administered with PBS and experimental fish administered with OmpC. Expression of some immune related genes viz., lysozyme C, lysozyme G, natural killer cell enhancement factor (NKEF), manganese superoxide dismutase (MnSOD), interleukin 1β (IL1β), complement factor 3 (C3), tumor necrosis factor α (TNFα), CxCa, toll like receptor 22, IgM were measured using head kidney samples collected at 1, 3, 6, 12, 24 and 72 h and 10, 28, 42 and 140 day post-injection. Fish administered with OmpC or PBS showed a significantly higher lysozyme activity compared to naïve fish whereas total antiprotease level was significantly higher in fishes that were administered OmpC. However, there was no significant alteration found in myeloperoxidase activity, ceruloplasmin level and serum haemolysin titre between control and vaccinated group fish. Fishes injected with OmpC showed a higher degree of IgM, lysozyme C, MnSOD, NKEF, IL1ß, C3, and TNFα expression, each showing an increase at different post-administration intervals. The OmpC injected fishes also showed a significant elevation in TLR22 and CXCa expression. However, there was no variation in expression pattern of lysozyme G in the fish administered with OmpC.

PP-F5

Estimation of heritability and genetic correlation of immune parameters screened as putative markers for selection against *Aeromonas hydrophila* resistance in rohu, *Labeo rohita*

S. Das[1], *P.K. Sahoo*[1], , *J.Mishra*[1], *K. Das Mahapatra*[1], *J.N. Saha*[1], *J. Ødegård*[2], *N. Robinson*[2,3] *and M. Baranski*[2]

[1]Cental Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
[2] Nofima, PO Box 5010, 1432 Ås, Norway.
[3]Flinders University, Sturt Road, Bedford Park, SA 5042, Australia
Email: pksahoo1@hotmail.com

Four selected immune parameters viz., serum ceruloplasmin, myeloperoxidase and antiprotease activities and natural antibody titre were investigated in 86 naïve full-sib families, generated from a selection program of rohu carp (*Labeo rohita*) for growth enhancement over three different year-classes i.e., 2003, 2004 and 2009 spreading over two generations. Sub-samples of fish from the same families were also subjected to a challenge test against *Aeromonas hydrophila* (with LD_{50} dose, intraperitoneal route). The correlation obtained between mean of immune parameters of different families studied in naïve fish and the survivals of the same families in challenge test was found to be significant. Serum ceruloplasmin and myeloperoxidase activities showed positive correlations (0.49 and 0.07, respectively) with survival, while serum hemaglutination titre and antiprotease activities showed negative correlation (-0.136 and -0.073, respectively) with survival. Heritabilities were also estimated for the four immune parameters, including data of all three year classes. The ceruloplasmin and myloperoxidase and antiprotease activities showed substantial estimated heritabilities (0.50 ± 0.22, 0.25 ± 0.09 and 0.37 ± 0.09, respectively). For hemolysin titre, the estimated heritability was low (0.0001). As negatively correlated parameters are usually discouraged to be used in selection programs for disease resistance and the positive correlation of myeloperoxidase activity was too low to be considered, the ceruloplasmin activity may be used as an indirect immunological marker for selection against aeromoniasis resistance in the selective breeding program of rohu. Furthermore, these traits can be recorded on live breeding candidates without exposure to the pathogen, which is a substantial advantage in selective breeding.

PP-F6

Estimation of genetic correlation of body length with innate immune status and resistance to *Aeromonas hydrophila* infection in rohu, *Labeo rohita*

***Sweta Das*[1], *K. Das Mohapatra*[1], *J. N. Saha*[1], *J. Ødegård*[2] *and P. K. Sahoo*[1]**

[1]Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India.
[2]Nofima, P.O. Box 5010, NO-1432 Ås, Norway.
Email: swetabt@gmail.com

Full-sib families of rohu (*Labeo rohita*) of four year classes (2003, 2004, 2008 and 2009) produced during the on-going selective breeding program at CIFA, Bhubaneswar were subjected to *Aeromonas hydrophila* intraperitoneal challenge as a part of the breeding program for raising disease resistance stock. The fingerlings were tagged individually with passive integrated transponder (PIT-tag) prior to challenge. The numbers of challenge-tested fish were 906, 1208, 2000 and 1355 for the year classes 2003, 2004, 2008, and 2009, respectively. The mortality was recorded on hourly basis over 10 days post-challenge during each challenge test. In addition, total length was measured for individual fish prior to challenge. Body length of all challenge-tested fish was analyzed using a univariate statistical model. Mean body lengths of survived and dead fish were significantly different (<0.05) for the year-classes 2004, 2008 and 2009, whereas for 2003 there was no marked difference. A positive correlation of 0.1 was found between length and survival for the above three year classes. However, the length of 2003 year-class fish showed a negative correlation (-0.065) with survival. Some of the serum immune parameters were also analyzed, such as hemolysin titre, ceruloplasmin and myeloperoxidase assays from naïve individuals of each family. Family means of all immune parameters showed positive correlations with body length of the same family (0.13, 0.29 and 0.11 for hemolysin, ceruloplasmin and myeloperoxidase, respectively) in three year classes i.e., 2003, 2004 and 2009. The positive correlation of length with survival and immune response traits indicated that body length might have some value as a possible co-trait for selection for improved resistance to *A. hydrophila* infection.

PP-F7

Outer membrane protein K as a recombinant protein vaccine against *V. anguillarum*

Mohammed Abdullatif Hamod[1, 2] *and Indrani Karunasagar1*

[1]Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore- 575 002, India.
[2]Department of Biotechnology, College of Sciences, University of Baghdad, Baghdad, Iraq.
Email: mohammedmohammed2@yahoo.com

Vibrio anguillarum has been described as a cause of disease in various species of aquatic animals from many parts of the world. Fish vaccination is a useful strategy for several disease problem in aquaculture. Outer membrane proteins of bacteria are considered ideal candidates for vaccine development. In the present study, *ompK* gene of *V. anguillarum* was cloned and sequenced. The recombinant OmpK was over expressed and purified by affinity chromatography on Ni-NTA Superflow resin. Immunoreactivity of the pure OmpK was studied by western blotting. The fresh water Indian major carp *Labeo rohita* (Hamilton, 1822) was well protected when challenged with *V. anguillarum* after vaccination with OmpK in comparison to non vaccinated fish. Specific antibody against OmpK and bacterial inhibition was observed in the vaccinated fish. Our results suggest OmpK as a candidate vaccine molecule against *V. anguillarum*. The molecule was immunogenic and demonstrated protective efficacy even when used in the freshwater fish.

PP-F8

Evaluation of biofilm of *Aeromonas hydrophila* for oral vaccination of *Channa striatus* (blotch)

Naveen Kumar B.T*, Prabhugouda Siriyappagouder, Shankar K.M, Omkar V. Byadgi , Adil B, Shilpa S.R and Iqlas Ahmed*

Fish Pathology and Biotechnology Laboratory, Department of Aquaculture, College of Fisheries, Karnataka Veterinary, Animal and Fisheries Sciences University, Mangalore 575002, Karnataka, India

E- mail: naveenkumar504@gmail.com

Oral vaccination is considered ideal for commercial vaccination as it is simple and economical for mass immunization. However, oral vaccines in general give poor protection due to their destruction in gut before reaching immune responsive sites. Our laboratory has developed a biofilm oral vaccine of *Aeromonas hydrophila* to facilitate improved antigen delivery in oral vaccination of fish, which has given significant higher antibody titer and protection in herbivorous carps and omnivorous catfish compared to free cell vaccine. Against this back ground in the present experiment *Channa striatus* a carnivorous model was fed with Biofilm (BF) and Free cell (FC) of *A. hydrophila* vaccine at 10^{10} cells / g fish /day for 20 days and monitored for serum antibody production and protection upto 60 day post vaccination. BF vaccinated *Channa* group showed significantly higher antibody titer and relative percentage survival (88) than FC (29.6) and control group.

PP-F9

Outer membrane proteins, Aha1 and OmpW of *Aeromonas hydrophila* are potential vaccine candidates for common carp

***Biswajit Maiti**, Mahesh Shetty, Malathi Shekar and Indrani Karunasagar*

UNESCO-MIRCEN for Marine Biotechnology, Department of Fishery Microbiology, Karnataka Veterinary, Animal & Fisheries Sciences University, College of Fisheries, Mangalore-575002, India
E-mail : maititbiswajit@yahoo.com

Aeromonas hydrophila is considered as an important fish pathogen and responsible for huge economic losses in aquaculture. The outer membrane proteins (OMPs) of Gram negative bacteria play a key role in the adherence to the target host cell a vital step of bacterial pathogenesis. They are conserved and highly immunogenic in nature due to their exposed epitopes on the cell surface and therefore considered as potential vaccine candidate. In this study, we characterized and studied the diversity of two important outer membrane proteins namely Aha1 and OmpW of *A. hydrophila.* The genes encoding these proteins were cloned and over-expressed in *Escherichia coli* host cell. The recombinant proteins were purified, characterized and used for the vaccination of common carp. Study of sequence analysis revealed that these proteins are an adhesin (>0.7 of pad value) consisting of conserved active antigenic sites. Common carp immunized with recombinant Aha1 and OmpW proteins showed high antibody production and a relative percentage survival (RPS) of 52 and 71 respectively. Results suggest a significant protective efficacy against *A. hydrophila* infection.

PP-F10

Cloning, sequencing and expression analysis of one novel apolipoprotein M in *Labeo rohita* in response to *Aeromonas hydrophila* infection

***M. Mishra**, A. Mohapatra, Sweta Das, B. Kar, M. Biswas and P.K. Sahoo*

Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
Email: mitali.mishra.mm@gmail.com

Apolipoproteins, the protein moieties of lipoproteins, play critical roles in lipid metabolism and fish innate immune system. Many fish apolipoproteins (Apo) are found to be acute phase proteins showing antimicrobial activity against Gram +ve and Gram -ve fish pathogens. Apo-AI, Apo-AII, Apo E, Apo 14 and Apo M are some of the fish specific apolipoproteins so far detected in rainbow trout, common carps and other teleosts. This study focussed on recently described Apo M for its presence in one of the important Indian major carp species, *Labeo rohita* (rohu) and its cloning, sequencing and expression analysis in response to a bacterial pathogen *Aeromonas hydrophila* infection. Rohu juveniles were injected intraperitoneally with LD_{50} dose of 5×10^6 live cells of Aeromonas hydrophila per gram body weight of fish. Liver tissues were collected at 0, 1, 3, 6, 12, 24, 72, 168 and 360 hours post challenge to measure the level of expression of Apo M. The partial sequence information (530 bp) of Apo M mRNA was generated using gene specific primers. The sequence showed highest (82%) similarity with Apo M of *Hemibarbus mylodon*, an endangered Korean fish species. The expression of ApoM gene in naïve rohu was found predominantly in liver tissues. However, heart and spleen tissues also showed faint expression, and the expression was below detectable limit in all other tissues studied. The real time expression analysis of Apo M revealed up-regulation at 24 hours post-challenge in liver and subsequent down-regulation till day 15. More in-depth analysis may reveal the significance of Apo M in innate immune response of Indian major carps.

PP-F11

Cloning, sequencing and expression analysis of hepcidin gene of *Labeo rohita* to *Aeromonas hydrophila* infection

***A. Mohapatra**, M. Mishra, Sweta Das, A. Das, M. Biswas and P.K. Sahoo*

Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751 002, India
E.mail: amruta.may16@gmail.com

Antimicrobial peptides (AMPs) are important mediators of the immune response against bacteria. Hepcidin, pardaxins, misgurin, pleurocidin, and moronecidin are the main antimicrobial peptides that have so far been isolated from teleosts. Hepcidin is a cysteine rich member of this family with known functions in iron regulation and the innate immune response. The present study was carried out to clone, sequence and measure the expression of hepcidin gene in one of the important Indian major carp species, *Labeo rohita* (rohu) in response to a common bacterial pathogen *Aeromonas hydrophila*. A partial sequence information (129 bp) of hepcidin mRNA of rohu was generated. The partial sequence revealed the highest (78%) similarity with hepcidin of *Puntius sarana*, a medium carp species. The expression of the hepcidin in various tissues of naive healthy rohu juveniles was examined and observed that hepcidin is highly expressed in liver than any other tissues examined. However, its expression was also noticed in spleen, gill, heart, stomach, intestine, brain, skin, eye, muscles, anterior and posterior kidney. The juveniles of rohu were challenged with LD_{50} (5×10^6 cfu/ml) dose of *A. hydrophila* per gram body weight of fish intraperitoneally and liver tissues from infected fish were collected after 0, 1, 3, 6, 12, 24, 72, 168 and 360 hours post-challenge (hpc). A real time quantitative PCR analysis revealed the up-regulation of hepcidin gene at 3 and 6 hpc, which then declined afterwards. The potential role played by hepcidin in innate immune response during bacterial infection is discussed.

PP-F12

The immune response against bacterial antigens in Atlantic cod (*Gadus morhua L.*) using microarray analysis

Anita Müller[1], *Marije Booman*[2], *Tiago Hori*[2], *Tim Kahlke*[3], *Marit Seppola*[4], *Helene Mikkelsen*[4], *Matthew Rise*[2] *and Roy Dalmo*[1]

[1] Norwegian College of Fishery Science, University of Tromsø, 9037 Tromsø, Norway.
[2] Ocean Sciences Centre, Memorial University of Newfoundland, A1C 5S7, NL, Canada.
[3] Institute for Medical Biology, University of Tromsø, 9037 Tromsø, Norway.
[4] Nofima Marin, Muninbakken 9-13, 9291 Tromsø, Norway.
Email:anitamu@nfh.uit.no

For many centuries fresh or dried Atlantic cod (*Gadus morhua L.*) has been an important food source and trade product in several countries. Its popularity led to its demise and wild cod stocks declined during the last century. While feral populations are still recovering, aquaculture is an important alternative to satisfy market demands. However, cod fish farming is still in its infancy and is faced with numerous challenges. One such challenge is how to deal with infectious diseases, such as those caused by bacterial pathogens.

Although vaccines are either available or under development their efficacies are basically evaluated by vaccination followed by challenge with the respective pathogen. Measuring the antibody response indicates the strength of the immune response. However, the antibody response does not always correlate with the protective effect of the vaccine. A better measure would be to find molecular markers, other than antibodies, that positively correlate to vaccine efficacy. This may especially be relevant for cod due to the absence of MHC class II genes. This may also lead to the development of new rational vaccine strategies to induce higher level of protection. The aim of the project described herein is to identify gene markers for immunity with the ultimate goal of evaluating vaccine efficacies.

Fish were injected with three different inactivated bacteria emulsified in oil (atypical *Aeromonas salmonicida*, *Vibrio anguillarum, Francisella noatunensis*) and spleen tissues were sampled 8 h, 2, 7, 21 and 49 d post immunisation (pi). After RNA isolation, individual samples were analysed using the microarray technique and the 20K Atlantic cod oligonucleotide microarray.

While there are one or no differentially expressed genes found 8 hpi, 7 and 21 dpi, respectively, the gene expression profiles 2 and 49 dpi show a varying number of up and down regulated genes. A fraction of the identified genes are common between the immunised groups. Further analyses of the data are underway and will be presented. The findings are discussed in respect to their significance in the immune response of cod against bacterial antigens.

PP-F13

Foundation for optimization of vaccination and first feeding regimes in Atlantic halibut: studies on the development of the specific immune system

Aina-Cathrine Øvergård[1,2], *Ingrid Uglenes Fiksdal*[1], *Audun Helge Nerland*[1,2], *Elin Sørhus1 and Sonal Patel*[1]

[1]Institute of Marine Research (IMR), PO Box 1870 Nordnes, 5817 Bergen, Norway
[2]The Gade Institute, University of Bergen, N-5021 Bergen, Norway
Email: aina-cathrine.oevergaard@imr.no

Marine larvae hatch in an environment where they can be exposed to a variety of pathogens, and often high mortality has been observed during the early stages when the immune system is not fully developed. This emphasizes the need to establish adequate preventive counter measures such as vaccination and the use of probiotics. However, immunization before the fish is able to mount an effective immune response might induce tolerance. Since, high mortality during the first feeding and the transition to dry feed stages has been observed during halibut farming; we were interested in studying the development of lymphoid organs and the expression of B-and T-cell markers during developmental stages. This will enable us to estimate earliest possible time point to activate the specific immune system and thereby vaccinate halibut fry.

Regular samples were taken of fertilized eggs, larvae and juveniles up to 159 days post hatching (dph). All three lymphoid organs, spleen, anterior kidney and thymus, appeared to be morphologically well developed at the end of metamorphosis. Molecular biological analysis (real-time RT-PCR and *in situ* hybridization) showed that IgM transcripts (B- cell marker) could be detected at 66 dph and later. Using immunohistochemistry, the presence of IgM protein was found in both kidney and spleen by 94 dph, while in thymus it was seen at 108 dph. *In situ* hybridization analysis detected genes that are essential for early T-cell development as early as 42 dph within the thymus anlage, while positive cells likely to be mature T- cells were seen at 87 dph. Interestingly, despite a general trend where all the investigated immune markers showed a clear increase in early metamorphosis stage, they were down-regulated around the period of transition to dry feed.

We can thus conclude that the halibut specific immune system is probably not fully developed until the latter part of metamorphosis. Vaccination of Atlantic halibut larvae before 94 dph could possibly lead to tolerance instead of protection. Probable optimal timing for vaccination would be later than 94 dph, since the strength of the immune response will be decisive for adequate protection.

PP-F14

Inducible nonspecific immune responses in striped murrel, *Channa striata*

S. Kalaivani Priyadarshini[1] *and R. Dinakaran Michael*[2]

[1]PG Department of Zoology and Biotechnology, Lady Doak College, Madurai 625 002, Tamil Nadu, India.
[2]Centre for Fish Immunology, School of Life Sciences, Vels University, Chennai-117, India.
Email:shekma_skp@yahoo.co.in

It is a well-known fact that the innate immune system in fish serves as the primary source of defense mechanism against wide divergent of pathogens. Several examples indicate that the innate immune responses are more pronounced in fish than higher vertebrates. While considering its nature, a question arises whether these innate immune mechanisms are inducible by an enhanced expression by the pathogen entry or does the host machinery have a ready resource of immune components to tackle the pathogen. In order to get answer to this question an experiment was designed to find out the possible modulation of the innate non-specific immune responses in the murrel, *Channa striata* upon experimental challenge with either live virulent or heat killed *Aeromonas hydrophila*. An unchallenged control group was also maintained for comparison. Several non-specific immune responses both humoral (serum lysozyme, total peroxidase, antiprotease and ACH_{50} activity) and cellular parameters (intracellular ROS, RNS and peroxidase enzyme production) were analysed. It was remarkable that most of the non-specific immune responses tested were substantially enhanced in both the experimental groups when compared to the unchallenged control group. This indicates that most of the innate non-specific immune responses are inducible though they are constitutive in fish system.

PP-F15

Effect of the macroalga, CFI MA 04 on the expression of immune response genes in the head kidney cells of the carp, *Cyprinus carpio*

Priyatharsini R[1] *and R. Dinakaran Michael*[2]

[1]PG and Research Department of Zoology and Biotechnology, Lady Doak College, Madurai-625002.
[2]Centre for Fish Immunology, School of Life Sciences, Vels University, Chennai-600117
Email: rajendranpriya@yahoo.com

In Fish immunity, serum factors like lysozyme and Interleukins play very important roles. The extracts and fractions of the marine macroalga, CFI MA-04 was found to have immunostimulatory effect on the specific and non-specific immune parameters in the common carp, *Cyprinus carpio*. The present work was done to study the effect of administration of the macroalgal (CFI MA-04) fractions on gene expression of IL-1β and lysozyme C using reverse transcription - polymerase chain reaction (RT-PCR). Fractionation of the macroalgal extract was done following the method of Harborne (1988) The fractions used for the study are Terpenoids and phenolics (TP), Polysaccharides (PS) and Methanol extract (ME). Intraperitoneal administration of 20mg kg^{-1} bodyweight of the different fractions of the macroalga was done, with four groups of eight fish each, with one group as control. At 24 hour post injection the fish from each group were killed, dissected and head kidney removed. RNA was extracted from the tissue and cDNA was synthesised . A PCR with primers for βactin was performed with all samples as a positive control for RT-PCR. The study provides a clear indication that the macroalga has the ability to modulate the genes responsible for the immune parameters.

PP-F16

The role of neuromedin U during inflammatory response in the common carp

Masahiro Sakai[1]*, Shogo Hamasuna*[1]*, Hiroki Korenaga*[1]*, Toshiyuki Iizasa*[2]*, Takanori Ida*[2] *and and Tomoya Kono*[2]

[1]Department of Marine Biotechnology, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuenkibanadai-Nishi, Miyazaki, 889-2192 Japan
[2]Interdisciplinary Research Organization, University of Miyazaki, 1-1 Gakuenkibanadai-Nishi, Miyazaki, 889-2192 Japan
Email: m.sakai@cc.miyazaki-u.ac.jp

Neuromedin U (NMU) has variety of functions which includes smooth muscle contraction of uterus, regulation of arterial blood pressure, stress response, alteration of ion transport in jejunum, reduction of food intake and immune regulation. However, the understanding of NMU in teleost fish is limited only to food intake to date and the other functional aspects of fish NMU is unclear. Therefore, we identified NMU gene from common carp *Cyprinus carpio L.* and investigated its role in immune response.

We identified five transcript variants of NMU gene (NMU1 - NMU5) from common carp and investigated the tissue trophism of NMU mRNA transcript. Results revealed that NMU genes were expressed in various tissues like brain, gill, intestine, spleen, head, kidney, liver, skin and muscle. Moreover, we analyzed the effect of feeding status on the expression of NMU mRNA in carp tissues. The expression pattern of carp NMU genes significantly differ between hunger and repletion stages. The NMU 1, 2, 3 and 4 genes were highly expressed in repletion status; on the other hand, in hunger status NMU 5 gene expressed high in intestine. This indicates the involvement of NMU in food intake regulation. Expression of NMU genes in intestine stimulated by immunostimulants (bacterial or viral mimics) showed up-regulation in NMU3 and 5 genes after 3h post stimulation. Moreover, up-regulation of inflammation-related cytokine genes (TNF-α, IL-1β and IL-10) was observed in intestine after treating with synthetic peptide of NMU (1 nmol/ml) and activation of phagocytic cells was confirmed. These studies suggest that carp NMU plays an important role in regulation of immune responses by interaction with inflammation-related molecules. This work was financially supported by the Special Coordination Fund for Promoting Science and Technology from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

PP-F17

Studies on immune response of *Labeo rohita* against experimental infection with *Aeromonas hydrophila*

***Shameem U** and Gowri Devi K*

Department of Zoology, Andhra University, Visakhapatnam- 530 003
Email: ummey.shameem@gmail.com

Experiments were carried out to study the immune response of *Labeo rohita*, inoculated with *Aeromonas hydrophila*, responsible for causing Haemorrhagic septicaemia in carps.

Fish were separated into two groups, with 15 fish in each group. First group of fish were treated as experimental and were inoculated with 200µl of formalin killed *A. hydrophila*; second group of fish were treated as control and were given 200 µl of PBS. A booster dose was given on 14th and 21st day of the experiment. 24 hours after every booster dose blood was analyzed for immunological parameters like cell viability, neutrophil activity, phagocytic activity, antibody titer values, myeloperoxidase activity, lysozyme activity and antiprotease activity in both control and experimental fish.

A significant rise was noticed in cell mediated immune response parameters like cell viability, neutrophil activity and macrophage activity in the experimental fish compared to control fish. Highest antibody titre value was recorded in experimental fish after the second booster, on 21st day. Similar variation was also noticed in the myeloperoxidase activity, lysozyme activity and antiprotease activity, between control and experimental fish.

The present study shows enhanced levels of immune response in fish inoculated with *A. hydrophila* when compared to unvaccinated control fish.

PP-F18
TLR mediated immune enhancement in fishes

***Sujit Kumar**, Amit Ranjan, Kishor Kunal and Uday Kumar*

Fisheries College and Research Institute, Thoothukudi
Email: sushanfcri@gmail.com

Aquaculture is the fastest growing food sector but at the same time is faced with several problems such as sudden outbreak of diseases. Treatment of disease with traditional methods involve complications such as development of resistant strain, bioaccumulation, damage to the indigenous beneficial microbes etc. Prevention is better than cure and immune enhancement by stimulation is one of the most efficient strategy to prevent disease outbreak in aquaculture industry.

Toll like receptor (TLR) one of the pattern recognition receptors (PRRs), a part of innate immunity activates immune system by recognising pathogen associated molecular pattern (PAMP) the signature molecules of pathogens. These signature molecules are considered to be an essential component for the survival of the pathogen. Therefore, PAMPs are considered to be conserved among a range of pathogens, including virus, bacteria, and, fungi. TLRs recognize various PAMPS in various compartments and trigger immune system by the release of inflammatory cytokines and type 1 interferons for host defence. Furthermore, the responses of the innate immune system are important not only to eliminate pathogens but also to develop pathogen-specific adaptive immunity, which is mediated by B and T cell. Thus TLRs along with other PRRs represent a central component of the innate immune response as well as a link between natural and adaptive immunity. 17 TLR types (TLR1, 2, 3, 4, 5, 5S, 7, 8, 9, 13, 14, 18, 19, 20, 21, 22, 23) have so far been identified in more than a dozen teleost species. PAMPS recognized by these TLRs are triacylated lipoprotein for TLR1, peptidoglycan for TLR2, dsRNA and ssDNA for TLR3, lipopolysaccharide for TLR4, flagellin for TLR5, diacylated lipoprotein for TLR6, imidazoquinoline and its derivatives R-848 for TLR7, and bacterial unmethylated CpG DNA for TLR9. Ligands analogous to PAMPs can be administered to fish to activate different TLRs and ultimately enhances immune system . Drugs or molecules incorporated with ligands able to interfere with TLRs involvement may represent new therapeutic approaches for the control of several bacterial, viral and fungal diseases.

PP-F19

Characterization of immunoglobulin M (IgM) of Family Pangasiidae and crossreactivity with anti-*Pangasius hypophthalmus* IgM monoclonal antibodies

***Wanna Sirimanapong*[1,2], *Kim D. Thompson*[1], *Kan Kledmanee*[2], *Prawporn Thaijongrak*[2], *Bertrand Collet*[3] *and Alexandra Adams*[1]**

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom.
[2]Faculty of Veterinary Sciences, Mahidol University, Salaya campus, Nakornpathom, Thailand 73170.
[3]Marine Scotland-Science, Scottish Government, Marine Laboratory, Aberdeen, AB11 9DB, Scotland, United Kingdom.
Email : ws7@stir.ac.uk

Pangasius hypophthalmus immunoglobulin M (IgM) was purified from plasma by affinity chromatography and sodium sulphate (Na_2SO_4) precipitation (14, 16 and 20%). The IgM of other Asian species *Pangasianodon gigas, Pangasius larnaudii, Pangasius Sanitwongsei, Hamibragus filamentus, Clarias bactracus, Clarias macrocephalus* and *Cyprinus carpio* were prepared using 14% Na_2SO_4 precipitation. Purified IgM was analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 12.5%) and the results indicated that the heavy and light chains of IgM from *P. hypophthalmus* were 70-72 kDa and 25-26 kDa, respectively. The light (L) chains of IgM from the other fish species were similar to *P. hypophthalmus*, while the heavy (H) chains varied (*P. gigas* and *P. larnaudii* 76 kDa, *P. sanitwongsei* 69 kDa, *H. filamentus* 73 kDa, *C. bactracus* 74 kDa, *C. macrocephalus* 73 kDa and *C. carpio* 70 kDa). Western blot analysis of the IgM from the different fish species, performed using six anti-*P. hypophthalmus* IgM monoclonal antibodies (mAbs) produced using affinity purified IgM, showed that two of the antibodies (mAbs 23 and 28) reacted with the heavy chains from all the fish species tested. The other antibodies (mAbs 1, 2, 7 and 18) reacted with the light chains of most species tested, with the exception of mAbs 2 and 7 which did not react with the *C. carpio* light chains.

PP - F20

Immune response of barramundi (*Lates calcarifer*) to *Streptococcus iniae* bacterin by intraperitoneal injection and anal intubations

Hich Tran Vi,[1] *Dzung Nguyen Huu*[1] *and Heidrun Inger Wergeland*[2]

[1]Department of Aquaculture, Nha Trang University, Vietnam
[2]Department of Biology, University of Bergen
Email: tranhich@gmail.com

The immune responses of barramundi (*Lates calcarifer*) were investigated via intraperitoneal injection by *Streptococcus iniae* bacterin. Both specific and non-specific immune parameters such as phagocytic ratio, phagocytic index, lysozyme activity and the specific serum antibody titre against *S. iniae* were recorded for 8 weeks. The data showed that the concentration of specific serum antibody in barramundi reached their peak levels 3 weeks after immunization and the same was significantly ($P<0.05$) increased in agglutinating antibody titre. There was no significant difference in lysozyme activity, phagocytic ratio, and phagocytic index recorded at the time of sampling.

PP - F21

Molecular cloning, expression and characterization of recombinant outer membrane protein F2 (OmpF2) as a vaccine candidate for *Edwardsiella tarda*

***Mahesh Shetty**, Biswajit Maiti and Indrani Karunasagar*

Department of Fisheries Microbiology, UNESCO-MIRCEN for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore -2, India.
Email: mahesh.shetty18@gmail.com

Edwardsiella tarda, a Gram-negative bacterium of the family Enterobacteriaceae is recognized as the causative agent of the systemic disease, edwardsiellosis in fresh water and marine fish species. Outer membrane proteins of Gram negative bacteria have a potential role in development of diagnostics and vaccines. During the study, E. tarda was isolated from moribund catfish (*Pangasianodon hypophthalmus*) from a culture farm situated in Andhra Pradesh on the east-coast of India. The gene encoding OmpF2 protein of E. tarda was cloned in a pQE-30UA vector and expressed in *Escherichia coli* (SG 13009). The size of the recombinant protein was ~40 kDa as seen in a 12% SDS-PAGE. Polyclonal antibodies were raised in rabbit against the purified OmpF2 protein and the specificity of the antibody was confirmed by Western blotting. The Indian major carp, *Labeo rohita* (Hamilton) was immunized by intraperitoneal injection using the purified protein in oil based adjuvant (Freunds incomplete adjuvant) and protein in phosphate buffered saline. The antibody response was assessed by Sandwich-ELISA which demonstrated the protein to be highly immunogenic in fish. To test the protective efficacy, fish were challenged with a virulent *E. tarda* strain and relative percent survival (RPS) was 62 and 75 for oil and water based injection respectively. The results show, recombinant OmpF2 to be a promising candidate for use in vaccination against edwardsiellosis.

PP-F22

Molecular characterization and protective efficiency of recombinant *Fenneropenaeus indicus* translationally controlled tumor protein (TCTP) against WSSV infection

Rajesh Subramanian, *C.Kiruthika, S.Rashika . J.Priya and R.B. Narayanan*
Centre for Biotechnology, Anna University, Sardar Patel Road, Chennai-600025, India.
E-mail: raajesh.bio@hotmail.com

The gene coding for Translationally controlled tumor protein (TCTP) was PCR amplified from haemocyte cDNA of Indian shrimp, *Fenneropenaeus indicus*, and sequenced. Phylogenetic analysis suggested a close relatedness of TCTP from *F. indicus* to *F. chinensis* compared with other isolates. Translationally controlled tumor protein gene expression was found to be elevated in the haemocytes of WSSV-infected shrimps compared with the uninfected ones.

Translationally controlled tumor protein (TCTP) is a highly conserved protein expressed ubiquitously in all the organisms from prokaryotes to eukaryotes. TCTP has been implicated to have multiple functions such as anti-apoptotic, calcium homeostasis, cell growth, cell cycle regulation etc. Shrimps with higher levels of TCTP were reported to sustain WSSV infection. In the present study, the putative antioxidant activity of *F. indicus* TCTP (Fi-TCTP) is tested invitro and the possibility of using recombinant TCTP to protect shrimp against WSSV infection was assessed. Further, the immunomodulatory effects of recombinant TCTP during WSSV infection were studied. The antioxidant activity of shrimp TCTP is evident by the growth of *E.coli* in the presence of hydrogen peroxide. Shrimps injected intramuscularly with purified recombinant TCTP, on subsequent WSSV infection exhibited 42% survival and those provided with oral supplementation had 15% survival. Results from immunomodulatory studies show that the TCTP treated shrimps exhibited elevated levels of THC, a reduction in phenol oxidase activity, and respiratory burst that concomitantly decreases the stress levels thereby decreasing mortality.

PP- F23

Immune enhancement of *Oreochromis mossambicus* (peters) in relation to different doses of *Lactobacillus sporogenes* given as a feed additive

S.Venkatalakshmi [1], *J. Ebanaza*[2] *and Michael Dinakaran*[3]

[1]Department of Zoology, Government College for Women, Kumbakonam, India.
[2]PG Department of Zoology & Biochemistry, Government Arts, College (Autonomous), Kumbakonam, India
[3]Director, Centre for Fish Immunology, VELS University, Chennai, India
Email: vittallakshmi@yahoo.co.in

The significance of a suitable diet in preserving the health of living organism is widely recognized. In this present study, the microbial probiotic *Lactobacillus sporogenes* was assessed for its immunostimulatory properties when given as feed additive. The probiotic was given in three different doses - $2.5X10^5$, $5X10^5$ and 106 CFU as a feed supplement in the form of spores. All the three doses enhanced the specific antibody response to heat killed. *Aeromonas hydrophila,* activated neutrophils, total and differential white blood cell count significantly. 100% survival was observed in 106 CFU fed groups and the other two lower doses gave 80% survival against *Aeromonas hydrophila* infection. The gut colonization was also tested in the treated groups. A dose dependent survival of *Lactobacillus sporogenes* was recorded in the tank water and gut of *Oreochromis mossambicus*. From the result of study *Lactobacillus sporogenes* can be prescribed as an efficient microbial feed supplement.

PP-G1
Bioinformatics based Protein-Interaction-Network study of the predicted Type III secreted Repeat toxin of *Vibrio vulnificus*

Rama Adiga *and Indrani Karunasagar*

Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore, India.
Email: rama_adiga@yahoo.com

Several bacterial pathogens use the needle shaped Type III secretion system (TTSS) to inject effector proteins into the host cell. The effector proteins evade the immune system of the host and help in bacterial colonization. Many effector proteins alter the host cellular target like the cytoskeleton or membrane and bring about necrosis. Thus the identification of effectors is a crucial step in understanding the pathogenesis of the organism. The Type III secretion system of *Vibrio vulnificus*, a marine bacteria causing infection in animals and human beings, has not been sufficiently addressed.

The vast repertoire of TTSS effectors in *V. vulnificus* were predicted. The repeat toxin (Rtx) known for its cytolytic/hemolytic activity is the most virulent, even though the mechanism of action is not known. The Rtx having GD-rich repeats form pores on the host cell membrane causing cell lysis. Recent studies suggest that the toxin may promote colonization. The signal peptide region was studied and predicted as a TTSS effector. Its prediction as a TTSS effector protein having insecticidal domain was unique. Protein-protein interaction network prediction studies revealed that the Rtx toxin interacts with a chaperone protein. The chaperone protein showed structural similarity to phase I flagellin. Thus phase I flagellin in *V. vulnificus* with fibronectin domain for cell surface attachment may be the predicted capsular polysaccharide implicated in virulence. Capsular polysaccharides have been associated with phase variation in colony morphology of *V. vulnificus* with opaque encapsulated colonies being more virulent than the translucent variety. The flagellin protein had repeats similar to harpin domain. Protein interaction network studies throw light on the virulence of capsular polysaccharide related to Rtx.

PP-G2

Down-regulation of TLR 7 transcription by VHSV in olive flounder (*Paralichthys olivaceus*)

***Satheesha Avunje**, Myung-Joo Oh And Sung-Ju Jung.*

Department of Aqualife Medicine, Chonnam National University, Chonnam 550-749, Republic of Korea.
Email: s.avunje@gmail.com

Olive flounder (*Paralichthys olivaceus)* is an important aquaculture species of East Asian countries such as Korea and *viral haemorrhagic septicaemia* virus (VHSV, Rhabdoviridae) infection of the species has caused a large-scale economic loss in the region. Toll-like receptors (TLRs) are well-known pattern recognition receptors (PRRs) for viral pathogen associated molecular patterns (PAMPs), reported in mammals and in fishes. Among different TLRs, TLR 7 detects ssRNA viral nucleic acids and the information on TLR 7 response is scarce in fishes. With the objective of quantitative expression study of TLR 7 in VHSV infected olive flounder; we developed VHS in olive flounder and sampled for fish anterior kidney at 3, 6, 12 hour post infection (hpi), 1, 2, 4 and 7 day post infection (dpi). Quantitative reverse transcriptase polymerase chain reaction was performed for relative expression analysis of the TLR using β actin, as internal control. Study also included absolute quantification of viral transcript as well as negative ssRNA genome. The statistical significance was evaluated by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test in SAS 9.2. In the infected group viral transcripts were found from 3 hpi, were considerably increased at 12 hpi to reach maximum at 1 and 2 dpi, but reduced at 4 and 7 dpi. The viral genome copies showed a reduction from 3 to 12 hpi, but increased after that until the end of the experiment. Initially up-regulated TLR 7 expression in VHSV infected olive flounder was lowered when the viral transcripts were high. Nevertheless, TLR 7 expression regained when pathogen load was reduced at a recovery stage of infectious period. We conducted another experiment with TLR 7 ligand, imiquimod. The fish were intra-peritoneally injected with 100ug of imiquimod/ fish and sampled for kidney at 6, 12 hpi, 1, 2 and 4 dpi to analyse TLR 7 expression. Imiquimod stimulation resulted an elevated expression of the TLR at 1 and 2 dpi thus confirming a down-regulatory mechanism of the gene by VHSV. Under expression of TLR 7 might be a factor for domination of the virus over host immunity ultimately leading to the mortality.

PP-G3

Intracellular survival of seafood associated nontyphoidal *Salmonella* in human epithelial cells and *Acanthamoeba*

Bhowmick P. P*, Devananda D, Shabarinath S, and Karunasagar I*

Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore- 575 002, India.
E. mail: patit_micro@yahoo.co.in

Salmonella possess an unique type three secretion system (T3SS) encoded by genes present in *Salmonella* Pathogenicity Islands 2 (SPI-2) which play an important role in survival of *Salmonella* in the host cells. Using HeLa cells, we studied the intracellular survival of seafood associated *Salmonella enterica* serovar Weltevreden, Newport, and Bareilly along with *S. Typhimurium* (ST14028). Results suggest that seafood associated *Salmonella* serovars are unable to replicate intracellularly. A significant difference was observed between survival of seafood associated *Salmonella* serotypes and *S. Typhimurium* ST14028. Real-time PCR analysis showed that expression of SPI-2 coded *sse*F and *sse*C gene were not statistically significant. The low expression levels of SPI-2 encoded genes among the different nontyphoidal *Salmonella* isolates (NTS) could be a possible reason for their inability to multiply in these cells. In addition we studied survival ability of seafood associated nontypoidal *Salmonella Acanthamoeba castellania* an saprophyte of the aquatic environment. Soil harbors a variety of free living amoebae especially *Acanthamoeba spp*. that act as environmental hosts to many species of intracellular bacteria like *Salmonella*. Results show that all seafood associated NTS isolates were able to survive and multiply inside *A. castellani* but were less efficient in survival and multiplication than ST14028. To determine the role of SPI-2 coded genes in intraamoebal survival of *Salmonella,* transcription level of the SPI-2 genes *sse*F and *sse*C was determined. Results indicate that expression of *sse*F and *sse*C genes do not play an important role in survival of seafood associated *Salmonella* inside the amoeba. We conclude that nontyphoidal *Salmonella* possibly has a symbiotic association with *Acanthamoeba* using SPI-2 independent pathway probably to keep its intracellular genes patent.

PP-G4

Culture conditions affecting ompK gene expression in *V. anguillarum* suggest a role for this protein in Iron uptake and resistance to bile salt

Mohammed Abdullatif Hamod[1,2] *and Indrani Karunasagar*[1]

[1]Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore- 575 002, India.
[2]Department of Biotechnology, College of Sciences, University of Baghdad, Baghdad, Iraq.
Email: mohammedmohammed2@yahoo.com

The outer membrane protein OmpK was first identified in *Vibrio parahaemolyticus* and has been shown to be a receptor for a broad host rage vibriophage KVP40 in members of *Vibrionaceae*. Though antibodies raised against this protein has been shown to react with members of Vibrionaceae and Photobacterium, the presence of gene encoding the protein and the role of this protein in the fish pathogen *V. anguillarum* has not been established. In this study, the ompK gene was amplified from *V. anguillarum* by using primers designed from the sequence of the gene in *V. harveyi.* The amplicon (792bp) was cloned, sequenced and the sequence analyzed using bioinformatics tools. The predicted three dimensional structure suggests a porin like shape with 12 strands and shear number 16. The effect of culture conditions on the expression of *ompK* was studied using Real Time PCR. The expression increased significantly in the presence of bile salts and iron chelating agent 2,2' bipyridine suggesting a role for this protein in resistance to bile and in iron acquisition by *V. anguillarum.*

PP-G5

Molecular cloning, mRNA variants and NO generation of the Nitric Oxide Synthase Gene, MjNOS, in Kuruma Shrimp *Marsupenaeus japonicus*

Mari Inada[1], *Junichi Nishi*[2], *Maki Yoshimine*[2], *Keisuke Kihara*[2], *Tomoya Kono*[3], *Raja Sdhakaran*[4,5], *Terutoyo Yoshida*[4], *Masahiro Sakai*[4] *and Toshiaki Itami*[4]

[1] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki Pref. 889-2192, Japan
[2] Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan
[3] Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuenkibanadai-nishi 889-2192, Miyazaki, Japan
[4] Faculty of Agriculture, University of Miyazaki, 1-1, Gakuenkibanadai-nishi, Miyazaki Pref. 889-2192, Japan
[5] Department of Biotechnology, Thiruvalluvar University, Serkadu - 632 106, Vellore, Tamilnadu, India
E-mail: mari_k_p_sky_melody@yahoo.co.jp

Nitric oxide (NO) signaling is involved in many physiological processes in vertebrates and invertebrates. In mammals, three types of NO synthase (NOS) were reported, namely neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). In crustaceans, NOS plays significant role in regulation of nerve system and innate immunity. Here, we describe the entire cDNA sequence (4,616 bp) and the open reading frame (1,187 amino acids) of the kuruma shrimp *Marsupenaeus japonicus* NOS (*Mj*NOS). When heat-killed *Vibrio* penaeicida cells were injected into the kuruma shrimp, *Mj*NOS was expressed in the brain, gill, heart, lymphoid organ, intestine and thoracic ganglion. *Mj*NOS expression in the gill reached its peak 12 h and decreased to its normal level 24 h after *V. penaeicida* injection. In *Mj*NOS mRNA, three variants were detected. Partial deletion of oxygenase domain sequence was found in variant 1, whereas complete deletion of reductase domain with or without novel 104 bp insertion were found in variant 2 and variant 3. The total NO concentration in hemocytes was determined using the Griess reaction and high concentration was found at 12 h after injection of heat-killed *V. penaeicida* cells. This study was supported, in-part, by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists, the Research and Development Program for New Bio-industry Initiatives and the University of Miyazaki's Program for the Support of Women in the Sciences.

PP-G6

Isolation and characterization of microsatellite loci using a CA repeat enriched library in rock bream, *Oplegnathus fasciatus*

Jong-Oh Kim[1]*, Sung-Ju Jung*[1]*, Jehee Lee*[2] *and Myung-Joo Oh*[1]

[1]Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Republic of Korea,
[2]Department of Aquatic Biomedical Sciences, Jeju National University, Jeju 690-756, Republic of Korea
Email: jongoh.kim77@gmail.com

Rock bream (*Oplegnathus fasciatus*) have gained attention as an industrial fish-food resource. However, since 1998, there have been huge losses in the farms caused by iridovirus disease during summer season. This study carries out experiments to isolate fish with iridovirus resistance. For this purpose, the development of genetic maps to facilitate localization of quantitative trait loci (QTL) for disease resistance traits and identifying candidate genes are necessary. A large number of genetic markers are required to improve map resolution. However, a search of the GenBank database revealed that only 49 microsatellite sequences have been published for rock bream. In this study, we developed additional microsatellite markers for the rock bream.

Genomic DNA was extracted from 1 g of muscle tissue of cultured rock bream. Extracted genomic DNA was treated with four restriction enzymes (*RsaI, HaeIII, AluI,* and *NheI*) and products were ligated with SNX linkers. The biotinylated repeat oligo (dCA_{16}) was hybridized to the digested DNA. The microsatellite enriched elutant was amplified with an oligo adaptor primer. The enriched library was purified using Qiaquick PCR Purification Kit (Qiagen), ligated into pBluescriptII SK(-) vector (Stratagene) and transformed into a prepared competent cell. Plasmids were sequenced and mass sequences were analyzed with a bioinformatical pipeline.

We analyzed 29 individuals from southern coastal areas of Korea. PCR products were electrophoresed on an ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). GeneScan Analysis (V.3.7, Applied Biosystems, USA) software was used to score microsatellite alleles, and allele size was manually checked. We calculate the number of alleles per locus (k), observed and expected heterozygosities (Ho and He), and polymorphic information content (PIC) using the Curvus Program. Linkage disequilibrium and the Hardy-Weinberg Equilibrium were determined using Genepop v4.0. These markers will be useful for population genetic studies and QTL for disease resistance traits of rock bream.

PP-G7

Importance of DNA markers to identify disease resistant Penaeus monodon for disease free shrimp aquaculture

Sourav Dutta, Usri Chakrabarty, Somanajana Khatua, Ajoy Mallik and ***Nripendranath Mandal***

Division of Molecular Medicine, Bose Institute, P-1/12 CIT Scheme VIIM, Kolkata-700054
Email : dmmandal@gmail.com

Shrimp aquaculture is one of the most important hard currency generating, job generating and better quality food production industry all over the world including India. However this industry is plagued by outbreak of pandemics by many shrimp viruses. Among these white spot syndrome caused by white spot syndrome virus (WSSV) is the major threat for huge economic loss each year. Despite serious efforts to control the disease, the damage continues. So, development of disease free shrimp for aquaculture is be a big challenge for scientific community.

Development of DNA markers to identify disease resistant shrimps would be useful approach for disease free shrimp aquaculture. For the first time we have found a 71 bp microsatellite DNA marker to identify disease resistant shrimps. This microsatellite provided a highly statistically significant ($p<0.01$) DNA band (71bp) present in disease susceptible and not found in disease resistant shrimp populations. Another DNA marker of 455 bp developed by RAPD method was present in most of the disease resistant population of *P. monodon* ($p<0.01$). Two more microsatellite DNA markers of 790 bp and 340 bp have also been developed to identify disease resistant *P. monodon*. The former one provided a highly statistically significant ($p<0.01$) DNA band (790 bp) present in disease resistant and not found in disease susceptible shrimp populations. A statistically significant ($p<0.01$) DNA band of 340 bp was present in most of the disease susceptible population but absent in most of the disease resistant population. We have developed total four DNA markers to identify disease resistant shrimps.

These DNA markers are also being used to study diversity of disease resistant shrimp among wild populations from various parts of coastal India. These DNA markers will be very helpful for the marker assisted selection (MAS) of breeding programme for development of Specific Pathogen Free (SPF) or Specific Pathogen Resistant (SPR) broodstock. Finally this will help overcome the huge economic losses of shrimp aquaculture industry due to viral disease.

PP-G8
Identification of the splice variants of RdRP and CP genes in betanodavirus

Tohru Mekata[1]*, Jun Satoh*[1]*, Hiroshi Sako*[1] *and Toshiaki Itami*[2]

[1]Aquatic Animal Health Division, National Research Institute of Aquaculture, Fisheries Research Agency, Oita, Japan
[2]Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
Email: mekata at affrc.go.jp

Betanodavirus are the causative agents of viral nervous necrosis (VNN), one of the most serious diseases affecting more than 40 marine fish species in the world. An infected fish exhibit abnormal behavior and histological investigations reveal vacuolations in brain and retina. The genome of betanodavirus consists of a bipartite, positive sense, single strand RNA molecules (RNA1 and RNA2). RNA1 encodes a non-structural protein, RNA-dependent RNA polymerase (RdRP) and RNA2 encodes a coat protein (CP). In this study, the splicing variants of RdRP and CP genes were identified from VNN infected fish samples, and expression analysis of these variants has been done *in vitro* and *in vivo* by exposure to betanodavirus isolated from seven-band grouper.

The sequence analysis of RdRP and CP gene transcripts revealed the presence of three alternatively spliced variants of each gene. The combination of expression pattern was classified roughly into six and four types in each RdRP and CP gene, which have been tended to be related to infection level of each sample. The gene expression of each splicing variant has not been found *in vitro* with grouper cultured cell constructed from embryo cells. On the other hand, in the gene expression analysis of larvae hatched from betanodavirus polluted eggs, the spliced variants expression of only CP gene was observed in larval fish five days after hatching.

PP-G9

Bioinformatic study reveals carcinogenic protein signatures in OMPs of *Edwardsiella* spp.

Neema M *and Indrani Karunasagar*

Department of Fishery Microbiology, College of Fisheries, Mangalore-575002, Karnataka, India
E-mail: neema_m_n@yahoo.co.in

The protein signatures associated with the outer membrane proteins (OMPs) of *Edwardsiella tarda* and *Edwardsiella ictaluri* was determined using finger print (FP) scan by PRINTS database. There is a tumour necrosis factor receptor (TNFR) 19-like signature in *E.tarda* OMP, YP_003294197.1. Activation of TNFRs can induce a range of disparate effects, including cell proliferation, differentiation, survival or apoptotic cell death, depending upon the receptor involved. In the *E. ictaluri* OMP YP_002934162.1 a mas oncogene signature was present. The mas oncogene which was discovered following co-transfection with DNA isolated from a human epidermal carcinoma was found to induce tumorigenicity efficiently. In *E. tarda* OMP YP_003296265.1, a xeroderma pigmentosum group B protein signature was found. In humans, xeroderma pigmentosum is characterized by a high incidence of sunlight-induced skin cancer. Again the presence of an acute myeloid leukemia 1 protein signature in *E. tarda* OMP, YP_003294661.1 and *E. ictaluri* OMP, YP_002932156.1 suggest that these OMPs might have the ability to induce the formation of cancerous cells and infection by the bacteria may induce cells to multiply indefinitely and cause cancer over a period of time. However the credibility of the statement can be substantiated only through extensive laboratory studies.

PP-G10

Kinetics of nervous necrosis virus (NNV) infectivity titer in sevenband grouper, *Epinephelus septemfasciatus* with Poly(I:C) administration

***Myung-Joo Oh**, Han-Gill Seo, Toyohiko Nishizawa and Wi-Sik Kim*

Email: ohmj@chonnam.ac.kr

Viral nervous necrosis (VNN) has become a serious disease in over 30 species of cultured marine fishes worldwide over the last 20 years. The causative agents of VNN are nervous necrosis viruses (NNVs) belonging to the genus *Betanodavirus* of the family Nodaviridae. It was recently demonstrated that Poly(I:C) immunization of sevenband grouper *Epinephelus septemfasciatus* with live NNV confers protection against VNN. The process of "Poly(I:C) immunization" involves immunization of fish with a pathogenic live virus following administration of polyinosinic-polycytidylic acid [Poly(I:C)], a synthetic double-stranded RNA that induces a transient, non-specific antiviral state. As a result, the fish in an antiviral state survive the initial immunization with live virus. We previously demonstrated that the degree of NNV infection must be similar to that of a fatal dose in order for fish to mount a specific protective immune response against the virus, and Poly(I:C) administration to achieve an antiviral state could protect fish from such intense viral infection. However, the kinetics of NNV infectivity titer in the fish with Poly(I:C) immunization have not yet been studied. Thus, in the present study, we investigated the change in the infectivity titer of NNV in sevenband grouper with Poly(I:C) administration. Upon challenge with NNV, the fish without Poly(I:C) administration began to die on the 5th day. The NNV titer in brain tissue was detectable the day after NNV challenge and increased up to $10^{8.27}$ TCID50 g-1 within 3 days. Although no mortality was observed in the fish administered Poly(I:C), an average NNV titer of $10^{5.80}$ TCID50 g^{-1} was detected on the 5th day of NNV challenge. Moreover, NNV multiplied up to $10^{6.13\pm1.85}$ TCID50 g^{-1} in the fish with Poly(I:C) administration even though those fish were injected with an equivalent fatal dose of NNV. Thus, it was suggested that multiplication of NNV was slow and delayed in the fish with Poly(I:C) administration. It was furthermore observed that the threshold level of NNV for fish with Poly(I:C) administration to mount a protective immune response against NNV was around $>10^4$ TCID50 g^{-1} .

PP-G11

A novel fasciclin 1 domain containing protein from disk abalone, *Haliotis discs discus*. Molecular characterization and temporal expression study

Premachandra, H.K.A.[1]*, Mahanama De Zoysa*[2] *and Jehee Lee*[1]

[1]Department of Marine Life science, school of Biomedical sciences, Jeju National University, Jeju Special Self-Governing Province,690-756, Republic of Korea.
[2]Laboratory of Aquatic Animal Diseases, College of Veterinary Medicine, Chungnam National University, Yuseong-gu, Daejeon, 305-764, Republic of Korea.
Email: ajithjnu@hotmail.com

Fasciclins are a cell adhesion molecule comparable with immunoglobulin, cadherin, integrin and selectin family which play key roles in embryonic development and morphogenesis and originally identified in insects. In this study, we identified a gene consists with fasciclin 1 domain from disk abalone (*Haliotis discus discus*) by expressed sequence tag (EST) analysis. The full-length cDNA of abalone fasciclin 1 was 1041 bp with 909 bp open reading frame (ORF), encoding for 303 amino acids which has predicted molecular weight of 33 kDa. A characteristic two domains of fasciclin 1 was identified between 23 to 148 and 152 to 283 amino acids, respectively. The conserved regions of FRa motif, FRb motif and H-box were identified in abalone fasciclin 1 amino acid sequence which are similar to other fasciclin 1 containing proteins. The abalone fasciclin 1 exhibited homology with other known fasciclin 1 domain containing proteins from vertebrates, invertebrates, algae and bacteria, and it was very closely related with the jellyfish (*Carukia barnesi*) in phylogenetic analysis.

The mRNA transcripts of abalone fasciclin 1 were mainly expressed in hepatopancreas, mantle, gill and muscle and also marginally detectable in hemocyte. After physical injuries to the mantle and shell of abalones, the relative expression level of abalone fasciclin 1 was upregulated in hemocyte nearly 3.5 fold ($P < 0.05$) at 6 h post-injury and mantle in ~10 fold ($P < 0.05$) at 12 h post-injury. After bacterial challenge, its mRNA expression level in hemocyte was upregulated to the peak at 6 h post-challenge (11 fold of that of control ($P < 0.05$)) and kept upregulation throughout the experimental period. These results collectively suggest that abalone fasciclin 1 is a member of fasciclin family proteins and may be a potent cell adhesion molecule and involved in immune response versus invading microorganisms.

PP-G12

Transcriptional up-regulation of caspase 3, involving in apoptosis signaling can be induced by microbe associated molecular patterns in rock bream (*Oplegnathus fasciatus*)

Premachandra, H.K.A, Elvitigala D.A.S , Ilson Wang and Jehee Lee

5373, Marine Molecular Genetics Lab, Department of Marine Life Sciences, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju 690-756, Republic of Korea.
E-mail: ajithjnu@hotmail.com

Caspase 3 is an effector caspase which exhibits Cysteine Aspartate protease activity. Upon activation by caspase 9, caspase 3 can execute the intrinsic apoptotic program leading to arise morphological characteristics of apoptosis. A putative caspase3 -like cDNA was isolated during homology search in our rock bream cDNA database and was characterized at cDNA level. Using two gene specific primers, Rbcaspase3 was cloned into pMAL c2x vector and was transformed into *E. coli* DH5α. Using qRT-PCR, the tissue specific distribution was evaluated. In addition acclimatized rock bream fish were subjected to time course challenge experiments, using LPS, poly I:C, *E. tarda* and rock bream irido virus (RBIV). Subsequently qRT PCR was performed in order to obtain the expression levels of Rbcaspase3 in liver tissue upon each induction.

The full length of Rbcaspase3 consists of 2422 nucleotides (nt), containing 849bp open reading frame (ORF) encodes 283 amino acids, 114bp 5' un-translated region (5'-UTR) and 1459bp 3' un-translated region [(3' UTR)]. The polyadenylation signal was located at 18 nt upstream of the poly A tail. Furthermore there were two RNA instability motifs, located at 259bp and 436bp upstream of the poly A tail, respectively. As typical caspase-3 domain architecture, Rbcaspase3 contained putative pro-domain (residues 1-36), a large subunit (residues 52-176) and a small subunit (residues 189-283). Phylogenesis indicated that Rbcaspase3 was closely related to caspase3 of large yellow croaker (ACJ65025). According to the qRT-PCR results, significantly higher expressions of Rbcaspase3 transcripts could be observed in blood and liver tissues. Moreover the relative mRNA expression pattern of Rbcaspase3 in liver tissue upon LPS, poly I:C, *E.tarda* and rock bream irido virus (RBIV), showed some up-regulations, in which upon poly I:C challenge it exhibited an early phase characteristic up regulation (6 hour post injection). However in other challenges, Rbcaspase3 transcription appeared to be up regulated in late phase (24 and 48 hours post injection). Excluding LPS challenge, in all other challenge experiments there were some fluctuation of the transcript levels of Rbcaspase3 until it reached to 48 h post injection. Based on the results we can suggest that *E. tarda,* RBIV, Poly:IC and LPS can activate the apoptotic signaling pathway executed by caspase3, in rock bream liver tissue, mounting an immune response.

PP-G13

Molecular cloning, transcriptional and functional analysis of *Cystatin B* gene from the disk abalone, *Haliotis discus discus*

Premachandra, H.K.A., *Wan Qiang and Jehee Lee*

Department of Marine Life science, School of Biomedical Sciences, Jeju National University, Jeju Special Self-Governing Province, 690-756, Republic of Korea.
Email: ajithjnu@hotmail.com

The cystatins are a family of natural tight-binding reversible inhibitors of cysteine proteases, which wide spread in all living organisms and involved in a broad spectrum of physiological and immunological responses in both vertebrates and invertebrates. In this presented study, a gene encoding cysteine protease inhibitor, designated as AbCyt B, was identified in disk abalone (*Haliotis discus discus*) by expressed sequence tag (EST) analysis and cloned from a previously constructed cDNA library. The AbCyt B gene contains three exons and two introns, consisting of a 303 bp open reading frame (ORF) encodes 101 amino acid residues with cysteine proteinase inhibitor signature and a conserved region of QXVXG sequence. The predicted molecular weight of AbCyt B is 11 kDa and the theoretical isoelectric point is 5.49. Analysis of deduced amino acid sequence revealed that AbCyt B shared up to 44.7% identity and 61.2% similarity with Cystatin B from other organisms. Phylogenetic tree analysis showed that AbCyt B is closely related with the Cystatin B in eastern oyster (*Crassostrea gigas*). Functional analysis of AbCyt B recombinant protein exhibited inhibitory activity against the papain, with almost 84% inhibition at 3.5 μmol/L of recombinant AbCyt B at the presence of casein as substrate. The tissue expression analysis demonstrated that AbCyt B transcripts were expressed predominantly in the hemocyte, gill, mantle and digestive tract, whilst marginally expressed in muscle and hepatopancreas. After the bacterial and viral challenges, AbCyt B expression level in hemocyte showed significant induction compared with control by qRT-PCR analysis. These results suggest that AbCyt B is a potent inhibitor of cysteine proteinases and is also involved in immune responses against bacterial and viral pathogens in abalone.

PP-G14

Molecular markers on evaluation of species diversity and phylogenetic relationship of penaeid species along the east coast of India

***Rajakumaran P**, Davidjayaseelan B, Esakkirajan M, Manju S, Prabhu NM and Manikandan R*

Department of Animal Health and Management, Alagappa University, Tamilnadu
Email: rajakumaranzoology@gmail.com

Penaeidae shrimps (Crustacea, Decapoda) are the most economically important cultured aquatic organism of all crustaceans. This family distributed world widely, highest diversity occurs in the Indo-West Pacific region; more than two hundred species are known and these have usually been grouped into seventeen genera. This group of animals exploited now a days by overfishing, diseases and habitat destruction through pollutant. Robust management strategies need to be protect penaeidae population. Molecular markers are mainly classified into three types; allozyme, mitochondrial and nuclear markers. The widely used mitochondrial DNA markers are 12S rDNA,16S rDNA, cytochrome b, control region (CR). The commonly used nuclear markers are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), RFLP (restriction fragment length polymorphism), SSCP (single-stranded conformational polymorphism), microsatellites and EST markers. Molecular marker on application in Penaeidae shrimp for species identification, and sex differentiation, genetic variation and assessment of demographic bottleneck and structure study of natural population, comparison between wild and hatchery populations, preserving genetic biodiversity and detecting genetic tags propagation assisted rehabilitation programmes. In the present study, random amplified polymorphic DNA markers (RAPD) accompanied with morphomertic analysis to detect species diversity and genetic variation among the Penaeidae species along the east coast of India, The result showed similar pattern of genetic diversity and phylogenetic relationship through both morphometry analysis and RAPD. These studies are very useful to evaluate phenotypic variability, regional availability of species, genetic and evolutionary relationship among the species. The increase in the number of molecular markers and the construction of high density genetic maps, as well as the implementation of genomic resources (including genome sequencing), are considered to provide tools for the genetic improvement of aquaculture species by marker assisted selection. Molecular markers are concrete tools for identification of populations with genetic crisis by comparing genetic diversities that in turn helps to resolve taxonomic uncertainties and to establish management units within these threatened species.

PP-G15

Population structure of *Penaeus monodon* srood stock based on mitochondrial D-Loop sequence variation along the coast of Andhra Pradesh, India

***A. Chandra Sekhar Reddy*[1],*M. Persis* [2] , *L. M. Rao* [3] *and G. D. Khedkar*[4]**

[1]State Institute of Fisheries Technology Jagannaickpur, Kakinada Andhra Pradesh, India
[2]Commissioner of Fisheries, Hyderabad, Andhra Pradesh, India
[3]Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India
[4]Aquacuture Research Laboratory, Dr. Babasaheb Ambedkar, Marathwada University, P.O.B. 02, Aurangabad, Maharashtra, India
Email: chandrakkd@yahoo.co.in

India occupies second position in the world aquaculture production contributing about 7 % of world production. The cultured shrimp dominated by *Peneus monodon* is currently contributing nearly 30% of the total fisheries GNP in India and it is much higher for Andhra Pradesh . In view of the severe disease problem by WSSV and other problems affecting the shrimp culture industry a study on the genetic diversity of *P. monodon* brood stock along the coast of Andhra Pradesh based on mitochondrial d-loop sequence analysis has been taken up, since the knowledge about the genetic diversity and population differentiation is imperative to construct an appropriate genetically based stock enhancement programme and also to identify over exploited regions where artificial recruitment is required. The study area included three regions as Vizag (North Andhra), Kakinada (Central Andhra) & Nellore (South Andhra), and 90 brooders 30 from each region were studied and 42 haplotypes were found with the mean haplotype diversity from the three populations of A.P was *0.959 +0.010*, with a mean nucleotide diversity of pi = 0.11453 and an average number of pair wise differences (k) of 32.756, The *P. monodon* population of Andhra Pradesh was highly structured and among the three regions the Kakinada population was highly diverse when compared to the other two populations.

PP-G16

Identification of novel molluscan IκB protein from manila clam: gene expression analysis against *Vibrio tapetis*

Saranya Revathy, K*, Youngdeuk Lee, Sukkyoung Lee and Jehee Lee*

[1]Department of Marine Life Science, Jeju National University, Jeju, 690756, Republic of Korea
Email: saranyarevathy@gmail.com

The manila clam, *Ruditapes philippinarum* represents one of the most economically-important shellfish species of the aquaculture industry in Korea. Over the last decade, clam landings have been decreasing dramatically. *Vibro tapetis* is the causative agent of brown ring disease (BRD), named as such for the obvious brown conchiolin deposits on the infected clam's inner shell surface. This phenotype is a result of *V. tapetis*-mediated disruption of normal periostracal lamina production, leading to anomalous deposition of peristracum. In addition to this morphological symptom, infected clams experience significant weight loss, altered biochemical composition, depressed defense-associated activities, and ultimately death.

Mollusks lack the adaptive immune system, relying solely on the innate immune response. The nuclear factor-kappaB (NF-κB) signaling pathway is one of the most important components of the innate immune system and its activity is regulated by physical interaction with the inhibitor of NF-κB (IκB). Upon binding, IκB protein masks the nuclear localization signal (NLS) of NF-κB, thereby sequestering inactive NF-κB in the cytoplasm. Thus, elucidating the expression profile of such NF-κB inhibitors in the agriculturally-important manila clam will advance our understanding of the species' immune response to pathogenic threats and provide potential therapeutic targets for preventing and controlling molluscan diseases.

Our investigations led to the identification a novel IκB (Rp-IκB) from the manila clam, *Ruditapes philippinarum.* The Rp-IκB cDNA is comprised of a 1032 bp ORF, which encodes 343 amino acid residues with a predicted molecular mass of 38 kDa. Rp-IκB protein exhibited typical features of IκB protein family members, including the IκB degradation motif, PEST sequence and six ankyrin repeats. Phylogenetic analysis showed that manila clam and other known molluscan IκB proteins grouped together in the invertebrate cluster. Tissue specific expression analysis revealed that Rp-IκB was ubiquitiously expressed in all tested tissues. Significant up-regulation of Rp-IκBexpression was observed in gill and hemocytes following bacterial immune challenge with *Vibrio tapetis* and purified lipopolysaccharide endotoxin. These results indicated that as a key regulator of NF-κB in mammals, Rp-IκB might play an important role in manila clam defense against bacterial infection.

PP-G17

Molecular identification and expression analysis of the MASP-2 gene from rock bream *Oplegnathusfasciatus*

Saranya Revathy. K*, Ilson Whang[1], Youngdeuk Lee[1],Sukkyoung Lee[1], Hyowon Kim[1], MinyoungOh[1], and Jehee Lee[1,2]*

[1]Department of Marine Life Science, College of Ocean Science, Jeju National University, 1, Ara [1]-dong, 102 Jejudaehakno, Jeju-si, Jeju Special Self-Governing Province, Republic of Korea, 690-756.

[2]Marine and Environmental Institute, Jeju National University, Jeju Special Self-Governing Province 690-814, Republic of Korea

Email: saranyarevathi@gmail.com

The complement system is a major effector system of innate immunity. Complement activation via mannose-binding lectin (MBL) is accomplished through activated mannose-binding protein-serine protease (MASP). Three different MASPs (MASP1, 2 and 3) have been identified in humans to be associated with MBL (or ficolins, which have also been demonstrated to activate complement through the lectin pathway). After activation by auto-catalytic cleavage, MASP-2 cleaves complement factorsC2 and C4 and leads to the formation of C3 convertase C4b2a.

In this study, MASP-2 from the rock bream fish *Oplegnathusfasciatus* was identified and characterized using theGS-FLX™ technique. The cDNA of rock breamMASP-2 was composed of 1358 bp with a 912 bp open reading frame that encodes 303 amino acids. The deduced amino acid sequence of MASP-2 possessed the signal peptide, calcium binding EGF domain and extracellular CUB domain. The MASP 2 amino acid sequence exhibited the highest level of identity (76%) with formosan land-locked salmon, *Oncorhynchusmasouformosanus.* The RT-PCR analysis revealed that rock bream MASP-2 mRNA was expressed constitutively in liver, blood, spleen, head kidney, gill, intestine, muscle, skin, and brain in a tissue specific manner. Expression levels of MASP 2 in head and kidney were up-regulated after stimulation with LPS in Gram-negative bacteria *Edwardsiella tarda* and Gram-positive bacteria *Streptococcus iniae* suggest that MASP 2 plays an important role in rock bream defenses against bacterial infection.

PP-G18

Quorum sensing regulation on expression of type three secretion system genes in vibrios belonging to the *Harveyi* clade and its relation to virulence

H.A. Darshanee Ruwandeepika[1], *Patit Paban Bhowmick*[2], *Iddya Karunasagar*[2] *and Indrani Karunasagar*[2]

[1]Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University, Belihuloya, Sri Lanka

[2]UNESCO MIRCEN for Marine Biotechnology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore-575002, Karnataka, India.

Email : ruwandeepika@hoo.co.uk

Vibrios belonging to *Harveyi* clade (*V. harveyi and V. campbellii*) are ubiquitous, bioluminescent pathogenic marine bacteria causing luminescent vibriosis in shrimp leading to severe losses in aquaculture industry. Several factors are involved in the virulence of these organisms including the type three secretion system (TTSS) and the expression of virulence genes is controlled by different regulatory mechanisms such as quorum sensing. The TTSS are specialized secretion apparatus used by pathogens to inject virulence factors directly into host cells. Quorum sensing, the bacterial cell-to-cell communication system, involves the production, secretion, and detection of extracellular signal molecules (autoinducers) to regulate the expression of certain genes. In this context, this study was aimed to investigate the quorum sensing regulation on expression of TTSS genes of *V. harveyi* and also to investigate its relation with virulence. The expression of three genes of TTSS (vopD: *V. harveyi* outer protein, *vcr*D: *V. harveyi* calcium response protein and vscP: *V. harveyi* secretion protein) was studied in virulent, moderately virulent and avirulent strains using reverse transcriptase real-time PCR with gene specific primers. To investigate the quorum sensing regulation of TTSS gene expression, quorum sensing maximally active mutant-JAF483 (QS+: high cell density conformation) and quorum sensing inactive mutant-JAF548 (QS-: low cell density conformation) have been used. The virulence of the strains was examined by infecting the brine shrimp larvae. Findings of this study revealed that expression of all three genes were negatively regulated by quorum sensing having significantly lower expression in QS+ (Fold-expression 1, 1.1 and 1.1 for *vopD*, *vcrD* and *vscP* respectively) compared to QS- mutant (Fold-expression 27.4, 19.7 and 13.4 respectively at $P < 0.05$). There were clear differences in expression levels between virulent (causing 29% Relative Percentage Survival/RPS) and avirulent strains (81-84% RPS), with significantly high expression in avirulent isolates (Fold-expression 27.4, 19.7 and 13.4 for *vopD*, *vcrD* and *vcsP* respectively) and lower in virulent isolates (Fold-expression 1 for all three genes). When bacteria are at low cell density conformation (autoinducer concentration is low) they will start expressing genes which are important for survival as individuals and same is seen in case of avirulent organism. Our identification of the virulence and QS regulation of TTSS gene expression can have significant consequences for the implementation of control measures for the devastating luminescent Vibriosis problem in the aquaculture industry. This is the first study showing the relationship between virulence and TTSS gene expression in *V. harveyi* and in *V. campbellii*.

PP-G19

Characterization and tissue specific expression of Immunoglobulin M heavy chain gene in rohu, *Labeo rohita* (Hamilton, 1822)

***K. Saravanan**, M. Makesh, K.V. Rajendran, Aparna Chaudhari and C. S. Purushothaman*

Central Institute of Fisheries Education, Panch Marg, Versova, Andheri West, Mumbai-400061, India.

Email: sarocife@gmail.com

Immunoglobulins (Igs) play a major role in mediating vertebrate humoral immunity. In fish, immunoglobulin M (IgM) appears to be the major Ig isotype. Focus on fish IgM stems from the requisite for protection of cultured fishes from infectious pathogens. The present study was aimed at characterization of the IgM heavy chain gene in rohu, *Labeo rohita* and its tissue specific expression. A 451 bp segment of IgM heavy chain gene of rohu (GenBank accession no.HM581639) spanning the variable region was sequenced using consensus primers designed using the conserved regions in related species. The partial nucleotide sequence of the rohu IgM heavy chain gene showed 91%, 87% and 86% homology with IgM of *Cyprinus carpio*, *Ctenopharyngodon idella* and *Danio rerio* respectively. The deduced amino acid sequence of the partial rohu IgM heavy chain gene was 85%, 82% and 72% identical to that of *Cyprinus carpio*, *Ctenopharyngodon idella* and *Danio rerio* respectively. Quantitative real-time PCR analysis of the IgM gene expression in various tissues revealed that the highest expression was observed in the lymphoid tissues namely kidney and spleen followed by heart, intestine, gill, liver and muscle. It was also observed that the IgM heavy chain gene expression increased with age, i.e. 6-month-old fish showed higher levels than 4-month-old & 2-month-old fishes. The results reveal that, as with most teleost fish, the lymphoid tissues are the major sites of antibody production in *Labeo rohita* and also the IgM heavy chain gene expression increases with age.

PP-G20

In silico identification and characterization of potential binding sites on the surface of thermostable direct hemolysin

Malathi Shekar *and Indrani Karunasagar*

Department of Fishery Microbiology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore, India.
Email: malathishekar@rediffmail.com

Vibrio parahaemolyticus, a Gram negative halophilic bacterium autochthonous to the aquatic environment is implicated in the serious disease 'Vibriosis' in fish and is also responsible for the acute gastrointestinal illness in humans due to ingestion of raw or undercooked seafood. Among the several putative virulence factors associated with pathogenesis of this organism, the thermostable direct hemolysin (TDH), a cytolytic exotoxin having the ability to lyse red blood cells, has long been considered a key virulence factor and a pathogenic marker in *V.parahaemolyticus*. Recent studies show that protein surface regions are carved by numerous concavities and projections, which offer the necessary environment for binding interactions, thereby fulfilling their cellular roles and other fundamental biological processes through interaction with other macromolecules. Against this background, we screened the surface topography of the TDH variant genes using computational tools, for identification of probable accessible surface pockets and/or cavities, which could give an insight into the functional interaction of this protein and hold clues to the ability of this toxin protein to adopt multiple biological functions. We report here pockets that are probable binding sites on surface of the TDH protein, the key residues involved in the formation of these pockets, their spatial patterns and physicochemical parameters. An association between the TDH phenotypes with regard to mutable residues forming the pockets will be presented.

PP-G21

Molecular cloning and characterization of DNA repair protein, Rad23 from kuruma shrimp *Marsupenaeus japonicus*

Raja Sudhakaran[1]*, Shogo Okugawa*[2]*, Tohru Mekata*[3]*, Mari Inada*[2]*, Tomoya Kono*[3]*, Masahiro Sakai*[1] *and Toshiaki Itami*[1]

[1]Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, 889-2192 Miyazaki, Japan

[2]Graduate School of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, 889-2192 Miyazaki, Japan

[3]Interdisciplinary Research Organization, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, 889-2192 Miyazaki, Japan

#Present address: Department of Biotechnology, Thiruvalluvar University, Serkadu, Vellore - 632 106, Tamilnadu, India.

Email: 2sudha@gmail.com

Lesions of DNA are removed by Nucleotide excision repair (NER) process in the living systems. NER process related host factors are believed to aid recovery steps during viral integration. Here we report identification and characterization of a DNA repair molecule Rad23 from kuruma shrimp *Marsupenaeus japonicus.* The full length cDNA of *M. japonicus* Rad23 gene (MjRad23) has 1149 bp coding for a putative protein of 382 amino acids with a 5' untranslated region (UTR) of 92 bp and 3' UTR region of 1116 bp. Quantitative expression analysis revealed MjRad23 is constitutively expressed in all the organs of healthy shrimp, whereas with high level in muscle tissue. Though MjRad23 expression is observed in every hemolymph samplings to post white spot syndrome virus (WSSV) infection, high expression is recorded at 2 hours post infection (h.p.i.). MjRad23 consists of putative functional domains including one Ubiquitin domain (UBQ), two Ubiquitin associated domains (UBA) and one heat-shock chaperonin binding motif (STI1). Multiple alignment of MjRad23 with Rad23 of other species showed highly significant identity ranging from 37% to 53%, however high homology is observed with Rad23 of *Bombyx mori* (BmRad23). UBQ domain region alignment revealed maximum of 66% homology with Rad23 of *Apis Melifera* (AmRad23). MjRad23 clustered with invertebrate sector along with insect species in evolution analysis. Three dimensional structural analyses demonstrated the highest identity between MjRad23 and human Rad23A (hHR23A).The present work revealed the presence of MjRad23 gene, which is essential in DNA repair process. Further studies are required to clarify the involvement of MjRad23 in nucleotide excision repair process.This is first report on identification and characterization of DNA repair protein in crustaceans, which will lead for further investigation to explore the molecular mechanisms behind the NER process.

PP-G22
CS/TPP nanoparticles: preparation, characterization and application as oral gene delivery

Vimal Sugumar, *Taju, Nathiga Nambi, Abdul Majeed, Sarath Babu, Ravi and Sahul Hameed*

Aquaculture Biotechnology Division, OIE Reference Laboratory for WTD, Department of Zoology, C.Abdul Hakeem College, Melvisharam - 632 509, Tamilnadu, India.
Email: vimalbiotechs@gmail.com

The present study examines the potential use of CS/TPP nanoparticles for delivery of gene in different tissues of fish through oral route. The porin gene of *Vibrio anguillarum* was used to construct DNA vaccines using pcDNA 3.1, a eukaryotic expression vector and the constructs were named as pVAOMP .The CS/TPP nanoparticles were synthesized by ionic gelation process and these particles were characterized. The morphology and particle size measurements of the nanoparticles were performed by field emission scanning electron microscopy (FE-SEM) and FTIR (Fourier Transform Infrared Spectra). The results of FTIR of CS/TPP nanoparticles revealed many peaks one at 3418 cm^{-1} representing a broad -OH stretching absorption and another peak between 1203 cm^{-1} and 1023 cm^{-1} representing the free amino group (-NH2) at C2 position of glucosamine. The FE-SEM of CS/TPP nanoparticles revealed a very homogeneous morphology with quite uniform particle size distribution and spherical in shape with the size of particles ranged from 30 to 80 nm. The encapsulation efficiency of plasmid was calculated. The stability of plasmid DNA was also determined after encapsulation using DNase I and chitosanase. The cytotoxicity of CS/TPP nanoparticles was evaluated by MTT assay using fish cell line and the results showed that the cytotoxicity of CS/TPP nanoparticles was quite low. *In vitro* and *in vivo* expressions of porin gene were observed in sea bass kidney cell line and in fish, respectively by fluorescent microscopy and ELISA. Distribution of gene in different tissues was studied in fish fed with the pVAOMP DNA encapsulated in CS/TPP nanoparticles by PCR and expression of genes by RT-PCR, Immunohistochemistry, ELISA and Real time PCR. The results indicate that DNA can be easily delivered into fish by feeding with CS/TPP nanoparticles.

PP-G23

Molecular cloning, tissue distribution and expression analysis of the peptidoglycan recognition protein (PGRP) from rock bream *Oplegnathus fasciatus*

Ilson Whang[1], *Youngdeuk Lee*[1], *Sukkyoung Lee*[1], *Hyowon Kim*[1], *Minyoung Oh*[1], *and Jehee Lee*[1,2]

[1]Department of Marine Life Science, College of Ocean Science, Jeju National University, Ara 1-dong, Jejudaehakno, Jeju-si, Jeju Special Self-Governing Province, 690-756, Republic of Korea
[2]Marine and Environmental Institute, Jeju National University, Jeju Special Self-Governing Province 690-814, Republic of Korea
Email: ilsonwhang@naver.com

Peptidoglycan recognition protein (PGRP), similar to antimicrobial lectins, specifically binds to the bacterial cell wall, kills bacteria, and plays an important role in the innate immunity.

The cDNA of PGRP from the rock bream fish *Oplegnathus fasciatus* (denoted as *OfPGRP*) was identified and characterized using the GS-FLXTM technique. The full length cDNA was composed of 1443 bp, an open reading frame encoding 480 amino acids with an estimated molecular mass of 53 kDa and a predicted isoelectric point of 6.5. *OfPGRP* shares significant identity (88%) with Channel bass, *Sciaenops ocellatus* PGRP. Quantitative real-time RT-PCR analysis results confirmed that *OfPGRP* transcriptional expression is constitutively expressed in various tissues from healthy rock breams. *OfPGRP* expression was analyzed in head kidney following immune challenge with bacterial lipopolysaccharide (LPS). Compared to non-injected control fish, a significant up-regulation (21.8-fold) of *OfPGRP* transcripts was observed in response to LPS, indicating a role for PGRP in innate immune response against LPS.

PP-G24

Phosphatidylethanolamine binding protein (PEBP): characterization and expression from rock bream *Oplegnathus fasciatus*

Ilson Whang[1]*, Youngdeuk Lee*[1]*, Sukkyoung Lee*[1]*, Hyowon Kim*[1]*, Minyoung Oh*[1]*, and Jehee Lee*[1,2]

[1]Department of Marine Life Science, College of Ocean Science, Jeju National University, Ara 1-dong, Jejudaehakno, Jeju-si, Jeju Special Self-Governing Province, 690-756, Republic of Korea
[2]Marine and Environmental Institute, Jeju National University, Jeju Special Self-Governing Province 690-814, Republic of Korea
Email: ilsonwhang@naver.com

The phosphatidylethanolamine-binding protein (PEBP) is a ubiquitously expressed and highly conserved protein with homologs, from bacteria to plants and mammals. In humans, PEBP (named Raf-1 kinase inhibitor protein, RKIP) is implicated in cancer, Alzheimer's disease, infertility, diabetes, etc. This protein seems to modulate important cell mechanisms including control of heterotrimeric G-proteins, inhibition of the MAP kinase and NF-kB signaling and also inhibition of seine proteases (thrombin, neuropsin, chymotrypsin). However, the molecular identification by which fish PEBP's act remain obscure.

In the present study, a novel PEBP gene was identified in the rock bream, *Oplegnathus fasciatus.* The full-length sequence of *OfPEBP* (907 bp) consists of a 564 bp coding region encoding a 187 amino acid protein. *OfPEBP* shares the PEBP family consensus signature which is part of the ligand binding pocket. When compared to the other PEBP family members, *OfPEBP* displays significant identity (87%) with channel catfish *Ictalurus punctatus*. The mRNA expressions of *OfPEBP* in healthy and LPS-challenged rock bream were examined using quantitative real-time polymerase chain reaction (qRT-PCR). *OfPEBP* transcripts were found to be constitutively expressed in heart, brain, liver, kidney, blood, gill, intestine, spleen, head kidney, skin, and muscle, particularly strong in heart, brain, liver, blood, kidney, gill, intestine, spleen but weaker in head kidney, skin and muscle. Expression levels of *OfPEBP* in liver were up-regulated after stimulation with bacterial lipopolysaccharide (LPS). This is the first report in which the possible role of PEBP in fish innate immunity.

PP-25

Cloning and characterization of two novel interferon inducible genes in rock bream (*Oplegnathus fasciatus*)

Wickramaarachchi,W.D.N.*, Qiang Wan, and Jehee Lee*

Marine molecular genetics Laboratory, College of Ocean Science, Jeju National University, Jeju, Republic of Korea
Email: niroshanwdn@yahoo.com

Interferons (IFNs) play an essential role in the host response to viral infection through the induction of numerous antiviral genes. In a recent study, scientists identified a novel interferon inducible gene from CAB cell of grass carp after treatment with GCHV virus and termed it as Gig2. Gig2 has been suggested to play a pivotal role in interferon antiviral response. Here, we report the cloning and expression analysis of OfaA and OfaB genes which show highest sequence similarity with Gig2 genes from other fish. The complete cDNA sequences of two genes were obtained from a normalized cDNA library constructed with mRNAs obtained from different tissues of rock bream *(Oplegnathus fasciatus*). OfaA and OfaB contain 501 and 489 bp open reading frames (ORF) encoding two polypeptides of 167 and 163 amino acids, respectively. Their protein sequences share 60% similarity with each other and over 50% similarity with other known Gig2 genes. A conserved poly (ADP-ribose) polymerase (PARP) catalytic domain is present in both OfaA and OfaB. The tissue distribution analysis by qRT-PCR showed two distinctive patterns for OfaA and OfaB transcripts. The highest mRNA level of OfaA was detected in gill tissue, while OfaB mRNA showed a blood specific expression. The responses of two genes in immune challenges were analyzed in rock bream blood and head kidney after injection of LPS, poly (I:C) or iridovirus. OfaA expression was dramatically induced after LPS and poly (I:C) challenges, with peaks at 6 and 12 hour post injection respectively. In contrast, its expression was significantly suppressed during iridodvirus challenge most of the time. Expression of OfaB was significantly induced upon all the tested samples, except for iridovirus challenged head kidney. Our results suggested that two novel interferon inducible genes of rock bream may share similar function with the known Gig2 genes and are probably involved in the immune defense of virus infection.

PP-G26

Molecular analysis of key pore-forming molecule of complement system in rock bream (*Oplegnathus fasciatus*)

Wickramaarachchi W.D.N*, Qiang Wan and Lee, J*

Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Special Self-Governing Province 690-756, Republic of Korea
Email: niroshanwdn@yahoo.com

The membrane attack complex and perforin (MACPF) superfamily is one of the largest families of pore-forming molecules. The 9th component of complement system (C9) is a single-chain glycoprotein that is involved in the formation of the membrane attack complex (MAC) on the surface of target cells. Rb-C9 gene was identified from a Bacterial Artificial Chromosome (BAC) clone database of rock bream (*Oplegnathus fasciatus*) by a BLASTX search of NCBI. The complete gDNA of rock bream C9 is 1852 bp and consists of 11 exons interrupted by 10 introns. An open reading frame (ORF) encoding a polypeptide of 408 amino acids (aa). The predicted molecular weight of the Rb-C9 polypeptide is 46 KDa and iso-electric point is 5.6. The deduced amino acid sequence of Rb-C9 showed 51% and 47% identity to puffer fish and Japanese flounder C9, respectively. In addition, it has a 30% identity with both cattle and pig, and 27% identity with human. Sequence analysis revealed that Rb-C9 contains a putative 22 aa signal peptide, a LDL receptor domain, two MACPF domains and a MACPF MAC/ Perforin domain. Phylogenetic analysis demonstrated that Rb-C9 is clustered in a same clade with Japanese flounder and puffer fish C9. The tissue expression profile of rock bream C9 gene was examined by quantitative real time RT-PCR (qRT-PCR). C9 mRNA was highly expressed in liver whilst in all other tissues only low expression levels were shown, similar to the expression pattern identified in other fish species including grass carp and rainbow trout. In bacterial challenge experiment, Rb-C9 transcripts were significantly up-regulated in liver at 12 hour post challenged in liver by *Edwardsiella tarda*. Similarly, during Iridovirus challenge, the highest elevation of C9 mRNA level was detected at 12 hour post injection. In conclusion, the complement component 9 gene is responsive to different immune challenges and possibly plays an essential role in the immune system of rock bream.

PP-G27

Akirin2, an NF-κB regulator: from *Oplegnathus fasciatus* - Molecular characterization and expression analysis

Navaneethaiyer Umasuthan, *Kasthuri Saranya Revathy and Jehee Lee*

5373, Marine Molecular Genetics Lab, Department of Marine Life Sciences, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju 690-756, Republic of Korea.
Email: navaumasuthan@gmail.com

Innate immune response involving mediator molecules like cytokines and antimicrobial peptides are crucial for host response to pathogenic challenges. Akirins are a recently described family of proteins involved in the regulation of NF-κB, in response to Gram-negative infections in drosophila. They are determined to be involved in Toll- and TNF- pathways in mouse. Studies related to akirins from fish are emerging.

In this report, a full length cDNA identified from GS-FLX sequencing rock bream cDNA database, showing similarity to akirin2 homologues by BLASTX analysis was taken and named *OfAkirin2* and has been cloned and characterized. The open reading frame was amplified using ORF specific oligos and cloned into pGEM T-Easy vector and sequence confirmed. A clone identified from the BAC library using the gene specific primers was sequenced. Phylogenetic tree was constructed using MEGA 5.0 program, using neighbor joining method. Multiple sequence alignment was performed using ClustalW. The transcriptional analysis was performed by qRT-PCR with βactin gene as an invariant control. The expression level of OfAkririn2 in adductor muscle was taken as a calibrator contrasting the other tissues.

The full length cDNA comprises of an ORF of 552 bp coding for a putative protein of 184 amino acids possessing molecular mass of 21 kDa and isoelectric point 8.9 and 5' UTR of 170 bp, 3' UTR of 508 bp (including stop codon). A putative nuclear localization signal was present in the N-terminal of the protein (^{19}PTSPKRRRCI^{28}). The genomic structure from BAC clone revealed that *Ofakirin*2 possessed five exons interrupted by 4 introns. The promoter site prediction revealed that 5' end possess the promoter (TATAAA) and a transcription initiation site 26 bps downstream of the promoter signal. The 3' end possessed a polyadenylation signal 611 bp downstream of the stop codon. The phylogenetic tree reveals the clustering of *OfAkirin2* along with other fishes. Multiple sequence alignment shows a highly conserved nature of *OfAkirin2*.The tissue expression studies revealed maximum level of expression in blood with the next level of expression in liver.

PP-G28

An acute phase reactant, serum amyloid A like 1 from *Oplegnathus fasciatus*: Molecular characterization and expressional analysis post immune challenges

Navaneethaiyer Umasuthan, *Kasthuri Saranya Revathy and Jehee Lee*

5373, Marine Molecular Genetics Lab, Department of Marine Life Sciences, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju 690-756, Republic of Korea.
Email: navaumasuthan@gmail.com

Acute phase response, a part of the innate immunity, comprises of a series of systemic changes to counter the homeostasis imbalance caused by injury or infection. During an acute phase reaction, a variety of plasma proteins named acute phase proteins, like C-reactive protein (CRP), serum amyloid A (SAA), complement components, are synthesized in liver. Serum amyloid A is a positive acute phase protein, upon injury or infection.

A full length cDNA sequence identified from rock bream cDNA database created by GS-FLX sequencing was named *OfSAA1*. The sequence was confirmed by cloning in pGEM T-Easy vector. A clone identified from the BAC library was sequenced to obtain the entire genomic structure of *OfSAA*1. The transcriptional analysis in different tissues was performed by qRT-PCR with beta-actin as an invariant control. The expression level of *OfSAA1* was used as the calibrator to analyze expression in other tissues. Also, transcriptional analysis post immune challenges were done using qRT-PCR. Tissues were collected from fish injected with lipopolysaccharide, *Irido virus, Streptococcus iniae, Edwardsiella tarda* and poly I:C. The expression level was compared with expression levels of PBS injected fish.

Molecular characterization of the sequence revealed an open reading frame of 1410 bp encoding for a putative protein of 470 amino acids of molecular mass 52 kDa and isoelectric point of 4. Genomic characterization revealed that OfSAA1 possessed 13 exons and 12 introns similar to *Danio rerio*, in contrast to the mammalian SAA1. The transcription initiation site was observed 22 bp downstream of the promoter signal (ATAAATA). The 3' UTR possessed a polyadenylation signal 406 bp, downstream of the stop codon. *OfSAA1* showed the highest level of expression in blood. The expression levels were significant in head kidney, liver, blood and spleen, from 12h-24 h post immune challenges. Inflammation occurs when pathogen breaches the first line of defense, and the immune system sends signaling molecules and defense cells to defend against invasions. Serum amyloid A1 has been demonstrated to repair damaged tissues, act as an antibacterial agent and signal the migration of germ-fighting cells to sites of infection. The expression patterns of *OfSAA1* post immune challenges suggest that it may be an important acute phase reactant up-regulated on infection.

PP-G29

Molecular analysis and transcriptional responses of Cathepsin D and S cDNAs from Manila clam, *Ruditapes philippinarum*

Mahanama De Zoysa[1] *and Jehee Lee*[2]

[1]College of Veterinary Medicine, Chungnam National University, Yuseong-Gu, Daejeon, 305-764, Republic of Korea
[2]Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju, 690-756, Republic of Korea
Email: mahanama@cnu.ac.kr

Cathepsins are lysosomal cysteine proteases of the papain family that play an important role in intracellular protein degradation. In the present study, complete coding sequences of cathepsin D (McCathepsin D) and S (McCathepsin S) of manila clam, *Ruditapes philippinarum* were identified after pyrosequencing and transcriptome analysis. We performed the molecular analysis of McCathepsin D and McCathepsin S cDNAs to confirm the sequences by comparing the similarity and evolutional relationship with other cathepsin counterparts of different invertebrates and vertebrates.

Molecular analysis results showed that McCathepsin D and McCathepsin S contain characteristic conserved domains of eukaryotic aspartyl protease and papain family cysteine protease, respectively. The pairwise analysis results revealed that McCathepsin D and S share 69.1% and 29.3% the highest identity with Penguin wing oyster (*Pteria penguin*) cathepsin D and *Homo sapiens* cathepsin S, respectively.

Vibrio tapetis is the marine bacterium responsible for the brown ring disease (BRD) affecting the manila clam. The transcriptional responses of both McCathepsin D and McCathepsin S were examined in healthy and *V. tapetis* challenged manila clams by quantitative real-time RT-PCR. Results showed that both McCathepsin D and McCathepsin S were expressed in gills at highest level followed by hemocytes. Transcriptional up-regulation of both McCathepsin D and McCathepsin S was mainly in gills than in hemocytes after the *V. tapetis* challenge.

Present study indicates that both McCathepsin D and McCathepsin S are constitutively expressed in different tissues and potentially inducible when exposure to *V. tapetis*, suggesting that they may be involved in immune response reactions against bacterial challenge.

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