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Welcome

On behalf of the Fish Health Section/Asian Fisheries Society, I am delighted to welcome all delegates to the 5th Symposium on Diseases in Asian Aquaculture here on the beautiful Gold Coast.

The previous four Symposia held throughout Asian counties have each brought together over 170 fish health scientists, students, government workers and industry practitioners from some 20 countries to discuss problems affecting aquaculture production and their solutions. The Symposium is an important event for the aquaculture industry in the Asia-Pacific region. This is the first time that the symposium has been held in Australia with the theme of Healthy, Wealthy and Wise. Biosecurity and risk assessment have particularly been included since they have become critical issues in aquaculture at all levels of production and marketing as well as for all aspects of fisheries world wide.

I would like to take this opportunity to thank you for all your kind contributions to the Symposium. Finally, I hope this meeting will be a time to renew your interaction among colleagues and friends and hope you all enjoy the social events and the beauty of Gold Coast.

Surpanee Chinabut
Chair, Fish Health Section /Asian Fisheries Society
(1999-2002)

AFS Fish Health Section Committee

FHS Executive Committee (1999-2002)

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Rohana Subasinghe	Food & Agricultural Organization of the UN, Italy
Peter Beers	Biosecurity Australia
Mike Hine	Ministry of Agriculture and Fisheries, New Zealand
Barry Munday	University of Tasmania, Australia
Barry Hill	Centre for Environment, Fisheries and Aquaculture Sciences, UK
Celia Lavilla-Pitogo	SEAFDEC, Philippines
Angus Cameron	AusVet, Australia

Keynote Speakers

Dr David Alderman

David Alderman is President of the European Association of Fish Pathologists, Senior Microbiologist at the CEFAS Weymouth Laboratory in UK and editor of the Disease Section of the international journal, Aquaculture. His research interests include a wide range of aquatic diseases and in more recent years have centred around pharmaceuticals and immunologicals in aquaculture. David has published more than 100 papers on these areas. A member of the Scientific Secretariat and Biologics Committees he provides expert advice to UK Veterinary Medicines Directorate on data submitted for Marketing Authorisations and has contributed extensively to international bodies such as FAO, WHO and ICES.

Dr Takashi Aoki

Takashi Aoki is a Professor in Fish Genetics and Biochemistry at the Tokyo University of Fisheries. Here he teaches graduate and undergraduate courses and directs a research program dealing with fish and shellfish diseases and immunology. Takashi Aoki has published extensively in the fish and shellfish diseases and immunology on topics including cloning and characterization of fish and shellfish immune-related genes, characterization of virulence and drug resistance genes of fish pathogenic bacteria, studies of vaccines for aquaculture and molecular diagnosis techniques for fish pathogens.

Dr Chris Baldock

Chris Baldock is one of Australia's leading veterinary epidemiologists. He is a specialist in the design and implementation of livestock disease control and surveillance programs within a business planning framework as well as in epidemiological research and training. He has undertaken numerous consultancies both in Australia and in Asia, has authored numerous scientific articles, is co-author for a number of epidemiology texts, and is on the editorial boards of two international scientific journals.

Dr Peter Beers

Peter Beers is Manager of Aquatic Animal Biosecurity at Biosecurity Australia in the federal Department of Agriculture, Fisheries and Forestry. He leads a team of seven professional staff who are responsible for developing Australia's aquatic animal health policies for the import and export of aquatic animals and their products. Peter has been closely involved in Australia's approach to risk assessment methodologies, quarantine legislation and the WTO's SPS Agreement. He has been directly involved in aquatic animal import risk analyses and the development of Australia's biosecurity policies for the importation of aquatic animals and their products since 1990.

Dr Franck C.J. Berthe

Franck Berthe, DVM, PhD, has been working, since 1995, at the IFREMER laboratory of La Tremblade, France. His responsibilities are: (a) research on marteiliosis, a parasitic disease of molluscs, (b) coordinator of disease research and control programs in collaboration with mainland and overseas IFREMER teams, (c) head of the Reference Laboratory for Mollusc Diseases for the OIE and European Union, and (d) member of the Aquaculture Department Directorate in IFREMER. Franck is also a regular observer at the Fish Diseases Commission of the OIE. He has published research articles; he has been involved in organising training courses, workshops, conferences, etc. on fish and shellfish health.

Dr Melba Bondad-Reantaso

Melba B. Reantaso, was Aquatic Animal Health Specialist of NACA from 1999 to 2002 before taking up a post as Aquatic Animal Research Pathologist at the Cooperative Oxford Laboratory, Maryland Department of Natural Resources in September 2002. Prior to that Melba retired after 20 years of service at the Fish Health Section of the Philippine Bureau of Fisheries and Aquatic Resources (BFAR). While at NACA, she was in charge of the Asia-Pacific Regional Program on Aquatic Animal Health and worked closely with Asian governments, APEC, ASEAN, FAO, OIE and SEAFDEC and other aquatic animal health institutes in Asia-Pacific developing national strategies and regional projects and policies on various aspects of responsible health management. She is also currently Secretary/Treasurer of the Fish Health Section of the Asian Fisheries Society. Melba initiated the Molluscan Health Management Program, did some work on pearl oyster health while at BFAR and now currently involved in an annual active targeted surveillance of Haplosporidium nelsoni in Chesapeake Bay. Melba earned her PhD at University of Tokyo and post-doctoral studies at Nippon Veterinary and Animal Science University in Tokyo, Japan.

Dr Pornlerd Chanratchakool

Pornlerd Chanratchakool is currently working as the pathologist at The Aquatic Animal Health Research Institute (AAHRI), Department of Fisheries, Thailand. He has more than 10 years experience in both research and field work, in shrimp diseases and farm management. He is the chief author of the shrimp farm manual "Health Management in Shrimp Ponds". He has been invited to visit many countries and participate in seminars, workshops and conferences.

Keynote Speakers

Dr Albert (Kwang-Sik) Choi

Albert Choi - 1979 to 1983 Department of Oceanography, Inha University, Inchon Korea, Bs
1984 to 1992; Ms and Ph.D. from Dept. Oceanography, Texas A&M University, USA.

1995 to Present; Associate Professor at Cheju National University, Cheju Korea at the Department of Aquaculture. Research Interest; reproductive physiology and parasitic disease problems in marine bivavles including oysters and clams.

Dr Barry Hill

Barry Hill is the Chief Advisor for Fish and Shellfish Health to the UK government's Department of Environment, Food and Rural Affairs. For over 30 years, he has played a lead role in advising on scientific aspects of new UK legislation and policy on fish and shellfish diseases. He also provides specialist advice to the European Commission on EU legislation on the animal health conditions for trade in aquaculture animals. Since 1988, he has been a member of the OIE Fish Diseases Commission and has represented OIE at numerous scientific and technical meetings with other international organisations, particularly FAO and NACA.

Dr Alex Hyatt

Alex Hyatt is a Senior Principal Research Scientist and Project Leader of Electron Microscopy & Iridoviruses at the CSIRO Australian Animal Health Laboratory. He is an OIE recognised expert in ranaviruses and has developed electron microscopy as a key and core activity within Australia for the identification of new and emerging viruses. He is internationally known for his work in immunoelectron microscopy, virus morphogenesis, identification of new and emerging viruses and the development of assays for the detection of ranaviruses and chytrids. Alex has approximately 156 publications including many contributed chapters and one book. He has also received three major awards including the CSIRO Chairman's Medal, 1995, CSIRO Medal 2000 and the 2000 AQIS Quarantine Award 's for Science and Research.

Dr Chu-Fang Lo

Chu-Fang Lo is Professor of Zoology at National Taiwan University. Respected by her academic colleagues and appreciated by the aquaculture industry, she has won acclaim both locally and internationally for her work on the white spot syndrome virus (WSSV). Over the last decade, Dr Lo and her associates have conducted research on the virus itself, on the associated disease and on the development of control measures. She is currently investigating gene-based strategies against the disease.

Dr Toshihiro Nakai

Toshihiro Nakai teaches a graduate course at the Hiroshima University, Japan and directs a research program dealing with fish & shellfish pathology. Particularly, Nakai and colleagues concentrated their works on betanodavirus infections of marine fish for 10 years, and published many papers on this subject in scientific journals. Dr. Kazuhiro Nakajima, co-author of this symposium's presentation, who belongs to Fisheries Research Agency, has extensively studied iridovirus infections of fish, published many papers, and contributed to establish the current vaccination system against RSIV infections in Japan.

Dr Sirirat Rengpipat

Sirirat Rengpipat is an Associate Professor in Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. Here she teaches both undergraduate and graduate courses dealing with Food Microbiology, Advance Bacterial Physiology and Metabolism, Microbial Enzyme, and Industrial Microbiology. Probiotic bacteria in shrimp feed is her interest of research topic for 8 years, she has isolated the effective probiont and demonstrated its benefits after shrimp taken as food supplement to Penaeus monodon. Competition exclusion, immune enhancement are the evidences found in shrimp fed probiotic fortified feed and using probiotic as prophylactic treatment is also possible.

Dr Rohana Subashinge

Rohana Subasinghe is an experienced aquatic animal health specialist, gaining experience from many years of research and development work in Asia. He is currently connected with the Food and Agriculture Organization of the UN (FAO) since 2000, in-charge of managing the aquaculture programme of the Fishery Resources Division of the FAO Fisheries Department, which includes a major programme on aquatic animal health management. He has been instrumental in developing an Asia Regional Programme on Aquatic Animal Health Management in Asia, in collaboration with the Network of Aquaculture Centres in Asia-Pacific (NACA) and the Office International des Epizooties (OIE), and recently in Latin America. His work now covers all regions of the world.

Keynote Speakers

Prof Dr Willem B Van Muiswinkel

Willem Van Muiswinkel is a professor in Cell Biology and Immunology at the Department of Animal Sciences, Wageningen University, The Netherlands. He is teaching undergraduate courses in Cell Biology and graduate courses in Comparative Immunology. For more than 25 years Willem and his group have performed extensive studies on the immune system of fish and shrimp. Special focus is on cell characterisation, ontogeny, mucosal immunity and neuro-endocrine regulation. The results have been used in genetic selection programs to increase disease resistance, development of oral vaccines or for understanding the impact of stress on animal health and welfare in co-operation with (inter)national scientists, agencies and industries.

Invited Speakers

Dr Ellen Ariel

Ellen Ariel is a senior consultant at the European Community Reference Laboratory for Fish Diseases. She co-ordinates the daily activities of the reference laboratory and is in charge of the planning and execution of the annual inter-laboratory proficiency tests and the epidemiological surveys for notifiable diseases in the EU. Ellen organises Annual Meetings for National Reference Laboratories in the EU and associated countries and provides consultancy for fish disease laboratories in the EU predominantly. She is also a member of the private expert group advising to the Commission on legislative matters relating to the health management and disease control in aquaculture.

Dr Flavio Corsin

Flavio Corsin is a post-doctorate research assistant at the Institute of Aquaculture of the University of Stirling (UK). After he completed an MSc in Aquaculture, he joined a collaborative project with the University of Liverpool aimed at identifying the risk factors for White Spot Disease outbreaks. He conducted epidemiological studies in both Vietnam and India. Some of his work on the epidemiology of White Spot Disease is already available in peer-reviewed journals, while other results are either in press or in preparation.

Dr Iain East

Iain East is a scientific specialist with the Aquatic Animal Health section within Agriculture, Fisheries and Forestry - Australia. Iain gained his PhD from the University of Melbourne and worked in research in both the United States and Australia for 20 years and has published over 70 scientific books and papers. Iain's current role involves provision of scientific advice on the prevention and management of emergency disease incursions in fisheries and aquaculture, the development of resources to assist management of emergency disease incursions and the design and conduct of training in emergency management.

Prof Timothy W Flegel

Tim Flegel is the head of the Center of Excellence for Shrimp Molecular Biology and Biotechnology at the Faculty of Science, Mahidol University, Bangkok, Thailand. He is primarily a shrimp pathologist and is particularly interested in the shrimp response to viral pathogens. His group has worked on a number of important pathogens of the black tiger shrimp, including viruses, bacteria and parasites, for which they have developed a number of rapid diagnostic techniques. Together with Leigh Owens' group in Australia, they have pioneered work on phage induced virulence in the shrimp pathogen, Vibrio harveyi.

Dr Luc Grisez

Luc Grisez is research manager for Intervet Norbio Singapore. He is responsible for research on diseases in Asian aquaculture as well as for the development of aquatic animal health products. At present 14 scientists are employed in Intervet Norbio Singapore. Luc has been working on fish diseases throughout his career first during his studies at the University of Leuven in Belgium mainly involving freshwater fish species and later involving the Mediterranean fish species such as European sea bass and sea bream. He is with Intervet, an animal health company with HQ in The Netherlands, for the last 5 years and for the last 3 years he is based in Singapore.

Dr Lachlan Harris

Lachlan Harris originally studied microbiology at the University of Queensland, Brisbane, Australia. He then continued at postgraduate level at James Cook University, Townsville, Australia with Dr Leigh Owens, specializing in the understanding and control of bacterial diseases of penaeid larvae, in particular the interaction between larvae and luminous strains of Vibrio harveyi. Lachlan then worked for three years as Head of Technical Services for Seafarm Pty Ltd, Australia's largest prawn farming operation, continuing to investigate the control of bacterial and viral diseases in order to improve production, and also supervising Seafarm's selective breeding program for Penaeus monodon and Penaeus merguiensis. In 2001, Lachlan travelled to Ecuador to commence working for Acuabiotec LLC, a company specializing in the control of bacterial diseases through the use of microbial technology. Between 2001 and 2002 Lachlan was responsible for implementing this technology and providing advice to a variety of shrimp farms in Ecuador severely affected by WSSV and bacterial diseases. Since July 2002 Lachlan has been working as the General Manager of Seaquest S. A., a program of maturation, genetic improvement and research and investigation responsible for an association of three shrimp producers in Ecuador, comprising 5000 hectares of extensive ponds cultivating Penaeus vannamei.

Invited Speakers

Dr Brian Jones

Brian Jones is senior fish pathologist at the Department of Fisheries, Government of Western Australia, Adjunct Professor of Fish Health at the Muresk Institute and Adjunct Associate Professor at Murdoch University School of Veterinary and Biomedical Sciences. Brian is author and co-author of over 80 scientific papers and technical reports and has broad international experience of both freshwater and marine shellfish aquaculture. For the past 7 years Brain has provided a disease diagnostic and surveillance service to the pearl oyster industry and was a key participant in a major 3 year survey to identify oyster diseases across northern Australia.

Professor Indrani Karunasagar

Indrani Karunasagar is an Associate Professor in Microbiology and Director of the UNESCO MIRCEN for Marine Biotechnology at the College of Fisheries of the University of Agriculture Sciences, Mangalore. She is also a scientific advisor to the International Foundation for Science, Sweden and a Member of the Task Force of the Department of Biotechnology, Govt. of India. Her research interests include: molecular diagnostics, bioremediation and probiotics, bacteriophages and shellfish toxins, use of biotechnological tools in health management, public health related pathogens.

Dr CV Mohan

CV Mohan is an Associate Professor of Fish Pathology at the College of Fisheries, University of Agricultural Sciences, Mangalore, India. He teaches several courses in the field of aquatic animal health to undergraduate and postgraduate students. Mohan and his associates, over the past 15 years, have completed several national and international funded research projects on various aspects of aquatic animal health. Mohan has published extensively in international (23) and national (25) journals on aquatic animal health topics including fish and shrimp pathology, diagnostics, immunology and epidemiology. He has been involved as an invited expert from India in many of the expert consultations and workshops organised by international agencies like NACA, DFID, FAO, World Bank and ACIAR.

Dr Barry Munday

Barry Munday is presently a Research Fellow in the School of Human Life Sciences at the University of Tasmania. Until 2000 he was a Reader in the School of Aquaculture with responsibility for the fish health program in that school. Since the early 1990s he has been involved in investigating disease problems in the Australian tuna Aquaculture industry and has also visited the Kinki University tuna research centre in Japan to obtain information on their propagation program.

Dr Antonius Suwanto

Antonius Suwanto is a Professor in the Department of Biology, Faculty of Science and Mathematics, Bogor Agricultural University, and a coordinator on Research Center for Microbial Diversity. In addition he is also the head of Molecular Biology Laboratory in the Southeast Asian Regional Center for Tropical Biology (SEAMEO-BIOTROP). He has been working on bacteriology and molecular genetics of Vibriosis in shrimp hatcheries in Indonesia for more than 10 years. He has published several papers on his work in this field. He is also a recipient for a number of scientific and academic awards from both national and international institutions, such as Rockefeller Foundation (USA), International Foundation for Science (Sweden), Indonesian Science and Technology Award (ITSF), and Kehati Award (Indonesian Biodiversity Award).

Prof Just M Vlak

Just Vlak is a Professor of Virology at Wageningen University, The Netherlands. He is Chairman of the Biology Program and Director of the Graduate School 'Production Ecology and Resource Conservation'. His research program includes studies on the biology and genetics of baculoviruses (insect viruses) and their application as biocontrol agents of insect pests and expression vectors for eukaryotic proteins. Furthermore, his group studies the genetics of nimaviruses (White Spot Syndrome Virus) of crustaceans. At present he is Vice-President of the Society for Invertebrate Pathology. Over the past 25 years he published over 300 papers in international journals and books.

Prof Jiang Yulin

Jiang Yulin is a Professor of Fish diseases and director of the key Lab of aquatic animal diseases in Chinese quarantine system. Over past 20 years, he directed and finished 23 research programs dealing with aquatic pathology, immunology and detection methods such as hemorrhagic disease of grass carp, pox disease of carp, white spot disease of shrimp, lymphocystics, turtle diseases etc. He published about 70 papers on these fields.

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* Please note that this program is subject to change.

Sunday 24 November 2002

4.30pm	Registration commences
6.30pm	Welcome Reception at the Gold Coast International Hotel proudly sponsored by CSIRO

Monday 25 November 2002

8.00am	Registration commences

Opening Session *proudly sponsored by CSIRO*
Chair: Peter Walker

8.30am	Opening Ceremony
8.35am	Dr Kamonporn Tonguthai (representing AFS)
8.40am	Dr Supranee Chinabut (representing FHS)
8.45am	Dr Richard Callinan (representing LOC)
8.50am	Keynote: *Aquatic animal health management in Asia*. **Melba Bondad-Reantaso**, Cooperative Oxford Laboratory, USA
9.25am	Keynote: *Food safety in aquaculture.* **David Alderman**, CEFAS Weymouth Laboratory, United Kingdom
10.00am	**Chief Veterinary Officer's Morning Tea**

Session 1:	***Biosecurity and Risk Assessment* – proudly sponsored by Biosecurity**
Co-chairs:	**Barry Hill and Chris Baldock**
10.30am	Keynote Address: Biosecurity: *A new word for an old concept.* **Peter Beers**, Biosecurity, Australia
11.05am	Invited Speaker: *Farm Level biosecurity in aquaculture.* **C.V Mohan**, College of Fisheries, India
11.30am	*'To hazard or not to hazard that is the question.' How unknowns in science affect the identification of hazards in an import risk analysis.* **Sarah Kleeman**, Aquatic Animal Biosecurity, Australia
11.45am	*The role of risk analysis and epidemiology in the development of biosecurity for aquaculture.* **Edmund Peeler**, Cefas, United Kingdom
12.00pm	*Import risk analysis: Philippine experience.* **Joselito Somga**, Fish Health Section, Bureau Of Fisheries And Aquatic Resources, Philippines
12.15pm	*A national survey to demonstrate freedom from white spot virus and yellow head virus in Australian crustaceans.* **Iain East**, Agriculture Fisheries & Forestry – Australia
12.30pm	Lunch

Session 2:	***Diseases of aquatic vertebrates***
Co-chairs:	**Jiang Yulin and Tina Hawkesford**
1.30pm	Keynote: *Recent advances of betanodaviruses and iridoviruses in Asian aquaculture.* **Toshihiro Nakai**, Hiroshima University, Japan
2.05pm	Invited Speaker: *Diseases of Thunnus spp.: Emerging aquaculture species.* **Barry Munday**, School of Human Life Sciences - University of Tasmania, Australia
2.30pm	*Susceptibility of marine fish species to Piscine nodavirus from orange-spotted grouper, Epinephelus coioides in the Philippines*. **Yukio Maeno**
2.45pm	*Characterisation of an iridovirus isolated from diseased marble goby Oxyeleotris marmoratus (Bleeker, 1852).* **Pongpun Prasankok**, Chulalongkorn University, Thailand
3.00pm	*Molecular charaterization of a novel ranacirus isolated from grouper, Epinephelus spp.* **Qiwei Qin**, National University of Singapore, Singapore
3.15pm	*Viral DNA sequences of genes encoding the ATPase and the major capsid protein of tropical iridovirus isolates which are pathogenic to fishes in Japan, South China Sea and Southeast Asian countries.* **Teruo Miyazaki**, Faculty Bioresourses, Mie University, Japan
3.30pm	Afternoon Tea

Session 3:	**Diseases of aquatic vertebrates – proudly sponsored by Queensland Department of Primary Industries**
Co-chairs:	**Kamonporn Tonguthai and Kishio Hatai**
4.00pm	Keynote: *Ranavirus of fish, amphibians and reptiles.* **Alex Hyatt**, CSIRO Livestock Industries, Australia
4.35pm	Invited Speaker: *Emerging diseases of soft-shell turtles.* **Jiang Yulin**, The Key Lab of Aquatic Animal Diseases, China
5.00pm	*New and emerging disease in reef and Estuarine fishes from North Queensland, Australia.* **Rachel Bowater**, Queensland Department of Primary Industries, Australia
5.15pm	*A virological survey in diseased grouper in Thailand using virus isolation and polynerase chain reaction (PCR) technique.* **Somkiat Kanchanakhan** Aquatic Animal Health Research Institute, Department of Fisheries, Thailand
5.30pm	*Isolation and identification of Edwardsiella ictaluri from diseased Pangasius hypophthalmus (Sauvage) cultured in Vietnam.* **Margaret Crumlish**, Stirling University, Scotland
5.45pm	*Viral hemorrhagic septicemia virus (VHSV) infection in Japanese flounder Paralichthys olivaceus.* **Kiyokuni Muroga**, Hiroshima University, Japan

6.00pm Sessions conclude

6.30pm EUS Workshop

Moderators: **Chris Baldock**
Melba Bondad-Reantaso

Invited Experts: **Vicki Blazer**
Richard Callinan
Kishio Hatai
Indrani Karunasagar
CV Mohan

The aim of the workshop is to achieve some agreement on "What is EUS?" Invited experts will each present their case definition with supporting evidence, followed by questions and answers from the audience. The workshop will (hopefully) conclude with an agreed case definition.

8.00pm Student Reception

Proudly sponsored by World Aquaculture Society

Tuesday 26 November 2002

Session 4: ***Epidemiology***
Co-chairs: **CV Mohan and Angus Cameron**

8.15am Keynote: *International trade and risk analysis.* **Chris Baldock**, AusVet Animal Health Services Australia

8.50am Invited Speaker: *Farm level risk factors for white spot disease outbreaks.* **Flavio Corsin**, University of Stirling, United Kingdom

9.15am *Application of epidemiology to support better health management in black tiger shrimp Penaeus monodon aquaculture: An experience from India.* **Arun Padiyar**, Network Of Aquaculture Centres In Asia-Pacific, Thailand

9.30am *Patterns of occurrence of the helminth parasites of the bullseye pufferfish (Sphoeroides annulatus) from Sinaloa, Mexico.* **Ana Roque**, Ciad,Ac, Mexico

9.45am *Epizootic haematopoietic necrosis virus – epidemiology and uncertainty.* **Richard Whittington** University of Sydney, Australia

10.00am Morning Tea ***proudly sponsored by INVE***

Session 5: ***Shrimp Health 1***
Co-chairs: **Guang-Hsiung Kou and Boonsirm Withychumnarnkul**

10.30am Keynote: *Key farm management issues to reduce loss from diseases.* **Pornlerd Chanratchakool**, Kasetsart University Campus, Thailand

11.05am Invited Speaker: *Phage induced virulence in the shrimp pathogen Vibrio harveyi* **Timothy W Flegel**, Centex Shrimp, Thailand

11.30am *Experimental transmission of Hepatopancreatic parvovirus (HPV) infection in Penaeus monodon postlarvae* **Elena Catap**, Fish Health Section, Aquaculture Department-Southeast Asian Fisheries Development, Philippines

11.45am *Does spawning stress trigger the replication of white spot syndrome virus in shrimp?* **Guang-Hsiung Kou**, Department Of Zoology, National Taiwan University, ROC

12.00noon *Polychaetes not carries of white spot syndrome virus.* **Pisit Poltana**, Mahidol University, Thailand

12.15am *Predicting outbreaks of White Spot Disease in a semi-intensive Penaeus monodon culture system in Karnataka, India.* **James Turnbull**, Institute Of Aquaculture, United Kingdom

12.30pm Lunch

Session 6: ***Shrimp Health 2***
Co-Chairs: **Tim Flegel and Indrani Karunasagur**

1.30pm Keynote: *White spot syndrome: What we have learned about the virus and the disease.* **Chu-Fang Lo**, National Taiwan University, Taiwan

2.05pm Invited Speaker: *Molecular genetics of white spot syndrome virus.* **Just M Vlak**, Laboratory Of Virology, The Netherlands

2.30pm *Breeding shrimp for disease resistance: A panacea or pariah* **Shaun Moss**, The Oceanic Institute, USA

2.45pm *B-type virus of Carconus mediterranmeus and WSSV of Penaeid shrimp: Similarities and possible relationships.* **Jean-Robert Bonami**, CNRS, France

3.00pm *Use of WSSV cDNA microarray for gene profilling during WSSV infection in shrimps.* **Jimmy Kwang**, The National University Of Singapore, Singapore

3.15pm *Variations in tandem repeat DNA segments in the ribonucleotide reductase gene of white spot syndrome virus (WSSV) isolates from Vietnam.* **Hoa Tran Thi Tuyet**, CSIRO Livestock Industries, Australia

3.30pm Afternoon Tea

Session 7: ***Emerging Technologies***
Co-chairs: **Jimmy Kwang and Peter Walker**

4.00pm Keynote: *Characterization of gene expression of biodefense related genes of Kuruma shrimp, Penaeus japonicus using real-time PCR technology.* **Takashi Aoki**, CSIRO Livestock Industries, Australia

4.35pm Invited Speaker: *Construction of recombinant Vibrio harveyi to study its adherence and pathogenicity in shrimp larvae.* **Antonius Suwanto**, Bogor Agricultural University Indonesia

5.00pm	*Recent advances of studies on molecular genetics concerning defense mechansim and control disease in the chinese shrimp, fenneropenaeus chinesis.* **Jianhai Xiang**, Chinese Academy of Sciences, China
5.15pm	*A hypothetical model for VHML bacteriophage conversion of vibrio harveyi.* **Jane Oakey**, Queensland Department Of Primary Industries, Australia
5.30pm	*Co-detection and differentiation of yellow head complex viruses using monoclonal antibodies.* **Chumporn Soowannayan** CENTEX Shrimp, Thailand
5.45pm	*Molecular approach to the identification of virulence genes involved in Edwardsiella tarda pathogenesis.* **Srinivasa Rao P S**, National University Of Singapore, Singapore
Chair:	**Supranee Chinabut**
6.00pm	Sixth Triennial General Meeting of the Fish Health Section – Asian Fisheries Society
8.00pm	Hard Rock Café – optional evening

Wednesday 27 November 2002

Session 8:	***Immunology***
Co-chairs:	**Ellen Ho and Barry Munday**
8.15am	Keynote: *Innate immunity in vertebrates and invertebrates.* **Willem van Muiswinkel**, Wageningen University, The Netherlands
8.50am	Invited Speaker: *Vaccine development for Asian aquaculture.* **Luc Grisez**, Intervet Norbio Singapore Pty Ltd Singpore
9.15am	*Immunostimulants induced immunity and its quantification in tiger shrimp, penaeus monodon (fabrics) through 'challenge' and 'pro-PO assay'.* **Sugantham Felix**, Fisheries Biotechnology Centre, India
9.30am	*Immunological properties of the phygocytosis and serum lectin in Scaphara subcrenata.* **Zhihong Liu**, Chinese Academy of Fishery Sciences, China
9.45am	*Vaccination against white spot syndrome virus in shrimp.* **Jeroen Witteveldt**, Wageningen University, The Netherlands
10.00am	Morning Tea
Session 9:	***Probiotics and Therapeutics***
Co-chairs:	**Celia Lavilla-Pitogo and Leigh Owens**
10.30am	Keynote: *Probiotic bacteria: Are they beneficial?* **Sirirat Rengpipat**, Chulalongkorn University, Thailand
11.10am	Invited Speaker: *Use of microbial technology to improve farm results in shrimp farm in Ecuador* **Lachlan Harris**, Gerante General de Seaquest SA, South America
11.30am	Invited Speaker: *Biocontrol of bacterial pathogens in aquaculture with emphasis on phage therapy.* **Indrani Karunasagar**, University of Agricultural Sciences, College of Fisheries, India
11.45am	*Treatment of bacillary necrosis of larval pacific oyster crassostrrrea gigas with bacteriophages.* **Toshihiro Nakai**, Hiroshima University, Japan
12.00noon	*The probiotic potential of vibrio alginolyticus (Val 1) in the oyster hatchery.* **Cheok Keong Tan** University of Technology Sydney, Australia
12.15pm	*Antagonistic activity of Aeromonas media strain A199 against Saprolegnia parasitica in two species of finfish, the eel Anguilla australis and silver perch Bidyanus bidyanus.* **Josie Lategan**, University Of Technology Sydney
12.30pm	Lunch
1.00pm	Optional tours: 1. Aquaculture Farm Tour 2. Golf at Sanctuary Cove 3. Currumbin Bird Sanctuary & Wildlife Park Free evening

Thursday 28 November 2002

Session 10:	***Mollusc Health 1***
Co-chairs:	**Franck Berthe and Robert Lester**
8.15am	Keynote: *Current Status of Perkinsis Infections in Asian water.* **Kwang-Sik Choi**, Cheju National University, Korea
8.50am	Invited Speakers: *Diseases of pearl oysters* **Brian Jones**, Department of Fisheries, Western Australia, Australia
9.15am	*Report on oyster mortality in Wonboyn Lake, Australia.* **Damian Ogburn**, NSW Fisheries, Australia
9.30am	*Transmission of perkinsus olseni among wild blacklip abalone in South Australia.* **Craig Hayward**, University of Queensland, Australia
9.45am	*Breeding for QX disease martelia sydneyi resistance in Sydney rock oysters saccostrea glomerata.* **John Nell**, NSW Fisheries, Port Stephens Australia
10.00am	Morning Tea ***proudly sponsored by University of Queensland***
Session 11:	***Mollusc Health 2***
Co-chairs:	**Kwang-Sik Choi and Mike Hine**
10.30am	Keynote: *Diseases in mollusc hatcheries: a paradox in health management.* **Franck C.J. Berthe**, IFREMER France

11.05am	Invited Speaker: *Survey on the ovarian parasite, Marteilioides chungmuensis in the cultured pacific oysters crassostrea gigas in Korea.* **Myoung-Ae Park**, National Fisheries Research & Development Institute, Republic of Korea
11.30am	*Marteilioides chungmuensis (paramyxea), an intracellular parasite of the ovocyte of Pacific oyster Crassostrea gigas: Isolation and sequencing of small subunit ribosomal DNA.* **Naoki Itoh**, The University of Tokyo Japan
11.45am	*Epizootiology and detection of nocardiosis in oysters.* **Susan Bower**, Fisheries and Oceans Canada Canada
12.00noon	*Diseases of cultured paua (Haliotis iris) in New Zealand.* **Ben Diggles**, National Institute Of Water & Atmospheric Research Ltd New Zealand
12.15pm	*Discovery of the early infective stages of the protozoan parasite marteilia sydneyi in oysters and the implications for disease detection and control.* **Sarah Kleeman**, Aquatic Animal Biosecurity, Australia
12.30pm	Lunch
Session 12:	***Trans-boundary and emerging diseases***
Co-chairs:	**Rohana Subasinghe and Richard Whittington**
1.30pm	Keynote: *Limitations to preventing increased international distribution of aquatic animal diseases.* **Barry Hill**, The Centre for Environment, Fisheries & Aquaculture Sciences, United Kingdom
2.05pm	Invited Speakers: *Ornamental disease vectors.* **Ellen Ariel**, EU Community Reference Laboratory For Fish Disease, Denmark
2.30pm	*Preliminary molecular and biological characterisation of Mourilyan virus (MoV): A new bunya-related virus of penaeid prawns.* **Jeff Cowley**, CSIRO Livestock Industries, Australia
2.45pm	*Fatal, virus associated peripheral neuropathy and retinopathy (PNR) in farmed penaeus monodon in Eastern Australia.* **Richard Callinan**, NSW Fisheries, Australia
3.00pm	*Zoning for marteiliosis in commercial rock oysters in Australia.* **Robert D Adlard**, Queensland Museum, Australia
3.15pm	*Field investigations on a serious disease outbreak and among common and koi carp in Indonesia.* **Agus Sunarto**, Central Research Institute for Aquaculture, Indonesia
3.30pm	Afternoon Tea - Proudly sponsored by NSW Fisheries
Session 13:	***The Future***
Co-chairs:	**Supranee Chinabut and Richard Callinan**
4.00pm	Keynote: *Improving aquatic animal health in Asia.* **Rohana Subasinghe**, Food and Agriculture Organisation of the UN, Italy
4.30pm	Invited Speaker: *Aquaculture health management: The Australian experience.* **Iain East**, Agriculture Fisheries & Forestry, Australia
4.50pm	*The role of extension in effecting on-farm practice change for controlling shrimp disease.* **Derek Foster**, Queensland Department of Primary Industries, Australia
5.10pm	Open Discussion Forum
5.40pm	Closing Ceremony – **Rohana Subasinghe**, Food and Agriculture Organisation of the UN, Italy
6.00pm	Symposium Concludes
7.00pm	Symposium Dinner – Sea World ***proudly sponsored by Queensland Department of Primary Industries***

PRESENTING AUTHOR	TITLE	THEME	POSTER BOARD#
Stephen Colquitt, Biosecurity Australia, AUSTRALIA	Quarantine detention of ornamental fish - Practical challenges involved in the implementation of a new policy	T1	1
Abu Tweb Abu Ahmed, Department of Zoology, BANGLADESH	Argulosis in Brood Carp Rearing Ponds of Bangladesh	T2	3
Kanit Chukanhom, Nippon Veterinary and Animal Science University, JAPAN	Freshwater fungi isolated from common carp (Cyprinus carpio) eggs in Thailand	T2	4
Gregoria Erazo-Pagador, Fish Health Section SEAFDEC-AQD, PHILIPPINES	Biology and pathogenicity of the gill monogenean (Pseudorhabdosynochus sp.) in grouper	T2	5
Susan Gibson-Kueh, Agri Food and Veterinary Authority of Singapore, SINGAPORE	Diagnosis of systemic iridoviral disease in fish	T2	6
Tiina Hawkesford, Fisheries and Aquaculture Development Queensland Department of Primary Industries, AUSTRALIA	Tasmanian Isolates of Streptococcus sp. biovar 1 and verified strains of lactococcus garvieae and enterococcus seriolicida compared by microbiological , molecular biological and "in vivo" techniques	T2	7
Mangalika Hettiarachchi, University of Kelaniya, Sri Lanka, SRI LANKA	A study on columnaris disease in guppy Poecilia reticulata	T2	8
Michael Kent, Oregon State University, USA	Diseases of Opakapaka held at the Hawaii Institute of Marine Biology	T2	9
Matthew Landos, NSW Fisheries, AUSTRALIA	Winter disease in farmed silver perch (Bidyanus bidyanus) in New South Wales, Australia	T2	10
Thitiporn Laoprasert, Aquatic Animal Health Research Institute Department of Fisheries, THAILAND	First report of systemic amoebosis in oscar, Astronotus ocellatus	T2	11
Thitiporn Laoprasert, Aquatic Animal Health Research Institute Department of Fisheries, THAILAND	Study on Tetrahymena infection in guppy (Poecilia reticulata)	T2	12
Ong-ard Lawhavinit, Department Of Veterinary Microbiology And Immunology, Faculty Of Veterinary Medi, THAILAND	Mycobacteriosis in ranchu, goldfish (Carassius auratus) imported from Japan	T2	13
Theerawoot Lerssutthichawat, Rajamangala Institute Of Technology, THAILAND	Diversity of freshwater monogeneans from siluriform fishes of Thailand	T2	14
Allan Mooney, University Of Queensland, AUSTRALIA	Seasonal variation in the ectoparasite assemblage of Pagrus auratus cultured in sea-cages off Eastern Australia	T2	15
Barry Munday, School of Human Life Sciences - University of Tasmania, AUSTRALIA	A Pentacapsula species inhibiting propagation of striped trumpeter - an aquaculture candidate	T2	16
Huu Dung Nguyen, University of Fisheries, VIETNAM	Hemorrhaging septicemia due to Aeromonas hydrophila in the Mekong catfish (Pangasius bocourti) cultured in An Giang province - Vietnam	T2	17
Qiwei Qin, National University of Singapore, SINGAPORE	Production and characterization of monoclonal antibodies to Singapore grouper iridovirus (SGIV)	T2	18
A Rayamajhi, Department Of Microbiology & Parasitology, NEPAL	Aquatic oomycetes from southeast Queensland	T2	19
Dalisay Ribu, The University of Queensland, AUSTRALIA	A new genus of dracunculoid nematode from the gills of the pufferfish Tragulichthys jaculiferus	T2	20

PRESENTING AUTHOR	TITLE	THEME	POSTER BOARD#
Somporn Roongkamnertwongsa, National Institute Of Coastal Aquaculture, THAILAND	Identification of a betanodavirus isolated from viral nervous necrosis-diseased redspotted grouper (Epinephelus coioides) cultured in Southern Thailand using PCR and sequence analysis	T2	21
Frances Stephens, Aquatilia Healthcare, AUSTRALIA	Health problems of captive West Australian dhufish	T2	22
Sivan Subburaju, Temasek Life Sciences Laboratory, SINGAPORE	Induction of caspase-dependent apoptosis by betanodaviruses GGNNV and identification of nucleolus localization signal of protein	T2	23
Kunika Wakita, Nippon Veterinary & Animal Science University, JAPAN	Histopathological study on tetrahymena infection in dwarf gourami (Colisa ialia)	T2	24
Yin-Geng Wang, Yellow Seas Fisheries Research Institute, CHINA	Turbot culture - a newly established industry in China and its disease problems	T2	25
Richard Whittington, The University of Sydney, AUSTRALIA	Pilchard herpesvirus in Australasia 1995-1999	T2	26
Arthur De Vera, Bureau Of Fisheries and Aquatic Resources, PHILIPPINES	Occurrence of hemic neoplasia in slipper oyster, Crassostrea iredalei (Faustino, 1928) in Dagupan City, Philippines	T2	27
Judith Handlinger, Department Of Primary Industries, Water And Environment, Tasmania, AUSTRALIA	Bacterial infection in Tasmanian farmed abalone: causes, pathology, farm factors and control options	T2	28
Craig Hayward, The University of Queensland, AUSTRALIA	Transmission of perkinsus olseni among wild blacklip abalone in South Australia	T2	29
Linsheng Song, Institute of Oceanology, Chinese Academy of Sciences, P.R.CHINA	Potential genes involved in immune response identified by expressed sequence tag analysis from scallop Chlamys farreri	T3	30
Serge Corbeil, CSIRO Livestock Industries, AUSTRALIA	Development of a real-time PCR assay for the detection of Piscirickettsia salmonis	T4	31
Mark Crane, ARDL, Australian Animal Health Laboratory, AUSTRALIA	Development of diagnostic antibodies specific for white spot virus	T4	32
Ikuo Hirono, Tokyo University Of Fisheries, JAPAN	Functional microarray analysis of Japanese flounder Paralichthys olivaceus immune related genes for selection of a disease resistance fish	T4	33
Thammanoon Jaturapahu, Aquatic Animal Health Research Institute, THAILAND	Detection and identification of Pseudomonas spp. by polymerase chain reaction-reverse cross blot hybridization (PCR-RCBH) with 16S-23S rRNA intergenic spacer probes.	T4	34
Pani Prasad Kurcheti, Central Institute Of Fisheries Education, INDIA	Development of onfarm diagnostics	T4	35
Rosalind George Mulloorpeedikayil, Fisheries College and Res.Institute, INDIA	Plasmid profile of bacterial isolates from white spot affected shrimps	T4	36
James Munro, James Cook University, AUSTRALIA	Experimental Bacteriophage-mediated virulence in strains of Vibrio harveyi	T4	37
Nakao Nomura, University of Tsukuba, JAPAN	Development and characterization of a monoclonal antibody against white-spot syndrome virus in Penaeid shrimp	T4	38
Myoung-Ae Park, National Fisheries Research And Development Institute, REPUBLIC OF KOREA	Detection and comparison of lymphocystis virus in flounder (Paralichthys olivaceus) and sea bass (Lateolabrax japonicus)	T4	39
Panarat Phadee, Nippon Veterinary and Animal Science University, JAPAN	Identification and diagnosis of Aphanomyces piscicida by PCR	T4	40
Suppalak Puttinaowarat, Aquatic Animal Health Research Institute, THAILAND	Development of a monoclonal antibody to hybrid catfish (Clarias macrocephalus x C. gariepinus) immunoglobulin	T4	41

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Pramoda Sahoo, Institute For Animal Health, UNITED KINGDOM	Cloning and expression of variable region of nucleocapsid gene of aquatic morbilliviruses for serological diagnosis	T4	42
Pattira Taweepreda, Department of Anatomy, Faculty of Science, Mahidol University, THAILAND	Ultrastructural changes in the sperm and eggs of the black tiger shrimp, Penaeus monodon, before and after fertilization	T4	43
Attaporn Taweetungtragoon, Center of Excellence for Shrimp Molecular Biology & Biotechnology (CENTEX SHRIMP, THAILAND	A rapid multiplex real time PCR for the early detection of double targets: white spot syndrome virus (WSSV) and yellow head virus (YHV), in the black tiger shrimp Penaeus monodon	T4	44
Kanokporn Chayaburakul, Mahidol University, THAILAND	Infectious hypodermal and hematopoietic necrosis virus infection in Domesticated P. monodon broodstock	T6	45
Li-Li Chen, National Taiwan University, ROC	Transcriptional analysis of the DNA polymerase gene of shrimp white spot syndrome virus (WSSV)	T6	46
Enrique De La Vega, Australian Institute Of Marine Science, AUSTRALIA	Potential indicators of stress response and their relation to survival in Penaeus monodon	T6	47
Zhenyu Guo, Institute of Oceanology, Chinese Academy of Sciences, P.R.CHINA	A novel antimicrobial peptide isolated from the shrimp Fenneropenaeus chinensis after bacterial challenge	T6	48
Nguyen Van Hao, Research Institute For Aquaculture N2, VIETNAM	White Spot Disease in Penaeus monodon: case definition, accuracy of clinical diagnosis and description of an epidemic	T6	49
Chiu-Jung Huang, National Taiwan University, TAIWAN, ROC	The PK1 protein of the white spot syndrome virus (WSSV) is a nuclear kinase with autophosphorylation activity	T6	50
Celia Lavilla-Pitogo, SEAFDEC Aquaculture Department, PHILIPPINES	Evaluation of pathogenicity of bacterial strains in crustacean larvae by static bath: significance of monitoring bacterial counts.	T6	51
Kok Leong Lee, University Putra Malaysia, MALAYSIA	Growth pattern of Vibrio parahaemolyticus, V. alginolyticus and V. harveyi isolates from Malaysia	T6	52
Masatoshi Matsumura, University Of Tsukuba, JAPAN	Electro-chemical processes for phytoplankton control and shrimp disease disinfection	T6	53
Yasunori Muraosa, Nippoh Veterinary And Animal Science University, JAPAN	Lagenidium thermophilum isolated from zoeae of black tiger (Penaeus monodon) in Thailand	T6	54
Dang Thi Hoang Oanh, Cantho University, VIETNAM	Prevalence of white spot syndrome virus (WSSV) and Monodon baculovirus (MBV) infection in Penaeus monodon postlarvae in Vietnam	T6	55
Songsak Roekring, Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shri, THAILAND	Comparison of penaeid shrimp and insect parvoviruses suggests that viral transfers may occur between two distantly related arthropod groups	T6	56
Sivan Subburaju, Temasek Life Sciences Laboratory, SINGAPORE	The white spot syndrome virus (WSSV) infects specific hemocytes of the shrimp Penaeus merguiensis	T6	57
Wasana Sukhumsirichart, Srinakharinwirot University, THAILAND	Prevalence of hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV) in stunted Penaeus monodon in Thailand	T6	58
Attaporn Taweetungtragoon, Center of Excellence for Shrimp Molecular Biology & Biotechnology (CENTEX SHRIMP, THAILAND	Comparison of various WSSV-PCR detection assays using naturally infected shrimp in Thailand and a fluorogenic WSSV probe as the gold standard	T6	59
Attaporn Taweetungtragoon, Center of Excellence for Shrimp Molecular Biology & Biotechnology (CENTEX SHRIMP, THAILAND	A dual, real time PCR for the simultaneous detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in the black tiger shrimp Penaeus monodon	T6	60
Eleonor Tendencia, Southeast Asian Fisheries Development Center, Aquaculture Department, PHILIPPINES	Effect of Tilapia Tilapia hornorum on luminous bacteria Vibrio harveyi	T6	61

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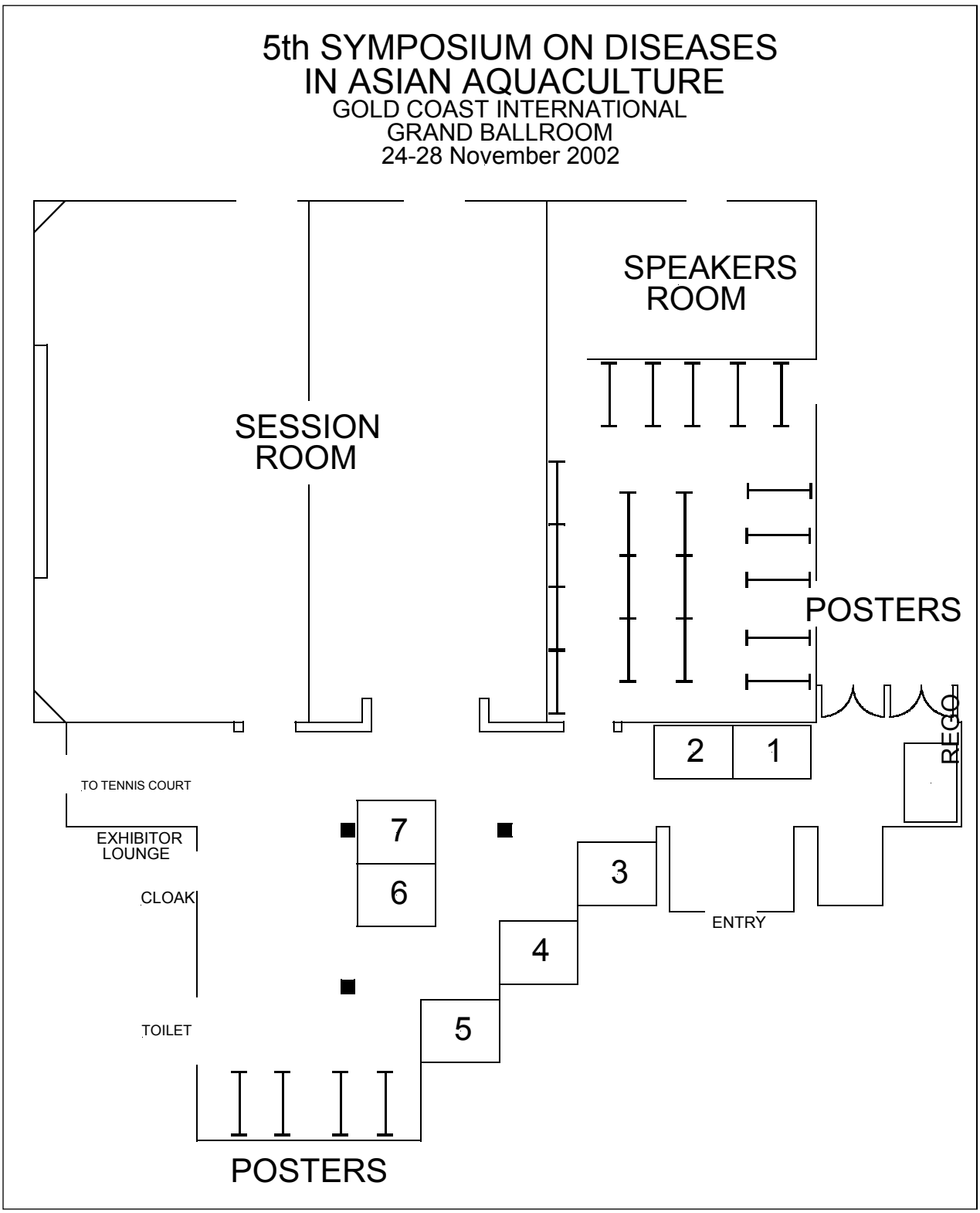
Han-Ching Wang, Department Of Zoology, National Taiwan University, TAIWAN, ROC	Does spawning stress trigger the replication of white spot syndrome virus in shrimp	T6	63
Priyanjalie Wijegoonawardane, Csiro, AUSTRALIA	Phylogenetic analysis of replicase (ORF1b) amplicon sequences reveals a fourth genotype of yellow head complex virus in P. monodon from India	T6	64
Lynette Williams, CSIRO Livestock Industries, AUSTRALIA	Attempts to establish continuous cell lines from prawn tissues	T6	65
Li Yu, Temasek Life Sciences Laboratory, SINGAPORE	Cloning and characterization of the White spot syndrome virus (WSSV) p25 gene and its promoter region	T6	66
Asmi Citra Malina A.R. Tassakka, United Graduate School Of Agricultural Sciences, JAPAN	Immunostimulatory effect of CpG oligodeoxynucleotides on the innate immune response of common carp, Cyprinus carpio L	T7	67
Victoria Alday - Sanz, INVE Technologies, BELGIUM	Industry's need of standard challenge tests for shrimp	T7	68
Pramoda Sahoo, Institute For Animal Health, UNITED KINGDOM	Dietary intake of levamisole enhances the immune response and disease resistance of the Asian catfish, Clarias batrachus	T7	69
Sivan Subburaju, Temasek Life Sciences Laboratory, SINGAPORE	The general protein secretion machinery of Aeromonas hydrophila is involved in fast-killing mechanism of c. elegans and mortality of fish	T7	70
James Torres, University of the Philippines in the Visayas, PHILIPPINES	Effects of B-glucan on non-specific immune response in grouper: hematological analysis	T7	71
Kjersti Gravningen, Alpharma Inc., UNITED STATES OF AMERICA	Bacterial disease prevention in high value marine fish culture by vaccination	T7	72
Clinton Chambers, University of Adelaide, AUSTRALIA	Orally administered praziquantel as a treatment for monogeneans infecting Seriola quinqueradiata: efficacy and practical considerations for a commercial fish farm	T8	73
Piyalai Hemtanon, Walailuk University, THAILAND	Studies on antiviral and antibacterial substances of Spirulina platensis for prevention the infectious diseases in black tiger shrimp (Penaeus monodon) caused by white spot syndrome virus and vibrio harveyi.	T8	74
Orapin Khongpakdee, Institute Of Agricultural Technology, THAILAND	Studies on the effect of antibacterial and antiviral substance of marine diatom Skeletonema costatum for prevention the infectious diseases in black tiger shrimp (Penaeus monodon) caused by Vibrio harveyi and white spot syndrom virus	T8	75
Seyed Saeed Mirzargar, Aquatic Animal Health,Faculty of Veterinary Medicine,University of Tehran, IRAN	Determination of minimum detectable limit (MDL) of residues of selected antibacterial agents in common carp (Cyprinus carpio) using thin layer chromatography-bioauthography (TLC-B)	T8	76
John Stephen Sampath Kumar, Fisheries College & Research Institute, INDIA	Herbal preparations for aquaculture bio-sexurity - Indian experiences	T8	77
Ke Wang, Chinese Academy Of Sciences, PR CHINA	The anti-virus effects of glucosamine and melaleuca oil on Chinese prawn (Penaeus chinesis)	T8	78
Shih-Chu Chen, National Pingtung University of Science and Technology, TAIWAN, ROC	Lactococcus garvieae, a bacterial infection in grey mullet Mugil cephalus	T9	79
CV Mohan, College of Fisheries, INDIA	Health status of cultured shrimp at harvest - epidemiological significance	T9	80

PRESENTING AUTHOR	TITLE	THEME	POSTER BOARD#
Kenton Morgan, University of Liverpool, UNITED KINGDOM	Feeding farmed shrimp with shrimp waste - the lessons for aquaculture from BSE	T9	81
Ana Roque, Ciad,Ac, MEXICO	Effect of methylparathion on the susceptibility of shrimp (Litopenaeus vannamei) on the development of vibriosis	T9	82
Judith Silapan, UP In The Visayas, Cebu College, PHILIPPINES	Acute toxicities of deltamethrin and lambda-cyhalothrin to the fry of green grouper (Epinephelus tauvina Forsskål) and milkfish (Chanos chanos Forsskål)	T9	83
Temdoung Somsiri, Aquatic Animal Health Research Institute, THAILAND	Contamination of mycobacterium spp. in live feed	T9	84
Kishio Hatai, Division of Fish Diseases, JAPAN	Mycobacteriosis in ranchu goldfish (Carassius auratus) imported from Japan	T10	85
Barry Munday, School of Human Life Sciences - University of Tasmania, AUSTRALIA	Fish kill of mullet Liza klunzingeri in Kuwait Bay: The role of Streptococcus agalactiae and the influence of temperature	T10	86
Indunil D.S.I.P Thilakaratne, Veterinary Research Officer, SRI LANKA	Seasonal prevalence of trichodina infection in guppies (Poecilia reticulata) and goldfish (Carassius auratus) cultured for export in Sri Lanka	T10	87
Jiang Yulin, Key Lab of Aquatic Animal Diseases, REPUBLIC OF CHINA	Isolation of IHN virus from imported turbot (Scophthalmus maximus)	T10	88

Trade Exhibition Floorplan



Fish Health Section - Asian Fisheries Society *(Booth 1)*

The Fish Health Section of the Asian Fisheries Society (FHS/AFS) is one of four global professional societies involved in aquatic animal health. The FHS has the following objectives: (a) promote effective interaction and cooperation among persons involved in fish health research; (b) encourage and promote investigation and advances in knowledge of fish health; (c) focus attention on fish health problems by disseminating technical and other information on all aspects of fish health; and (d) promote the proper implementation of proper implementation of effective fish health protection practices in the region. To achieve its objectives, the FHS organizes a triennial "Symposium on Diseases in Asian Aquaculture" (DAA), as well as other scientific seminars/workshops on topics of interest to the membership, independently or in conjunction with other organizations and implements projects relevant to aquatic animal health. The Section publishes the proceeding of DAA, a newsletter and other project related reports. The Section is administered by an Executive Committee (ExeCom) consisting of a Past Chairperson, Chairperson, Vice-Chairperson, Secretary-Treasurer, Newsletter Editor, and six elected members; governed by the FHS/AFS ByLaws; and holds a Triennial General Meeting of members in conjunction with DAA. Membership is open to any person interested in the furtherance of Fish Health Section objectives and who is a member in good standing of the Asian Fisheries Society. More detailed information about the FHS activities can be found at

http://afs-fhs.seafdec.org.ph/ http://afs-fhs.seafdec.org.ph/

Biosecurity Australia *(Booth 4)*

Biosecurity Australia is a one of the operating groups within Agriculture, Fisheries and Forestry- Australia. Our main objective is to ensure that Australian animal and plant industries have increased access to markets and are protected from diseases and pests. The tasks of facilitating the export of live aquatic animals and genetic products and the development of policies that allow the safe importation of aquatic animals and their products is carried out by Aquatic Animal Biosecurity.

Biosecurity Australia - AFFA
Agriculture, Fisheries and Forestry Australia
GPO Box 858 CANBERRA ACT 2601
Work Phone: 02 6272 5330 Fax: 02 6272 3399

CSIRO *(Booths 6 &7)*

CSIRO Livestock Industries conducts research for Australia's livestock and allied industries to facilitate their sustainability and long-term competitiveness. We create, develop and commercialise technologies for novel products, new production options, improved production efficiency, disease control and product quality throughout the livestock industry value chain. We have a significant aquaculture diagnostic capability through our Australian Animal Health Laboratory and are working with a range of research collaborators on prawn, yabby and finfish aquaculture.

CSIRO Livestock Industries
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Primo/INVE *(Booth 2)*

INVE is a multinational group of companies, active in agri and aquaculture. Our aquaculture Business Units provide nutritional and health solutions in fish and shrimp rearing. INVE are leaders in the development, production and commercialisation of a complete range of high quality products, comprising Artemia cysts; a Selco line of rotifer and Artemia enrichment products; an extensive range of compound starter and broodstock diets; a Health line; Standard and Specialty premixes and Concentrates for various stages/species. For more information, please visit us at www.inve.com

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QDPI *(Booth 3)*

The Queensland Department of Primary Industries (QDPI) is proud to be a major sponsor of the Symposium. Aquaculture is a growing industry in Queensland and aquatic animal health is an important aspect of the continued expansion of the industry. Prawns, barramundi, silver perch, crayfish and oysters are the peak industries in aquaculture in Queensland are. QDPI plays a major role in research and development to support the sustainable growth of aquaculture in Queensland.

Queensland Department of Primary Industries
Level 2, Primary Industries Building 80 Ann Street, Brisbane QLD 4000
PO Box 46 BRISBANE QLD 4000
Work Phone: 07 3234 0701 Fax: 07 3239 0439

University of Queensland *(Booth 5)*

The University of Queensland's Faculty of Biological and Chemical Sciences (BACS) is world-renowned for its strengths in teaching and research in the areas of biological and chemical sciences and since 1981 has been named as Australia's Number 1 Biological Sciences Research University.* Brisbane is fast becoming a centre for excellence in molecular biosciences and a major hub for bioindustries in the Asia-Pacific region, with innovative research institutes based at the University of Queensland. The development of the Institute of Molecular Biosciences (a major joint University / CSIRO / Government initiative) on the UQ campus, confirms this status. As well as underpinning various areas of study in the Faculty, Chemistry links strongly to Biology through new initiatives in Structural Biology. BACS also has the greatest breadth and depth of academic expertise in Ecology, Conservation Biology, Environmental Science, Marine Studies and Aquaculture of any university in Australia and is ranked as one of the best in the world in these fields.

Department of Microbiology and Parasitology
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Social Program

Welcome Reception

Sunday 24 November
6.30 – 8.30pm
Gold Coast International Hotel

Take the opportunity to relax with new and old friends at the official Symposium Welcome Reception. Attendance is included for all registrants.

Lunches

Lunches on Monday, Tuesday, Wednesday, and Thursday will be held in the Trade and Poster areas.

Official Symposium Dinner

Thursday 28 November 7.00pm
Sea World, Main Beach

Dinner promises to be the Symposium highlight! Take a Behind the Scenes Tour followed by the opportunity to relax and converse with colleagues over a sumptuous banquet. Included for all registrants. Please note that coaches will depart from the Gold Coast International Hotel at 6.30pm.

General Information

Symposium Venue

Gold Coast International Hotel
Cnr. Gold Coast Highway & Staghorn Avenue
Surfers Paradise QLD 4217

Telephone	Facsimile
(07) 5584 1200	(07) 5584 1280
Int: +61 7 5584 1200	Int: +61 7 5584 1280

Messages

A message board will be located in the Symposium registration area. Please advise potential callers to contact the Gold Coast International Hotel and ask for the 5th Symposium on Diseases in Asian Aquaculture registration desk.

All messages will be placed on the message board. Delegates are asked to check the board regularly throughout the symposium. The Symposium Secretariat takes no responsibility for messages not delivered to the delegate.

Telephones

All mobile phones and pages are to be turned off when delegates are in session. Public telephones are located throughout the hotel.

Prayer Room for Delegates Observing Ramadan

A dedicated room has been set aside as a prayer room for delegates observing Ramadan. This is located on level 2 of the Gold Coast International Hotel. Please enter via the Boardroom - turn left and it is the first door on the right. There will be a sign on the door indicating that this is the Prayer Room.

Symposium Registration Desk

The registration desk located in the foyer of the conference centre will be staffed at the following times:

Sunday 24 November	4.30pm – 6.30pm
Monday 25 November	8.00am – 6.00pm
Tuesday 26 November	7.45am – 6.00pm
Wednesday 27 November	7.45am – 1.00pm
Thursday 28 November	7.45am – 6.00pm

Name Badges

Delegates are requested to wear their name badge at all times during the Symposium.

Dress

Dress for all Symposium sessions and social events is smart casual. Smart casual means open neck shirt and trousers/shorts for gentlemen.

Personal Requirements

If you have any special dietary requirements such as Vegan or Halal and did not indicate this at the time of registration, please let us know so we can make the necessary arrangements.

If you have any medical, wheel chair access, or other special requirements, please let us know so we can assist you.

Personal Mail and Deliveries

Personal deliveries should be sent to your accommodation address.

Tickets

Tickets will be required for entry into the social functions. Delegates who have paid for "additional" reception, lunch and dinner tickets will be given the appropriate ticket(s). All tickets will be inside your registration envelope.

Ticket Refunds

Ticket refunds for social functions will be available if participation is cancelled more than 48 hours prior to the event.

Trade Exhibition

All morning and afternoon teas will be served in the trade area.

Sunday 24 November	6.30pm – 8.30pm
Monday 25 November	8.00am – 6.00pm
Tuesday 26 November	8.15am – 6.00pm
Wednesday 27 November	8.15am – 1.00pm
Thursday 28 November	8.15am – 4.00pm

Poster Exhibition

Posters will be displayed for the duration of the Symposium. Posters should be in place by no later than 5.30pm on Sunday 24 November and must be collected by 6.00pm on Thursday 28 November. Poster boards will be numbered as indicated in the poster program. Please refer to the poster program for further details. Delegates are encouraged to visit all the poster displays during coffee and lunch breaks and the welcome drinks.

Accommodation

Please ensure that the balance of your accommodation (if applicable) is paid directly to your hotel on departure, as well as any incidentals.

Disclaimer

All information disclosed in the Symposium Program is correct at time of printing. The Organising Committee reserves the right to alter the Symposium Program in the event of unforeseen circumstances.

All speakers were invited to provide abstracts for the Symposium Handbook, unfortunately not all were able to provide us with their papers.

Symposium Organisers

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Fish Health Section – Asian Fisheries Society Member Directory

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Opening Session 1

Aquatic animal health management in Asia

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In this new millennium, aquatic animal health management strategies in Asia expanded and adjusted to the current disease problems faced by the aquaculture sector. This presentation will briefly discuss some of the most serious trans-boundary pathogens affecting Asian aquaculture including a newly emerging disease and highlight recent regional and national efforts on responsible health management for mitigating the risks associated with aquatic animal movement. A regional approach is fundamental since many countries share common social, economic, industrial, environmental, biological and geographical characteristics. The implementation of the 'Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals', the establishment of the Asia Regional Advisory Group on Aquatic Animal Health, and the importance of regional and international cooperation to address health management issues will be discussed. Capacity and awareness building on aquatic animal epidemiology, science-based risk analysis for aquatic animal transfers, surveillance and disease reporting, disease zoning and establishment of aquatic animal health information systems to support development of national disease control programs and emergency response to disease outbreaks are highlighted. Molecular diagnostics with emphasis towards standardization and harmonization, inter-calibration exercises and quality assurance in laboratories, accreditation program and utilization of regional resource centers on aquatic animal health will also be discussed. Whilst most of these strategies are directed in support of government policies, implementation will require pro-active involvement, effective cooperation and strategic networking between governments, farmers, researchers, scientists, development and aid agencies, and relevant private sector stakeholders at all levels. Their contributions are essential to the health management process.

NOTES

Food safety in aquaculture

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Although the title of this presentation encompasses the whole range of actual and potential food safety problems associated with aquaculture products, the limited time available means that it concentrates primarily upon controls placed on the use of pharmaceuticals in aquaculture which are primarily in place to protect consumes of the aquaculture product. The reasons for these complex regulations will be discussed, their origin and development explained. This is an area with considerable interest to the Asia - Pacific producer, since the regulations developed in Europe and in N. America now mean that aquaculture product from areas where use of veterinary medicines is less regulated may be excluded from these large and high value markets. In the last twelve months China and SE Asia have felt the impact of these bans.

NOTES

Session 1 - Biosecurity and Risk Assessment

Biosecurity: A new word for an old concept

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Biosecurity is a fashionable word that is being used in a number of different circumstances. Its meaning in these situations is explored. The concept of biosecurity is used to cover the management of risks arising from biological organisms and agents that may cause harm to living organisms and other aspects of the environment. Following the spread of diseases such as whitespot and Taura syndrome in prawns, Akoya disease in pearl oysters and epizootic ulcerative syndrome in fish, the need for improvement to biosecurity for aquatic animals has been recognised. The principles underpinning the development of a biosecurity program are identified. Biosecurity programs should have a strong scientific basis and use risk assessment to evaluate risk management approaches to ensure that measures provide appropriate protection without unduly hindering business opportunities.

NOTES

Farm level biosecurity in aquaculture

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Economically and environmentally sustainable aquaculture is vital for global food security. Disease outbreaks caused by endemic Category-1 (C-1) pathogens are the greatest deterrents to successful aquaculture. For a disease to occur, the pathogen (necessary cause) should be able to gain entry into the culture system, through air, water, or land. Possible pathogen carriers include infected hosts (seed, brood, vectors, carriers, intermediate hosts, reservoir hosts), non-host biological carriers (birds, dogs, insects, other predators, human beings) and fomites (water, vehicles, buckets, shoes, nets, clothing). Biosecurity principles involve standard sets of practices that ensure the security of the cultured organism from exposure to dangerous C-1 pathogens. Biosecurity can be achieved by blocking all the possible routes of pathogen entry, by putting up external and internal barriers. Adherence to strict biosecurity protocols, seed and brood screening programs, proper disinfection strategies at defined critical control points, isolation and quarantine facilities, restricted access, etc are some of the measures of biosecurity. Identification and quantification of relative risks associated with each possible route of pathogen entry into the farms through epidemiological studies would help to target resources only to the main risks, to make biosecurity measures cost effective. Pathogens which are endemic and well established in a diverse host range may find their way into farms, despite the best biosecurity measures. It is well known that mere presence of necessary cause alone will not lead to disease outbreaks. Identification of farm level component causes (risk factors) through epidemiological studies and their effective management would help prevent disease outbreaks. In addition, co-ordinated herd health programs, rapid diagnostic systems and early warning surveillance strategies would reduce the probability of disease outbreaks. In the case of C-1 pathogen outbreak, damage control should be the only post-outbreak goal. Isolation of affected farm, removal of hosts, effective disinfection programs, early warning systems and co-operation of processors in avoiding contamination would help to contain the spread of the pathogen. Farm level biosecurity should be comprehensive with the broad objective of preventing the entry, establishment, amplification and spread of C-1 pathogens. Principles of biosecurity should be considered right from site selection stage till harvest and processing. Awareness creation among farmers on the importance of farm level biosecurity should be taken up on top priority.

NOTES

'To hazard or not to hazard that is the question' How unknowns in science affect the identification of hazards in an import risk analysis

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The process of hazard identification, in the context of an import risk analysis (IRA), involves the recognition of disease agents and pests that could be associated with trade in a commodity. For a pathogen to be classified as a potential hazard the following criteria should be met. The pathogenic agent should be: 1) appropriate to the imported commodity; 2) capable of producing adverse consequences in the importing country; 3) likely to be present in the exporting country and; 4) exotic to the importing country, or, if present, be subjected to mandatory control or eradication measures. Hazard identification is, in essence, a decision-making process resulting in the classification of a pathogen as 'a hazard' or 'not a hazard'. A pathogenic agent determined not to be a hazard is not considered further in an IRA. In this regard, the analysis must be transparent by providing clear reasons for the exclusion or the inclusion of a pathogen. However, it is often the case that the data available on a pathogen is incomplete or inconclusive, yet value judgements (and justifications) must still be made by analysts as to whether the above criteria are met. This paper discusses various solutions to tackling uncertainty and lack of knowledge in the identification of potential hazards, using Australia's current import risk analysis on non-viable bivalve mollusc as a practical example.

NOTES

The role of risk analysis and epidemiology in the development of biosecurity for aquaculture

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Biosecurity is a management strategy to minimise the risk of disease introduction, and is critical to development of a successful aquaculture industry. In this paper it is argued that the combination of epidemiological research and risk analysis methodology is required to develop appropriate biosecurity programmes. Risk analysis ensures that a logical, transparent approach is adopted to identify and prioritise disease hazards and pathways of introduction and exposure. In aquaculture it has been mainly used to assess risks of disease introduction at a country or regional level, and has been little used at the farm level. Risk analysis is only as good as the data it uses, and primarily epidemiological data is required. Epidemiological investigations that underpin risk analysis fall into two main categories: disease outbreak investigations and structured observational studies. The outbreak of infectious salmon anaemia in Scotland is used to illustrate how risk analysis and epidemiological investigations can be combined to develop improved biosecurity. Other epidemiological studies in a range of production systems have identified possible routes of disease introduction, and factors affecting the establishment of disease. The use of the results of these studies in risk analysis and the development of biosecurity is discussed. Finally it is shown that risk analysis can identify critical gaps in the data needed for the development of biosecurity, and, therefore, direct future epidemiological investigations.

NOTES

Import risk analysis : Philippine experience

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A rational approach to decision-making in the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) on the importation of live aquatic organisms started in 1992 with the creation of a committee for this purpose. Although the committee was short-lived, it planted the "seed" for the process of Import Risk Analysis (IRA) in BFAR. In 1993, with the increasing awareness of local shrimp industry and the government on the risk of irresponsible trans-boundary movements of aquatic organisms, the importation of live shrimp and prawn was prohibited. In 1998, a new Philippine Fisheries Code which strengthens BFAR's Fisheries Inspection and Quarantine Service (FIQS), among other things, was signed into law. In 1999 and 2001, BFAR enlisted the technical assistance of the Food and Agriculture Organization (FAO) and the Canadian Executive Service Organization (CESO) to help BFAR strengthen its FIQS. The FAO/Network of Aquaculture Centres in Asia-Pacific (NACA)/Australian Agency for International Aid (AusAID)/Agriculture, Fisheries and Forestry of Australia (AFFA) organized training/workshops on Import Risk Analysis and Aquatic Animal Disease Surveillance, Reporting and Contingency Planning for BFAR key officials. In 2000, an IRA Panel was finally created by the BFAR Director. Among its immediate tasks is to ensure the approval of an order regulating the importation of fish and fishery products, microorganisms and biomolecules. The said order categorizes fish species for importation based on their relative risks i.e., low risk, medium risk, high risk and prohibited or banned species and provides for their corresponding certification and quarantine requirements. Since the creation of the IRA Panel, it has already encountered several requests for importation. Among the special cases handled by the Panel are the proposed importation of: 1) Penaeus vannamei from Taiwan, 2) specific disease resistant-Litopenaeus stylirostris from the USA, 3) specific pathogen free- Penaeus vannamei from Hawaii, 4) Cherax quadricarinatus from Australia, and 5) Ictalurus punctatus from the USA. The IRA process in BFAR takes into consideration the potential impact of the proposed importation not only on fish health but also on public health and the environment as well. Institutionalizing the IRA process in a government bureaucracy like BFAR takes much time and effort. Political pressures will continue to be a challenge to the IRA Panel to maintain its objectivity. Although there is still much to be done to push IRA in BFAR to a higher level, we believe that the staff's commitment and continuing external support from NACA, FAO, Asia-Pacific Economic Cooperation (APEC) and other organizations, can make it possible.

NOTES

A national survey to demonstrate freedom from white spot virus and yellow head virus in Australian crustaceans

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In November 2000, mud crabs within the Darwin Aquaculture Centre and prawns within the Aquaculture School at the Northern Territory University reacted positively in polymerase chain reaction (PCR) tests specific for white spot virus (WSV). The facilities were immediately destocked and disinfected. In response to concerns that the WSV may have spread beyond the aquaculture facilities, a national survey was designed and conducted to assess the WSV status of Australia's wild crustacean populations. Over 3400 prawns, crabs and other crustaceans were collected from over 60 sites throughout Australia and tested by PCR for WSV. Simultaneously, samples were also examined for the presence of yellow head virus by PCR. No sample contained WSV or YHV, and Australia was shown to be free of these viruses. The synthesis of disease data from a range of sources to establish the disease status of Australian crustaceans will be demonstrated.

NOTES

Session 2 - Diseases of aquatic vertebrates

Recent advances of betanodaviruses and iridoviruses in Asian aquaculture

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Viral nervous necrosis (VNN) caused by betanodaviruses is highly destructive disease among a variety of cultured marine fish and has spread worldwide for this decade. Betanodaviruses are small, non-enveloped, icosahedral particles and the genome is composed of bipartite, single-stranded, positive-sense RNA molecules. There are some genetic and phenotypic variations among betanodaviruses and these variations seem to be closely related to the host specificity of the virus. Recent establishment of a reverse genetics system for SJNNV (the type species of the Betanodavirus) opened the way for molecular studies directed at virus multiplication and pathogenesis of the betanodaviruses. Although pathobiological data on the disease have been accumulated after finding of some susceptible cells, the infection mechanisms are still poorly understood in many cases of the disease, particularly in groupers. Iridoviruses are large, enveloped double-stranded DNA viruses. Two types of irodovirus infection are known in cultured fish including tropical ornamental fish. One is iridoviruses in the genus Lymphocystivirus which causes benign papilloma-like tumors, resulting in loss of commercial value, and the other is systemic infection with severe mortalities. These include members of the genus Ranavirus (EHNV), the erythrocytic iridoviruses, and a group of viruses that produce hypertrophic cells; sea bass iridovirus, grouper iridovirus, and red sea bream iridovirus (RSIV). Mortalities of commercially important fish caused by the last group of iridovirus have prevailed in East and Southeast Asian. Although a way to control the disease is urgently needed, transmission mechanisms remain unknown for the majority of these viruses. However, RSIV infection is only one exception that can be controlled by vaccination.

NOTES

Diseases of (Thunnus spp.) - Emerging aquaculture species

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Much is known about those aspects of tuna health which can be studeied in wild populations, eg helminth parasites. However, because aquaculture of these species is in its infancy knowledge on microbial, nutritional and environmental diseases is limited. This contribution is an attempt to bring together the available information on those diseases of Thunnas spp. which cause significant, morbidity, mortality or economic liss. In doing so it has become clear that much more research needs to be undertaken on the physiology of the species (southern, northern and Pacific bluefin tunas) currently used in aquaculture in order for the pathogenesis of some conditions to be properly understood. Also, attempts at hatchery culture of Pacific bluefin tuna has indicated that Thunnus spp. will be problematic to hatch and propagate.

NOTES

Susceptibility of marine fish species to Piscine nodavirus from orange-spotted grouper, Epinephelus coioides in the Philippines

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Viral nervous necrosis has a constraint on the seed production of marine fishes worldwide. In the Philippines, we found that mass mortality in hatchery-reared orange-spotted grouper Epinephelus coioides, was caused by a strain of piscine nodavirus using light and transmission electron microscopy, virus isolation in SSN-1 cells and RT-PCR. Considering the impact of this virus to aquaculture, the present study aimed to investigate the host range of this strain, focusing on the susceptibility of other species in Southeast Asia: orange-spotted grouper, Asian sea bass Lates calcarifer, mangrove red snapper Lutjanus argentimaculatus and milkfish Chanos chanos. The experimental fish were intraperitoneally injected with 0.05mL of the filtrate homogenate of infected organs from diseased grouper at 1 x 10 9.5, 1 x 10 8.5 and 1 x 10 7.5 TCID50/g, while the control group received 0.05mL of Hanks_f balanced salt solution. Clinical signs such as lethargy, anorexia and darkened pigmentation were observed in fish injected with high dose of the homogenate. Although few mortality occurred in the experimentally-challenged fish 10 days-post infection, severe necrosis and vacuolation of the brain and retina characteristic to nodavirus infection were produced in the four fish species. The virus was reisolated in SSN-1 cells inoculated with the homogenate of survivors in all doses for all four fish species. These results indicate that grouper, sea bass, mangrove red snapper and milkfish are susceptible to the piscine nodavirus from diseased grouper. The low mortality rate could be explained by possible differences in virulence of the virus to different species and size of fish.

NOTES

Characterisation of an iridovirus isolated from diseased marble goby Oxyeleotris marmoratus (Bleeker, 1852)

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A high mortality disease of culture marble goby, Oxyeleotris marmoratus occurred in Nakorn Pathom province, Central Thailand in March 2000. The diseased fish exhibited minor ulcer lesions on the body and around mouth area. No external parasites and blood parasites were observed. No bacteria could be isolated from internal organs, livers, kidneys and spleen. Three diseased fish were used for virological investigation. Each fish was sacrificed and internal organs and ulcers were pooled as one extract sample. The tissue extracts were filtered through 0.45 micron then inoculated on to Epithelioma papulosum cyprini (EPC) cell line at 25ºC. The viruses induced round plaque in EPC line during early infection. Virus titres were decreased over 6 Log10 TCID50/ml when incubated with IUdR or chloroform indicating DNA genome and enveloped virus particle. Transmission electron micrograph indicated numerous icosahedral symmetry of nucleocapsid with ~132 nm in diameter, which located in cytoplasm. Above properties indicate the new virus isolate can be classified as a virus member of the Family Iridoviridae. This virus propagated well in fish cell lines, BF-2, EPC, FHM, BB, SSN-1 and discuss tail (DT) and 2 reptile cell lines, Siamese crocodile embryo (SCE) and soft-shelled turtle embryo (STE) at 25-30ºC. The highest virus titres up to 9.2 Log10 TCID50/ml obtained from BF-2 line. New virion released from EPC cells about 15 hr post-infection. The virus isolate was sensitive to heat at 56ºC. Polymerase chain reaction amplification of the newly isolate using specific primers designed from major capsid protein gene of Ranavirus strain FV-3 gave positive PCR products. Sequence analysis 300 bp product this newly isolate exhibited 98-100% nucleotide homology to Ranavirus FV-3 and Rana trigrina ranavirus. Findings indicate that the virus is an Iridovirus and is most likely to be a strain of Ranavirus. This virus is named as Oxyeleotris marmoratus ranavirus or OMRV. Virulence and pathogenesis of OMRV needed to be identified. Keywords: Ranavirus, Rana trigrina ranavirus, Oxyeleotris marmoratus ranavirus, Iridovirus

NOTES

Molecular characterization of a novel ranavirus isolated from grouper, Epinephelus spp

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A large icosahedral virus was isolated from diseased grouper, Epinephelus tauvina. The virus grew well in several cultured fish cell lines, with stable and high infectivity after serial passages in grouper cell line (GP). The virus was sensitive to both acid and heat treatments. Virus replication was inhibited by 5-iodo-2-deoxyuridine (IUDR) indicative of a DNA containing genome. The virus infectivity was reduced with ether treatment suggesting that the virus was lipid-enveloped. Electron micrographs showed abundant cytoplasmic icosahedral virons in the virus-infected GP cells. The size of intracellular nucleocapsid was 154 nm between opposite sides, or 176 nm between opposite vertices with an inner electron-dense core of 93 nm. Virus particles were released through budding from plasma membranes with a size of 200 nm in diameter. SDS-PAGE of purified virus revealed 20 structural protein bands and a major capsid protein (MCP) of 49 kDa. A DNA fragment of ~500 nucleotides was successfully amplified by polymerase chain reaction (PCR) using the primers from conserved regions of major capsid protein gene of frog virus 3 (FV3) the type species of Ranavirus. Subsequent multiple alignment and phylogenetic analysis showed that the newly isolated grouper virus was closely related to largemouth bass virus (LMBV), FV3 and Regina ranavirus (RRV). Our data suggests that the virus isolate is a novel member of genus Ranavirus, family Iridoviridae. We tentatively name the virus as Singapore Grouper Iridovirus (SGIV). SGIV was able to cause serious systemic disease capable of killing 96% of grouper fry.

NOTES

Viral DNA sequences of genes encoding the ATPase and the major capsid protein of tropical iridovirus isolates which are pathogenic to fishes in Japan, South China Sea and Southeast Asian countries

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Tropical iridovirus infection causes severe epizootic resulting in mass mortalities and large economic losses in freshwater ornamental fishes cultured in Southeast Asian countries, in wild fish seedlings captured in South China Sea, and in marine fishes farmed in Japan, Singapore, and Thailand. All of tropical iridovirus-infected fishes histopathologically showed the systemic formation of inclusion body-bearing cells and necrosis of virus-infected splenocytes and hematopoietic cells. We designed primer sets for the ATPase gene and the major capsid protein (MCP) gene and sequenced the PCR products derived from 5 iridovirus isolates from sea bass in South China Sea (SBIV), red sea bream in Japan, brown-spotted grouper with a grouper sleepy disease in Thailand, dwarf gourami from Malaysia and African lampeye from Sumatra Island, Indonesia. The ATPase gene and the MCP gene of these 5 viral isolates were highly homologous (>95.8%, >94.9% identity, respectively) and the deduced amino acid sequences of the ATPase and the MCP were also highly identical (>98.1%, >97.2% identity, respectively). Based on the high homology, these 5 isolates of tropical iridovirus from various fishes in geographically different regions were determined to have a single origin and to be native to Southeast Asian regions. However, these sequences were far different from those of members of the genera Ranavirus (FV3, ECV, ESV), Lymphocystivirus (LCDV-1) and Iridovirus (CIV) in the Family Iridoviridae. The SBIV MCP amino acid sequence had 47% identity and 67% similarity to that of LCDV-1, 45% identity and 63% similarity to FV3, 45% identity and 68% similarity to ECV, 45% identity and 63% similarity to ESV, and 45% identity and 63% similarity to CIV. We propose a new genus 'Tropivirus' for tropical iridovirus in the Family Iridoviridae.

NOTES

Session 3 - Diseases of aquatic vertebrates

Ranavirus of fish, amphibians and reptiles

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Ranaviruses are a group of viruses belonging to the genus Ranavirus within the family Iridoviiridae. The genus contains viruses known to infect and cause disease in a range of ectothermic animals namely fish, amphibians and reptiles. The piscine viruses belong to three, possibly four sub-groups within this genus whilst those isolated from amphibians and reptiles belong to the same group. Another group of associated iridoviruses, namely the erythrocytic iridoviruses, are yet to be classified by the ICTV and are known to cause anemia in three classes of animals. Each group of viruses has their own characteristics and can be identified via an array of diagnostic tests. The various groups will be discussed within the presentation together with accepted (OIE) testing procedures. So what is the significance of ranaviruses? Ranaviruses have been described as the 'cold-blooded killers'. Viruses have been associated with fish kills in Asia, Australia, North America and Europe. Within North America, endangered tiger salamanders have undergone major population fluctuations and in the United Kingdom the common frog has undergone annual population crashes because of infection with ranaviruses. These viruses have also been isolated from healthy and diseased reptiles. The potential for ranaviruses to cross animal classes and species make them potentially lethal to the finfish industry and to piscine and herpetofauna biodiversity. In this presentation these viruses will also be discussed in terms of 'ecosystem health' i.e. do ranaviruses represent emerging viruses, are they a threat to aquaculture and the herpetofauna, and are they the cause of declines in amphibian populations?

NOTES

Emerging diseases of soft-shell turtles

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The soft-shelled turtle has been widely cultured in whole China in the recent 10 years because of its nutritional and medicinal values. Unfortunately, epizootic outbreaks of disease usually occurred in densely stocked young turtle ponds. Some symptoms, such as shell perforation (furuncle), red neck, red dots, ulcerated skin, blisters, white spots and white dots has been described and called as names of diseases by farmers, The major problem is that the pathogen causing the clinical signs has not been identified clearly. Frequently, same bacteria were isolated from sick turtle with different symptoms, or different kinds of bacteria were isolated from sick turtle with same symptoms. Besides, there exits secondary infection and mixed infection, It could cover up primary infection. This review describes in detail some diseases of soft shelled turtle in China according to pathogens, such as Trionyx sinensis virus (TSV), Soft shelled turtle iridovirus(STIV), Aeromonas hydrophila, Edwardsielliasis. Aspects of their etiology, epidemiology, immunology and control are summarized.

NOTES

New and emerging diseases in reef and Estuarine fishes from North Queensland, Australia

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The Department of Primary Industries Northern Fisheries Centre (NFC), Cairns, Queensland is undertaking research into reproduction and larval rearing technologies for a range of finfish including grouper species (barramundi cod, Cromileptes altivelis, flowery cod Epinephalus fuscoguttatus and estuary cod Epinephalus cuoides) and estuarine species (mangrove jack, Lutjanus argentimaculatus and barramundi, Lates calcarifer). Potential broodstock have been captured from wild stocks, as sources of captive-bred stocks are unavailable in Australia. This paper presents a synopsis of newly emerging diseases seen in these species. Disease investigations included a range of diagnostic aids and tests done at Oonoonba Veterinary Laboratory, Townsville, including gross pathology, histopathology, electron microscopy, bacterial and fungal culture, isolation and identification and viral isolation in cell culture. The clinical features and diagnostic findings for each disease are presented here. Emerging diseases include a new syndrome of hemangioma-like neoplasia of the gills with unknown aetiology observed in C. altivelis, a fungal infection in the swim bladder of C. altivelis with Cladosporium cladosporioides and Scopulariopsis brumptii, and a gill fluke infestation of E. fuscoguttatus with Allobenedenia n. sp. Wild-harvested, captive barramundi cod C. altivelis and coral trout Plectropomus leopardus have also shown infection with larval Terranova sp. Type II associated with visceral fibrosis, peritonitis and death. Other diseases encountered infrequently, are briefly mentioned and include White spot (Cryptocaryon irritans), Amyloodiniasis (Amyloodinium ocellatum), Vibriosis (Vibrio harveyi, Vibrio alginolyticus) and gill and skin fluke infestation.

NOTES

A virological survey in diseased grouper in Thailand using virus isolation and polymerase chain reaction (PCR) technique

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A virological survey had been conducted in brown spotted grouper, Epinephelus malabaricus, cultured in cages, earthen ponds and pens during April 2001 to January 2002 in East Coast and South Coast of Thailand. The adult grouper samples exhibited a wide range of clinical signs beginning from darkening body color with focal distension of the skin to red boil skin and to red spot ulcers on the body and head, while diseased fry to juvenile grouper showed darkening body color and swirling movement. Thirty-five isolates of viruses were discovered from using freshwater fish cell lines, SSN-1 and EPC. These isolates could be grouped into two different groups of fish viruses. One viral group was identified as a betanodavirus, which belonged to genotype RGNNV. As RT-PCR tests using specific primers to SJNNV revealed negative, while specific primers to RGNNV showed positive RT-PCR product. The second viral group could be identified as an Iridovirus. This grouper iridovirus had some level of similarities to red sea bream iridovirus. As one set of specific primers for RSIV could give positive PCR product while the second set of specific primers gave negative PCR result. The grouper iridovirus was found different to Ranavirus. Betanodavirus could infect both adult and fry to juvenile stage of the grouper and seemed to have more significant during November to January or during the dry and cold season in Thailand. Grouper iridovirus seems to cause disease in adult size of the fish with low level of mortality and seems to infect the fish predominantly in August and September or during rainy season in Thailand. Findings indicate that two different viruses are associated with diseased grouper cultured in Thailand that are identified as Betanodavirus and grouper iridovirus. There is a need to do more molecular studies of these new viral isolates as well as theirs pathogenesis.

NOTES

Isolation and identification of Edwardsiella ictaluri from diseased Pangasius hypophthalmus (Sauvage) cultured in Vietnam

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Farming of the indigenous freshwater catfish P.hypophthalmus in Vietnam has contributed to the livelihoods of rural families for decades. Many features of this fish make it an attractive option for culture. It has a relatively short production cycle of 8 months from stocking to harvest, there is a ready domestic and international market for the product and captive reproduction ensures that fish seed is available all year. Many families raise this omnivorous species extensively in polyculture ponds for family consumption or to sell locally on the domestic markets. Since 1995 there has been an increase in the number of families producing Pangasius spp. intensively in river-based cages, from where 80% of the total annual production is exported as frozen processed products. Fish farmers in Vietnam recently reported lower yields and high level mortalities in their systems due to disease outbreaks. These outbreaks occurred in fingerling and grow-out fish where diseased animals had few if any external signs of disease but in which white spots were observed on the internal organs. The disease was called bacillary necrosis of Pangasius (BNP) due to the presence of microcolonies of large rod-shaped bacteria within necrotic areas of tissue, as observed by light microscopy in tissue sections. Pure bacterial growth was recovered from P. hypophthalmus with clinical signs of BNP from various provinces of the Mekong Delta. The fastidious colonies required 48 hours at 280C before punctate, small, light coloured colonies were visible on solid agar. These colonies were identified as E. itcaluri by primary and biochemical tests, by immunohistochemistry and 16S rRNA analysis. The optimal growth temperature on solid media was 280C and colonies grew on agar selective for E. ictaluri. This presentation describes the findings in naturally occurring infections in farmed P.hypophthalmus due to E.ictaluri. To the authors' knowledge this is the first time that E. itcaluri has been identified from farmed Pangasius spp.

NOTES

Viral hemorrhagic septicemia virus (VHSV) infection in Japanese flounder Paralichthys olivaceus

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Viral hemorrhagic septicemia (VHS) is one of the most serious viral diseases of farmed rainbow trout and has been observed in some marine fishes in Europe but the disease had never been reported from salmonid or marine fishes in Asia until recently. In 1996, a viral disease occurred in Japanese flounder farmed in the Inland Sea of Japan and the disease has been prevailing in flounder farms since 1998 in western part of Japan. The causative virus was identified as VHSV in 2000. The disease prevails in farmed fish of 200-500 g body weight at water temperatures of 8-15?, usually chronically but sometimes daily mortlity reaches 10%. The diseased fish show darkened body color, ascites, congestion of the liver, enlargement of the spleen and kidney, and sometimes petechiae in the viscera and muscle. Histopathologically, myocardial necrosis is most prominent. Interestingly, VHSV had been isolated from apparently healthy wild Japanese flounder caught not only in the Inland Sea but also in the Sea of Japan since 1999. The VHSV isolates from farmed and wild flounder were closely related to American isolates (Genogroup 1) and they were pathogenic to flounder but not to rainbow trout.

NOTES

Session 4 - Epidemiology

International trade and risk analysis

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The importation of animals and animal products involves a degree of disease risk to the importing country. This has been highlighted by the pandemic of white spot disease of shrimp and very recently by the outbreak of disease in koi carp and common carp in Indonesia suspected to have resulted from imports of live animals. Because of the serious impacts of infectious diseases, particularly in farmed aquatic animals the process of import risk analyses (IRA) to prevent the entry and spread of unwanted pathogens is assuming increasing importance. The principal aim of IRA is to provide importing countries with an objective and defensible method to assess the disease risks associated with the importation of animals, animal products, animal genetic material, feedstuffs, biological products and pathological material. It forces a thorough and logical approach to be adopted in considering the likelihood of undesirable events, and identifies gaps in our current knowledge. In undertaking an import risk analysis, a country must be guided by the International Aquatic Animal Health Code (Code) of the Office International des Epizooties (OIE). The OIE Code provides guidelines for national authorities to assist them in addressing the principles laid out in the WTO's Agreement on the Application of Sanitary and Phytosanitary Measures. Facilitating trade while at the same time managing the associated disease risks is a challenge for all those associated with aquatic animal health. The coming years are likely to see an increasing need for skills and experience in this very important area.

NOTES

Farm level risk factors for white spot disease outbreaks

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White spot disease (WSD) is a pandemic disease of crustaceans caused by White Spot Syndrome Virus (WSSV). The scientific community has acted promptly in an attempt to limit its devastating impact on the shrimp farming industry. In the earlier stages of the epidemic, a number of potential risk factors for WSD outbreaks were hypothesised from information on other diseases and circumstantial evidence; as a result recommendations were distributed to farmers. In addition, highly sensitive diagnostic methods were developed that allowed the detection of WSSV in shrimp broodstock, post-larvae and wild animals. This led to further interventions aimed at reducing the risk of introducing the virus into populations. The effect of some of the hypothetical risk factors for WSD was tested through experimental trails. These, however, used WSD at the animal level as an outcome. Field investigations were also carried out and provided further information on risk factors for outbreaks at the pond level and the information generated has been used to investigate WSSV transmission using mathematical modelling. In this presentation, the farm level risk factors for WSD are discussed paying particular attention to the effects of the presence or introduction of WSSV into the farming system; shrimp stocking density and other management practices that might be associated with WSD outbreaks. The information presented is derived from manuscripts published in peer-reviewed journals, reports and the experience gathered by the authors during longitudinal epidemiological studies of WSD carried out in Vietnam and India. In addition to the information from field studies, results from experimental trials and mathematical modelling are also examined for their potential value at the farm level.

NOTES

Application of epidemiology to support better health management in black tiger shrimp Penaeus monodon aquaculture: an experience from India

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In 2001, a longitudinal epidemiological study of 365 randomly selected shrimp ponds was carried out in two districts of Andhra Pradesh, India to identify risk factors for shrimp disease outbreaks (emphasising the economically devastating white spot disease) and low pond production. Risk factors significantly associated with disease outbreaks were then used to identify locally relevant risk management practices. During 2002, demonstration sites were established in the two districts to support and evaluate the practical implementation of these better management practices (BMPs) on private farms. In this paper, experiences from the demonstrations in two villages in West Godavari district (8 ponds on 4 farms) are presented. Risk and protective factors identified from 184 epidemiological study ponds in this district were used to design and implement BMPs relevant to the local modified extensive farming system. Although farmers experienced disease outbreaks, demonstration ponds had better performance, in terms of days of culture (DOC), mean body weight (MBW), production compared to 2001 in the same ponds and with nearby non-demonstration ponds during 2002. The average DOC, MBW, production was 93, 18.5 grams and 417 kg/ha respectively. The demonstrations provided further understanding of risk factors, BMPs, shrimp disease occurrence and farm performance. Timely implementation of BMPs by farmers was a major factor in crop performance, which was dependent on farmers' understanding and willingness to adapt BMPs and their financial status. Experiences from this study show the epidemiological approach provided understanding of shrimp disease risks, but constraints to implementation of BMPs at farm level need to be understood and addressed in research and extension if such findings are to contribute to adoption of better health management practices at farm level.

NOTES

Patterns of occurrence of the helminth parasites of the bullseye pufferfish (Sphoeroides annulatus) from Sinaloa, Mexico

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The Mexican shrimp culture industry is starting to diversify into culturing other species. The bullseye puffer (Sphoeroides annulatus) has good aquaculture potential and is under pilot culture. However, parasitic infestations are a common problem for the bullseye puffer. The aim of this study was to determine patterns of occurrence of the helminth parasites from wild pufferfish populations used as potential broodstock. A total of 226 specimens were collected from two locations (Teacapan and Mazatlan) in Sinaloa state and were examined for the presence of helminth parasites. Two monogenea (Neobenedenia melleni and Heterobothrium ecuadori) and four trematodes (Bianium plicitum, Lintonium vibex, Homalometron longisinosum and Phyllodistomum mirandai) were identified. The most prevalent species were H. ecuadori (44%), B. plicitum (28%) and N. melleni (19%). Generally fish from Mazatlan had 2 helminthic species and fish from Teacapan 5 species. A relative risk analysis showed fish from Teacapan were at higher risk of presenting parasites (RR=1.85) than fish from Mazatlan. B. plicitum had higher probabilities (RR= 1.5) of being present in temperatures of 21-25 C than 26-30 C. H. ecuadori had higher probabilities (RR=1.72) of being present in fish from Teacapan than Mazatlan, in temperatures of 23-24.5 C (prevalence=67%, mean abundance =3 parasites/fish); and when other parasites were also present on the gill (RR=1.34). N. melleni had higher probabilities of being present when other parasites were present, such as Lepeophtheirus simplex (RR=1.31), trichodines (RR=1.47), Udonella sp.(RR=1.34). This study is one of the first contributions to the knowledge of the parasitic biology of this fish species.

NOTES

Epizootic haematopoietic necrosis virus: Epidemiology and uncertainty

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EHNV is one of only a handful of finfish viral agents requiring notification to OIE. Although closely related to other ranaviruses found in north America and Europe, it is distinguishable from these agents at the molecular level and is biogeographically isolated within south east Australia. Responsibility for control and field research lies with Australia. Two species are known to become infected naturally, free-living redfin perch Perca fluviatilis and farmed rainbow trout Oncorhynchus mykiss. Epidemiological evidence will be presented to support a view that neither of these is the natural host; the origin of the virus and its reservoir in Australia remains a mystery. However, slow continued expansion of the geographic range, and experimental evidence of a broader range of susceptible hosts is cause for concern related to future trade in finfish and effects on the aquatic ecosystem.

NOTES

Session 5 - Shrimp Health 1

Key farm management issues to reduce loss from diseases

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1. Aquatic Animal Health Research Institute

Among the diseases in cultured shrimp, bacterial and viral infection has proved to be the most serious cause of loss. Many attempts have been tried in order to reduce the loss. Currently closed system with liner pond and more intensive farming has been tried in Latin America whereas less intensive, low input system has been used in Asian country. Screening of post larvae for low prevalence of various viral infections is commonly used with various results. The post larvae sampling techniques still requires further study in order to ensure more accurate result. Formalin treatment for post larvae prior to stock does seem to be effective in eliminating infected/weak animals from the population and therefore minimizing the risk of a serious outbreak later. However, basic farm management including pond preparation, water and pond soil management as well as the stocking schedule is seemed to play an important role in trigger an outbreak. In order to reduce the loss from serious disease outbreak, some steps on basic pond management with emphasized on the water quality and pond soil monitoring were discussed, in particular on oxygen requirement in the system, relationships between pH, ammonia and hydrogensulfide.

NOTES

Phage induced virulence in the shrimp pathogen Vibrio harveyi

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Vibrio species comprise the most frequently encountered bacterial pathogens of cultivated shrimp, and V. harveyi is amongst the most virulent. Most strains are luminescent on agar media and also in infected shrimp that are suffering from luminescent disease or luminous bacteriosis. However, not all isolates of V. harveyi are highly virulent. Some can be injected at high dose (105-107 cells per g shrimp body weight) without causing shrimp mortality, while other isolates are lethal at 103 per g shrimp body weight or less. In addition, virulence is often lost upon continuous subculture. Simple differentiation of virulent and avirulent isolates has not been successful, although virulence factors including various enzymes (e.g., proteases and lipases), siderophores and proteinaceous toxins have been identified. Because of this and the genetic diversity of V. harveyi, it has been suggested that virulence is acquired via mobile genetic elements. Indeed, recent work has revealed that 2 quite different bacteriophages, one from the family Myoviridae and the other from the family Siphoviridae, can change the phenotype of V. harveyi isolates from non-virulent to virulent. The host range for both bacteriophages is narrow. A similar phenomenon occurs in V. cholerae, where conversion to virulence is mediated by a filamentous phage (Inovirus) from the family Inoviridae. Altogether, the current information suggests that there may be diverse groups of phages and complementary Vibrio hosts that could make the process of phage mediated virulence in V. harveyi quite complex. It also suggests that virulence of other Vibrio pathogens of shrimp may be influenced by bacteriophages. Narrow host ranges and the propensity to alter virulence suggest that the practical use of bacteriophages for biological control of pathogenic Vibrio species may be limited.

NOTES

Experimental transmission of Hepatopancreatic parvovirus (HPV) infection in Penaeus monodon postlarvae

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Hepatopancreatic parvovirus (HPV) infection in penaeid shrimps was first reported in various countries of the Asia-Pacific region in mid-1980's. The virus affects the hepatopancreas of postlarvae and juveniles, usually leading to slow growth and mortality during the early stage of culture. At present, there is no established experimental model of infection since there has not been any report of successful HPV transmission under laboratory condition in any known susceptible penaeid shrimp. Therefore, experiments were undertaken to induce HPV infection by feeding Penaeus monodon postlarvae (PL's) with virus-infected PL's. P. monodon PL's (PL-15), initially examined to be free from HPV, were found HPV-positive 24 hours after they were fed with the infected material. Percentage of infection was from 30% (day 1) to 100% (day 7) based on the examination of wet mounts of hepatopancreas (squashed tissue) stained with malachite green and through histopathology. This is the first report of a successful horizontal transmission of HPV in P. monodon PL's. This infection model could be used to study the pathogen further and would permit controlled experiments to be undertaken in order to identify prevention and control against the pathogen.

NOTES

Does spawning stress trigger the replication of white spot syndrome virus in shrimp?

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In shrimp, a persistent viral infection can be converted to productive viral infection by stressors such as poor water quality and overcrowding. Here we investigate whether the stress associated with spawning can also trigger virus replication in shrimp. Quantitative real-time PCR was used for a temporal analysis of WSSV loads in a batch of 14 wild-caught black tiger shrimp (Penaeus monodon) brooders. For these specimens, four basic patterns emerge: Group 1 was comprised of 4 specimens which had a relatively high initial virus load (approximately 800 ~ 8000 viral DNA copies/per µg total DNA) and in these specimens, the virus replicated rapidly up to the time of spawning. After spawning the virus levels remained high and all of these shrimp died within a few hours. Two other groups (II and III) both had similar initial virus loads (approximately 10~ 600 viral DNA copies/per µg total DNA), but in group II (5 specimens), as in group I, the virus replicated rapidly up to and beyond spawning and all the shrimp died soon after spawning, whereas in Group III (3 specimens), the virus load increased only relatively slightly (approximately a 10-fold increase) and the shrimp survived well beyond spawning. The Group IV (2 specimens) shrimps did not spawn at all during the observation period, and no viral replication was triggered. At this time, it seems clear that spawning stress can trigger WSSV replication, but it is not known why or how this does not occur in some (25%) specimens.

NOTES

Polychaetes not carries of white spot syndrome virus

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Polychaetes are one of the important live feeds for Penaeus monodon broodstock, especially the domesticated ones. Polychaetes rich contents in arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid are believed to help increase fecundity of the broodstock. There has been some concern that polychaetes might contain white spot syndrome virus (WSSV), which could be transmitted to the broodstock and subsequently to the postlarvae. The purpose of this study was to determine if polychaetes could be infected with WSSV and, if so, whether they could transmit the virus to the shrimp. The study was divided into two experiments. In the first experiment, natural polychaetes (Perinereis nuntia)(n = 240), 8-10 cm long, were stocked in four 100 l-aquaria, with 60 polychaetes in each aquarium. At day 0, three polychaetes from each aquarium were examined for the presence of WSSV by using nested and single-step polymerase chain reaction (PCR). Eight out of 12 polychaetes were found to have light positive nested PCR reactions, but all were -ve by the single-step. The polychaetes were fed for one day with fresh shrimp meat from WSSV-infected P. monodon, and randomly sampled for PCR detection of WSSV, as well as for histology, searching for evidence of cellular infection by the virus. At day 1, the nested PCR revealed that 9/12 polychaete were +ve, with two severe and three moderate levels of infection, and the rest were light or very light +ve. At day 4, 11/12 polychaetes were +ve, all of them were light or very light +ve. Similar results were observed at days 7, 10 and 13, with all except one showing light and very light +ve by nested PCR. Under light microscopy, no cells with hypertrophic nuclei typical of WSSV infection were detected in any of the polychaetes studied. From day 13 to day 60, the polychaetes were continually examined for the presence of WSSV by nested PCR, which again showed light or very light reactions. When the +ve PCR polychaetes were fed to healthy shrimp, the shrimp did not develop white-spot disease, which was confirmed by PCR and histology. In the second trial, five WSSV-infected shrimp were stocked together with 80 polychaetes for one week. After all the shrimp died of white-spot disease, as confirmed by PCR and histology, the polychaetes were periodically sampled for 42 days for the nested and single-step PCR. The nested PCR showed +ve cases varying from 3/10 to 9/10, with all light or very light +ve, but all of them were -ve by single-step PCR. Ten shrimp were then stocked in the aquarium with the polychaetes and no mortality was observed after one week, with all the shrimp nested PCR –ve. The two experiments suggested that WSSV that entered the polychaete might not be able to replicate and remained in such a low amount that it could not infect the shrimp.

NOTES

Predicting outbreaks of White Spot Disease in a semi-intensive Penaeus monodon culture system in Karnataka, India

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4. Research Institute For Aquaculture

A longitudinal study of 70 semi-intensive shrimp farms was undertaken in Karnataka, southwest India. White Spot Disease (WSD) was defined as the observation of mortality by farmers and detection of White Spot Syndrome Virus (WSSV) by 1-step PCR in harvested shrimp. Samples were collected from 62 ponds at harvest and of these 37 (59%) fulfilled the case definition. In this system WSD had a significant effect on both the length of production cycle and productivity. Farmers have tried to reduce losses from WSD through avoiding risks and harvesting in the face of an outbreak. However, the information available on which to base such strategies can be misleading. Typical white spots are not highly predictive of a WSD outbreak and in this study neither was the presence of the virus (nested 2 step PCR). Stocking PCR positive post-larvae was not significantly associated with the length of the production cycle, yield, the presence of WSSV at harvest or the risk of WSD. Nor was the presence of WSSV in shrimp from the pond (1- or 2-step PCR) associated with length of production cycle, yield or the risk of WSD. The findings indicate that WSSV and shrimp with WSD histopathology can be present in the pond without progressing to a full outbreak of WSD. However, there were significant associations between WSD and both observation of typical histopathology in dead shrimp from the pond and the pattern of mortalities. The implications of these findings for informing decisions on harvest strategy in various farming systems will be discussed.

NOTES

Session 6 - Shrimp Health 2

White spot syndrome: What we have learned about the virus and the disease

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One of the lessons ultimately learned from the first dramatic outbreaks of white spot syndrome virus (and other shrimp viral diseases) in the early 1990's was that aquaculture management practices needed to be improved. Subsequent research revealed that critical factors included: water quality and sourcing, culture density, broodstock sourcing, postlarva sourcing, screening techniques and strategies, diverse transmission pathways, critical infection levels, disease susceptibility, and stressors. This paper reviews the work done in the last decade by several research groups, and shows how studies on the key aspects of the biology of WSSV infection have led to improved disease management solutions that are now widely used by the shrimp aquaculture industry.

NOTES

Molecular genetics of white spot syndrome virus

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White spot syndrome virus (WSSV) is a major disease agent of penaeid shrimp and other crustaceans. The virus particle has an ovoid-to-bacilliform shape with a tail-like appendage. Its rod-shaped striated nucleocapsid contains a large circular double-stranded DNA molecule with a size of about 300 kbp and is wrapped in an envelope. With this DNA size WSSV is among the largest animal viruses. The virus replicates and assembles in the nucleus of infected cells. Its DNA is further characterized by the presence of multiple regions with homologous repeats dispersed around the genome. Isolates differ primarily in a single region where extensive insertions / deletions have occurred. The viral DNA encodes about 180 open reading frames, most of which have not been described elsewhere in data bases. The largest ORF encodes a putative protein of over 660 kDa. Five major and many minor structural virion proteins have been analyzed and their genes identified. Genes involved in nucleotide metabolism, DNA replication and protein modification have also been found. The WSSV sequence was further analyzed in silico using the computer program MEME and a set of computer scripts for the presence of conserved promoter motifs. This analysis was complemented by 5' RACE analyses of a multitude of ORFs. Transcription initiation sites within the nucleotide sequence TCAYTC for early and TMMTRACM for late transcripts were identified and suggest a unique transcriptional regulation machinery. On the basis of its unique morphological and genetic features WSSV has been accommodated in a new virus family (Nimaviridae).

NOTES

Breeding shrimp for disease resistance: A panacea or pariah

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After the 1992 Taura Syndrome Virus (TSV) epizootic in Ecuador, shrimp farmers in the Western Hemisphere began implementing a variety of strategies to mitigate crop loss from this pathogen, including selective breeding programs to develop TSV-resistant shrimp using between-family selection. Although TSV-resistant shrimp initially improved production and profitability for those farmers who were experiencing a TSV outbreak, genetically improved stocks were not a panacea to the broader disease problems plaguing the industry. In fact, breeding shrimp for resistance to a single viral pathogen, using current selective breeding strategies, may not be the most prudent course of action for the long-term viability of the shrimp farming industry. There are a number of concerns associated with current breeding strategies. Similar to other organisms, there appears to be a trade-off between disease resistance and shrimp growth. Disease-challenge tests typically used to estimate shrimp breeding values are inefficient, and there are concerns that results from laboratory challenge tests may not be predictive of survival in commercial ponds. There are growing concerns about viral mutations, whereby previously resistant shrimp strains may become susceptible to evolving viruses. Effective strategies for selecting disease resistant shrimp do exist, as do biosecurity protocols which exclude specifically listed pathogens from the production environment. However, the decrease in revenue caused by 'living with' shrimp diseases and the increased operating costs due to genetic improvement and/or pathogen exclusion have qualitatively similar effects on profit, and the proper choice between these two strategies should be based on quantitative estimates of their relative cost-effectiveness.

NOTES

B-type virus of Carcinus mediterraneus and WSSV of Penaeid shrimp: Similarities and possible relationships

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2. IFREMER

3. UM2

4. UnimaFrance

Nuclear envelopped rod-shaped viruses were first reported in marine crabs. The first one was found in Carcinus maenas, in 1974, and named B virus. Later, two similar viruses were described in Callinectes sapidus in 1977, named Baculo-B, and a third virus named B2 in 1987 from Carcinus mediterraneus. All the data available on these viruses are based on morphology and morphogenesis. Moreover, they are all located in connective cells and in non circulating hemocytes. We regrouped them under the name of B-type viruses. Since, numerous morphological similarities were noted between these B-type viruses from crabs and the White Spot Syndrome virus from shrimp. Morphologically, in TEM sections, the B-type viruses exhibit similar size and structure as the WSSV. After purification, the two possess a tail-like structure at one extremity of the enveloppe. Their nucleocapsids are asymetric and segmented in appearance. Spacing of the WSSV nucleocapsid segments is the same as that of B2 (20 to 22 nm) but greater than that of the B virus from C. maenas. The number of nucleocapsid segments reported varies from 14 in B2 to 16 in B virus, compared to 16 in WSSV. Many morphological data are available on the ultrastructure, morphogenesis and first steps of cell infection (viral penetration) of the B-type viruses. At the opposite, no data was reported about WSSV early stages of infection. Therefore, we can hypothesize some steps in WSSV development using known data observed in the B-type viruses.

NOTES

Use of WSSV cDNA Microarray for gene profilling during WSSV infection in shrimps.

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White spot syndrome virus (WSSV) is currently a major shrimp pathogen causing high mortality resulting in huge economic losses. Although the entire genome of WSSV, which environs approximately 180 open reading frames (orfs), has been determined, the mode of infectivity is still unknown. We have approached this subject by employing microarray technology which allows one to profile the gene expression patterns of thousands of genes simultaneously under different experimental conditions. We have created clones derived from AluI restricted WSSV genome which theoretically generates about 744 fragments with an average size of 400bp to 500bp. More than 3000 of such fragments have been cloned into pbluescript plasmid and spotted on the microarray thus statistically covering the entire genome. Using fluorescent labelled probes, we compared the gene expression of commercially obtained specific pathogen free and WSSV infected shrimps. We identified genes which displayed expression ratios greater than 2.0 fold. Interestingly, 1% of the analysed data showed over-expression in SPF shrimps which indicates that these shrimps were carrier of the virus. A BLAST search revealed that these differentially regulated genes share similarity to proteins involved in signal transduction and ubiquitination. These exciting findings may shed light on the molecular mechanisms of WSSV-induced mortality.

NOTES

Variations in tandem repeat DNA segments in the ribonucleotide reductase gene of white spot syndrome virus (WSSV) isolates from Vietnam

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White spot syndrome is a viral disease that affects most commercially cultivated marine shrimp species. The disease first emerged in East Asia in 1991 and has since spread throughout most shrimp farming regions of Asia and the Americas. Disease outbreaks usually result in high mortalities in affected ponds. However, shrimp may also be infected chronically with no signs of disease and often obtain the infection in hatcheries from infected broodstock. A wide range of other crustaceans can also act as asymptomatic carriers of infection. In this report, we describe the application of a 54 nucleotide tandem repeat sequence in the WSSV ribonucleotide reductase gene as a strain-specific genetic marker. We use the marker to examine the extent of variation among WSSV isolates from hatcheries different areas of Vietnam and to trace the progression of infection in ponds during grow-out. The analysis of approximately 200 WSSV isolates has shown common variations in number of repeats among these strains, with some broodstock and postlarval batches harbouring more than one genotype. In healthy ponds and in healthy broodstock or postlarval samples collected from hatcheries, viral strains containing 4-, 5-, 6- 7-, 8- and 9-repeat sequence genotypes were detected with no evidence of any predominant genotype. However, amongst ponds sampled during disease outbreaks, the 7-repeat sequence was dominant. Of ten diseased ponds sampled from three provinces in the Mekong Delta, eight were infected with the 7-repeat genotype, one with the 4-repeat genotype and one with the 9-repeat genotype. In one pond in Bac Lieu Province, several genotypes were detected in shrimp sampled at 30 days after stocking but only the 7-repeat genotype was detected in samples collected at 60 days during a disease outbreak. In another pond in Soc Trang Province, the 7-repeat genotype was detected in postlarvae with disease only 4 days after stocking. Studies in progress are investigating the possibility that the 7-repeat sequence is a reliable marker of viral virulence.

NOTES

Characterization of gene expression of biodefense related genes of kuruma shrimp, Penaeus japonicus using real-time PCR technology

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The outbreak of infectious diseases within the shrimp industry cause significant economic losses in all over the world. Characterization of immune system of shrimp is important for development of infectious diseases prevention methods including immunostimulants, probiotics, etc. However, the biodefense and immune related system of shrimp are poorly understood at the molecular level. In view of the above reasons, we used kuruma shrimp, Penaeus japonicus for characterization of defense system in the molecular level. An expressed sequence tag analysis was conducted for discovery of biodefense-related genes of kuruma shrimp haemocytes. We sequenced 635 clones and 370 clones from healthy and white spot syndrome virus (WSSV) infected shrimp cDNA libraries, respectively. Of 635 clones (healthy shrimp), 284 clones (44.7 %) had significant homology to known genes in DNA/protein databases. 174 (47.0%) of 370 clones (WSSV infected shrimp) were significantly matched with sequences in DNA/protein databases. There were 27 EST for immune- and defense-related genes including prophenoloxidase system, antibacterial peptides and protein, membrane associated proteins, and soluble proteins. Expression of biodefense-related genes (prophenoloxidase, 2 types of prophenoloxidaseactivating factor, Masquerade protein, TGase, clottable protein, lysozyme,a-2-macroglobulin and penaeidin) in the kuruma shrimp affected by the oral administration of peptidoglycan (PG), which is an immunostimulant derived from Biofidobacterium thermophilum, were determined at the transcriptional level by quantitative real-time RT-PCR. Results from the real-time RT-PCR suggested that all but not the clottable protein dramatically increased following PG stimulation. Results also suggested that proteins involved with innate defense mechanisms react against pathogens synchronously. Nearly all of the biodefense genes analysed were expressed solely in haemocytes, suggesting the highly significant defensive responsibility of haemocytes.

NOTES

Construction of recombinant Vibrio harveyi to study its adherence and pathogenicity in shrimp larvae

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Gene for green fluorescent protein (gfp) was inserted into a broad-host range plasmid, pBBR1MCS2 to generate recombinant plasmid pWG01. This constructed recombinant plasmid was introduced into a pathogenic Vibrio harveyi M employing triparental mating. V. harveyi M (pWG01) resulted in green-fluorescent colonies due to the expression of gfp. Growth curve analysis showed that the recombinant V.harveyi exhibited almost identical profiles to that of the wild type parental strain. However, after 48 h of incubation in antibiotic-free medium, approximately 50% of the cells had lost their recombinant plasmid carrying gfp. Expression of gfp in V. harveyi and its application for adherence or pathogenicity assays in shrimp larvae will be presented.

NOTES

Recent advances of studies on molecular genetics concerning defense mechanism and control disease in the Chinese shrimp, fenneropenaeus chinesis

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Since 2000, a National Key Fundamental Program ¡ Study on Occurrence and Control of the Major Diseases in Mariculture Organisms¡± have been carried out. Interactions between host, pathogen and environment have been studied. We especially focuses on molecular genetics in the Chinese shrimp to understand defense mechanism and to find new way to control the disease. By sequencing the cDNA library of F. chinensis, 10,446 high quality ESTs have been obtained, then 1373 Contigs/ESTs annotated by BLAST. ESTs matched to known genes were categorized into different functional groups. At least 20 novel gene sequences implied to be related to defense/immunity proteins. Among them antibacterial peptide£¨AP£©and heat shock protein£¨HSP£©are important immunity factors. We successful cloned a novel AP gene and a novel HSP70 gene by RT-PCR and/or 3¡¯-RACE methods. The deduced AP and HSP70 protein have 71 and 652 amino acids respectively. Furthermore using proteomics technology the antibacterial peptide has been purified by Sep-Pak C18 extraction, reverse-phase HPLC, and native gel electrophoresis. The bioassay demonstrated that the AP was active against both gram-positive and -negative bacterial. Effects of various stressors on the expression of HSP70 in various tissues such as muscle, hepatopancreas, eyestalk, hemolymph, and gills were studied. To make Marked-Assist Selection, microsatellite sequences were isolated by PCR and/or from ESTs. This presentation reports preliminary results on transgenic experiments in shrimp by ribozyme gene of the WSSV with a reported GFP gene too.

NOTES

A hypothetical model for VHML bacteriophage conversion of vibrio harveyi

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2. Department Of Microbiological And Immunology, James Cook University

The bacteriophage VHML (Vibrio harveyi Myovirus Like) originates from a toxin-producing strain of Vibrio harveyi. It has been demonstrated previously that the presence of the VHML prophage can induce virulence to V. harveyi in the laboratory. Here, a hypothetical model for the mode of action of the phage virulence conversion of V. harveyi will be presented. Through the nucleotide sequence determination of the entire VHML genome (43,193 bp), we have identified the putative structural genes of the phage virion and these were consistent with the physical characteristics of the virions as observed by TEM. We have also identified putative genes consistent with integration of the genome, supporting the theory that VHML integrates the host V. harveyi genome as a prophage. In addition, based upon nucleotide sequence of the phage DNA, we have identified a potential toxin gene on the VHML genome. This gene includes DNA sequence that is similar to the reported active site of the ADP-ribosylating group of toxins. These ADP-RT's include also toxins from other bacteria that have been previously reported to be a result of bacteriophage conversion. This presentation will illustrate how the genes upon the phage genome could cause infection of the Vibrio harveyi host cells, integration of the phage genome into the hosts' chromosome and subsequent production of the potential toxin, thereby conferring virulence to Vibrio harveyi.

NOTES

Co-detection and differentiation of yellow head complex viruses using monoclonal antibodies

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Three monoclonal antibodies (MAb) raised against yellow head virus from Thailand (YHV) were tested against tissues of shrimp from Thailand, Australia, Eduador and India purported to be infected with viruses in the yellow head virus complex. As a preliminary step, the site of reactivity of the 3 MAb in YHV nucleocapsids and mature virions was also determined by immuno-electron microscopy using ultra thin sections of YHV-infected shrimp tissue and negatively stained, semi-purified YHV particles. With MAb Y-19, gold particles deposited on YHV nucleocapsids and the inner core of mature, enveloped virions in ultra-thin sections but not on negatively-stained YHV particles, confirming its capsid protein specificity. With MAb V-3-2B, gold particles deposited on the surface of negatively stained YHV particles, confirming its specificity to the 135 kDa YHV structural glycoprotein. MAb Y-18 did not react with YHV particles either in sections or in semi-purified preparations. Immuno-histochemistry using Penaeus monodon infected with either YHV or GAV showed that MAb Y-19 and Y-18 reacted with both while MAb V-3-2B reacted with YHV only. All these tissue samples also gave positive in situ hybridization reactions with a cDNA probe specific to the ORF1b gene of YHV. They also gave the expected differential RT-PCR results for YHV and GAV. By contrast, 2 natural Thai shrimp specimens with no gross signs of disease gave positive immuno-histochemical reactions with MAb Y-19 and Y-18 but not with MAb V-3-2B. Although they also gave positive in situ hybridization reactions, their RT-PCR products were suggestive of GAV. Indeed, sequencing of the RT-PCR products showed that they shared 90% identity to GAV but only 80% identity to YHV. Specimens from Eduador and India that displayed histopathology suggestive of YHV infection gave negative immunohistochemical reactions with all 3 MAb and negative in situ hybridization results. Additional work is required to determine whether a virus from the yellow head complex was responsible for the observed histopathology. These preliminary data suggest that the 3 YHV MAb could be used in diagnostic situations to identify and differentiate some viruses in the yellow head virus complex.

NOTES

Molecular approach to the identification of virulence genes involved in Edwardsiella tarda pathogenesis

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2. Tropical Marine Science Institute

Edwardsiella tarda causes systemic infection in fish and gastro- and extra-intestinal infections in humans. Survival and replication within phagocytes and in fish are the two in vitro and in vivo infection models we use to examine bacterial virulence. A genome-wide analysis to identify the genes responsible for the above properties in E. tarda was carried out using TnphoA transposon mutants. We obtained 5 mutants able to stimulate reactive oxygen intermediate production by phagocytes and 15 mutants that are attenuated in fish. Characterization of these mutants resulted in identification of 17 different genes required for bacterial virulence. Distribution of these genes in pathogenic and non-pathogenic E. tarda indicated that some of them were specific to virulent strains and few were also present in other pathogenic enteric bacteria such as Salmonella and Vibrio species. Further characterization of genes such as katB and pstSCB indicated their respective role in E. tarda pathogenesis. This study would not only help in understanding the pathogenesis but also to develop diagnostic kits for rapid identification of pathogenic E. tarda.

NOTES

Session 8 - Immunology

Innate immunity in vertebrates and invertebrates

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Two systems providing internal defence against infectious agents have been selected during evolution: the innate (natural) and the acquired (adaptive) immune system. The innate immune system can be found in all multicellular animals (both invertebrates and vertebrates) and consists of cellular and humoral elements. The most prominent cellular defence reactions against invading micro-organisms are phagocytosis, encapsulation, non-specific cytotoxicity and clotting. The humoral defence factors, such as clotting proteins, agglutinins, proteases, anti-proteases and anti-microbial peptides are often produced by the defence cells. The acquired immune system is phylogenetically younger and is found exclusively in vertebrates, including fish. The acquired response is characterised by specificity and memory formation, but is usually slower than the innate response. Both responses must start with the recognition of invading micro-organisms. The innate defence system presumably recognises molecular patterns shared by large groups of pathogens, rather than specific structures. The important role of the subsequent innate response will be illustrated by data derived from functional studies using haemocytes from black tiger shrimp (Penaeus monodon) and macrophages or granulocytes from common carp (Cyprinus carpio).

NOTES

Vaccine development for Asian aquaculture

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1. Intervet Norbio Singapore

Although more than 90% of the world's aquaculture takes place in Asia, less than 10% of the vaccine sales in aquaculture are accounted for in Asia. The reason for this marked discrepancy is not that Asian aquaculture is a recent development; in fact it could be considered as one of the pioneers with in aquaculture. It is also not because the species cultured are free from diseases. In general, the development of effective vaccines and vaccination strategies follow the development and intensification of the industry. Prerequisites for vaccine development are: 1) intensified production of a fish species with optimized management practices; 2) identification of the disease causing agents, 3) an understanding of the epidemiology of the disease, the available windows for vaccination and duration of protection needed, 4) knowledge of the immune development of the fish species and 5) a vaccine that ultimately will profit both the fish farmer and the vaccine company. Asian aquaculture is still thought of as a hit and run operation with regard to the fish species produced. Farms are mostly small, low-cost, family-owned operations. Some disease causing agents are described but comparative studies between isolates from different countries and different fish species are lacking. Epidemiology data are generally missing as are basic data on the immune system of Asian fish species. However, several universities and private companies are recently engaging in these fields and it is clear that vaccines will soon become increasingly available in Asia. The present status of 'vaccines for Asia' will be discussed with reference to specific examples.

NOTES

Immunostimulants Induced Immunity and its Quantification in Tiger Shrimp, Penaeus monodon (Fabrics) through ' Challenge ' and ' Pro-po Assay '

Felix Sugantham [1]*

1. Fisheries College and Research Institute

While immunostimulants play an important role in shrimps, their relative efficiency in terms of enhancing immunity in shrimps is still not understood. This study aims to test the relative efficiency of immunostimulants viz., ß-1,3-Glucan and Lipopolysaccharide (LPS) on the giant black tiger shrimp, Penaeus monodon by incorporating immunostimulants in the basal shrimp diet. Glucan at 10, 20, 40 and 60 mg/kg levels and Lipopolysaccharide at 10, 20, 30 and 40 mg/kg levels for 10 days treatment were attempted. The challenge study and 'pro-PO activity' (in haemocytes and plasma) were carried out using Vibrio parahaemolyticus and Microplate Reader (ELISA Reader) respectively. The maximum immune enhancement (in challenge and pro-PO) was recorded for Glucan - 10 and LPS - 30 mg/kg followed by LPS - 10, LPS - 20, Glucan - 20 and Glucan - 40 mg/kg of feed. Further, all other doses except Glucan - 60 showed higher immune enhancement (in challenge and pro-PO) than that of control.

NOTES

Immunological properties of the phygocytosis and serum lectin in Scaphara subcrenata

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2. Chinese Academy of Fishery Science

Bivalves do not possess an acquired immunity equivalent to that of vertebrates. Innate immunity reaction, including phagocytic, cytotoxic action or inflammatory responses by the circulating cells, some lysosomal enzymes, lytic and cytotoxic molecules in the serum plays an important role in the defense mechanism of bivalves. Scaphara subcrenata is a kind of bivalve distributed broadly in the coast of China, Korea and Japan. In this paper, the phygocytic reaction of the haemocytes, the properties of the serum lectin and effects of challenge by vibrio sp. on the immune activities were reported. 1. Properties of lectin Lectin was shown here to exist in the serum of Scaphara subcrenata. The lectin showed an agglutinating activity against human A B O, chicken and mouse erythrocytes, the agglutinating titers were 256,256,8,16,64 respectively. The serum of Scaphara subcrenata could also agglutinate microorganisms, such as Candida albicans, Saccharomyces cerevisiae, Vibrio alginolyticus. The experiment showed that the lectin was stable at a wide range of temperature and pH degree. The activity of the lectin could be inhibited by L-rhamnose, L-arabinose, maltose, D-galactose and sucrose. When the animals were challenged by vibrio, the agglutinating titers of the serum increased markedly and could keep the superiority within 13 days after injection. 2 Phygocytic properties of the haemocytes Flow cytometry and optical microscope were used to study the classification of the haemocytes. It was proved that there were three main types of haemocyte. (1) Erythrocyte with the size of 18.4±0.97mm is dominant type in the blood. (2) Hyaline leukocyte, 5.63±1.49mm, possesses a nucleus heavily stained and no granule in the cytoplasm. (3) Granulocyte, possesses optical microscope-visible granules in the cytoplasm, which could be stained into different colors with wright's dye: one is red with the size of 8±1.06mm; and the other is blue, 6.3±1.25mm. There is also another kind of haemocyte, 5.17±1.69mm, with a nearly naked nucleus, which is very little in the hemolymph. The experimental results showed that haemocytes of Scaphara subcrenata could phagocytize Candida albicans cells in vitro. The optimum temperature of phagocytosis was 30 C, and the phagocytosis rate attained 62.11%. While the bivalve was challenged by Vibrio anguillirum, the phagocytic activity of the haemocytes increased dramatically, which was much higher than that of injected by physiological saline. Then the phagocytic activity decreased, and turned to be close to the level of control at 72h after injection.

NOTES

Vaccination against white spot syndrome virus in shrimp

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1. Wageningen University

Since the 1990's shrimp culture has been hampered by mass mortalities in ponds throughout the world. Penaeid shrimp are affected by many infectious agents, mainly of bacterial and viral origin. Especially white spot syndrome virus (WSSV) has had a major impact on shrimp culture since its discovery in 1992 and remains a problem up to the present day. WSSV has a broad host range including all cultured shrimp species, but also other crustaceans like crab and crayfish. No adequate measures to control WSSV, other than rigorous sanitation and adequate chain management practices, are available yet. Due to the current aquaculture practices this is not a viable option in the foreseeable future. Therefore, alternative strategies such as vaccination or other intervention strategies need to be explored. However, the potential of shrimp vaccination is equivocal, as the immune system of crustaceans has not been studied in great detail. Many parallels have been identified between the innate immunity in vertebrates and invertebrates, whereas an adaptive immunity seems to be restricted to vertebrates. Nevertheless, quasi-immune responses have been observed in shrimp upon WSSV infection, resulting in increased protection. Recent research on the molecular characteristics of this virus resulted in the identification of the major structural proteins of the virion. For one of the structural proteins vaccination experiments have showed it to have potential use as a subunit vaccine. The correct formulation for the structural WSSV proteins and the immunological background for the protection obtained in shrimp is currently subject to further investigation.

NOTES

Session 9 - Probiotics and therapeutics

Probiotic bacteria: Are they beneficial?

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1. Chulalongkorn University

Probiotic bacteria are now widely used as prophylactic treatments with poultry, swine, and other land animals for protection against pathogenic microorganism. More recently, probiotics have been developed for aquaculture crops, but are still not widely used in aquaculture. There are, however, substantial advantages for probiotic use with shrimp and fish culture. These benefits include protection against bacterial pathogens for which there are no medications. Probiotics also do not create chemical residues that can taint animals intended for human consumption. We have demonstrated the live weight gain and more survival on Penaeus monodon after fed a probiont as a feed supplement and their resistance to luminescent disease infection by challenge tests including the greater immune response compared with control shrimp not fed probiotic bacterium in laboratory tanks. Possible transferring a probiont via artemia by bioencapsulation for nursing P. monodon larvae in hatchery was confirmed and more effective benefits on post larvae shrimp were obtained. The assessment of our probiont to earthen pond settings more similar to commercial growthout of P. monodon was extensively studied. Survival and growth of shrimp fed probiotic feeds were significantly greater . Disease challlenge tests confirmed that probiotic feed could delay disease onset and reduce disease severity.

NOTES

Use of microbial technology to improve farm results in shrimp farms in Ecuador

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2. Bravito SA, Machala, Ecuador
3. Marecuador SA, Machala, Ecuador
4. Acuabiotec LLC, Brisbane, Australia
5. Acuabiotec LLC, Milwaukee, USA

It has been a difficult struggle for Ecuadorian shrimp farmers since 2000, attempting to recover production levels since the devastating effects resulting from the spread of WSSV. However WSSV has been only one of Ecuador's disease problems. Bacterial diseases have always been present and important in Ecuadorian shrimp culture. Overcoming bacterial disease problems, using sustainable technology, is one of the major keys to producing in the presence of serious viral problems.

Coupled with improved pond management, the administration of species of *Bacillus*, used in culture water for juveniles, and applied to feed throughout the culture cycle, has been effective in controlling bacterial diseases without requiring the application of antibacterials. *Bacillus* isolates, resembling the administered strains in colonial morphology, have been re-isolated from shrimp mid-gut and hepatopancreas in concentrations sufficient to competitively inhibit pathogenic bacteria such as *Vibrio species*.

The experiences of several Ecuadorian producers will be presented, comprising more than 1000 hectares of extensive ponds. These producers stopped using antibiotics between February to July 2001 and have now produced four cycles using solely microbial technology for bacterial control. Their production has increased more than 200% since July 2001, reaching pre-WSSV levels. Key factors involved in applying microbial technology have been the control of feeding and method of stocking of juveniles, the bacterial concentration (not less than 10^8 CFU/g) of administered *Bacillus* in feed pellets, and continual monitoring of the population of shrimp in order to augment bacterial doses during times of environmental stress or viral challenge.

NOTES

Biocontrol of Bacterial Pathogens In Aquaculture With Emphasis on Phage Therapy

Karunasagar Indrani [1], Karunasagar Iddya [1]*

1. University of Agricultural Sciences, College of Fisheries

One of the major constraints for the development of aquaculture is the mortality due to diseases which affect both hatcheries and farms. Presently to control diseases a numbeer of antibiotics, sanitisers and other chemicals are being used by the aquaculture industry. However, these have adverse environmental effects and emergence of antibiotic resistance, persistance of chemical residues in animal tissue are the major problems. In this context, biological control of pathogens would be a very useful strategy to prevent diseases. In this paper, our experience in controlling the major shrimp pathogen, Vibrio harveyi which causes luminous bacterial diseases in hatcheries and farms is described. Some aquatic bacteria such as Pseudomonas and Bacillus have been found to produce anti-Vibrio compounds.In the case of Pseudomonas, the compound has been found to be chloroform soluble and present in the culture supernatant. In seawater, both these bacteria were observed to reduce the levels of Vibrio harveyi. Bacteriophages capable of lysing several V. harveyi strains have been isolated from shrimp culture environments. These bacteriophages have been found to be very efficient in reducing levels of V. harveyi in water. Ability of bacteriophages to control luminous bacterial diseases in shrimp hatcheries were tried. Bacteriophage treatment was observed to reduce luminous bacterial counts in water and in larvae and tremendously improved larval survival. Bacteriophages acting on other vibrio pathogens in aquaculture such as V. alginolyticus, V. parahaemolyticus and V. vulnificus have also been isolated and found effective in reducing the respective vibrio numbers in microcosms. The results show promise. These results suggest that biocontrol of pathogens would be very environment friendly approach to manage disease problems in aquaculture.

NOTES

Treatment of Bacillary necrosis of larval Pacific oyster Crassostrea gigas with bacteriophages

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2. Hiroshima Prefectural Farming Fisheries Association

Bacteriophages (phages) can be therapeutic and prophylactic agents for bacterial diseases in aquaculture as well as in other fields. The feasibility in scientific demonstration of the causal sequence distinguishes phage-therapy or phage-control from other biological controls like probiotics. Here we report on successful phage treatment of bacillary necrosis in triploid larvae of Pacific oyster Crassostrea gigas under laboratory conditions. In larval production at the HPFFA hatchery, mortality occurs regularly in 2- to 8-day-old larvae and Vibrio splendidus biovar II was identified as one of the causative agent of the disease. Lytic phages (Podoviridae) specific to virulent strains of V. splendidus were isolated from adult oyster or the rearing water by enrichment and double-agar-layer techniques. Phages significantly lowered mortalities of 5-day-old triploid larvae, which were challenged experimentally with virulent strains of V. splendidus in 6-well-tissue culture plates. Inoculation of phages at 106 to 107 PFU/ml was effective to prevent the infection of V. splendidus at 105 CFU/ml. A similar protective effect of phage was also observed when 0-day-old larvae were exposed to naturally-occurring bacterial infection in a small scale (2-L beaker). Mortality of larvae in the control group without phage treatment was 99% while that in the experimental group with phage treatment was 32%. This was equivalent to the effect of streptomycin (mortality: 26%). These results suggest that it may be possible to use phage to control the bacterial infection in oyster larvae culture, though the efficacy on the production scale remain for the future study.

NOTES

The probiotic potential of Vibrio alginolyticus (Val 1) in the oyster hatchery

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1. University Of Technology, Sydney

The present study investigated the probiotic potential of a strain of Vibrio alginolyticus (Val 1), for use in oyster hatcheries. The results found that rearing Sydney Rock Oyster (Saccostrea glomerata) larvae in autoclaved seawater and in the presence of V. alginolyticus improved larval growth and survival by 15µm and 40% respectively, than when compared to larvae reared in 1µm filtered seawater with the absence of V. alginolyticus. Larval survival and growth were also compared for different seawater treatment, with and without V. alginolyticus. The treatments were 1µm filtered; 0.2µm filtered; activated carbon filtration; pasteurisation at 65oC and 85oC; chlorination; U.V.; and chlorination followed by activated carbon filtration. The results showed larval growth and survival in 1µm-filtered seawater without V. alginolyticus were higher than the larvae reared in the other seawater treatments, irrespective of the presence or absence of V. alginolyticus. This suggests that in the absence of an outbreak, treatment of seawater and the presence of V. alginolyticus provided no beneficial effect on the growth and survival of S. glomerata larvae. When S. glomerata larvae reared in 1µm-filtered seawater were subjected to a challenge with the pathogen Vibrio tubiashii, larval survival decreased from 62% in the unchallenged control to 8% in the challenged control. In contrast, larval reared in the presence of V. alginolyticus challenged with V. tubiashii demonstrated 35% improvement in survival to that of the challenged control. Thus, indicating a substantial beneficial effect. These findings indicate the potential of V. alginolyticus as a probiotic for the prevention of bacterial epidemics in oyster hatcheries.

NOTES

Antagonistic activity of Aeromonas media strain A199 against Saprolegnia sp. in two species of finfish, the eel Anguilla australis and silver perch Bidyanus bidyanus

Lategan Josie [1]*

1. University Of Technology Sydney

Antagonistic activity of Aeromonas media strain A199 against Saprolegnia sp. in two species of finfish, the eel Anguilla australis Richardson and silver perch Bidyanus bidyanus Mitchell. Aeromonas media strain UTS A199, an organism isolated from fresh water is a potential probiotic for use in the aquaculture industry against Saprolegnia sp, the oomycete associated with "winter kill", environmental stresses and farming practices that may cause the loss of fish stock. Aeromonas media produces a bacteriocin-like inhibitory substance (BLIS) that has shown a wide spectrum of in vitro antagonistic activity against various fish pathogens. In this study, BLIS inhibited in vitro the growth of Saprolegnia sp. isolated from affected fish. Inhibition was observed against both the vegetative state of the aquatic mould and cyst germination. A number of separate in vivo tank observations involving affected eels revealed that the daily addition of A199 to tank water contributed to the subsequent swift recovery of affected hosts from invasion by this opportunistic pathogen. While, in a small scale, pilot trial, to test the effectiveness of A199 during an outbreak of saprolegniosis, silver perch displaying early lesions of the disease were maintained in tank water containing A199 for a period of 3 weeks. Eleven percent (11%) accumulated mortality was observed for fish exposed to A199 for the three-week treatment, increasing to thirty-three percent (33%) in the week post treatment. In contrast, 77%-accumulated mortality was obtained for fish not exposed to A199, with the majority of saprolegniosis related deaths occurring during the first two weeks of the study. The discovery of both in vitro and in vivo antifungal activity by A. media appears to be a promising novel approach in the use of probiotics for the management of the opportunistic oomycetes that lead to the rapid death of affected fish.

NOTES

Session 10 - Mollusc Health 1

Perkinsus disease in Korean waters; taxonomy, distribution, diagnostics and their effects on clam ecology

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Perkinsosis is a shellfish disease caused by parasitic protozoan in some commercially important mollusks including oysters, clams, abalones and scallops. Heavy infection with Perkinsus often results in mass mortalities and commercial loss. Currently Perkinsus is classified as a molluscan disease in the OIE. In Korean waters, Perkinsus sp. has been found in the Manila clams, Ruditapes philippinarum. Perkinsus is also believed to be responsible for the decline in clam landings for the past decade in Korea. Perkinsus trophozoites were distributed commonly in gills, digestive glands, mantle and gonadal connective tissues. They were relatively scarce in foot and siphons. Heavy infection with Perkisus often caused white nodule formation on gills and mantle, as well as massive concentration of haemocytes around the infected tissues. Microscopic features of different life stages and 5.8S rRNA nucleotide sequences read from the non-transcribed spacer and internal transcribed spacer indicated that Perkinsus sp. discovered in the clam in Korea could be P. atlanticus in European waters. Ray's fluid thioglycollate medium (RFTM) with Choi's NaOH lysis method was used in identification of Perkinsus in the clams. The prevalence was mostly 80 to 100% in commercial clam beds on the west and south coast of Korea. The infection intensity was found to be highest in October when most clams completed spawning and mass mortalities where observed in the fields. Quantification of clam eggs using enzyme-linked immunosorbent assay with rabbit anti-clam eggs protein IgG demonstrated that the amount of eggs produced during spawning was negatively correlated with infection intensity of Perkinsus. In conclusion, high level of Perkinsus infection in the clam could precipitate reduced growth and reproduction, as well as mass mortalities in the field, resulting in decreases in clam harvesting in Korean and possibly in other Asian waters.

NOTES

Diseases of pearl oysters

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The aquaculture of pearl oysters is an expanding multi-million dollar industry in the tropical marine environment of many countries, including Australia, French Polynesia, the Middle East, SouthEast Asia and Japan. Despite the size and extent of the industry there is remarkably little known about the diseases and parasites of the genus Pinctada. There is a growing awareness among the industry that, as with other molluscs under cultivation, disease can be an important constraint and that translocation of shellfish poses a serious risk. This paper will review known diseases caused by pathogenic agents as well as those with a non-infectious aetiology. Management techniques, which can be used to minimise the impact of disease, will also be discussed.

NOTES

Report on oyster mortality in Wonboyn Lake, Australia

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2. University Of Tasmania

In March 2002, a major mortality of Sydney rock oyster (Saccostrea glomerata) was reported on aquaculture leases in Wonboyn Lake (latitude 37o south) in southern NSW. This paper describes the investigations surrounding the mortality event and the subsequent conclusions. Gross lesions in the stomach epithelium of oysters was observed. The dinoflagellate Prorocentrum minimum has been implicated, together with a changing ecology in the lake resulting from a restricted sea entrance due to sand accretion. The strategy for ongoing management and monitoring for the oyster industry in Wonboyn Lake and potentially elsewhere is discussed.

NOTES

Grouper hatchery health in the APEC region: surveys of hatcheries and nurseries and regional experts and institutions involved in grouper health

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An APEC Fisheries Working Group workshop, entitled "Development of a Regional Research Program on Grouper Virus Transmission and Vaccine Development", was held in Bangkok in October 2000. To assist in implementing some of the workshop's recommendations, four surveys were prepared at the Network of Aquaculture Centres of Asia-Pacific (NACA) and the Aquatic Animal Health Research Institute (AAHRI) of Thailand. Questionnaires were designed to assess the impacts of diseases among grouper hatcheries and nurseries, and to inventory the scientists and laboratories working on grouper diseases in the region, to help pinpoint expertise and capacity for contribution to the proposed Collaborative Resource Centre. Surveys were distributed to representatives of 13 APEC economies involved in the production of groupers. Four respondents to the hatchery and nursery health questionnaire listed 7 cases of disease in the last 12 months in 4 countries. These included one diagnosed and two suspected cases of infection with a nodavirus (VNN), one occurrence of vibriosis, one case of leech infestation, and a disease of unknown aetiology characterized by deformed skeletons and floating larvae. A further two respondents reported no diseases among larvae in their hatcheries. These responses will be accessible in EpiInfo databases.

NOTES

Breeding for QX disease martelia sydneyi resistance in Sydney rock oysters saccostrea glomerata

Nell John [1], Hand Rosalind [1]*

1. NSW Fisheries

The Sydney rock oyster, Saccostrea glomerata, breeding program was established by NSW Fisheries in 1990 to provide the industry with a faster growing oyster. The Georges River component of the breeding program was severely interrupted by the appearance of QX disease in this estuary in 1994. Selection for QX disease resistance Marteilia sydneyi in Sydney rock oysters, in Georges River commenced in 1997. Wherever QX disease has appeared, the local oyster industry has severely declined, slowly in three northern NSW rivers, taking 30 years for a 56% decline in production, but quickly in Georges River, Sydney, taking only 7 years after the parasite was detected for a 97% decline in production. Selection for QX disease resistance is important in case yet another estuary should suffer this fate. The progeny of second generation Sydney rock oyster breeding lines were tested for resistance to QX disease Martelia sydneyi against a non-selected control. Mortality was reduced from 86% for the controls to 64% for the most improved breeding line. This is a reduction in mortality of 22% after only two generations of selection. These partially QX disease resistant oysters in which M. sydneyi was found were also 21% heavier than controls. Selection for resistance to M. sydneyi is feasible and may be improved through further selection.

NOTES

Session 11 - Mollusc Health 2

Diseases in mollusc hatcheries: A paradox in health management

*Berthe Franck CJ [1]**

1. IFREMER

The world aquaculture of molluscs is still increasing, reaching 10.73 millions of tonnes in 2000. Among the 42 cultivated mollusc species, 5 species are dominating the global production, which the Pacific cupped oyster, Crassostrea gigas, is leading. In many countries, there is a traditional mollusc aquaculture, usually based on wild stocks. However, these natural populations often do not fulfil the market demand - because of their poor value, over-fishing of the resource or impact of diseases - and an answer to this has frequently been the introduction of new stocks or new species. Furthermore, genetic improvements, availability of juveniles, as well as species diversification for aquaculture do increase the demand for international movements and transfers of live molluscs. Diseases have become a primary constraint to mollusc aquaculture growth and sustainability, given their severe impact on socio-economic development in many countries. Diseases are a major threat to aquaculture, natural resources alike. Pathogen transfers via transfers of live molluscs are currently recognised as a major cause of disease outbreaks and epizootic. In this prospect, hatchery production may be seen as a way to provide disease-free batches of juveniles and therefore a pivotal tool in effective programs to prevent the transfer of infected stocks in disease free areas. On the other hand, several diseases are known to occur in hatcheries that could be disseminated with release of hatchery products in grow-out areas. After reviewing the importance of hatcheries of molluscs and molluscs diseases in hatcheries, their paradox in health management approach will be discussed.

NOTES

Survey on the ovarian parasite, marateilioides chungmuensis in the cultured Pacific oysters Crassostrea gigas in Korea

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In Korea, oyster culture began in early 1900's and commercial oyster culture using hanging culture system started in 1960's in the southern coast. After 1960's, production amount of cultured oyster has been increased every year until late 1980's and the highest production amount as shellstock was recorded 288,078 metric ton in 1987. But increment of the production amount in 1990's has shown a stagnant status. Production amount recorded 174,117 metric ton as shellstock in 2001. From 1990's, oyster culture industry in Korea has faced hard circumstances because of insufficient seed collection and mass mortality of the cultured oyster. National Fisheries Research and Development Institute (NFRDI) presumed contaminants from inland side, self contamination in the growing area and recessivation of broodstock induced the results. The ovarian parasite of oyster has been supposed a cause of the bad seed collection and mass mortality. In Korea, the parasite was reported first time in 1970 and then presumed belonged to Acanthamoaba (Chun, 1979). Comps et al. (1986) performed morphological identification of the parasite isolated from the Pacific oyster collected at Chungmu area in Korea and named the parasite Marteilolioides chungmuerisis. The infection rate of M. chungmuensis in Korea has been increased every year and appearance period of the parasite also also has extended from spawning season to all the year round. The regulatory authorities such as Minister of Maritime Affairs & Fisheries (MOMAF) and provincial government have performed growing area cleaning project for a better condition keeoing. And also NFRDI recommend oyster industry triploid oyster that is not infected by the parasite.

NOTES

Marteilioides chungmuensis (paramyxea), an intracellular parasite of the ovocyte of Pacific oyster Crassostrea gigas: isolation and sequencing of small subunit ribosomal DNA

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Marteilioides chungmuensis is the intracellular parasite of the ovocyte of Pacific oyster Crassostrea gigas in Japan, causing irregular enlargment of the infected ovary. Infected oysters lose their marketability due to the unaesthetic appearance. Although the parasite causes a serious economical impact on oyster fisheries, biological aspects of the parasite including the infection route, mutiplication stages and early infective stage remain unknown. Recently, molecular biological techniques have become powerful tools to detect microorganisms or discover unknown stages of parasites. We developed a molecular detection method for M. chungmuensis using parasite DNA as follows. Infected gonads were frozen at -20oC to rupture parasitised ovocytes and homogenized with glass homogenizer. Sporonts were collected with nylon meshes to remove host cells and debris, and purified with discontinuous sucrose and Percoll gradients. Further, we extracted parasite DNA from the sporonts and sequenced partial 18s small subunit ribosomal DNA (ca. 1200 bp). Two specfic probes were desinged based on the sequence, and in situ hybridization was applied on histological sections. Positive signals were recognized only on parasite cells, confirming the sequenced DNA derived from M. chungmuensis. These results provide basic molecular tools which would be helpful to elucidate the life cycle and phylogenetical position of this economically important parasite.

NOTES

Epizootiology and detection of nocardiosis in oysters

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Nocardiosis is a bacterial disease of oysters caused by Nocardia crassostreae. This disease has many common names, some of which describe its pathology, including: fatal inflammatory bacteraemia, focal necrosis, multiple abscesses and Pacific oyster nocardiosis (PON). Infection with N. crassostreae induces a massive accumulation of haemocytes resulting in the formation of green coloured lesions or pustules up to 1 cm in diameter in the mantle, gill, adductor muscle, and heart of oysters. However, histological evidence suggests that some oysters are capable of ridding themselves of infection by the process of diapedesis (haemocyte migration through intact epithelium). First described as a fatal disease among Pacific oysters (Crassostrea gigas) in Matsushima Bay, Japan in the1960s, it has since been reported from various locations along the west coast of North America but the true geographic distribution is not known. Recent investigations in British Columbia clearly indicate that this bacterium is also pathogenic and lethal to flat oysters (Ostrea edulis). Infection and mortalities seem to be highest among beach cultured oysters, especially those on a muddy substrate. However, other environmental factors such as reduced water circulation in shallow embayments and warm temperatures also increase the prevalence of infection and severity of nocardiosis. Although eradication of nocardiosis is not feasible, off-bottom culture seems to mitigate the disease. The potential negative impact of this disease on oyster culture around the world warrants further investigations and precautions against the transplantation of infected oysters. A polymerase chain reaction (PCR) assay for management and study of nocardiosis is currently undergoing validation.

NOTES

Diseases of cultured paua (Haliotis iris) in New Zealand

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Mortalities of cultured paua (Haliotis iris) in New Zealand have been associated with a variety of disease agents. During the summers of 1999/2000 and 2000/2001, mortalities of between 82.5 and 90% of juvenile paua (Haliotis iris) were reported in a commercial culture facility. Affected paua exhibited behavioural abnormalities including lethargy, loss of righting reflex, and easy detachment from surfaces. Histology of moribund paua showed heavy infections of a novel haplosporidian. Laboratory experiments failed to transmit the haplosporidian horizontally by cohabitiation or by injection of healthy paua with hemolymph containing haplosporidian plasmodia. Spore formation was not observed in juveniles, but sporocyst-like bodies containing acid-fast putative spores were observed in the right kidney of poorly performing adult paua collected from the wild. The epidemiology of the haplosporidian disease in affected culture facilities remains poorly understood. A survey of 1094 paua collected from 5 spat producing farms and 3 grow out farms during the summer of 2001/2002 failed to detect the haplosporidian. A number of other potential disease agents and syndromes were detected, however, including rickettsial inclusions in the gut, haemocytic neoplasia, granuloma-like lesions in internal organs, and erosion of external epithelia associated with bacterial infection and ectocommensal ciliates. Other disease agents which affect the shell of cultured paua include a mycosis and mudworm infestation. The possible effects of these disease agents on paua farming in New Zealand are discussed.

NOTES

Discovery of the early infective stages of the protozoan parasite Marteilia sydneyi in oysters and the implications for disease detection and control

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Marteilia sydneyi, the aetiological agent of QX disease, causes persistent mortalities in commercial rock oysters on the east coast of Australia. In order to diagnose QX in oyster tissue, the OIE recommends various examination procedures involving surveillance, presumptive and confirmatory techniques. Specifically: histological examination is considered the most suitable technique for surveillance of disease; tissue imprints allow rapid presumptive analysis of disease following mass mortality outbreaks, and; electron microscopy and in situ hybridisation are often required for species identification following detection by the aforementioned means. However, most of these techniques have relied on the presence of sporulating stages residing in the digestive gland of the oyster host, which, until recently, were the only lifecycle stages known. The discovery and characterisation of the initial infective stages of M. sydneyi in the gills and palps of oysters, as well as presporulating stages in the digestive gland and connective tissue, has not only presented new opportunities for disease diagnosis and detection in Marteilia species but has also identified limitations in some existing methods. This paper discusses the possibility of improved protocols, as well as the value of existing protocols, in the surveillance, monitoring and species confirmation of M. sydneyi in light of these new data.

NOTES

Limitations to preventing increased international distribution of aquatic animal diseases

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The belief that serious diseases of aquatic animals have been, or could be, introduced into their territory from abroad has led some countries to impose strict controls on imports of live and dead aquatic species. Although national quarantine and health certification requirements for imports are certainly a valid part of first line defence against introduction of exotic diseases, they must be developed within the context of international standards (WTO SPS Agreement and the OIE Code and Manual) and should not be used as an unjustified barrier to competitive trade from other countries. Even so, it is important also to recognise that such legal import safeguards alone may not necessarily prevent the sudden appearance of a serious disease in a country from which it was previously believed to be absent. Reasons include failure of an import risk analysis, ineffective surveillance for the disease/pathogen in the exporting country and inadequate enforcement of the import restrictions (e.g. illegal imports). Furthermore, there are other possible pathways for pathogen introduction than just the importation of live or dead aquatic animals (or their products for human consumption) e.g. dead wild fish as fresh feed for farmed fish or as fishing bait, live fish transport vehicles that have been used in other countries and possibly ships' ballast water. Finally, rather than recent importation of the pathogen being the cause of a first-time occurrence of an 'exotic' disease, there is the possibility of the emergence of a more virulent strain of the agent that has existed benignly in a local reservoir without previously being detected. This is particularly relevant to marine diseases and the author will present some possible examples.

NOTES

Ornamental disease vectors

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The ornamental fish industry transfers large quantities of fish between many countries around the world thereby presenting significant potential for trans-boundary transfer of disease agents. Although tropical ornamental fish are usually kept in indoor aquaria and are unlikely to survive for long if released into the natural environment in temperate regions, they are capable of carrying viruses that pose a threat to temperate fish species. Of particular concern are the ranaviruses which have been reported in several tropical ornamental species in Australia, the USA and Israel. Some of these ranaviruses are highly pathogenic to certain temperate fish species, both farmed and wild. The so-called 'cold water ornamentals' are fancy varieties of common species, mostly cyprinids, and do survive and breed in temperate regions as well as in tropical countries. The most widely traded of these species internationally are goldfish (Carassius auratus) and koi carp (Cyprinus carpio koi) and both species have been implicated in trans-boundary transfer of viral diseases to farmed fish species such as carps and also to wild fish populations. A major example is koi herpes virus disease, which has been spread to several countries via this trade and is causing considerable economic damage to the industry. More so, in Indonesia this disease is currently suspected to be responsible for recent largescale mortalities not only in koi carp but also in common carp varieties farmed as food, and imports of koi carp are suspected to be the route of entry. Such events are likely to lead to increased demands for health certification for ornamental fish, which may in the short term cause restrictions on trade, however, in the long term it is likely to aid the sustained development of the industry.

NOTES

Preliminary molecular and biological characterisation of Mourilyan virus (MoV): A new bunya-related virus of penaeid prawns

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Mourilyan virus (MoV) is a new bunya-related virus that infects penaeid prawns. Spherical to ovoid MoV particles (85 x 100 nm dia.) possess classical bunyavirus-like morphology. RT-nested PCR testing has indicated that natural MoV infections occur commonly in black tiger (Penaeus monodon) and Kuruma (Penaeus japonicus) prawns from the wild or farmed commercially in Queensland. In each species, low-level MoV infections can be detected by in situ hybridisation (ISH) in vacuolated 'spheroid bodies' within the lymphoid organ. In heavily infected prawns, MoV is detected throughout the lymphoid organ and in connective tissues of other organs. In some P. japonicus, MoV has been identified in midgut and nerve tissues displaying histopathology consistent with gut-and-nerve syndrome (GNS). However, MoV infection has not been consistently observed within these abnormal tissues and additional studies are required to determine the relevance of MoV to this syndrome. Preliminary data suggests that the MoV genome comprises 4 segments of (-) sense single-stranded RNA. BLAST searches using open reading frames (ORFs) encoded in 3 segments identified distant relationships to proteins encoded by the L (RNA-dependent RNA polymerase), M (G1/G2 glycoprotein) and S (N nucleoprotein) RNA segments of Uukuniemi virus and other viruses within the genus Phlebovirus of the Bunyaviridae. In phleboviruses, the S RNA segment also contains a small non-structural protein (NSs) gene that is encoded in ambisense. In the MoV S1 RNA there is no ambisense coding strategy but a somewhat larger protein not obviously related to NSs is encoded in the small (S2) fourth RNA segment. Evidence of elevated levels of virus infection associated with disease episodes suggests that MoV may be a significant pathogen of farmed prawns in Queensland and elsewhere in the region.

NOTES

Fatal, virus associated peripheral neuropathy and retinopathy (PNR) in farmed Penaeus monodon in Eastern Australia

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Outbreaks in farmed Penaeus monodon of a previously unreported, fatal disease, 'peripheral neuropathy and retinopathy' (PNR) are described. These outbreaks, associated with minor to heavy mortalities, occurred in most ponds on an eastern Australian farm during two consecutive growout periods. Moribund prawns, 5-26 g mean body weight, were typically reddish in colour, lethargic, with partially amputated appendages. Histologically, mild to severe degeneration and necrosis of axons and their sheaths, together with associated glial cell apoptosis, were consistently present in peripheral nerve fibres. Mild to severe, acute to chronic retinitis, associated with degeneration and necrosis of retinular cells and their axons, also occurred in most clinically affected prawns. Intracytoplasmic nucleocapsids and enveloped virions, morphologically consistent with a yellow head related virus, were present in peripheral nerve and eye lesions. Immunohistochemical examinations were conducted using monoclonal antibodies reacting with both yellow head virus, considered exotic to Australia, and with the closely related gill-associated virus (GAV), widely endemic in P. monodon in eastern Australia. Positive reactions were consistently observed in lesions, but not histologically normal tissues, in peripheral nerves, eyes, lymphoid organ and vas deferens. Findings strongly suggest that a yellow head related virus, most probably GAV, is the causal infectious agent of PNR, an emerging disease of P. monodon. It is also likely that PNR is a component disease within the ill-defined 'mid crop mortality syndrome'. Although confirmatory transmission trials remain to be done, measures to prevent further spread of GAV, both within Australia and beyond, should be seriously considered.

NOTES

Zoning for marteiliosis in commercial rock oysters in Australia

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1. Queensland Museum

Marteilia sydneyi, the causative agent of marteiliosis (QX disease), has been responsible for significant mortality in commercial rock oysters (Saccostrea glomerata) in Australia for many years. This pathogen is listed by the OIE as notifiable and is therefore included on the Australian National List of Reportable Diseases of Aquatic Animals. The OIE recognises the concept of zoning whereby areas recognised as free from a disease can be established within a country, and trade from these free areas may continue unaffected by the presence of the disease elsewhere in the country. A zoning strategy must be based on scientifically-defensible principles with such parameters as sample design and diagnostic technique quantified to allow assessment of an appropriate level of detection. Two projects funded by the Fisheries Research Development Corporation and the Federal Government's Building a National Approach to Animal and Plant Health initiative are being undertaken to establish baseline data for marteiliosis. The major objective of these projects is to field test the zoning policy framework in a practical context in Australia, and to facilitate the development of further zoning policies for other significant diseases of aquatic animals. Subsidiary objectives include validation of molecular diagnostic procedures to provide best cost/benefit for ongoing monitoring and surveillance, while maintaining appropriate standards for detection specificity and sensitivity. Outcomes from these projects will include a reduction in the risk of translocating marteiliosis into disease-free production areas, and facilitate domestic and international market access for the industry.

NOTES

Field investigations on serious disease outbreak among Koi and Common Carp (Cyprinus carpio) in Indonesia

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Since April 2002, disease outbreaks have occurred in cultured Cyprinus carpio (koi and common carp) in Java Island, Indonesia. The outbreak caused huge economic losses and significant social impact. For instance, in Blitar regency, East Java, it has wiped out koi carp belong to 5,000 fish farmers with economic losses more than Rp. 5 billions (US$ 5 millions) within 3 months periods. To prevent the spread of the outbreak to other island, the government of Indonesia has closed Java Island from any movement of koi and common carp. This paper described the clinical history, gross signs, histopathology, PCR detection and experimental infection of the disease. The outbreak occurred after heavy rain, movement of adult fish to other pond or transportation of fry to other area. This phenomenon leads to the hypothesis that the virus was latent and becoming active under particular circumstances, such as stress of transportation and handling and environmental changes particularly temperature fluctuation. Another scenario was that the disease occurred through trans-boundary movement of infected koi carp from Hongkong. The disease occurred in on-growing fish of all ages and in all culture system including stagnant, running water and cage culture system. A variety of symptoms have been reported from infected fish. Infected fish may be lethargic, show loss of balance and gasp for breath. Sloughing off the epithelium with loss of mucus, and rough, hemorrhage of operculum, fins, tail and abdomen are common symptoms. However, the only consistent clinical sign of the disease is gill necrosis. In the early stage of infection, the gill filaments showed typical focal necrosis. In the late stage, the necrotic gill filaments fused and badly damaged. However, the symptoms of the disease may be complicated by secondary infection of debilitated fish by opportunistic organisms such as bacteria, fungi and parasites. Flexibacter columnaris and Aeromonas hydrophila have been isolated from necrotic gill filaments and skin ulcer, swollen kidney and liver, respectively. Dactylogyrus sp., Trichodina sp, Ichthyophthyrius multifiliis and Argulus sp has also been found in some infected fish. Attempts have been made to treat the infected fish with Kalium Permanganat and antibiotics (Enrofloxacin, Erythromycin, Amoxicylin and Oxytetracyclin), but it has no prevail. Histopathological study revealed necrotic changes in the gill, fin, skin, kidney, spleen, liver, heart and intestine. Prominent basophilic intranuclear inclusion bodies were observed in the gill and kidney of infected fish. Experimental infection by cohabitation test and injection with 0.45 _m-filtered homogenate resulted 100% and 70% mortality, respectively. Based on the clinical history, gross signs and histopathological changes, experimental infection and polymerase chain reaction (PCR) detection of naturally and experimentally diseased fish, it is strongly suspect that Koi Herpesvirus (KHV) is involve on the serious outbreak on koi and common carp in Indonesia. This is the first KHV outbreak reported in Asian region.

NOTES

Session 13 - The Future

Improving aquatic animal health in Asia

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Asian aquaculture has expanded, diversified, intensified and advanced technologically. Its growth and contribution to national economic and societal goals are expected to continue, provided enabling environments for sustainable development are established. Bitter experiences and substantial economic losses have demonstrated that good health management is a key to success in aquaculture. Prevention and control of disease, avoidance of introductions and transfers of pathogens, development and adoption of better farm management practices, capacity building, and targeted research, are a few vital components of good health management. Although, progress has been made in Asia, much remains to be done to achieve adequate capacity in aquatic health management.. Rapid sector growth increases the risk of unsustainable development, thus the urgency for achieving necessary management capacity. Globalization and liberalization of international trade will also require adherence to international health guidelines and standards if Asian aquaculture is to maintain its place in the international market place. Increased consumer awareness of food quality and safety, animal welfare and the environment will also exert pressure in local and regional markets. These are some of the issues that need to be addressed. The development and implementation of integrated, practical health management strategies to this end, which include appropriate regulatory frameworks and enforceable laws, will only be possible with relevant national policy and appropriate institutional arrangements. While researchers and scientists must continue to provide the necessary scientific base, through targeted research and dissemination of information, ensuring institutional, financial and human capital will continue to depend on the political will.

NOTES

Aquaculture health management: The Australian experience

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1. Aquatic Animal Health, Agriculture, Fisheries & Forestry - Australia

Aquaculture is of increasing economic importance in Australia. The gross value of production (A$750 million in 2000-01) now exceeds 30% of the total value of fisheries, with major contributing sectors being pearl farming, tuna cage culture, salmon culture, edible oysters and prawn culture . Recognising the critical importance of aquatic animal health for overall profitability, competitive trade advantages and public health, Australia in 1998-99 signed on to 'AQUAPLAN', the National Five-Year Strategic Plan for Aquatic Animal Health. AQUAPLAN is a world-first as a joint industry-government strategic initiative in aquatic animal health. AQUAPLAN provides a framework for health management that is underpinned by both legislation and policy. It is a coordinated approach that emphasises stakeholder consultation and industry participation. The major aims of AQUAPLAN are to support industry profitability and maintain the quality of the aquatic environment. Because Australian waters are contiguous with Asia, international linkages and regional cooperation are a key component of AQUAPLAN. Recent key achievements include the implementation of the Building a National Approach to Animal and Plant Health initiative, the establishment of the Fisheries Research and Development Corporation's Aquatic Animal Health Subprogram, and the steps taken to establish the Aquatic Animal Health Consultative Committee as the primary government/industry interface committee for policy, communication and awareness related to aquatic animal health issues. A critical outlook towards the future will also be given.

NOTES

The role of extension in effecting on-farm practice change for controlling shrimp disease

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The history of participatory extension methods throughout Asia and the Pacific illustrates the effectiveness of these methodologies in creating sustainable changes to farming practices. By involving farmers in research on farms it is possible to construct appropriate technologies to improve farming practice. The ACIAR project 'Development and Delivery of Disease Control Programs to Small Scale Shrimp Farmers in Indonesia, Thailand and Australia (FIS/2000/061)' is designed to create effective processes for controlling shrimp diseases such as white spot and yellow head. The project builds on technologies developed in a previous project and focuses on developing more sustainable aquaculture systems for each of the participating countries. This contextual relevance for specific extension approaches and resulting technologies means that there is a fusion between scientific and social systems in play. The paper will describe extension processes used and the role of extension in creating on farm adoption and adaptation of technologies In outlining the main methodologies used in each country, the paper will define the theoretical underpinnings of these methods and explain the reason why different methods suit different countries. The paper contends that without effective social research and extension program design there will be little adoption of appropriate technologies. No matter how good the scientific research, there will be little change if people participating in the aquaculture industry do not contextually apply technologies. Extension is one key factor for successful control of shrimp disease in the Asia/Pacific region.

NOTES

Theme 1

Quarantine detention of ornamental fish - Practical challenges involved in the implementation of a new policy

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Based on the findings of an import risk analysis (IRA), Australia introduced new biosecurity requirements for the importation of ornamental finfish in November 1999. All ornamental finfish imported into Australia must meet pre-export and post-arrival biosecurity requirements before release into the aquarium trade, including the satisfactory completion of post-arrival detention in quarantine facilities approved by the Australian Quarantine and Inspection Service. These import controls address risk factors identified in the IRA and are designed to reduce the likelihood of exotic fish diseases and pests establishing in Australia, to an acceptable level. Although these controls are amongst the most stringent in the world, their practical implementation has not been without problems. This presentation will describe some of the challenges that Australia has faced in the 3 years following the introduction of new import controls in 1999, and based on these experiences, explore ways to better manage biosecurity risks associated with imported ornamental fish in the future.

NOTES

Theme 2

Argulosis in Brood Carp Rearing Ponds of Bangladesh

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Recent investigation on the infection of a new species of Argulus Muller shows that Argulosis is a common problem in almost all carp hatcheries of Bangladesh, and the farmers are using the pesticides- Cymbush and Sumithion at regular intervals. As a result, the pond ecology as well as the abundance of zooplankton were found to be adversely affected. All the 3 major carps viz. Catla catla, Labeo rohita and Cirrhinus mrigala are infected and shows the clinical signs of agitation, lethargy, sporadic movement, cessation of feeding, shortage of ovulation, opaqueness and darkness of skin, shrunken eyes and skin erosion. The infected brood fish becomes unfit for breeding as well as table fish thus causing economic loss. Out of 1440 fishes studied from two infected ponds of BRAC hatchery at Rajendrapur near Dhaka, the overall prevalence of Argulus infestation on L. rohita, C. mrigala and C. catla was 50%, 36.66% and 12.91% respectively in pond no.3 and 26.66%, 20% and 10.41% respectively in pond no.1.Whereas the overall intensity of infestation was 13.78, 11.37 and 9.6 in pond 1 and 10.6, 9.97 and 9.22 respectively in pond 3. The prevalence and intensity of infestation in all the 3 species of fish of both the ponds were highest in the largest length group of 60-74.9cm in the month of July, and female fishes were more susceptible to infestation. To overcome the hazards of using pesticides, a mechanical means of using pyrex sheets to collect and destroy Argulus eggs and biological control measures of culturing predatory organisms like Macrobrachium with the brood carps are being tried

NOTES

Freshwater fungi isolated from common carp (Cyprinus carpio) eggs in Thailand

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Fungal infection in eggs of freshwater fishes is well known as problematic disease. We had a chance to examine the fungal infection in common carp (Cyprinus carpio) eggs at fish farms in Khon Kaen Province, Northeast of Thailand in February 2002. We attempted to isolate fungi from the eggs with fungal infection at three fish farms. Each egg with a fungal infection was placed directly on a GY agar plate, and then a small amount of streptomycin sulphate and ampicillin was scattered on the medium to retard bacterial contamination. All agar plates were incubated at 25?. Nineteen fungi from the farm A, two fungi from the farm B and two fungi from the farm C were isolated. The fungi from the farms A, B and C were identified as Saprolegnia diclina, Achlya sp. and Allomyces arbuscula, Achlya sp., and Saprolegnia diclina, respectively. S. diclina and Achlya sp., and A. arbuscula grew well at 25-30? and pH 5-7, and at 37? and pH 6-8, respectively. Artificial infection to platy (Xiphophoras maculates) was made using the selected fungi. For isolates S. diclina and Achlya sp., the mortality in injured fish challenged with 104 zoospores/ml was 100%. But the mortality was 0% in the other experiments

NOTES

Biology and pathogenicity of the gill monogenean (Pseudorhabdosynochus sp.) in grouper

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Eggs of the gill monogenean (Pseudorhabdosynochus sp.) were collected from adult parasites and were subjected to different test salinity levels(0, 10, 20, 30 and 60 ppt) to determine the effect on hatching and development until oncomiracidial stage. Results showed that the rate of egg hatching was 75% at 10 ppt, 92% at 20 ppt and 97% at 30 ppt. No hatching was observed at 0 and 60 ppt. Monogenean eggs were collected from adult parasites and were incubated at 25 degrees C, 30 degrees C and 35 degrees C to determine the effect of different temperature levels on hatching and development until the oncomiracidial stage. Preliminary results showed that egg hatching at 25 degrees C occurred on day 3, 30 degrees C on day 2, 85.33% at 30 degrees C and 64.33% at 35 degrees C. Epinephelus coioides juveniles were experimentally exposed to adult monogenean (Pseudorhabdosynochus sp.) at various levels (0, 100, 500, 1000 and 5000 per 10 fish) to study the effect of different levels of infection on healthy grouper juveniles. Results showed that a level of 5000 adult monogenean/10 fish resulted in 100% within 72 h of exposure. A level of 100 adult monogenean/10 fish caused 36.66% mortality, 500 adult monogenean/10 fish caused 60% mortality and 1000 adult monogenean/10 fish, 87% mortality until the experiment was terminated after 15 days. Histopathological response of the gills of infected grouper (5000 adult monogenean/10 fish showed diffuse hyperplasia of the gill lamellae. The hematocrit of the highest infection group (5000 adult monogenean/10 fish) was lower than those of the other treatment groups.

NOTES

Diagnosis of systemic iridoviral disease in fish

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Iridovirus infections have been implicated as the cause of severe disease and economic loss in a great variety of freshwater and marine fish species. Although iridovirus infections were observed in 3 marine fish species and 4 freshwater ornamental fish species, success in viral isolation and propagation was achieved only with the Malabar grouper (Epinephelus malabaricus Bloch & Schneider). The iridovirus of mullet (Mugil cephalus Linnaeus) and dwarf gourami (Colisa lalia Hamilton) were associated with rapidly declining infectivity during serial passages on tissue culture. Since virus isolation had been difficult, diagnosis had been based mainly on LM and EM examination of infected fish tissues. Lightly to intensely basophilic hypertrophied virus-infected cells were consistently observed in spleen, kidney and intestine from all 7 species under LM. Under EM, the localization of infected cells suggested that systemic iridoviruses are mesotheliotropic. Better success with vital isolation on fibroblastic cell lines further supported the hypothesis that systemic iridoviruses of fish are mesotheliotropic in nature. Immunohistochemistry (IHC) and direct immunofluorescent antibody tests (IFAT) using a rabbit polyclonal antiserum as well as a monoclonal antibody against the iridoviral isolate from Malabar grouper (SGIV) directly on fish tissues, suggested these as potential tools in the diagnosis of iridovirus infections. IFAT, using a monoclonal antibody (Mab10) to the Japanese red sea bream iridovirus (RSIV), suggested an antigenic relationship between the 'Sleepy Grouper' iridovirus-like agent and RSIV.

NOTES

Tasmanian Isolates of Streptococcus sp. biovar 1 and verified strains of lactococcus garvieae and enterococcus seriolicida compared by microbiological , molecular biological and "in vivo" techniques

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A gram-positive coccus was isolated from diseased, farmed rainbow trout and ocean-caged trout (Oncorhynchus mykiss) in Tasmania in 1988. This disease caused significant commercial loss to the trout farming industry at that time. Based on morphological, physiological and biochemical characteristics of the Tasmanian isolates, the initial identification placed them in the genus Streptococcus and they were hence given the epithet Streptococcus sp. biovar 1. Subsequent studies indicated that these organisms were more closely related to the genus Enterococcus, especially the fish-pathogenic bacterium known as Enterococcus seriolicida, than the genus Streptococcus. Further investigation has indicated that these fish pathogenic isolates could be identical to the species Lactococcus garvieae isolated from cattle. In order clarify the situation, studies were undertaken using molecular biology to investigate the possibility of some genetic similarity. The technique used for this investigation was the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). Pathogenicity of the isolates was also performed using rainbow trout (Oncorhynchus mykiss). The results of all these studies showed that the Tasmanian type strains (Streptococcus sp. biovar 1) were closely related to, if not identical with, both Lactococcus garvieae and organisms previously named Enterococcus seriolicida. Therefore Streptococcus sp. biovar 1 should be reclassified as Lactococcus garvieae, which must now be considered a major fish pathogen worldwide.

NOTES

A study on columnaris disease in guppy Poecilia reticulata

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2. Confifi Aquaculture Ventures (Pvt) Ltd

About 65% of ornamental fish exported from SriLanka is guppy and guppy breeders in the country have been trying to produce fish with wider caudal fin to suit the requirement in the export market. This attempt has been hampered by the presence of larger proportion of male guppies with eroded margins of caudal fins and the occurrence of subsequent mortalities. Therefore the present study was undertaken to investigate the cause of fin erosion and mortality in guppy. Monthly samples of guppy were obtained from a guppy farm in the Western Province of SriLanka that had the experience of having fish with eroded caudal fins. These fish were maintained and observed in the laboratory under simulating conditions to those of the farm. Within 48 hours, erosion of the margin of caudal fin progressed gradually leaving the fin rays bare and lesions developed on the caudal peduncle, body surface and on other fins which rapidly progressed in to yellow to orange coloured ulcers. Tips of gill lamellae of some fish showed necrosis which progressed rapidly reaching the base of the gill arch. Histopathology revealed that the ulceration could spread in to deeper tissues. In wet mounts of lesion, long thin rods (0.60 to 1.0 µm width; 5.0 to 10.0 µm length) with characteristic gliding motion of Flexibacter columnaris were present. The bacteria produced yellow-green flat colonies with uneven margins on cytophaga agar. Percentage prevalence of the disease was increased during the months with water temperature above 28.5 oC causing 85 - 91% mortality within a period of 8 days. Water quality management together with prophylactic treatment is suggested to control F.columnaris in culture facilities of guppy.

NOTES

Nodavirus infections as an emerging disease in aquaculture

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1. National Agriculture Quarantine and Inspection Authority

[Please note: The summary below is done as a requirement for necessary funding to attend the symposium. It was necessary for me to do it in order for my funding agency to allocate finance. It is hoped that I can learn a lot more than what I know now, from attending the symposium. It is based on an incomprehensive review of recent literature on the subject] ABSTRACT/SUMMARY Several RNA and DNA viruses of lower vertebrates have induced diseases resulting in important economic losses, especially in fish aquaculture. One of the emerging RNA virus is the nodavirus, also called nervous necrosis virus (NNV). The disease caused is called viral nervous necrosis (VNN), sometimes also called viral encephalitis and retinopathy (VER). It is one of the OIE's notifiable fish pathogenic RNA viruses. The same viral diseases have been observed in barramundi, sea bass, turbot, groupers and other fish in the world. It mainly affects larvae and juveniles via vertical or horizontal transmission and causes a vacuolating encephalopathy and retinopathy. The virus strains have adapted to different geographic locations and environments and could possibly extend its host range easily. Diagnostic procedures reccomended are at present electronic microscopy, immuno histochemistry, fluorescent antibody techniques, ELISA, PCR and viral isolation. Control measures include prevention of vertical spread by selection of non-infected spawners, sterilisation of eggs and larvae, and possibly vaccination of juveniles at the end of the nursery period. There is still need for continued epidemiological surveillance of fish nodavirus and the development of effective control measures.

NOTES

Diseases of Opakapaka held at the Hawaii Institute of Marine Biology

Kent Michael L [1]*

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Opakapaka (Pristipomoides filamentosus), a highly valued commercial deep-water snapper, is under investigation at the University of Hawaii through support of Department of Land and Natural Resources. One endeavor is to develop aquaculture methods for enhancement of wild stocks of this fish and related species as well as for commercial culture of opakapaka. We conducted a proactive study to elucidate potential diseases that could cause problems in opakapaka held in captivity. A total of 143 moribund and healthy opakapaka were examined in July-August 2001. Fish were wild-caught and then held in seawater netpens or in flow through circular aquaria. The most common and problematic infection was Crytocaryon irritans. Other external parasites included Diplectanum opakapaka (Monogenea), and a Caligus sp. (Copepoda). Common internal parasites were the Metanematobothrioides opakapaka (Didymozoidae; Digenea) and a new Goussia sp. (Coccidia). Ichthyophonus sp. and epitheliocytsis were observed in a few fish. Bacterial infections of the swim bladder were detected in several fish, due most likely to degassing of this organ at capture (i.e., deliberate puncture). Exophthalmia was frequently observed, most likely due to barometric imbalances as opakapaka is captured in deep waters. We concluded that the external monoxenous parasites, especially Cryptocaryon, and possibly the coccidian, pose the greatest potential health issues for this species for aquaculture.

NOTES

Winter disease in farmed silver perch (Bidyanus bidyanus) in New South Wales, Australia

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Health related losses of silver perch cultured in earthen ponds are the subject of a three year research project which began in 2001. Winter disease, characterised by severe dermatitis and branchitis associated with Saprolegnia parasitica infection, is responsible for a significant proportion of these losses. Outbreaks of winter disease cause mortalities between 1% and 100% in fish >80g. Typically affected fish have large, irregular, pale areas on skin and/or gills, with attached fungal hyphae. S. parasitica has been isolated consistently from these lesions. The fungus does not provoke a significant inflammatory response in the skin and does not penetrate the stratum compactum. Where gill is infected, there is extensive destruction of gill lamellae by invasive fungi. Moderate to severe proliferative branchitis, unrelated to fungal infection, is always present in fish prior to outbreaks. A temporal association between infestation by the monogenean gill parasite Lepidotrema bidyana and this gill lesion is being investigated. No significant lesions have been identified in internal organs of affected fish. Timing and severity of outbreaks varies within and between farms. Outbreaks occur below 16 oC water temperature and often coincide with rapid drops in water temperature. Physical damage to skin from handling has also preceded some outbreaks. Occurrence does not appear to be correlated with fluctuations or absolute levels of pH, hardness, alkalinity, DO, or TAN. Improved understanding of causal factors and therapeutic agents are required for cost-effective control.

NOTES

First report of systemic amoebosis in oscar, Astronotus ocellatus

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2. Division of Fish Diseases, Nippon Veterinary and Animal Science University,Japan

Systemic amoebosis was found in oscar (Astronotus ocellatus). Diseased fish showed lose of appetite, emaciation, skin darkening, small lesion on the body surface, aggregation on the bottom of the tanks. Mortality rate at 80-100 percent was found within 3-5 days after disease on set. Causative pathogen was isolated and maintained in pure culture. Morphological study by light microscope was conducted and found two distinctive forms, trophozoites which were spiny-liked pseudopodium and cysts which showed in polygonal shape. Application of Polymerase Chain Reaction technique with specific primer and nucleotide analysis of PCR product indicated that this organism is an amoebic protozoa belonging to the genus Acanthamoeba. Keyword: Amoebosis, oscar (Astronotus ocellatus), Acanthamoeba

NOTES

Study on Tetrahymena infection in guppy (Poecilia reticulata)

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Recently Tetrahymena infection is serious problem in guppy (Poecilia reticulata). Infected fish were collected from guppy farms in Bangkok and the other areas nearby. The clinical sign of infected fish is whitish patch associated with lesion on the body. Fish scales are bristled, in serious infection, outer skin and muscular tissues was lost. The infection results in mortality within short time. Diseased fish were observed under light microscope and histopathologically studied. Large number of ciliated protozoas are presented on skin and in scale pocket. It was identified as Tetrahymena sp. Histopathological studies showed severe extensive necrosis occur in subdermal and muscular tissues. These ciliated protozoa could invade through skin into muscle and also internal organs. Inflammatory reaction was rarely observed. It was possible to conclude that these ciliated protozoa were the main causative agent of the heavy mortality in guppy. The endemic of this ciliated protozoa may relate to the importation of ornamental fish into the country without the proper quarantine system. The suitable health management and quarantine of new stock before introducing into the farms is recommended for preventing of this disease.

NOTES

Mycobacteriosis in ranchu, goldfish (Carassius auratus) imported from Japan

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Ranchu, Japanese goldfish, is very famous for culturing among Thai people. Each year, many company have imported ranchu from the famous farm in Japan. In the past winter, January 2002, 30 percent of imported juvenile fish showed inactive swimming, anorexia and lethargy. Therefore, the six diseased fish were collected to investigate the cause of diseases. The clinical signs of the fish showed asymmetrical swelling of abdomen, pale discoloration of gills, swelling and discoloration of head and trunk kidneys. Abdominal organs were attached to peritoneal wall, indicating peritonitis, associated with numerous white nodules of various sizes. These nodules were also found on the abdominal organs, head and trunk kidneys and heart. The isolation was done with Ogawa egg medium and identified as Mycobacterium chelonae by the PCR method at Aquatic Animal Health Research Institute, Department of Fisheries, Thailand. The histopathological features showed that these white nodules on the affected organs were chronic proliferative lesions composed of multiple caseous epithelioid cell granulomata with surrounding granulation tissue. In the central caseous area, a number of colonies of slender, long rod, Gram positive were found to be acid-fast by Ziehl-Neelsen method. These acid-fast bacteria were also observed in the epitheliod cells and in the macrophages of the granulation tissue. From these findings, this case was diagnosed as serious systemic mycobacteriosis.

NOTES

Diversity of freshwater monogeneans from siluriform fishes of Thailand

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This study is to document the monogeneans on freshwater siluriform fishes in Thailand. Of the hundred catfish species in Thailand, 44 were examined for gill monogeneans and 40 were found to be infected. Eighty-three monogenean species found in this study belong to seven genera, Bifurcohaptor (2), Bychowksyella (8), Cornudiscoides (13), Hamatopeduncularia (2), Mizelleus (1), Quadriacanthus (2) and Thaparocleidus (55). All these seven genera are placed in Ancylodiscoididae. Assuming an average infection of three and two monogeneans per host species, the expected monogenean diversity on Thai freshwater siluriforms is about 294 and 196 species, respectivley. The present observed diversity represents only 33% - 50% of the expected diversity. The majority of the monogeneans on Thai siluriforms (76%) are host-specific, while 24% are found on two or more related host species (at generic or family levels). About 9% of the fish hosts are without monogeneans, while 25% have one, 28% have two, 18% have three, and 20% with four or more co-existing species: indicating that co-existing species are common. Co-existing species can be congeners or non-congeners and the number vary from one to eight depending on host species, such an example of Hemibagrus neemurus having eight species belonging to three genera, H.wyckioides harbours seven species from two genera, while Pangasius larnaudii and Pteropangasius pleurotaenia have six species of Thaparocleidus each.

NOTES

Seasonal variation in the ectoparasite assemblage of Pagrus auratus cultured in sea-cages off Eastern Australia

*Hayward CJ [1], Lester RJG [1], Mooney Allan [1]**

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High stocking densities and a reduced water quality are two environmental factors that provide a favourable environment for diseases and parasites to flourish within an aquaculture system. The smaller living area associated with high stocking densities increases the risk and extent of infection. Fouling on the net reduces water quality by confining a greater proportion of potentially damaging parasitic eggs, excess food and fish waste products within the cage. Changing nets regularly is one husbandry practice used to maintain water quality and so limit infestation of diseases and parasites. A rise in mortalities of Pagrus auratus peaking approximately 12 days after a net change during summer 2000 was observed in commercial sea cages off the coast of central New South Wales. Preliminary observations on moribund fish suggested death was associated with large numbers of Bivagina pagrosomi, a blood-sucking monogenean. This research documents the population dynamics of this monogenean and four others, Lamellodiscus pagrosomi, Anoplodiscus cirrusspiralis, Choricotyle australiensis, Benedenia sekii and a copepod, Lernanthropus atrox, to determine whether detrimental increases in parasite numbers are primarily linked to net changes at this location or whether other factors are more important.

NOTES

A Pentacapsula species inhibiting propagation of striped trumpeter - an aquaculture candidate

Munday Barry [1], Grossel G [1], Ellis J [2], Dykova I [3] Handlinger J [4], Whipps C [5], Kent M [5]*

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5. Center for Fish Disease Research, Oregon State University

Striped trumpeter Latris lineata is a premium eating fish much sought after for the restaurant trade, especially for sashimi. In order to meet the demand for this fish a propagation program to develop striped trumpeter as an aquaculture species has been initiated at the Tasmanian Aquaculture and Fisheries Institute. Conditions such as failure of swim bladder inflation and bacterial enteropathy have caused losses in larval fish but are being overcome. However, fish surviving over 30 days of age have frequently developed nervous aberrations and are the subject of this poster. A Pentacapsula myxozoan was detected in the central nervous system and was accompanied by a severe host reaction. Measurements made of isolated spores indicated that the organism was distinct from all previously described Pentacapsula spp. The 18S rDNA gene has been sequenced and, as no other Pentacapsula spp. sequences are available, will be compared with sequences of other multivalvulid, histozoic myxozoans.

NOTES

Hemorrhaging septicemia due to Aeromonas hydrophila in the Mekong catfish (Pangasius bocourti) cultured in An Giang province - Vietnam

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Hemorrhaging septicemia disease has been observed in the Mekong catfish Pangasius bocourti cultured in earth ponds and floating net cages in An Giang province since the early 1990's. The infection caused serious losses to the industry due to high mortalities and/or reduced value of chronically infected fish. In order to identify the causative agent, 497 infected fish were collected from various fish farms in the province in four years from 1997 to 2000. The samples were used for histopathological, parasitological and bacteriological examinations. Various parasitic and bacterial species were detectable from the collected samples. However, the motile Gram-negative strains of Aeromonas hydrophila showed to be the causative agent as it was isolated at highest frequency from the liver, spleen and kidney of all of the infected fish. The infection was also successfully reproduced in experimental fish of 15g in body weight being injected with 0.1ml of the isolated A. hydrophila strains with LD50 = 106,41CFU/fish. The pathogenic isolates showed resistance to common antibiotics i.e. penicillin, ampicillin, oxytetracycline, streptomycin, sulfamethoxazol, and chloramphenicol. Drug resistance of the isolates has resulted in difficulty for controlling the disease. However, initial study on vaccination development using the isolated strains has showed that water-based heat- and formalin-killed vaccines did not produce effective protection in the experimentally vaccinated fish.

NOTES

Infestation and Effects of Nematode Parasites on Xenentodon Cancila (Hamilton-Buchanan)

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Xenentodon cancila, a commercially important freshwater fish obtained from Pechiparai reservoir, Kanyakumari District, Tamil Nadu was found infested by nematode parasites of the genus Philometra inflicting severe tissue damage to host and causing great economic loss to reservoir fishery. Detailed investigations were made on the distribution, infestation and effects of Philometra on X. cancila. The nematode parasites were distributed in the body cavity, musculature, alimentary canal, gonad, liver and air bladder with maximum distribution in musculature (37.6%) and minimum in gonad (3.0%). Distribution of parasites in relation to different periods, size and sex of host was also studied. The overall prevalence was 87.8% and the mean intensity was 8.4. The percentage prevalence was generally very high ranging between 70 and 100 and the intensity ranged between 3.4 and 15.8 with a maximum number of parasites on a single fish was 63. The infestation was greater in males (P. 90.67% MI 8.4) than in females (P. 86.2% MI 8.3). Detailed studies were also made on the effects of parasites on the length weight relationships, feeding intensity and reproductive potential of X. cancila.

NOTES

Production and characterization of monoclonal antibodies to Singapore grouper iridovirus (SGIV)

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A panel of 6 monoclonal antibodies (mAbs) against a grouper iridovirus (SGIV) were produced by immunization of Balb/c mice with purified virus preparations. Isotyping test revealed all the mAbs were IgG1. None of the mAbs possessed ability to neutralize SGIV in cell cultures but all reacted with the cytoplasm of SGIV-infected grouper cells (GP) as determined by an indirect immunofluorescence test (IIF). Western blot assay showed that 4 mAbs reacted with 2 SGIV proteins at molecular mass of approximately 100 and 117 kDa in gradient-purified virus. Fractionations of the iridovirus in a 20-60% sucrose gradient were successfully detected by all the six mAbs using immunodot blot. These mAbs will facilitate the development of more specific and standardized diagnostic techniques for marine fish iridovirus.

NOTES

Aquatic oomycetes from southeast Queensland

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The occurrence of two oomycetes, Aphanomyces sp. and Achlya sp. in freshwater fish, Scortum barcoo, from a local fish farm led to an investigation of the moulds that were present in local waterways. Oomycetes were initially cultured on Czapek Dox Agar and later on split autoclaved chickpeas in autoclaved pond water with antibiotic (Penicillin G, 100 units/ml and oxolinic acid 100 mg/ml). Four Achlya species were identified, A. americana, A. bisexualis, A. caroliniana and A. proliferoides, and two Saprolegnia species, S. luxurians and S. terrestris, each by the morphology of their sporangia, oogonia and antheridia. Aphanomyces sp. was frequently isolated but cultures failed to develop sexual stages and therefore isolates were not identifiable to species. Goldfish from which 3 scales had been removed were exposed to an isolate of Aphanomyces sp. or an isolate of Achlya sp. for 2 hr. then kept at 18.5, 24.5, or 27.5 C. No infection developed over the next 3 weeks.

NOTES

A new genus of dracunculoid nematode from the gills of the pufferfish Tragulichthys jaculiferus

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2. University Of Queensland

A new dracunculoid species belonging to the Guyanemidae is described from gill filaments of the pufferfish Tragulichthys jaculiferus Cuvier (Tetraodontiformes: Diodontidae, 'green porcupine fish') from Moreton Bay, Queensland. The species has a reduced buccal capsule and divided oesophagus. Females have a functional vulva and single ovary; males have caudal alae and spicules. These characters are typical of Guyanemidae. Of the three known genera, Guyanema and Travassosnema are exclusively parasites of freshwater fishes in South America while Pseudodelphis is a parasite of the tidepool sculpin in Canada. All existing species are parasitic in the body cavity except for T. travassosi which occurs in the eye. The new species differs from members of these genera in that it has fine cuticular transverse striations, two forwardly protruding cephalic elevations, a circumoral elevation, a small oval mouth surrounded by a peribuccal membrane, 6 internal cephalic papillae arranged in two clusters of three papillae each and a pair of large oval external cephalic papillae. The males have only two pairs of pedunculated caudal papillae supporting the caudal alae. Gills of all 69 green porcupine fish contained mobile larvae in the gill filament between the epithelial basement membrane and the efferent artery. Abundant larvae resulted in mild oedema in the filament. Eleven fish harboured adult nematodes in the same location with little apparent tissue response.

NOTES

Identification of a betanodavirus isolated from viral nervous necrosis-diseased redspotted grouper (Epinephelus coioides) cultured in Southern Thailand using PCR and sequence analysis

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A viral agent was successfully isolated from redspotted grouper seeds (Epinephelus coioides) exhibiting viral nervous necrosis (VNN) clinical signs in Southern Thailand in March 2001. Grouper seeds, ~1.5-2 cm in total length, had been collected from the wild and maintained in private nursing farms. The VNN disease occurred within a few days after stocking. Eyes and brains tissue of diseased fish were pooled, extracted and filtered through 0.45  disposable filters. The filtrates were inoculated on to striped snakehead whole fry tissue or SSN-1 cells and incubated at 28C. Cytopathic effect (CPE) was first observed on day 3 post-inoculation which recognized as shrunken and rounded shape of cells. Cells continued to aggregate and the CPE completed on day 7. Viral particles in virus-infected SSN-1 cells were seen using a transmission electron microscope. The isolated virus possessed icosahedral nucleocapsid with ~25 nm in diameter. Viral propagations were not sensitive to chloroform or IUDR, which indicated that they were naked virions and contained RNA genome. These viral characteristics were primarily classified as a viral member of Nodaviridae family. Viral identification had been conducted using polymerase chain reaction (PCR) and sequence analysis of PCR product. PCR amplification using specific primers designed from Betanodavirus genotype RGNNV gave a better PCR product intensity in agarose gel than an amplification using specific primers designed from genotype SJNNV. One 730 bp PCR product was sequenced in both directions. Sequence analysis of this product using Blast program showed 720/730 or 98% nucleotide homology to dragon nervous necrosis virus and redspotted grouper nervous necrosis virus. Only a 400 bp part of the sequence was most similar to genotype SJNNV with 357/400 bp or 89% homology. Findings indicate that this first isolated Betanodavirus in Thailand can be identified and grouped as nodaviral genotype RGNNV.

NOTES

Health problems of captive West Australian dhufish

*Stephens F J [1] *, Raidal S R [1], Jones B [1], Thomas T [1], Jenkins G [2], Cleary J [1]*

1. Aquatilia Healthcare

The dhufish Glaucosoma hebraicum is a potential aquaculture species but captive dhufish experience several health problems, including exophthalmos in otherwise apparently normal fish and infestation of gills with a monogenean parasite, Haliotrema abaddon. Gas and haemorrhage was present in the choroid of exophthalmic eyes, with haemorrhage in retrobulbar tissues resulting from perforation of the sclera in some eyes. Oxygen content of gas in eyes with recently developed exophthalmos was high (up to 73%). In some eyes with retrobulbar haemorrhage, oxygen tension approached zero, indicating severe disruption of blood supply to the eye. Oxygen tension at the retinal-vitreal junction of normal dhufish eyes was high (344 ± 26 mm Hg), with oxygenated blood supplied to the choroid body from the gills via the pseudobranch. The finding of a single haemoglobin with pronounced Root and Bohr effects in dhufish was significant and may contribute to the susceptibility of the species to exophthalmos. Investigations suggest that exophthalmos is physiological in origin and is related to the environmental differences between the natural habitat of the fish and the conditions that are experienced in aquaculture. Rapid changes of temperature or blood acid-base characteristics may precipitate the development of exophthalmos. The monogenean parasite, Haliotrema abaddon, was described and stages of its life-cycle identified. Potential treatments were investigated using in vitro and in vivo studies. Praziquantel was identified as the most effective 'in water' treatment of fish infested with H. abaddon. Other useful but less effective and safe treatments were low salinity baths (<1.5 ppt for ninety minutes) and 0.5 mg L-1 trichlorphon for 36 hours.

NOTES

Induction of caspase-dependent apoptosis by betanodaviruses GGNNV and identification of nucleolus localization signal of protein

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Apoptosis is the process whereby individual cells of multi-cellular organisms undergo systematic self-destruction in response to a wide variety of stimuli. For many viruses, the careful induction of apoptosis during lytic infection may represent the basis for cytotoxity and important outlet for dissemination of progeny virus. Fish betanodaviruses (GGNNV) infection of asian seabass (SB) cells appeared to induce a typical cytopathic effect (CPE). The infected SB Cells showed typical DNA fragmentation, positive TUNEL assay and activation of capase-3-like proteases pathway. All of these are hallmark of apoptosis. Furthermore, expression of protein a in SB cells confirmed that protein a was a apoptotic inducer. To search for the mechanism of GGNNV infection, enhanced green fluorescent protein (EGFP) was fused with protein a to study it's subcellular localization in transferred cells. Protein a was detected in nucleolus and cytoplasm of both SB and Cos-7 cells. Deletion mutants of protein a demonstrated that N-terminal amino acids RRRANNRRR of protein a was a nucleolus localization signal that functioned in both fish and mammalian cells. Although the localization of protein a was excluded from the nucleolus when the amino acids RRRANNRRR was deleted, the apoptosis could not be prevented in transfected cells, indicating there is no relationship between nucleolus localization of protein a and apoptosis induction. Since protein a is located in both cytoplasm and nucleolus in transfected and infected cells, it's cytoplasm localization might be involved in it's apoptosis.

NOTES

Histopathological study on tetrahymena infection in dwarf gourami (Colisa ialia)

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Tetrahymena infection in guppy (Poecilia reticulata) caused by Tetrahymena corlissi is well known as the most problematic disease in Southeast Asian countries. Recently, the similar parasitic ciliate was found in dwarf gourami (Colisa lalia), which was imported from Southeast Asian countries to Japan. The fish sometimes had ulcer lesions on the body surface. The fish usually died of the infection within several days after their arrival. We had a chance to examine some dwarf gourami with ulcer lesions on the body surface, which were imported from Singapore to Japan, and attempted to isolate the parasite from the lesion and fixed in 10% phosphate buffered formalin solution for histopathological examination. The cultured ciliates were stained with Protargol method for morphological characteristics. As a result, the ciliate was identified as T. corlissi, which has been reported as a pathogen of guppy. In histopathological examination, the parasites were mainly found in the connective tissues between muscle fibers, and a few ciliates invaded into abdominal cavity. The inflammatory reaction in dwarf gourami mainly consisted of macrophages and neutrophils, and was intensive than that in guppy.

NOTES

Turbot culture - a newly established industry in China and its disease problems

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1. Chinese Academy of Fishery Sciences

Turbot (Scophthalmus maximus) is one of the most important culture fish species in Europe, from where it has been introduced into China in 1992. With the consequential successes in artificial breeding from 1998, the commercial culture has rapidly spread along the coast of North China. In 2001, about 3 to 4 million juveniles were produced, and yielded more than 3000 MT marketable fish with the culture area of 600,000M2, which totally valued about 140 million US dollars. The turbot was mostly cultured in indoor cement tank, which is an intensive culture system equipped with oxygen generator and flowing water. At such conditions, 60-90% survival rate and biomass of 15-20kg/ M2 (less than 18-month old) were normally achieved. In addition, general husbandry for broodstock, hatchery and on-growing aspects were described in the present paper. The rapid expansion of turbot culture led to the occurrence of diseases. There were several diseased conditions have been found, some of the syndromes were firstly recognized in turbot culture. In which, the non-infectious disease included depigmentation and deformities, and infectious disease were displayed with viral, bacterial and parasitic infections. The epidemiology and prevention skills were provided for each particular condition, as well as the limiting factors in the sustainable development of turbot culture in China were also discussed. Key Words: turbot (Scophthalmus maximus) fish aquaculture disease

NOTES

Pilchard herpesvirus in Australasia 1995-1999

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Two epizootics have occurred in populations of the Australasian pilchard Sardinops sagax neopilchardus in waters of southern Australia. The first occurred between March and September 1995, and the second in 1998-1999. In 1995 mortalities occurred along more than 5000 km of the Australian coastline and also affected pilchards in New Zealand. It was thought to be the largest fish kill ever recorded. The disease front spread from its origin in South Australia at about 30 km/day, often against prevailing currents and was not impeded by storm events. Thus it was not caused by planktonic toxins/pathogens. Affected fish died within minutes of signs of respiratory distress. Fish were sampled before, during and after the advancing mortality front. Relevant lesions were confined to gills and were unlike lesions associated with known gill pathogens or toxins in other species of fish. Lesions were initially focal but became locally extensive then generalised, with inflammation then being replaced by epithelial hypertrophy and hyperplasia over about 4 days. The pathology in affected fish across the distribution of the disease was similar, suggesting a common aetiology. A herpesvirus was the only factor consistently associated with lesions, including those in early stages of the disease. The herpesvirus was not isolated in fish cell lines but has been detected by PCR. Approximately 60% of the total pilchard biomass in Southern and Western Australian waters was lost in the 1998-1999 epizootic, but spread was slower than in 1995. The source of the virus remains controversial. Studies of the virus and its relationship to lesions are on-going.

NOTES

Theme 3

Occurrence of hemic neoplasia in slipper oyster, Crassostrea iredalei (Faustino, 1928) in Dagupan City, Philippines

de Vera Arthur [1], McGladdery Sharon [2], Stephenson Mary [2], Maillet Michelle [2], Bourque Daniel [2]*

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Cases of hemic neoplasia in the Philippine slipper oyster (Crassostrea iredalei) have been confirmed from a one-year histology-based disease survey conducted in Dagupan City. Samples were collected on a quarterly basis from the BFAR-NIFTDC demonstration farm and processed for histopathology. Slides were stained with hematoxylin and eosin (H & E) or Feulgen picromethyl blue (FPM) as required. Of 210 oysters examined, 3% showed the presence of hemic neoplasia. Histopathological features of the disease condition are described. This is the first documented case of this disease in slipper oysters in the Philippines.

NOTES

Bacterial infection in Tasmanian farmed abalone: causes, pathology, farm factors and control options

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Abalone aquaculture based on artificial feeds and intensive generally land-based systems is increasing in Australia. Intensive systems are vulnerable to diseases of poor environment control. This paper reports several types of bacterial diseases investigated in grow-out stock of Halioitis rubra, H. laevigata and their hybrids over approximately 10 years, their pathology, farm factors precipitating disease outbreaks, and the potential for disease control using antibiotics. Vibrio species infections have been the most common cause of infection, with a variety of species isolated from moribund animals, generally irrespective of the primary insult. Specific disease outbreaks, with differing pathology, have been associated with two species, Vibrio harveyi and V. splendidus I. Specific pathology has also been associated with Flavobacterium-like species. In most cases stress factors precipitating disease has been identified, and control has been largely directed to eliminating this stress. Antibiotics have also been used, with equivocal results, precipitating preliminary studies of antibiotic absorption and efficacy in these species. The limited potential for antibiotic use, and the requirement for understanding and control of on-farm stress, is discussed.

NOTES

Transmission of perkinsus olseni among wild blacklip abalone in South Australia

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The protozoan Perkinsus olseni was recently confirmed to be widespread among molluscs in the Pacific Ocean by rRNA NTS sequence data; the parasite also occurs in the North Atlantic. In temperate waters off southern Australia, P. olseni infects aggregations of abalone in the wild. Individuals that develop lesions in response to the parasite are unmarketable; at other localities, infections may be acute, leading to dieback of abalone populations. To develop appropriate management strategies, we aim to model the infection dynamics of the parasite. Samples of healthy blacklip abalone were tagged and translocated to a site east of Taylor Island, a known ?hotspot? for the disease in South Australia, in two consecutive summers. In 2002, the proportion of abalone acquiring Perkinsus three months after translocation was markedly lower than in 2001 (11% cf. 85%); infections were also lighter. Similarly, resident abalone were infected at a lower rate this year, both at the beginning and end of the experiment (19% cf. 50% in late January; 30% cf. 57% in early May). This fall in prevalence is consistent with a cooling of the maximum sea surface temperature this summer by almost 3 C, to below 20 C. Other than blacklip abalone and one Roe?s abalone, no molluscs in the vicinity of the Taylor Island site were hosts of P. olseni, and this will simplify construction of the epidemiological model.

NOTES

Investigations on Microbial Pathogens Associated with Diseased Oysters

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The pearl and edible oysters, collected from Tuticorin of Tamil Nadu and Astamudi Lake of Kerala, India were subjected to pathological investigations. A total of 94 specimens of pearl oysters (Pinctada fucata) and 130 specimens of edible oysters (Crassostrea madrasensis) were examined. A few pearl oysters were found to have mantle discolouration and damaged tissues on the mantle and some edible oysters were characterized with abnormal yellowish ulceration on the mantle. Bacterial samples were taken from the diseased sites of both pearl and edible oysters. A total of 3 bacterial samples from pearl oysters and 2 from edible oysters were isolated. The isolated bacterial strains were tested for their pathogenicity (Koch's postulate). Among the three bacterial isolates, only one bacterium was found to be pathogenic against pearl oyster and one among the two of edible oysters was pathogenic. These two pathogenic bacterial isolates were subjected to several biochemical tests. Based on the biochemical properties, the bacteria, pathogenic for pearl oysters were characterized to be Vibrio sp., and that of edible oyster was Bacillus sp. These two bacterial pathogens were tested against ten antibiotics for their susceptibility. The most effective antibiotic against Vibrio sp., was Chloramphenicol and that of Bacillus sp., was Kanamycin. Invitro experiments were also carried out to test extracts of certain natural products for their bioactivity.

NOTES

Potential genes involved in immune response identified by expressed sequence tag analysis from scallop Chlamys farreri

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Chlamys farreri is one of the most important species of cultured scallop in China. In an effort to identify the genes related to cell or organism defense in the scallop, high quality cDNA library was constructed from an individual of Chlamys farreri infected by the pathogen of Vibrio anguillarum. A total of 6935 successful sequencing reactions yielded 4980 virtual sequences with the average length of 392.52 bp. All of the sequences were clustered into 581 contigs and 2751 single-ests. Blast analysis showed that 3750 ESTs, which accounts for 75.3%, are novel sequences without significant homology to any genes (e-value<5.0´10-2). 216 ESTs are matched to the genes of unknown function. 1014 ESTs are significant homology to the genes of known functions such as cell division, cell signaling or cell communication, cell structure or motility, gene or protein expression, metabolism and so on. Of these sequences, 200 ESTs are related to cell or organism defense including heat shock protein, cathepsin, thymosin, chitinase, selenium-binding protein, cyclophilin, glucan binding protein etc. The result provided a well-characterized EST resource for the genomics community study of scallop and also an approach for the discovery of new genes involved in immune defense. Key words: Chlamys farreri, expressed sequence tag, immunity, gene

NOTES

THEME 4

Development of a real-time PCR assay for the detection of Piscirickettsia salmonis

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1. CSIRO Livestock Industries

Rickettsia are a group of gram-negative bacteria which are obligate, intracellular parasites that can infect a range of vertebrate and invertebrate hosts, including finfish, and aquatic invertebrates. Piscirickettsia salmonis epizootics in several countries have been responsible for significant economic loss to the salmonid aquaculture industry. Recently, there have been a number of reports of Rickettsia-like organisms (RLOs) associated with disease in other farmed fish (e.g. tilapia, white sea bass) and marine organisms (e.g. abalone, crayfish). The threat of the possible introduction of P. salmonis to Australia has prompted the development of an RLO-specific, real-time PCR assay that provides a rapid means of identifying RLO-infected animals. In this report we describe the use of real-time PCR as a diagnostic tool for the specific detection of laboratory grown exotic strains of P. salmonis. This study was partly funded by FRDC project number: 2001/624

NOTES

Development of diagnostic antibodies specific for white spot virus

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1. CSIRO Livestock Industries

White spot disease (WSD) of penaeid prawns, first reported from farmed Penaeus japonicus in Japan in 1993, has since spread throughout the prawn farming regions of Southeast Asia and beyond. Over this period, there has been a huge effort throughout the region to develop diagnostic tools for the detection and identification of white spot virus (WSV) infected prawns, including a number of PCR assays as well as immunoassays based on WSV-specific polyclonal and monoclonal antibodies. At the Australian Animal Health Laboratory (AAHL), polyclonal and monoclonal antibodies have been raised against white spot virus and it is anticipated that such antibodies will underpin development of a number of diagnostic tests. Progress on this project is reported here. White spot virus was imported from Thailand and expanded using experimental infections of penaied prawns. Following infection, prawns were bled, and virus was purified from the prawn haemolymph using sucrose gradient centrifugation. Purity of viral fractions was monitored using SDS-PAGE and electron microscopy. Following purification, viral fractions were used to immunise rabbits and mice for production of specific antibodies. Out of a total of 1500 hybridoma supernatants, screened by ELISA, 20 cultures were demonstrated to contain antibodies with high activity. Preliminary characterisation of the polyclonal and monoclonal antibodies specific for white spot virus is presented. This study was partly funded by ACIAR project number FIS9698

NOTES

Functional microarray analysis of Japanese flounder Paralichthys olivaceus immune related genes for selection of a disease resistance fish

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The use of DNA markers to define the genotype and predict the performance of an animal is a powerful aid to animal breeding. One strategy is known as marker-assisted selection (MAS). Recently, a new technology Microarrays has developed in parallel with the genome projects of humans and other model organisms. Microarrays allow the gene expression profiles of tens of thousands of genes to be compared in a single experiment. The use of high throughput DNA microarray technology is thought to be revolutionize animal breeding in near future. In view of the above reasons, we constructed cDNA microarray and to assess a possibility of using fish cDNA microaary for selection breeding. We used about 850 different cDNA clones from more than 3,000 cDNA clones which were identified by the expressed sequence tag (EST) analysis of Japanese flounder, Paralichthys olivaceus liver, spleen, skin, hirame rhabdo virus infected Japanese flounder leukocytes and Ig positive cells, and ConA/PMA or LPS treated leukocytes and kidney cells cDNA libraries for making cDNA microarray. The target mRNAs were prepared from LPS or ConA/PMA treated peripheral blood leukocytes (PBLs) and kideney and rhabdovirus infected PBLs of Japanese flounder. The target mRNAs were labeled with either Cy3 or Cy5 using the commercial labeling kit. After hybridization, the slide was scanned using the Genepix400B Scanner. The expression patterns and the amount of expressed mRNAs of these genes were different in the various genes examined, stimulations and time points. These gene-expression profiling studies of Japanese flounder have suggested some key molecular players in a large number of immune-regulated genes with unknown immunological function. All of our results suggest that the using microarray technology will be enable for selection breeding in aquaculture.

NOTES

Detection and identification of Pseudomonas spp. by polymerase chain reaction-reverse cross blot hybridization (PCR-RCBH) with 16S-23S rRNA intergenic spacer probes

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Pseudomonas causing haemorrhagic bacteraemia is frequently found in fish with a moderate to high mortality. Three species are commonly isolated from tropical fish including P. fluorescens, P. putida and P. aeruginosa. A variety of methods have been used to identify Pseudomonas spp. including biochemistry and immunological-based methods. However, these methods are unable to differentiate between different species of Pseudomonas. Polymerase chain reaction (PCR) followed by reverse cross blot hybridization (RCBH) was adapted in this study to speciate Pseudomonas. Primers were designed for an amplification of the 16S-23S rRNA spacer regions of Pseudomonas. The PCR products were analysed in a reverse cross blot hybridization assay with four probes specific to the genus and three species. The specificity and sensitivity of these probes were examined by testing them against a collection of 10 Pseudomonas spp. and 11 strains from other bacteria. The method was highly specific for Pseudomonas spp. and identified the bacteria to species level with a detection limit of 200 cells/ml.

NOTES

Development of onfarm diagnostics

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As industrialization and human activity has expanded and intensified, the microbes and diseases have also increased exponentially, putting tremendous pressure to find ways and means for early detection and control of these pathogens. The first step in the successful control is the early detection of microbes and immediate control measures. The different methods of disease detection are: (i) Gross symptoms (ii) Post mortem examination (iii) Wet mount (iv) Electron microscopy (v) Histopathology (vi) Microbiology (vii) Immunodiagnostics (viii) DNA based diagnostics. The need of the hour is to have rapid, on-site immunodiagnostics which do not need sophisticated equipments and trained manpower. Antibodies are host proteins produced in response to the presence of foreign molecules in the body by the immune system. Functionally they are characterised by their ability to bind to specific antigens. These antibodies are specific for each antigen and are used for the development of immunodiagnostic kits like 1.ELISA test 2. Dot-ELISA test 3. Latex agglutination test The paper describes the development of these rapid techniques for identification of bacteria (Vibrio alginolytcus) and viruses (Yellow head virus), the advantages of each method and their stability.

NOTES

Plasmid profile of bacterial isolates from white spot affected shrimps

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Samples of white spot infected shrimps (Penaeus monodon) were collected from the East coast of India. The samples were analysed and 84 presumptive vibrio isolates were collected from shrimps exhibiting various degree of infection. These isolates were examined for their antibiotic resistance. Presence of plasmids was found in strains exhibiting multiple drug resistance. Restriction analysis of the plasmids indicated that isolates contained plasmids belonging to different groups. The study demonstrated that the plasmid profiles of the isolates could be used as a useful indicator to investigate the strain variability of the Vibrio strains. Plasmid curing studies conducted revealed that some of the strains have antibiotic resistance associated with R factor connected with plasmids. This could imply that the resistance transfer could happen from the farm isolates to microbes of public health significance.

NOTES

Experimental Bacteriophage-mediated virulence in strains of Vibrio harveyi

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1. James Cook University

Vibriosis is a major disease of prawn aquaculture. Until now there has been no clear explanation why some strains of Vibrio are pathogenic, while others are not. This study demonstrated that the presence of the bacteriophage, Vibrio harveyi Myo-Like virus (VHML) may confer virulence in Vibrio harveyi strain 642. This was demonstrated by infecting naive avirulent V. harveyi strains 12, 20, 45 and 645 with the bacteriophage and converting them into virulent strains. The previously naive strains of Vibrios infected with the bacteriophage, VHML, from V. harveyi strain 642 demonstrated up-regulation of haemolysin, up-regulation of protein excretion, additional proteins which were recognised as toxic proteins from strain 642 by monoclonal antibodies specific to the exotoxin sub-units and a significant increase in mortality of larval Penaeus monodon. It was concluded that the bacteriophage VHML conferred virulence to V. harveyi strain 12, 20, 45 and 645 and that the bacteriophage VHML either fully or partly confers virulence in V. harveyi strain 642.

NOTES

Development and characterization of a monoclonal antibody against white-spot syndrome virus in Penaeid shrimp

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White spot syndrome virus (WSSV) is a major viral disease agent of penaeid shrimp. Number of studies have been done for basic research and development of a diagnostic kit for WSSV. One of the promising diagnostic tools to detect viral pathogens in shrimp farms must be the immunological methods by using monoclonal antibody because it doesn't need any expensive equipment and time for the analysis can be very short. However, since WSSV is a virus encapsulated with envelope protein and the envelope protein are very fragile and easily destroyed by ordinary virus purification procedure including the steps that gives physical stress to WSSV. Purification of pathogen is an essential step for a manipulation of monoclonal antibody to develop a diagnostic kit. This work will focus on the development of a new method to purify the virion of WSSV and to obtain IgG monoclonal antibodies against envelope proteins. The monoclonal antibodies that recognize WSSV envelope proteins, VP29 and VP19 specifically were successfully isolated from this work. The antibodies obtained here can be used for detection of WSSV in infected Penaeus japonicus. After confirmation of the antibodies specificity against WSSV from different countries, these antibodies would be a great help to develop a diagnostic kit for detection of WSSV in shrimp farms in several countries. Moreover, some of the antibodies obtained in this study must have the activity to block the viral infection because it is known that envelope proteins play an important role in WSSV infection. The investigation on the blocking activity of the obtained antibodies is now undergoing. The antibody that can block viral attachment to the host shrimp cell will be a powerful tool for the farther study on the infection mechanism of the WSSV.

NOTES

Detection and comparison of lymphocystis virus in flounder (Paralichthys olivaceus) and sea bass (Lateolabrax japonicus)

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2. The Japanese Society of Fish Pathology

Lymphocystis disease has occurred in various kinds of marine fin fish in the world. This study was performed both to explore the host spectrum of lymphocystis virus (LV) among cultured marine fishes and to examine the phylogenic position of the LV in Korea. LV-infection was confirmed on the basis of histopathological examination, analysis of structural protein and nucleotide sequence analysis of partial gene, then LV was identified from fishes including flounder (Paralichthys olivaceus) and sea bass (Lateolabrax japonicus). LV-Infected fishes developed lymphocystis on the body surface, fin, gill and intestine. Electron microscopic observation revealed that the virus was large icosahedral structure, 150-200nm in diameter that assembled in cytoplasm of infected cell. Infected cell was encapsulated by hyaline extracellular matrix and contained basophilic intracytoplasmic inclusion and condensed nucleus. Viral proteins of the two LVs isolated from flounder and sea bass were analyzed by SDS-PAGE, and the resulting electrophoretic profiles were different from each other. The result of nucleotide sequencing for a partial gene of LVs from olive flounder and sea bass showed 98% and 88% homology respectively with those of LCDV-1 in GenBank. There was phylogenetic difference between LV from seabass and LV from flounder.

NOTES

Identification and diagnosis of Aphanomyces piscicida by PCR

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Some primers were designed from the sequence data of Aphanomyces sp. 84-1240 in Gene Bank to determine the sequence of ITS1, 5.8S and ITS2 genes of Aphanomyces piscicida, which is pathogen of mycotic granulomatosis in fishes. The sequence identity of this region was 100% among samples from diseased fishes in several countries. The specific primers were designed from sequence data of fish pathogenic Aphanomyces piscicida. The PCR using the specific primers detected only Aphanomyces piscicida, but not Saprolegnia spp., Achlya spp., the other Aphanomyces spp. Lagenidium spp. and Dictychus sp. The PCR method was also useful for the detection of the pathogen in the lesion of goldfish, Carassius auratus, artificially infected with A. piscicida. It was demonstrated that the primer set designed in this study was effective for identification of A. piscicida and diagnosis of mycotic granulomatosis in fishes.

NOTES

Development of a monoclonal antibody to hybrid catfish (Clarias macrocephalus x C. gariepinus) immunoglobulin

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Monoclonal antibodies (Mab) are commonly used to examine the immune response of fish. A Mab was developed here against the immunoglobulin of hybrid catfish (Clarias macrocephalus X C. gariepinus) for use as a probe to monitor the antibody response of this fish species against different pathogens. The Mab, referred to as B6 was of an IgG 2a subclass with k light chain. The Mab reacted strongly with the immunoglobulin of hybrid catfish when used in both enzyme linked immunosorbent assay (ELISA) and Western blot (WB). The Mab was found to react with the heavy chain of the hybrid catfish immununoglobulin in WB analysis, identifying a band at 66 kDa. The cross reactivity of the MAb was examined by ELISA against eight different fish species, including snakehead (Channa striata), giant snakehead (Channa micropeltes), Nile tilapia (Oreochromis niloticus), common carp (Cyprinus carpio), silver barb (Babodes gonionotus), rohu (Labeo rohita), grouper (Epinephelus malabaricus) and seabass (Lates calcarifer). The Mab was found to react with the immunoglobulin of common carp (Cyprinus carpio) and Silver barb (Babodes gonionotus) at 61% and 45% respectively, when compared to the hybrid catfish immunoglobulin.

NOTES

Cloning and expression of variable region of nucleocapsid gene of aquatic morbilliviruses for serological diagnosis

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Morbilliviruses constitute a major threat to the health of animals and man; they group in the Morbillivirus genus in the family Paramyxoviridae The more commonly known members are measles virus (MV), canine distemper virus (CDV), rinderpest virus (RPV), and peste-des-petits-ruminants virus (PPRV). In the last 14 years, newly recoznised members of the morbillivirus family have caused many deaths among marine mammals, specifically pinipeds and cetaceans. These viruses are phocine distemper virus (PDV) found in pinnipeds and two closely related viruses isolated from cetaceans: dolphin morbillivirus (DMV) from striped dolphins and porpoise morbillivirus (PMV) from harbour porpoises. Serological and molecular studies have shown that cetaceans of many species have been exposed to these latter two viruses. The cetacean morbilliviruses are thought to spread to other species by means of the pilot whale which is considered to be the main vector of the virus. Altered migration patterns in animals caused by environmental changes is thought to be the origin of the epizootic of PDV in European seal in 1988. In order to determine the prevalence and understand the epidemiology and of these marine morbilliviruses it is necessary to develop simple, inexpensive and rapid differential diagnostic tests. The nucleocapsid (N) protein of the virus has a highly variable C-terminal region, which is also highly immunogenic. In the present study we have cloned and expressed the variable region of N protein gene from aquatic morbilliviruses (DMV and PDV), as well as from all other species of morbillivirus (RPV, PPRV, CDV and MV), to produce virus-specific antigens for developing specific serological diagnostic tests.

NOTES

Ultrastructural changes in the sperm and eggs of the black tiger shrimp, Penaeus monodon, before and after fertilization

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3. Shrimp Culture Research Center, Charoen Pokphand Foods Public Company Limited

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This series of studies were aimed at revealing the changes of the sperm and eggs before and after fertilization of the black tiger shrimp Penaeus monodon. The first study was to investigate the morphological events of the sperm at the time of acrosomal reaction. The sperm were incubated with egg water and the acrosomal reaction was observed under SEM and TEM. The sperm of P. monodon were nonmotile and composed of posterior spherical main body, central cap and anterior single spike extending from the cap. In the acrosomal reaction, the sperm underwent two phases of ultrastructural changes, acrosomal exocytosis and acrosomal process formation. It began with a gradual degenerative change of the spike. While the spike was degenerating, the cap region became enlarged; the outer and inner membrane swell and burst. Finally, the subacrosomal region was polymerized to form an acrosomal process. The second study was to compare the acrosomal reaction of the sperm between those from the male spermatophore and those from the female thelycum, following contact with egg water. It was found that 95% of the sperm from the female thelycum had an acrosomal reaction, while only 30% of sperm from the male spermatophore did. The third experiment was aimed at revealing the morphological changes of the eggs upon contact with seawater, the process known as egg activation. As soon as the oocyte was released from the gonopore into seawater, the cortical rods that appeared in the peripheral cytoplasm were released out from the oocyte surface. At the early stages of egg activation, the rods began to emerge from the cortical crypts of the oocyte and elevated the thin investment coat that encompassed the oocyte from its surface. Several sperm with the first phase of acrosomal reaction were observed on both the oocyte and the surface of the investment coat. The rods protruded from the surface and were completely expelled, within 45 sec after leaving the gonopore. Immediately after the completion of cortical rods extrusion, the rods began to dissipate and formed the jelly layer around the egg. By this time, the interaction between the sperm with second phase of acrosomal reaction and egg took place. Then the hatching envelope was formed at 1 min post-spawning, and the hatching envelope elevation was completed within 13-15 min post-spawning. The first and second polar body were extruded from the egg at 2-5 and 5-8 min post-spawning, respectively. During egg activation, exocytosis of three types of cortical vesicles; high-density vesicles, low-density vesicles, and granular vesicles occurred.

NOTES

A rapid multiplex real time PCR for the early detection of double targets: white spot syndrome virus (WSSV) and yellow head virus (YHV), in the black tiger shrimp Penaeus monodon

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5. Center for Excellence for Shrimp Molecular Biology and Biotechnology

WSSV and YHV cause high and rapid mortality in shrimp (Penaeus monodon). Recently, PCR has been commonly used for screening specific pathogen-free (SPF) shrimp in many hatcheries. However, performing PCR and RT-PCR assays take time; new quicker, multipurpose detection methods are being demanded by shrimp producers. The multiplex real time PCR is one route to classify quality of shrimp. Briefly, total nucleic acid of the viral and shrimp genetic materials were extracted, preserved and amplified with specific primer pairs for DNA virus, WSSV and RNA virus, YHV. The designed primers were optimized by annealing to two targets in one tube with fluorescent dye. The RT-PCR and PCR reaction were performed in one tube. Comparison between conventional and real time PCR was carried out. The results demonstrated that sensitivity was 10 times higher and 12 times faster than that of conventional PCR. By using the advantage of melting curve analysis, the optimized conditions could simultaneously generate two peaks of amplification products showing the different curve of both viruses based on their Tm. Analysis of the melting curve could demonstrate WSSV, YHV infection and no peak from uninfected organisms. Moreover, this method was tested with naturally infected with WSSV and YHV infected shrimp samples collected in Thailand. It works well with no gross sign shrimp from field. This detection system is rapid and sensitive for simultaneous dual detection. It should be a useful early detection system for shrimp culturists, especially for screening SPF broodstock and larvae in the hatchery.

NOTES

Theme 6

Infectious hypodermal and hematopoietic necrosis virus infection in Domesticated P. monodon broodstock

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About 30% of a domesticated Penaeus monodon broodstock line of the fourth generation (F4) were infected with infectious hypodermal and hematopoietic necrosis virus (IHHNV), as detected by polymerase chain reaction (PCR). The growth rate of these broodstock was normal, compared to non-infected cohorts. Tissues from female broodstock were prepared for examination by routine histology using H&E staining and by in situ hybridization using a DNA probe specific for IHHNV. The tissues examined included hematopoietic tissue, lymphoid organ tissue, connective tissue, muscle tissue, ovarial tissue, subcuticular epithelium and neural tissue. In the H&E stained sections, Cowdry A Type inclusion (CAI) bodies frequently observed in IHHNV-infected cells of P. vannamei and P. stylirostris, were not observed in any of these tissues. By contrast, in situ hybridization with IHHNV-PCR positive broodstock gave positive hybridization reactions for all these tissue types except neural tissue while IHHNV-PCR negative brooders gave no positive hybridization reactions. The most abundant staining reaction was observed in hematopoietic tissue and lymphoid organ tissue. In the ovary, positive hybridization reactions were absent in oocytes and confined to the ovarian capsule and some follicular cells. Several degenerated/resorptive oocyte were observed. The results suggested some difference in the tissue trophism of IHHNV infection in P. monodon when compared P. vannamei and P. stylirostris. They also indicated possible detrimental effects of IHHNV infection on reproductive performance of the domesticated P. monodon broodstock.

NOTES

Transcriptional analysis of the DNA polymerase gene of shrimp white spot syndrome virus (WSSV)

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The white spot syndrome virus DNA polymerase (DNA pol) gene (WSSV dnapol) has already been tentatively identified based on the presence of highly conserved motifs, but it shows low overall homology with other DNA pols and is also much larger (2,351 amino acid residues versus 913 ¡V 1244 aa). In the present study we perform a transcriptional analysis of the WSSV dnapol gene using the total RNA isolated from WSSV-infected shrimp at different times after infection. Northern blot analysis with a WSSV dnapol-specific riboprobe found a major transcript of 7.5 kb. 5¡_ RACE revealed that the major transcription start point is located 27 nucleotides downstream of the TATA box, at the nucleotide residue A within a CAGT motif, one of the initiator (Inr) motifs of arthropods. In a temporal expression analysis using differential RT-PCR, WSSV dnapol transcripts were detected at low levels at 2-4 h p.i., increased at 6 h p.i. and remained fairly constant thereafter. This is similar to the previously reported transcription patterns for genes encoding the key enzyme of nucleotide metabolism, ribonucleotide reductase. Phylogenetic analysis showed that the DNA pols from three different WSSV isolates form an extremely tight cluster. In addition, like an earlier phylogenetic analysis of WSSV protein kinase, the phylogenetic tree of viral DNA pols further supports the suggestion that WSSV is a distinct virus (likely at the family level) that does not belong to any of the virus families that are currently recognized.

NOTES

Potential indicators of stress response and their relation to survival in Penaeus monodon

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There is growing evidence describing the links between environmental stressors and the on-set of disease in many marine organisms including crustaceans. However, there is very limited data on the relationship between environmental conditions or stressors and variation of the immune system in marine invertebrates. Among the multiple environmental variables, temperature is potentially of great importance in ectothermic organisms since it directly affects their metabolism. A stress condition leads to an onset of molecular and physiological modifications, some which can be measured and used as molecular biomarkers. These have been used as an indication of cellular status, and hence of a stressed condition. Certain biomarkers, such as heat shock proteins (HSPs), have also been linked to the immune response in invertebrates. An analysis of these molecular biomarkers should provide information not only on the general health status of the organism but might be used as an alert system to help prawn farmers identify any environmental stressor which might induce the outbreak of a disease in the farm. As a test for the impact of temperature on prawns, four groups of 15 animals were exposed to 25, 27.5, 30 and 35 ¢ªC water temperatures, respectively. Survival ranged from 20% to 100% with highest mortalities in 35 C. Changes in expression levels of different molecular biomarkers in P. monodon were examined by Western Blotting and ELISA using invertebrate specific antibodies. The relationship between expression patterns in these molecular biomarkers will be discussed.

NOTES

A novel antimicrobial peptide isolated from the shrimp Fenneropenaeus chinensis after bacterial challenge

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Antimicrobial peptides are important for non-specific host defence in many animals. Here we report the presence of a novel antimicrobial peptide, active against both gram-positive and gram-negative bacterial, in the shrimp Fenneropenaeus chinensis. CZE (capillary zone electrophoresis) was applied to analyse hemolymph samples from shrimp before and after an immune challenge with the bacteria strains Vibrio anguillarum. The analysis of these spectra led to the following observation: the peptides relative concentration was found to increase by the first 3 hours post-challenge; and reached a maximum at 6 hours; after 24 hours, the plasma content of peptides appeared to be similar to that observed in unchallenged animals. On the basis of CZE and antimicrobial assay, the antimicrobial peptide was purified to homogeneity by Sep-Pak C18 extraction and reverse-phase HPLC. The partial N-terminal amino acid sequence obtained via Edman degradation revealed that it was proline rich and shared more than 60% identity in a 15-amino-acid overlap with the penaeidins, a family of shrimp Litopenaeus vannamei. The classification and differential count of circulating hemocytes have also been studied at different time after microbial challenge. The results showed that bacterial challenge triggered a plasmatic increase of the antimicrobial peptide concentration and gave implication of the simultaneous release of the peptide from the shrimp granulocytes.

NOTES

White Spot Disease in Penaeus monodon: case definition, accuracy of clinical diagnosis and description of an epidemic

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This paper presents a case definition for WSD. It is developed from a detailed longitudinal study of 24 ponds growing P. monodon in a rice-shrimp farming system in Vietnam. At pond level WSD is defined by the occurrence of any dead shrimp in which WSSV is detected. Using this definition the sensitivity and specificity of white spots on the carapace as a diagnostic sign for WSD was 84.6% and 81.8% respectively. The sensitivity of mortality was by definition 100% but the specificity was 45.5%. This low sensitivity was reflected by the fact that a third of 'emergency harvested' crops in this study were negative for WSSV. This case definition highlights the importance of farmer observation and rapid detection of WSSV in limiting the economic impact of disease. We suggest that, in open farming systems, WSSV PCR resources would be better targeted at rapid diagnosis than at screening post-larvae. WSD was detected in 54% (13/24) ponds in this study. The pattern of disease suggested size, development stage or density of P. monodon as important in initial disease occurrence and subsequently an incubation period of 7 days. There was evidence of clustering in space and time. The temporal clustering appeared to be associated with a fall in temperature and water exchange at spring tides. Cases were clustered spatially in the area nearest the sea and we propose that water exchange may be important not just in lowering the temperature but also in introducing infection into a pond. These results suggest that manipulating water exchange may be a strategy for preventing disease. In addition to its effect on spring tides, the lunar cycle may also have a direct effect on the susceptibility of shrimp by synchronising moulting. These hypotheses need to be tested in larger epidemiological studies. UK government DFID research project 7051.

NOTES

The PK1 protein of the white spot syndrome virus (WSSV) is a nuclear kinase with autophosphorylation activity

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White spot syndrome virus (WSSV) is a virulent pathogen causing high mortality in cultured shrimp, and its genome encodes a serine/threonine protein kinase (PK1) that contains the major conserved subdomains. To determine the cellular localization of PK1, EGFP-PK1 fusion protein was expressed in Sf9 cells. Full-length PK1 protein was found to localize in the nucleus of transfected cells suggesting that PK1 contains a nuclear localization signal (NLS). To assay for PK1 activity, the pk1 gene was expressed as a GST-fusion in E. coli and purified by glutathione-agarose beads. The fusion PK1 catalyzed an autophosphorylation activity when incubated with gamma-32P [ATP]. When domains VIB and VII were deleted, the autophosphorylation and the MBP phosphorylation activity were abolished. Subdomain VII of PK1 contains the highly conserved DFG triplet. When D586 was replaced with an R residue, the autophosphorylation and MBP phosphorylation activity were abolished. Subdomain VII includes an Mg2+-binding loop, and it was experimentally confirmed that PK1 phosphorylation activity was Mg2+-dependent; Mg2+ was not replaceable by Mn2+ or Ca2+. Taken together, the results show that the PK1 protein of WSSV is a nuclear kinase with autophosphorylation activity.

NOTES

Evaluation of pathogenicity of bacterial strains in crustacean larvae by static bath: significance of monitoring bacterial counts.

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Although pathogenicity test has become the accepted method to explain the course of disease, differences in protocol under which tests are performed may result in strong variations and cause misinterpretations. Bacterial challenge in crustacean larvae by means of static bioassay may be complicated by their associated microflora or feed, if it is given. Therefore, in studies where only the initial inoculum dose was determined, effects of possible growth and replication of bacterial pathogen in the test chamber were not accurately measured and reported. In the course of our study on microbial diseases of hatchery-reared crab larvae, several tests were simultaneously conducted to prove the pathogenicity of luminescent Vibrio harveyi or the benign effect of potential probiotic bacteria associated with the alga Chlorella sp. In both tests, inoculation of 102 bacterial colony-forming-units (cfu)/ml in UV-sterilized seawater always resulted in increase of bacterial numbers by at least 2 logs after 24 h, proving that in static bath bacterial bioassays, growth and replication of test bacteria occurs in the test chamber. However, when the bacterial inoculum is 105 to 106 cfu/ml, no significant change in bacterial numbers occur. Furthermore, control chambers with no bacteria inoculated, were found to harbor between 104 to 105 cfu/ml of mixed bacterial population after 24 h, part of which is luminescent Vibrio. As these tests were conducted without feeding, the results prove that bacteria associated with the test larvae also proliferated in significant numbers inside test chambers. Thus, continued monitoring of bacterial population in the test chambers needs to be done to show its interaction with the already established microbiota in the larvae. Meaningful results of static bioassay of bacteria on crustacean larvae need to present a correlation of bacterial flora in tests chambers with survival of larvae.

NOTES

Growth pattern of Vibrio parahaemolyticus, V. alginolyticus and V. harveyi isolates from Malaysia

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Vibrio parahaemolyticus, V. alginolyticus and V. harveyi isolated from the shrimp cultures in Malaysia were studied for their growth pattern for 24 hours post incubation (hpi). Tryptone Soya Broth incorporated with 1.5% sodium chloride was used for growing with orbital shaking. A 1 ml of broth was sampled from each isolate hourly for 24 h. Optical density was read at 600 nm using a spectrophotometer. Results showed that V. parahaemolyticus had the fastest growth rate within 5 hpi followed by V. alginolyticus and V. harveyi. Vibrio parahaemolyticus entered log phase at _ hpi, followed by V. alginolyticus at 3 hpi and V. harveyi at 4 hpi. Vibrio alginolyticus however overcame V. parahaemolyticus at 5 hpi while V. harveyi was still far lagging behind. Vibrio harveyi and V. parahaemolyticus entered the stationary phase earlier at 7 hpi compared to V. alginolyticus at 10 hpi. At 24 hpi, all three species were in stationary phase. Generation time for V. alginolyticus, V. parahaemolyticus and V. harveyi were also determined. The present findings explain the observation of dominance of V. alginolyticus in the Vibrio flora.

NOTES

Electro-chemical processes for phytoplankton control and shrimp disease disinfection

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Some species of phytoplankton produce compounds that are extremely toxic to aquatic animals. Mass mortality of shrimp was observed to coincide with blooms of some species of Oscillatoria. Even in the case of beneficial phytoplankton massive algal die-off triggered the increase in pathogenic bacteria resulting in the mass mortality of shrimp. The control of phytoplankton over-blooming remains to be one of the serious problems in intensive shrimp culture. Electro-chemical processes offer several promising approaches for the prevention and remedy of pollution problems, and it has been widely applied in the drinking water industry and wastewater treatment. However, its application in the field of aquaculture is still very limited. We found that electro-chemical processes like electro-flotation and electro-oxidation are effective not only for controlling phytoplankton over-bloom but also for ammonia removal and inactivation of pathogenic bacteria.

NOTES

Lagenidium thermophilum isolated from zoeae of black tiger (Penaeus monodon) in Thailand

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Zoeae of black tiger, Penaeus monodon, have been produced at many hatcheries in Chachemgsao Province, Thailand. Fungal infection caused by lower fungi, however, sometimes occurred in the zoeae, and it has known as the problematic disease. We had a chance to examine the fungal infection in the black tiger zoeae in Aug 2000. As a result, all fungi isolated from the moribund zoeae were classified into the genus Lagenidium, because the fungi formed vesicle at the top of discharge tube. A typical isolate (NJM 0031) was used in all experiments. Zoospore liberation of the isolate occurred after the vesicle was separated from the discharge tubes. Based on the morphological characteristics, the isolate NJM 0031 was identified as Lagenidium thermophilum which was reported by Nakamura et al. (1995). The phylogenic relationships between Lagenidium spp. isolated from crustaceans and some fungi in other Oomycetes were explored by comparing the sequences of their 18S rDNA. As a result, L. thermophilum was clearly separated from the fungi of the order Saprolegniales.

NOTES

Prevalence of white spot syndrome virus (WSSV) and Monodon baculovirus (MBV) infection in Penaeus monodon postlarvae in Vietnam

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A survey on the nature and prevalence of white spot syndrome virus (WSSV) and Mondodon Baculovirus (MBV) infection in Penaeus monodon postlarvae collected in hatcheries and nurseries in the centre and the south of Vietnam was conducted from the period between mid October 2001 to mid May 2002. Four hundred seventy-one samples were collected and subjected to two-step nested ploymerase chain reaction (PCR) analysis for WSSV using the IQ 2000 WSSV kit (Farming IntelliGene Technology Corporation, Taiwan). Of these, three hundred and eighty-eight samples were also tested for MBV using rapid staining method with Malachite green (lightner et al. 1996). The prevalence of WSSV infection in postlarvae collected from hatcheries and nurseries ws 20.6% whereas 46.4% prevalence was found in samples tested for MBV. In addition, it was found that prevalence infection of WSSV fluctuated according to sampling month and was the highest in February (37.7%) while prevalence infection of MBV remained the same throughout sampling months. Postlarvae samples from the central region, where hatcheries supply approximately 80% postlarvae for grow-out ponds in southern provinces, displayed a significantly higher prevalence of WSSV infection (21.1%) than those from the south (15.7%) ($P<0.05$). However , prevalence of MBV infection on postlarvae from the central part was not significantly different compared with samples from the south (44.3% and 46%, respectively) ($P<0.05$). The statistical significance of the differences in prevalence indicated that quality of postlarvae varies by production points and sources of supply. Therefore, the implications of the findings for shrimp health management in Vietnam will be discussed in this paper.

NOTES

Comparison of penaeid shrimp and insect parvoviruses suggests that viral transfers may occur between two distantly related arthropod groups

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The DNA and putative amino acid sequences of representative insect and shrimp parvoviruses (subfamily Densovirinae) were analyzed using computer programs. Shrimp viruses included hepatopancreatic parvovirus (HPV) of Penaeus monodon (HPVmon) and P. chinensis (HPVchin), spawner-isolated mortality virus from P. monodon (SMVmon) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) from P. vannamei. Insect viruses included Aedes aegypti densovirus (AaeDNV), Aedes albopictus densovirus (AalDNV), Junonia coenia densovirus (JcDNV), Galleria mellonella densovirus (GmDNV), Bombyx mori densovirus 5 (BmDNV), Diatraea saccharalis densovirus (DsDNV) and Periplaneta fuliginosa densovirus (PfDNV). Virion size for all these viruses ranged between 18 and 30 nm diameter and ssDNA genome length was between 4-6 kb. Using BLAST or Clustal W with the sequence fragments available, no significant DNA homology was found except for 77% DNA identity between HPVmon and HPVchin. However, phylogenetic trees constructed by comparing DNA genome sequences for putative viral polypeptides, capsid proteins and nonstructural proteins placed the parvoviruses into two Clades: Clade 1 with SMVmon, PfDNV, DsDNV, GmDNV, JcDNV, and BmDNV; and Clade 2 with HPVmon, HPVchin, IHHNV, AalDNV and AaeDNV. The 4 shrimp parvoviruses fell into two different groups that grouped in different insect parvovirus clusters.

NOTES

The white spot syndrome virus (WSSV) infects specific hemocytes of the shrimp Penaeus merguiensis

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The white spot syndrome virus (WSSV) was specifically detected by PCR in Penaeus merguiensis hemocytes, hemolymph and plasma. This suggested a close association between the shrimp hemolymph and the viruses. Three types of hemocytes from shrimp were isolated using flow cytometry. Dynamic changes of the hemocyte subpopulations in Penaeus merguiensis at different times after infection were observed, indicating that the WSSV infection selectively affected specific subpopulations. Immunofluorescence assay (IFA) and Wright-Giemsa double staining were performed to confirm this observation and to identify the hemocyte types susceptible to WSSV infection. These results revealed the cellular localization of the virus in the infected hemocytes. Electron microscopy showed that virus particles were found in both vacuoles and the nucleus of the semigranular cells (SGC) and vacuoles of the granular cells (GC). However, no virus could be detected in the hyaline cells (HC). Our results indicated that the virus infected two types of shrimp hemocytes: SCGs and GCs. Since SGCs express higher virus loads and exhibit faster infection rates, these cells are apparently more susceptible to WSSV infection.

NOTES

Prevalence of hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV) in stunted Penaeus monodon in Thailand

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The prevalence of hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV) in cultured P. monodon in the central and southern part of Thailand were examined using both single PCR and multiplex PCR techniques. The stunted shrimp samples consisting of juvenile and adult (45-120 days of culture) were collected during 2000-2001 and screened for HPV and MBV infection. It was found that the prevalence rate of HPV infection in Phuket, Pattani, Ratchaburi, and Pathum Thani provinces were 93.3% (14/15), 80.0% (12/15), 80.0% (12/15), and 46.7% (14/30), respectively. The MBV infection was investigated in Ratchaburi, and Pathum Thani provinces where the rate of infection were 3.4% (1/29) and 53.3% (16/30), respectively. The concomitance infection of HPV and MBV was also determined in Ratchaburi and Pathum Thani provinces. The data revealed that the multiple infection was detected in both areas with the prevalence rate of infection at 31.0% (9/29) and 43.3% (13/30), respectively. These result was preliminary information for the prevalence of HPV and MBV infection in Thailand.

NOTES

Comparison of various WSSV-PCR detection assays using naturally infected shrimp in Thailand and a fluorogenic WSSV probe as the gold standard

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Recently, PCR has been used to screen for White spot syndrome virus (WSSV)-free shrimp in many hatcheries. However, different PCR assays may give different results. In this study, four PCR assay methods were compared using shrimp naturally infected with WSSV. One method performed on ORF91 region (named primers as 91F-91R), another used the P1-P2 primer developed in Thailand and yet another used a method developed in Japan. A set of fluorogenic probe and primers was developed and used as the gold standard for these methods since it could be quantified to determine the number of target copies. The probe used was designed to target a region of the WSSV genome which corresponded to that targeted by the OIE assay. Total nucleic acid (TNA) was extracted by our protocol before the PCR. Results demonstrated that the probe was more sensitive and more rapid than conventional PCR followed by gel analysis. The time required for the assay was 20 min. Sensitivity was higher than that for the first PCR amplification round of the OIE method. It was 100 times higher than that for the primers 91F-91R and P1-P2. In addition, it was 100 times higher than that for the first step and 10 times higher than that for the second step of the Japanese method. The probe assay showed no cross-reaction with TNA extracts from YHV, MBV, HPV and IHHNV infected shrimp samples. This rapid, quantitative method will be useful for studying WSSV thresholds in shrimp under various environmental or stress situations.

NOTES

A dual, real time PCR for the simultaneous detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in the black tiger shrimp Penaeus monodon

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White spot syndrome virus (WSSV) and yellow head virus (YHV) cause high and rapid mortality in shrimp (Penaeus monodon) and PCR has been commonly used for their detection in hatchery broodstock and in post larvae before stocking in shrimp ponds. However, performing PCR and RT-PCR assays is time consuming so that more rapid and simple, multipurpose detection methods are in demand by shrimp producers. Thus, we developed a duplex, real time PCR method to screen shrimp for WSSV and YHV. Briefly, total shrimp nucleic acid was extracted, preserved and amplified with specific primer pairs for the DNA virus WSSV and the RNA virus YHV. The RT-PCR and PCR reactions were performed in one tube. By taking advantage of melting curve analysis, simultaneously generated products gave different curves based on their Tm. Comparison of sensitivity between conventional PCR and the real time PCR was carried out using standard plasmid DNA for positive controls. The sensitivity of the dual detection method was 10 times higher than that of conventional PCR and could be completed 12 times faster. The method was tested with shrimp samples naturally infected with WSSV and YHV and it worked well with lightly-infected shrimp that showed no gross signs of disease. This detection system is rapid and sensitive and suitable for early viral detection in shrimp ponds or for screening broodstock, larvae and post larvae in the hatchery.

NOTES

Effect of Tilapia Tilapia hornorum on luminous bacteria Vibrio harveyi

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In this study, the effect of tilapia Tilapia hornorum, stocked at different biomass, on Vibrio harveyi was investigated using 3-ton concrete tanks filled with micro-filtered water (5 micron) with salinity of 24 ppt, covered with black plywood to prevent sunlight penetration. All tanks were stocked with Penaeus monodon (20g) at 4 pcs/ton. Tanks for treatment 1 were stocked with 1 kg/10 m3 tilapia, treatment 2 at 3kg/10m3, and 5 kg/m3 for treatment 3. No tilapia was stocked in tanks that served as the control. Tanks were inoculated with luminous bacteria, V. harveyi to a final concentration of 103 cfu/ml 6 hours after stocking with tilapia. There were two replicates per treatment. Luminous bacteria, total bacteria, fungal, and algal count were monitored regularly. Results showed that no luminous bacteria could be detected in treatments 2 and 3 from day 6 until termination on day 28. Luminous bacteria can still be observed in treatment 1 and the control on day 28. Total bacterial count and fungal count were comparable in all tanks. No algal growth was observed in all treatments by microscopic examination using a haemacytometer. Results suggest that the presence of Tilapia hornorum, in a shrimp culture system, at a density not lower than 3kg/10 m3 can control the occurrence of luminous bacteria. This culture system is sustainable, easy to manage, and cost less and therefore could be an alternative method to prevent the occurrence of luminous bacterial disease in shrimp

NOTES

Identification of Penaeus monodon baculovirus (MBV) in cultured P. semisulcatus in Islamic Republic of IRAN

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Shrimp aquaculture in I. R. Iran has a short history. Farmed shrimp production grow slowly from 1992 until 2000 when a number of large farm started to come production. In the 2000 the production was 4,500 MT and estimated that more than 7,000 MT will be produce by the 2001. During the period from August 1997 to March 1998, two thousand samples of cultured Penaeus semisulcatus postlarvae and subadults were collected from 5 hatcheries and 20 growout farms distributed in 3 province along the cost of Persian Gulf and Oman Sea. Based on grows sign, Histopathology, LM and TEM, Penaeus monodon baculovirus (MBV) have been recorded from the samples. The MBV is a rod-shaped baculovirus, DsDNA virus and the virion size is 300±75 nm in diameter. The target organ of virus is hepatopancreas and midgut epithelium

NOTES

Application of PCR to study the prevalence of white spot syndrome virus (WSSV) and Monodon Baculovirus (MBV) in penaeus monodon hatcheries in South East Coast of India

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Diseases caused by white spot syndrome virus (WSSV) and monodon baculovirus (MBV) are a major problem for shrimp aquaculture in India and other countries of Asia. The major routes of entry of these viral pathogens into the aquaculture systems are through the infected broodstock and through infected larvae. Screening of shrimp broodstocks and postlarvae for the presence of WSSV and MBV by a sensitive method that can detect the infection status will greatly reduce the risk of disease outbreak in the pond. A sensitive assay of the carriers/ water entering into the culture system will also help evolve suitable management measures for eliminating these pathogens and subsequent outbreak of diseases in the culture systems. Polymerase chain reaction (PCR), a highly sensitive method that has been proven beyond doubt as the most suitable method to detect the viral pathogens when present even in low levels was employed in this study. During the period March 2000 to May 2002, postlarvae and broodstocks collected randomly during different months from the shrimp hatcheries in Southeast coast of India were screened for WSSV and MBV by PCR. Nested PCR was performed for detection of WSSV while one step PCR was carried out for MBV. Prevalence of WSSV ranged from 10-13% in postlarvae and 25-50% in the broodstocks while the prevalence of MBV ranged from 34-39% in the postlarvae and 25-60% in the broodstocks. The success of the culture in relation to the PCR results obtained will also be discussed.

NOTES

Does spawning stress trigger the replication of white spot syndrome virus in shrimp

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In shrimp, a persistent viral infection can be converted to productive viral infection by stressors such as poor water quality and overcrowding. Here we investigate whether the stress associated with spawing can also trigger virus replication in shrimp. Quantative real-time PCR was used for a temporal analysis of WSSV loads in a batch of 14 wild-caught black tiger shrimp (Penaeus monodon) brooders. For these specimens, four basic patterns emerge: Group 1 was comprised of 4 specimens which had a relatively high initial virus load (approximately 8 x 102 ~ 8 x 103 viral DNA copies/per microgram total DNA) and in these specimens, the virus replicated rapidly up to the time of spawning. After spawning the virus levels remained high and all of these shrimp died within a few hours. Two other groups (II and III) both had similar initial virus loads (approximately a 1 x 101 ~ 6 x 102 viral DNA copies/per microgram total DNA), but in group II (5 specimens), as in group I, the virus replicated rapidly up to and beyond spawning and all the shrimp died soon after spawning, whereas in Group III (3 specimens) the virus load increased only relatively slightly (approximately a 10-fold increase) and the shrimp survived well beyond spawning. The group IV (2 specimens) shrimps did not spawn at all during the observation period, and no viral replication was triggered. At this time, it seems clear that spawning stress can trigger WSSV replication, but it is not known why or how this does not occur in some (25%) specimens.

NOTES

Phylogenetic analysis of replicase (ORF1b) amplicon sequences reveals a fourth genotype of yellow head complex virus in P. monodon from India

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Yellow head virus (YHV) and gill-associated virus (GAV) are closely related pathogens of farmed shrimp in the Asia-Pacific region. Yellow head disease was first reported in Thailand in 1990. Although there have since been reports of yellow head disease in other Asian countries, YHV has been positively identified only in diseased P. monodon from Thailand and Taiwan. GAV was first isolated from diseased shrimp from northern Australia in 1996 but the virus is also known to occur commonly as a chronic infection in healthy Australian P. monodon. We have recently reported that a third genotype in the yellow head complex occurs commonly in healthy P. monodon broodstock from Thailand and in postlarvae from Vietnam. The third genotype is more closely related to GAV than YHV and has not yet been detected in diseased shrimp. In this paper, we describe the identification of a fourth virus of the yellow head complex in healthy postlarvae from Andra Pradesh in India. The new virus was detected by phylogenetic analysis of nucleotide sequences amplified from a 612 nucleotide region of the replicase (ORF1b) gene. The Indian virus is a new genetic lineage that is distant from all 3 previously known genotypes in the complex and is not detected by diagnostic PCR tests currently in use. In the amplified region, the new virus shares approximately 81-82 % nucleotide sequence identity with YHV, GAV and the third genotype from Thailand and Vietnam. By comparison, GAV and YHV share approximately 83% identity. The third genotype shares 82-83% identity with YHV and approximately 92% identity with GAV. We are continuing to investigate the distribution of all 4 genotypes in the Asian region and seeking evidence of the association of the new genotypes with outbreaks of disease.

NOTES

Attempts to establish continuous cell lines from prawn tissues

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Virus isolation in cell culture is considered the most sensitive and reliable technique for the detection of viral pathogens. Moreover, the ability to grow virus in culture should provide a good source of virus for research purposes including characterisation of the virus and development of diagnostic procedures. The current lack of continuous prawn cell lines is a major limiting factor not only for diagnosis of viral diseases of prawns but also for progress on research into these diseases. In the past, numerous attempts to obtain continuous cell lines from a number of prawn species have been made, with limited success. This report describes attempts to establish continuous cell lines by mutagenesis of primary cell cultures of Penaeus monodon and P. japonicus prawn tissues. Primary cell cultures from various organs of post larval prawns, as well as embryonated eggs, were initiated. Although primary cultures were established from lymphoid organ, very few, if any, cells in these cultures were actively replicating, and thus would not be susceptible to mutagenesis. While continuous cell lines were not established, this study has identified specific areas for further research which may enable the development of continuous prawn cell lines. Research may include, identification of prawn tissues which, under normal conditions, retain the potential for cell division; analysis of prawn haemolymph and identification of growth factors to aid the development of specific cell culture media; transformation by chemical mutagenesis of actively replicating prawn cell cultures and the use of somatic cell genetics to facilitate cell line development.

NOTES

Cloning and characterization of the White spot syndrome virus (WSSV) p25 gene and its promoter region

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A complement DNA (cDNA) encompassing a 672bp open-reading-frame (ORF) was isolated from the pooled heads of WSSV-infected shrimp by RT-PCR. The ORF encodes 224 amino acids with estimated pl of 4.69 and calculated molecular mass of 25.0 kDa, bearing no significant homology to any known genes and thereby being called p25 gene . The deduced amino acid sequence contains a putative EF-hand calcium-binding domain and a C2H2 Zinc finger domain, predicting a possible involvement in DNA-protein interaction and transcription regulation. The transcript of the gene was determined using 5' RLM-RACE and 3' RACE. The transcription appeared to be initiated from a conserved CAGT motif consistent to that of early genes of many baculovirus and insects. 5' non-coding region of the gene was inserted before the luciferase gene in phRG-b vector to investigate the promoter efficiency. Transient transfection assay performed on insect sf9 and monkey Marc145 cells revealed that this non-coding region has a remarkable promoter activity. The minimal sequence required for basal promoter efficiency was mapped with a panel of 5' end-truncated fragments of the non-coding region, and the promoter activity was also investigated in various cells including human TK143, monkey Marc145 and Vero, porcine PK15, chicken embryo-fiboblasts, carp EPC, insect sf9 and shrimp haemocytes. In addition, p25-coding protein was expressed in sf9 and Marc145, and its localization was characterized.

NOTES

Theme 7

Immunostimulatory effect of CpG oligodeoxynucleotides on the innate immune response of common carp, Cyprinus carpio L

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Bacterial DNA and synthetic oligodeoxynucleotide (ODN) containing unmethylated CpG motifs within certain flanking base pairs are recognized as a danger signal by the innate immune system of vertebrates. However, such effects in fish are poorly described. Using phagocytic activity, production of superoxide anion and lymphocyte proliferation response (LPR) assays, a panel of synthetic oligodeoxynucleotide was screened for immunostimulatory activity on the innate immune response of common carp (Cyprinus carpio L) kidney cells. In vitro treatment of fish by CpG-ODNs, resulted in increased responses of phagocytic activity and production of superoxide anion in kidney phagocytic cells. The CpG-ODNs also stimulated lymphocyte proliferation in the fish kidney cells. Intraperitoneal injection of CpG-ODNs into fish, resulted in enhanced responses of phagocytic activity and production of superoxide anion in kidney phagocytic cells. The serum lysozyme activity also increased in fish treated with CpG-ODNs. ODN composed of multiple CpGs were highly stimulatory in all assays, and CpGs located at the terminus of an ODN were ineffective. These results show that CpG-ODNs are effective immune stimulators for fish immune cells, suggesting that CpG-ODN may be useful in enhancing the innate immune response in veterinary applications for fish.

NOTES

Industry's Need of Standard Challenge Tests for Shrimp

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There are many products available in the shrimp market such as vaccines, immunostimulants or anti-viral components that claim to have an effect on the better survival or disease resistance of shrimp to viral and bacterial pathogens. However, no standard methodology has been available till now (to perform the challenge tests required in order to prove it). Results from bacterial and viral challenges commonly performed in shrimp lack confidence due to the difficulty to repeat and reproduce them consistently. In order to service the indusrty with reliable products, standard methodology should be developed. In this poster, we refer to the standardization already in place for fish and terrestrial animals and discuss the different variables that affect the challenges proposing alternatives to solve them.

NOTES

Dietary intake of levamisole enhances the immune response and disease resistance of the Asian catfish, Clarias batrachus

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2. Institute For Animal Health

In order to determine the immunomodulatory effect of the dietary intake of levamisole in Asian catfish (Clarias batrachus), specimens were fed four different diets for 10 days: a commercial diet as control and the same diet supplemented with 50, 150 and 450 mg levamisole kg-1 feed. The serum bacterial agglutination titre against A. hydrophila as a measure of specific immunity, serum haemolysin titre, lysozyme activity, globulin level and oxidative radical production by neutrophils as a measure of non-specific immunity as well as disease resistance against A. hydrophila challenge to vaccinated and non-vaccinated fish were evaluated at 0, 1, 2 and 3 weeks after last administration of levamisole. The results demonstrate that levamisole supplements at lower level (50 mg/kg), significantly ($p<0.05$) enhanced oxidative radical production, serum lysozyme and globulin levels to peak immediately after 10 days of feeding except for haemolysin titre which was enhanced after 3 weeks of feeding. The bacterial agglutination titre was raised at 50 and 150 mg/kg of levamisole feeding after 0 and 1 weeks. To examine the disease resistance capacity, fish were challenged with a virulent strain of A. hydrophila on 0, 1, 2 and 3 weeks after levamisole feeding and the percent mortality was recorded up to 10 days after challenge. Feeding of levamisole at 50 mg/kg significantly reduced the mortality in non-vaccinated fish up to 3 weeks than any other groups. There was no difference in mortality pattern in vaccinated groups of fish. On the contrary, higher level of levamisole feeding (450 mg/kg) caused immunosuppression in catfish as noticed from the reduction in most of the immune parameters along with increase rate of mortality. The present result supports the possible use of levamisole as immunostimulant in catfish farming.

NOTES

The general protein secretion machinery of Aeromonas hydrophila is involved in fast-killing mechanism of c. elegans and mortality of fish

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C. elegans has been demonstrated to be an ideal host for virulent strains of A. hydrophila in this study. The bacteria as well as toxins that they produce bring about significant mortality of nematodes by a fast-killing mechanism and also inhibit egg-laying. We investigated if bacterial infection of the worm is needed for killing or if death occurs due to intoxication. For this we tagged wild-type 134/91 with pGFPuv which confers a green fluorescence to the bacteria. Worms harboring infection in the intestine would show fluorescence. However, our studies using confocal micrsocopy did not show infection in the intestine suggesting that killing of C. elegans by A. hydrophila is caused due to toxins released into the extracellular environment. Transposon mutagenesis using TnphoA was successfully employed to identify virulence factors of A. hydrophila that makes C. elegans susceptible to mortality. Bacterial supernatants from the wild-type (134/91) and the exeE mutant of A. hydrophila obtained in this study were shown to differ significantly in their ability to lyse RBC and EPC cells and kill nematodes, mice and fish. The mutant was thus characterised to be deficient in cytolysis as well as hemolysis. exeE is involved in the secretion of proteases, and is a component of the GPS machinery of A. hydrophila which also serves to regulate secretion of hemolysin and perhaps a hemolytic cytotoxin.

NOTES

Effects of B-glucan on non-specific immune response in grouper: hematological analysis

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Groupers, Epinephelus coioides were injected intraperitoneally with curdlan, a B-1-,3 glucan from bacterium Alcaligenes faecalis, at different doses of 0.5 mg, 5 mg, 10 mg, 20 mg, and 50 mg/kg body weight to determine the effect of immunostimulants on the non-specific immune response. Various hematological and non-specific immune response parameters were determined. A significant increase in the total leukocyte count was noted in immunostimulated fishes. It was observed that the percentage of granulocytes and monocytes increased while the lymphocyte numbers decreased in the differential leukocyte count in fishes treated with curdlan. These changes in the leukocyte composition was significant when compared with unimmunostimulated fish. It was only hematocrit determination which showed no significant difference between immunostimulated and unimmunostimulated fish. The production of superoxide anion, determined by NBT reduction and plasma lysozyme levels showed significantly increased values in immunostimulated fishes. Groupers injected with curdlan at a dose of 5 mg/kg body weight exhibited the most significant changes in the in vitro determinations. Doses higher than 5 mg/kg body weight have no more significant effect. This shows that curdlan at this dose may be used to enhance immunocompetency and disease resistance in grouper culture.

NOTES

Bacterial disease prevention in high value marine fish culture by vaccination

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This presentation describes vaccination in high value marine fish culture; such as sea bass (Dicentrarchus labrax and Lates calcarifer), great amberjack (Seriola dumerili), yellowtail (Seriola quinqueradiata) etc., those become economically important for aquaculture lately. In the first experiment, sea bass (D. labrax) are immunized with Vi-brio anguillarum and Photobacterium damsela vaccine either by double immersion (Imm-Imm) or immersion followed by intraperitoneal injection (Imm-IP) at 15 weeks post primary vaccination. When fish are challenged with V. anguillarum, the mortality are 0.0 and 1.4 % for Imm-Imm and 0.0 and 0.0 % in Imm-IP vaccination compare to 62.3 and 46.7 % in non-immunized group at 7 and 26 weeks post primary vaccination respectively. The mortality, when challenge with Ph. damsela, are 1.4 and 8.7 % for Imm-Imm and are 0.0 and 10.0 % in Imm-IP vaccination compare to 55.1 and 50.0 % in non-immunized group at 7 and 26 weeks post primary vaccination respectively. The sec-ond experiment, great amberjack are immunized by intraperitoneal injection (IP) with Ph. Damsela and Lactococcus garvieae vaccine and are challenged 37 days later. At chal-lenge, the mortality are 36.2 and 38.8 % in immunized group compare to 93.5 and 89.2 % in non-immunized for Ph. damsela and L. garvieae challenge respectively. In third ex-periment yellowtail are immunized by IP with Ph. damsela and L. garvieae vaccine but challenged with L. garvieae only. The mortality are 0.0 and 9.8 % in immunized group compare to 92.0 and 92.5 % in control when challenge 14 and 60 days post vaccination respectively.

NOTES

Theme 8

Orally administered praziquantel as a treatment for monogeneans infecting Seriola quinqueradiata: efficacy and practical considerations for a commercial fish farm

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Parasitic diseases are a serious concern for sea cage aquaculture and in some instances they may threaten industry viability. In Japan, cultured Seriola spp. are vulnerable to outbreaks of the monogenean parasites Benedenia seriolae, Heteraxine heterocerca, Neobenedenia melleni and Zeuxapta seriolae. Control of monogeneans is currently achieved by bathing fish in freshwater or hydrogen peroxide. This procedure significantly increases production costs because it is labour and infrastructure intensive, stresses fish and may result in lost growth. In-feed treatment for monogenean infections provides a desirable alternative to bathing. Presently, only one in-feed medication is licensed in Japan for use against monogenean parasites of Seriola spp. The active ingredient of this product is praziquantel, which has demonstrated therapeutic activity against a broad range of helminths. We assessed the efficacy of orally administered praziquantel as a treatment for B. seriolae and H. heterocerca infections in a commercial yellowtail farm. Treatment was found to reduce total abundance of H. heterocerca by up to 97.9% and juvenile abundance by up to 99.5%; total abundance of B. seriolae were reduced by up to 62.9% and juvenile abundance by up to 70.5%. Methods of application may have a significant effect on the efficacy of orally administered praziquantel. The key practical considerations for praziquantel treatment are discussed, recommendations for improving efficacy made and areas requiring further research identified.

NOTES

Studies on antiviral and antibacterial substances of Spirulina platensis for prevention the infectious diseases in black tiger shrimp (Penaeus monodon) caused by white spot syndrome virus and vibrio harveyi.

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Experiments were designed to 3 parts; the first, the effect of crude extract from S. platensis against white spot syndrome virus (WSSV) and application to post larvae 15 and adult stage of P. monodon ; the second, the effect of crude extract against Vibrio harveyi and application to P. monodon and the final was the effect of S. platensis on shrimp cellular immunity. The crude extract 1, 0.1, 0.01 and 0.001 mg/ml were mixed with WSSV. After 14 days of injection, all of concentrations could inhibit WSSV infection. For post larvae 15, fed with the steamed egg containing 0, 0.5 and 5 % of S. platensis and challenged with WSSV, the survival rate were 73.75+10.34, 86.50+8.80 and 83.17+3.87 %. The results from PCR diagnosis, the infection were 100, 0 and 16.67 %. Application for adult, the survival rate were 61.00+38.74, 96.00+8.94, 100.00+0.00 and 92.00+10.95 %. The effect of crude extract on the growth of three strains of V. harveyi. The result showed that it could inhibit the growth of all strains and the minimum concentration was 2.5 mg/ml. The application for adult, fed with the diet containing 0, 0.5 and 1 % of S. platensis. The result showed that bacterial could clearance higher in treatments than those control group, there was significant different ($P<0.05$). For cellular immunity, phenoloxidase activities in the haemolymp were not significant different between treatments and control group ($P>0.05$). This study indicated that S. platensis could control WSSV and V. harveyi infection in shrimp.

NOTES

Studies on the effect of antibacterial and antiviral substance of marine diatom Skeletonema costatum for prevention the infectious diseases in black tiger shrimp (Peanaeus monodon) caused by Vibrio harveyi and white spot syndrom virus

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The effect of Skeletonema costatum was investigated in three sections (in vitro antibacterial and antiviral activity, application for prevention of bacterial and viral infection and toxicity) .Studies on antibacterial and antiviral activity were tested in vitro by using extracellular and intracellular products. Extracellular product was tested by using S. costatum cells immobilized in sodium alginate beads. The results indicated that the number of 1114GL and VH. 046 decreased after 24 hrs. and VH. 1526 decreased after 6 hrs, of the total 96 hrs. The incubation time of.intracellular product was measured by using crude extract of S. costatum and the result showed that three strains of bacterial were decreased after 24 hrs of the total inoculation time.at 96 hrs. In the case of antiviral activity, there was no significant difference between tested and control groups ($p>0.05$) from intracellular product using crude extract at 0, 10, 100 and 1,000 ppm. The application of antibacterial and antiviral activity were investigated by using S. costatum supplemented diet to inhibited V. harveyi and WSSV. The effect of S. costatum on antibacterial was investigated by bactericidin method. The result of bactericidin of hemolymph showed that V. harveyi highest cleared from hemolymph of shrimps, fed 50 g/kg S. costatum supplemented diet, about 99.38%. However there was no significant difference between tested and control groups ($p >0.05$) of antiviral activity. Studies the toxicity of crude extract of S. costatum were examined in 7 concentrations (0, 0.1, 1, 10, 100, 1,000 and 10,000 ppm). LD50 that calculated using probit analysis was 2.56 x 107 ppm., it was very safely to black tiger shrimp From all of results indicated that S. costatum contained only antibacterial substance to inhibited V. harveyi.

NOTES

Determination of minimum detectable limit (MDL) of residues of selected antibacterial agents in common carp (Cyprinus carpio) using thin layer chromatography-bioauthography(TLC-B)

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Minimum Detectable Limit(MDL) of selected antibacterial agents:oxytetracycline(OTC), chlortetracycline(CTC),trimethopim(TMP) and flumequine(FLM) were measured in liver tissue extracts of common carp(CYPRINUS CARPIO) and standard solutions in order to evaluate suceptibility of TLC-B tests. Standard dilutions of selected antibacterials in steril distilled water(3.125-50 microgram per milli liter) and fortified-liver extraction were prepared.Five microliters of a graded concentration of each antibacterials was applied in a similar manner to that described for TLC-B and TLC-B plates were developed ascendingly with 3% NH4CL solution as the developing solvents (for OTC,CTC,TMP) and combined solvent(propanol,acetic acid and sterill distilled water)for FLM. The minimum amount of each antibacterial showing a inhibition clear zone on the Muller Hinton Agar medium(seeded with BACILLUS SUBTILIS) was taken as' MDL'. The resultant supernatant of fortified liver extraction was chromatographed as the solution to be chromatographed.Five microliter portions of this solution were spotted on TLC plates. As results revealed based on TLC-B procedure MDL of selected antibacterals agents: OTC,CTC,TMP and FLM resulted in standard solutions 0.0625,0.0157,0.1250 and 0.2500 micrograms,respectively and in fortified liver 0.0665,0.0313,0.1330 and 0.2500 respectively. The amount of Minimum Detectable Limit(MDL) of chlortetracycline(CTC) was the least among other antibacterials. MDL of standard solutions were more than fortified tissues but flumequine(FLM).MDL of trimetheoprim(TMP) was equal both standard solution and fortified liver extraction.

NOTES

Herbal preparations for aquaculture bio-security - Indian experiences

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Annual growth rate of Indian aquaculture sector (4.87%) is a fair indicator of the optimum development of this enterprise in India during the past decade. But the actual potential (6.0 million tonnes) could not be reached so far. This has been primarily attributed to the poor growth of cultivable species and uncontrolled and poorly estimated mortality in the ponds. Herbal preparations when used in the production system was found to enhance growth, feed conversion, meat quality and disease resistance among Indian Major Carps (Catla catla, Labeo rohita) Common carp (Cyprinus carpio), catfish (Clarias batracus) and freshwater prawns (Macrobrachium rosenbergii). Laboratory as well as field trials conducted with liver stimulant (IHF 500) exhibited the growth promotion varying from 20 to 172% for the above mentioned species. The effect was above the control maintained in the trial (Results are individually tabulated). Another herbal preparation called Magacal helped M. rosenbergii broodstock to overcome 'moult entrapment syndrome' in the tank. In the production pond also M. rosenbergii had 40% growth increment over control because of this herbal supplement. Wide scale adoption in the field also proved to be useful as it is seen from the farmers' response. There is a scope for the development of more herbal medicines for use in aquaculture of different species. Herbal preparations were also used in the shrimp (Penaeus monodon) production and found useful. The discussion is focusing the large-scale adoption of the herbal medicines for different purposes like breeding and immune development in fishes and prawns in Asia. The advantages and the future scope of the herbal medicines are highlighted in the paper.

NOTES

The anti-virus effects of glucosamine and melaleuca oil on Chinese prawn (Panaeus chinesis)

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Glucosamine and Melaleuca oil were added in diets for juvenile Chinese prawn to test their anti-virus function. Feeding trials are conducted for 5 weeks in Prawn Hatchery Factory. Mortality, growth performances, tissue structure and ultra-structure were recorded to compare the anti-virus effects among the groups. Juvenile prawn fed 15-mg/kg glucosamine and 0.05mg/kg Melaleuca oil showed the highest surviving rate and Specific Growth Rate, and less falciform virus was found in the liver and pancreas. The surviving rate was 3 times higher than that of the control group. The anti-virus effects were Mulaleuca oil dose dependent. All the experimental groups had better growth performances than that of control groups. Tissue structure and immunological reaction showed that glucosamine and Mulaleuca oil both could advance the general immunity ability in early stage of Chinese prawn. The further use and marine medicine development of these two natural substances were also discussed.

NOTES

Theme 9

Lactococcus garvieae, a bacterial infection in grey mullet Mugil cephalus

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An epizootic with a cumulative mortality approximately 5-8% in grey mullet Mugil cephalus in Taiwan was caused by Lactococcus garvieae. The diseased fish were approximately 2 months old and 12-16cm in length. Diseased fish showed exophthalmia, ascites, pericarditis and enlarged spleen with diffuse pinpointed white spots. Histopathologically, necrosis and granulomata were found in the spleen, eye, kidney and brain. The morphology of bacteria isolated from brain heart infusion medium or tryptic soy agar was ovoid and Gram-positive. Eight strains L. garvieae were collected from diseased fish at seven farms and evaluated for API 20 Strepsystem, conventional tests and PCR assay. The LD50 value of L. garvieae FLG4 was 1.2x106 colony forming units/fish. The 16S r DNA sequence of L. garvieae FLG2, FLG4, FLG5 and FLG12 isolated from grey mullet gave 100% sequence identity to Enterococcus seriolicida (synonym L. garvieae, GenBank accession number AF061005). The major polypeptides of ECPs of Lactococcus garvieae FLG4 are 78, 54, 45, and 41 kDa.

NOTES

Health status of cultured shrimp at harvest - epidemiological significance

Mohan CV [1], Sashikala G [1], Jaganath V [1], Ahmed I [1], Corsin F [2], Padiyar PA [1], Madhusudan M [1], Turnball JF [3], Hao NV [4] Morgan KL [2]*

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A longitudinal observational study was undertaken in 70 shrimp farms in Kundapur, Karnataka, India from September 1999 to April 2000 to identify risk factors associated with outbreaks of White Spot Disease (WSD) in cultured Penaeus monodon. As a part of the larger study, 400 shrimp/pond were randomly collected from 62 ponds at the time of harvest and observed for the presence or absence of white spots on the carapace. From this, 100 shrimp were randomly selected for recording the length and weight and for detailed clinical examination. Per pond, 10 each of clinically sick and clinically healthy looking shrimp were selected and fixed in 10% neutral buffered formalin for histopathological studies. In 21 (33.9%) ponds, only white spot syndrome virus (WSSV) inclusion bodies were detected. Samples from 21 (33.9%) ponds showed evidence of only chronic inflammatory lesions (CIL) characteristic of chronic bacterial infections. In 13 (21%) ponds, both WSSV inclusion bodies and CIL were observed. Samples from 7 (11.3%) ponds did not have any pathology. In total, WSSV inclusions were recorded in 55% (34/62) and CIL in 55% (34/62) of the ponds. On a pond basis, there was no significant difference in the health status of sick and healthy looking shrimp. If WSSV ($p<0.001$) or CIL ($p=0.001$) was present in clinically sick shrimp in a pond, it was also more likely to be present in the healthy looking shrimp in the same pond. Ponds with clinical white spots on shrimp, were more likely to have WSSV inclusions in the samples ($p<0.001$), while there was no association with CIL ($p=0.120$). In 77% of the ponds (48/62), dead shrimp had been observed and collected by the farmers prior to harvest. Ponds where dead shrimp were positive for WSSV inclusions ($p=0.001$) or CIL ($p=0.008$) were also more likely to have the same condition at harvest. Ponds positive for WSSV inclusions at harvest were significantly associated with high mortalities prior to harvest ($p<0.001$) and shorter rearing period ($p<0.001$). On the other hand, ponds positive for CIL at harvest, were significantly associated with low or no mortalities prior to harvest ($p=0.01$) and longer rearing period ($p=0.002$). Monitoring pond side mortality and rapidly confirming WSD can assist shrimp farmers in proper decision making.

NOTES

Feeding farmed shrimp with shrimp waste - the lessons for aquaculture from BSE

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In 1993, outbreaks of White Spot Disease (WSD), appeared in shrimp in Japan and China and in the ensuing years reached pandemic proportions. WSD is characterized by rapid onset, high mortality and white spots on the carapace associated with an unclassified virus known as White Spot Syndrome Virus (WSSV). The method of transmission of WSSV has not been identified. Oral infection has been demonstrated experimentally and vertical transmission is also thought to occur. In a longitudinal study, designed to identify the risk factors for WSD, a simple random sample of 70 shrimp (P.monodon) farms were monitored from stocking to harvest. The study was carried out in South West India, between September 1999 and April 2000. A feed sample was taken at the start of the study and subsequently every time the type, or brand, of food was changed. Approximately 2-3g of feed were collected on each occasion. Samples were stored for up to 2 months at room temperature and subsequently at -20 0 C until analysed. At the end of the study, a nested PCR was used to detect WSSV in PL, wild shrimp, feed and P. monodon collected 6 weeks after stocking and at harvest. WSSV PCR products were detected in 42.9% (30/70; 95%CI 31.1- 55.3) commercial shrimp feeds. In this is the first population based study, between and third and a half of commercial shrimp feed used in the Kundapur estuary of India was PCR positive for WSSV. Furthermore, there were distinct differences between brands in the frequency with which WSSV was detected. These findings have important real and potential implications for shrimp farming worldwide.

NOTES

Effect of methylparathion on the susceptibility of shrimp (Litopenaeus vannamei) on the development of vibriosis

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Under increasing pressure of environmental concerns, a replacement of persistent pesticides with more rapidly degradable products has been observed over the past two decades. However, these pesticides can still affect non-target species and can be associated with slow growth and increased susceptibility to infections. In this study juvenile shrimp (Litopenaeus vannamei) were exposed to combinations of methylparathion and a Vibrio sp. There were five treatments, each with five replicates and 10 shrimp per replicate. In one treatment shrimp were fed pellets containing 0.080 mg of methylparathion g-1 for 4 days and injected intramuscularly with Vibrio parahaemolyticus on day 5 (MPAR/V). The other treatments were shrimp fed with methylparathion and injected with sterile saline solution (MPAR), shrimp fed with pellets containing acetonitrile (solvent for methylparathion) and injected with V. parahaemolyticus (V), shrimp fed with pellets containing acetonitrile and injected with a sterile saline solution (A) and a group that only received uncontaminated pellets (C). Mortality was recorded and the surviving shrimp were sampled for histology on the fourth day after injection. Analyses of the incidence rate for mortalities (IR) in the treatments revealed that feeding methylparathion was significantly associated with an increase in IR. The injection of the Vibrio sp. was significantly associated with increased IR and histopathological lesions. The combination of feeding methylparathion and injection of the Vibrio sp. was also associated with a significant increase in IR when compared with either treatment alone. The study provides strong experimental evidence that exposure to methylparathion can increase susceptibility to Vibrio infection.

NOTES

Acute toxicities of deltamethrin and lambda-cyhalothrin to the fry of green grouper (Epinephelus tauvina Forsskål) and milkfish (Chanos chanos Forsskål)

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A static 96-hr acute toxicity test was used to assess the potential ecological effects of deltamethrin and lambda-cyhalothrin to the fry of green grouper Epinephelus tauvina and milkfish Chanos chanos. The deltamethrin and lambda-cyhalothrin LC50 of the grouper fry (0.23 g mean body weight) were 3.6 and 8.25 µg/L, respectively. For both pesticides, the NOAEC (No Adverse Effect Concentration) value was 2.5 µg/L. The deltamethrin and lambda-cyhalothrin LC50 of the milkfish fry (0.06 g mean body weight) were 0.14 and 0.006 µg/L, respectively. The NOAEC values were .025 and .0025 µ/L for deltamethrin and lambda-cyhalothrin, respectively. The two species are the leading fin fishes cultured in cages and pens that are located in estuarine and semi-exposed bays in the Philippines. Moreover, these areas are the collecting grounds for wild grouper and milkfish fry. On the other hand, the two toxicants are pyrethroid insecticides that have gained wide use in the country for the control of major insect pests of common Philippine crops. There is a growing concern among mariculture farmers on the risk of transient agricultural run-offs. Reports of fish kills in these facilities have been recorded following first rainfall of the wet season. The basic effects data provided by this study may be used as input for risk assessment of run-off related pesticides on mariculture organisms. Key words: deltamethrin, lambda-cyhalothrin, effects assessment, acute toxicity, fry of Epinephelus tauvina and Chanos chanos

NOTES

Contamination of mycobacterium spp. in live feed

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The importance of moina, mosquito larvae and blood worm as live feed for tropical fish, especially carnivorous fish, is well known. These live feed always has contaminate with pathogens that cause diseases in aquarium fish. Mycobacteriosis is a chronic bacterial disease infecting aquarium fish. The detection of Mycobacterium spp contaminated in live feed in Bangkok and 4 provinces around Bangkok by isolation and polymerase chain reaction technique revealed four pathogenic bacteria, namely Mycobacterium spp., M. fortuitum, M. marinum and M. chelonae. Contamination of M. fortuitum showed high prevalence. Live feed are recognized as one of the important sources of mycobacteria that cause diseases in aquarium fish. Feeding of contaminated live feed should be avoided. The practical on producing live feed free from mycobacteria is recommended. Key word : Mycobacterium spp., Moina, Mosquito larvae, Blood worm

NOTES

Theme 10

Mycobacteriosis in ranchu goldfish (Carassius auratus) imported from Japan

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Ranchu, Japanese goldfish, is very famous for culturing among Thai people. Each year, many company have imported ranchu from the famous farm in Japan. In the past winter, January 2002, 30 percent of imported juvenile fish showed inactive swimming, anorexia and lethargy. Therefore, the six diseased fish were collected to investigate the cause of diseases. The clinical signs of the fish showed asymmetrical swelling of abdomen, pale discoloration of gills, swelling and discoloration of head and trunk kidneys. Abdominal organs were attached to peritoneal wall, indicating peritonitis, associated with numerous white nodules of various sizes. These nodules were also found on the abdominal organs, head and trunk kidneys and heart. The isolation was done with Ogawa egg medium and identified as Mycobacterium chelonae by the PCR method at Aquatic Animal Health Research Institute, Department of Fisheries, Thailand. The histopathological features showed that these white nodules on the affected organs were chronic proliferative lesions composed of multiple caseous epithelioid cell granulomata with surrounding granulation tissue. In the central caseous area, a number of colonies of slender, long rod, Gram positive were found to be acid-fast by Ziehl-Neelsen method. These acid-fast bacteria were also observed in the epitheliod cells and in the macrophages of the granulation tissue. From these findings, this case was diagnosed as serious systemic mycobacteriosis.

NOTES

Fish Kill of Mullet Liza klunzingeri in Kuwait Bay: The Role of Streptococcus agalactiae and the Influence of Temperature

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A massive fish kill occurred in Kuwait Bay during August and early September 2001. Wild mullet Liza klunzingeri represented 99% of the fish kill, whereas the remaining 1% consisted mainly of silvery croaker Otolithes argenteus, giant sea catfish Arius thalassinus and striped grunt Rhonciscus stridens. The average seawater temperature during the incident was 32.3∞C. Affected fish showed erratic swimming, hemorrhages around the mouth, abdomen, pectoral and pelvic fins. They also exhibited internal hemorrhage and exophthalmia. Bacterial isolates obtained from the four wild fish species, one cage-cultured species (sea bream Sparus auratus) and sewage samples from two beach locations were used to identify the causative organism. The biochemical, biophysical and API 20 Strept tests identified the bacterium as b-hemolytic, group-B Streptococcus agalactiae. S. agalactiae can grow in a temperature range from 18 to 43 ∞C, but not at 5, 12 or 45 ∞C. Also, it can grow in a salinity range between 0.5 to 6.0% NaCl, but not at 6.5 %. To investigate the effect of temperature on the susceptibility of fish to infection, healthy mullet kept at two seawater temperatures (25 and 33∞C), were intraperitonealy injected with four bacterial doses (102,104,106 and 108 CFU/mL). At 33∞C, 100% mortality was produced after 24h, when fish were injected with 108, whereas the other doses produced the same mortality after three to four days. At 25 ∞C, the mortality varied between 20 and 60% among the different doses. The results clearly implicate the significant role of high temperature in increasing the virulence of S. agalactiae causing the massive mullet mortality. The possible relationship of this outbreak to another in August and September 2000 in cultured silver pompfret Pampus argenteus is discussed.

NOTES

Seasonal prevalence of trichodina infection in guppies (Poecilia reticulata) and goldfish (Carassius auratus) cultured for export in Sri Lanka

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Trans-boundary pathogen movement has been a concern in the international ornamental fish trade. To implement policies preventing movement of pathogens it is essential to establish the epidemiological features of fish pathogens. Trichodina infection is one such potential protozoan encountered in fresh water guppies and goldfishes, which represents more than 80% of the ornamental fish exports from Sri Lanka. A total of 1488 guppies and 270 goldfishes cultured in 4 ornamental fish export farms were observed monthly, starting from September 2001 for a period of 9 months in a area where the annual rainfall range from 1875 – 2500 mm and Temperature vary from 24-35 degree centigrade. Wet mounts of scrapings of body surface mucus and excised gills of freshly killed fish were examined microscopically and parasites were enumerated. More than 99% of the Trichodina species were recovered from body surface mucus. The prevalence of Trichodina species recorded during September, October, November, December, January, February, March, April and May were 14.5%, 6.5%, 12.4%, 13.9%, 5.5%, 0.9%, 1.7%, 8.3%, 7% respectively for guppies and 33.3%, 50%, 50%, 30%, 10%, 17%, 26%, 21% respectively for goldfishes. The mean monthly intensity of the parasite ranged from (1.3 -118.2) for guppies and (1.3- 156) for goldfishes. The lower prevalence of Trichodina species in the months of February, March and April may be related to comparatively higher ambient temperature and lower rainfall during these months. Funding from Council of Agricultural Research Policy of Sri Lanka under CRP/12/450/336 grant is acknowledged.

NOTES

Isolation of IHN virus from imported turbot (Scophthalmus maximus)

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At end of May 2001, turbot fry were imported from France through Hong Kong. 5 fry were sent to Lab for routine detection. The liver, spleen, kidney and brain of fish were collected and homogenized, some of them were inoculated into EPC, CO, RTG-2, CHSE cell monolayers and cells were incubated at 15 C, and 25 C for isolation of viruses. The RNA in some organs were extracted for detection of viral RNA using PCR according to Diagnostic Manual for Aquatic Animal Diseases (OIE, 2000). CPE appeared in EPC cell culture at 15 C in 1 week, which indicated that there was a virus in fish organs. 786 bp segment and 323 bp sigment were amplified respectively after RT-PCR and nested -PCR. DNA sequence of RT_PCR products from this virus (2000-479) and IHNV strain were compared. The sequence homologies between 2000-479 and IHNV_WRAC, RB, 11290 etc. was 91.7% - 98.6% . It is suggested that the virus isolated from turbot fry a closely related with IHNV. These results give reasonable evidence that the turbot may be infected with IHNV. The susceptibility of turbot to IHNV was demonstrated experimentally (I/P inoculatin) iby a French workers some years agol However, natural infection of IHNV in a marine fish species has not been demonstrated yet. It is the first finding of natural infection of IHNV in turbot.

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