

# Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development (APEC FWG 02/2000)



Asia-Pacific  
Economic Cooperation



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**Foreword**

We are pleased to bring this report from the Workshop of the project APEC FWG 02/2000 “Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development” to the attention of the Asia Pacific Economic Cooperation (APEC), economies of APEC and member governments of the Network of Aquaculture Centres in Asia-Pacific (NACA), research institutes, universities, non-government organizations, the private sector involved in grouper aquaculture and trade, regional and international agencies and other stakeholders concerned about sustainable grouper aquaculture in the Asia-Pacific region. The workshop was held in Bangkok, Thailand on the 18th-20th October 2000. It was supported by APEC FWG and jointly implemented by the Fish Health Section of the Asian Fisheries Society (FHS/AFS), the Aquatic Animal Health Research Institute (AAHRI) of the Department of Fisheries of Thailand (as Project Overseer), and NACA that is collaborating in coordination of the Asia-Pacific grouper aquaculture network.

The FHS/AFS was formed in 1989. Its aim is improving regional knowledge of fish health management and developing awareness of Asian aquaculturists towards establishing a sustainable aquaculture industry. The Section promotes interaction and cooperation among involved parties in fish health research, encourages studies and development of knowledge in fish health area, focuses attention on aquatic animal health problems through dissemination of technical and other information on all aspects of aquatic animal health; and promotes proper implementation of effective aquatic health protection practices in the region. The Society is honoured to implement APEC 02/2000 and we are grateful to APEC for an opportunity to contribute to sustainable grouper aquaculture development.

The “Grouper Health Impact Survey” workshop was held on 27-29 May 2000, and subsequently followed by the country surveys determined and evaluated the status of grouper diseases and their impact on Asian aquaculture. The Workshop Proper, with thirty-seven participants from twelve APEC economies and NACA member governments, representatives from research institutes, universities and the private sector discussed and prepared a regional framework on grouper health and production. The framework contains a number of important issues and recommendations on improving grouper health management in the Asia-Pacific region. This would be accomplished by research, capacity building and implementation of strategies to minimise the risk of disease spread through responsible trade of grouper.

We would like to take this opportunity to thank APEC and NACA, APEC economies and NACA member governments, non-government organizations, scientists from universities and research institutes and the private sector that supported the survey and the workshop. We also thank Dr Temdoug Somsiri of AAHRI for assistance in formatting of the report and Mr Sih Yang Sim of NACA who scanned most of the photos used in this report. We hope that the spirit of regional cooperation towards sustainable development of grouper aquaculture will prevail and the recommendations contained in this report will be jointly implemented by various stakeholders.

**Supranee Chinabut**  
Chairperson  
FHS/AFS

**Somkiat Kanchanakhan**  
Project Overseer  
APEC 02/2000

**Abstract**

A workshop on APEC FWG 02/2000 “Development of a Regional Research Programme Grouper Virus Transmission and Vaccine Development”, was convened at the NACA Headquarters in Bangkok, Thailand on 18th-20th October 2000.

This project was implemented by the Fish Health Section of the Asian Fisheries Society (FHS/AFS) in close cooperation with the Aquatic Animal Health Research Institute (AAHRI) of the Department of Fisheries of Thailand as Project Overseer and with the Network of Aquaculture Centres in the Asia-Pacific (NACA) who is cooperating in the coordination of the Asia-Pacific Grouper Aquaculture Network.

The two workshops (Grouper Health Impact Survey Workshop on 27-29 May 2000 and the Workshop Proper on 18-20 October 2000) successfully fulfilled the objectives of APEC FWG 02/2000 to determine the impact of grouper diseases, update current knowledge on grouper diseases and techniques for disease diagnosis and to develop a regional research framework which addresses research needs on grouper health and strategies which minimize the risks of disease spread through responsible movement of groupers within the region.

The workshop brought together representatives from twelve economies of APEC and member governments of NACA (Australia, Brunei Darussalam, China, Chinese Taipei, Hong Kong China, Indonesia, Korea RO, Malaysia, Philippines, Singapore, Thailand and Vietnam); technical experts/specialists on grouper diseases, import risk analysis and aquatic animal vaccines from national governments, research institutes and universities and the private sector. A Framework of a Regional Research Programme on Grouper Health and Production was drafted. The framework contains 9 major components. It includes sub-projects and specific recommendations on the following major components:

- (a) health and production at hatcheries
- (b) regional collaborative disease resource centers
- (c) regional disease monitoring and surveillance
- (d) improving regional diagnostic capabilities
- (e) responsible trans-boundary movement
- (f) farm health management
- (g) vaccines and vaccination
- (h) funding mechanisms
- (i) *ad-hoc* working group.

**List of Acronyms**

AAHRI	Aquatic Animal Health Research Institute of the Department of Fisheries of Thailand
AAPQIS	Aquatic Animal Pathogen and Quarantine Information Systems
ACIAR	Australian Centre for International Agricultural Research
AFFA	Agriculture, Fisheries and Forestry of Australia
AFS	Asian Fisheries Society
ALOP	Appropriate Level of Protection
APEC	Asia-Pacific Economic Cooperation
AQIS	Australian Quarantine Inspection Service
ASEAN	Association of Southeast Asian Nations
ATTC	American Type Tissue Culture Collection
AVA	Agri-food and Veterinary Authority of Singapore
BFAR	Bureau of Fisheries and Aquatic Resources of the Philippines
BKD	Bacterial Kidney Disease
CPE	Cytopathic effect
CSIRO	Commonwealth Scientific International Research Organization of Australia
CVL	Central Veterinary Laboratory of Singapore
ELISA	Enzyme-linked immunosorbent assay
EU	European Commission
FAO	Food and Agriculture Organization of the United Nations
FAT	Fluorescent antibody test
FBS	Foetal bovine serum
FHS	Fish Health Section of the Asian Fisheries Society
FRI	Fisheries Research Institute of the Department of Fisheries, Penang, Malaysia
FWG	Fisheries Working Group of APEC
GATT	General Agreement on Tariffs and Trade
GNNV	Grouper nervous necrosis virus
GRSCF	Gondol Research Station for Coastal Fisheries of Indonesia
IPN	Infectious pancreatic necrosis
IPPC	International Plant Protection Convention
IRA	Import Risk Analysis
JICA	Japan International Cooperation Agency
JIRCAS	Japan International Research Center for Agricultural Sciences
NACA	Network of Aquaculture Centres in Asia-Pacific
NFRDI	National Fisheries Research and Development Institute of Korea RO
NNV	Nervous necrosis virus
NRIA	National Research Institute of Aquaculture of Japan
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
RGNNV	Red-spotted grouper nervous necrosis virus
RPS	Relative percentage survival
RT-PCR	Reverse transcriptase-polymerase chain reaction
SEAFDEC-AQD	Aquaculture Department of the Southeast Asian Fisheries Development Center
SJNNV	Striped-jack nervous necrosis virus
SPF	Specific Pathogen Free
SPS	Sanitary and Phytosanitary Measures of WTO
TMSI	Tropical Marine Science Institute of the National University of Singapore
UPM	Universiti Putra Malaysia
VER	Viral encephalopathy and retinopathy
VHS	Viral haemorrhagic septicemia
VNN	Viral nervous necrosis
WSSV	White spot syndrome virus
WTO	World Trade Organization

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## Introduction

The APEC FWG 02/2000 “Development of a Regional Research Programme for Grouper Virus Transmission and Vaccine Development” was approved for implementation at the APEC FWG 10<sup>th</sup> meeting held in Cairns, Australia in May 1999.

The Fish Health Section (FHS) of the Asian Fisheries Society (AFS) has been awarded the contract to implement this project. The Section worked closely with the Aquatic Animal Health Research Institute (AAHRI) of the Department of Fisheries of Thailand (the Project Overseer) and the Network of Aquaculture Centres of the Asia-Pacific (NACA), which is cooperating in coordination of the APEC grouper aquaculture network in the region.

The project was implemented by a ‘Project Team’ headed by Dr Supraanee Chinabut, Chairperson of the FHS/AFS and Director of AAHRI; two consultants, Dr John Humphrey of the Department of Primary Industry and Fisheries, Northern Territory, Darwin, Australia and Dr Angus Cameron of AusVet, Australia; two staff members of the FHS/AFS, Dr Melba B. Reantaso of NACA and Dr Sataporn Direkbusarakom of Walailuk University for technical support; and Dr Somkiat Kanchanakhan of AAHRI as Project Overseer of APEC FWG 02/2000.

The first major activities were the organization and conduct of a “**Grouper Diseases Impact Survey Workshop**” held at the NACA Headquarters in Bangkok, Thailand on 27-29 May 2000. Representatives from Australia, Indonesia, Korea RO, Malaysia, Philippines, Singapore, Thailand and Vietnam participated in the workshop. The representatives and the ‘Project Team’ discussed and finalized the survey, which included development of the survey questionnaire and database, sampling methods, data entry, management and analysis using EpiInfo software. The participants from the above-mentioned economies were trained on the use of the EpiInfo<sup>1</sup> software. The survey was subsequently carried by above mentioned economies. Additionally, the ‘Project Team’ discussed with representatives from Brunei Darussalam, China PR, Chinese Taipei, Hong Kong SAR China and Singapore their participation in the grouper survey and the Workshop Proper on “Development of a Regional Research Framework on Grouper Virus Transmission and Vaccine Development” in October.

The **Workshop Proper** held at the NACA Headquarters, in Bangkok, Thailand on 18-20 October 2000, was attended by a total of 41 participants from 12 APEC economies and NACA member governments (Australia, Brunei Darussalam, China PR, Chinese Taipei, Hong Kong, China, Indonesia, Korea RO, Malaysia, Philippines, Singapore, Thailand and Vietnam) ; technical experts/specialists on grouper diseases, import risk analysis and aquatic animal vaccines from representatives of national governments, research institutes and universities and the private sector.

The programme consisted of 12 country presentations on grouper disease impact survey and status of grouper diseases, one regional synthesis, seven technical presentations, one APEC FWG project update, three working group discussions and presentations, and development of a framework of a regional research programme on grouper health management.

This report contains the proceedings of the Workshop Proper and is presented in two parts. Part I contains the objectives, expected outcomes, workshop mechanics, brief summaries of the economies papers and expert presentations, the main recommendations which resulted from the working group discussions, workshop programme and list of participants; Part II contains the complete papers (economy reviews and technical presentations) presented during the workshop.

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<sup>1</sup> EpiInfo is an integrated database / data management / statistical analysis / word processing package that was designed and produced by the Centres for Disease Control (CDC) and World Health Organisation. It is specifically designed to support the rapid implementation and analysis of field surveys, and to support in-depth analysis of data related to biological systems.

**PART 1**

**The Workshop**

**Opening Ceremonies**

The workshop was opened by welcome remarks and opening speeches by various representatives of agencies/institutes.

Dr Somkiat Kanchanakhan of AAHRI, Project Overseer of APEC FWG 02/2000 “Development of a Regional Research Program on Virus Transmission and Vaccine Development” welcomed the guests, experts and participants to the workshop. He noted that there are now 21 countries in APEC, which had become members since 1991, when the Fisheries Working Group (FWG) was initially formed. He observed that since that time, the FWG had distributed funding for training and for the promotion of trade in aquatic species and products. He noted that the present workshop was one of APEC funded projects to promote grouper culture and to overcome problems in grouper culture. This workshop was an attempt to address and resolve the grouper disease problems in individual countries and the region. For these reason experts from both the governments and private sector were brought together.

Dr Supranee Chinabut, Chairperson of the FHS/AFS, and Director of the AAHRI of Thailand’s Department of Fisheries and also Project Team Leader of APEC FWG 02/2000 welcomed participants and briefed on the role of the FHS/AFS. Dr Supranee noted that the FHS was originally formed in 1989 at the Universiti Pertanian Malaysia (now Universiti Putra Malaysia - UPM). Its objective was improving regional knowledge on aquatic animal health and sustainable aquaculture and promoting scientific exchanges. Dr Supranee noted that the FHS was actively involved in the FAO/NACA Regional Programme on Aquatic Animal Health Management where past Chairpersons served as members of the Regional Working Group (RWG) of the regional programme. She noted that, thanks to APEC, the Society was involved in implementing this major project, which is the subject of the current workshop. Dr Supranee noted that participants are from private sector and member economies. She stressed that this was a regional project in response to the needs of grouper aquaculture. She noted that the objective of the workshop was closely aligned with the objectives of the FHS in promoting fish health activities and that the FHS would be pleased to participate in future projects.

Dr Maitree Duangsawasdi, representative of the APEC FWG and Deputy Director General of Thailand’s Department of Fisheries, welcomed participants to the workshop. Dr Maitree noted that groupers are particularly important in aquaculture and that the APEC FWG considered grouper aquaculture a priority issue for the region. He expressed the hope that measures on disease control and improving grouper health will result from this workshop. He wished the meeting every success in defining outcomes of the workshop for fish health future in grouper. Finally, Dr Maitree wished all participants success in their endeavours and a pleasant stay in Thailand.

Mr Hassanai Kongkeo, NACA Coordinator, extended the most cordial welcome to all participants on behalf of the NACA Secretariat. He was pleased that NACA has achieved an important role in convening the workshop. He observed that this was a result of a long working relationship with FHS/AFS and AAHRI, countries represented at this workshop, and particularly with APEC on a number of projects. He observed that grouper had emerged as a major commodity, especially in the live fish trade. He expressed concern over the exploitation of the species from the wild and the increasing demand that can only be met by culture. He noted the considerable potential to meet the need for grouper by small-scale farmers. He also noted that production is severely threatened by diseases. Mr Hassanai remarked on the wide range of participants in the workshop, which included representatives from the private sector. He expressed the hope that the participants of the workshop will contribute significantly to grouper health and production in the region. Finally, Mr Hassanai welcomed all delegates to Thailand and expressed the hope that all enjoy their stay.

**Objectives**

The objectives, expected results, workshop mechanics and terms of reference were presented by Dr Melba B. Reantaso of NACA, currently Secretary/Treasurer of the FHS/AFS, and technical staff of the Project Team.

Dr Reantaso provided a background on the issues leading to the workshop. Live grouper trade has been growing and this has been, associated with increased consumption, increased preference and a growing live seafood market and restaurant trade. She described the concerns regarding the increasing number of diseases affecting grouper, especially Nodavirus and Iridovirus, as well as bacterial and parasitic diseases. Several viruses affecting species of cultured grouper were listed, namely (a) nodavirus – Viral Nervous Necrosis (VNN) or Viral Encephalopathy and Retinopathy (VER), (b) iridovirus – GIV-1, GIV-2 and TGIV, (c) lymphocystis, (d) Herpes virus, (e) golden eye disease (astro-like virus) and (f) red grouper reovirus disease. It was also noted that a number of bacterial diseases, parasitic infections and undiagnosed diseases had impact on grouper culture.

The severe economic impacts of diseases on grouper production were tabled and the extensive losses due to diseases in marine fish in Japan, Thailand, Malaysia and Singapore noted. Dr Reantaso stressed the impact of disease on small-scale farmers. A Philippine survey indicated that 75 % of farmers reported reduction in income due to health/disease problems, and 19.44% reported increased household debt.. In Thailand more than 80% of farmers reported losses ranging from 30% to 50% due to diseases.

It was noted that the workshop was a response to various recommendations of several regional meetings towards enhanced health management for a sustainable grouper aquaculture industry. A number of previous workshops, which influenced the implementation of the current workshop, were described.

The original project proposal was prepared by Thailand, and presented during the 10th APEC FWG Meeting in Cairns, Australia. Funding was approved for a one year period to undertake a disease impact survey, conduct a workshop to determine the status of grouper health, to evaluate existing diagnostic techniques and to develop a regional programme on grouper health that will assist in reducing losses by identifying research needs and strategies to minimise the risks of disease incursion. The project depended on collaboration between APEC, AAHRI, the AFS-FHS, the Grouper Aquaculture Network and NACA.

The objectives of the project were:

To update the current knowledge on grouper health, particularly viral diseases, their impact, including standard and rapid techniques for viral disease diagnosis

To develop a regional program on grouper health that will assist in reducing losses due to grouper diseases, initially by identifying research needs that will address the following:

- Development of suitable cell lines for grouper viral isolation
- Development of techniques for grouper viral identification and diagnosis
- Development of protocols for grouper viral disease induction and investigation on modes of virus transmission
- Prevention and control of viral diseases of grouper, for example, VNN, iridovirus at the hatchery stage

To develop strategies to minimise risks of pathogen transfer through responsible movement of live grouper

To identify funding mechanisms that will support the implementation of the regional program on grouper health

To strengthen the network of aquatic animal health scientists working on grouper and other marine fish diseases in the APEC region.

### **Expected Outcomes**

- Report and proceeding of APEC FWG 02/2000 “Development of a Regional Research Program on Grouper Virus Transmission and Vaccine Development”, which will include papers on disease issues in grouper in represented economies, papers on disease issues from resource persons, and major discussions and recommendations from the workshop participants.
- Strengthened regional cooperation and networking of aquatic animal health scientists working on grouper and other marine fish diseases within and outside the Asia-Pacific region

### **Workshop Mechanics**

The program for the workshop was described. It included dividing participants into three groups to further discuss the following:

- Group I: Identification and prioritization of research needs on grouper health
- Group II: Strategies for minimising the risks of pathogen transfer through responsible movement of live aquatic animals
- Group III (in plenary): Regional framework, funding options, mechanism for implementation.

The working groups designated a Chair and Co-chair to facilitate the discussions and ensure that the terms of reference of the working groups are met. These individuals were also responsible for presentation of the results of the working group discussions. Rapporteurs were also designated. They recorded all the discussion points and conclusions agreed upon by the working group members.

### **Terms of Reference of the Working Groups**

The working groups were instructed to focus on activities within a regional framework rather than within an institutional framework. It was noted that the focal point for the regional program should have a government-mandated framework for development, which would act as a foundation of programs and projects. It was stressed that regional cooperation offers significant economies of scale, opportunities for synergies, and promoting complementing activities rather than duplication and competition.

In terms of research needs, the workshop was advised that priorities should be based on systematic evaluation of needs and impact rather than career interests or scientific preoccupations, thus avoiding capacity led research within institutes. It was stressed that the workshop was directed at avoiding competition between research groups, enhancing cooperation and maximising use of limited research resources. A key element was the identification of systems for monitoring and uptake of research results.

In considering strategies to minimise disease transfer, emphasis was placed on the *Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals* and recognising other regional and international agreements.

### **Terms of Reference: Working Group I**

The terms of reference were specified as follows:

- Determine the most significant grouper diseases that need priority research attention (viruses, bacteria, parasites, undiagnosed diseases)
- Development of suitable cell lines for grouper viral isolation, determining which agencies/countries represented in the workshop have successfully established cell lines for grouper viral as well as performance and accessibility of the cell lines for viral diagnostic work
- Determine the most significant grouper diseases that need priority research attention
- Development of techniques for grouper viral identification and diagnosis considering recommendations of the Expert Consultation on DNA-based techniques for VNN and noting which countries/agencies have capability for grouper viral disease identification and diagnosis
- Standardisation of techniques
- Development of protocols for grouper viral disease induction and investigation on modes of transmission, considering whether such methodologies already exist and which agencies are currently working in this area
- Develop a current understanding on viral disease transmission
- Identify other research areas for consideration
- Prevention and control of viral and other diseases affecting grouper, identifying areas of disease prevention and control which need investigation, and at what stage (grow-out or hatchery level)
- Identify what research studies (intervention studies with farmers, observational studies, on farm-trials) are applicable to grouper health management
- Consider vaccination, especially information available to develop vaccine for grouper diseases and identify areas that need to be considered, determine the feasibility of vaccination and identify target diseases for a vaccination program

**Terms of Reference: Working Group II**

Working Group II was instructed to consider the *Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals* and discuss mechanisms on how it might assist in the implementation of the '*Technical Guidelines*' focusing on the following areas:

- Aquatic animal disease surveillance and reporting
- Diagnostics
- Import risk analysis
- Information systems: Contribution to the Aquatic Animal Pathogen and Quarantine Information Systems (AAPQIS)

The group was also instructed to consider the responsibilities, criteria and candidate institutes for Resource Centers for Grouper Diseases.

**Terms of Reference: Working Group III**

Within a regional framework, Working Group III was expected to consider discussions that promote cooperation between participating economies, relevant research institutes, the private sector. It was requested to focus on reducing on-farm losses from grouper disease, and supporting responsible movement and trade in live groupers to minimise risks of pathogen introduction and transfer along with their fish hosts. Implementation mechanism, including roles and responsibilities of key regional institutions and networking strategy, linkages with other on-going projects were key elements of the Working Group discussions.

Specific activities for the Working Group included consideration of

- Private sector needs and potential contribution
- Funding options for research, follow-up workshops, training (ACIAR, APEC, EU, FAO, JICA, JIRCAS, private sector)
- Links with other on-going projects
- Mechanism for dissemination/extension of research results to private sector/small and medium sized enterprises
- Networking of scientists especially through the FHS/AFS and the Grouper Aquaculture Network

**Workshop Program**

The workshop program is summarised in Annex 1. Delegates to the workshop are listed in Annex 2.

A summary of the Workshop program follows:

- 12 Country presentations
- Regional Synthesis
- 7 Technical presentations
- 1 APEC project update
- 3 Working Group Discussions
- Working Group Presentations
- Discussion and Recommendations

In conclusion, Dr Reantaso thanked all countries, institutes and private sector representatives for participating and declared the workshop open.

**SESSION 1: PRESENTATION OF GROUPEL DISEASE IMPACT SURVEY**

The following section provides a brief summary of the presentations and discussion points which followed after each session. The full paper of the presentations are presented as Part II of this Report.

**Survey on the Impacts of Grouper Viral and other Health Problems in Indonesia (Ms Erna Dewi, Medan Provincial Fisheries Service)**

Grouper culture in the aquaculture regions of Indonesia during the period from 1995 to 1999 was described. Diseases caused termination of culture. The following diseases, disease syndromes, parasites and pathogens were identified in the survey: encapsulated gill didymozoid (uncharacterized), iridovirus, deaths of unknown aetiology, skin fluke, and vibriosis. It was concluded that mortality among grow-out fish was a problem in grouper culture. The causes included iridovirus as well as other diseases. Dependence of seed supply on the wild sources remains a significant problem. Sumatra has no facility for grouper hatchery production.

**The Status of Grouper Culture and Disease in Korea (Dr Myoung Park, South Sea Fisheries Research Institute, National Fisheries Research and Development Institute, NFRDI)**

Grouper is cultured in the Korean coastal area. The environmental characteristics of the Korean coastal sea were described. Korean marine fish aquaculture is highly profitable, but high profit fish production is limited to a few farms. Farming is conducted in net cages, enclosure ponds and land-based tanks. The disease status of seven band grouper was described in terms of clinical signs, results of pathogenicity studies, experimental infection, PCR tests and the role of temperature in viral infectivity.

Marine fish diseases have become more diverse and complicated. As disease occurrence is closely related to management practices, it is important that aquaculture farmers have sufficient knowledge, and that appropriate husbandry methods and disease control measures are implemented. In order to decrease the impact of diseases and to ensure safety of cultured fish for human consumption, the National Fisheries Research and Development Institute (NFRDI) together with other universities in Korea are conducting research on disease control.

**Survey of Grouper Diseases in Malaysia (Mr Chuah Toh Tye, National Fish Health Research Center, Fisheries Research Institute)**

A description of cage farming practices in Malaysia was presented. Fry are imported from Chinese Taipei or Indonesia while eggs are imported into local hatcheries from Chinese Taipei or Singapore. Production has dropped between 1994 and 1998, and farmers are very concerned about disease. In Malaysia, major efforts have been made in barramundi farming. The production has been successful during first few years but has been limited due to occurrence of diseases. A similar pattern is now emerging with grouper. At present, Malaysia still depends on imported fry. There are problems with diseases introduced by host movement.

A list of reported diseases was presented. They included the following: black body disease (suspect viral; the fish body turns black and then fish die), red body disease (possibly bacterial, as antibiotics can be used to manage disease), fin rot syndrome (characterised by eroded fins), ulcerative lesions on body, bacterial ulceration, and gill rot. Other classified syndromes include bacterial vibriosis, black viral disease, and cryptocaryoniasis. Studies at sea bass farms have shown Iridovirus infection. It is suspected that black body disease syndrome may be of viral aetiology. Liver disease problem reported by farmers has been resolved. The need to confirm presence of virus in grouper aquaculture in Malaysia was emphasized. This has not been done yet.

**Survey on the Impacts of Grouper Viral and Other Diseases in the Philippines (Dr Sonia Somga, Fish Health Section, Bureau of Fisheries and Aquatic Resources)**

The results of the survey conducted in the Philippines were presented. There are six significant disease syndromes affecting grouper culture in the Philippines, as reported by farmers during this survey. Three of the reported disease syndromes are likely to be of viral origin. These include syndromes described as strong putrid smell, corkscrew swimming and pop eye syndromes. Bacterial diseases (*i.e.* hemorrhagic ulceration and white patch syndrome) and parasite (*i.e.* marine leech) problems were also commonly reported. Production losses due to these disease syndromes had a great economic impact on farmers. Some farmers switched to culturing other species, others decided to terminate culture operations.

Based on the information gathered and observations made during the survey, there are a number of risk factors that may directly or indirectly affect the grouper health. These include: (a) quality of seed stock, (b) management practices, (c) sudden changes in the environment, and (d) increasing and unregulated aquaculture activities.

Disease remains a threat to the flourishing grouper industry of the Philippines. Though major disease syndromes are gradually documented, additional studies on the aetiology of these syndromes are required. Additional research is required to assess the role of risk factors identified in the development of the disease.

#### **Grouper Viral Impact Survey in the South and East Coasts of Thailand (Mr Somporn Rongkamneardvong, National Institute for Coastal Aquaculture (NICA), Department of Fisheries)**

An overview of grouper production in Thailand was presented. The survey covered 82 farms out of 1100 (52 in Southern Thailand and 20 in Eastern Thailand). The species cultured are *E. malabaricus* (4.5%) and *E. coiodes* (95.4%). Grouper aquaculture is based on trapping with growth of 12-18 months to market size. Fish of the market size over 700 g is priced at US\$ 8-10. The major export markets are Chinese Taipei and, to a lesser extent, Singapore. The culture methods of culture include cage systems (87.8%) and pond culture (12.2%). The survey revealed variations in occurrences of diseases for different culture types.

Reported diseases and disease syndromes were classified into 7 broad categories such as (a) viral infection (VNN, Iridovirus), (b) bacterial infection (*Streptococcus*, *Flexibacter*, red spot, others), (c) parasites, (d) EUS like syndrome (ulcerative necrosis and bad smell), (e) starvation (flat abdomen), (f) tumour, and (g) unknown (no clinical signs with high mortalities).

#### **Diseases of Cultured Grouper in Khanh Hoa Province, Vietnam (Dr Nguyen Dung, University of Fisheries, Na Thrang)**

An introduction to grouper culture in Vietnam was presented. It was noted that the survey had concentrated on the south-mid coast of Vietnam. Fry are captured from wild fish at 5-15 cm, and low stocking densities are used. Trash fish (tilapia) is used as feed in ponds and cages. Size sorting is generally performed on a bi-monthly basis. Farms are mostly small scale and usually family run. The family assists in sorting and moving fish and cleaning nets and cages. Annual production is 400-450 metric tons. A relationship between culture type and disease was identified. The disease occurred more frequently at nursery farms. The disease syndromes reported by farmers included sea-lice infections, sinking death fish, skin lesions and whirling syndrome. Other classified syndromes included parasitic infection, oxygen depletion, vibriosis and VNN-like infection.

#### **Grouper Health and Production in Australia (Dr John Humphrey, Department of Primary Industry and Fisheries, Darwin, Northern Territory)**

Grouper aquaculture in Australia is still in its infancy with only 2 species being candidates for aquaculture (barramundi cod or hump-backed grouper *Cromileptes altivelis*, and estuary cod *Epinephelus coiodes*). The occurrence of reported pathogens, parasites and diseases in grouper was presented. There is no record of viral diseases among grouper. However, VNN was a major limiting factor in the barramundi/sea bass industry in Australia. Other iridovirus, notably epizootic haematopoietic necrosis virus in other fish was also reported. *Vibrio harveyi* and *Photobacterium damsela* had been associated occasionally with peritonitis in barramundi cod. Isolated occurrences of swim bladder mycosis in barramundi cod were reported. In one case the fungus appeared to belong to the Cladosporium – Xylohypha group. Microsporidian cysts in the abdominal cavity of barramundi cod have been recorded. The digenean *Pseudorhabdosynochus* sp. was recorded in *Epinephelus quoyanus* as an isolated occurrence. Its pathogenic significance is unknown. Similarly, the monogenean *Sprostonia longiphallus* was recorded in *Epinephelus tauvina* and *Epinephelus malabaricus* as isolated cases. Its pathogenic significance is also unknown. Copepods recorded include *Caligus epinephali* in *Epinephelus merra*, *Dissonus manteri* in *Epinephelus fario*, and *Lepeophtheirus epinephali* in *Epinephelus hoedti* and *Epinephelus gilberti*. These are isolated records of unknown pathogenic significance. Nematode larvae identified as *Terranova* sp. type 11 has been associated with a syndrome of visceral fibrosis and peritonitis in mature barramundi cod. This syndrome has resulted in the death of 17 broodstock fish and is a serious problem in broodstock management. Other disease syndromes include : visceral fibrosis and peritonitis associated with *Terranova* sp. Larvae, exogenous mortality feeding syndrome, “Shock” syndrome, and cannibalism mortality syndrome. The latter three occur in fry at around 1 month of age and appear to be associated with feeding behavior and possibly nutritional deficiencies.

**Cage Culture of Grouper in Brunei Darussalam (Mrs Laila Hajah Hamid, Department of Fisheries, Ministry of Industry and Primary Resources)**

An overview of current activities relating to grouper aquaculture and health in Brunei Darussalam was presented. Imported groupers are examined for parasites and bacterial infections. Some of the recognized diseases of grouper in Brunei included bacterial tail rot, bacterial gill diseases, other bacterial diseases, trichodiniasis, diplectanid and capsalid monogeneans. For this purpose, fish are held in holding tank and closely monitored. It was noted that there are a number of key issues and constraints in grouper aquaculture. It was suggested that consideration be given to the joint production of seed stocks free from disease.

**Status of Grouper Culture, Fry Production and Grouper Disease in Guangdong, China (Mr Zhang Haifa, Guangdong Fishery Technical Extension Center)**

The status of seed production, fry supply, grow-out culture, and diseases of grouper in Guangdong Province, People's Republic of China was reported. China, the biggest producer of grouper, contributes about 50% to the total world aquaculture production. Guangdong province is the largest producer in China. The mean survival rate of net-caged cultured groupers is 30-40%. The mortality of larvae and juvenile of hatchery-reared groupers due to VNN can reach as high as 100%. The survey results indicate that diseases seriously threaten grouper culture. Prevention of further spreading of the grouper diseases in People's Republic of China is a critically important and urgent matter.

**Diseases of Grouper in Chinese Taipei (Dr Shau Chi Chi, National Taiwan University)**

A survey of VNN amongst cultured grouper in Chinese Taipei was presented. VNN was recognized as a world-wide epizootic disease amongst marine fish causing high mortalities in larvae and juveniles. The four genotypes of the virus were described. The different diagnostic techniques used for GVNN were also described. They include histopathology, electron microscopy, PCR, virus isolation and *in-situ* hybridization. The important role of temperature in viral proliferation was noted. Results of studies on effect of temperature on cell proliferation were presented. GNNV has been detected from several species of cultured fish in Chinese Taipei and a wide variety fish already infected, including barramundi. It was noted that either high or low mortalities may occur. It was thought that GNNV has spread to other species of fish and there was a need to find means of controlling spread of GNNV. The presentation noted that (a) hatchery hygiene can be effective in controlling the disease and eggs can be sterilised before hatching, (b) transmission is considered to be both horizontal and vertical, and (c) the virus has been detected in trash fish and this feed may be a source of viral infection.

**Grouper Disease Impact Survey in Hong Kong China (Dr Roger Chong, Agriculture, Fisheries and Conservation Department)**

An overview of grouper production and health in Hong Kong China was presented. It covered three main species (*Epinephelus tauvina* - Green grouper, *Epinephelus areolatus* - Brown spotted grouper, and *Epinephelus malabaricus* - Malabar grouper). Fingerlings are imported from a number of countries in the region including China, Chinese Taipei, Indonesia, Malaysia, Philippines and Thailand.

Listless disease, vibriosis, glugeosis were described including clinical signs, histopathology, diagnostics, associated risk factors and control measures. The presentation concluded that poor water quality in the mariculture zones is the basic problem that precipitates the whole range of serious diseases among cultured grouper in Hong Kong China. This is associated with the following: (1) trash fish feeding, (2) poor site location, (3) inadequate sea bed management, (4) stocking in excess of the holding capacity of the culture zone, (5) stocking based on market price of fish which leads to overstocking, (6) limited sea area which resulted to overcrowding of rafts and poor water exchange, and (6) lack of a rotational system of zone usage which promoted sea bed degradation. The problem has surfaced during the last 5 years, because the natural limit of the culture zone ecosystem had been exceeded. For the first 10 years, losses due to disease were approximately 10-20% of stocked fish. They have skyrocketed to 60-70%.

**Grouper Viral Diseases and Research in Singapore (Dr Siow Chang Foong, Central Veterinary Laboratory, Agri-Food and Veterinary Authority of Singapore)**

Dr Foong presented an overview of grouper production and the role of viruses diseases in Singapore. Grouper production comprised 11% of finfish production in Singapore. Fry are imported from Indonesia and Chinese Taipei. It was reported that three viral diseases are of importance to grouper aquaculture in Singapore. They are iridovirus (reported in 1998 affecting Malabar grouper), sleepy grouper disease (reported in 1992 with high losses) and VER or VNN (first described in 1996, with last reported case among seabass in 1997). Further studies are being undertaken to characterise the nature of grouper iridoviruses and nodavirus. The presentation was concluded with an observation that outbreaks of viral diseases are not common and do not appear to be a major problem.



**Regional Synthesis of Grouper Health Impact Survey (Dr Angus Cameron, Aus. Vet Animal Health Services)**

The report focussed on a regional synthesis of survey results collected by participating countries over the past 3-4 months. It attempted to draw conclusions about priorities in grouper disease research. The syndromes reported during the survey were classified into 7 broad categories such as parasitic trematodes, viral, bacterial, fungal, parasitic protozoan, other syndromes and non-specific syndromes. The list is a mixture of differential diagnosis and syndrome descriptions, and laboratory confirmation was not available except in only one case (*i.e.* Iridovirus in Indonesia). The listed syndromes should, therefore, be interpreted as shorthand descriptions of disease syndromes, presenting signs consistent with those caused by the nominated pathogens. With respect to disease impact, it was revealed that there has been an increase in disease problems over the last 5 to 10 years, with most countries reporting that 90% of farms had disease problems. Statistical analysis of survey results showed that there are unlikely to be any real differences between countries, except for Vietnam where the number of farms reporting disease problems was significantly lower. This was probably due to fewer farms suffering from disease, different survey methodology or a lower awareness of diseases amongst farmers.

On a regional basis, 365 diseases or diseases syndrome occurrences were reported. Diseases have been broadly classified into four main putative causes: viral, bacterial, parasitic and others. The classification was based on the assumed cause of the disease, which in turn was based on farmers' reports of clinical signs. The commonly reported diseases, VNN and Vibriosis, have been reported separately, as well as combined into *All viral* and *All bacterial* disease categories. Bacterial diseases constitute 40% of problems reported, and viral diseases - 26%. Vibriosis is the most commonly reported disease. The apparent importance of vibriosis was noted. This refers to a syndrome characterised by skin lesions and or tail rot. It is apparent that, while *Vibrio* organisms are commonly isolated from disease outbreaks, they may not be the primary cause. Other factors that have been suggested as primary or contributing causes include parasites, feed quality, water quality and overstocking. If vibriosis is mainly a secondary or opportunistic pathogen, then the relative importance of bacterial diseases, compared with viral, parasitic or other causes (*e.g.* environmental or management) should be greatly diminished.

With respect to estimates of mortality for key diseases, it was noted that there is a large amount of variation in the estimates of mortality, both between individual farms, and between country averages. However, as a broad estimate, these four key diseases result in an average of approximately 30% disease mortality. Data on mortality, morbidity and economic loss indicated that those viral diseases tend to affect a greater proportion of fish (higher morbidity) and result in more deaths (higher mortality) than bacterial, parasitic or other diseases. The economic loss data appear to be somewhat misleading, as different definitions may have been used in different countries. Bacterial diseases tend to result in greater residual damage and loss in value in surviving fish than other diseases, but VNN causes the highest estimated loss for an individual disease. These conclusions should be treated with caution.

**General discussion**

- It was noted that the survey results indicated a number of pathogens, not only viral and that it is important to interpret syndromes correctly.
- A concern was expressed over the correlation of clinical signs, syndromes and etiological agents in the absence of confirmed laboratory findings and the issue of lacking laboratory diagnostic capabilities to accurately identify syndromes.
- It was indicated that the absence of samples for laboratory examination during the survey was one of the reasons for the lack of a laboratory data. It was agreed that there is a need for research to further characterize the syndrome/s and there was a need for increased application of diagnostic methods and improvement in diagnostic skills.
- Examination of multiple specimens by laboratory means was identified as important in correlation of syndromes with disease.
- It was noted that while the project was focusing on viral diseases, its scope covers other disease investigations and research. It was also noted that the workshop would be a very good foundation for further work of this nature.
- The discussion was concluded with the observation that since 1996 production dramatically declined It is associated with widespread sourcing of seed from other countries, transfer of pathogens from some species to others and possibly with spreading of pathogens into the environment. It was further noted that over the past 10 years, pathogens rapidly expanded and that an urgent need exist to prevent further spread of diseases.

## SESSION II: TECHNICAL PRESENTATIONS

### **Parasites and Bacterial Diseases of Grouper and other Cultured Marine Finfishes and Control Strategies (Dr Leong Tak Seng, LTS Consultancy, Penang, Malaysia)**

Dr Leong's presentation focused on his extensive experience and research into parasitic diseases, especially the monogenean gill parasites, which emerged as major pathogens at commencement of sea cage culture of grouper in Malaysia. He provided a brief history of disease occurrences in Malaysia. Dr Leong noted that the main problems were vibriosis and monogenean infection, swim bladder disease, baldness disease, tail rot, and diseases of uncertain aetiologies. In 1991 and 1992, sleepy grouper disease occurred among *E. coiodes* as well as scale drop disease in *L. calcarifer*. Dr Leong reported that the scale drop affected fish turned dark without any other signs and died. Subsequently, ulcerative lesions "boil disease" emerged as a problem. The parasites *Benedenia* and *Neobenedenia* also represented a problem. A comprehensive review of disease conditions in farmed fish in Malaysia was presented. It was noted that if fish survive for the first week, then they are generally not susceptible to diseases. *Vibrio* spp. can also be isolated in healthy fish, but also pathogenic *Vibrio* spp. belonging to Groups 1 and 2. He presented a list of parasites identified in farmed fish in Malaysia, noting the common occurrence of monogeneans. The presentation was concluded with a statement that disease is multifactorial in the culture environment. Malaysia had developed a production system which can overcome problems with vibriosis, the primary cause of which was suspected to be predisposed by high monogenean population in gills.

### **Recent Developments on Identification and Control of Viral Nervous Necrosis (VNN) of Grouper (Dr Toshihiro Nakai, Hiroshima University, Japan)**

Dr Nakai gave a brief overview of the history of VNN occurrence worldwide, noting its distribution in the Mediterranean region, Europe, Asia and the Pacific and in California. Other species are susceptible, especially striped jack, which are very susceptible up to 10 days of age (which is different for grouper).

The histology and molecular biology of the virus were presented. The difficulty in studying fish nodaviruses resulted from unavailability of cell lines, which limited molecular characterization of the virus. Two cell lines are available for fish nodavirus: SSN-1 from snakehead fish and SBL from sea bass. Information on infectivity and growth or culture conditions for nodavirus on SSN-1 cell line was also presented. The disadvantage of the cell lines was its being composed of mixed cells, and possible spontaneous infection with retrovirus. Attempts to clone SSN-1 cell line have been undertaken. Clone cells have given rise to an E11 clone, which gives very clear CPE, with contraction of cells. Cloned cell line also gives very high titers of virus, especially compared to RTG cell line which did not grow virus.

Using the E11 cell line, Dr Nakai demonstrated that all strains of virus tested grew between 25-30°C. A disadvantage of this method is that the cell line takes a long time (up to 10 days) to express CPE.

The presentation included results of viral isolation and use of RT-PCR technique for viral diagnosis, as well as their advantages and disadvantages. With respect to disease transmission, the importance of the carrier state was emphasized. Current control measures for VNN being carried out in Japan was described including alternative methods such as development of SPF brood-stock and potential of vaccination technology.

Dr Nakai concluded his presentation by describing principles of health maintenance, which included choice of proper facility site, avoidance of exposure to pathogen, segregation of brood-stock and larvae, and stress reduction.

### **Present Situation of Occurrence of Viral Nervous Necrosis (VNN) in Indonesian Grouper Hatcheries and Control Measures for VNN (Dr Kei Yuasa, JICA, Indonesia)**

Dr Yuasa gave a brief history of the occurrence of VNN in Indonesia, where VNN was detected first in 1997 in barramundi. The history of humpback grouper production at the Gondol Research Station for Coastal Fisheries (GRSCF) was provided. Juvenile groupers were successfully produced until 1998, after which the production failed. The cause was viral nervous necrosis (VNN). It was noted that other research stations had reported mass mortalities due to VNN.

Diagnostic methods for VNN detection were described; control measures at the GRSCF were presented. Further studies were recommended which would include vaccine development, use of immunostimulants, virus habitats using cell lines and environmental factors examining relationship of temperature, salinity, pH and bacterial flora on development of VNN.

The presentation was concluded with a note that VNN is the most serious disease in grouper production. Most species are affected and the disease has a major impact on hatcheries. Oral administration of Prefuran may reduce virus for unknown reasons and vaccination may improve survival. There may be a relationship between water temperature and occurrence of VNN.

### **Structure and Transmission Cycles of Nodaviruses and Iridoviruses Infecting Fish (Dr Peter Walker, CSIRO, Australia)**

Dr Walker reviewed the history of nodavirus diseases in a range of fish species and under different names. He described the clinical syndromes associated with infection, noting similar disease characteristics in most fish species, primarily the abnormal swimming behaviour (inverted, corkscrew-like, floating near surface, discolouration). Histologically, the disease is characterised by vacuolation and necrosis in the grey matter of the brain and spinal chord, and in the granular layers of retinal tissue, the presence of basophilic intra-cytoplasmic inclusions. The genetic relationships of Nodaviruses were also discussed. The extent of diversion between isolates was approximately 30%. The considerable diversity between host fish species suggests that horizontal transfer between species is part of the normal evolutionary history of nodaviruses.

Transmission mechanism was also described. There is an evidence of vertical transmission only through spawners, which suggests that virus may be replicating in internal organs. It was suggested that multiple spawning might increase disease transmission and that ozone disinfections appears to be effective (an indication that virus is present on the surface of eggs).

Dr Walker pointed to the potential to spread virus as a consequence of aquaculture. He noted the need for screening in hatcheries, the availability of clean or PCR negative brood-stock, the combinations of true horizontal and vertical infection in the epidemiology and pathogenesis of the disease. He noted that an increasing prevalence of virus in brood-stock may occur, making selection of virus-free brood-stock important. He stressed the need to be far more cautious in movements of fish from one location to another.

The iridoviruses infecting fish were briefly reviewed. The genus Lymphocystivirus, results in benign tumours in a range of fish hosts. The genus Ranavirus is found in systemic infections of fish reptiles and amphibians. Members of the genus are emerging as important pathogens of cultured fish. They are a large virus, double stranded DNA, circularly permuted, terminally redundant and highly methylated. A recent project in Australia for iridovirus control of cane toad was also reviewed. It was indicated that this program might be useful in iridovirus vaccine development. Transmission cycles of iridoviruses are complex, with a variety of hosts, carriers of infection, spread across species between fish, frogs and reptiles. There is no evidence of vertical transmission.

The presentation was concluded with a note that it may be possible to use viral vaccines in feed, but process and cost of vaccine development and registration are important considerations. While there may be effective vaccines it is not clear if they can intervene in vertical transmission situations.

### **Immunological Methods for Disease Control in Aquaculture (Dr Somsak Vinitnantharat, Alpharma, USA)**

Dr Somsak discussed the application of immunology to diagnostic methods and vaccination. It was noted that diagnostic applications utilise the specificity of antigen and antibody. There is a range of such methods, *e.g.* ELISA, fluorescent antibody tests and others. He noted that diagnostic immunological tests helped to diagnose disease, to ensure appropriate treatment, to control disease and to implement eradication programs. The need to have agreement on interpretation of detecting antigen or antibody was stressed. It was noted that the use of diagnostic immunological methods requires understanding of meaning of results. Dr Somsak emphasised a need to address this issue prospectively with written protocols.

In discussing vaccination, Dr Somsak emphasised the appropriate use of vaccines in different aquaculture systems, exemplified by vaccination in channel catfish production in USA under pond systems, hybrid bass in recirculating systems, cage culture systems in Norway, raceway culture systems in USA. Vaccine delivery methods were also discussed including the advantages and disadvantages of such methods. The commercially available vaccines and those under experimental development were described. Dr Somsak noted the differences between bacterial and viral vaccines and that a high proportion of viral vaccines is still at the experimental stage.

Requirements for commercial vaccine development and benefits of vaccination were discussed. The significant reduction in antibiotic use was emphasized. The example of Norway controlling Hitra disease was cited.

Dr Somsak concluded the presentation with a note that immunological methods provide fast and accurate diagnosis and are good tools for disease control in aquaculture. He noted that while vaccines are a cost effective method for controlling certain infectious diseases, they should be used only as part of a comprehensive and integrated program on aquatic animal health management.

**Vaccine Development and Potential for Disease Control (Mr Stuart Miller, Institute of Aquaculture, Stirling University, Scotland)**

Vaccine development and registration mechanisms were discussed. Mr. Miller cautioned about the real costs of developing vaccines. He spoke on the importance of disease control through good husbandry practices, health monitoring, chemical treatment, use of antibiotics and vaccines.

A disease should be sufficiently serious and of such economic importance to warrant vaccine development. A vaccine must fulfil certain criteria: (a) be effective in preventing disease or mortalities, (b) be inexpensive to produce, (c) create long-term immunity, (d) be readily administered and (e) prevent virus persistence.

Mr Miller spoke on studies on the sea bass nodavirus undertaken by Stirling University and France. He noted that most of the work was performed on a range of European isolates of nodavirus. The studies included chemical and physical factors, antigenic and genetic variance, improved diagnostic tools for detection of virus and pathogenicity studies. Results of these studies indicated that nodavirus has high degree of chemical and physical stability in aquaculture systems, which supports the need for vaccine development.

With respect to vaccine development, Mr Miller noted that vaccine development requires considerable capital investment. Development of even a crude vaccine requires extensive and, thus, expensive laboratory and field trials. Effect of the vaccine on target and non-target fish should be researched. Another issue is the complex and daunting regulatory controls and the process of vaccine registration, which is time consuming and expensive. Finally, Mr Miller stressed that the economic viability of developing a vaccine should be determined. Technical and regulatory issues and other considerations regarding vaccine development were presented. They include the following: (a) isolation and characterisation of the agents, (b) comparative studies, (c) antigen analysis, (d) immune response, (e) crude vaccine laboratory trials, (f) field trials and (g) commercial involvement.

The presentation was concluded with a note that the workshop might find that vaccination is the right answer. However, the need for a vaccine and administration mechanisms must be given a serious thought.

**Import Risk Analysis in Minimising Trans-boundary Movement of Infectious Disease Agents (Dr Ian Peebles, AQIS, Australia)**

The import risk process and the importance of the mechanisms surrounding the fish import risk process were described. Dr Peebles emphasized the need to define the hazards involved in the process and to decide on what constitutes a hazard in terms of international trade. The definition for 'hazard' was presented and the need for consistency in domestic and international disease management was stressed. The hazard list and international and regional disease lists (particularly the OIE and the FAO/NACA disease lists) should be developed. The national databases would form a basis for management of the import risk process.

Dr Peebles stressed that risk management should be implemented by aquatic animal health scientists. He noted that the import risk assessment process was defined in the OIE Code. He discussed the concept of release assessment and exposure assessment as part of the overall risk assessment process.

The release assessment factors were tabled and discussed. They include biological factors, country factors and commodity factors. It was noted that country factors include the definition of free regions.

**Development of a Health and Husbandry Manual for Grouper Farming (Dr Erlinda Lacierda, SEAFDEC-AQD, Philippines)**

Dr Lacierda reviewed the manual background. Development of the manual is also an APEC FWG funded project, which is undertaken by the Philippine Bureau of Fisheries and Aquatic Resources (as Project Overseer) and SEAFDEC-AQD (as Project Implementor).

The manual was required because of the major problems concerning diseases and production practices in sustainable grouper culture. The problems included limited seed supply, reliance on wild caught seed, and high demand for fish. The high demand resulted in unsuitable practices, including cyanide poisoning for fish collection. In addition, mortalities in farmed fish were frequent. It was decided to develop a practical manual on grouper culture to assist in resolving

these problems.

The objective was to produce a practical health and husbandry management manual for grouper culture, which would be simple, practical, farmer friendly and contain many illustrations. The intention is to publish it initially in English and then translate the manual into Thai, Bahasa, Filipino and Chinese. The manual will be based on extracts from books and journals and will rely on participating and collaborating institutions, especially for unpublished material. Participating organisations and institutions include (a) QDPI and the Aquaculture Development Foundation Inc. in Australia, (b) GRSCF in Bali, Indonesia, (c) the Fisheries Research Centre in Malaysia, (d) Guangdong Daya Bay Fisheries Development Centre in Guangzhou, China, (e) Fisheries Research Institute in Chinese Taipei and (f) National Institute of Coastal Aquaculture and the Department of Fisheries of Thailand.

The format of the manual was briefly outlined. The manual contains sections on the following: (a) site selection, (b) culture systems, (c) collection and handling and transport of wild seed, (d) operations and management protocols, (e) health management, and (f) harvesting and post-harvest handling.

The deadline for completing the manual was June 2001 with translated versions being available by the end of this year.

### General Discussions

- The availability of suitable cell lines was discussed. It was mentioned that cells line are currently available in the following laboratories: (a) James Cook University (Dr Leigh Owens), (b) Tasmania University (Dr Barry Munday), and (c) AVA of Singapore.
- Diagnostic techniques were discussed intensively. The discussions included the use of PCR techniques which were strongly recommended by the recently held Expert Consultation on DNA based methods for use in aquatic animal diseases (Walker and Subasinghe 2000). The discussions also focused on more careful evaluation of PCR methods currently used for diseases of grouper and use of viral isolation.
- The workshop discussed the need to properly diagnose diseases of groupers other than the viral diseases (which appear to be the most serious).
- With respect to vaccine development, the workshop strongly recommended to evaluate the vaccines currently used in Malaysia and private sector (for example, Alpharma) for the grouper vaccine development. An on-going project on vaccine development funded by EU, which involves a number of APEC economies, was also discussed.

**SESSION III WORKSHOP DISCUSSIONS and Presentation of the Framework of a Regional Research Programme on Grouper Health and Production**

**Working Group 1: Research Needs on Grouper Health**

**Chair:** Dr T. Nakai

**Co-Chair:** Dr Peter Walker

**Rapporteurs:** Dr Gilda Lio-Po and Mr Stuart Millar

**Members:** Dr Shau Chi Chi, Dr Myoung Ae Park, Mrs Erna Dewi, Dr Sonia Somga, Mr Zhang Haifa, Mr Sompong Roungkamneardvong, Dr Kei Yuasa, Ms Kjersti Garvningen, Dr Erlinda Lacierda, Dr Nguyen Huu Dung, Dr Somkiat Kanchanakan, Dr Sataporn Direkbusarakom, Dr Supalak Putinaowarat, Dr Ong-ard Lawhanit.

**Working Group 1.** Based on the findings and presentations at the workshop and considering the research needs to support existing and future grouper health and production, the group made the following observations, recommendations and conclusions.

**1. Most significant grouper diseases**

**Viruses:** VNN, Iridoviruses

**Bacteria:** *Vibrio* spp., *Flexibacter* spp., *Pasteurella* spp., *Streptococcus* spp.

**Parasites:** Monogeneans (*Benedenia/Monogenea*, *Cryptocaryon*, *Trichodina*)

**Unknown or uncharacterised diseases:**

- Unknown viral infections may be an important cause of unknown disease problems.
- “Putrid smell syndrome” reported from several countries but aetiology unknown

**2. Suitable cell lines for virus isolation**

**Viral Nervous Necrosis (VNN).** Two good relevant cell lines: SSN-1 – available at EACCC, high titre (10<sup>10</sup>) for VNN, acceptable but not ideal for growth rate and robustness, infected with retrovirus, mixed cell population (E11 is a subclone of SSN-1 to be deposited in Japanese Type culture collection, more consistent characteristics but still has retrovirus.) GF-1 appears to be more robust, lower viral titres (10<sup>8</sup>), retrovirus status unknown, available soon through ATTC. Maintenance and distribution from reference laboratory in Japan or Italy is desirable.

**Iridovirus.** A number of cell lines is available. However, there is a need to continue development of additional cell lines for detection and growth of new and unrecognised pathogens.

**3. Development of techniques for viral identification and diagnosis**

**Recommendations of Expert consultation on DNA-based diagnostic technologies<sup>2</sup>.** Due to WSSV problem, availability of thermal cycles and familiarity with PCR use has increased in Asia. There is a need for additional training in the use of DNA-based technologies for screening and some diagnostic applications. Antibody or histological methods are suitable for disease diagnosis in other diseases.

**Need for manual of standardized methods for grouper disease diagnosis and for training.** The manual should be developed following consultation to identify standardized methodologies.

**Capacity Building.** There are considerable variations in the capabilities in histology, immunodiagnosis, cell culture and DNA-based methods throughout the region. Specific needs for capacity building should be identified.

**4. Protocols for disease induction**

**VNN.** Disease can be induced by injection or immersion but the process of natural disease emergence and vertical transmission is not well understood. Additional work is required to understand differences between the SJNNV disease model and observations on VNN.

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<sup>2</sup> Walker, P. and R. Subasinghe (eds). 2000. DNA-based molecular diagnostic techniques: research needs for standardisation and validation of the detection of aquatic animal pathogens and diseases. Report and proceedings of the Expert Workshop on DNA-based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation of the Detection of Aquatic Animal Pathogens and Diseases. Bangkok, Thailand, 7-9 February 1999. FAO Fisheries Technical Paper. No. 395. Rome, FAO. 93 p.

**Iridoviruses.** Information on iridoviruses in grouper, particularly on disease transmission, pathogenesis and possibility of multiple strains/species, is insufficient. This disease will likely emerge as a problem

**Bacterial Diseases.** The epidemiology of bacterial disease, particularly the role of environmental factors, is not clear. Additional work is required.

**Unrecognised Pathogens.** Unrecognised pathogens should be classified and characterized.

### **5. Prevention and control of viral diseases**

More information is required on items 1-4 before effective prevention and control methods can be developed. Effective treatments for parasitic infections (e.g. bath treatments) should be also evaluated.

#### **Vaccination**

**VNN.** Antigen development is progressing successfully. *E coli*-expressed antigen looks very promising. There is a need for more information on vaccination strategies based on an understanding of the disease cycle, the association between infection and immunity, and the development of the immune response in grouper. No real alternatives exist at this time.

**Iridovirus.** There is an existing iridovirus vaccine for red sea bream developed in Japan, which appears to be cross protective. There is a need for more information on the genetic and antigenic structure of grouper iridoviruses. Live attenuated vaccine may be effective but reservations about regulatory requirements were raised.

**Bacteria.** The existing vaccines should be tested after the pathogens in Asian grouper are better identified. The vaccines are unlikely to be cross protective but it may be possible to apply technology to vaccine development.

### **Working Group 2: Strategies to Minimise Risks of Pathogen Transfer Through Responsible Movement of Live Aquatic Animals**

**Chair:** Dr John Humphrey

**Co-Chair:** Dr Leong Tak Seng

**Rapporteurs:** Dr Ian Peebles and Dr Roger Chong

**Members:** Mrs Hajah Laila Haji Hamid, Dr Edward Dannahutana, Dr Angus Cameron, Dr Kamonporn Tonguthai, Dr Chang Soiwoong, Mrs Phan Thi Van, Mr Sih Yang Sim, Mr M. Sanggam, Mr Chuah Toh Tye, Mr Somsak Vinintharat, Dr Melba B. Reantaso

**Working Group II.** The group reviewed the spread of diseases through live grouper movements and identified a number of major issues that subsequently formed the basis for a series of recommendations.

#### *General Considerations*

#### ***Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals***<sup>3</sup>

The group noted that this document was a set of guiding principles for adoption by regional nations. It was an important benchmark as a basis for future regional disease control and quarantine.

#### ***General Questions***

A number of questions were raised. They included the definition of diseases, the geographic occurrence and distribution of the diseases, mechanisms for disease reporting and surveillance, diagnostic capabilities, distribution patterns of movement of live fish, application of risk assessment processes and risk minimisation issues.

#### ***General Observations***

The Group made several general observations to assist in developing recommendations. These included the following.

- Trade in live grouper fry and fingerlings are essentially unregulated.
- A large amount of fish is translocated and the number is increasing.

<sup>3</sup> FAO/NACA. 2000. Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals and the Beijing Consensus and Implementation Strategy. FAO Fisheries Technical Paper No.402.Rome, FAO.2000, 53 p.

- Majority of trade is in wild-harvested fish. Few hatcheries exist in the region.
- Sources of imports are a good starting point for minimisation strategies.
- Multiple factors are involved in the spread of pathogens or parasites.
- Implementation of strict or excessive controls will drive trade underground.
- Legislation and specific mechanisms for disease control exist in some jurisdictions, *e.g.* requirements for health certification, inspection, but these are often difficult to implement or enforce.
- In some cases, there is confusion over nature of certificates and requirements.
- There are no uniform regional requirements to support movements.
- Hatcheries play a key role in the industry.

### **Diseases to be considered**

VNN and parasitic blood flukes were considered the priority diseases. The first was on the OIE and NACA/FAO lists. The second disease has indications that they had not yet widely disseminated.

### **Reference or resource center for grouper viral disease &/or other diseases**

The group noted regional deficiencies in skilled staff and laboratory resources and supported the concept of a regional resource centre, which would assist in addressing these limitations. The group considered that a network of collaborating institutions might be more appropriate than one facility. Issues regarding reporting and confidentiality of information and financial support for this centre were discussed. The need for confidentiality in reporting of results to submitting nations was stressed. It was considered unreasonable to expect existing institutions to take on a major role in acting as a resource capacity without additional funding.

### **Diagnostic capabilities**

The group emphasised the need for diagnostic capabilities to underpin issues relating to movement of aquatic animals and act as a basis for disease surveillance and monitoring. Causes of disease should be identified. Screening of hatcheries and possibly wild fish prior to movements of live grouper needs to be undertaken.

The group agreed that laboratory capabilities and scientific expertise in both exporting and importing countries are a minimum requirement to support responsible regional movement of grouper.

### **Import Risk Assessments**

The group concluded that application and implementation of risk assessments to proposed movements of live grouper was a major factor in controlling the spread of disease. It was noted, however, that an extensive knowledge base was necessary for this process to be meaningful, and that knowledge in this was rapidly changing.

### **Reporting**

The group discussed the potential difficulties that could be faced as a result of trade sanctions imposed if a notifiable disease is officially reported. While the group recognised that the process of formal disease notification played a major role in prevention of international spread of diseases, the possibility of trade restrictions was an active deterrent to official reporting.

### **Recommendations**

#### **General Recommendations**

**Recommendation 1.** The group strongly recommended the use of and compliance with the Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals in live grouper movements. It was also recommended that the Guidelines be brought to the attention of APEC member economies not participating in the FAO/NACA Regional Programme (*e.g.* Brunei Darussalam and Chinese Taipei).

**General Recommendation 2.** The group recommended that Regional Reference Laboratories or Resource Centers for Grouper Diseases be established as a network of collaborating centres, noting that terms of reference, criteria, responsibility and a candidate list of institutions would require identification.

**General Recommendation 3.** Regional monitoring and surveillance systems should be established for the detection of grouper diseases.



**General Recommendation 4.** Functional links between national OIE delegates and fisheries authorities should continue to be promoted in regard to OIE reporting.

**General Recommendation 5.** Regional standards on disease risk assessments for grouper movement should be established.

**General Recommendation 6.** Consideration should be given to determining infected zones or free zones as a basis for decision making (based on monitoring and surveillance data).

### *Specific Recommendations*

**Specific Recommendation 1.** Improved monitoring, surveillance and reporting systems for grouper diseases should be implemented at country levels within the region. Such systems should focus on upgrading skills at extension level or district staff level (the staff who have direct contact with farmers). Consideration should be given to establishing a network of sentinel farms to assist in disease surveillance.

**Specific Recommendation 2.** Health screening program should be established for existing and future grouper hatcheries as hatcheries play a central role in supporting aquaculture industries and can disseminate diseases.

**Specific Recommendation 3.** Health certification procedures for grouper receive high priority. They should be urgently implemented because of the high value of the industry, the large numbers of fish translocated and the established possibilities of disease transfer.

**Specific Recommendation 4.** Hatcheries should be surveyed to further evaluate the biological and economic impact of disease at the hatchery level.

**Specific Recommendation 5.** Risk assessment procedures should be implemented for trans-boundary movement of grouper. Projects related to building capacity in conducting an IRA should receive priority.

### **Working Group 3: Frameworks, Collaboration, Linkages, Conclusions and Funding Options (Plenary Session)**

**Chair:** Dr Kamonporn Tonguthai

**Co-Chair:** Dr John Humphrey

**Rapporteurs:** Drs Peter Walker and Sonia S. Somga

### *Introduction and General Considerations*

The workshop considered the observations, discussions, findings, conclusions and recommendations from Workshop Groups 1 and 2, as well as the country information and technical papers presented during the course of the workshop. The workshop agreed that it was appropriate to draft a number of priority subjects for research and development activities, which would support existing and future regional grouper health and production.

The workshop was vitally concerned about the problems relating to disease in grouper production and the spread of disease associated with regional movements of grouper. The workshop was further concerned that future aquaculture in the region was being seriously threatened by the spread of disease through uncontrolled and unregulated movements of live fish.

The workshop emphasised a need for regional governments to act harmoniously at both national and regional levels to control movements and improve diagnostic capabilities. This matter has a high priority as it would assist in disease control and support sustainable grouper aquaculture.

The workshop agreed that a case existed to prepare a comprehensive regional research program on grouper health and production. In determining the nature and projects within this regional program, the workshop identified a number of key factors for incorporation into the program and stressed the need of establishing this program within a regional framework.

The workshop identified a need to link the program framework to regional social and environmental priorities.

The workshop agreed that grouper disease research would benefit in the disease diagnosis, control, prevention, quarantine and health certification procedures in other aquaculture species.

The workshop emphasised that proposed research, development and extension activities must be linked to programs or projects on aquatic animal health within the region and noted that several such programs exist including the following:

- APEC Grouper R&D network
- NACA/FAO Regional Program on Aquatic Animal Health Management
- Other regional programs on aquatic animal health

The workshop identified the need for information systems to support regional programs on health and noted that several systems existed within the region. These systems can be linked for disease surveillance, monitoring, reporting and collecting disease survey data. The systems include as follows:

- AAHIS: Aquatic Animal Health information System
- AAPQIS: Aquatic Animal Pathogen and Quarantine Information System<sup>4</sup>
- AFS Fish Health Section Newsletter
- FAO/NACA and OIE Asia-Pacific Quarterly Aquatic Animal Disease Reporting System<sup>5</sup>

The workshop identified a need for networking between regional scientists, laboratories, institutions and organisations in the implementation and application of any research program, as well as the need for a transparent and dynamic exchange of information between them and between economies.

The workshop agreed that the practical application of results at the farm level to improve farm productivity should be high priority for any research program.

The workshop identified a need for private sector involvement in the regional program and identified specific areas for this involvement. This includes links to existing or future regional programs, participation in future workshops, provision of core funding support for research in specific areas of interest. The workshop recognised that the private sector may be an excellent source of disease information and that they provided a valuable extension role and acted as an entry point for disseminating appropriate information to farmers. It was clear that the private sector had a major role to play in vaccine development and also in future hatchery development.

**The workshop identified the following components of a regional research program on grouper health and production.**

### ***Project 1. Hatchery Health & Production***

The workshop recognised the central role of hatcheries in supporting current and future grouper aquaculture, noted the catastrophic impacts of disease in some existing hatcheries and noted the potential of sub-clinically infected hatchery reared fingerlings and fry to spread disease.

The workshop agreed with the need for hatcheries and hatchery development to redress the problem of seed supply and agreed that prompt attention be given to understanding and controlling disease in hatcheries as a basis for future sustainability of regional grouper aquaculture. The workshop identified a number of areas for study.

**Sub-project.** Health screening and certification programs for grouper hatcheries to ensure quality of seed and seed availability through hatchery production, definition of “good quality seed” standards and qualitative measures of “high health”.

**Sub-project.** Survey of hatchery diseases and disease impacts.

**Sub-project.** Research on VNN transmission cycles in brood-stock and larvae to support development of effective disease management strategies.

**Sub-project.** Production of SPF stocks for distribution/sale on a regional basis

**Sub-project.** Hatchery management and husbandry practices; impacts on health and survival.

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<sup>4</sup> available at internet via <http://www.enaca.org>

<sup>5</sup> available by request from NACA, e-mail: [Melba.Reantaso@enaca.org](mailto:Melba.Reantaso@enaca.org)

**Project 2. Regional Collaborative Grouper Disease Resource Centres**

The workshop agreed on the need to establish a network of regional grouper disease resource centres. The development, implementation and operations of such centres would need to be subject to definition and agreement on a number of issues including;

- Terms of reference
- Role and function
- Participating institutions
- Diagnostic specialities
- Epidemiological data and regional monitoring: links with disease database
- Non-grouper species
- Links to the FAO/NACA Regional Programme on Aquatic Animal Health which will develop mechanisms for the designation of resource centers for aquatic animal diseases in the region
- Links to OIE regional reference laboratories for VNN & Iridoviruses
- Links to UPM library
- Technical training programs for key resource people
- Funding arrangements

**Project 3. Regional Disease Monitoring & Surveillance**

The workshop agreed that disease monitoring and surveillance were key components of programs to minimise disease transfers and that such data played a key role in import risk analysis processes. It was also noted that disease occurrence and distribution data was an essential basis, on which decisions relating to control and eradication programs must be based.

The workshop formulated a list of activities that may be included in the overall project as sub-projects.

**Sub-project.** On-farm monitoring, surveillance.

**Sub-project.** Action plans for disease control/eradication dependent on detailed knowledge of disease occurrence.

**Sub-project.** Regional reporting: Reporting relationships and systems: OIE, FAO/NACA; linkages to other reporting databases; Animal Health and Production Information System for ASEAN and linkages to OIE reference laboratories. Further strengthening relationships with national level OIE authorities with respect to disease reporting.

**Sub-project.** Education, training and extension: at various levels with particular emphasis at district level staff/extension staff who have direct contact/interaction with farmers.

**Sub-project.** Sentinel farms; farmer participation in monitoring and surveillance.

**Sub-project.** Establishment of epidemiological risk factors.

**Project 4. Improved Regional Diagnostic Capabilities**

The workshop agreed that diagnostic capabilities for the identification and confirmation of pathogens, parasites and diseases within regional countries were fundamental to supporting current and future grouper aquaculture. A need exists for the capability to identify the full spectrum of pathogens and parasites that occur in the region. Specific diagnostic areas and disciplines were identified as sub-projects, in which improved capabilities were necessary.

The workshop noted that in relation to disease diagnostic capabilities, OIE reference laboratories were present in the region for VNN and iridoviruses, and that within reason; these laboratories may be able to support research and development efforts in improving diagnostic capabilities. The workshop noted that Chinese Taipei has significant VNN Research and Development program in place and expects to solve many of the problems in the future.

**Sub-project.** Review of regional laboratory diagnostic capabilities and resources, including specialist discipline scientists.

**Sub-project.** Pathology/histopathology - development of specialist capabilities in pathology and histopathology.

**Sub-project.** Immuno-diagnostics - development and implementation of immunodiagnostic tests and procedures in regional laboratories.

**Sub-project.** Cell culture methods - development of cell lines for viral growth and distribution linked to resource centre and regional laboratories.

**Sub-project.** Development of diagnostic tests focussing especially on the implementation of the recommendations of the expert consultation on DNA-based diagnostic technology.

**Sub-project.** Improved training and development of expertise in the identification and characterisation of specific groups of protozoan and metazoan parasites.

**Sub-project.** Standardisation and inter-calibration of diagnostic tests.

**Sub-project.** Regional diagnostic manual for aquatic animal health practitioners, with links to the Asia Aquatic Animal Diagnostic Health Guide under the FAO/NACA Regional Programme on Aquatic Animal Health, the OIE Code and Diagnostics Manual, and the APEC Grouper Health and Husbandry Manual.

**Sub-project.** Training and education. The need for on-going training of scientific and technical staff was identified as central to improvement in diagnostic capabilities. A factor in training was the need to impart knowledge from current active regional scientists who were approaching retirement, *i.e.*, succession planning and training. The need to utilise the expertise of these scientists was stressed. A need for training courses in development and implementation of standardised techniques was also stressed.

#### ***Project 5. Responsible Transboundary Movements***

The workshop agreed that this issue was central to minimising disease transfer accompanying translocation of live grouper. The meeting endorsed the ***Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals*** in this regard and stressed the need to for member economies to comply with these guidelines and provide national level support for the implementation of the different components outlined in the '***Technical Guidelines***'. The workshop proposed a number of sub-projects within the project.

**Sub-project.** Development of regional standards including the establishment and harmonisation of import/export protocols, health certificate and general health certification requirements.

**Sub-project.** Import risk assessment processes and hazard identification.

**Sub-project.** Disease Zoning - establishment of zoogeographic zones and national boundaries with respect to disease occurrence and distribution.

#### ***Project 6. On-Farm Health Management***

The workshop agreed that the development and implementation of cost-effective and practical disease control strategies for on-farm application was a high priority issue in view of the high prevalence and adverse economic impacts of disease on farms. The workshop acknowledged that the aquatic environment played a major role in the development of disease and an understanding of the environment was essential to resolve disease issues.

The workshop identified several areas for investigation, as follows:

**Sub-project.** Development of on-farm management strategies for control or prevention of disease.

**Sub-project.** Studies on pathogenesis and epidemiological risk factors focussing on sources and mechanisms of infection and environment/disease interactions, including issues relating to water re-circulation and water quality management.

**Sub-project.** On-farm disease control trials and studies for bacterial, viral and parasitic diseases.

**Sub-project.** Isolation and characterisation of new or previously unrecognised pathogens.

### ***Project 7. Vaccines and Vaccination***

The workshop agreed that given the current successful use of vaccines in farmed fish in other regions sufficient justification existed to investigate the role of vaccines within the region to mitigate against disease and disease transfer in grouper.

The workshop noted that in terms of vaccine development, interactions with private sector were an essential component in order to achieve success in this area.

The workshop also noted difficulties and impediments to vaccine manufacture and use. The workshop noted the need for economic returns on investment by vaccine manufacturers when considering vaccine development.

The workshop identified a number of areas for further investigation.

**Sub-project.** Identification and characterisation of regional pathogens, parasites and diseases of grouper and the identification of diseases suitable for vaccination strategies.

**Sub-project.** Assessment of the need for new vaccines and development of new vaccines as appropriate, including identification of possible collaborative projects in vaccine development with the private sector, possible financial inputs from this sector and mechanisms for promotion of a regional vaccine program for vaccine production, and registration for use of vaccines in the regional countries.

**Sub-project.** Use of vaccines and the implementation of vaccination strategies for disease control, including the use of existing vaccines, confirmation of applicability of existing vaccines to existing diseases

**Sub-project.** Preliminary research investigations for vaccine development

### ***Funding of Program***

The workshop noted that the report and recommendations for further research would be tabled at the next APEC meeting. It was envisaged that different sections of the program might be identified for funding by different funding agencies.

### ***Future Directions: An Ad-hoc Working Group***

The workshop agreed to a proposal that an ad-hoc working group be established to further progress the project discussed during the workshop and that this working group focus on:

- Development of a program with sub-projects for presentation to funding agencies
- Building components of research, training/capacity building and standardisation of tests and procedures into the program
- Framing the program in a broader context of socio-economic and environmental impacts of fish diseases, especially benefits to farmers, their families and communities, and benefits to the environment

### **General Discussions**

- The issue of disease reporting was discussed in great length; with a mention that this is being currently addressed in the Asia-Pacific Regional Programme on Aquatic Animal Health Management. It is starting to work through cooperation with FAO and OIE, where in some countries there is closer working relationship with veterinary authorities.
- The process of disease surveillance, reporting, import risk assessments should be promoted. Ways on how to further develop skills on aquatic animal disease surveillance should be identified.
- There are a number of valuable sources, farmer knowledge, and extension knowledge. Information should be collected in a systematic manner, for example, through structured surveys. It is important to document all data that can describe disease situation. There are various ways and approaches of collecting information and these should be promoted in the region for grouper diseases.

- Getting quality information is extremely difficult but there are a number of ways to get information of reasonable quality. One of these ways is to establish a reasonably extensive network of gathering information at farm and district levels. Farmers will cooperate and should be convinced of the benefits, which can go to a wide range of procedures and data management and interpretation. Some models are available in the livestock sector, and some countries are starting to develop aquatic animal health information systems.
- There is an urgent need to get information regarding trade in live groupers. While INFOFISH has some information, it is still necessary to get more information from other countries of the region. Bulk of trade is still in wild catch of different sizes. It can be difficult to determine whether fish were cultured or wild caught.
- With respect to establishing regional resource centers for grouper diseases, it was mentioned that there are 2 laboratories in Japan designated as OIE Reference Laboratory for VNN (Hiroshima University) and Iridovirus (NRIA). Candidate resource centers for grouper diseases could be the Australian Animal Health Laboratory in Geelong, Australia.

### **Closing Ceremonies**

The closing remark was provided by Mr Glenn Hurry, Lead Shepherd of the APEC FWG and Assistant Director, Agriculture, Fisheries and Forestry of Australia (AFFA). Mr Hurry noted that it was a successful workshop, with a number of recommendations resulting from three working group discussions. He was pleased to see the workshop so well represented by a range of economies in the region. He indicated that the APEC FWG meeting held in Seattle last July 2000 raised the issue concerning the number of projects on grouper currently being funded and the issue of raising the living standards of communities. He emphasized that there is potential to present the results of the workshop to the next meeting in May in 2001. There is an APEC trust fund that will consider projects with potential impact on trade and liberalization. It is uncertain whether some of the required work resulting from this workshop can fit into this fund and will require development of proposals. The Australian Agency for International Development (AusAID), which had funded a number of projects during the last several years, with NACA, could also be another source of funding. Women in aquaculture, women in developing countries, particularly in rural areas is a current priority and a number of economies including Australia and Canada had expressed interest on funding women involvement in some of the work. He emphasized what has been learned during the past years concerning wide ranging consultation and private sector involvement, which he was quite glad to see carried out in this particular project. He indicated that the report is required for the next meeting in May 2001 in Hong Kong, China and requested that the report be produced in timely manner. He proposed to involve NACA in the further development of the programme. He reminded all the economies to send a copy of the final papers, economy reviews and presentations to the Project Team. In conclusion, he thanked the member governments of NACA and economies of APEC, the Project Team and all the participants. He concluded that through NACA, APEC could continue to fund projects that will benefit people from communities.

## Annex 1: Workshop Programme

*Programme*

<b>15 October 2000, (Sunday)</b>	Arrival of Participants
<b>16-17 October, 2000 (Monday-Tuesday)</b>	Preparation of economy reviews by survey participants
<b>17 October, 2000 (Tuesday)</b>	Arrival of Workshop Participants
<b>18 October, 2000 (Wednesday)</b>	
0830-0930	<b>Registration and Opening Ceremony</b> Welcome speeches and opening remarks from: <b>AAHRI:</b> <i>Dr Somkiat Kanchanakhan</i> , Project Overseer <b>FHS-AFS:</b> <i>Dr Supranee Chinabut</i> , Project Team Leader, Chairperson, FHS-AFS, Director of AAHRI <b>Department of Fisheries:</b> <i>Dr Maitree Duangsawasdi</i> , Deputy Director General <b>NACA:</b> <i>Mr Hassanai Kongkeo</i> , NACA Coordinator
0930-1000	<b>Background, Objectives, Expected Outcomes and Workshop Mechanics</b> - <i>Dr Melba B. Reantaso</i> , FHS-AFS Secretary/Treasurer (NACA)
1000-1030	Coffee Break
1030-1050	<b>Session Ia: Presentation of Grouper Disease Impact Survey</b>
1050-1110	<b>Indonesia</b> ( <i>Ms Erna Dewi</i> , Provincial Fisheries Service, Medan, Indonesia) <b>Korea RO</b> ( <i>Dr Myoung Ae Park</i> , National Fisheries Development Research Institute, South Sea Fisheries Research Institute )
1110-1130	<b>Malaysia</b> ( <i>Mr Chuah Toh Tye</i> , National Fish Health Research Center, Fisheries Research Institute, Penang, Malaysia)
1130-1150	<b>Philippines</b> ( <i>Dr Sonia S. Somga</i> , Philippine Bureau of Fisheries and Aquatic Resources, Department of Agriculture)
1150-1210	<b>Thailand</b> ( <i>Mr Somporn Roungkamneardvong</i> , National Institute for Coastal Aquaculture)
1210-1230	<b>Vietnam</b> ( <i>Dr Nguyen Huu Dung</i> , Na Thrang University of Fisheries and <i>Mrs Phan Thi Van</i> , Research Institute for Aquaculture No. 1)
1230-1330	Lunch
1330-1350	<b>Session Ib: Country Presentations</b>
1330-1350	<b>Australia</b> ( <i>Dr John Humphrey</i> , Department of Primary Industry and Fisheries, Darwin, Northern Territory )
1350-1410	<b>Brunei Darussalam</b> ( <i>Mrs Hajah Laila Haji Hamid</i> , Department of Fisheries, Ministry of Industry and Primary Resources )
1410-1430	<b>China PR</b> ( <i>Mr Zhang Haifa</i> , Guangdong Fishery Service Center, Guanzhou, China PR )
1430-1450	<b>Chinese Taipei</b> ( <i>Dr Shau-Chi Chi</i> , Department of Zoology, National Taiwan University)
1450-1520	Coffee Break
1520-1540	<b>Hong Kong China</b> ( <i>Dr Roger Chong</i> , Agriculture, Fisheries and Conservation Department)
1540-1600	<b>Singapore</b> ( <i>Dr Chang Siow Foong</i> , Agri-Food and Veterinary Authority (AVA) of Singapore)
1600-1630	<b>Regional Synthesis of Grouper Health Impact Survey</b> ( <i>Dr Supranee Chinabut</i> , FHS/AFS and AAHRI)
1630-1730	Discussion
<b>19 October 2000 (Thursday)</b>	<b>Session II: Technical Presentations</b>
0830-0900	<b>Parasites and Bacterial Diseases of Marine Finfishes</b> ( <i>Dr Leong Tak Seng</i> , Private Consultant, Malaysia)
0900-0930	<b>Recent Development on Rapid Identification of Viral Diseases of Grouper</b> ( <i>Dr Toshihiro Nakai</i> , Hiroshima University, Japan)
0930-1000	<b>Viral Nervous Necrosis in Larvae/Juveniles of Grouper</b> ( <i>Dr Kei Yuasa</i> , Japan International Cooperation Agency (JICA)
1000-1015	Coffee Break

- 1015-1045      **Antigenic Structure and Transmission Cycles of Nodaviruses and Iridoviruses Infecting Fish** (*Dr Peter Walker*, Commonwealth Scientific Industrial Research Organization, Australia)
- 1045-1115      **Immunological Methods to Disease Control**  
(*Dr Somsak Vinitnantharat*, ALPHARMA, USA)
- 1115-1145      **Vaccine Development and Potential for Disease Control**  
(*Mr Stuart Millar*, Institute of Aquaculture, Stirling University)
- 1145-1215      Import Risk Analysis in Minimising Trans-boundary Movement of Infectious Disease Agents (*Dr Ian Peebles*, Australian Quarantine Inspection Service (AQIS))
- 1215-1230      **Update on APEC FWG “Development of a Grouper Health Manual”**  
(*Dr Erlinda Lacierda*, Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC-AQD))
- 1230-1330      Lunch Break
- 1330-1730      **Session III: Workshop Discussions**  
**Group I:** Research Needs on: Development of suitable cell lines for grouper viral isolation Development of techniques for grouper viral identification and diagnosis Development of protocols for grouper viral disease induction and investigation on modes of transmission Prevention and control of viral and other diseases affecting grouper.  
**Group II:** Strategies to minimize risks of pathogen transfer through responsible movement of live aquatic animals (IRA, diagnostics, disease reporting, emergency responses, resource centers, *etc.*)

**20 October 2000 (Friday)**

- 0830-0930      **Group I Presentation and discussion**
- 0930-1030      **Group II Presentation and discussion**
- 1030-1100      **Coffee Break**
- 1100-1230      **Group III (Plenary Session):** Funding options, responsibilities of different cooperating agencies, linkages with other on-going projects, framework of the regional programme
- 1230-1330      Lunch Break
- 1330-1500      **Continue Group III discussion**
- 1500-1530      **Coffee Break**
- 1530-1630      **Summary of Recommendations**
- 1800-            **Closing Ceremony APEC :** *Mr Glenn Hurry*, Assistant Director, Agriculture, Fisheries and Forestry of Australia (AFFA) and APEC FWG Lead Shepherd  
Farewell Dinner

**21 October 2000 (Saturday)      Departure of Participants**



## Annex 2: List of Participants

Economy Representatives	Name and Contact Address
Australia	<p><b>Dr John Humphrey</b>            Manager, Aquatic Animal Health/Fish Pathologist            Darwin Aquaculture Centre Berrimah Veterinary Laboratory            Department of Primary Industry and Fisheries Darwin,            Northern Territory, NT 0801 Australia            Tel: 08-8924-4264, 08-8999-2249            Fax: 08-8924-4277, 08-89992024            E-mail: <a href="mailto:John.Humphrey@nt.gov.au">John.Humphrey@nt.gov.au</a>; <a href="mailto:John.Humphrey@dpif.nt.gov.au">John.Humphrey@dpif.nt.gov.au</a></p>
Brunei Darussalam	<p><b>Mrs Hajah Laila Haji Hamid</b>            Head of Aquaculture            Department of Fisheries Ministry of Industry and Primary Resources            Bandar Seri Begawan BB3910            Brunei Darussalam            Tel: 673 2 770 736/383067-8            Fax: 673 2 382069/770065            E-mail: <a href="mailto:laila_hamid@fisheries.gov.bn">laila_hamid@fisheries.gov.bn</a></p>
China	<p><b>Mr Zhang Haifa</b>            Guangdong Fishery Service Center            Guangdong Fishery Technical Extension Center No. 10 Nancun Rd.,            Guangzhou Province            China PR            Fax: 86 20 84246210            E-mail: <a href="mailto:zhhaifa@163.net">zhhaifa@163.net</a></p>
Chinese Taipei	<p><b>Dr Shau Chi Chi</b>            Department of Zoology            National Taiwan University            Chinese Taipei            Fax: 886 2 2367 3852            E-mail: <a href="mailto:shauchi@ccms.ntu.edu.tw">shauchi@ccms.ntu.edu.tw</a></p>
Hong Kong China	<p><b>Dr Roger Chong</b>            Fish Health Officer Agriculture,            Fisheries and Conservation Department            5-8/F, Cheung Sha Wan Road Kowloon,            Hong Kong China            Tel: 852 2510 7087            Fax: 852 2311 3731            E-mail: <a href="mailto:vfhoafd@netvigator.com">vfhoafd@netvigator.com</a></p>
Korea RO	<p><b>Dr Myoung-Ae Park</b>            Pathology Division National Fisheries Research and Development Institute            South Sea Regional Fisheries Research Institute            347 Anpo-ri, Hwayang-myun, Yeosu-City Chullanam-do, 556-820            Republic of Korea            Tel: 82-662-690-8989            Fax: 82-662-685-9073            E-mail : <a href="mailto:mapark@haema.nfrda.re.kr">mapark@haema.nfrda.re.kr</a></p>
Indonesia	<p><b>Mrs Erna Dewi</b>            Provincial Fisheries Service of North Sumatra            Sei Batubingging No. 6 Medan, Indonesia            Tel: 62 61 4551508            Fax 62 61 4563722, 62 61 4535153</p>

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## Part II - Technical Proceeding

### Survey on the Impacts of Grouper Viral and other Health Problems in Indonesia

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#### Abstract

This paper described grouper culture in the aquaculture region of North Sumatra in Indonesia during the period from 1995 to 1999. Diseases and environmental pollution caused termination of culture. The following diseases, disease syndromes, parasites and pathogens were identified in the survey: encapsulated gill didymozoid (uncharacterized), Iridovirus, deaths of unknown aetiology, skin fluke, and vibriosis. It was concluded that mortality among grow-out fish was a problem in grouper culture. The causes included Iridovirus as well as other diseases. Dependence of seed supply on the wild sources remains a significant problem. North Sumatra has no facility for grouper hatchery production.

#### Introduction

Mariculture is one of the most important fisheries industry in North Sumatra Province of Indonesia particularly in Tapian Nauli Bay, Nias and Batu Island in the west coast and Langkat in the east coast. Mariculture was developed in Tapian Nauli Bay since 1989 initially as a small-scale venture; currently there are 375 units of floating cages in the area. The most common species of grouper cultured is *Epinephelus coiodes* due to high market demand, availability of seed and ease of culture as compared to other species. In the east coast, grouper culture began in Jaring Halus village in Langkat district where cages are under house poles using the same species of grouper. Grouper culture gradually spread to other areas such as Sembilan Island, Kampey Island and Perlis, where currently there are about 1931 cage units in operation. Singapore, Malaysia and Hong Kong China are important markets for grouper produced in North Sumatra.

#### Cage Culture Methods

Site selection is an important criteria for cage culture. Two types of net cages are used for grouper culture in North Sumatra: fixed net cages and floating net cages whose application depends on the characteristics of the coastal environment. In coastal areas with low current and less wave such as in Tapian Nauli Bay, Nias and Perlis, the preferred culture method is the floating net cage. Areas with strong current and waves such as in Sembilan Island, Kampey Island, Bukit Jennkol, Pangkalatan Siata and Jaring Halus, the methods used are the fixed net cages. Table 1 shows a summary of grouper culture methods in North Sumatra.



**Table 1.** Grouper culture production methods in North Sumatra.

	Fixed cage culture method	Floating cage culture method
Size of cage	4 x 4 x 5 m <sup>3</sup> ; 3 x 3 x 5 m <sup>3</sup>	3 x 3 x 3 m <sup>3</sup> ; 4 x 4 x 3 m <sup>3</sup>
Seed supply	Wild	Wild
Species	<i>E. coioides</i>	<i>E. coioides</i>
Size of seed	> 100 g to < 300 g	> 100 g to < 300 g
Stocking density/cage	300 to 500 pcs	300 to 500 pcs
Grow out period	6 months	6 months
Feeding	Trash fish, 6.5 kg/cage (during high tide)	Trash fish
Size at harvest	> 400 g	> 400 g
Production	Average of 92.2 kg/cage	Average of 72 kg/cage

Tables 2 and 3 show the total number of fixed or floating net cages in operation and production data during last five years. As indicated in Table 3, development of grouper culture in the province grew steadily until 1999 due to high market demand. Exporters usually come directly to the farms, so market is good which strongly encouraged farmers. Table 4 shows several districts with no production in 1997 and 1998 particularly in Deli Serdang and Sibolga districts. Since 1997 the coastal area of Poncan Gadang in Sibolga was developed for tourism and industrial (*i.e.* ship and speed boat landings) purposes, which caused pollution from oil/lubricant leaching, thus making it an unsuitable site for grouper culture. The farmers moved to Central Tapanuli. In Deli Serdang, on the other hand, grouper culture experienced severe disease problems, which diminished or totally terminated culture operations.

**Table 2.** Development of fixed and floating net cage grouper culture in North Sumatra East and West Coasts from 1995 to 1999.

District	Total number of fixed or floating net cages (unit)				
	1995	1996	1997	1998	1999
Nias	2	-	16	16	30
Central Tapanuli	298	320	350	370	375
Sibolga	34	116	-	-	-
Langkat	230	240	268	1258	1931
Deli Serdang	300	44	-	-	-
<b>TOTAL</b>	<b>864</b>	<b>720</b>	<b>634</b>	<b>1644</b>	<b>2336</b>

Source: Buku Tahunan Statistik Perikanan DP. 1995-1999.

**Table 3.** Production of grouper culture in North Sumatra Province during 1995 to 1999.

District	Total Production (tons)				
	1995	1996	1997	1998	1999
Nias	54.6	70.3	80	76	74.6
Central Tapanuli	6.5	65	68.3	78.8	77.4
Sibolga	23.8	23.8	-	-	-
Langkat	98.2	23.8	118.2	129.7	131
Deli Serdang	100	20	-	-	-
<b>TOTAL</b>	<b>283.1</b>	<b>202.9</b>	<b>266.5</b>	<b>284.5</b>	<b>283</b>

Source: Buku Tahunan Statistik Perikanan DP. 1995-1999.

**Discussion**

*Problems in Grouper Culture in North Sumatra*

During the survey, the following diseases, disease syndromes, parasites and pathogens were identified in the survey: encapsulated gill didymozoid (uncharacterized), iridovirus, deaths of unknown aetiology, skin fluke, and vibriosis. It was concluded that mortality among grow-out fish was a problem in grouper culture. The causes included Iridovirus as well as other diseases. Another significant problem apart from diseases is the limited quantity and poor quality of seeds. Pollution from industry and from oil/lubricant leaching is also seen as a major constraint to grouper culture development in the province.

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**Status of Diseases of Seven Band Grouper, *Epinephelus septemfasciatus*, in Korea RO***Myoung Ae Park*<sup>1</sup> and *Sang-Gyu Sohn*<sup>2</sup>

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**Abstract**

Marine fish culture in Korea RO has grown rapidly following development of artificial seed production in the 1980s. At the same time, mass mortalities have frequently occurred among cultured fishes due to environmental pollution, overstocking and outbreaks of infectious diseases. Losses have been increasing every year. Among the pathogens, virus has been one of the major causative agents of diseases of cultured marine fishes in Korea RO.

Relying on fry collected from the wild, the net cage culture of seven band grouper, *Epinephelus septemfasciatus*, has expanded along the southern coast of Korea RO. Mass mortality caused by virus has occurred among adults as well as fry during the summer season. Mortality among fingerlings usually reached over 80% within a few weeks.

This paper describes the current status of grouper culture and the results of a survey of viral disease of seven band grouper in Korea RO.

**Introduction**

The southern coast of the Korean peninsula is suitable for various types of aquaculture activities. Most of the marine fish cultured by net-cage is concentrated in this area. Net-cage is the most common type of culture system for grouper. Culture grounds for grouper are located in Tongyeong, Yeosu on the southern coast.

The main cultured species in Korea RO are flounder (*Paralichthys olivaceus*), rockfish (*Sebastes schlegeli*), seabass (*Lateolabrax japonicus*), red seabream (*Pagrus major*), parrotfish (*Oplegnathus fasciatus*), grouper (*Epinephelus septemfasciatus*) and tiger puffer (*Takifugu rubripes*). Total production of cultured marine fish in 1999 was 33,453 tons. Flounder comprised 21,368 tons or 63.9% of the total marine fish production. Grouper contributed 19 tons or 0.14%.

Seven species of grouper are distributed in Korea RO such as seven band grouper, kelp grouper (*E. moara*), red grouper (*E. akaara*), black tipped grouper (*E. fasciatus*), brown spotted grouper (*E. chlorostigma*), black saddled grouper (*E. fario*) and yellow grouper (*E. awoara*) but the main cultured species is sevenband grouper.

Grouper fry are caught in the wild, as there is still no hatchery. Fry are collected from May to November. This is closely related to the spawning season of grouper and the Kuroshio Current. The grow-out period is from 2 to 2.5 years. In terms of the growth rate and market value, groupers are one of the most important mariculture species in Korea RO. The current focus of research is on understanding the reproductive biological characteristics.

**Methodology***Fish samples*

Seven band grouper were sampled from six cage farms at Tongyong and Yeosu on the southern coast of Korea in August 2000. Fish were cultured at a density of 25 to 35 kg/m<sup>3</sup> at 25-28°C. Grouper were co-cultured with the other fishes in the same or nearby cages. Live samples were fixed for observation by light and electron microscopy, and the rest were stored at -85°C.

*Histopathology*

Tissues of gill, brain, eyes and other internal organs from moribund fish were fixed in Bouin's fixative and processed for standard histological preparations. Paraffin sections (4  $\mu$ m in thickness) were stained with haematoxylin-eosin (H & E) and observed by light microscopy.

*Electron microscopy*

Tissues of brain from moribund fish were fixed in 2.5% glutaraldehyde solution (pH 7.2), postfixed in 1% osmium tetroxide solution (pH 7.2) and embedded in Epon 812. Ultrathin sections were stained with 1% uranyl acetate and 1% lead citrate, and examined with a Hitachi-7200 electron microscope at 80 kV acceleration voltage.

*Virus preparations*

Pooled organs of diseased fish were homogenized with sea sand and 9-volumes of Dulbecco's Modified Eagle's Medium (DMEM) at low temperature, and centrifuged at 5000g for 15 min. The supernatant was recentrifuged at 12000g for 2 min and filtered with a 0.45 $\mu$ m membrane filter. The filtrate was used as virus inoculum in artificial infection.

*Polymerase chain reaction (PCR)*

PCR was conducted in order to confirm the presence of the causative virus in the various organs of the diseased fish such as brain, eyes, kidney, spleen and liver. Each organ of infected fish was homogenized with RNA zol (Biotex) followed by chloroform extraction and centrifuged at 12000g for 15 min. The total nucleic acids were precipitated by addition of isopropanol and RNA Tack Resin (Biotex), washed with 75% ethanol twice and dried in a speed vacuum (Heto). The pellet was dissolved in distilled water. For the reverse transcription and PCR (RT-PCR) amplification, a primer set designed for T4 region (about 430bp) in the open reading frame of SJNNV coat protein gene by Nishizawa *et al.* (1994) was used. For reverse transcription, total nucleic acids from tissues of diseased fish was pre-heated at 90°C for 5 min and incubated at 42°C for 30 min in 20°C of PCR buffer (10 mM Tris-HCl, Ph 8.3, 50mM KCl) containing 2.5 U of M-MLV reverse transcriptase (USB), 1 U of ribo-nuclease inhibitor (Toyobo), 0.5  $\mu$ M of reverse primer, 1 mM each of 4 deoxynucleotide triphosphates (dNTP), 5 mM of MgCl<sub>2</sub>. Following cDNA synthesis, the mixture was incubated at 99°C for 10 min to incubate the reverse transcriptase and then diluted 5-fold with PCR buffer containing 0.1  $\mu$ M of forward primer (F2), 2.5U of Tth Version 2.0 DNA polymerase and 2 mM of MgCl<sub>2</sub>. The mixture was incubated in an automatic thermal cycler (Perkin Elmer) programmed for 1 cycle at 95°C for 5 min and 35 cycles at 5°C for 40 sec and at 72°C for 5 min. Amplified product was electrophoresed in 1.5% agarose gel and stained with ethidium bromide.

*Virus pathogenicity*

Characteristics of the causative virus were tested by pathogenicity test using artificial infection according to the method described by Iida *et al.* (1989).

One ml of the filtrates was intramuscularly and intraperitoneally injected into each of 10 healthy grouper (20 to 100g) and fish were dipped in 5 l seawater contained homogenate of grouper infected with VNN for 1 hr. Grouper were transferred to 250 l tanks with moderate aeration at 25°C for 2 weeks. The mortality rate was monitored. DMEM and mock-treated filtrate were also injected as controls.

## Results and Discussion

Viral nervous necrosis occurred at two farms during the survey period with mortality of approximately 20 000 fish. Clinical signs of diseased fish included anorexia, dark coloration, loss of equilibrium, spiral swimming behaviour, vertebral deformity and inflation of the swimbladder. Exophthalmus was observed in some fish. *Trichodina* sp. and *Vibrio* sp. were detected of the diseased fish. However, these parasites and bacteria were not likely to be direct causative agents of the cultured grouper mortality, because these were also observed in normal grouper, red sea bream and sea bass co-cultured with the diseased grouper in same or near cages.

Significant histopathological changes were observed mainly in the brain and retina of the eye of diseased fish. Changes in moribund samples included vacuolar degeneration of the eye retina and severe necrosis and/or vacuolation of the brain.

The morphology of the virus was observed under electron microscopy where virus particles were found in the cytoplasm of the nerve cells of the brain of infected fish. The virions were about 30 nm in size, unenveloped, and icosahedral.

Based on RT-PCR results, PCR product of about 430 bp was obtained from both naturally and artificially infected fish. Virus particles were detected only in the brain and eye tissue of affected fish. These results indicate that the causative virus of the disease in grouper belongs to the nodavirus VNN as the main targets organs of this virus are the brain and eye tissues.

### *Pathogenicity*

Pathogenicity experiments revealed that groupers with weights ranging between 20 to 100g were the most susceptible to VNN. In terms of infectivity of the virus using artificial infection methods, fish were successfully infected using intramuscular, intraperitoneal and dip methods, although there were slight differences in the mortality rates among the different infection methods used.

Marine fish disease problems have become more diverse and complicated. As disease occurrence is closely related to management practices, it is important for fish farmers to have sufficient knowledge and to implement appropriate husbandry methods and disease control measures.

At the present time, there are no effective antibiotics or chemotherapeutic compounds for the treatment of viral diseases. Culture of noninfected healthy fish fry and disinfection of the facilities continue to be the main methods used to control viral diseases.

In order to decrease the impact of viral diseases and to ensure safety of cultured fish for human consumption, NFRDI is actively conducting research on the rapid diagnosis using PCR-based method, strict quarantine, epidemiological studies and development of vaccines.

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## Survey of Grouper Diseases in Malaysia

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### Abstract

Finfish cage culture has been a flourishing industry in the coastal waters of Malaysia, with an annual net revenue of approximately US\$ 6.9 M. High value food fishes such as groupers, seabass, snappers, coral fish and other exotic varieties are the main cultured species. Besides technical problems, losses due to diseases remain a major challenge for the industry. This survey revealed that grouper diseases in Malaysia were attributed mainly to diseases of viral origin (50%) and bacterial origin (47%). Infection by ectoparasites such as monogeneans, *Trichodina*, etc. were commonly associated with the diseased fish. Farmers describe fish affected with viral infection as dying without showing any clinical symptoms except for black coloration of body, which indicates iridovirus as the causative agent (confirmed in a case in Langkawi). The dominant bacterial group is the *Vibrio* spp. followed by *Flexibacter* spp. To control and reduce disease impact to the grouper culture industry improving diagnostic and research capabilities on health management are required.

### Introduction

Finfish cage culture was introduced into Malaysia in 1973 when a floating cage farm was established in Jelutong, Penang. Since then, it has spread throughout the country. The main grouper culture areas are in the states of Sabah (43%), Perak (21%), Selangor (17%), Penang (11%) and Johor (8%). From 1994 to 1998, these areas contributed an average annual production of 777 metric tons with a wholesale value of RM 26 million (US\$6.8 million) (see Table 1).

Though the main species under culture is still *Epinephelus coioides*, other species which may be more disease resistant are being tried. Some of the new species such as the giant grouper *E. lanceolatus*, green grouper *E. awoara*, tiger grouper *E. fuscoguttatus* and red-spotted grouper *E. bleekeri* were imported from Chinese Taipei which could air-freight the fry according to demand. At this time, three local hatcheries can not produce enough fingerlings to meet the market demand.

The floating cage farms are usually situated outside a river mouth or along the banks of tidal rivers. Generally they are made of wooden structures with styrofoam-drums as float support. A utility house and shed unit are used to shelter workers, netting and other materials used for operations. Most farms are family-owned with foreign workers employed at some larger farms. However, some farms in Pulau Ketam are merging to operate as a company. The corporate style of culture based on adopted technology from Norway is also being tried in Langkawi waters.

Farm size varies from 30 to 2000 culture units, each measuring about 12 x 12 x 8 cu.ft. (4 x 4 x 2.6 m<sup>3</sup>). Trash fish is commonly used as feed as it is much cheaper than moist or dry pellets. Usually about 1000 tails (3 to 4 inch fry) would be stocked into a culture unit which is fixed with 1-inch mesh net. After a month, it is changed to a 2-inch mesh net followed by regular grading and changing of nets. In 8 to 12 months, the fish would have grown to a marketable size of 600 to 1000 g.

Diseases are often encountered during the culture period. High mortality frequently occurs during the first few weeks. Because of the fisheries economic importance, a survey to study the impact of losses due to viral agents and other diseases was carried out in a number of regional economies.

## Materials and Methods

A standard questionnaire was used to gather information from grouper aquaculturists in Malaysia. The main grouper culture areas in the country were identified and a list of culturists obtained from the State Fisheries Department. Farmers involved in grouper culture were randomly selected. Appointments with the Fisheries Assistants of the respective districts were made and the survey was carried out in a participatory manner in order to understand the grouper disease situation during the past 12 months.

The collected data were analyzed using EpiInfo 6 software to determine species composition under culture, disease incidence, classified syndrome descriptions, average morbidity and mortality experienced during the culture period. The clinical symptoms were presumptively classified into bacterial, parasitic or viral disease based on our experience from cases diagnosed during regular diseases monitoring work in the laboratory.

## Results and Discussion

Based on the culturists' description of clinical signs observed, most of the grouper diseases could be classified as bacterial, parasitic, viral or unknown aetiology. Though ectoparasites such as monogeneans were noted in some cases after freshwater treatment, they were not considered by farmers as a serious problem since no obvious symptoms nor mortality were observed. However, large numbers of these parasites on the gills had been implicated as a primary cause of disease outbreak in cultured greasy grouper (Leong & Wong 1990). The only incidence of parasitic mortality recorded was in a private hatchery where some of the fingerlings succumbed to cryptocaryoniasis. The survey found that 50% of the grouper diseases were of viral origin while 47% were caused by some bacteria such as the *Vibrio* spp. and *Flexibacter* spp. (Table 2). Report on fish viral disease is generally lacking except a case of "sleepy grouper disease" in Singapore and Malaysia (Chua *et. al.* 1994) as compared to bacterial diseases which are well known in causing high mortality of fishes cultured in cages in Malaysia (Chua and Teng 1978; Ong 1984). These diseases were easily distinguished by the discoloration (blackening) of the fish body without any other external symptoms, whereas bacteria-associated disease would show clear haemorrhages and reddening of the body as well as some ulceration or rotting of fins and tail. In this survey, the average morbidity due to viral, bacterial and parasitic syndromes was 78%, 59% and 50% (Table 3) and the average mortality was 64%, 38% and 20% (Table 4), respectively. It was suspected that the "black viral disease" could be similar to the iridovirus outbreak (May/June 2000) in the deep-sea cage culture of grouper in Langkawi island. The spleen samples of these moribund grouper showed cytopathic effect on BF-2 and EPC cell-lines (Oseko, JICA Expert, per. comm).

As disease is still rampant with no effective treatment the culturists are trying to overcome the problem by diversification with the hope that some new species introduced would be more disease resistant. This intention is not without risk as some exotic strains of pathogens might be brought in as well since they usually do not follow proper entry protocols nor quarantine procedure. In fact, some of the fingerlings such as the giant groupers are suffering mortalities due to diseases soon after stocking into the cages. The extent and seriousness of such disease incidence remain to be seen.

A concerted effort in the Asia-Pacific region to prevent and control grouper disease outbreak is urgently needed to minimize its threat to the industry. Multi-disciplinary approach to upgrade diagnostic capabilities in molecular techniques, cell-line and vaccine development, enforcement of responsible trans-boundary movement of live aquatic animals would be required to safe-guard the sustainable culture of grouper, which is of significant importance to the region.

**Table 1.** Grouper production and wholesale value in Malaysia (1994-1998).

Year	Production (Mt)	Wholesale Value (RM'000)	US\$('000)
'94	930.71	27,863.28	7,332.00
'95	833.74	27,351.14	7,198.00
'96	857.10	29,962.40	7,885.00
'97	798.42	28,398.88	7,474.00
'98	465.66	16,806.62	4,423.00
Ave.	777.13	26,076.46	6,862.00

Source: Malaysia Annual Fisheries Statistics (1994-1998).

**Table 2.** Classified syndrome descriptions.

Classified Syndromes	Frequency	Percent	Cummulative
Bacterial Vibriosis	17	47.2%	47.2%
Black Viral Disease	18	50.0%	97.2%
Cryptocaryoniasis	1	2.8%	100.0%
Total	36	100.0%	

\* Analysis by EpiInfo 6 software

**Table 3.** Average morbidity due to different syndromes.

Classified Syndrome	Average Morbidity	Count
Bacterial Vibriosis	59.41%	17
Black Viral Disease	77.78%	18
Cryptocaryoniasis	50.00%	

\* Analysis by EpiInfo 6 software

**Table 4.** Average mortality due to different syndromes.

Classified Syndromes	Average Mortality	Count
Bacterial Vibriosis	38.24%	17
Black Viral Disease	63.89%	18
Cryptocaryoniasis	20.00%	

\* Analysis by EpiInfo 6 software

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## Survey on the Impacts of Grouper Viral and Other Diseases in the Philippines

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### Abstract

A survey on viral diseases and other health problems of grouper (*Epinephelus* spp.) culture in the Philippines was conducted from July to September 2000. Sixty farmers from four provinces (Mindoro, Palawan, Aklan and Samar) were interviewed using a semi-structured survey questionnaire.

Groupers are cultured in floating or fixed cages, which are the most popular culture methods used in the Philippines. A grouper farm usually has a minimum of one module that consists of four cages with a usual dimension of 3 x 3 x 3 m<sup>3</sup> per cage. Each cage can usually accommodate 300-350 pcs of fish. The culture period ranges from 6 to 12 months depending on the initial size of fish at stocking. Trash fish is used as feed. Marketable sized-grouper ranges from approximately 400 to 800 g with a price of US\$ 5.60- 6.40 (US\$ 1 = PHP 50.00) per kilo.

Various diseases and other health related problems are considered a major constraint to grouper production. There are six significant fish disease syndromes observed by farmers. Presumptive diagnosis of these syndromes was made based on the clinical signs. These are as follows:

- (1) hemorrhagic ulceration (diagnosed as most likely vibriosis)
- (2) leech infestation (diagnosed as most likely marine leech, *Zeylanicobdella arugamensis*)
- (3) strong putrid smell (diagnosed as possibly of viral or bacterial origin)
- (4) corkscrew swimming (diagnosed as most likely viral nervous necrosis)
- (5) white patch (diagnosed as likely a bacterial infection), and
- (6) pop-eye (diagnosed as a possible viral or bacterial infection)

The economic impact of disease syndromes particularly during the grow-out period are heavily felt by farmers because of considerable operational expenses (e.g. cost of fingerlings, feed, labor and other maintenance expenses). Farmers with loans from private and unregistered lending institutions shift to culturing other species to pay their debt, while other farmers ceased operation. Diseases during the nursery stage are also critical since the industry still relies on wild seeds. Mortalities at this stage will eventually reduce the supply of fingerlings, thereby increasing the price of fingerlings.

Diseases and other health related problems of cultured groupers in the Philippines appear to be associated with a multitude of factors. These include: (a) quality of seed stock; (b) management practices (i.e. stocking density, irregular feeding regime and poor quality trash fish, handling and sanitary practices such as sorting/grading and changing/cleaning of nets); (c) sudden changes in the environment; and (d) continuously increasing aquaculture activities in the area.

**Introduction**

Groupers (*Epinephelus* spp.) are abundant in Philippine waters and have been cultured since the early 1980s. Two species, *Epinephelus coioides* and *E. malabaricus* are the most common with good growth in cages and pens, usually in bays and estuaries, as well as in ponds. They have become an important part of the country's coastal aquaculture production.

Grouper is also an important live fish export species of the country. Large volume of groupers are being exported to Hong Kong SAR China, Chinese Taipei, Singapore, and to a lesser extent other countries in the region (Johannes and Riepen 1995). In 1997, the total production in pond, cages and pens is 496 mt valued at PhP 184 388 000 (US\$ 4 727 897.44) (BAS 1997). Total live grouper exported in 1999 was 3 721 mt valued at PhP 186 282 000 (US\$ 4 623 000; Philippine Fisheries Profile 1999).

Reduced seed supply is a major limitation for the industry. The devices used to collect seeds also affect the quantity of catches and its survival (Ogburn and Johannes 1999). Research and development on artificial breeding, nursery and nutrition is being undertaken by different institutions such as the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) and the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC-AQD).

Diseases in groupers are a major constraint for the industry. Significant mortalities due to diseases (*i.e.* vibriosis and monogenetic trematodes) have been recurring for the past few years. Impact of these diseases is intensely felt by farmers. In addition, farmers are discouraged when they encounter disease problems of unknown etiology. Farmers find it wise to temporarily stop operations when disease occurs. During the past years, farmers did not seek support from the government when they encounter disease problems (Somga *et al.* 2001). This lack of information exchange between the government and the grouper operators may be one of the major factors why disease problems in grouper has not been well-addressed in the past. Currently, the Fish Health Sections of BFAR and SEAFDEC-AQD are now focusing attention on significant diseases and health related problems of grouper through research and development, improvement in diagnostic capability and increased interaction with grouper farmer through frequent consultation and provision of informal basic grouper health training services.

A survey was conducted to determine the viral diseases and other health problems in grouper. Specifically the survey was aimed to: 1) determine the disease syndromes recognized by farmers; 2) to assess the impacts of the disease syndromes; and 3) to determine possible risk factors in the occurrence of disease and other syndromes in grouper aquaculture.

**Methodology**

The survey was conducted from July to September 2000 covering four provinces using a questionnaire provided by the APEC FWG 02/2000 "Development of a Regional Research Program on Grouper Virus Transmission and Vaccine Development". The questionnaire was developed during the workshop held in Bangkok on 29-31 May 2000. The questionnaire consisted of three parts: 1) a cross-sectional study of grow-out farms to identify important disease syndromes recognized by farmers; 2) consultation with experts or experienced staff to associate the identified syndromes with known diseases; and 3) assessment of impacts of the disease syndromes and identification of possible associated risk factors.

Sixty farmers were randomly selected from the list of 165 farmers from four provinces using EpiCalc program. These four provinces are: Palawan (Puerto Princesa City, n=3; and Taytay, n=18), Mindoro (Caminawit Bay, n=13), Aklan (Numancia, n=1; Makato, n=3; and New Washington, n= 4) and Western Samar (Hinabangan, n=6; Tarangnan, n=2; Daram, n=4; and Catbalogan, n=6). The gathered data were based on the last completed production cycle or on the last 12 months for those without clear cycle. The clinical signs enumerated by farmers were discussed with four experts (BFAR=2; SEAFDEC-AQD=2) for differential diagnosis. The information was analyzed using the Epi Info program.

**Results and Discussion***Cage culture operation*

Groupers are commonly cultured in floating or fixed bamboo cages in coves, bays and along the mouths of the rivers. A farm has a minimum of one module (with 4 cages) with a usual dimension of 3x3x3 m<sup>3</sup>. Stocking density is about 300-350 pcs/cage. The size at stocking ranges from 0.5 inch to 5 inches (termed as "tiny-extra large"). The price ranges between US\$ 0.1 to US\$ 1.20. The culture period is from 6 to 12 months based on the size of fry and fingerling

at stocking. It has been a practice of farmers to sort/grade the stocks every two weeks (until the fish reaches juvenile stage) and once a month thereafter (until reaching an ideal marketable size of 400g to 800g/piece). Trash fish are used as feed at satiation once a day. Others feed on alternate days, especially during periods of shortage in supply of trash fish. It is estimated that one grouper consumes 5 kg of trash fish until it reaches marketable size. The farmgate price of marketable-sized grouper ranges from US\$ 5.60 to US\$ 6.40/kg.

#### *Disease syndromes*

Out of the sixty farmers interviewed, 88.30% experienced disease syndromes during the last production cycle. Six disease syndromes determined based on the clinical signs recognized by farmers are described below.

##### 1. Hemorrhagic ulceration

The syndrome is characterized by red ulceration along the body surface of fish penetrating the muscle. Observations include hemorrhages at the base of fins, and fin and tail rot. Some fish showed cloudiness of the eyes. Affected fish floated while others swam up and down. These symptoms were commonly observed in fish up to 3 inches but it can also occur throughout the culture period. This condition may last from 2 weeks to one month and may affect all stocks. The recorded average mortality is 21.57%.

##### 2. Leech infestation

Leeches were attached to the body surface, fins and gills. Hemorrhages at the site of attachment were observed. The parasite can easily reproduce and may infest all stocks of any size. It occurs during rainy season. The average mortality is 23%. Manual removal of the leech and changing the net reduces infestation.

##### 3. Strong putrid smell

Fish affected by this syndrome exhibit a strong putrid smell with softened and swollen muscles. Fish also showed abdominal distention. Dissection of the abdominal cavity showed that affected fish had ruptured gallbladder. Other symptoms included loss of sense of balance, swimming upside down and inappetence. The size of affected fish was about 4 inches and above. This condition is fatal and affected fish do not usually survive. The syndrome lasts for one month, occurring during summer and at onset of rainy season an average mortality of 63.75%.

##### 4. Corkscrew swimming

Fish exhibited dark discoloration of the body. There were no other external lesions observed. The fish manifested corkscrew swimming behavior before dying. The syndrome can occur anytime during the culture period. Generally, low percentages of fish were affected (22.22%) and all affected fish do not survive. Few farms have experienced mortalities as high as 90%.

##### 5. White patch

This syndrome was characterized by white patches along the body surface. The white patch lesion does not penetrate the muscle and was commonly observed during the first two months of culture period (8-15 g). The disease lasted for one month and the average mortality was 35%.

##### 6. Pop eye

Fish affected by this syndrome showed bulging eyeball. Fish became weak, emaciated with darkened body coloration, and inappetent. The base of the operculum separated or detached from the trunk. Gills were eroded and pectoral fin rot observed. These clinical signs were commonly observed at nursery stage. Affected fish do not survive and the average mortality was 15.90%.

Table 1 shows a summary of the different disease syndromes reported by farmers including frequency of occurrence, average morbidity, average mortality and average economic lesion. The syndrome “haemorrhagic ulceration” had the highest percentage of occurrence of 37.6%. Although 48.14 % of the fish population were affected by this syndrome, the average mortality was only 21.57%.

The “strong putrid smell” was the most serious among the six disease syndromes reported. The disease affects 67.50% of the population and almost all of the affected fish died (63.75%). The “corkscrew swimming” syndrome had a lower percentage of occurrence (19.40%) but it is fatal since all of the affected (22.22%) fish died. The “pop eye” syndrome

was moderately low in occurrence (11.80%) but the morbidity and mortality were significant (15.90 %). The “white patch” syndrome affected 66.25% of the fish population but the mortality was only about 35%.

### *Differential diagnosis of disease syndromes*

Four experts were consulted (BFAR=2; SEAFDEC-AQD=2). They made presumptive diagnosis of the disease syndromes based on the clinical signs present. No laboratory examination was performed due to samples being unavailable during the survey. Three possible viral diseases (two of which also demonstrated clinical signs of bacterial diseases), two bacterial diseases and one parasitic infestation were identified. The differential diagnosis for each syndrome is presented in Table 2.

### *Impact of disease*

Diseases during the grow-out period have a significant economic impact on the farmers because of the considerable operational expenses (cost of fingerlings, feed, labor and other maintenance expenses), not to mention the effort and time spent by farmers to complete the whole culture cycle. Farmers who had loans from private and unregistered lending institutions shifted to culturing other species to reduce their debt. However, culturing of other species (snapper and siganids) provide lower profit than culturing grouper. Some farmers that experienced fish mortalities had to terminate operation..

Diseases at nursery stage are also critical since the industry still rely on wild seedstocks. Mortalities at this stage eventually reduce the supply of fingerlings, thereby increasing the price of fingerlings.

## **Conclusions**

There are six significant disease syndromes affecting grouper culture in the Philippines, as reported by farmers during this survey. Three of the reported disease syndromes are likely to be of viral origin. These include syndromes described as strong putrid smell, corkscrew swimming and pop eye. However, bacterial diseases (*i.e.* hemorrhagic ulceration and white patch syndrome) and parasite (*i.e.* marine leech) problems are also commonly reported.

Based on the information gathered during the survey, there are a number of risk factors that may directly or indirectly affect grouper health. These include: (a) quality of seed stock, (b) management practices, (c) sudden changes in the environment, and (d) increasing aquaculture activities. These factors are further described below.

**(a) Quality of seedstock.** Seed supply of grouper is still dependent primarily on wild sources. The practice of fry/fingerling gathering can be stressful for the fry. The catching gear used (brush piles, rock mounds), and the transportation methods of fry/fingerlings (usually placing the fry in a small bucket or basin) to the holding facilities put further stress on fish. Fish may experience such a high level of stress that they do not survive the first two months of the culture period.

**(b) Management practices.** Most farmers have inadequate technical knowledge of proper husbandry particularly health management practices.

- Stocking density has not yet posed a problem in grouper culture.
- Lack of an appropriate feeding regime. Fish are fed usually once a day or on alternate days depending on availability of trash fish. This kind of practice may result to overfeeding or underfeeding. In addition, the quality of trash fish has always been overlooked by farmers as a possible source of infection.
- Sorting/grading is also stressful for fish and sometimes causes minor mortalities.
- Changing/cleaning of nets and the manner of changing could easily cause skin irritation or scale loss which could become an entry point for opportunistic pathogens.

**(c) Changes in environmental conditions.** Sudden changes in environmental condition such as heavy rain which causes run-off, temperature and salinity fluctuations, may cause mortalities. Fish that survive the changes may consequently succumb to disease.

**(d) Continuously increasing aquaculture activities.** The intensification and diversification of aquaculture activities (grouper, milkfish, mollusc) in the coastal areas could strain the environments carrying capacity. Overcrowding, together with improper management practices, can cause deterioration of the aquatic environment and promote pathogen

proliferation.

Production losses due to the above mentioned disease syndromes had a great economic impact on the farmers. Some farmers switched to culturing of other species, while others decided to terminate culture operation.

Diseases remain a threat to the flourishing grouper industry of the Philippines. Though major disease syndromes have been documented, additional studies are required to determine the aetiology of those syndromes. Additional research is also needed to assess the role of risk factors associated with the development of the disease.

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**Table 1.** Summary of the disease syndrome experienced by farmers.

Syndrome name	Frequency of Occurrence (%)	Average Morbidity (%)	Average Mortality (%)	Average Economic Lesion (%)
Hemorrhagic Ulceration	37.60	48.14	21.57	16.14
Leech infestation	5.40	50.00	23.00	17.00
Strong putrid smell	12.90	67.50	63.75	1.66
Corkscrew swimming	19.40	22.22	22.22	None
White patch	12.90	66.25	35.00	18.75
Pop eye	11.80	15.90	15.90	None

**Table 2.** Summary of presumptive diagnosis of different disease syndromes in grouper.

Syndrome Name	Expert Diagnosis		
	Differential Diagnosis	Level of Confidence	Basis for Diagnosis
Hemorrhagic ulceration	Vibriosis	3	DO
Leech infestation	Marine leech, <i>Zeylanicobdella arugamensis</i>	3	DO;UO
Strong putrid smell	Viral infection	1	REF
	Swimbladder stress syndrome	2	DO
	Bacterial infection	1	REF
Corkscrew swimming	Viral Nervous Necrosis	1,2	DO;UO
White patch	Flexibacterial infection	3	UO
Pop eye	Viral infection	1	REF
	Bacterial infection (Streptococciosis)	2	UO

\*Level of confidence :

- (1) Possible, with some clinical signs in agreement with signs known for the disease; no previous experience
- (2) Possible, with some clinical signs in agreement with signs known for the disease; previous experience with unconfirmed outbreak
- (3) Possible, with all clinical signs in agreement with signs known for the disease based on previous confirmed outbreaks

\* Basis for diagnosis:

- (DO) previous experience with confirmed outbreak  
 (UO) previous experience with unconfirmed outbreak  
 (REF) no previous experience but matches descriptions in reference material

## Grouper Viral Impact Survey in the South and East Coasts of Thailand

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### Abstract

A grouper viral impact survey was conducted on fish farms located in the South and East coasts of Thailand from August to October 2000. A questionnaire was used to interview grouper fish farmers. Eighty-two farms were selected randomly from 1 100 farms. Data were stored and analysed using EpiInfo programme. All interviewed farms were grow-out farm types and most of them were classified as small-scale farms. Seventy-two of 82 farms were involved in cage culture while the rest used the earthen pond culture type. Brown-spotted grouper, *Epinephelus malabaricus*, was the main cultured grouper followed by orange-spotted grouper, *E. coioides*. All farmers (100%) reported losses due to diseases. The farmers reported 10 different disease syndromes, however, after expert consultations, all 10 syndromes could be grouped as 7 diseases or factors leading to fish losses. These diseases include viral disease, bacterial disease, parasitic disease, EUS-like disease, starvation, tumor and unknown causes. Most farmers experienced losses due to diseases and the main disease was of viral origin followed by the bacterial disease. Findings from this survey suggest that efforts should be directed at resolving the viral disease problems experienced by small-scale fish farmers. This would support farmers' livelihood derived from grouper culture and help to sustain grouper aquaculture.

### Introduction

Grouper is one of the main economically important cultured fishes in Southeast Asia. Usually Hong Kong, China, Chinese Taipei, China PR and Singapore are major importing countries. Sources of the grouper in the market include wild catch and aquaculture. Due to the increasing awareness of fishing, pollution and illegal fishing issues, the development of grouper aquaculture is receiving more attention from many countries in the region. Grouper aquaculture has been in existence for over 20 years along the South and East coasts of Thailand. Available records indicated that cultured marine finfish production, including groupers, was 4 800 metric tons in the year 1996 (Fisheries Statistics of Thailand 1996). Recently, as the failure of shrimp culture in some areas continued, some shrimp farmers shifted to grouper culture using earthen ponds (Yashiro *et al.* 1999). This shift in cultured species resulted in increased grouper production in Thailand. Unfortunately, neither the government, nor private hatcheries, has consistently produced large amounts of grouper seeds. Most seeds are presently collected from the wild. Apart from seed supply, health problem is another major concern to farmers. Many grouper diseases have been well documented (Bondad-Reantaso *et al.* 2001). Viral diseases are the major cause of high losses. Two different viruses, Iridovirus (Danayadol *et al.* 1994; Kasornchandra and Khongpradit 1997) and Nodavirus (Danayadol *et al.* 1995) have been identified as pathogens, which are capable of causing high mortality. Many countries in this region and other parts of the world also report these two kinds of viruses.

With the increasing importance of grouper aquaculture and growing awareness of viral disease problems, this survey study was an attempt to gain better understanding of the impact of viral diseases on grouper aquaculture in Thailand.

## Materials and Methods

The viral impact survey was conducted on the East and South coasts of Thailand. It used a semi-structured questionnaire developed during a 'Grouper Impact Disease Survey Workshop' held in Bangkok, Thailand 27-29 May 2000. The workshop represented a part of the implementation of APEC FWG 02/2000 "Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development".

Eighty-two fish farmers were selected randomly from 1 100 grouper fish farmers and interviewed during the period from August to October 2000. Sixty-two farmers were from the South while 20 farmers were from the East Coast. The farmers were asked for clinical signs or syndromes of grouper diseases. All syndromes were reviewed by fish disease experts (Ms Yaowanit Danayadol, Fish Pathologist, NICA and co-authors of this survey) to identify diseases or causative agents. The questionnaire data were stored electronically and analysed using EpiInfo 6 programme.

## Results and Discussion

All interviewed grouper farmers were involved in grow-out culture. Seventy-two farms were involved in cage culture while 10 farms used earthen pond culture. Most farms were small-scale operations (2-4 fish cages) and mainly employed members of the family. Grouper seeds were collected from the wild or bought from collectors.

Only two main species of grouper are cultured in Thailand. One is the brown-spotted grouper, *Epinephelus malabaricus*, which is grown at 95% of the fish farms. The other is the orange-spotted grouper, *E. coioides*, which is cultured at 5% of the interviewed farmers. It normally takes 8-18 months for grouper to reach market size (in excess of 700 g). Diseases appeared to be one of the main problems as 77 out of 82 farms reported losses during the culture period in 1999.

An analysis of the relationship between culture type and disease revealed that 95% of the cage culture farms and 80% of the pond culture farms experienced diseases. Findings from this survey indicated that 10 syndromes occurred during the last culture period or last 12 months. After consultation with fish disease experts, all 10 syndromes could be identified as 7 groups of diseases or factors leading to fish losses (as shown in Table 1). According to the experts, 32.8% of the farms recorded losses due to viral diseases. Both Nodavirus and Iridovirus caused high economic losses of 62.44% of the production value. The Nodavirus alone caused mortality in grouper of up to 69.2% of the stocking density, and frequency of 23%; while Iridovirus caused mortality of approximately 26.25% with frequency of 10%. Economic loss due to Nodavirus infection alone was 40.44%, whereas that caused by Iridovirus was 22%.

Fish farmers also experienced bacterial disease problems (31.9%) with a cumulative economic loss of approximately 35.2%. Loss due to *Streptococcus* infection was incurred at 18.75% of stocking density. This average number was reported by 3.3% of the fish farmers. The economic loss was at 7.5%. The occurrence of *Flexibacter* infection seems to be associated with poor handling conditions and appears to be related to other infections. Fish mortality due to *Flexibacter* disease was about 15% with an economic loss of about 15%, (reported by only one farmer). Losses due to unclassified bacterial diseases were recorded by 27% of the fish farms with the average fish mortality of 14.18%. The average economic loss caused by the unclassified bacteria was 12.7%.

Parasitic infections were common in grouper culture. Eighteen percent of fish farmers reported that the fish had clinical signs related to parasitic diseases. However, fish mortality due to parasites was low, 6.4%, and an economic loss of 3.18%. The diseased fish usually exhibited darkened body coloration, swimming near the water surface and loss of appetite.

The epizootic ulcerative syndrome (EUS) or EUS-like syndrome was recorded by 2 farms or 1.6% frequency. Fish had ulcerative necrotic lesions on the body and near caudal peduncle with bad smell and high mortality of up to 30%. The economic loss was 10%.

Only one farmer reported starvation of grouper with 5% mortality, and economic loss of about 2%. Diseased tumor was also recorded at one farm with 1% fish mortality. The fish had tumors on skin and fins. There was no figure for economic loss.

In many cases, fish died without any clinical signs and the farmers could not provide any details regarding the cause. The unknown diseases were reported by 13.9% of fish farms, with mortality of up to 12.94%, and economic loss of 7.44%.

This survey also revealed that farmers generate substantial profit (53% return on investment) from the grouper culture. The costs of grouper production consist of trash fish feed, grouper seed, labor, fuel/electricity and drugs/chemicals, in descending order.



## Conclusions and Recommendations

Grouper aquaculture in Thailand mainly consists of small-scale cage culture operations. Most farmers own 2-4 cages. Some old shrimp earthen ponds have been converted for grouper culture in the South (Yashiro *et al.* 1999). Grouper seeds are usually bought from the collectors or traders while some farmers collect seed from the wild. Although farmers receive substantial profit from this aquaculture activity, a great number of farmers experienced losses due to diseases. The main disease problems appear to be viral diseases followed by bacterial diseases. As noted in this paper, Nodavirus causes more severe losses than Iridovirus. Although the bacterial disease had similar frequency, the fish mortality and economic losses were low in comparison to the viral disease. The survey findings indicate that the viral disease is a major problem in grouper aquaculture. Efforts are to be made to solve viral disease problems in order to help small-scale fisheries and to attain sustainability of grouper aquaculture.

**Table 1. Groups of diseases or factors leading to fish losses identified from the survey.**

Group	Diseases/Syndromes	Clinical signs
<b>1</b>	<b>Viral Diseases:</b>	
	Viral nervous necrosis (VNN)	Paralysis, corkscrew movement, abdominal distension, floating near the water surface
	Iridovirus	Tiny spot of hemorrhage on body with abscess
<b>2</b>	<b>Bacterial Diseases:</b>	
	Streptococcus	Exophthalmia, weak movement, hemorrhage at the base of the fins
	Flexibacteria infection	White patches on the body, tail and fin rot, wound lesion on the skin
	Unclassified bacteria	Red spot
<b>3</b>	<b>Parasitic Infection:</b>	
	Protozoa (Cryptocaryon and Trichodina)	Severe fin rot, white spot on the skin, irregular swimming
	Monogenea, leech, isopod	Irregular swimming, slight fin rot
<b>4</b>	<b>EUS-like syndrome</b>	Ulcerative necrosis of body with bad smell
<b>5</b>	<b>Starvation</b>	Flat abdomen
<b>6</b>	<b>Tumor</b>	Tumor like lesions on fins, tail and mouth
<b>7</b>	<b>Unknown</b>	No clinical signs

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## Cage Culture of Grouper in Brunei Darussalam

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### Abstract

Three of more than 40 species of groupers in tropical waters are commercially cultured in Brunei Darussalam. They are marketed locally to restaurants and hotels. The Department of Fisheries has policies/regulations for aquaculture development which includes zoning of aquaculture areas, specific technical requirements for aquaculture and licensing of farms. This paper presents information on disease (parasites and bacteria) of cultured groupers and their treatment based on diagnostic cases received by the Department of Fisheries. The paper is concluded with a number of recommendations for the prevention and control of disease outbreaks.

### Introduction

Marine finfish culture in floating cages in Brunei Darussalam is a recently developed industry. It showed a remarkable growth during the last few years. Asian sea bass, *Lates calcarifer* (Bloch), estuarine grouper, *Epinephelus suillus* (Valenciennes), black-spotted grouper, *Epinephelus malabaricus* (Bloch), yellow spotted grouper, *Epinephelus bleekeri* (Vaillant), mangrove snapper, *Lutjanus argentimaculatus* and jacks, *Caranx melampygus* are the major species cultured.

Similar to other countries, Brunei Darussalam was drawn to grouper culture by strong market demand and high prices in export markets, particularly Hong Kong China. Grouper fingerlings are caught using a fish trap locally known as "bubu". A survey of grouper fingerlings in Brunei waters was conducted in 1993. Results of the survey indicated that grouper fingerlings are abundant in Brunei Bay. However, as the bay is surrounded by high-quality mangrove vegetation, aggregating or attracting grouper fingerlings to the traps is difficult. In the coral areas attempts to collect grouper fingerlings by fish traps did not yield good results.

With the intensification of culture practices, diseases have become a threat to the industry causing severe losses to the farmers. Due to shortages of grouper fingerlings caught by the traps, fingerlings were imported from Indonesia, Malaysia, Philippines, and Thailand. Fingerlings were susceptible to bacterial, fungal and parasitic infestation as they were stressed from transportation. Control of these diseases is essential for the sustainable development of the industry and for ensuring a stable high yield from the culture systems.

There are around 40 species of grouper in tropical waters. Only a few species, (estuarine grouper, black-spotted grouper, and yellow-spotted grouper) are cultured commercially in the country. For the most part they marketed locally to restaurants and hotels.

### Site Selection and Net Cage Specifications

The Department of Fisheries has a plan to sustain the productivity of aquaculture areas in the country. The Department zones areas for aquaculture, allocating specific areas for particular aquaculture activities and determining the number of farms and their allowed sizes. Licensing of aquaculture farms provides for better control of aquaculture areas.

The country has several government-approved sites for aquaculture. A floating cage module, for example, usually has a maximum of forty compartments supported by a framework in a 0.2 ha area. The requirements for cage culture in the country are:

- Cage frame. Cage frames are usually made of wood with dimensions of either 4 m x 4 m x 2.5 m or 5 m x 2.5 m x 5 m. The construction should be durable enough to withstand stress caused by wave action and increased weight during the culture period.
- Sinkers. These are made of either concrete blocks, plastic containers filled with sand or galvanized pipes, which are suspended by ropes and placed at the bottom of the four corners of the net cage for rigging.
- Floatation materials. These are plastic drums tied to the frame of the cages and serve as floats.

### ***Present Status of Grouper Cage Culture and Diseases in Brunei Darussalam***

Currently, there are sixteen floating cages in operation with estimated 3108 groupers being cultured.

Imported grouper fingerlings are examined for parasites and bacterial infestation. Infected fish are treated with chemicals and/or held in tanks prior to stocking. Healthy or good quality fingerlings are stocked directly in nursery or in cages. The water used during transportation (water in polyethylene bags) is treated with strong concentration of formalin prior to discarding. Newly stocked fingerlings are closely monitored by the Department of Fisheries in order to assist the farmers if the fingerlings require to be further treated with chemicals or bathed with freshwater.

Common grouper diseases discussed below are based on diagnostic cases received and experiences at the Brunei Darussalam Department of Fisheries.

#### **(a) Bacterial Diseases in Grouper**

*Causative agent:* *Vibrio parahaemolyticus* and *Streptococcus* sp.

*Affected stages:* Groupers at all stages can be affected but the disease is more common in fry and fingerling stages (from 5 to 15 cm).

*Disease Transmission:* It appears that the bacteria are always present in the environment on the carrier fish in the cages or wild fish. However, spread of the disease is closely related to other factors, such as presence of parasites, handling stress, sub-optimal water quality. It has been observed that infected grouper always harbour a large number of monogeneans and protozoans on the gills and skin.

*Treatment:* (a) antibiotics such as oxalonic acid mixed with feed at 20 mg/kg of fish; (b) Prefuran bath treatment for 1 hr at 2 ppm concentration.

#### **(b) Tail Rot Disease**

*Causative agent:* Myxobacteria (*Flexibacter* sp.) and *Vibrio* sp.

*Affected stages:* Fingerlings (from 3 to 8 cm)

*Clinical signs:* The disease starts with an erosion at the tip of the caudal fin and could destroy the whole tail within two days. Bacteria then invade the muscle fibres of the tail area. Severely infected fish will have only the bones of the spinal column in the tail. Mortality can reach about 80% within few days if the infected fish are not treated.

*Disease transmission:* It was observed that the spread of the disease has close correlation with the water salinity. The disease normally starts when the high salinity seawater (30–35 ppt) gets flushed into the cage culture sites. Bacteria invades fish through a damaged area on the tail. Therefore, rough handling of fish could trigger the infection.

*Treatment:* Freshwater bath for about 10–15 min

**(c) Bacterial Gill Disease**

*Causative agent:* *Cytophaga* sp., *Flexibacter* sp. and *Flavobacter* sp.

*Affected stages:* Fingerlings and medium sized fish (from 8 to 20 cm)

*Clinical signs:* Affected fish become lethargic and dark in colour. They tend to remain near the surface and may be flaring their operculum.

*Disease transmission:* The disease generally starts with the deterioration of water quality after heavy rains. The silt and organic particles that flush to the area could irritate the gills and increase susceptibility to the disease. The low dissolved oxygen level and high ammonia levels are often observed during disease outbreaks.

*Treatment:* Antibiotic such as oxolinic acid mixed with feed at 20 mg/kg of fish

**(d) *Trichodina* spp. infection**

*Causative agent:* *Trichodina* spp.

*Affected stages:* Juveniles and adult fish. Parasite infection can develop to outbreak levels among fry and fingerlings, as those stages are more prone to the infection. Adult fish act as carriers.

*Clinical signs:* Parasites are found on the gills, skin and fins of the infected fish. When there are a large number of parasites, they can severely irritate the fish. The most serious syndrome is the disruption of the respiratory process due to physical presence of the parasites. Infected fish will have a thin whitish coating of mucus on the skin and may exhibit abnormal behaviour and dark colouration. The fish become sluggish, lose weight and become moribund.

*Disease transmission:* Parasites multiply rapidly by binary fission. They can survive in the water for up to two days without a host. Therefore, they can easily spread from one fish to another through the water.

*Treatment:* (a) formalin bath for thirty minutes at 200 to 250 ppm; (b) potassium permanganate bath for five to ten minutes at 100 ppm concentration; and (c) formalin bath for thirty minutes at 200 to 250 ppm.

**(e) Diplectanid monogenean infection**

*Causative agents:* *Pseudorhabdosynochus epinepheli*, *P. monosquamodiscusi*, *P. latesi* and *Diplectanum penangi*

*Affected stages:* Fry, fingerlings and adults

*Clinical signs:* When the infection is intensive, fish scratch against the net to get rid of the parasites. This often causes injuries to the skin and allows secondary bacteria to invade the fish. During feeding, parasites continuously irritate the fish and cause severe damage to the gills and skin, particularly when their number is high. During severe infection, infected fish may lie still with open but rapidly moving operculum. Large numbers of monogeneans can be seen in the gills of these fish when they are dissected out and observed under a microscope.

*Disease transmission:* Infection usually remains at low levels without effect on the fish. Intensity of these parasites can increase rapidly under stressful conditions such as high concentration of organic pollutants in water, high stocking densities, unclean net cages, which can lead to high mortality.

*Treatment:* (a) Formalin bath treatment at 250 to 300 ppm for fifteen to thirty minutes with strong aeration repeated for three days; (b) overnight bath treatment of 25 ppm formalin and 0.15 ppm malachite green; and (c) antibiotics to control secondary bacterial infections.

**(f) Capsalid monogenean infection**

*Causative agent:* *Benedenia* sp.

*Affected stages:* Fingerlings and adults

*Clinical signs:* Infected fish scratch against the cage netting to get rid of the parasites on their skin. This behaviour damages the skin and secondary bacterial infection can enter. They cause large lesions on the body. The body surface becomes cloudy due to excessive secretion of mucus

*Disease transmission:* Infection usually remains at low level without effect on the fish. Intensity of these parasites can increase rapidly under stressful conditions such as high concentration of organic pollutants in water, high stocking densities, unclean net cages, which could led to high mortality.

*Treatment:* (a) freshwater bath for three to four minutes; and (b) antibiotic treatment to control bacterial infection.

### **Conclusions and Recommendations**

Cage culture of grouper is forecasted to increase the export of marine fish. Its economic value may be low but it could provide other benefits to the farmers in terms of income generation and employment opportunities. The profitability of cage culture can be improved. Further development of the local fish trap “bubu” will help augment the income of traditional fishers.

Joint regional efforts to refine and develop reliable seed production technology would assist in development of the grouper culture industry. Formulated feeds should be developed. Regular information on market prices and demand could be useful to the producers.

Parasitic infections in Brunei Darussalam are caused by protozoans (particularly ciliates), by monogeneans (mainly capsalids, diplectanids and dactylogyrids) and flukes. The floating net cage systems can act as huge reservoirs of pathogens.

In order to prevent and control outbreak of diseases, the following is recommended:

- An integrated health management system needs to be developed. It must cover all levels of aquaculture production at the farm, district and national levels.
- There should be regional collaboration to standardize grouper health certificates.
- Quarantine procedures and facilities for imported grouper should be improved to prevent entry of exotic diseases.
- The surveillance, monitoring and reporting systems for grouper diseases should be improved by focusing on improvement in diagnostic capabilities of extension workers and developing a network for the monitoring programs.
- The use of chemicals to treat fish diseases should be decreased to prevent development of antibiotic resistance and environmental pollution from chemical residues.
- A program to eradicate grouper diseases in the hatcheries should be developed by improving the operators’ knowledge of good farming practices, stress management, sanitation and hygienic measures, and food and trash feed management.
- More research on possible biological and economic impacts of diseases.
- Improve the knowledge, diagnostics and research skills of those working in aquaculture.
- In Brunei Darussalam, pathogen screening, disease diagnosis and research are still very limited in terms of manpower and facilities and should be addressed.

## Status of Grouper Culture, Fry Production and Grouper Diseases in Guangdong, China PR

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### Abstract

This report focuses on the status of seed production, fry supply, grow-out culture, and diseases of grouper in Guangdong Province, China PR. China, the biggest producer of grouper, contributes about 50% to the total world aquaculture production. Guangdong province is the largest producer in the country. The mean survival rate of net-caged cultured groupers is 30-40%. The mortality of larvae and juvenile of hatchery-reared groupers due to viral nervous necrosis (VNN) can reach as high as 100%. The survey results indicate that diseases seriously threaten grouper culture. Prevention of further spreading of the grouper diseases is a critically important and urgent matter.

### Introduction

The cultivation of groupers in China is concentrated in the four coastal provinces of Guangdong, Fujian, Zhejiang and Hainan. China produced an estimated 8 000 tons in 1997 (Anon. 1997). Guangdong Province, the largest producing area, yielded 2 500 tons (Anon. 1998). About 12 species of groupers are currently cultivated in Guangdong Province (Anon. 1999). The orange-spotted grouper (*Epinephelus coioides*) and three-spotted grouper (*Epinephelus trimaculatus*) are two most commonly cultured species. However, humpback grouper (*Cromileptes altivelis*), red-spotted grouper (*Epinephelus akaara*) and giant groupers (*Epinephelus lanceolatus*) are the species which command higher prices.

### Fry supply

There are four main sources of grouper fry used for culture. These are as follows:

- Locally captured wild fry, about 50%
- Fry imported from Southeast Asian countries, about 20%
- Hatchery produced fry imported from Chinese Taipei, about 20%, and
- Hatchery produced fry from Guangdong Province, about 10%

Hatchery breeding of groupers in Guangdong Province includes orange-spotted grouper and red-spotted grouper. The survival rate of all species is between 40-60%. The quality of imported fry varies greatly and the survival rate is at 20-40%. Imported fry has been a source of diseases introduced to the domestic grouper.

### Grow-out culture

Grow-out culture of groupers mainly utilizes floating cages in shallow region. There are about 12 species of groupers cultured using net-cages in Guangdong. These are malabar grouper (*Epinephelus malabaricus*), brown-spotted grouper (*E. chlorostigma*), red-spotted grouper, kelp-grouper (*Epinephelus moara*), three-spotted grouper, sixbar grouper (*Epinephelus sexfasciatus*), orange-spotted grouper, yellow grouper (*E. awoara*), giant grouper, banded grouper (*Epinephelus amblycephalus*), greasy grouper (*Epinephelus tauvina*), and humpback grouper (Anon. 1997, 1998, 1999).

Guangdong Province has a coastal line of 3 300 km with many gulfs. There are about 20 000 cages involved in grouper culture, with sizes of 3 m<sup>3</sup> or 4 m<sup>3</sup>, stocking density of about 40-60 pcs/m<sup>3</sup> (3-5 cm), or 5-10 pcs/m<sup>3</sup> (1-2 kg). They use trash fish as feed. Production in 1997 was 2 500 tons. The area still has sufficient space for further fish

culture development. According to the study, of the 110 000 net cages for marine fish culture in Guangdong, 40% of the producers tend to raise groupers. Guangdong province has a great potential and advantages in developing grouper culture although there are also some limiting factors.

#### *Artificial reproduction techniques of grouper fry*

At the beginning of the 1980s, research departments in Zhejiang, Fujian and Guangdong provinces conducted extensive research on the artificial reproduction of orange-spotted grouper and red-spotted grouper (Liufu *et al.* 2000). Studies were confined to the laboratory. As funding was limited, the number of spawning fishes bought was small, and the amount of fertilized eggs was far from what was the required for large scale production. Therefore, it was impossible to provide the amount of fry needed by the producers.

In 1997, the Guangdong Daya Bay Fisheries Development Center (GDFDC) cooperated with the Overseas Fishery Cooperation Foundation (OFCF) of Japan to carry out research on the artificial reproduction of red-spotted grouper and orange-spotted grouper. The natural spawning of red-spotted grouper succeeded in 1998. An estimate of 150 000 fingerlings of orange-spotted groupers and 30 000 red-spotted grouper up to 5 cm were successfully produced and were offered to fish farmers in 1999.

The present technical problems of artificial reproduction of groupers include the following: (a) synchronization of spawning and getting a large amount of fertilized eggs at the same time, (b) prevention of the cannibalism at the earlier stage, (c) development of the technology for large scale production of fry and (d) control strategy for Viral Nervous Necrosis (VNN) (Lin Li *et al.* 2001).

#### *Survey of Grouper Virus and other Diseases*

Diseases have been seriously threatening grouper culture in Guangdong Province in recent years. Because of various diseases, the survival rate of net-caged cultured groupers is about 30-40%. The number of diseases affecting grouper has increased with the expansion of grouper culture in Guangdong Province.

A study of the grouper diseases at 20 farms was carried out in Guangdong by staff of GDFDC from April to October 2000. The survey used the semi-structured questionnaires provided by APEC FWG 02/2000. Based on farmers' responses and experts' consultation (Dr. Lin Li provided valuable information), the survey revealed 14 kinds of diseases affecting 7 species of groupers which were categorized as bacterial diseases, parasitic diseases, viral diseases and diseases of unknown origin as indicated in Table 1.

### **Conclusions and Recommendations**

The survey results indicate that diseases seriously threaten grouper culture in Guangdong province. The survival rate of net-caged cultured groupers in Guangdong Province is very low mainly due to diseases.. Among these diseases, viral nervous necrosis (VNN) caused by betanodaviruses (piscine nodaviruses) is one of the most devastating diseases in hatchery-reared groupers (Lin Li *et al.* 2001, He *et al.* 1998). The number of diseases affecting grouper has increased with expansion of grouper culture in Guangdong Province.

Development of disease control measures is an important strategy to increase the survival rate and quantity of production in aquaculture. Establishing disease control research centers at both regional and national levels, strengthening and upgrading research and diagnostic capabilities at various institutions, developing practical techniques in the prevention and control of diseases would provide strong support for grouper culture development.

### **Acknowledgement**

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**Table 1.** Results of survey of grouper viral and other diseases in Guangdong Province.

Farmer ID	Farm type/ Culture type	Species	Classification of Disease	Mortality/ Morbidity	Economic Loss	Farmer's Description of Disease
1	Grow-out, cages	<i>E. coioides</i>	Bacteria	50% 80%	50%	External haemorrhagic lesions
2	Grow-out, cages	<i>E. moara</i>	Unknown	30% 50%	50%	Black body disease
3	Grow-out, cages	<i>E. bleekeri</i>	Bacteria	40% 100%	40%	Ulcerations of fins
4	Grow-out, cages	<i>E. fuscoguttatus</i>	Unknown	20% 20%	20%	<i>Neobenedenia</i> sp.
5	Grow-out Pond	<i>E. coioides</i>	Parasite	10% 50%	20%	<i>Neobenedenia</i> sp.
6	Nursery, grow-out	<i>E. coioides</i>	Parasite	20% 30%	30%	<i>Pseudorhabdosynochus epinepheli</i>
7	Grow-out, pond		Parasite	10% 50%	10%	White-spot disease Lymphocystis disease
8	Grow-out, cages	<i>E. bleekeri</i>	<i>Trichodina</i> sp.	5% 10%	5%	<i>Trichodina</i> sp.
9	Grow-out cages		Virus	2% 5%	10%	Lymphocystis disease
10	Hatchery, nursery, tank	<i>E. coioides</i> <i>E. akaara</i>	VNN	98% 98%	98%	Lethargy, swimming near surface of water, abrupt whirling and sinking to bottom
11	Grow-out cages	<i>E. akaara</i>	Unknown	20% 10%	20%	Bulging eyeball
12	Grow-out cages	<i>E. akaara</i>	Bacteria	30% 30%	30%	Dark body color, extensive haemorrhagic erosion
13	Hatchery Nursery tank	<i>E. akaara</i>	Parasite	20% 90%	40%	White spot disease
14	Grow-out Cages		Viral	2% 10%	15%	Lymphocystis disease
15	Grow-out cages		Bacteria	50% 80%	50%	Body rotten disease
16	Grow-out cages	<i>E. moara</i> , <i>E. coioides</i> , <i>E. akaara</i>	Unknown	5% 20%	10%	Bulging eyeball
17	Grow-out cages	<i>E. fario</i> , <i>E. coioides</i> , <i>E. akaara</i>	Viral	2% 5%	10%	Lymphocystis disease
18	Hatchery nursery, Tank, pond		VNN	100% 100%	100%	Lethargy, swimming near surface of water, abrupt whirling and sinking to bottom
19	Grow-out cages	<i>E. coioides</i> , <i>E. akaara</i>	Parasite	20% 30%	30%	Microsporida
20	Grow-out cages	<i>E. lanceolatus</i>	Unknown	25% 40%	40%	Black body disease

## A Review of the Occurrence of Viral Nervous Necrosis (VNN) Among Cultured Groupers in Chinese Taipei

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### Abstract

Mass mortality of hatchery-reared grouper larvae and juveniles repeatedly occurred during the past few years in the southern part of Chinese Taipei. Non-enveloped icosahedral to spherical particles with diameter of 20-25 nm were found in the cytoplasm of infected cells. The virus was identified by RT-PCR using nervous necrosis virus (NNV)-specific primers as a fish nodavirus, and was designated as grouper nervous necrosis virus (GNNV). By *in situ* hybridization using specific dig-labeled probe, GNNV appeared to be systematically distributed in the moribund larvae. GNNV can induce specific cytopathic effect (CPE) in GF-1 cell line at 24-32°C. The infectivity of GNNV can be blocked after one hr treatment at temperature higher than 60°C. GNNV was sensitive to pH lower than 3 or higher than 10. Purified GNNV has two structural proteins with molecular weights of 43 and 41 kDa containing carbohydrate, and consists of two species of single stranded RNA with molecular weights of  $1.02 \times 10^3$  and  $0.5 \times 10^3$  kDa. The viral etiology was demonstrated by experimental transmission. Larvae immersed with GNNV showed 100% mortality on day 3, and juveniles intramuscularly injected with GNNV began to die 9 days post infection. Recently, VNN was also detected among other species of hatchery fish in the country. So far, all the VNN isolates in the country were identified as RGNNV (red-spotted grouper nervous necrosis virus) genotype due to similarities of T2 sequence, which was higher than 97%.

### Introduction

Viral nervous necrosis (VNN) is a worldwide disease affecting many species of marine fish, and causing high mortalities of affected larvae and juveniles (Munday *et al.* 1992; Nakai *et al.* 1995; Chi. *et al.* 1997; Breton *et al.* 1997; Munday *et al.* 1997). The reported fish hosts are at least 19 in 10 families (Muroga 1995). The etiologic agent of VNN has been identified as a new member of Nodaviridae based on the properties of the viral genome and proteins (Mori *et al.* 1992). Fish nodaviruses are classified into four genotypes, such as: (a) tiger puffer nervous necrosis virus (TPNNV), (b) striped jack nervous necrosis virus (SJNNV), (c) barfin flounder nervous necrosis virus (BFNNV); and (d) red-spotted grouper nervous necrosis virus (RGNNV) (Nishizawa *et al.* 1997). The nerve tissues are the target organs for VNN infection, but other tissues are infected as well. The distribution of VNN in other tissues varied according to fish species and ages (Comps *et al.* 1996; Nguyen *et al.* 1996; Grotmol *et al.* 1997; Grotmol *et al.* 1999).

Grouper aquaculture is a vital industry in the country. In recent years, VNN disease caused high mortality among grouper larvae and juveniles, and resulted to considerable economic losses. In this paper, we briefly review the studies on VNN disease in Chinese Taipei.

### Clinical Pathology

Diseased grouper larvae collected from hatchery farms in the southern part of the country showed lethargy and swimming in a corkscrew fashion, but responded to alarm or physical stimulation. The earliest occurrence of VNN syndrome is 17 days post hatch. The highest mortality was 100%, while the mortality among naturally infected juveniles with a body length of 6-10 cm was about 50%.

Under light microscopy, many vacuoles were found in the brain tissue. Electron microscopy, on the other hand, revealed viral particles in the cytoplasm of affected cells closely associated with the intracytoplasmic cell membranes. Mature viral particles are packed inside the membranous organelles, and then released into the cytoplasm (Chi *et al.* 1997).

## Diagnosis

### *Polymerase Chain Reaction (PCR) Amplification*

Deproteinized nucleic acids extracted from diseased grouper larvae and juveniles were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers specific to SJNNV RNA2 gene. The separate nucleic acid preparations consistently produced a major band with a size similar to that of the T2 region (875 bp) of SJNNV RNA2, and the results of the two-step PCR amplification using an internal primer set (F2-R3) can amplify a product similar to the target region T4. Therefore, primers set (F1, R3) and (F2, R3) are suitable to amplify the NNV isolates in Chinese Taipei.

Specific cytopathic effect (CPE) was induced in GF-1 cell line, derived from the fin tissue of the grouper *Epinephelus coioides*, 3 days after inoculating with the filtrate of diseased grouper larvae. CPE developed initially as rounded, granular, refractive cells and, then, spread to the complete cell sheet and, finally, the cell degenerated and floated. The same CPE was reproduced in three passages, and the titer increased to  $10^9$  TCID<sub>50</sub>/0.1 ml (Chi *et al.* 1999).

### *Immunofluorescent staining*

Viral protein was initially detected in the GNNV-infected GF-1 cells 3 hours after infection, and 80% of the infected cells showed positive reaction 12 hours post infection even though no CPE appeared at that time.

### *In-situ hybridization*

Dig-labeled T4 probe was used to hybridize the tissue sections of GNNV-free and GNNV-infected grouper larvae. No reaction was found in the tissue of GNNV-free larvae but positive signal was found in the brain, retina, gill, skeletal muscle, liver, pyloric gland, stomach, intestine, and in blood cells of the heart of the GNNV-infected larvae. Therefore, GNNV can cause systematic infection among heavily infected larvae.

## Characterization of pathogen

GNNV is resistant to 40°C and 50°C for 1 h, but its infectivity can be totally blocked following treatment at 60°C for 1 h. GNNV was resistant to pH 5, but sensitive to exposure in pH 3 and pH 10-12 for 30 min, and the titer dropped over 4 log<sub>10</sub> compared with virus titer at pH 7.

Purified GNNV stained with Coomassie Blue revealed two coat proteins with molecular weights of 43 and 41 kDa. GNNV coat proteins are glycoproteins because they can be stained by periodic acid silver as another glycoprotein ovalbumin.

The molecular weights of GNNV RNAs are  $1.02 \times 10^6$  Da and  $0.5 \times 10^6$  Da. The multiple alignments of the nucleotide sequence (T2 region) of GNNV isolates with four fish nodavirus genotypes were compared, and the similarity among GNNV isolated in the country and RGNNV isolated in Japan was as high as 99%.

## Challenge Test

Larval groupers with size of 1 cm were bath-challenged with GNNV in titer of  $10^5$  TCID<sub>50</sub>, and then reared at water temperature ranging between 26 and 28°C. The accumulated mortalities of challenged larvae reared at both temperatures reached 100% three days post infection. However, during the first two days the accumulated mortality of the larvae cultured at 28°C is much higher than the mortality of the larvae cultured at 26°C.

The transmission of GNNV among juvenile groupers, 6-10 cm in total length, was tested by intramuscular injection with GNNV. Clinical signs of VNN disease appeared 9 days post infection, and the accumulated mortality reached 80% on day 15. The time for the outbreak of high mortality of juveniles is much longer than that of larvae.

## Transmission Pathway

In two grouper hatchery farms in the southern part of the country, 17 batches of grouper eggs were collected, and examined by RT-PCR using NNV-specific primers. Ten lots of the egg samples showed positive results. The larvae hatched from GNNV-infected eggs began to die 17-19 days post hatching, and the mortality increased to 80-90% after further 10 days. Viral particles were also observed in the hatched larvae by electron microscopy examination. These data suggested the

possibility of viral transmission vertically to the larvae via the virus-contaminated eggs (Chi and Lo 1998).

### **Epidemiology**

We examined VNN infection by PCR among several species of cultured fish other than groupers. The collected larvae and juveniles were both with and without clinical signs of VNN. By two-step PCR, we detected NNV on six species of cultured fish other than groupers, among which barramundi (*Lates calcarifer*), yellow-wax pompano (*Trachinotus falcatus*) and eel (*Anguilla anguilla* L.) showed clinical signs of VNN disease, and occurred mortality. Analysis of T2 sequence similarity among GNNV and the NNV isolates from barramundi, yellow-wax pompano and eel revealed that these isolates belong to RGNNV genotypes. The VNN disease in barramundi and yellow-wax pompano was reported only during the years 1998-2000, while mortality of NNV-infected eel only happened once.

### **The Future**

The introduction of VNN to these three new host species of fish was suggested to originate from the GNNV-infected groupers. Therefore, it is important to prevent the further spread of this disease by sterilization of water, utensils and tanks, and reduction of stress factors for the fish.

Monoclonal antibodies against GNNV were established in our laboratory and are used for NNV diagnosis and epitope analysis (unpublished data). The pathogenesis of NNV isolates from different species of cultured fish in the country will be compared in the near future.

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## Grouper Disease Impact Survey in Hong Kong China

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### Abstract

A farmer survey of management practices and diseases in farmed grouper in Hong Kong China was conducted using a standardized questionnaire. The species of grouper currently farmed in the country are *Epinephelus tauvina* and *E. coioides* (54%), *E. areolatus* and *E. bleekeri* (45%) and *E. malabaricus* (1%). Fingerlings are sourced from China PR, Chinese Taipei, Indonesia, Malaysia, Philippines, and Thailand. Grouper production was 160 tons in 1999 which is 13% of the current total mariculture production volume and is valued at US\$ 2.4 M.

Diseases reported in the survey as having major impacts were Vibriosis (68% prevalence) and "listless disease" (17%). Other diseases such as tumours, yellow eye disease and gallbladder disease were reported by 10% of farmers. Only 5% of farms reported no disease in the previous 12 months. Mortalities were more frequently reported in fingerling and juvenile fish after translocation and during the establishment period. Further investigation of diseases in farmed grouper will involve detailed investigation of selected farms through a production cycle to determine specific aetiologies of the diseases and identify any contributing factors.

### Introduction

In Hong Kong China, there are 26 fish culture zones occupying a total sea area of 209 ha. The majority of licensed farms are small and family-based consisting of 1 to 2 rafts with an average total area of about 250 m<sup>2</sup>. In 1999, imports of fry for aquaculture were valued at US\$ 3 M. The cost of production is about 50% of the farm gate value of fish estimated at US\$ 14-15/kg. Grouper production for the year 1999 was about 160 tons, valued at US \$ 2.4 M, equivalent to 30% of the total value of mariculture production. The estimated mariculture production (including non-grouper species like snapper, sea bream and pompano) in 1999 was approximately 1 250 tons (US \$ 8 M), 3 060 tons (US \$ 22 M) in 1996 and a peak of 3 860 tons (US \$ 28.5 M) in 1991.

Various diseases have been recognized in farmed grouper in Hong Kong China and survival rates as low as 10-20% have been seen in some batches of grouper introduced to fish farms (Wong 1998). A severe red tide in 1998 also had a major impact on fish stocks. The mariculture industry also had to cope with a decline in the market price of cultured grouper, a result of the competition from frequent imports of air-freighted or shipped fish.

Local fish farms supply about 7.8% of local demand for live marine fish. Total aquaculture production for 1999 was 5 807 tons valued at US \$ 17 M. Mariculture comprised 21.5% of total aquaculture production with 77.5% coming from freshwater aquaculture. However, in terms of unit value, mariculture products (US \$ 6,400/ton) were over three times more expensive than freshwater fish products (US \$ 1 974/ton).

Table 1 shows the number of licensed fish farms in Hong Kong from 1989 to 2000. The average age of farms is between 15 and 20 years, and the number of workers/farm is about 2 to 3 persons, usually consisting of family members.

The aim of this study was to obtain more information on the management practices and types of diseases seen in grouper mariculture in Hong Kong China through a survey of randomly selected fish farmers. Non-grouper species were not considered in the survey.

**Table 1.** Number of licensed farms engaged in mariculture culture from 1989 to 2000.

Year	Number of licensed farms	Year	Number of Licensed Farms
1989	1,810	1996	1,580
1990	1,770	1997	1,544
1992	1,654	1998	1,480
1993	1,648	1999	1,454
1994	1,636	2000	1,428
1995	1,622		

*Source* : Compiled from Agriculture and Fisheries Department (AFD) Annual Reports 88/89-98/99 and from Fisheries Branch of AFCD for the year 1999 and 2000 figures (AFCD, pers. comm.).

## Materials and Methods

The farmer survey of grouper disease was conducted using the questionnaire format developed by the APEC/FWG 02/2000 workshop on grouper diseases (Anon. 2000). Sixty grow-out farmers were interviewed using the questionnaire in September 2000. The farmers represent 13 out of 26 mariculture zones.

## Results

### *Species of Farmed Grouper and Importation of Fingerling (1999/2000)*

There are 5 species of grouper currently farmed in Hong Kong China. These are the green grouper (*Epinephelus tauvina*, *E. coioides*) – 54%; the brown-spotted grouper (*E. areolatus*, *E. bleekeri*) – 45%; and Malabar grouper (*E. malabaricus*) – 1%. All survey respondents imported their fingerlings. Fingerlings of green groupers are imported from China, Chinese Taipei, Indonesia, Malaysia, Philippines, and Thailand. Brown-spotted groupers come from Indonesia, Philippines, Thailand and Malabar groupers from Thailand (see Table 2).

**Table 2.** Estimates of grouper fingerling importation for the period 1999 to 2000.

Fish	Imports (average quantity; tails/farm/year) for 1999/2000	Annual Imports (estimates)
Green grouper	8 600	4.33 M
Brown spotted grouper	6 400	3.22 M
Malabar grouper	4 000	22.0 M

*Source*: Compiled from survey results reported in APEC FWG 02/2000 (Anon. 2000)

### *Management Practices*

The number of cages kept by each farmer ranged from 2-70 with an average of about 16 and they used trash fish as a major food source for fish stocks. Stocking densities ranged from 900 to 3 300 per cage. Growth rates and time to market are detailed as follows:

Brown spotted grouper: Fingerlings introduced at weights ranging from 20-30 g can reach market size of about 0.6-1.2 kg in 1.5 to 2 years, with average stocking density of 1800 fish/cage and cage sizes ranging from 10-40 m<sup>2</sup>. Peak growth occurs the warmer months (March to October), which is the period when fingerlings are stocked.

Green grouper: The growth of this species varies from 20-30 g fingerling to 1.2 kg (in 2 years), 3-4 kg (in 3-4 years) and 5-6 kg (in 5-7 years) with average stocking density of 1500 fish/cage and cage sizes ranging from 10-40 m<sup>2</sup>. Growth does not occur from November to February (water temperatures around 15 –17°C) and few fingerlings are stocked during this period.

Minced locally caught trash fish (storage time of <1 day) is used as the major feed. Food conversion ratio is estimated by farmers to be approximately 10:1. Most farmers do not monitor the physical parameters of the water environment. Net cages are cleaned with pressurized water jet once every 10-30 days to remove biofouling. Fish are only sold as live product to restaurants or fish markets.

### *Diseases*

In the survey, 95% of farmers reported disease in at least one cage of fish during the current growing cycle, with morbidity rates ranging from 2-3% to 100% and mortality rates ranging from 2% to 100%.

The following main syndromes were reported.

1. Approximately 68% of farmers described a disease with clinical signs of skin ulcers and lesions (ulcerative disease), exophthalmos (“popeyes”), red spot and white patch/spot, skin oedema and scale loss. This was reported in 55% of farms raising brown-spotted grouper and 45% of farms raising green grouper. The frequency of similar outbreaks in these farms was 1.8 times per year (range 1-5 times). According to the farmers, the disease lasted from 2 weeks up to 2 months causing a morbidity of 68% and mortality in affected fish of 72%. Some farmers reported that bath treatment with a yellow powder antibiotic (possibly oxytetracycline) and freshwater bath was ineffective unless employed during the first 24 hours when fish showed inappetence. Once skin lesions occurred the disease spread quickly within 2-3 days. Some farmers employed formalin bathing but this increased the morbidity and mortalities.

2. Another disease syndrome was described by 17% of farmers as “listless disease”. Approximately 83% of these cases occurred in green grouper and 17% in brown-spotted grouper. In this disease, fish became sick very rapidly and died within 12 hours of showing signs. Affected fish were listless and responded poorly to external stimuli, (farmers describe the fish as “mentally retarded”, and refer to the eyes as having “no life in them”). Fish float in the water and turn a dark colour, cease feeding and die within 12 hours of the appearance of clinical signs. They don’t have skin lesions. Several farmers reported that the disease appeared to spread from one batch of fingerlings to another, with mortalities peaking after 1 week of onset and ending rather abruptly in the 2<sup>nd</sup> week. Fish that survive an outbreak do not seem to succumb again to the disease. It was mainly seen from April to June and causes a morbidity of 72% and a mortality of 70% in affected fish.

The other diseases reported by farmers were tumours of fish, “slim built”, yellow eye disease and gall bladder disease. These constituted 10% of responses, while 5% of farmers surveyed reported that they did not experience fish diseases during the period covered by the survey.

### **Discussion**

The signs of the major disease reported by the farmers were highly suggestive of vibriosis. This disease has been recognized as a major cause of disease in Hong Kong China mariculture (Hong Kong Country Paper 1998, Woo *et al.* 1999). From laboratory records on diseased fish examined by AFCD (S. Everitt, pers. comm.) over the period from 1997 to 1999, the following *Vibrio* species were isolated from affected tissues including skin ulcers, fin/tail rot, eye socket, kidney and spleen : *V. alginolyticus* (52%), *V. vulnificus* (41%), and *V. parahaemolyticus* (7%).

Outbreaks of vibriosis in Hong Kong China have previously been linked to poor water quality (Lam 1990). Fish farm investigations conducted by our laboratory have revealed that vibriosis is particularly severe among fingerlings in the post-translocation and acclimatization period. Some of our investigations have shown very high levels of total vibrio counts in water in the fish farms which may be related to feeding trash fish, lack of tidal flushing, environmental deterioration of seabed in culture sites (Wong 1998) or high water temperatures (between 25-30°C), run-off after heavy rainfall, high stocking densities (*e.g.* >1000 fish/10m<sup>2</sup> cage) (unpublished data).

The second condition reported by farmers was described as “listless disease”. Investigations of cases of listless disease by our laboratory have indicated that at least some cases have been the result of acute septicaemia with *Vibrio* spp. and other investigations including virological examination have not identified alternative causes (unpublished data).

Further studies are underway. The selected fish farms in each of the major fish culture regions are being closely monitored for a 6-8 week period post-stocking. These will be used to further identify the causes of diseases observed by fish farmers and to determine the key factors that precipitate these outbreaks. In addition, studies are underway to assess the value of vaccination in prevention and control of vibriosis.

### **Acknowledgements**

We would like to thank Ms. Suzanna Everitt and her staff in the Aquaculture Development Division of the Fisheries Branch in the AFCD for performing the questionnaire survey as part of the study into Grouper diseases in Hong Kong China mariculture and the Aquatic Animal Health Research Institute, Department of Fisheries, Thailand for assistance in testing fish samples in virology and molecular techniques.



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## Grouper Viral Diseases and Research in Singapore

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### Abstract

The significant viral diseases affecting grouper in Singapore are briefly described. The most common viruses associated with diseases in grouper are iridoviruses and nodaviruses. Iridovirus was found to be the cause of "sleepy grouper disease" in 1992 as well as the cause of sporadic mortalities in a batch of grouper fingerlings in 1998. Vacuolating encephalopathy and retinopathy (VER) nodavirus was detected as early as 1988 in groupers. Although the incidence of viral infections in groupers is low, both viruses are capable of causing significant economic losses. Current research into marine fish viruses in Singapore is focused on these two viruses.

### Introduction

Grouper production in 1999 was 106 metric tons, which is equivalent to 3.1% of the total marine aquaculture production in Singapore and contributed 11% of total finfish production. The major grouper species farmed are the Malabar grouper (*E. malabaricus*), the greasy grouper (*E. tauvina*). Other finfish species cultured include milkfish (*Chanos chanos*, 378 metric tons), Asian seabass (*Lates calcarifer*, 239 metric tons), snapper (various species, 68 metric tons) and other species of finfishes (133 metric tons). In terms of value, grouper production accounts for S\$2.05 million representing 32% of finfish production or 21.6% of total production.

This paper presents a brief account of the status of viral diseases of grouper in Singapore based on publications and diagnostic cases observed at the Central Veterinary Laboratory (CVL) of the Agri-Food and Veterinary Authority (AVA) of Singapore.

#### *Viral Diseases of Groupers in Singapore*

##### 1. Grouper iridovirus

The most recently confirmed grouper iridovirus infection occurred in 1998. It affected Malabar grouper (*E. malabaricus*) fingerlings and caused sporadic losses in a single batch of fish. Mortalities (before the fish were culled) were greater than 50%. Clinical signs included lethargy and anorexia. Affected fish did not show any gross lesions.

Histological sections revealed splenic degeneration with large, roundish basophilic cells showing displaced nuclei. Similar lesions were observed in the kidney. A mononuclear infiltrate was observed in the heart tissue.

A cytopathic effect (CPE) producing agent was isolated on sea bass and grouper cell lines. The agent was initially characterised as an enveloped, DNA virus. Electron microscopy revealed icosahedral virions between 150-180 nm. Enveloped virus particles were approximately 200 nm in size.

##### 2. Sleepy grouper disease

An outbreak occurred from April to August 1992, causing severe losses to fish farms. Mortalities of up to 50% were reported in greasy grouper (*E. tauvina*). Affected fish became lethargic and inappetent. No gross lesions were observed except in cases affected by secondary bacterial parasites (Chua 1994).

Histologically, the primary lesions were seen in the spleen. These consisted of degeneration and necrosis of splenic tissue. Basophilic inclusion bodies were observed in some cells. Other affected tissues include the heart and kidney, which showed degenerative changes.

Virus isolation on sea bass cell line and BF2 were negative. However, virus particles of 130 nm to 160 nm were detected in spleen, kidney and heart tissues. Co-habitation studies showed disease transmission from diseased fish to healthy fish. Based on physical characteristics, the virus was preliminarily classified as an iridovirus.

### 3. Vacuolating encephalopathy and retinopathy (VER)

First described in 1991 in Singapore as viral nervous necrosis (VNN) among groupers (Chua *et al.* 1995), the disease was also detected by PCR in tissue culture fluid (TCF) samples collected in 1988 (Lim *et al.* 1997). The last confirmed case was in sea bass in 1997 (Chang *et al.* 1997). There were no confirmed cases in groupers recently.

The VER nodavirus is neurotropic, causing extensive vacuolative lesions in the nervous tissue of the brain, spinal cord and eye. Affected fish become anorexic and exhibit abnormal swimming behaviour, typically seen as corkscrewing or whirling swimming patterns. Mortality rates are usually high, especially in fry, often reaching 90-100%. In Singapore, VER has been implicated in several episodes of mass mortalities involving greasy grouper *E. tauvina* (Chua *et al.* 1995) and Asian sea bass *Lates calcarifer* since 1991. Affected fish range from fry to adult fish.

#### *Socio-economic impact of diseases*

Although viral disease outbreaks among groupers are sporadic, based on data from case records, estimates of losses at each outbreak are up to 50% for iridovirus infection and about 80 to 100% among fry and fingerlings for VER nodavirus infection. Chua (1993) described the “sleepy grouper disease” causing more than S\$ 100 000 in direct losses in 10 farms over a five-month period.

#### *Research and Development*

Two institutes are conducting research and development work on grouper diseases. These are the Tropical Marine Science Institute (TSMI), doing further characterisation and study of grouper iridovirus, and the Department of Biological Sciences, National University of Singapore, which is studying nodavirus and other marine fish viruses.

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## **Regional Synthesis of Grouper Health Impact Survey**

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### **Abstract**

A survey of the impact of grouper diseases using a standardized questionnaire based on a random sample of between 6 to 82 farms per country was conducted throughout Southeast Asia. The results from six countries (Indonesia, Korea RO, Malaysia, Philippines, Thailand and Vietnam) were compared. The survey found that about 90% of farms reported disease problems, primarily due to bacteria (especially Vibriosis) and viruses (especially VNN), and to lesser extent parasites. The impact of the diseases was assessed through morbidity, mortality, and the loss in value of recovered fish ('economic loss'). Reported mortality was remarkably consistent across all diseases, at about 30%. There is some evidence that viral diseases cause higher morbidity and mortality than bacterial disease, but bacterial diseases result in greater residual loss of value in recovered fish. The overall conclusions of the survey at a regional level are that diseases are commonly recognized by farmers; viral and bacterial diseases (particularly VNN and Vibriosis) are amongst the most common diseases reported; and that the losses due to these diseases are significant.

Both bacterial and viral diseases are major factors in production losses suffered by grouper farmers in Southeast Asia.

### **Introduction**

Grouper culture is practiced throughout Southeast Asia, and is of increasing economic importance. Until recently, there has been little information on the impact of disease on this industry at a regional level. Based upon the results of a series of national surveys, this paper presents a synthesis of the results from the six countries (Indonesia, Korea RO, Malaysia, Philippines, Thailand and Vietnam) participating in the data analysis workshop. Two other countries (i.e. China PR and Hong Kong China) conducted a survey of the impact of grouper diseases but results (see Haifa, Chong, elsewhere in this report) were not included in the regional synthesis.

### **Materials and Methods**

A survey of the nature and impact of grouper diseases was conducted using a standardized questionnaire<sup>1</sup> in countries throughout Southeast Asia. The survey methodology varied slightly from country to country but was based on the use of random sample of between 6 and 82 farms per country. Farmers' descriptions of disease syndromes were interpreted by local experts to provide a most likely diagnosis and causative agent. During a two day workshop held for six of the participating countries in Bangkok from October 16-17, 2000 the survey data was entered into a standard database, developed using EpiInfo 6.04 (Dean *et al.* 1994). The data from each country was analyzed separately, using an automated sequence of analyses to ensure uniformity.

### **Results**

#### *Disease syndromes reported*

A list of the classified syndromes reported by all participating countries during the survey is shown in Table 1. Many of these syndromes were expressed in terms of a pathogen. However during interpretation of all survey results, it was

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<sup>1</sup> The standardized questionnaire was developed during a 'Grouper Disease Impact Survey Workshop' held in Bangkok, Thailand in May 26-29, 2000 as part of APEC FWG 02/2000 "Development of a Regional Research Programme on Grouper Virus Transmission and Vaccine Development".

important to maintain an awareness of the survey methodology. Farmers were asked to report (retrospectively) their descriptions of disease. These took the form of a variety of clinical signs. Farmers and interviewers were then asked to group clinical signs and disease patterns into a limited number of syndromes. A single identified syndrome may have many separate causes, or may be caused by the confluence of many factors. Researchers then consulted a number of experts to generate a list of differential diagnoses for the syndromes described, and to nominate a 'most likely' diagnosis. The list of diseases in Table 1 is, therefore, a mixture of differential diagnoses, and syndrome descriptions.

**Table 1.** Disease syndromes reported.

Type	Syndrome/presumed cause	Countries identifying syndrome
<b>Parasites</b>	Sea lice	Vietnam
	Crustaceans	Thailand
	Leeches	Thailand, Philippines
	Encapsulated didymozoid	Indonesia
	Skin Fluke	Indonesia
<b>Viral</b>	VNN-like syndrome	Vietnam, Thailand, Philippines, Korea RO
	Iridovirus (GIV1)	Thailand, Indonesia
	Swim bladder stress syndrome	Philippines
	Black viral disease	Malaysia
	Non-differentiated viral infection	Philippines
<b>Bacterial</b>	Vibriosis	Vietnam, Philippines, Malaysia, Korea RO, Indonesia
	Flexibacter	Thailand, Philippines
	Streptococcosis	Thailand
	Non-differentiated bacterial infection	Thailand, Philippines
<b>Fungal</b>	EUS-like syndrome	Thailand
<b>Protozoan</b>	Cryptocaryoniasis	Malaysia
	Trichodiniasis	Korea RO
<b>Others</b>	Oxygen depletion	Vietnam
	Haemorrhage due to transportation	Thailand
	Starvation	Thailand
	Tumours	Thailand
<b>Non-specific</b>	Abdominal distension	Thailand
	Lethargy	Thailand
	Paralysis	Thailand
	Ulcerative necrosis	Thailand

Where specific pathogens were nominated as the cause of a syndrome, it should be remembered that laboratory confirmation was available in only one case (Iridovirus in Indonesia). Each of the diseases listed above should therefore be interpreted as shorthand descriptions of disease syndromes, presenting signs consistent with being caused by the nominated pathogen. For example, the EUS-like syndrome clearly describes the type of ulcerated lesions observed in the fish. However EUS does not occur in grouper, so this should not be interpreted as meaning that the syndrome is, in fact, EUS.

#### *Disease impacts*

##### Proportion of farms reporting disease problems

Presentations from other countries during the workshop indicated that there has been an increase in disease problems over the last 5 to 10 years. During the survey, farmers were asked if they recognized any disease problems on their farms. Table 2 summarizes the results by country.

Most countries found that around 90% of farms reported disease problems. Statistical analysis shows that there are unlikely to be any real differences between countries, except for Vietnam. In Vietnam, the number of farms reporting disease problems was significantly lower (around 40%). This may be due to fewer farms suffering from disease problems, different survey methodology, or a lower awareness of disease amongst farmers.

**Table 2:** Proportion of farms reporting disease problems.

Country	Disease	Total	Percent	95% CI
Vietnam	28	71	39.4	(28.2 - 51.8)
Thailand	77	82	93.9	(85.9 - 97.7)
Philippines	53	60	88.3	(76.8 - 94.8)
Malaysia	37	38	97.4	(84.6 - 99.9)
Korea	4	6	66.7	(24.1 - 94.0)
Indonesia	57	57	100.0	(92.1 - 100.0)

## Relative frequency of different disease types

On a regional basis, 365 diseases or disease syndrome occurrences were reported in the survey. The relative frequency of reports of major classes of disease is shown in the Table 3. Diseases have been grouped into four main putative causes: viral, bacterial, parasitic and other. The commonly reported diseases, VNN and Vibriosis, have been reported separately, as well as combined into all *viral* and all *bacterial* disease categories. It should be noted that this broad classification is based on the assumed cause of the disease, which in turn is based on farmer reports of clinical signs. These figures can therefore be used to provide an indication of rankings, but may not be reliable. For instance, VNN may commonly be used to describe a syndrome of whirling or lethargy. However, other viruses with neurological effects, or indeed other non-viral pathogens may conceivably cause such a syndrome.

**Table 3:** Relative frequency of disease reports.

Disease type	Reports	Percent
VNN	39	10.7
Other Viral	54	14.8
<i>All Viral</i>	93	25.5
Vibriosis	95	26.0
Other Bacterial	50	13.7
<i>All Bacterial</i>	145	39.7
Parasitic	71	19.5
Other	56	15.3
<b>Total</b>	<b>365</b>	

Bacterial diseases make up 40% of problems reported, and viral diseases make up 26%. Vibriosis is the single most commonly reported disease. Based on frequency of reporting, the ranking of priority groups of disease should be:

- 1) bacterial
- 2) viral and
- 3) parasitic

It is worth noting the apparent importance of vibriosis in this table. This really refers to a syndrome characterized by skin lesions and or tail rot. It is apparent that, while vibrio organisms are commonly isolated from disease outbreaks, they may often not be the primary cause. Other factors that have been suggested as primary or contributing causes include parasites, feed quality, water quality and overstocking. If, in fact, vibriosis is mainly a secondary or opportunistic pathogen, then the relative importance of bacterial diseases, compared with viral, parasitic or other causes (e.g. environmental or management) should be greatly diminished.

## Estimates of mortality for key diseases

During the survey, farmers were asked to estimate the proportion of fish that die due to particular disease syndromes. Table 4 shows country and combined estimates of mortality for four key disease syndromes.

There is a large amount of variation in the estimates of mortality, both between individual farms, and between country averages. However, as a broad estimate, these four key diseases result in an average of approximately 30% disease mortality. It should be noted that the high mortality recorded for VNN in Vietnam was due to the inclusion of a number of nursery farms in the survey.

**Table 4.** Mortality rate estimates in four key disease syndromes

<b>Disease</b>	<b>Country</b>	<b>Mortality</b>	<b>n</b>
<b>VNN</b>	Vietnam	70.7	6
	Korea	30.0	1
	Thailand	22.6	14
	Philippines	22.2	18
	<i>Weighted Average</i>	<i>30.0</i>	<i>39</i>
<b>Iridovirus</b>	Indonesia	80.0	1
	Thailand	26.3	12
	<i>Weighted Average</i>	<i>30.4</i>	<i>13</i>
<b>Vibriosis</b>	Indonesia	42.2	25
	Malaysia	38.2	17
	Vietnam	25.0	16
	Philippines	21.6	35
	Korea	4.0	2
	<i>Weighted Average</i>	<i>30.2</i>	<i>95</i>
<b>Flexibacteria</b>	Philippines	35.0	12
	Thailand	15.0	1
	<i>Weighted Average</i>	<i>33.5</i>	<i>13</i>

## Summary of mortality, morbidity, and economic loss

Table 5 shows estimates of morbidity, mortality and economic loss for different disease categories, as described above. Morbidity measures the proportion of fish that become affected (show clinical signs) due to the presence of disease. Mortality measures the proportion of fish that die. The term economic loss was used in this survey to describe a measure of the impact of disease on fish that have recovered from the disease. It is the decrease in market price for recovered fish, due to the longer term effects of disease. Equivalently, it may also be the increased costs of production resulting in longer production cycles to allow affected recovered fish to reach market size or condition.

The table indicates that viral diseases tend to affect a greater proportion of fish (higher morbidity) and result in more deaths (higher mortality) than bacterial, parasitic or other diseases.

The economic loss data may be somewhat misleading, as different definitions may have been used in different countries. Based on the definition provided above, bacterial diseases tend to result in greater residual damage and loss in value in surviving fish than other diseases, but VNN causes the highest estimated loss for an individual disease, however these conclusions should be treated with caution.

**Table 5.** Morbidity, mortality and economic loss due to different disease categories.

<b>Disease type</b>	<b>Average Morbidity</b>	<b>Average Mortality</b>	<b>Average Economic Loss</b>
VNN	31.9	30.0	18.9
<b>Other Viral</b>	52.2	46.0	6.9
<i>All Viral</i>	43.8	39.3	11.9
<b>Vibrio</b>	45.0	30.2	14.8
<b>Other Bacterial</b>	35.1	19.6	13.8
<i>All Bacterial</i>	40.9	26.5	14.4
<b>Parasitic</b>	19.2	30.5	5.7
<b>Other</b>	13.8	24.9	42.0

### Conclusion

A number of key conclusions may be drawn from the survey. On a regional basis, diseases are a very widely recognized problem amongst grouper farmers, with about 90% reporting the presence of disease. The range of diseases reported in each country was quite variable. This could be due to the different distribution of different diseases (due to the distribution in the causative agent, or differences in culture systems or environment). Alternatively, as the survey was based on farmer reporting and local expert interpretation of their descriptions, the difference in diseases could be due to differences in extension messages that have been provided to farmers, in the awareness or research focuses of local experts.

The survey methodology of attempting to assess causative agents based on farmers' descriptions of the disease is clearly prone to some misclassification error. However, it is felt that a combination of farmers' descriptions and the use of expert opinion are able to classify broad disease categories with reasonable reliability. More detailed diagnoses may be less reliable. Using this broad classification, bacterial diseases accounted for about 40% of reported problems, while viral diseases represented about 25%. It is likely that in a proportion of the diseases identified as involving bacteria, the bacteria were not the primary cause, but simply acting as opportunistic pathogens following some other insult, perhaps environmental. However, in the case of the viral diseases, the viruses were more likely to be critical in the causal pathway, although many other factors may have also been playing a role. This means the relative difference between viral and bacterial diseases may be less than reported, as a proportion of the bacterial diseases may be attributed to other causes.

The impact of diseases was assessed through morbidity, mortality, and the loss in value of recovered fish (economic loss). Reported mortality was remarkably consistent across all diseases, at about 30%. There is some evidence that viral diseases cause higher morbidity and mortality, than bacterial disease, but bacterial diseases result in a greater residual loss of value in recovered fish.

The overall conclusions of the survey at a regional level are that diseases are commonly recognized by farmers; viral and bacterial diseases (particularly VNN and Vibriosis) are amongst the most common diseases reported; and that the losses due to these diseases are significant.

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## Parasitic and Bacterial Diseases of Grouper and other Cultured Marine Finfishes and Control Strategies

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### Abstract

In Malaysia, mariculture of marine fishes expanded rapidly in the early 1980s with successful commercial hatchery production of sea bass, *Lates calcarifer*. In the early 1970s, only grouper, *Epinephelus coioides*, and golden snapper, *Lutjanus johni*, were cultured. At the present time, cultured species include other species, particularly those that are successfully hatchery produced in Chinese Taipei. Rapid expansion and concentration of fish farms, intensification using polyculture systems and the large-scale international movement of fingerlings or juveniles may have been causes of severe disease problems. These problems result to parasitic and bacterial infections. Bacterial infections are mainly caused by *Vibrio* spp. and *Flexibacter* sp., both of which are naturally present in the marine environment. Parasitic infections are caused by protozoans (particularly the ciliates - *Trichodina* spp. and *Cryptocaryon irritans*), monogeneans (mainly capsalids, diplectanids and dactylogyrids), and sanguinicolid blood flukes. The mortality rate of parasitic and bacterial diseases depends on a stage in the culture period as well as fish species involved. Once parasites are introduced into the culture system, it is very difficult to eliminate them. They would transmit successfully including even blood flukes that require a second intermediate host to complete the life cycle. Prophylactic, chemical and/or freshwater treatments helped reduce parasitic infection, but cannot completely eliminate them. The floating net cage system acts as a huge reservoir of pathogens. Vibriosis in grouper can be prevented by vaccination. Outbreak of vibriosis could be successfully treated by injection of affected fish with sulphate drug. The tail rot disease in sea bass and benedeniid monogenean infection in grouper can be controlled by freshwater dip treatment. To prevent, control and treat diseases in floating net cage systems, practical methods need to be developed. These methods would be part of an integrated health management system that includes a knowledgeable farm manager, application of prophylaxis, adequate and appropriate nutrition, sanitation, and immunization. In addition, market sized farmed fish need to be harvested as quickly as possible to reduce the number of reservoir pathogen hosts.

### Introduction

Mariculture of marine finfishes in floating net cages was successfully initiated in Japan during the 1950s and in Malaysia during the 1970s. In those years, the estuarine grouper, *Epinephelus coioides* and golden snapper, *Lutjanus johni*, which were wild-caught, were the main species cultured. Mariculture in floating net cages expanded rapidly during the early 1980s due to the success in hatchery production of sea bass fry in Thailand. Since then, many more species have been cultured, particularly those that have been successfully produced in hatcheries in Chinese Taipei. Because of the large number of fish species suitable for culture in warm tropical water and the increasing demand, polyculture is more widespread than the monoculture system. The common species of marine finfish cultured in floating net cages are listed in Table 1.

The mariculture industry in Southeast Asia experienced serious disease problems since the late 1980s. The types of diseases and mortality rates are greatly influenced by host fish, environmental conditions, stage of grow-out, husbandry management and technical knowledge of the fish farmers.

Almost all species of fish are affected by vibriosis, but groupers are more susceptible than other types of fish, and it can occur at any stage of the grow-out cycle. Epizootic diseases, which have been reported in cultured marine finfishes, are described in Table 2. The chronological occurrence of these epizootic diseases revealed that whenever a new species is introduced into the culture system, serious disease outbreaks could occur.

**Table 1.** Major marine finfishes cultured in floating net-cages in Malaysia.

Scientific Name	Common Name
<b>GROUPEL</b>	
<i>Epinephelus coioides</i>	Estuarine grouper
<i>E. malabaricus</i>	Black-spotted grouper
<i>E. bleekeri</i>	Yellow-spotted grouper
<i>E. fuscoguttatus</i>	Tiger grouper
<i>E. amblycephalus</i>	White-spotted green grouper
<i>E. chlorostigma</i>	Brown-spotted grouper
<i>E. lanceolatus</i>	
<i>Cromileptes altivelis</i>	Humpback grouper
<i>Plectropomus leopardus</i>	Blue-dotted coral trout
<b>SNAPPER</b>	
<i>Lutjanus johni</i>	Golden snapper
<i>L. argentimaculatus</i>	Mangrove snapper
<i>L. russelli</i>	Russel's snapper
<i>L. erythropterus</i>	Red snapper
<i>L. sebae</i>	Emperor red snapper
<i>Pinjalo-pinjalo</i>	Pinjalo snapper
<b>SEABASS</b>	
<i>Lates calcarifer</i>	Asian seabass
<i>Lateolabrax japonicus</i>	Japanese seabass
<b>JACKS</b>	
<i>Caranx ignobilis</i>	Giant trevally
<i>Trachinotus blochii</i>	Snub-nose pompano
<i>Carangoides sp.</i>	Trevally
<i>Gnathanodon speciosus</i>	Golden trevally
<b>MILKFISH</b>	
<i>Chanos chanos</i>	Milkfish
<b>MULLET</b>	
<i>Mugil cephalus</i>	Mullet
<b>GRUNT</b>	
<i>Pomadourys kaakan</i>	Silver grunter

**Table 2.** Summary of epizootic diseases among cultured marine finfishes in Malaysia.

Year	Host Species	Disease/Disease Syndrome Reported	Remarks
1973	<i>E. coioides</i>	Vibriosis	Start of mariculture in Malaysia
	<i>L. johni</i>	Monogenean infection	
1978-1979	<i>L. calcarifer</i>		Fry from local coastal areas
1982-1983	<i>L. calcarifer</i>		Imported fry
1985	<i>E. coioides</i>	Vibriosis	Very high mortality
		Swim bladder disease	
	<i>L. johni</i>	'Boldness' disease	
	<i>L. calcarifer</i>	Tail rot	Few infected
1988	<i>L. calcarifer</i>	Tail rot epidemic	Imported; mortalities as high as 90% occur frequently at every stocking of fry
1989	<i>L. argentimaculatus</i>		Fry imported
	<i>T. blochii</i>		Fry imported
1990-1991	<i>L. argentimaculatus</i>	Tail rot	Increasingly larger number of affected fishes
1990-1991	<i>E. coioides</i>	Sleepy grouper disease	Occurs 3 to 6 weeks after stocking 200 to 300 g fish affected; very smelly
	<i>L. calcarifer</i>	Scale drop disease	Occurs 5 to 7 months after stocking 150 g to 250 g fish affected; very smelly
1993-1994	<i>E. coioides</i>	'4 weeks grouper disease'	Large number of monogeneans Gastro-enteritis, very smelly, no other clinical signs
	<i>L. erythropterus</i>		Susceptible fish develop eye infection, eyes become enlarged followed by blindness
1996-1997	<i>L. argentimaculatus</i>	Ulcerative disease	Appearance of chronic ulcers; no response to treatment
1997-1998	<i>E. bleekeri</i>	Cluster red boil disease	Epizootic proportion; rapid mortality; responds to treatment at early stage of disease
1999-2000	<i>E. coioides</i>	Benedeniid infection	Very few die, freshwater treatment immediately at onset of disease
	<i>E. lanceolatus</i>	Benedeniid infection	Newly introduced species; very susceptible; high mortality
	<i>E. fuscoguttatus</i>	Lymphocystis	Tumour-like black masses of tissue, mainly on fins. Very few die, slow grow.
	<i>Cromileptes altivelis</i>		Growth rate slow

The culture of marine finfishes in floating net cages is intensive. Disease frequently occurs during initial stocking as well as the grow-out period. During the initial stocking period, diseases are caused mainly by stress arising from handling, transportation, acclimatization and adaptation to the new environment. Frequently, fry and juveniles were infected with the monogenean *Diplectanum* spp. and *Pseudorhabdosynochus* spp., the protozoan *Cryptocaryon irritans* and *Trichodina* spp. and concurrently with vibriosis (Chong and Choo 1986; Leong 1994; Leong and Wong 1986, 1990; Regidor and Arthur 1992; Wong and Leong 1986, 1990). Presence of several generations of cultured fish in the culture system creates a large reservoir for pathogens to infect any new fish introduced into the net cage system. The occurrence of diseases is the result of various factors, many of which are beyond the control of the fish farmers and some are inherent in the culture system.

### Bacterial Diseases

A great number of aquatic bacteria are opportunists and do not cause diseases under normal environmental conditions. However, under the stressful conditions of intensive fish farming, they become pathogenic. The same fish species kept in different net cages responded differently to the same environmental conditions (disease may occur in one cage, but not in another). However, once bacterial infection occurs in a net cage and no preventive treatment is undertaken, there is high probability of its spreading to the same fish species kept in other net cages at the same farm as well as to other fish farms in the same vicinity. Many clinical signs of bacterial diseases of cultured marine finfish are similar. Definitive diagnosis requires isolation, *in vitro* culture of the organisms involved and infectivity experiments. Due to difficulties in research of bacterial diseases, very few bacterial diseases of cultured marine finfish in Southeast Asia are reported (Tables 3 and 4).

**Table 3.** Bacterial flora from healthy and diseased cultured fishes (%).

Family	Health		Diseases	
	Seabass	Grouper	Snapper	Grouper
Vibrionacea	81.6%	81.2%	61.5%	74.0%
Pseudomonadocea	2.8%	0.9%	1.5%	2.0%
Enterobacteriaceae	1.9%	2.2%	7.7%	10.0%
Others	13.7%	15.7%	27.7%	14.0%
Types of Vibrios				
Group I	10.6%	16.3%	2.7%	21.6%
Group II	1.6%	4.1%	9.0%	27.0%
Group III	9.5%	6.2%	15.4%	0%
Group IV	37.0%	31.9%	38.5%	8.1%
Group V	41.3%	41.5%	43.4%	43.3%

Reference: Leong and Wong (1987)

**Table 4.** Important bacterial diseases among marine finfishes cultured in floating net-cages.

Diseases	Causative Agents	Species Affected
Vibriosis	<i>Vibrio alginolyticus</i>	All fish species
	<i>V. parahaemolyticus</i>	Groupers highly susceptible
	<i>Vibrio</i> spp.	
Crater-like boil disease	<i>Vibrio</i> / <i>Streptococcus</i> ?	Grouper
Bristle-like boil disease	Vibrios/Virus ?	Grouper
Sleepy grouper disease	Vibrios/Virus/Parasite	Grouper
Streptococcosis	<i>Streptococcus</i> spp.	Grouper
Tail rot disease	<i>Flexibacter</i> sp.	Asian seabass
Myxobacteriosis	<i>Flexibacter</i> sp.	Asian seabass
Ulcerative disease	<i>Vibrio</i> / <i>Aeromonas</i> spp.	Mangrove snapper

Vibriosis is the most significant, common and widespread bacterial disease, which affects all fish species cultured in floating net cages, regardless of fish age, and sex (Sano and Fukuda 1987; Leong 1992, 1996; Sako 1996; Arthur and Ogawa 1996; Shariff and Arulampalam 1996). This disease is characterized by haemorrhagic septicemia and two forms are recognized. The first form produces external haemorrhage referred to as the dermatitis form of vibriosis; the second form has no external symptoms, is less common, and referred to as gastro-enteritis vibriosis (Muroga *et al.* 1990; Egusa 1992). Groupers are the most susceptible marine finfish to vibriosis (unpublished data), which can occur throughout the grow-out cycle.

Leong and Wong (1993) first reported the “sleepy grouper syndrome”, which occurred in 1991 to 1992 in Malaysia. In this case, a high number of monogeneans as well as concurrent infection with gastro-enteritis vibriosis (virus not examined) were found. Diseases of groupers in Singapore and Indonesia were attributed to a virus infection, with vibriosis as secondary invaders, and no parasites were found (Chua *et al.* 1995; Arthur and Ogawa 1996). The most common pathogenic species of vibrios recovered from diseased fish were *Vibrio parahaemolyticus* and *V. alginolyticus*. Groupers suffering from vibriosis were successfully treated with sulphate drug.

Frequently, newly stocked juvenile groupers died within the first week, with symptoms of crater-like red boil like lesions on the body surface. Affected grouper had systemic infection with *Vibrio* spp., which could be the main pathogen. The clear-cut pin sized ulcers on the epidermis were unusual, suggesting that other organism(s) maybe involved.

In 1998 to 1999, a new disease symptom was observed in red grouper, *Epinephelus bleekeri*. Affected fish had clusters of bristle red boils on the body surface and very often enlarged, opaque eyes during advanced stage of the disease. Fish were not examined for virus, however, systemic vibrios were found in affected fish. Groupers with bristle boils had been reported to be infected with virus (Khongpradit *et al.* 1997). Once all fish in the net-cage were treated with sulphate drug, the disease ceased.

Fish with a single red boil on the body surface were also commonly observed. Examination of these red boils showed the presence of *Streptococcus* sp. which is not a serious disease.

The other group of bacteria, which causes serious chronic disease among cultured marine finfishes, is the gliding bacteria. Asian sea bass are highly susceptible to this bacterium. Two types of syndromes were observed in affected sea bass; the type depended on the size of fish involved. Sea bass fry and juveniles less than four inches were highly susceptible to this bacterium. At this size, the bacterium could destroy the caudal fin and invade the adjacent musculature resulting to eventual disintegration of the muscle fibers. This disease is referred to as tail rot disease and is caused by the bacterium, *Flexibacter* sp. (Pergmark 1992). Very few numbers of other fish species are affected by this bacterium. Dipping affected fish in freshwater for 10 to 15 min can control this disease.

Sea bass could overcome this bacterial infection, if there were enough fish left after the initial infection. On reaching 150 g to 200 g, sea bass would again be infected by the bacterium, *Flexibacter* sp. At this size, the proteolytic enzymes produced by the bacterium did not destroy the muscle fibers of affected fish. Instead, the scales of affected fish would gradually drop off from the fish or easily come off when touched. Histological examination of affected fish showed that the epidermis separated from the underlying dermis (Win 1999). This disease occurs throughout the year with the peak occurrence from November to March (coinciding with the beginning of the cold months of October and November each year). This is a chronic disease, which can cause substantial financial losses. There is no known treatment for this infection.

The mangrove snapper was introduced for culture in the floating net-cages in 1989 to 1990. In 1991, large numbers of mangrove snapper were imported for culture. The snappers were to replace sea bass lost due to epizootics of tail rot and scale drop diseases. They were selected due to strong resistance of this species to diseases. In 1996 to 1997, large size mangrove snappers over 1 kg developed ulcers on the muscle, the jawbone, and manifested reddish swellings at the pectoral fin. Affected fish continued to consume feed but continuously lost weight; its eyes became opaque; affected fish swam sluggishly on the surface and eventually died. Presently, this disease affects all sizes of the mangrove snapper. Systemic vibrios were found in the affected fish, as well as other bacteria isolated around the ulcer area. The disease has not responded to treatments such as oxytetracycline and sulphate drug.

#### *Parasitic Infection*

A variety of parasites infect marine finfishes. Some parasites may be transmitted from wild fishes within the vicinity of the cages, but the majority is introduced into the cage environment along with hosts. Most of the parasites are normally not pathogenic and do not cause diseases. When large numbers are present in the fish host, they can cause diseases and/or can become a major contributing factor to disease development. Quantitative data on parasites are scanty and additional research needs to be done to determine the role of parasites in causing diseases in the cage environment. Based on publications, three groups seem to cause diseases among cultured fishes. The dominant parasites infecting cultured marine fish at various locations of the same system of culture (pond or net cage) are similar (Chong and Choo 1986; Leong 1994; Leong and Wong 1987, 1990; Ruangpan 1985).

Protozoans are a large heterogeneous group of organisms, some of which are ectoparasites while others are endoparasites. Many of these organisms are not specific in their host preferences and can cause severe damage to any marine fish in intensive culture systems. Protozoans have been reported to be pathogenic to grouper, sea bass and snapper fry

and fingerlings at the nursery phase or grow-out phase during the first week after stocking in the cages (Chong and Choo 1986; Leong 1994; Leong and Wong 1987, 1988, 1990). The ciliated protozoans, *Cryptocaryon irritans* and *Trichodina* spp., are very pathogenic to newly introduced fish fry and juvenile in the cage environment. These protozoans were introduced into the cage environment through fingerlings brought in for stocking in the cages (Leong and Wong 1986, 1990).

Cultured marine finfishes in Malaysia are infected with a variety of capsalid, dactylogyrid and diplectanid monogeneans, which are listed in Table 5 (Leong and Wong 1986, 1987, 1988, 1990a, b, 1992a, b, 1995, Hla Bu *et al.* 1998). All fishes examined for parasites were infected with one or more species of monogenean. Capsalid monogeneans are not fish host specific and infect all cultured fish species, whereas dactylogyrid monogeneans are confined to species of snapper fish. Capsalid monogeneans are found under the scales. Dactylogyrid and diplectanid monogeneans are found on the gills. The capsalids are pathogenic to grouper, and grouper mortality has been attributed to these monogeneans (Ogawa *et al.* 1995a, b; unpublished data). Groupers infected with the monogenean have no symptoms except cessation of feeding. The body of affected fish turns dark and dies within a few days after the onset of these symptoms. Different species of groupers have different levels of susceptibility to capsalid monogeneans, with the estuarine grouper, *Epinephelus coioides*, being the most susceptible. The species affected with the ‘sleepy grouper syndrome’ were also infected with a large number of monogeneans. The newly introduced species, *E. lanceolatus*, were highly susceptible to capsalid monogenean infection (unpublished data). Frequently, diseased fish had more dactylogyrid and diplectanid monogeneans than healthy or wild fish, and their mortality was attributed to secondary bacterial infection (Leong and Wong 1987, 1988, 1989, 1990a). The role of these parasites (particularly when they are present in large numbers) in disease development is unclear.

**Table 5.** Monogeneans found among commonly cultured marine finfishes.

	<b>Grouper</b>	<b>Seabass</b>	<b>Snapper</b>
	<i>Epinephelus</i> spp.	<i>Lates calcalifer</i>	<i>Lutjanus</i> spp.
<b>CAPSALID</b>			
<i>Benedenia epinepheli</i>	+	+	+
<i>B. lutjani</i>	+	+	+
<i>Benedenia</i> spp.	+	+	+
<i>Megalocotyloides epinepheli</i>	+	-	-
<i>M. convoluta</i>	+	-	-
<i>Neobenedeniagirellae</i>	+	+	+
<i>Neobenedenia</i> spp.	+	+	+
<b>DIPLECTANID</b>			
<i>Pseudorhabdosynochus epinepheli</i>	+	+	-
<i>P. lanteuensis</i>	+	-	-
<i>P. latis</i>	-	+	-
<i>P. monosquamodisci</i>	-	+	-
<i>P. coioidesis</i>	+	-	-
<i>Diplectanum penangi</i>	-	+	-
<i>D. grouperi</i>	+	-	-
<b>DACTYLOGYRID</b>			
<i>Haliotrema johni</i>	-	-	+
<i>H. noncalcaris</i>	-	-	+
<i>Haliotrema</i> spp.	-	-	+

Parasitic trematodes normally do not cause diseases in cultured marine finfishes, even though they infect a large numbers of fish species. The sanguinicolid blood flukes do cause mortality among cultured marine finfishes (Ogawa and Egusa 1986; Ogawa and Fukudome 1994). Blood flukes of the genera *Curilocola*, *Pearsonellum* and *Cardicola* were found among cultured grouper, seabass and snapper, but no mortalities have been attributed to them (Herbert *et al.* 1994; Leong and Wong 1986, 1990b). Herbert *et al.* (1994) reported a 100% infection rate of the blood fluke *Curilocola latis* in a sample of sea bass. Fishes cultured in floating net-cages have been observed dying and floating upside down at the surface with rapid movements of the operculum without any symptoms of disease. Blood flukes were the probable cause of the mortalities.

#### *Prevention and Control Strategies*

Diseases caused by parasites and bacteria in intensive fish culture have become a serious problem. Prevention, treatment and control, as well as proper health management of cultured marine finfishes in floating net-cage system are needed to ensure that this form of mariculture remains economically viable.

Disease factors are inherent in the operational activities of the floating net-cage culture system, as well as the quality and conditions of the fish fry and juveniles on arrival at fish farms. The first one to four weeks is the most crucial period that will determine successful survival of a particular batch of fish in the new culture environment. This first four-week period can be referred to as the adaptive phase of the culture cycle. Most fish farmers do not give prophylactic treatment and even when it is given, it does not guarantee a high survival rate. Chong and Choo (1984) reported survival of only about 30% of groupers provided with prophylactic treatments during pre-shipment, trans-shipment and post-shipment. The floating net cages act as a reservoir of pathogens. Prophylactic treatments may fail to reduce the severity of disease, as the fish may have already been weakened due to prolonged periods of handling and poor water quality during transportation. Fish in the culture system have a large number of various parasites. Often diseased cultured fish are found having even larger numbers of parasites (Leong and Wong 1987,1988,1990a).

The quality and condition of fry on arrival at the fish farms are extremely important. Fry of mangrove snapper, red snapper and emperor red snapper that are hatchery produced tend to have better chances of overcoming diseases upon arrival at the fish farms. Among the hatchery produced fish fry, sea bass fry were found to be highly susceptible to diseases. The low survival could be due to the inherent operational problem as sea bass fry are cultured in the low salinity environment and placed immediately into the high salinity environment at the culture site. The conditions of packaging are also critical to the survival of newly arrived fish. It can be concluded that good health and reduced stress factors during transportation are important considerations that determine the fish susceptibility to diseases on arrival. Field observations of wild caught fish (grouper and golden snapper) showed high mortality within the first week of stocking in the cage.

In order to prevent and control diseases, a pathogen(s) involved must be identified. For instance, in newly introduced groupers affected by vibriosis, high number of monogeneans, resulted in diseases (4-week disease and sleepy grouper disease) during the period from a few days to six to eight weeks. The characteristics of the diseases were systemic *Vibrio* spp. infection as well as presence of a large number of monogeneans. The population of monogeneans increased very rapidly within the first four weeks, followed by outbreak of vibriosis. Groupers that survived this period had fewer monogeneans, which suggests that the surviving fish develops immunity to both *Vibrio* spp. and monogenean infection. Experimental treatments (Liang and Leong, 1992) of monogeneans indicated that the monogeneans on the gills cannot be easily removed, but monogeneans on the body surface or under the scales can (unpublished data). The monogeneans found under the scales are benedenid monogeneans, which are known to be pathogenic to cultured marine fish. If groupers could be immunized against *Vibrio* spp. infection, diseases observed in grouper could be overcome. Experimental studies (Leong *et al.* 1997) indicated that vaccinated grouper have high survival rates, and no serious diseases occurred within the six to eight week period. Even if vibriosis did occur, haemorrhages on the body surface were not severe and fish easily responded to treatment. The most important strategy in the control of diseases is to know the pathogen(s) that caused the disease and other factors that may contribute to the onset of the disease. To implement this strategy, there must be at least one competent, knowledgeable farm manager at the fish farm.

We will see more diseases with the introduction of new species for culture. Some of these species are very susceptible to the pathogens already present in the floating net-cage environment.

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## Recent Developments in Identification and Control of Viral Nervous Necrosis (VNN) of Grouper

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### Abstract

We recently demonstrated that the SSN-1 cell line is highly permissive for all genetic variants of piscine nodaviruses (the Betanodavirus), the causal agent of viral nervous necrosis (VNN). A clonal cell line (E-11) derived from SSN-1 cells was also useful for both qualitative and quantitative analyses due to its stable, clear CPE expression and high productivity of infectious virus particles. In addition, a combination of the culture system and RT-PCR proved useful as a rapid and sensitive method for detection of piscine nodaviruses. This technique will be of use to determine the transmission mode of the virus in groupers. Immunization of seven band grouper *Epinephelus septemfasciatus* with the *Escherichia coli*-expressed recombinant coat protein also induced neutralizing antibodies, and immunized fish showed significantly high protection against experimental virus challenge. This suggests the potential for vaccination against VNN in groupers, which are susceptible to piscine nodaviruses at young to adult stages.

### Introduction

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by piscine nodaviruses have been reported in a variety of marine fish in different countries during this decade (Munday and Nakai 1997, Office International des Epizooties 1997). The disease was first described in Japanese parrotfish *Oplegnathus fasciatus* in Japan (Yoshikoshi and Inoue 1990) and barramundi *Lates calcarifer* in Australia (Glazebrook *et al.* 1990), and thereafter has been reported in the Indo-Pacific region, Mediterranean, Scandinavia and North America. At present, the reported host fish species number more than 19 in 10 families, including 7 species of grouper: *Epinephelus akaara*, *E. moara*, *E. septemfasciatus*, *E. malabaricus*, *E. tauvina*, *E. fuscogutatus*, and *Cromileptes altivelis* (Table 1). The disease usually occurs among larvae and/or juveniles resulting in high mortality rates, but infection at grow-out stages often occur in some species of grouper and European sea bass *Dicentrarchus labrax* (Fukuda *et al.* 1996, Le Breton *et al.* 1997, Tanaka *et al.* 1998).

### Piscine Nodavirus

Nodavirus is a non-enveloped, icosahedral particle. It has a bipartite genome of positive-sense RNAs, RNA1 encoding RNA-dependent RNA polymerase and RNA2 encoding coat protein, both of which are capped but not polyadenylated. During RNA replication, a subgenomic RNA3, which is co-terminal with RNA1 and encodes small proteins, is synthesized. The family Nodaviridae consists of two genera: the Alphanodavirus, which primarily infects insects and the Betanodavirus, which infects fish (Van Regenmortel *et al.* 2000). Piscine nodaviruses (betanodaviruses) can be divided into four genotypes based on partial sequences of the coat protein gene: SJNNV (striped jack nervous necrosis virus), RGNNV (redspotted grouper nervous necrosis virus), TPNNV (tiger puffer nervous necrosis virus), and BFNNV (barfin flounder nervous necrosis virus) genotypes (Nishizawa *et al.* 1997). All nodavirus isolates from diseased groupers belong to RGNNV genotype.

**Table 1.** Grouper species affected by viral nervous necrosis (VNN).

Fish species	Location	References
Redspotted grouper <i>Epinephelus akaara</i>	Japan, Taiwan	Mori <i>et al.</i> (1991), Chi <i>et al.</i> (1997)
Kelp grouper <i>E. moara</i>	Japan	Nakai <i>et al.</i> (1994)
Sevenband grouper <i>E. septemfasciatus</i>	Japan, Korea	Fukuda <i>et al.</i> (1996) Sohn and Park (1998)
Brownspotted grouper <i>E. malabaricus</i>	Thailand	Danayadol <i>et al.</i> (1995)
Greasy grouper <i>E. tauvina</i>	Singapore	Chua <i>et al.</i> (1995)
Carpet cod <i>E. fuscogutatus</i>	Taiwan	Chi <i>et al.</i> (1997)
Humpback grouper <i>Cromileptes altivelis</i>	Indonesia	Zafran <i>et al.</i> (2000)

### Cell culture for piscine nodaviruses

Several techniques to detect piscine nodaviruses from diseased fish have been developed. They include enzyme-linked immunosorbent assay (ELISA), fluorescent antibody technique (FAT), reverse transcription-polymerase chain reaction (RT-PCR), and *in situ* hybridization (Arimoto *et al.* 1992, Nguyen *et al.* 1994, Nishizawa *et al.* 1994, Comps *et al.* 1996). Although RT-PCR and other methods are suitable for detecting the virus from diseased fish, they have relatively lower sensitivities and, therefore, are not effective in investigating the epidemiology of the disease or pathogenicity of the virus.

Early studies on isolation of piscine nodaviruses using established fish cell lines such as RTG-2, CHSE-214, FHM, EPC, and BF-2 reported unsuccessful results (Breuil *et al.* 1991, Mori *et al.* 1991, Munday *et al.* 1992, Nguyen *et al.* 1994, Grotmol *et al.* 1995). That is why fewer studies on the betanodaviruses RNA replication, gene expression and virion assembly have been performed in comparison with well-researched alphanodaviruses (Ball and Johnson 1998). The first successful isolation of a piscine Nodavirus was made from diseased European sea bass using the SSN-1 cell line, that had been established from whole fry tissue of striped snakehead *Ophicephalus striatus* (Frerichs *et al.* 1996). Subsequently, Chi *et al.* (1999) reported that a new cell line (GF-1) derived from grouper *E. coioides* is useful for the isolation and proliferation of a piscine Nodavirus (GNNV: grouper nervous necrosis virus).

We recently demonstrated that the SSN-1 cell line is highly permissive for all examined genetic variants of piscine nodaviruses (Iwamoto *et al.* 1999). However, the SSN-1 cell line has practical disadvantage. This cell line is composed of a mixed population of cells, causing inconsistencies in the cytopathic effects (CPE). This problem was resolved by cloning the SSN-1 cells (Iwamoto *et al.* 2000). Six cell clones were derived from the SSN-1 cell line; all were susceptible to four piscine nodavirus strains belonging to different genotypes (SJNNV, RGNNV, TPNNV, and BFNNV). These cell clones (especially a clone designated E-11) were useful for qualitative and quantitative analyses due to their stable, clear CPE expression and high productivity (up to  $10^{10}$  TCID<sub>50</sub>/ml) of infectious virus particles. Virus titration using the E-11 cell line clearly revealed differences in the optimal growth temperature among the four genotypes: 25 to 30°C for RGNNV, 20 to 25°C for SJNNV, 20°C for TPNNV, and 15 to 20°C for BFNNV.

Although the E-11 cells are highly permissive to all genotypic variants of piscine nodaviruses, it takes long time (up to 10 days) to detect the virus at lower number based on the CPE. In contrast, the RT-PCR is generally a rapid and convenient method to examine a large number of samples but its sensitivity seems to be lower. Accordingly, we developed a procedure by combining advantages of both methods, *i.e.* high permissivity of E-11 cells and rapidity of RT-PCR. As a result, 24 h-cultivation in the E-11 cells prior to RT-PCR was very effective to detect the virus at the lowest titer (Iwamoto *et al.*, unpublished data). This preculture in cells and RT-PCR will be useful as a rapid and sensitive method for detection of piscine nodaviruses, particularly from asymptomatic carriers.

### Control measures for VNN

In VNN of striped jack *Pseudocaranx dentex*, the virus carrying broodstock were the most important inoculums source of the virus to their larvae (Arimoto *et al.* 1992, Mushiake *et al.* 1994). This finding led to the successful control of VNN of larval striped jack, where elimination of virus-carrying brood stock by RT-PCR and disinfection of fertilized eggs by ozone were applied (Mori *et al.* 1998). However, it is still unclear in VNN of other fish species whether this vertical route is the major transmission mode of the virus in other species, though piscine nodaviruses

have been also detected in the gonadal materials of brood stock from kelp grouper *E. moara* or European sea bass (Nakai *et al.* 1994, Comps *et al.* 1996).

Strict hygiene within hatcheries assisted in the control of VNN. Anderson *et al.* (1993) reported that non-recycling of water; chemical sterilization of influent seawater and disinfection of half of the tanks during each hatching cycle was successful in a barramundi hatchery. Arimoto *et al.* (1996) recommended the following measures: (a) disinfection of eggs (iodine or ozone) and materials (chlorine); (b) rearing of each batch of larvae/juveniles in separate tanks supplied with sterilized (UV or ozone) seawater; and (c) rigorous separation of larval and juvenile striped jack from brood fish.

Recently, we detected Nodavirus-neutralizing antibodies in the serum of seven band grouper *E. septemfasciatus* that survived intramuscular injection with the virus. This indicates establishment of acquired immunity among survivors and may explain why survivors from natural infection are resistant to recurrence of the disease. Immunization with the *Escherichia coli*-expressed recombinant coat protein prepared using a RGNNV genotype strain also induced neutralizing antibodies for at least 110 days. Immunized fish showed significantly higher resistance to the experimental virus challenge (Tanaka *et al.* 2001). Vaccination with this recombinant coat protein also proved effective against experimental challenge in VNN of humpback grouper *Cromileptes altivelis* (Dr. K. Yuasa, JICA, pers. com.). These results suggest the potential for the VNN vaccination of groupers, which are susceptible to piscine Nodavirus from young until adult stages. Further studies, including cross protection experiments with recombinant coat protein from a variety of piscine nodaviruses, will be required for the VNN vaccine development.

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## Present Situation of Occurrence of Viral Nervous Necrosis (VNN) in Indonesian Grouper Hatcheries and Control Measures for VNN

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### Abstract

In 1997 and 1998, several national and private hatcheries in Indonesia including the Gondol Research Station for Coastal Fisheries (GRSCF) succeeded in the production of thousands of seeds of humpback grouper, *Cromileptes altivelis*, the species which has the highest economic value among marine finfish. However, the amount of production at each hatchery has been gradually affected by mass mortalities during larval and juvenile stages. This paper describes the present situation of grouper seed production in Indonesia and the impacts of viral nervous necrosis (VNN) occurrences and control measures for VNN at the GRSCF.

By the end of 1998, our project 'Research and Development for Multi-Species Hatchery Project' investigated the cause of the mass mortalities by histopathological and PCR techniques, and revealed that the cause in all cases was Nodavirus infection. To control the mortality due to the viral infection, the GRSCF attempted several counter measures as follows : selection by Polymerase Chain Reaction (PCR) of VNN-negative brood stock or the virus-negative larvae; disinfections of tanks and equipment for seed production; removal of small or weakened or dead fish from rearing tanks; increasing the amount of intake water and administration of antibiotic. All of these measures did not completely eliminate the infection but significantly decreased the mortality. As a result of the application of these countermeasures, the Station successfully produced the seeds during the following years.

### Introduction

A project 'Research and Development for Multi-Species Hatchery Project' started in 1994 at the Gondol Research Station for Coastal Fisheries (GRSCF), a national research institute located in Bali, Indonesia in cooperation with the Japan International Cooperation Agency (JICA). The initial aim of the project was to develop technologies for seed production of several kinds of marine finfish. At present, the project is involved in the seed production of groupers, particularly humpback grouper (*Cromileptes altivelis*) because of their high economic value. Since 1998, the project has successfully produced several thousands of humpback grouper seeds. However, mass mortalities have often occurred after several successful productions, which completely interrupted activities. Investigation on the cause of mass mortalities was initiated. Using the Polymerase Chain Reaction or PCR technique introduced by JICA. It appeared that viral nervous necrosis (VNN) due to a Nodavirus has been the main cause of the mortalities.

VNN, the most dangerous disease among larval and juvenile marine finfish at present, was first reported in Japanese parrotfish (*Oplegnathus fasciatus*) in Japan as a viral infection caused by Nodavirus (Yoshikoshi and Inoue 1990). Since then, VNN and VNN-similar diseases known as fish encephalitis (Breuil *et al.* 1991) or viral encephalopathy and retinopathy or VER (Munday *et al.* 1992) have been reported throughout the world, causing severe losses in

hatcheries of marine finfish. The causative agent of the disease has been recognized as a piscine Nodavirus that includes several genomic types (Nishizawa *et al.* 1997). The range of fish species susceptible to the virus is very wide, including more than 19 species in 10 families (Munday and Nakai 1997).

In Indonesia, VNN infection was initially reported in barramundi (*Lates calcarifer*) in a hatchery located in East Java in 1997 (Zafran *et al.* 1998). It spread to private hatcheries of barramundi in Bali Island in 1998. In the same year (1998), several private hatcheries and national institutes succeeded in the seed production of humpback grouper. However, mass mortality of 100% occurred among larvae and juveniles of humpback grouper at a private hatchery in Bali Island in November 1998. The Nodavirus could be detected from moribund fish by PCR (Zafran *et al.* 2000). At present, with the exception of the operation at GRSCF, all national institutes and private hatcheries involved in seed production of groupers have encountered severe mortalities among larvae and juveniles due to Nodavirus infection. This resulted in failures in mass production of seeds. This paper describes the present situation of grouper seed production in Indonesia and the impacts of VNN occurrences and control measures for VNN at the GRSCF.

### General infectious diseases of groupers in Indonesia

Grouper diseases are categorized according to their occurrence at various stages of culture, namely: brood stock, larvae and juveniles and grow-out fish (see Table 1). They are described below.

**Table 1.** Main diseases of grouper broodstock in Indonesia.

Species	Grow-out	Brood stock	Larvae/juvenile
Humpback grouper <i>Cromileptes altivelis</i>	VNN Parasites (capsalid monogenean)	Parasites (e.g. <i>Cryptocaryon</i> , <i>Trichodina</i> , <i>Caligus</i> , capsalid monogenean, gill trematodes, nematodes, cestodes)	VNN
Coral trout <i>Plectropomus leopardus</i>	Bacterial gill disease (gill rot)	Parasites (capsalid monogeneans, nematodes, <i>Lepheophtheirus</i> ,	not produced
Orange-spotted grouper <i>Epinephelus coioides</i>	Vibriosis Iridovirus	Parasites (capsalid Monogeneans, gill trematodes, <i>Lepheophtheirus</i> )	VNN
Tiger grouper <i>E. fuscoguttatus</i>	Vibriosis Parasite (capsalid monogenean)	Parasite (capsalid monogeneans, gill trematodes, <i>Lepheophtheirus</i> )	VNN
Marbled grouper <i>E. polyphkadion</i>	not conducted	Parasites (capsalid monogeneans)	VNN
Brown-spotted grouper <i>E. malabaricus</i>	not conducted	Parasites (capsalid monogeneans, <i>Lepheophtheirus</i> )	not produced

*a. Broodstock* : Diseases of grouper brood stock were investigated at the GRSCF since 1997. The brood stocks included six species: humpback grouper (*Cromileptes altivelis*), orange-spotted grouper (*Epinephelus coioides*), tiger grouper (*E. fuscoguttatus*), marbled grouper (*E. polyphkadion*), brown-spotted grouper (*E. malabaricus*) and coral trout (*Plectropomus leopardus*). The most widespread disease in each species was infection by ectoparasites including *Cryptocaryon irritans*, *Trichodina* sp., *Benedenia* spp., *Neobenedenia girellae*, *Haliotrema* sp., *Pseudorhabdosynochus* sp., *Caligus* sp., and *Lepheophtheirus* sp. Among them, *Cryptocaryon* infection showed the highest pathogenicity, but *Benedenia* or *Neobenedenia* was the most frequently observed parasite (Koesharyani *et al.* 1999). Humpback grouper and coral trout were also infected by internal parasites such as nematodes and cestodes, but their pathogenicities have not been confirmed. Bacterial infections were sometimes encountered in some species and were secondarily observed with parasitic infection. Mortalities due to viral infection have not been encountered among brood stock.

*b. Larvae and juveniles* : Until recently, the GRSCF succeeded in producing juveniles of humpback grouper, marbled grouper, orange-spotted grouper and tiger grouper. However, all of four species were affected by VNN resulting in mass mortalities. The other two species, coral trout and brown-spotted grouper will possibly be affected by the virus if they are produced in the future. Other pathogens have not caused mass mortalities during the seed production. VNN is the major disease problem in the seed production of groupers.

*c. Grow-out fish:* Disease outbreaks in grouper cage culture farms around Indonesia were investigated from 1998 to 2000. The most popular disease was vibriosis due to several kinds of *Vibrio* spp. Vibriosis in orange-spotted grouper was characterized by ulcers on the body surface, while fingerlings of tiger grouper showed swellings with hemorrhages on the body surface. The isolated *Vibrio* sp. showed yellowish colonies on TCBS agar, but detailed identification was not conducted. In coral trout, gill rot was the most frequently observed clinical sign and caused high mortalities. Bacterial isolation from gills failed, but histopathologically, numerous long rod-shape bacteria in gills of affected fish were detected, which may possibly belong to gliding bacteria. Recently, iridovirus infection was found in orange spotted grouper reared in pen cages in West Sumatra, where cumulative mortalities reached 80 to 90 %. Iridovirus infection appears to be another potentially significant disease for grouper cage culture in the future. Parasitic infection with capsalid monogeneans and gill trematodes were also commonly observed among cage-cultured fish, but proper management can solve such problems.

### General information about VNN in groupers

#### *a. Susceptible species and age/stage*

The cause of mortalities at grouper hatcheries and net cages in Indonesia during disease outbreaks in 1999 and 2000 were investigated by PCR. Results of PCR examination showed that all of the species were affected by the Nodavirus (Table 2). The susceptible ages among humpback grouper were between 10 day-old and 4 month-old, where mass mortalities frequently occurred particularly from 20 day-old to 30 day-old. Mortalities rapidly decreased after 3 months, and the virus could be rarely detected from 1 year-old moribund fish. In the other grouper cases, the most susceptible ages were between 20 and 30 day-old fish (similar to the case for humpback grouper).

#### *b. Clinical signs of affected fish*

Clinical signs of affected fish differ among the ages of affected fish (Table 2). Prior to the 20<sup>th</sup> day, affected fish did not show any symptoms except for inappetence (as indicated by the number of remaining rotifers in the rearing water). From 20 to 45 days, affected fish showed weakened swimming near the water surface. At the same time, dead fish were observed at the bottom. From 45 days to 4 months of age, during nursery stage and early stages of cage culture, affected fish rested at the bottom for a few days, which was followed by mortality. After 4 months, affected fish, with expanded swim bladder, floated near the water surface.

**Table 2.** Stages susceptible to Nodavirus and clinical signs of VNN in humpback grouper.

Fish stage	Susceptibility to virus*	Clinical signs
Larvae (10 to 20 day old)	++	Inappetence
Post-larvae (21 to 30 day old)	++	Weakened swimming
Juvenile/fingerling (31 to 120 day old)	++	Resting at bottom
Grow-out (121 days)	+	Floating near water surface
Brood stock	-	-

\*

-:No mortality due to the virus infection was observed

+:Mortalities due to the virus infection were sometimes observed possibly after fish was stressed by environmental factors.

++:Mass mortalities due to the virus infection were frequently observed.

#### *c. Histopathological features of affected fish*

Histopathologically, vacuolation and degeneration of nerve cells in the brain and retinas were observed among moribund larvae and juveniles. Recovery from viral infection was also observed, where 5 month old or older fish did not show these histopathological features in spite of being PCR-positive for the virus.



## 2. History of humpback grouper seed production in each hatchery

Field surveys on the seed production of humpback grouper were conducted at four national hatcheries in Indonesia (Table 3).

**Table 3.** Chronology of humpback grouper seed productions and VNN occurrences at four national hatcheries in Indonesia.

Hatchery	Seed Production Status	
	Month/Year	Production (# of juveniles)/Observations
Gondol Research Station for Coastal Fisheries	January to February 1998	2 000
	May to July 1998	5 000
	August to October 1998	3 000
	November 1998 to March 1999	None; unknown mortalities
	April 1999	Follow-up project commenced
	June to August 1999	All larvae dead before reaching one month of age; Nodavirus detected by PCR; larval rearing temporarily terminated; all equipment and tanks for larval rearing disinfected by chlorine; VNN-negative fish selected as spawners by PCR
	October to December 1999	82 000
	Jan to March 2000	All larvae were dead before 24 days; virus detected by PCR
	April to May 2000	Larval rearing was temporarily halted to disinfect equipment and tanks for 2 months
	June to July 2000	30 000
East Java Hatchery	August to October 2000	10 000
	January to June 1999	2 000 to 10 000/ month
	July to November 1999	Hundreds of juveniles; virus detected from moribund fish by PCR
	November to December 1999	2 000
East Sumatra Hatchery	January to September 2000	Hundreds of juveniles
	October to December 1999	2 000
	January to February 2000	20 000; virus detected from moribund fish
Batam Hatchery	March to September 2000	Hundred of juveniles
	April to June 2000	1 000
	May to August 2000	All larvae dead before 15 days

*Gondol Research Station for Coastal Fisheries (GRSCF)*

The first successful seed production at the GRSCF was achieved with the production of 2 000 juveniles in 1998. Subsequently, 5 000 and 3 000 juveniles were produced in July and October 1998, respectively. However, succeeding production continuously failed until March 1999. In April 1999, the 'follow-up project' started where several new techniques for seed production were introduced. This resulted in a rapid increase in the initial survival rate of the larvae (unpublished data). However, no seeds could be produced due to mass mortalities at post-larval stages from June to August 1999. Examination of moribund or dead larvae by PCR in all trials of the production showed positive results for VNN. Based on these results, the GRSCF concentrated its activities on eradication of VNN through selection of VNN-negative brood stock and complete disinfections of tanks and equipments used for seed production (to prevent vertical and horizontal transmission). Additionally, seed production in September was terminated to complete the disinfections process. As an outcome of the above measures, 70 000 and 12 000 juveniles were successfully produced in November and December 1999, respectively. Subsequent production in January, February and March 2 000 failed due to VNN occurrences, however, about 30,000 in July and 10,000 juveniles in October were successfully produced.

*Hatchery in East Java*

This Station has a great potential to produce several hundreds of humpback grouper seeds since 1997, and the number of production gradually increased. About 2 000 to 10 000 juveniles were produced monthly from January to June 1999. However, mass mortalities occurred in June 1999. PCR examinations conducted at the GRSCF revealed that 20 day to 4 month-old fish sampled were infected by the Nodavirus. This station could produce only hundreds of juveniles for each of the following months except for 2 000 juveniles produced in December 2000.

*Hatchery in West Sumatra*

This hatchery produced 2 000 juveniles for the first time in December 1999, and followed by about 20 000 juveniles produced in February 2000. However, mass mortalities occurred in the post juvenile stages of the second production. Results of PCR tests conducted at the GRSCF revealed that the cause of mass mortalities was the Nodavirus. Since then, the numbers of seeds produced monthly were reduced to only a few hundreds.

*Hatchery in Batam Island*

This hatchery attempted humpback grouper seed production for the first time in April 2000, resulting in a successful production of about a thousand juveniles. However, subsequent productions failed until August 2000.

**3. Present control measures of VNN at the Gondol Station**

The project applied the following six procedures as counter measures for VNN.

*a. Selection of VNN-negative brood stock by PCR*

All humpback grouper brood stocks (VNN-negative and VNN-positive) were separated in July 1999 when VNN spread in the Station hatchery. Gonads were sampled by canulation, and sperms were sampled using 1 ml syringe. Samples were put into 1.5 ml micro tubes and immediately used for PCR examination. Viral RNA was extracted and amplified as described by Mushiake *et al.* (1994). The target sequence was a 426-bp sequence referred to as T4 (Nishizawa *et al.* 1994). As a result, the viral nucleic acid was detected from 5 females in 21 females examined, but not from 8 males (Table 4). Brood stocks, which were PCR-negative for VNN were used during the next seed production, but spawning was delayed one month because of the effects of canulation. PCR-positive brood stocks were quarantined in a separate tank, and then used for the PCR examination in August. Interestingly, all quarantined fish turned to be VNN-negative in August and October.

**Table 4.** Number of Nodavirus carrier brood stock reared at the Gondol Station.

Sex	Number of VNN positive fish
Male	0/8 (0%)
Female	5/21 (24%)
<b>Total</b>	<b>5/29 (17%)</b>

*b. Disinfection of tanks and equipment for seed production*

All facilities and equipment were disinfected using 100 ppm chlorine for 1 to 4 weeks. The concentration of chlorine used was twice the virucidal concentration (Arimoto 1995).

*c. Detection of the virus by PCR from hatched larvae*

Detection of virus from eggs or 1-day-old larvae was not successful; therefore, 2 or 3 day-old larvae were used as the target for viral detection by PCR. For the preparation of 2 or 3 day-old larvae, about 300 fertilized eggs in 1 l seawater were transferred to a 2 l plastic bottle with the addition of a pinch of the antibiotic streptomycin. The bottle was kept in incubator at 26 to 27°C for 2 to 3 days. About 200 hatched larvae were then collected using a 1.5 ml micro tube for PCR examination. The PCR procedure used was the same as that for viral detection for brood stock. The results are shown in Table 5. The virus could be detected from hatched larvae in July 1999 and February 2000, and as expected, all larvae in each case were dead before reaching 20 days. In March 2000, the result of PCR test was negative, but mass mortality occurred among 24 day-old larvae. This case can be possibly explained by a viral contamination from intake water, since mass mortality occurred among juveniles of barramundi and humpback grouper in a cage located near the water inlet. In June 2000, PCR test results were negative and 30 000 juveniles were successfully produced.

**Table 5.** Detection of Nodavirus by PCR from hatched larvae.

Date	PCR Test Result	Seed Production Status
July 1999	Positive (Day 2 larvae)	Failed
January 2000	Positive (Day 2 larvae)	Not conducted
February 2000	Positive (Day 3 larvae)	Failed
March 2000	Negative (Day 2 larvae)	Failed
June 2000	Negative (Day 2 larvae)	Successful

*d. Removal of small, weakened or dead fish from rearing tank*

Removal of small or non-vigorous fish that may be a source of the viral multiplication is possibly an effective way to reduce mortalities due to the viral infection among larvae and juveniles.

*e. Increasing the amount of intake water*

Increasing water exchange rate is an effective counter measure to reduce the pathogens in rearing water and to maintain good water quality. At the Station, a maximum of 500% water exchange per day after 30 days of culture is being undertaken.

*f. Administration of Prefuran (10 % nifurpirinol compression, Argent Laboratories)*

When affected larvae were observed, Prefuran was added into the rearing water at a concentration of 1 ppm of the water volume with usual water exchange. At the nursery stage, Prefuran (1 g/kg food) was orally given to fish when mortality was observed. The administration was potentially effective to reduce mortalities, although scientific data to support such observations are still lacking.

#### 4. Further studies on control measures of VNN

The Station will concentrate the next activities on the following four areas:

- Investigation of the viral habitats by using cell line
- Administration of immuno-stimulant to post larvae
- Development of vaccine and its application to juveniles and fingerlings
- Study on the relationship between VNN-occurrences and environmental factors such as temperature, salinity, pH, ammonia level and bacterial flora.

**Discussion and Recommendations**

VNN is characterized by its wide host range and limited affected ages of susceptible host fish. Grouper species affected by VNN included 5 species: red-spotted grouper, kelp grouper, sevenband grouper, brown-spotted grouper and greasy grouper (Munday and Nakai 1997). Our studies showed that 4 other species at the Station had also been affected by the Nodavirus. There are now 9 species of groupers considered to be susceptible to the virus. This indicates that groupers showed the highest sensitivity to the Nodavirus among marine finfish. In the future, VNN will be also observed in the other species of groupers when seeds of such species are produced. Seed production of groupers will always be accompanied by VNN problems. Susceptible stages to the virus in groupers are generally larvae and juveniles, as already suggested by many reports on VNN in groupers (Mori *et al.* 1991, Mori *et al.* 1992, Munday and Nakai 1997, Chi *et al.* 1997) except in the case in seven band grouper in Japan (Fukuda *et al.* 1996). However, we also observed mass mortalities due to Nodavirus among humpback grouper fingerlings in net cages with continuous waves on the water. Suitable location of cage with good quality water, calm water surface and moderate tide are essential for rearing the fingerlings, especially when the fish has a latent viral infection. Stressful conditions can also be lethal to the virus carriers at juvenile and fingerling stages. Viral infection was rarely observed among 1 year-old fish, which were also concurrently infected with a large number of capsalid monogenetic parasites. Mortality due to the virus at this stage may occur under the existence of concurrent infections with other pathogens. Clinical signs in fish affected with VNN are very few. In particular, when VNN is observed among larvae younger than 20 day-old, mass mortality occurs without any symptoms. In this case and when fish appetite has decreased, samples of inactive larvae near the water surface should be taken for PCR examination. At post-larval stage (25 day-old to 30 day-old), it takes a few days for mass mortalities to occur after the initial mortality. In this case dead fish should be sampled to detect VNN by PCR test. At the nursery stage, mass mortality does not occur among juveniles during short periods and mortalities continue daily. Characteristic of affected fish include resting at the bottom. The cause of mortality can be presumptively diagnosed from this symptom, however PCR test is essential for confirmatory diagnosis. Affected fish older than fingerling stage floats near the water surface. As this symptom is also common in other diseases such as those caused by external parasitic infections, the cause of disease should be diagnosed by PCR test.

At present, the GRSCF utilizes three diagnostic methods (*i.e.* PCR, histopathology and cell culture) for confirmatory diagnosis of VNN. Histopathological observation such as vacuolation of nerve cells in the brain or retina is the best way to diagnose VNN (Muroga *et al.* 1998). However, this method is not suitable for routine diagnosis because of difficulty in treatment of small sample and long preparation period required. The PCR test can deal with any size of sample and it takes less than 6 hours to complete the examination, thereby allowing rapid diagnosis. The level of sensitivity of PCR is inferior to cell culture, but sufficient to detect the virus from a moribund or dead fish. The PCR test has been adopted as a routine test for the diagnosis of VNN at the Station. Cell culture needs a maximum of two weeks to diagnose VNN, and is not suitable for rapid diagnosis. Because of its high sensitivity this method is useful for the detection of the virus from carriers and environmental water.

In Indonesia, four national hatcheries and several private hatcheries have succeeded in seed production of humpback grouper in the past, but only the GRSCF can continually produce the seeds at present. Epidemiological investigation with PCR proved that the spread of VNN caused failures in the production at almost every hatchery. The GRSCF has made every effort to prevent the spread of the virus during every seed production, thereby attaining continuous seed production. The route of VNN-infection in groupers may possibly include vertical transmission from brood stock and horizontal transmission within the environmental water (to be further investigated by research). The virus could be detected from gonads of humpback grouper brood stock at the Station by PCR, which suggested the possibility of the vertical transmission of the virus. The virus could also be detected from hatched larvae, following VNN outbreaks in hatcheries for several months. This suggests the importance of vertical transmission for the spread of VNN. Selection of VNN-negative brood stock is an effective method to control VNN among striped jack (Mushiaki *et al.* 1994). This method was used at the GRSCF once, and no viral infection was observed during the subsequent seed production, which indicates its efficacy as a preventative method against viral contamination. However, castration for sampling of gonads provides additional stress to fish resulting in inability to spawn during that month. In addition, the fact that the viral positive brood stock turned to be negative the following month indicates the fluctuation of viral numbers in brood stock during short periods. Thus, examination should be conducted prior to spawning period. In consideration of these factors, brood stock selection with castration is not recommended. There is possibility for viral contamination from intake water even if vertical transmission of the virus may be completely avoided. In these circumstances, the methods to reduce the viral numbers in rearing water are also important. When moribund or dead larvae are observed in post juveniles, they should be removed immediately and used for virus detection by PCR. In case of VNN-positive results, water exchange rate should be increased and dead and weakened larvae should be removed by siphoning. Control measures at nursery stage also include removal of moribund or dead fish and increasing water exchange rate. Additionally, oral administration of Prefuran appears to be effective in reducing mortality.

The most important work in the future is to determinate the route of the viral infection, which can provide vital information for prevention of viral contamination. To investigate the route, it is essential to use viral detection using cell line that is more sensitive than PCR. Application of immunostimulant or vaccine is also an important area to consider. The most sensitive stage of fish to the virus infection is between 20 and 30 day-old larvae. Since rotifers and brine shrimps are given as feed, the water exchange rate cannot be increased much during this stage. One possible measure to decrease mortality during this period is to feed brine shrimp supplemented with immunostimulant. Vaccination should also be considered for juveniles at nursery stage and fingerlings in the early cage-culture stage. Recently, it was experimentally proven that *E. coli*-recombinant vaccine could reduce mortality due to Nodavirus infection in certain species of groupers (Tanaka *et al.* 2001). An experiment conducted at the Station also indicated the efficacy of the *E. coli*-recombinant vaccine in humpback grouper fingerlings (unpublished data).

Currently, grouper hatcheries in Indonesia adopt a running water system for water supply, where the rearing water is exchanged as much as possible. This method can be applied on the condition that inlet water is of high quality and not contaminated by the virus. However, when mortality due to the virus occurs in this system, outlet water contains numerous viruses. When it is released to the sea, it will eventually contaminate inlet water. Circulating water system may possibly be an ideal method to prevent this situation, which is expected to be developed in the future. Circulating water system is superior to running water system with respect to environmental factors in the rearing water which may be associated with VNN-occurrence and which can be controlled in the former system.

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## Structure and Transmission Cycles of Nodaviruses and Iridoviruses Infecting Fish

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### Abstract

Nodaviruses have been reported to cause viral nervous necrosis (VNN) in a wide range of marine fish. The gross signs and histopathology of VNN appear to be similar in all infected species. In general, mortalities occur in larval or juvenile fish and there appears to be a reduced risk of disease with age. Nodaviruses are small (25-34 nm), non-enveloped; icosahedral particles containing 2 segments of (+) sense single-stranded RNA. The coat protein is the site of neutralizing epitopes and is the obvious target for subunit or recombinant vaccines. Coat protein genes of nodaviruses isolated from a diverse range of marine fish share homology at the nucleotide and amino acid levels, and there is little correlation between host species and viral genotype. This is consistent with observations that nodaviruses are transmitted horizontally within and between species. There is also evidence of vertical Nodavirus transmission in some marine fish but it is not known if the virus is present within or external to the egg.

Iridoviruses are large, enveloped double-stranded DNA viruses that infect invertebrates and ectothermic vertebrates with an aquatic stage in their life cycle. Iridoviruses in the genus *Ranavirus* infect fish; reptiles and amphibians, causing systemic infections that may be unapparent or severe with mortalities reaching 100%. The disease was first described in Australia as epizootic haematopoietic necrosis (EHN) in red fin perch. Other systemic iridovirus infections of cultured marine fish have since been associated with similar conditions elsewhere. The ranavirus virion is a 160-200 nm icosahedral particle containing a 150-170 kb, highly methylated, linear, double-stranded DNA genome. Although there is evidence of a high degree of antigenic cross-reactivity between ranavirus capsid proteins, their antigenic structure is largely unknown and there is a need to identify antigens suitable for vaccine development. Ranaviruses can be transmitted horizontally by immersion or co-habitation between different susceptible hosts. There is also evidence that ranaviruses isolated from diverse hosts in the same geographic location are similar or identical, suggesting a complex transmission cycle that involves reptile or amphibian reservoirs of infection.

### Introduction

There is increasing evidence of the emergence and spread of aquatic animal pathogens as a result of the rapid worldwide expansion of intensive aquaculture. This is partly because these farming industries provide a concordance of factors conducive to a shift in the balance between pathogen, host and environment. High stocking densities increase stress on the host and promote the rapid transmission of disease. Land-based and sea-cage cultures often change the natural habitat of the host, presenting the opportunity for exposure to new pathogens. The growing international trade in brood stock and seed has also contributed to the transfer of pathogens to new sites, spreading disease and providing opportunities to extend the natural host range. In warm water finfish (including grouper), newly emergent viral pathogens in the Nodavirus and Iridovirus families are of particular concern. These viruses were unrecognized prior to the mid-1980s but have since been reported in association with mass mortalities in wild and cultured fish in Asia, Australia and Europe. This short paper describes the current knowledge of these pathogens, focusing on their structural properties and transmission cycles.

## Nodaviruses

*Host range and pathology.* Nodaviruses are known to infect and cause fatal encephalopathies in a wide range of marine fish, including several species of cultured grouper. The first report of viral nervous necrosis (VNN) was in cultured Japanese parrotfish (*Oplegnathus fasciatus*), in which mass mortalities in larvae and juveniles caused severe production losses at 2 facilities in Nagasaki from 1985-1987 (Yohsikoshi and Inoue 1990). Diseased fish often became darker in colour and displayed loss of swimming activity with loss of equilibrium and spiral swimming near the surface prior to death. The only consistent histopathological feature was necrosis in the nervous tissues with extensive vacuolation, pyknosis, basophilic intracytoplasmic inclusions and shrinkage of affected cells in the spinal chord, spinal ganglia and brain. Gross signs and histopathology characteristic of viral nervous necrosis were subsequently reported in a wide range of tropical and coldwater fish including barramundi (*Lates calcarifer*), sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), striped jack (*Pseudocaranx dentex*) and grouper (*Epinephelus* spp.) (Glazebrook *et al.* 1990, Bloch *et al.* 1991, Breuil *et al.* 1991, Munday *et al.* 1992, Mori *et al.* 1992) and several species of grouper (Mori *et al.* 1991; Boonyaratpalin *et al.* 1996, Fukuda *et al.* 1996, Chi *et al.* 1997, Tanaka *et al.* 1998, Zafran *et al.* 2000). Although not originally reported in Japanese parrotfish, retinal lesions are also common in most affected species (Munday and Nakai 1997). Mortalities usually occur in larval or juvenile fish and there appears to be a reduced risk of disease with age. However, in cultured sevenband grouper (*Epinephelus septemfasciatus*) and sea bass, mass mortalities associated with VNN have been reported in young and adult fish (Fukuda *et al.* 1996), and disease has been reproduced experimentally in adult fish of the same species (Tanaka *et al.* 1998, Le Breton *et al.* 1997, Skliris and Richards 1999).

*Structure and gene expression.* Nodaviruses are small (25-32 nm), non-enveloped, icosahedral particles containing a genome comprising 2 segments of (+) sense single-stranded RNA. Nodaviruses have been classified into 2 genera. Viruses in the genus *Alphanodavirus* infect primarily insects. The genus *Betanodavirus* comprises viruses infecting fish that are the agents of VNN. The betanodavirus virion comprises two coat proteins ( $M_r$  42 x 10<sup>3</sup> and 40 x 10<sup>3</sup>) that appear to be generated by co-translational processing of a single polypeptide. The virion also contains 2 segments of (+) RNA that lack 3' poly(A) tails. RNA 1 (3.1 kb) encodes protein 1a (110 kDa) that is not packaged into virions and appears to function in cells as an RNA-dependent RNA polymerase. RNA2 (1.4 kb) encodes the coat proteins 2a and 2b. A third sub-genomic RNA is also present in infected cells but is not packaged into virions. The product encoded in RNA 3 (0.4 kb) has not yet been identified (Mori *et al.* 1992, Comps *et al.* 1994, Delsert *et al.* 1997, Ball *et al.* 2000).

*Variation and relationships between betanodaviruses.* Seven betanodavirus species and two tentative species are currently recognised by the International Committee on Taxonomy of Viruses (Ball *et al.* 2000; see also Table 1). Striped jack nervous necrosis virus (SJNNV) is the designated type species. Species demarcation is based on comparisons of the nucleotide sequences of the coat protein gene. The coat protein sequences of betanodavirus species share at least 75% identity at the nucleotide level and 80% identity at the amino acid level (Nishizawa *et al.* 1995).

Analysis of coat protein gene sequences of nodaviruses isolated from a diverse range of marine fish has indicated little correlation between host species and viral genotype (Nishizawa *et al.* 1997). BFNNV was isolated in Japan from barfin flounder (*Verasper moseri*) in 1993 and from Pacific cod (*Gadus macrocephalus*) in 1996. As these hosts are members of different superorders (*Acanthopterygii* and *Paracanthopterygii*), BFNNV appears to have a very broad range of host susceptibility amongst teleost fish. It has also been reported that 2 different nodaviruses infect sea bass populations in the Mediterranean and Atlantic coasts of France (Thiery *et al.* 1999) and, between 1993 and 1995, 3 different nodavirus species (BFNNV, TPNNV and RGNNV) were isolated from flounder in Japan. This indicates that the transfer of nodaviruses between fish species may be common. Furthermore, in 1995, closely related isolates of RGNNV were obtained from flounder (*Paralichthys olivaceus*) and sea perch (*Lateolabrax japonicus*) in Japan, and from Ombrina (*Umbrina* sp.) and sea bass in Italy (Nishizawa *et al.* 1997). This indicates that the translocation of nodaviruses between distant geographic locations may have occurred recently. This evidence suggests that the growth of intensive aquaculture and the translocation of brood stock and seed are disturbing the ecological relationship between Nodavirus and their natural hosts, and underscores the importance of controlling the translocation of aquaculture species.

*Transmission cycles.* Genetic studies of the relationship between Nodavirus isolates are consistent with observations that nodaviruses may be transmitted horizontally within and between species. Transmission of SJNNV and associated disease has been demonstrated experimentally by immersion of striped jack larvae in a diseased tissue extract or by cohabitation with infected larvae (Arimoto *et al.* 1993). SJNNV could not be transmitted experimentally to juvenile red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*) or goldstriped amberjack (*Seriola lalandi*), suggesting a limited host range. However, as very closely related SJNNV isolates have been recovered from striped jack and red sea bream in Japan (Nishizawa *et al.* 1997), the host range and susceptibility to experimental infection should be examined more closely. Experimental transmission of RGNNV to juvenile grouper and of LcEV to barramundi by immersion and cohabitation has also been reported (Glazebrook *et al.* 1990, Mori *et al.* 1991, Boonyaratpalin *et al.* 1996, Munday and Nakai 1997). Tanaka *et al.* (1998) have reported that a nodavirus isolated from moribund adult sevenband grouper could be transmitted by intramuscular injection to young seven band grouper and juvenile redspotted grouper. VNN has been transmitted to



juvenile sea bass by oral infection, bath exposure, cohabitation or injection (Peducasse *et al.* 1999). Experimental transmission of DIEV to adult sea bass by cohabitation has also been reported (Skliris and Richards 1999).

There is also evidence of vertical Nodavirus transmission in striped jack and in barfin flounder but it is not yet known if transmission is transovarian. Arimoto *et al.* (1992) used indirect ELISA to detect SJNNV in ovaries and in fertilized eggs but the location of the virus was not determined. Watanabe *et al.* (1998) have reported the use of PCR to detect BFNNV in egg fluids. Regardless of the site of infection, there will be opportunity for shedding of virus during and after spawning, resulting in early larval mortalities which most frequently occur 2-10 days after hatching (Ahne *et al.* 1997, Mori *et al.* 1998). For other betanodaviruses, the role of vertical transmission in the disease cycle is less clear. Munday *et al.* (1992) have suggested that, as LcEV has an incubation period of 4 days and lesions do not occur prior to 9 days after hatching, horizontal transmission within the hatchery is the most likely source of infection in barramundi larvae. This was supported by observations that removal of juveniles from the hatchery greatly reduces the risk of disease in subsequent batches of larvae. In grouper, mortalities commonly occur in juvenile fish (Chi *et al.* 1997, Zafran *et al.* 2000) and so an environmental source of infection appears most likely. Exclusion of infected spawners by RT-nested PCR screening in grouper hatcheries should prevent both vertical and subsequent horizontal Nodavirus transmission in culture and reduce the risk of VNN in larvae. However, there is a need to better understand the transmission cycle and potential sources of infection in order to implement more comprehensive disease control strategies.

*Vaccine potential.* The coat protein of betanodaviruses is the site of neutralizing epitopes and is an obvious target for the development of subunit or recombinant vaccines. The development of an SJNNV vaccine based on a recombinant coat protein is in progress (Ahne *et al.* 1997). It will be important to determine if the SJNNV vaccine protects against nodavirus infections in other species. The relatively recent availability of cell lines that support the growth of piscine nodaviruses (Chew-Lim *et al.* 1994, Iwamoto *et al.* 1999, Chi *et al.* 1999, Watanabe and Yoshimizu 1999) will assist the development of subunit and attenuated vaccines. There is also potential to engineer rationally attenuated live vaccines that should produce durable, cross-protective immunity after a single vaccination.

### Iridoviruses

*Host range and pathology.* Iridoviruses are large, enveloped double-stranded DNA viruses that infect invertebrates and ectothermic vertebrates with an aquatic stage in their life cycle. Iridoviruses in the genus *Lymphocystivirus* infect a wide range of freshwater and marine fish (including grouper), causing benign papilloma-like tumors that regress spontaneously and little or no mortality (Wolf 1988). Iridoviruses in the genus *Ranavirus* infect fish, reptiles and amphibians, causing systemic infections. Ranaviruses are emerging as important pathogens of cultured freshwater and marine fish. Ranavirus infection may be unapparent, or severe with mortalities reaching 100% in fry and 30% in adult fish (Ahne *et al.* 1997). Ranavirus disease was first described as epizootic haematopoietic necrosis (EHN) in wild redbfin perch (*Perca fluviatilis*) and in farmed rainbow trout (*Oncorhynchus mykiss*) in Australia (Langdon *et al.* 1986; Langdon and Humphrey 1987, Langdon *et al.* 1988). A disease with similar gross signs and histopathology was subsequently reported as "sleepy grouper disease" in brown-spotted grouper (*Epinephelus tauvina*) in Singapore (Chua *et al.* 1994). Other systemic Iridovirus infections of cultured marine fish have since been associated with similar conditions elsewhere in Asia (Inouye *et al.* 1992, Kasornchandra and Khongpradit 1995, Chou *et al.* 1998, He *et al.* 2000, Jung and Oh 2000). Typically, diseased fish display extreme lethargy and abnormal swimming, but there may be few visible external clinical signs. Histological examination of moribund fish commonly reveals necrosis of haematopoietic tissue and enlarged cells with basophilic inclusions in the spleen, kidney, heart, liver and gills.

Individual ranaviruses often have a very wide range of host susceptibility amongst teleost fish and amphibians. Matsuoka *et al.* (1996) reported systemic Iridovirus disease occurred in 19 species from 3 taxonomic orders of cultured marine fish in Japan between 1991 and 1995. A comparison of isolates from Japanese red sea bream, amberjack (*Seriola dumerili*), striped jack and albacore (*Thunnus thynnus*) indicated each was pathogenic for red sea bream, and that red sea bream isolates were pathogenic for yellowtail (Nakajima and Maeno 1998). EHNV has been transmitted experimentally to eight species representing 4 families of teleost fish. Some other fish species are apparently resistant to infection (Langdon 1989). Bohle iridovirus, a highly virulent pathogen of the burrowing frog (*Lymnodynastes ornatus*) in Australia, has been shown to infect and cause systemic disease in frogs and barramundi, causing necrosis of haematopoietic tissue of the kidney and spleen (Moody and Owens 1994). It has also been reported that the ranavirus type species (*Frog virus 3*), although not causing disease, could be detected in the various organs of experimentally infected sheatfish (*Silurus glanis*) and rainbow trout (Ahne *et al.* 1997). The available data suggests that, although the host range may be very broad, the pathogenicity of individual ranaviruses can vary significantly between hosts.

*Structure and gene expression.* The ranavirus virion is a 160-200 nm icosahedral particle containing a 150-170 kb, highly methylated, linear, double-stranded DNA genome. The particle may be naked or enveloped but the envelope is not essential for infectivity. The genome is circularly permuted and approximately 30% terminally redundant. The unit genome size is approximately 110 kb. Virions are complex structures that contain at least 29 polypeptides

including two proteins (VP44 and VP63) in the intermediate lipid layer and one protein (VP58) in the membrane of the envelope. The major capsid protein (MCP) of FV3 comprises 463 amino acids with an estimated molecular weight of 49 860 (Mao *et al.* 1996). FV3 proteins do not undergo extensive post-translational processing but phosphoproteins ( $M_r$  10-114 x 10<sup>3</sup>) are present in virions. Various enzyme activities have also been associated with FV3 virions (Elliott and Kelly 1980, Willis *et al.* 1977, Williams *et al.* 2000), including protein kinase, nucleotide phosphohydrolase, endonucleases, phosphatase and integrase-recombinase. Less is known of the detailed structure of other ranaviruses and rana-like viruses. EHNV has been shown to contain at least 19-20 polypeptides in the range  $M_r$  18-128 x 10<sup>3</sup>. The sizes of three major capsid proteins were estimated to be 32, 53 and 88 kDa, of which the 53 kDa protein appeared to be associated with core particles (Eaton *et al.* 1991). A similar number of proteins have been reported in the virions of rana-like viruses infecting sheatfish and catfish, and in Bohle iridovirus from burrowing frogs (Hendrick *et al.* 1992, Hengstberger *et al.* 1993). The DNA of all ranaviruses examined to date is highly methylated.

*Variation and relationships of ranaviruses.* The current taxonomic classification of iridoviruses infecting marine fish is shown in Table 2. Due to the absence of adequate data for definitive classification and demarcation of species, the genus *Ranavirus* presently comprises only one recognized species (*Frog virus 3*) and 5 tentative species. Alternative names are recorded for many of these viruses. The recognized ranaviruses and rana-like viruses have significant variations in the structural, antigenic and biological properties. Hendrick *et al.* (1992) demonstrated the virion proteins of EHVN and FV3 and systemic iridoviruses isolated from sheatfish and catfish (*Ictalurus melas*) from Europe were similar in number, but there were some distinct variations in  $M_r$  of major and minor capsid proteins. Similar but distinct protein profiles have also been reported for Bohle iridovirus and EHNV isolates from redfin perch and rainbow trout (Hengstberger *et al.* 1993). Mao *et al.* (1997) also observed similarities in the profile of proteins and used the profiles to assign the viruses into 4 groups that reflected the geographic origin of the isolates. According to this analysis, the viruses clustered as sheatfish and catfish iridoviruses from Europe, EHNV from redfin perch and rainbow trout from Australia, doctor fish (*Labriodes dimidatus*) iridovirus and guppy (*Poecilia reticulata*) iridovirus from south-east Asia, and North American iridovirus isolates from box turtle (*Terrapene c. carolina*), tortoise (*Testudo horsfieldi*), tadpole (*Rana aurora*) and frog (*Rana pipiens*).

Antigenically, Bohle iridovirus and EHNV isolates cross-react in ELISA and western blot analysis (Hengstberger *et al.* 1993). EHNV also cross-reacts in indirect fluorescent antibody tests with European sheatfish and catfish systemic iridoviruses and with grouper iridovirus from Thailand, but red sea bream iridovirus from Japan appears to be unrelated (Hendrick *et al.* 1992, Nakajima *et al.* 1998). Red sea bream iridoviruses isolated from various cultured marine fish in Japan are closely related, although some differences between isolates were detected by screening with a panel of monoclonal antibodies (Nakajima *et al.* 1998).

At the genetic level, there is a relatively high degree of homology between systemic iridoviruses from diverse sources. EHNV isolates from fish and Bohle iridovirus from frogs cross-hybridize under conditions of high stringency indicating an overall identity of approximately 90% (Hengstberger *et al.* 1993). A comparison of deduced amino acid sequences of an 85 amino acid N-terminal portion of the 50 kDa major capsid protein of FV3 with other iridoviruses indicated 100% identity with turtle and tadpole iridoviruses from North America and 95-100% identity with sheatfish and catfish iridoviruses from Europe and EHNV isolates from Australia. There was a lower level of identity with guppy and doctor fish iridoviruses from Southeast Asia (Mao *et al.* 1997). Restriction fragment length polymorphisms (RFLPs) can be detected between individual isolates of systemic iridoviruses but, in general, RFLP patterns indicate similarities between viruses from the same geographic location rather than a specific association between virus and host (Hengstberger *et al.* 1993, Mao *et al.* 1997, Mao *et al.* 1999).

*Transmission cycles.* There are various reports that iridoviruses causing systemic infections in fish can be transmitted horizontally by immersion or co-habitation between different susceptible hosts (Langdon 1989, Ahne *et al.* 1990, Ogawa *et al.* 1990, Chua *et al.* 1994, He *et al.* 2000). In some cases, there is evidence of infection without visible signs of disease and adult fish that survive viral challenge may act as carriers of infection (Langdon 1989, Ahne *et al.* 1991). There is also evidence that systemic iridoviruses isolated from diverse hosts in the same geographic location are similar or identical, suggesting a complex transmission cycle that involves reptile or amphibian reservoirs of infection (Hengstberger *et al.* 1993, Mao *et al.* 1997, Nakajima *et al.* 1998, Mao *et al.* 1999). The role of vertical transmission in the ranavirus disease cycle is not yet clear.

*Vaccine potential.* Although there is evidence of antigenic cross-reactivity between systemic iridoviruses of diverse origins (Hendrick *et al.* 1992, Nakajima *et al.* 1998) and a high level of sequence identity between the major capsid proteins (Mao *et al.* 1997), their antigenic structure is largely unknown and there is a need to identify antigens suitable for vaccine development. The major capsid protein (MCP) gene of FV3 has been cloned but there is no indication that MCP antibody will be neutralizing or protective (Mao *et al.* 1996). It is also of some concern that rabbit antisera raised to purified preparations of EHNV and iridoviruses isolated from sheatfish and catfish failed to neutralize even the homologous viruses *in vitro* (Hendrick *et al.* 1992).

## Conclusion

The emergence of new, virulent viral pathogens of fish is a predictable consequence of the disturbance to viral ecology that has occurred with the rapid growth of aquaculture. The common practice of translocation of brood stock and fry is contributing to the rapid global spread of these pathogens, generating a difficult, high-risk farming environment. Translocation is of particular concern for viruses such as nodaviruses and iridoviruses that have a wide range of available susceptible hosts as they move to new locations. To halt the spread of pathogens and to improve the sustainability and profitability of fish aquaculture, translocations must be carefully controlled and more effective diagnostic and control strategies must be developed. This will be only achieved through a better understanding of the viruses, their structural and antigenic properties, and their transmission and disease cycles.

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**Table 1.** Taxonomic classification of nodaviruses infecting fish (Ball *et al.* 2000).

Family	Genus	Species
Nodaviridae	Betanodavirus	Barfin flounder nervous necrosis virus (BFNNV)
		<i>Dicentrarchus labrax</i> encephalitis virus (DIEV)
		Japanese flounder nervous necrosis virus (JFNNV)
		<i>Lates calcarifer</i> encephalitis virus (LcEV)
		Redspotted grouper nervous necrosis virus (RGNNV)
		Striped jack nervous necrosis virus (Type sp.) (SJNNV)
		Tiger puffer nervous necrosis virus (TPNNV)
		<b>Tentative</b>
	Atlantic halibut nodavirus (AHNV)	
	Malabar grouper nervous necrosis virus (MGNNV)	

**Table 2.** Taxonomic classification of iridoviruses infecting fish (Williams *et al.* 2000).

Family	Genus	Species
Iridoviridae	Ranavirus	Frog virus 3 (Type species) (FV-3)
		(Box turtle virus)
		(Lucke triturus virus)
		(Tadpole edema virus)
		(Tortoise virus 5)
		<b>Tentative</b>
		Bohle iridovirus (BIV)
		Epizootic haemopoietic necrosis virus (EHNV)
		(Rainbow trout virus)
		(Redfin perch virus)
		Redwood Park virus (RPV)
		(Tadpole virus 2)
		(Stickleback virus)
		Regina ranavirus (RRV)
		(Tiger salamander virus)
	( <i>Ambystoma tigrinum stebbensi</i> virus)	
	Santee-Cooper ranavirus (SCRV)	
(Largemouth bass virus)		
(Doctor fish virus)		
(Guppy virus 6)		
Lymphocystivirus		Lymphocystis disease virus 1 (Type species) (LCDV-1)
		(Flounder lymphocystis disease virus)
		(Flounder virus)
		<b>Tentative</b>
		Lymphocystis disease virus 2 (LCDV-2)
		(Dab lymphocystis disease virus)

## Immunological Methods of Disease Control

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### Abstract

Over the past twenty years, aquaculture has grown to become a significant industry in many parts of the world. Production husbandry has changed from extensive or semi-intensive to intensive or super-intensive. Given high population densities, infectious diseases pose a constant and very costly threat to successful husbandry. Even when environmental conditions are good and fish are healthy, certain infectious agents are so virulent that mass mortality can and does occur. Diseases are one of the major constraints to aquaculture. Antibiotics provide a useful means of helping to control many bacterial diseases but are not the complete answer. There are many problems associated with antibiotics including the development of antibiotic resistance and recurrent outbreaks necessitating further costly treatments. Management of fish diseases can be best accomplished by a combination of long-term planning such as diagnostic testing for broodstock disease, and vaccination programs.

The successful use of vaccines in warm-blooded animals, the lack of registered and approved therapeutic chemicals and antibiotics for aquaculture, and the decreasing effectiveness of antibiotics in controlling diseases in fish have led to the development of vaccines for fish. This paper describes disease controls in aquaculture using immunological methods, diagnostic and vaccination. Methods of vaccine administration, vaccine efficacy, and benefits from using vaccine in fish are discussed.

### Introduction

Aquaculture has become a significant industry in many parts of the world. Production of channel catfish in the USA increased from 21 120 metric tons in 1980 to 214 601 metric tons in 1996 (Vinitnantharat *et al.* 1997). Salmonid production in Norway increased from 50 000 metric tons in 1986 to 460 000 metric tons in 1999 (Vinitnantharat *et al.* 1997). Japan produced 145 773 metric tons of yellowtail in 1996 (FAO 1999). Thailand increased production of tiger prawn from 106 metric tons in 1985 to 211 110 metric tons in 1997 (*pers. comm.*). With increased aquaculture activities, production husbandry has evolved from extensive to intensive. Given high population densities, even under good environmental conditions certain infectious agents can cause mass mortality if introduced onto the farm. There are many approaches to disease control. This paper describes two immunological methods of disease control: vaccination and diagnostics.

- *Disease Diagnostics*

As an immunological method of disease control, diagnostics uses the principle of specificity of the antigen to antibody. As a disease management strategy, diagnostics is a good tool for disease control; as exemplified by the successful management of bacterial kidney disease (BKD) or the eradication program for viral haemorrhagic septicemia (VHS). Many specific immunological methods are used as diagnostic tools, including enzyme-linked immunosorbent assay (ELISA), and fluorescent antibody test (FAT) (Stolen *et al.* 1990). The successful use of diagnostics for disease control is based on the correct interpretation of the diagnostic results and determination of the best intervention. The key in using diagnostics is to have written guidelines on each specific disease with diagnostic results.

- *Vaccination*

Vaccination is one of the most powerful tools used in aquaculture disease control programs. The successful use of vaccines in warm-blooded animals and the decreasing effectiveness of antibiotics in controlling diseases in fish has

led to the development of fish vaccines. In 1976, the first commercial vaccine for fish (against *Yersinia ruckeri*) was licensed in the United States. Since then, many fish vaccines have been commercialized (Vinitnantharat *et al.* 1997). Table 1 lists commercially available vaccines and those being researched for aquaculture use. Methods of administration, benefits from using vaccine in fish and its efficacy are discussed in the following section.

**Table 1.** Vaccines for aquaculture: commercially available and under research.

Commercially available	Under research
<b>Bacterial Vaccines</b>	
<i>Yersinia ruckeri</i> (ERM)	<i>Flexibacter maritimus</i>
<i>Vibrio anguillarum</i> serotype O1	<i>Flavobacterium psychrophilum</i>
<i>Vibrio anguillarum</i> serotype O2	<i>Flavobacterium columnaris</i>
<i>Vibrio ordalii</i>	<i>Vibrio</i> sp.
<i>Aeromonas salmonicida</i>	<i>Renibacterium salmoninarum</i>
<i>Vibrio salmonicida</i>	
<i>Pasteurella piscicida</i>	
<i>Vibrio viscosus</i>	
<i>Aerococcus viridans</i>	
<i>Lactococcus garvieae</i>	
<i>Streptococcus</i> sp.	
<b>Viral vaccines</b>	
Infectious Pancreatic Necrosis	Infectious Hematopoetic Necrosis
Iridovirus	Viral Hemorrhagic Septicemia
	<i>Piscirickettsia salmonis</i>
	Pancreas Disease
	Infectious Salmonid Anemia
	Viral Nervous Necrosis
<b>Parasite vaccines</b>	
None	<i>Ichthyophthirius multifiliis</i>

- *Immunization methods in fish*

There are three methods for vaccinating fish: immersion, orally and by injection. Each method has its own advantages and disadvantages (Table 2).

- *Immersion vaccination*

Immersion vaccination is an easy and effective immunization method. Fish are immersed in a dilute vaccine solution for a short period of time, thirty seconds to two minutes, and released into the culture unit (typically ponds, raceways, or net pens). This method is limited to operations where fish will not be moved after stocking as the immersion vaccination can only be used during stocking time. Immersion vaccination is more costly for larger sized fish.

- *Oral vaccination*

Oral vaccination is the most convenient way to immunize fish because the vaccine can be administered on any size fish, anytime during the culture cycle, and in all types of culture systems. The vaccine is either incorporated into or adhered to the feed and then fed to the fish. It is the least stressful method because handling is not required. Similar to immersion, oral vaccination is not cost effective when immunizing larger fish. Oral vaccination provides the lowest efficacy compared to the other two methods. The main problem appears to be the destruction and absorption of antigens by the fish digestive system. Further research is needed to develop methods to protect antigens from being destroyed by the digestive system and improve absorption in order to improve efficacy of oral vaccination.

- *Injection vaccination*

Intra-peritoneal injection (IP) vaccination is the most effective way of immunizing fish. The injection method allows the use of adjuvants, especially oil adjuvant, which provides longer lasting protection than immersion. Fish are anaesthetized, injected with vaccine and returned to clean water. Commercial operations use repeating injection guns. The injection guns can be manually or automatically operated and allow each operator to inject 1 000 to 2 000 fish per



hour. The typical system consists of an anaesthetization tank, an injection table, an injecting gun connected to a vaccine bottle, and a recovery tank. Injection vaccination is very labor intensive. The fish size limits the use of injection. In general, for a fish less than 5 g, the injection method is not practical. Other disadvantages of injection include adhesion formation, temporarily off feed or reduced feeding, inadvertent puncture of the intestine, and creating a wound that could provide a portal for disease.

	Immersion	Oral	Injection
<b>Application</b>	Easy	Very easy	Moderate
<b>Stress</b>	Light	None	Moderate
<b>Labor</b>	Inexpensive	None	Intensive
<b>Effectiveness</b>	Good	Fair	Excellent
<b>Duration</b>	3-12 months	2-4 months	12-24 months

**Table 2.** Comparison of three different methods for fish vaccination.

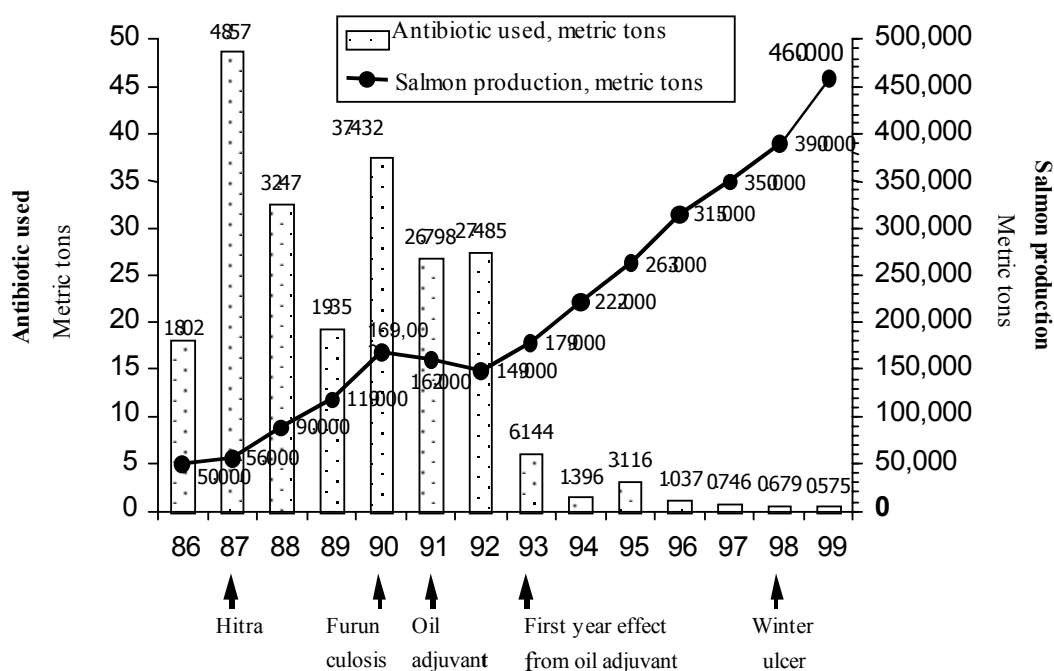
### Benefits of using vaccine

Efficacious vaccines protect immunized fish against specific diseases, and reduce losses due to disease. Once fish acquire protection against certain diseases, production becomes more predictable. Efficacy of fish vaccine is tested by actual challenge with live organism at a specific intervals after vaccination. Relative percent survival (RPS) is used to evaluate vaccine efficacy (Ellis 1988). The higher are RPS values, the better the protection.

$$\text{RPS} = 1 - \frac{(\% \text{mortality in vaccinated fish}) \times 100}{\% \text{mortality in control fish}}$$

Vaccines are a preventative measure as opposed to antibiotic treatment which is used after a disease occurs. Antibiotics have been useful in controlling many bacterial diseases but are not the definitive solution. Chemotherapy is less successful than desired, because highly infected fish do not eat and cannot be medicated. There are many problems associated with antibiotics including the development of antibiotic resistance and recurrent outbreaks necessitating further costly treatments.

Vaccines for aquaculture have been successful in reducing the use of antibiotics. An example is the case in Norway (Fig. 1). In 1987, Norwegian aquaculture experienced an outbreak of Hitra disease, caused by *Vibrio salmonicida*. Antibiotic use reached 48.5 mt compared to 18.0 mt in 1986. Vaccine against *Vibrio salmonicida* was introduced in late 1987. In 1990, the amount of antibiotic used was 37.4 mt, while salmonid production was 169 000 mt. In 1991, oil adjuvant vaccine was introduced to aquaculture in Norway. In 1999, the amount of antibiotic usage dropped to 0.57 mt with an increase in salmonid production to 460 000 mt.



**Fig 1.** Use of antibiotic relative to salmon and trout production in Norway from 1987 to 1999.

Since the first commercial aquaculture vaccine (against ERM) was introduced, many vaccines have become commercially available including vaccines against the viral disease infectious pancreatic necrosis (IPN), and vaccine against bacterial diseases caused by a number of bacterial species such as *Photobacterium damsela* subspecies *piscicida*, *Vibrio anguillarum*, *V. ordalii*, *V. salmonicida*, *V. viscosus*, and *Aeromonas salmonicida*.

#### *Vaccine against Vibrio anguillarum*

*Vibrio anguillarum* is the causative agent of vibriosis. It is a gram negative bacteria of the family Vibrionaceae. It is a short curved rod, motile, and cytochrome oxidase positive. It is sensitive to vibriostat 0/129 and novobiocin. This disease is distributed worldwide in cultured fish, principally in marine environments, but sporadic outbreaks have occurred in freshwater. The disease is normally characterized by generalized septicemia with clinical signs indistinguishable from other bacterial septicemias. The disease ranges from peracute (mortalities without gross lesion) and acute (hemorrhaging of the eyes, gills, vent, skin and internal organs, ascites fluid in body cavity) to sub-acute and chronic (hemorrhagic ulceration of the skin and underlying muscle).

Vaccine against vibriosis is very effective. An experiment using hybrid striped bass (average size of 6.8 g) showed a very good protection group (Rogers and Xu 1992). Hybrid striped bass were immunized by twenty second immersion (Imm) in vaccine and challenged at twenty-five and thirty-five days post vaccination. When challenged by immersion at twenty-five days post vaccination, the mortality rate in the control group was 70% vs. 40% in the vaccinated group. With intra-peritoneal injection challenge at 25 days post vaccination, the mortality rate in the non-vaccinated control was 85% vs. 25% in the vaccinated group in the first replicate. For the second replication, the mortality rate in the control non-vaccinated group was 80% vs. 5% in the vaccinated group. The average relative percent survival (RPS) was 70.2% when challenged at twenty-five days post vaccination, and the average RPS was 66% when challenged at thirty-five days post vaccination (Fig 2).

**Fig 2.** Laboratory trials on vaccine against *Vibrio anguillarum* in striped bass.

**Fig 3.** Potency of vaccine against *Vibrio viscosus* in rainbow trout.

#### *Vaccine against Vibrio viscosus*

Winter ulcer was first identified in Norwegian Atlantic salmon operations in the early 1980s (Lunder 1990, Anon. 1991). The disease typically occurs at sites in Iceland and Norway from February to April when water temperatures are below 8°C. Survivors typically recover in the spring when water temperatures rise above 8°C. The disease infects juvenile and adult salmon and trout raised in salt water. It also occurs at freshwater hatcheries when seawater is added for smolt acclimation (Lunder 1992). The disease typically involves shallow, superficial lesions on scale-covered tissue, which develop into penetrating ulcers. Early investigations suggested that winter ulcers resulted from mechanical disruption of vesicles formed after vascular thrombosis of dermal vessels, and were influenced by high levels of dietary iron (Salte *et al.* 1994). An infectious etiology was strongly suggested as two *Vibrio* species, *Vibrio viscosus* and *Vibrio wodanis*, were frequently isolated from kidneys of Atlantic salmon during winter ulcer outbreaks and identified in situ in degenerative muscle tissue using immunohistochemistry (Lunder 1992, Lunder *et al.* 1995). The disease could be transmitted horizontally. Mechanical disruption of the skin was a predisposing factor (Lunder *et al.* 1995). Though mortality rates during winter ulcer outbreaks are typically in the range of 0% to 10%, up to 20%, mortality has occurred within a one-month period at certain farms. The disease has been reported in up to 50% of

adult fish prior to slaughter (Lunder 1992). Lesion formation necessitates downgrading from “superior” to “ordinary” or “production” quality fish, and a reduction in market value. Infection of adult fish and losses due to mortality and downgrading represent a significant economic loss in affected Atlantic salmon farms in Norway and Iceland.

An intra-peritoneal injection challenge model for *V. viscosus* was developed in rainbow trout. Injection of live bacteria produced signs typical of septicemia and concentration-dependent mortality. Bacteria could be re-isolated from liver tissue of affected fish. The challenge model was used to evaluate potency of a multivalent, formalin killed, mineral oil adjuvanted vaccine. Rainbow trout (9.5 g) were vaccinated with 0.2 cc of a vaccine containing *V. viscosus*, *V. anguillarum* (serotype O1 and O2), *V. salmonicida*, and *Aeromonas salmonicida*. Vaccinated fish were held in 32 gal fresh water tanks (10°C) at a 25 gal per hour flow rate and fed 1/16” pelleted feed at 2% body weight.

At twenty-one and forty-three days post-vaccination, 10.1g and 19.1g fish were injected intra-peritoneally with *V. viscosus* cultured in a specialized media to an optical density of 2.2 to 2.4 at 560 nm (2% inoculum, 100 ml broth in a 250 ml shake flask for nineteen to twenty hrs at 175 rpm, 15°C. Injections were performed with a 1.0 cc syringe using a 26 gauge, 3/8” needle. Four replicate vaccinated and control groups of 25 fish each were placed in flow-through 16.6 l tanks supplied with 10°C fresh water at a 5 gph flow rate. Mortality was recorded daily for twenty-one days. Challenge at twenty-one days post-vaccination produced a cumulative mortality of 2% in vaccinated and 52% in control fish, with a relative percent survival of 96%. At forty-three days, a challenge that killed 83% of control fish produced a 1% mortality in vaccinated fish, and a relative percent survival of 98% (Fig 3). The high relative percentage survival in vaccinated fish challenged with *V. viscosus* demonstrates that winter ulcer can be successfully prevented by vaccination.

#### *Polyvalent vaccine against Vibrio anguillarum serotype I & II, V. salmonicida, and A. salmonicida*

At the beginning of fish vaccine era, most of the commercial vaccines were introduced by immersion of monovalent vaccine. By the early 1990s the oil adjuvant technology was incorporated into fish vaccines, and the fish were immunized by intraperitoneal injection with polyvalent vaccines instead of monovalent vaccine. This chapter will briefly discuss the etiological agents and the efficacy of the polyvalent vaccine against *Vibrio anguillarum*, *Aeromonas salmonicida* and *V. salmonicida*.

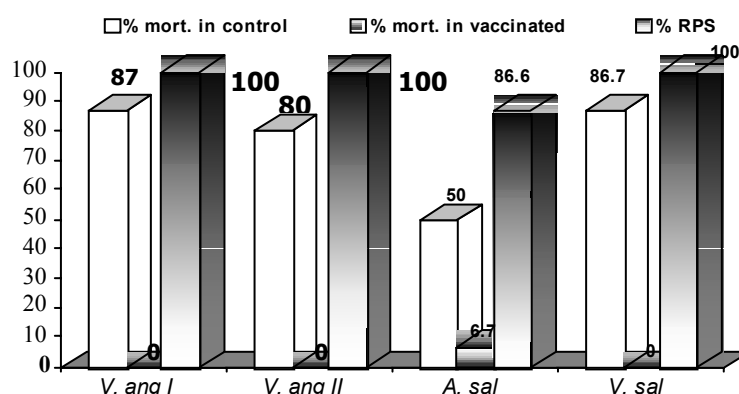
*Vibrio salmonicida* is the causative agent of cold-water vibriosis or Hitra disease (Egidius *et al.* 1981). It is classified under the Family Vibrionaceae. It is a short, 0.3-1.0 µm by 1.5-1.8 µm, curved rods. The bacterium is halophilic and requires at least 0.5% NaCl in the medium for growth. It is sensitive to vibriostat 0/129 (2,4-diamino 6,7-diisopropyl pteridine phosphate) and novobiocin. Hitra disease was one of the most serious diseases in Norwegian fish farming in the 1980s. The disease derives its name from heavy outbreaks in the island of Hitra in 1979 and 1980. Outbreaks of cold water vibriosis have also been reported in the Shetland Islands, Faroe Islands, and Canada. The typical external signs include hemorrhaging of the skin, the area around the gills, and the vent. Internally, hemorrhaging may be evident in all organs including muscles. The liver may be pale. Histologically, necrosis can be observed in the kidney, muscle, gastro-intestinal tract, spleen, and gills. These clinical signs are different from the clinical signs of disease caused by *Aeromonas salmonicida*.

*Aeromonas salmonicida* is a gram-negative, non-motile and cytochrome oxidase positive bacteria. The majority of the strains produce a brown diffusing pigment. *Aeromonas salmonicida* is the causative agent of furunculosis disease (Bullock and Stuckey 1975). The disease is named after the raised liquefactive muscle lesions (furuncles). Furuncles sometimes occur in chronically infected fish although these lesions are rarely seen in acute infection (McCarthy and Roberts 1980). The clinical signs include darkening and going off feed. Internally, the viscera are hemorrhagic, the kidney tissue is very soft, the spleen is enlarged and the liver is very pale or mottled with petechiae. In sub-acute form skin lesion are present. Internally, there is intestinal inflammation and hemorrhaging in various organs.

*Vibrio anguillarum* is a gram-negative bacteria of the family Vibrionaceae. It is a short curved rod, motile, and cytochrome oxidase positive. It is sensitive to vibriostat 0/129 and novobiocin. *Vibrio anguillarum* is the causative agent of vibriosis. This disease is distributed worldwide in cultured fish, principally in marine environments, but sporadic outbreaks have occurred in freshwater. The disease is normally characterized by generalized septicemia with clinical signs indistinguishable from other bacterial septicemias. The disease ranges from peracute (mortalities without gross lesion and acute (hemorrhaging of the eyes, gills, vent, skin and internal organs, ascites fluid in body cavity) to subacute and chronic (hemorrhagic ulceration of the skin and underlying muscle).

Vaccines against vibriosis, cold water vibriosis, and furunculosis were available to fish farmers as monovalent and immersion vaccine. This following section discusses the efficacy of the polyvalent oil adjuvant injectible vaccine against *Vibrio salmonicida*, *Aeromonas salmonicida* and *V. anguillarum*. Four hundred rainbow trout, with the aver-

age size of 5.36 g, were immunized by intra-peritoneal injection with the polyvalent oil adjuvant commercial product, Bioject 1900. They were kept at 15°C in a flow through circular tank with the flow rate of 24 gal per min. The other four hundred fish were kept as control, non-immunized. Three weeks post vaccination, fish were challenged with *Vibrio anguillarum* serotype O1 & O2 (causative agent of vibriosis), and *V. salmonicida* (causative agent of Hitra disease) by intra-peritoneal injection. Fish challenged with *V. anguillarum* were kept in a flow through tank at 15°C. The flow rate was 5 gal per minute. Fish challenged with *V. salmonicida* were kept in the flow through tank at 10°C and the flow rate of 5 gal per minute. Mortality was recorded for a period of fourteen days. Dead fish were confirmed for specific mortality with antiserum. At the end of 14 days, mortality in the control fish challenged with *V. anguillarum* serotype O1 was 87 % and there was no mortality in vaccinated fish. The relative percentage survival (RPS) was 100%. The results of the challenge with *V. anguillarum* serotype O2 also gave an RPS of 100% (80% mortality in control fish and no mortality in vaccinated fish). The RPS from *V. salmonicida* challenged was 100% (86.7% mortality in control and no mortality in vaccinated fish). The fish were challenged with *Aeromonas salmonicida* (causative agent of furunculosis) five weeks post immunization by intramuscular injection. They were kept at 15°C in a flow through circular tank with the flow rate of 5 gal per minute. Mortality was recorded for a period of fourteen days. The specific mortality was confirmed with antiserum. At the end of fourteen days, mortality in control fish due to *A. salmonicida* was 50% and the mortality in vaccinated fish was 6.7%, resulting in the RPS of 86.6%.



**Fig. 4.** Potency of polyvalent vaccine in rainbow trout challenged with four bacterial pathogens.

The above data indicate that the oil adjuvant polyvalent vaccine protects fish from each disease. The effectiveness of vaccine from different commercial companies varies depending upon their methods of growing organisms. Media composition, growth conditions, and the downstream processing affect the efficacy of final product.

#### *Vaccine against Virus*

Currently there are only two commercially available viral vaccines for aquaculture. They are vaccine against Infectious Pancreatic Necrosis (IPN) and vaccine against Iridovirus in red sea bream. The vaccine against IPN shows a strong dose response when fish are immunized with low, medium, and high concentration of viral particle.

#### **Summary**

Fast and accurate diagnostics is a good tool for disease control in aquaculture. The interpretation of results is important for the success of using diagnostics in disease control. The key is to have written guidelines on results for each specific disease.

Fish vaccines can be delivered in the same manner as that for warm-blooded animals. Fish can be immunized by immersion in vaccine for a short period of time (thirty seconds to two minutes). They can be immunized by injection (intra-muscularly or intra-peritoneally) and orally by mixing vaccine with feed (either by top dressing or by incorporating into feed as an ingredient). Fish respond to vaccine the same way as other animals do, but since fish are cold-blooded animals, their response to vaccine depends largely upon water temperature. In general, the higher the temperature, the faster the immune response of fish to the vaccine.

Over the past 20 years, fish vaccine have become an established, proven, and cost effective method of controlling certain infectious diseases in aquacultured animals worldwide. Fish vaccines can significantly reduce disease related losses and decrease the use of antibiotics. The final result is the reduction in unit costs and more predictable production. Fish vaccines are preferable to antibiotics because they are natural biological materials that leave no residue in the product or environment. Fish vaccines will not induce a resistant strain of the disease organism. Fish vaccines are licensed by the federal government and closely regulated in the same manner as other veterinary vaccines to assure safety, potency, and efficacy.

While commercial vaccines for aquaculture protect fish against certain diseases, they should be used only as part of a comprehensive and integrated fish health management program. Fish vaccines are not a cure to all deceases. Proper husbandry still is the key to successful aquaculture.

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## **Vaccine Development and Potential for Disease Control**

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### **Abstract**

Various types of vaccines have been commercially produced against fish pathogens with the majority of them against bacterial pathogens. Successful viral vaccines have been produced but the development, testing and licensing of a vaccine for commercial use remains very time-consuming and expensive. The disease condition has to be serious and have economic consequences. The scientific background for production of a safe, efficient, and cost effective vaccine must be validated.

The ideal viral vaccine for aquaculture must be effective in preventing fish mortalities, be inexpensive to produce and license, be easily administered and provide long-term immunity. These vaccines must not only provide protection against the lethal effects of virus infection but also prevent the formation of virus persistence.

This paper examined what is required for the process of developing an effective and acceptable vaccine for Nodavirus (or any virus) and discussed some recent work on Nodavirus isolates carried out at the Institute of Aquaculture, University of Stirling. The understanding of the agent, its properties, composition and characteristics is the essential first stage towards possible vaccine development.

### **Introduction**

Disease control in cultured fish species can be achieved by a variety of methods, either through prevention or "cure". Proper husbandry, regular health monitoring, chemical treatment, use of antibiotics and vaccines can be effective in reducing losses in farmed fish. On-going processes of disease management such as examination of stocking densities, removal of mortalities, monitoring of water quality, early diagnosis, and screening of stocks are extremely important as well as "treatments". Chemical treatment of water supplies and the fish may be used and if suitable, antibiotics may provide a good level of control. Prevention, however, is often better than treatment after a disease has occurred. As a preventative measure, vaccination is becoming an increasingly important area of health management in aquaculture, as it has in human and veterinary medicine.

#### *Vaccines*

A successful vaccine must have certain characteristics. It must be effective, relatively cheap to produce, have a long-term effect, be simple to administer and prevent the virus from persisting in the fish. Vaccines can be of various types including live vaccines, killed vaccines, purified sub-units, purified proteins, recombinant DNA and multivalent vaccines. All types have advantages and disadvantages. For instance, live vaccines are relatively easy to produce but may pose a threat to wild fish stocks. Multivalent vaccines are useful in control of viruses that have many antigenic variants, but there may be problems of antigenic competition and immunodominance as occurs with bacterial antigens (Killie and Jørgenson 1994). The newest developments are in the area of DNA vaccines, which are very specific and do not involve the use of whole virus. However, there are many unanswered questions in terms of how long they persist in the fish in addition to the public and licensing authorities' perception of using this type of product in animals.

If a vaccine is to be produced against a virus, the antigen itself must be looked at closely. It is essential that the virus is detected in the fish and the relative sensitivities of different diagnostic methods used for detection evaluated. The virus should be capable of being isolated. In the case of Nodavirus, there are, at present, only two possible cell lines

that can support replication. These are SSN-1 (Frerichs *et al.* 1996) and GF-1 (Chi *et al.* 1999). It is also necessary to know the route of infectivity; the virus or sub-units require purification. Further, the immune response of the fish has to be studied in order to understand how it is stimulated and how it offers protection to the animal. Not all Nodavirus isolates are antigenically similar; therefore, the genetic diversity of the isolates must also be examined.

#### Recent Nodavirus Studies

Recent work at the University of Stirling in Scotland in conjunction with French scientists at Institut Francais de Recherche Pour l' Exploration de la Mer (IFREMER) and Agence Francaise de Securite Sanitaire Des Aliments (AFSSA) in France has resulted in the collation of a range of Nodavirus isolates, primarily of European origin. The study has been examining the effects of physical and chemical treatments on nodaviruses well as looking at the isolates' antigenic and genetic variation. Further work will focus on improving diagnostic tools for detection and investigating pathogenicity. There must be an infection model, which includes sensitive detection if possible vaccines are to be tested.

#### Physical and Chemical Treatments

The following parameters were studied with two European Nodavirus strains from diseased juvenile fish from Malta and Greece. The viruses were isolated in striped snakehead cell line (SSN-1) cultures using Leibovitz L-15 medium supplemented with 5% foetal bovine serum (FBS) and antibiotics at 20°C (Frerichs *et al.* 1996). Working preparations were obtained by further propagating each culture on SSN-1 monolayer cultures at 25°C and harvesting cell culture fluids when the infected monolayer were completely destroyed 5-6 days later. Some important findings include the following:

- No viable virus detected after 4 days at 37°C
- Viable virus detected after 4 weeks at 25°C
- No reduction in titre of virus after 6 months at 15°C
- Viable virus recovered after 1 year at 4°C

A 1:00 dilution of clarified Nodavirus cell culture harvest was prepared in a range of electrolytic solutions. All diluted virus preparations were held at 15°C and samples removed for infectivity assay at selected intervals over a period of 6 months. Virus strains used were from diseased juvenile sea bass from Greece and Malta. The mean infectivity titres of the two strains following incubation are shown in Table 1.

	1 Day	4 Days	16 days	32 Days	3 Months	6 Months
<b>HBSS</b>	8.0	8.0	7.5	7.0	6.5	4.5
<b>HBSS+ FBS</b>	9.0	8.5	9.0	7.0	7.0	4.5
<b>Freshwater</b>	6.5	7.0	7.0	5.0	4.5	0
<b>Seawater 37%</b>	6.5	6.5	7.0	6.0	5.5	4.5
<b>Seawater 20%</b>	7.0	8.5	5.5	5.0	5.5	4.0

#### Heat and UV radiation

Nodavirus was rapidly killed at 60°C. Following exposure to UV radiation at 44 $\mu$ W/cm<sup>2</sup> for 8 min there was a 3-log reduction in Nodavirus titre and a 99.99% reduction in Nodavirus titre after 10 min exposure to ultraviolet (UV) treatment.

This is relevant to possible treatment of hatchery waters as it is not possible to vaccinate young fry by injection and Nodavirus problems are common in hatchery systems.

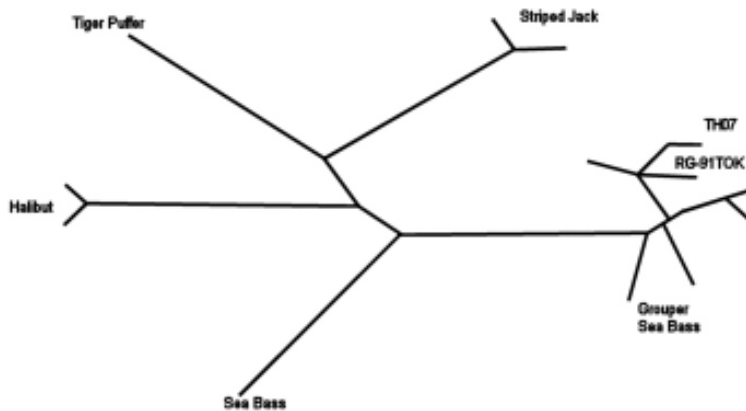
#### Chemical treatment

Formalin was not particularly effective and gave less than a 2-log reduction in virus titre after 30 min treatment with 0.025% formalin. However, with a 2% formalin solution, a 4-log reduction in Nodavirus titre was achieved in 30 min. Chlorine and iodine were found to be effective in inactivating Nodavirus but not in the presence of FBS. This protection of virus with the addition of FBS was also found with chlorine where there was less than a 2-log reduction in Nodavirus activity after 30 min treatment with 50 ppm chlorine when FBS was present. Peroxide was also not particularly effective in killing Nodavirus.

These results illustrate the stability of Nodavirus and re-inforce the requirement for a more effective method of control, *i.e.* a vaccine, against the agent.

*Phylogenetic analysis*

Genetic variation has to be considered when a vaccine is developed. The variation may cause problems in development of molecular based diagnostic tests. Our recent work has indicated that there are five distinct phylogenetic groups of Nodavirus present in fish. Results from phylogenetic analysis are shown in Fig 1. Although this analysis was carried out on a limited number of strains, the grouper nodaviruses RG-91TOK (Nishizawa *et al.* 1997) and TH07 included in this study, both fall within the same phylogenetic group.



**Fig 1.** Phylogenetic diagram of nodavirus relationship

*Towards a Vaccine – Evaluation and Strategies*

When a decision is made to develop a vaccine, all required information and processes need to be carefully considered. Initial work will include crude vaccine development followed by extensive laboratory and field level testing and evaluation. Trials must include target and non-target fish. Any vaccine produced must be quality controlled and tested, *e.g.* for sterility. Regulatory controls and financial constraints are possibly the biggest difficulties that development of any potential vaccine would face. Any product produced commercially must be economically viable to the company producing it. This type of work is unfortunately financially dependant, but the rewards in terms of protection to a high priced and valuable fish such as grouper could justify this. A vaccine should demonstrate cost effectiveness in terms of protecting the fish given that development costs are high. The protection rate should be evaluated. Areas such as immunosuppression should be examined closely before any product becomes available for use. Environmental issues should also be examined and data made available to illustrate that there would be no harm to humans or other fish species.

Consideration must also be given to appropriate strategies that will be used if a suitable and effective vaccine can be produced. The best route of administration must be determined. This route must be practical to carry out and is not restricted to farms with specific facilities or capabilities. There would possibly be special equipment required. A vaccination programme may require farmers to be trained in practical and technical methods of vaccine administration.

**Summary**

A cheap oral vaccine which confers 100% protection to grouper after a single feed is not currently available. A more realistic goal is a practical, cost-effective vaccine, which reduces losses to an acceptable level. There is a need for a vaccine against Nodavirus and this need will probably increase as the disease problems caused by Nodavirus spread to more species and to more countries. A large amount of work is required before we can have a suitable vaccine available for use by farmers and aquaculturists.

Work must be carried out on the isolation and characterization of the different nodaviruses present in grouper, along with comparative studies to determine the relationship of these to each other. The immune response of grouper following infection has to be studied. If a vaccine were found to offer protection in the laboratory, then comprehensive field trials would be required. Commercial involvement of vaccine companies in all stages of the process would be extremely beneficial and crucial during the later stages of development.



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## **Import Risk Analysis in Minimizing Trans-boundary Movement of Infectious Disease Agents**

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### **Abstract**

The General Agreement on Tariffs and Trade (GATT) commenced in 1948 and provided international rules for trade in industrial products. In 1994, GATT ("the Uruguay Round") members agreed to the replacement of GATT by a new international forum for trade, the World Trade Organization (WTO). The WTO introduced a number of new agreements for international trade including the Agreement on the Application of Sanitary and Phytosanitary Measures (the "SPS Agreement"). The principal objective of the SPS Agreement is to ensure that governments do not use food safety and quarantine requirements as unjustified trade barriers to protect their domestic agricultural industries from competitive imports.

The SPS Agreement ensures that governments can give health protection priority over trade. Governments explicitly have the right to impose restrictions on international trade when these are necessary to protect human, animal or plant health from certain risks. However, governments should be able to demonstrate that the trade restriction is indeed necessary to protect health, that is, that there is scientific evidence of potential health risks in the absence of a protective measure. Essentially two options are available to governments in order to provide a scientific justification for a trade barrier. The first, and the most encouraged by the WTO, is for governments to make use of internationally developed standards, guidelines and recommendations. Where such international standards do not exist, or in cases where a government chooses not to use an international standard, the importing country must be able to show that its measure is based on an assessment of the potential health risks. Where there is not sufficient scientific evidence to demonstrate a health risk, a government can nonetheless take a precautionary approach and provisionally impose a measure. In these cases, the government must seek further scientific evidence and review its provisional measure within a reasonable period of time.

This paper describes the general import risk analysis (IRA) framework used by the Australian Quarantine and Inspection Service (AQIS) to evaluate quarantine risk presented by the importation of commodities derived from animal (including fish) and plant sources. It outlines the basic import risk analysis parameters that must be addressed to meet international obligations as a member of the WTO. The paper also includes definitions provided by the Office International des Epizooties (OIE) for import risk analysis terminology and a brief discussion of some limitations to the application of import risk analysis.

### **Introduction**

Import risk analyses (IRAs) carried out by Agriculture Fisheries and Forestry Australia (AFFA) follow the principles laid out in the following publication, *The AQIS Import Risk Analysis Process: A Handbook* (AQIS 1998). This process is consistent with Australia's obligations under the SPS Agreement, and relevant recommendations of the Office International des Epizooties (OIE). Copies of the Handbook may be obtained from AFFA, or viewed on the AFFA homepage (<http://www.affa.gov.au>). IRAs carried out by AFFA are:

*Conducted in a consultative framework*  
*A scientific process and therefore politically independent*  
*A transparent and open process*

*Consistent with both government policy and Australia's international obligations*  
*Harmonized through taking account of international standards and guidelines*  
*Subject to appeal on the process*

Proposals requiring an IRA - those involving significant variations in established policy are generally addressed via a *non-routine* process that incorporates direct involvement in the IRA process of specialist scientists external to AFFA. Less complex changes to or reviews of established policy are generally handled through a *routine* process conducted by a team of personnel within AFFA.

### **The World Trade Organization (WTO) Environment for Import Risk Analysis**

Risk analysis methodologies utilised by AFFA are intended to comply with the obligations imposed on Members of the WTO by the WTO Agreement on the Application of Sanitary and Phytosanitary Measures, the SPS Agreement. The SPS Agreement provides that quarantine measures may only be applied for the protection of human, animal or plant life or health.

The SPS Agreement contains 14 articles and three annexes. Of the many principles outlined in these articles and annexes, the following are considered pivotal to these guidelines for import risk analysis:

*Basic rights and obligations of WTO Members*  
*Harmonisation*  
*Equivalence*  
*Risk assessment*  
*The appropriate level of protection*  
*Consistency in risk management*

### **BASIC RIGHTS AND OBLIGATIONS**

Article 2 of the SPS Agreement states that:

*“Members have the right to take sanitary and phytosanitary measures necessary for the protection of human, animal or plant life or health, provided that such measures are not inconsistent with the agreement.*

*Members shall ensure that any measure is applied only to the extent necessary to protect human, plant or animal health or life, is based on scientific principles and is not maintained without sufficient scientific evidence, except as provided in paragraph 7 of Article 5 (Risk Assessment).*

*Members shall ensure that their sanitary and phytosanitary measures do not arbitrarily or unjustifiably discriminate between members where identical or similar conditions prevail, including between their own territory and that of other Members. Sanitary and phytosanitary measures shall not be applied in a manner which would constitute a disguised restriction on international trade.”*

These statements underpin the fundamental WTO requirement that SPS measures adopted by Members should be no more restrictive to trade than is necessary to protect human, animal or plant life or health, and that such measures should not be used as disguised barriers to trade. These ‘basic rights and obligations’ establish the need for consistency in risk management, national treatment (all things being equal, imported goods should not be treated differently to domestically produced goods), and the scientific justification of trade restrictions based on SPS measures. The other articles and annexes to the SPS Agreement elaborate on the basic rights and obligations as well as containing machinery provisions for the implementation of the Agreement.

### **HARMONIZATION**

Article 3 of the SPS Agreement states that:

*“To harmonize sanitary and phytosanitary measures on as wide a base as possible, Members shall base their sanitary and phytosanitary measures on international standards and guidelines or recommendations.*

*Sanitary or phytosanitary measures which conform to international standards shall be deemed to be necessary to protect human, animal or plant life or health, and presumed to be consistent with the relevant provisions of this Agreement and with GATT 1994.*

*Members may introduce or maintain sanitary or phytosanitary measures which result in a higher level of sanitary or phytosanitary protection than would be achieved by measures based on the relevant international standards, guidelines or recommendations, if there is a scientific justification, or as a consequence of the level of sanitary or phytosanitary protection a Member determines to be appropriate in accordance with the relevant provisions of paragraphs 1 through 8 of Article 5”.*

Standards for sanitary measures for animal health are provided in the OIE Code (OIE 1999), the OIE Aquatic Animal Health Code (OIE 1997a) and the OIE Manuals (OIE 1996; OIE 1997b). Standards for phytosanitary measures for plant health are outlined in the IPPC (International Plant Protection Convention) series of International Standards for Phytosanitary Measures (FAO 1996).

The WTO principle of harmonisation thus requires that SPS measures adopted by a Member shall be based on these standards (where they exist), unless that country can demonstrate that there is scientific justification or that the relevant international standard does not provide an appropriate level of protection from particular animal or plant diseases or pests.

## **EQUIVALENCE**

Article 4 of the SPS Agreement states that:

*“Members shall accept the sanitary or phytosanitary measures of other Members as equivalent, even if these measures differ from their own or from those used by other Members trading in the same product, if the exporting Member objectively demonstrates to the importing Member that its measures achieve the importing Member’s appropriate level of sanitary or phytosanitary protection”.*

WTO Members must accept the SPS measures of other Members as equivalent to their own if the latter can demonstrate objectively that their measures provide the level of protection required by the importing country. Often there are a number of alternative measures that may either singly or in combination achieve the appropriate level of protection, or ALOP, (for example, treatment, quarantine or increased inspection). In choosing among such alternatives, a WTO Member should put in place measures that are no more trade restrictive than required to achieve its health protection objectives, provided those measures are technically and economically feasible. In doing so, the importing country should remain open to approaches from exporting countries with regard to alternative measures that may meet its ALOP.

## **RISK ASSESSMENT**

Article 5, paragraphs 1 to 3, of the SPS Agreement outlines the requirements that Members should follow when carrying out import risk assessments. A definition of import risk assessment is also provided in Annex A to the SPS Agreement.

*“Members shall ensure that their sanitary or phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to human, animal or plant life or health, taking into account risk assessment techniques developed by the relevant international organisations”.*

This paragraph requires Members to take into account the standards developed by the ‘relevant scientific organisations’ when evaluating risk. A standard methodology for import risk analysis for terrestrial animals and animal products is provided in the OIE Code, while that for aquatic animals is given in the OIE Aquatic Animal Health Code. The OIE Code chapter on import risk analysis was comprehensively revised and expanded, and accepted by the OIE International Committee in May 1999. The OIE Aquatic Code chapter was revised in-line with the OIE Code in May 2000.

The second paragraph of Article 5 outlines factors that should be considered when assessing the risks associated with a proposed importation. Specifically, it is stated that *“in the assessment of risks Members shall take into account available scientific evidence, relevant processes and production methods, relevant inspection, sampling and testing methods, prevalence of specific diseases or pests, existence of pest- or disease-free areas, relevant ecological or environmental conditions, and quarantine or other treatment”*. This paragraph is particularly pertinent since it places an emphasis on the need to consider a wide range of ‘factors’ in both the *importing* and *exporting* country. That is, it will not be satisfactory to examine only the potential for a disease agent or pest to be transmitted in a given commodity.

The third paragraph of Article 5 describes the need to include a consequence assessment in a risk assessment, and lists dimensions that should be considered when assessing ‘potential damage’ arising from a disease or pest incursion. Specifically, it is stated that:

*“Members shall take into account as relevant economic factors, the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease, the cost of control or eradication in the territory of the importing Member”.*

This list of ‘relevant economic factors’ may be viewed as the bare minimum that must be considered if an analysis is to be compliant with the terms of the SPS Agreement. The OIE Code outlines factors that should be considered when assessing consequences and the need to consider the ‘likely magnitude’ of consequences - that is, to base an assessment of consequences on the likelihood of establishment and spread in the importing country. Finally, Members need also to consider *“the relative cost-effectiveness of alternative approaches to limiting risks”*. This is an issue that should be explored during risk management. Among factors that may not be taken into account are those relating to import competition.

The following definition is generally applicable to quarantine risk assessments:

*“The evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing Member according to the sanitary or phytosanitary measures which might be applied, and of the associated potential biological and economic consequences”.*

From this definition a risk assessment must as a minimum:

Identify the diseases whose entry, establishment or spread a Member wants to prevent within its territory, as well as the potential biological and economic consequences associated with the entry, establishment or spread of these diseases

Evaluate the likelihood of entry, establishment or spread of these diseases, as well as the associated potential biological and economic consequences

Evaluate the likelihood of entry, establishment or spread of these diseases according to the SPS measures that might be applied

Paragraph seven provides for the use of precaution when information is insufficient. This paragraph states that:

*“In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organizations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time”.*

In adopting provisional measures, members must base these on available information including the approach of other countries and international standards. Countries adopting provisional measures have the obligation to seek additional information in a timely manner, in order to objectively assess risks.

### **APPROPRIATE LEVEL OF PROTECTION (ALOP)**

The SPS Agreement defines *“appropriate level of sanitary or phytosanitary protection”* as the level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory. The SPS Agreement notes that many Members also refer to this concept as the *“acceptable level of risk”*. In setting their ALOP, WTO Members are to take into account the objective of minimising negative trade effects (Article 5, paragraph 4, SPS Agreement).

ALOP can be illustrated using a *risk estimation matrix* (refer to Table 1). The cells of this matrix describe the product of likelihood and consequence - termed ‘risk’, or ‘expected impact’ - in the same terms as the criteria used in the original consequence assessment (see above). When interpreting the risk estimation matrix, it should be remembered that although the descriptors for each axis are similar (‘low’, ‘moderate’, ‘high’, *etc.*), the vertical axis refers to likelihood and the horizontal axis refers to consequence. The implication of this is that a ‘negligible’ probability combined with an ‘extreme’ consequence, is not the same as an ‘extreme’ probability combined with a ‘negligible’ consequence - that is, that the matrix is not symmetrical.

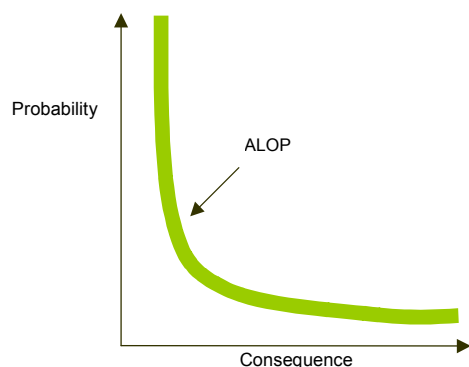
**Table 1.** Risk estimation matrix

Likelihood of entry and exposure	<u>High</u>	Very low	Low	Moderate	High	Extreme	Extreme
	<u>Moderate</u>	Negligible	Very low	Low	Moderate	High	Extreme
	<u>Low</u>	Negligible	Negligible	Very low	Low	Moderate	High
	<u>V. Low</u>	Negligible	Negligible	Negligible	Very low	Low	Moderate
	<u>E. Low</u>	Negligible	Negligible	Negligible	Negligible	Very low	Low
	<u>Negligible</u>	Negligible	Negligible	Negligible	Negligible	Negligible	Very low
		<u>Negligible</u>	<u>Very low</u>	<u>Low</u>	<u>Moderate</u>	<u>High</u>	<u>Extreme</u>

Consequence of entry and exposure

In the sample matrix provided in Table 1, the band of cells marked ‘very low’ is intended to represent the ALOP for a given country (note: the ALOP may vary between countries such that another country’s ALOP may be represented by the band of cells marked “low”). This band of cells represents an approximation of a continuous ‘iso-risk curve’ - a curve that will be asymptotic at the minimum level of consequence considered to be ‘acceptable’ and at a likelihood that tends toward zero. The principle of an iso-risk curve is illustrated in Figure 1.

**Figure 1.** Theoretical iso-risk curve



**CONSISTENCY IN RISK MANAGEMENT**

The fifth paragraph of Article 5 states:

*“With the objective of achieving consistency in the application of the concept of appropriate level of sanitary or phytosanitary protection against risks to human life or health, or to animal and plant life or health, each Member shall avoid arbitrary or unjustifiable distinctions in the levels it considers to be appropriate in different situations, if such distinctions result in discrimination or a disguised restriction on international trade”.*

This paragraph has the objective of consistent application of the ALOP by countries imposing SPS measures. Countries are under an obligation to avoid ‘arbitrary or unjustifiable distinctions’ between the level of risk considered to be appropriate in different situations if this results in discrimination or a disguised restriction on trade.

## THE IMPORT RISK ANALYSIS PROCESS

To support the carrying out of import risk analyses that are science-based, objective, defensible and transparent, the OIE Aquatic Animal Health Code contains a standardized sequence of tasks or procedures (Fig 2). Collectively, these procedures comprise the relevant ‘international standard’ for the conduct of import risk analyses for aquatic animals and their products.

**Fig 2.** The OIE approach to import risk analysis.

These steps are defined as follows in the OIE Code:

*Hazard identification:* The process of identifying the pathogenic agents that could potentially be introduced in the commodity considered for importation.

- Exposure assessment

*Risk:* The likelihood of the occurrence and the likely magnitude of the consequences of an adverse event to animal or human health in the importing country during a specified time period.

- Consequence assessment
- Risk estimation

*Risk assessment:* The evaluation of the likelihood and the biological and economic consequences of entry, establishment or spread of a pathogenic agent within the territory of an importing country.

*Release assessment:* A description of the biological pathways necessary for an importation activity to ‘release’ (that is, introduce) pathogenic agents into a particular environment, and an estimation of the probability (qualitative or quantitative) of the complete process occurring.

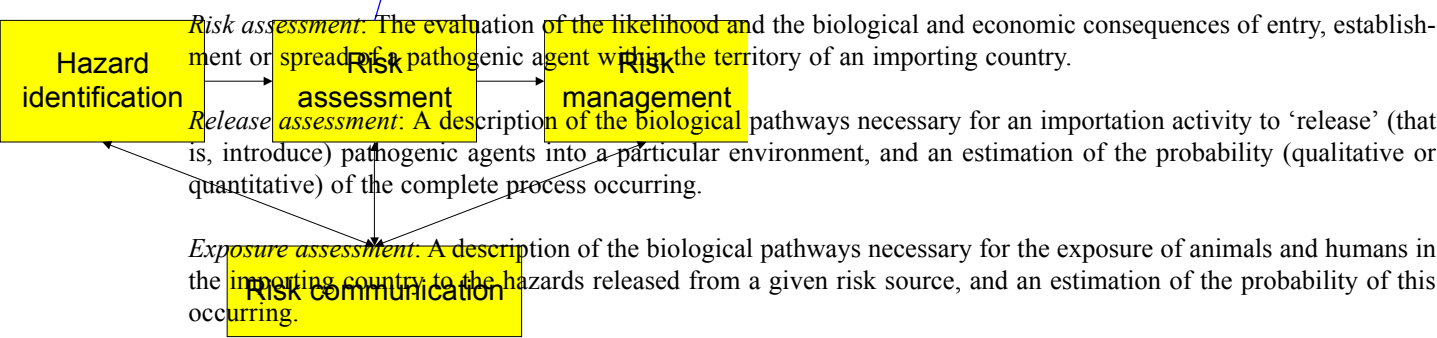
*Exposure assessment:* A description of the biological pathways necessary for the exposure of animals and humans in the importing country to the hazards released from a given risk source, and an estimation of the probability of this occurring.

*Consequence assessment:* A description of the potential consequences of a given exposure and an estimate of the likelihood that each will occur. The consequence assessment may be qualitative or quantitative.

*Risk estimation :* An integration of the results of the release assessment, exposure assessment and consequence assessment to produce an overall measure of the risk associated with each identified hazard.

*Risk management:* The process of identifying, selecting and implementing measures that can be applied to reduce the level of risk.

*Risk communication:* The process by which information and opinions regarding hazards and risks are gathered from potentially affected and interested parties during a risk analysis, and by which the results of the risk assessment and proposed risk management measures are communicated to the decision makers and interested parties in the importing and exporting countries.



**Discussion and Recommendations**

Import risk analysis is used to identify and classify potential quarantine risks and develop risk management procedures to address these risks. The SPS Agreement encourages Member countries to base their national SPS measures on relevant international standards, guidelines and recommendations. Governments may choose national measures that provide a higher level of protection than relevant international standards subject to conformity with obligations relating to risk assessment and a consistent approach to risk management. In assessing risks, WTO Members are required to take into account available scientific evidence; relevant processes and production methods, inspection, sampling and testing methods; prevalence of specific diseases and pests, existence of disease/pest free regions, relevant ecological and environmental conditions, efficacy of quarantine and/or other treatment measures. The approach adopted by Australia to import risk analysis is highly consultative to ensure broad opportunity for scientific comment of all stakeholders; accordingly an IRA will often take at least 18 months to complete. AQIS has completed IRAs on salmonid and non-salmonid finfish products while experience in import risk analysis on live aquatic animals is limited to live ornamental finfish. Final reports are available electronically on the AFFA website at the following address: [http://www.affa.gov.au/docs/market\\_access/biosecurity/animal/irafnl.html](http://www.affa.gov.au/docs/market_access/biosecurity/animal/irafnl.html).

The availability of substantive disease surveillance information in an importing country is a general prerequisite to demonstrate country/regional freedom from specific disease agents as the benchmark that demonstrates a potential need to apply sanitary measures. Surveillance, monitoring and disease reporting for finfish in Australia is coordinated under a national strategic plan for aquatic animal health – AQUAPLAN (<http://www.affa.gov.au/docs/animalplanhealth/aquatic/aquaplan.html>). States and Territories provide quarterly reports to the Office of the Chief Veterinary Officer on the occurrence of listed diseases during each month of the quarter. In addition, a legislative basis must be available in an importing country to enable enforcement of sanitary measures recommended in an import risk analysis. In the absence of adequate disease surveillance information to justify the application of sanitary measures on imported finfish, the development of appropriate voluntary sanitary standards/guidelines for export hatcheries that supply fry/fingerlings to the Asia-Pacific region may help to minimise the risk of trans-boundary movement of infectious pathogens. Scientific input from fish disease specialists could be used to develop guidelines for hatchery management to minimise the risk of introduction, establishment and/or transmission of infectious agents of concern to finfish aquaculture in the region.

With respect to training on IRA for aquatic animal diseases, AFFA may be able to conduct short-course (e.g. 2 days) training workshops for NACA members and APEC economies in IRA methodology and implementation should there be sufficient demand/interest. A minimum number of 8 participants would be required to enable AFFA to conduct a training workshop. Course work will essentially cover the OIE approach to Import Risk Analysis (hazard identification, risk assessment, risk management and risk communication) and will focus on qualitative risk assessment methodology.

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## Review of Grouper Diseases and Health Management Strategies for Groupers and other Marine Finfishes

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### Abstract

Grouper has recently become one of the most important aquaculture and trade commodities in the Asia-Pacific region. The expanding trade in live grouper of different ages and stages (whether for aquaculture or for seafood restaurants) increases the risks of moving pathogens. The number of diseases affecting grouper has expanded steadily with expansion and intensification of grouper aquaculture. These include infectious (virus, bacteria and parasites) and non-infectious (environment, management, nutritional) diseases and a number of diseases yet undiagnosed or of unknown origin. Apart from some virus problems in Southeast Asia, very little is known about the impact of major diseases which may go beyond direct mortalities and production losses. They affect all levels of aquaculture activity and are profoundly felt by small-scale farmers who represent the backbone of many rural communities in Asian aquaculture. Their livelihoods are threatened through reduction in food availability, loss of income and employment, social upheaval and increased vulnerability.

Unless appropriate measures are implemented, major epidemics will continue to threaten the industry. It may cost more to contain them later. This paper presents available information on infectious and other diseases affecting grouper aquaculture in the Asia-Pacific region and current disease control practices. It also provides some recommended health management options, which may be applied or which can be further explored for attaining high health status to sustain grouper aquaculture.

### Introduction

Grouper has recently become one of the most important aquaculture and trade commodities in the Asia-Pacific region. It is also an important fish in the livelihoods of small and large-scale coastal fish farmers. The intensified trade in live groupers resulted from a number of recent developments: increased consumption and high cultural and social preference for this fish; the growing live seafood market and restaurants in China PR, Hong Kong China, Chinese Taipei and Singapore (and to a lesser extent in most economies of the Asia-Pacific region); and intensified aquaculture due to high economic returns. Groupers are now considered a high-value species with a high potential for contributing to the economic development of these countries. Hong Kong China and China PR are the main markets for live grouper, other smaller markets include Chinese Taipei and Singapore. The main suppliers of live grouper to Hong Kong SAR China are Indonesia, Philippines, Malaysia, Thailand, Vietnam and Australia, with a large portion eventually shipped to China PR (Pawiro 1999).

This paper reviews the reported diseases, which affect grouper belonging to the genera *Epinephelus*, *Cromileptes* and *Plectropomus* (Table 1). It also covers health management strategies available for grouper and other marine finfish. The review of health management strategies is based on published reports and the scientific literature available to the authors at the time of writing. The paper provides some additional recommendations on dealing with trans-boundary aquatic animal diseases. It also includes an update on APEC FWG<sup>1</sup> 02/2000 "Development of a Regional Research Program on Grouper Virus Transmission and Vaccine Development".

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### *Impacts of Trans-boundary Diseases*

Trans-boundary diseases are epidemic diseases which are highly contagious or transmissible and have the potential for very rapid spread across national borders. These diseases cause serious socio-economic and possibly public health consequences (Baldock 2001).

Some examples of recent trans-boundary aquatic animal diseases which had major social and economic impacts in the Asia-Pacific region are the epizootic ulcerative syndrome or EUS of freshwater and brackish water fish, and the viral diseases of shrimp (white spot syndrome virus, yellow head virus and taura syndrome virus). The impact of these diseases goes beyond direct mortalities and production losses and affect all levels of aquaculture activity. They are also profoundly felt by small-scale farmers who represent the backbone of many rural communities in Asian aquaculture. Their livelihoods are threatened by reduction in food availability, loss of income and employment, social upheaval and increased vulnerability. In large-scale aquaculture, the impact on investor confidence is considerable, with a further impact on the employment and income of rural communities where such operations exist. There is also increasing concern regarding newly emerging diseases facing the Asian region. These include marine finfish mortalities due to viral diseases among seabass and groupers; epizootics in shellfish (scallop mortalities in China, pearl oyster mortalities in Japan, Indonesia, Philippines); and the spawner mortality virus (SMV) in shrimp.

Among grouper diseases, a disease of viral origin, a monogenean parasite, and a variety of other parasites were reported to be associated with importing of live fish. *Neobenedenia girellae*, one of the most commonly reported parasites of grouper, has been introduced to Japan by imports of amberjack fry from Hainan, China and Hong Kong (Ogawa *et al.* 1995a). The other disease of viral origin, 'red grouper reovirus', was reported by Chew-Lim *et al.* (1992) in red grouper (*Plectropomus maculatus*) imported from Indonesia for culture in Singapore. Earlier reports by Leong and Wong (1990) showed that grouper imported from Thailand and Philippines for cage culture in Malaysia were heavily infected with a wide variety of parasites. Chong and Chao (1986) reported that import mortalities are the single most important source of loss to local fish farmers in Singapore.

Statistics on economic impact of marine finfish diseases including diseases of groupers are scarce. In Japan, losses due to marine fish disease amount to approximately US\$ 114.4 M (1992). In Thailand, losses in marine cage-cultured seabass and grouper due to diseases were about US\$ 1.9 M in 1989. In Malaysia, vibriosis in sea-caged farms resulted in losses equivalent to MR\$ 20 M in 1990 (more than US\$ 5 M). In a survey of the disease situation of marine fish in Singapore, Chua *et al.* (1993) estimated losses due to diseases at two farms at S\$ 126 800 and S\$ 233 700.

In a survey on the impact of fish health problems on rural small-scale farmers involved in grouper culture in the Philippines, 75% of respondents experienced reduction in income due to fish health and disease problems, while 19.44% incurred increased household debt, particularly those who borrowed capital for investment (Somga *et al.* 2001). In Thailand where finfish cage culture of seabass and grouper is mostly comprised of small farms (one to five cages), more than 80% of farmers reported losses ranging from 30% to 50% due to fish diseases (Kanchanakhan *et al.* 2001).

The expanding trade in live grouper of different ages and stages for aquaculture or for the seafood restaurants without appropriate quarantine and health considerations increases the risks of moving pathogens that may come along with the movement of host fish. Unless measures are implemented, major epidemics continue to threaten the region. It may cost more to contain them and would cast doubt on the sustainability of grouper aquaculture.

**Table 1.** Grouper species: scientific names and common names included in this review

Scientific Name	Common Name <sup>1</sup>	Country <sup>2</sup>
1. <i>Epinephelus akaara</i>	Hong Kong grouper (FB 96, FAO)	China PR, Japan
	Red-spotted grouper (Hawaii, FB 96)	
	Kijihata (Japan, FB 96)	
	Red grouper (Hong Kong, FB 96)	
2. <i>E. awoara</i>	Yellow grouper (FB 96, FAO)	China PR
	Green-spotted grouper (China, Chen 1995)	
	Aohata (Japan, FB 96)	
3. <i>E. bontoides</i>	Pale margin grouper (FB 96)	Indonesia
4. <i>E. coioides</i> = <i>E. suillus</i> (synonym)	Orange-spotted grouper (FB 96)	Indonesia, Philippines, Thailand
	Estuary grouper (Fish Base 96)	
	Estuary cod (Australia, FB 96)	
	Chi hou (Singapore, Malay/Indonesia, FB 96)	
	Estuary grouper (Hong Kong, FB 96)	
	Chairomaruhata (Japanese)	
5. <i>E. chlorrostigma</i>	(Hua <i>et al.</i> 1994)	China
6. <i>E. cyanopodus</i>	Speckled blue grouper (FB 96)	Japan
7. <i>E. fuscoguttatus</i>	Brown-marbled grouper (FB 96, FAO)	Indonesia, Malaysia
	Flowery cod (Australia, FB 96)	
	Tiger grouper (Malaysia)	
8. <i>E. malabaricus</i>	Malabar grouper (FB 96)	Indonesia
	Brown-spotted grouper (Thailand, Danayadol <i>et al.</i> 1996)	Thailand
9. <i>E. moara</i>	Kue (Japanese, FB 96)	Japan
	Kelp grouper (Nakai <i>et al.</i> 1994, Muroga 1995)	
10. <i>E. salmoides</i>	(Ong 1988)	Malaysia
11. <i>E. septemfasciatus</i>	Convict grouper (FB 96)	Japan Korea RO
	Sevenband grouper (Japan, Fukuda <i>et al.</i> 1996)	
	Mahata (Japanese, FB 96)	
12. <i>E. tauvina</i>	Greasy grouper (FB 96)	Malaysia, Philippines Singapore
	Brown-spotted grouper (Malaysia, Chua <i>et al.</i> 1994)	
	Estuarine grouper (Malaysia, Nash <i>et al.</i> 1987)	
13. <i>Epinephelus spp.</i>		Philippines, Taipei China, Myanmar
14. <i>Cromileptes altivelis</i>	Humpback grouper (FB 96)	Indonesia
	Barramundi cod (Australia, FB 96)	
15. <i>Plectropomus leopardus</i>	Leopard coral grouper (FB 96, FAO)	Indonesia
	Common coral trout (Australia, FB96)	
16. <i>Plectropomus maculatus</i>	Spotted coral grouper (Lau and Li, 2000)	Singapore
17. <i>Plectropomus spp.</i>	Red grouper (Chua <i>et al.</i> 1993)	Singapore

<sup>1</sup> The common names indicated in this Table were mainly taken from ICLARM's Fish Base 96 (FB 96) or by another reference in some cases.

<sup>2</sup> Refer to the countries where reports of diseases are indicated in the other Tables

*Grouper Diseases in Asian Aquaculture*

Arthur and Ogawa (1996) overview of disease problems in the culture of marine finfishes in East and Southeast Asia identified the principal diseases (Box 1) in grouper (primarily *Epinephelus* spp.) cultured in Southeast Asia.

Box 1 (Arthur and Ogawa 1996)	
Environmental:	pollution, toxic plankton blooms
Management:	acclimatization/adaptation mortalities (juveniles); handling mortalities (juveniles and grow-out); transport mortalities (juveniles)
Nutritional:	spinal curvature
Viruses:	golden eye disease; Red grouper reovirus; Sleepy grouper disease (iridovirus); Spinning grouper disease (picorna-like virus)
Bacteria:	red boil disease, streptococcosis ( <i>Streptococcus</i> sp.); Vibriosis, hemorrhagic septicaemia ( <i>V. alginolyticus</i> , <i>V. parahaemolyticus</i> )
Parasites:	Protozoa ( <i>Amyloodinium</i> , so., <i>Brooklynella</i> sp., <i>Cryptocaryon irritans</i> , <i>Trichodina</i> sp.) Monogenea ( <i>Benedenia</i> sp., <i>Megalocotylodes epinepheli</i> , <i>Pseudorhabdosynochus epinepheli</i> )
Fungi:	<i>Ichthyophonus</i> sp.
Diseases of unknown aetiology: popeye, blindness, swimbladder syndrome	

The following section (updated from Arthur and Ogawa, 1996) presents information on infectious diseases (viral, bacterial and parasitic) and diseases which are either undiagnosed or of unknown origin but of increasing importance.

**Viral Diseases**

The first grouper viral disease was reported by Mori *et al.* (1991) as a disease similar to Viral Nervous Necrosis (VNN) affecting larval and juvenile stages of *E. akaara* in Japan and which caused 80% mortality. In 1994, Nakai *et al.* reported hatchery mass mortalities among kelp grouper, *E. moara*. This time it was diagnosed as VNN. Since 1995, reports of viral infection (mainly of Nodaviruses) in Singapore, Thailand, Japan and Korea have been published. Chua *et al.* (1995) reported a 'spinning grouper disease' which caused heavy mortalities among *E. tauvina* fry for two weeks in 1991 in Singapore. There was no visible external or internal lesions but affected fry exhibited loss of equilibrium, uncoordinated and weak swimming movements, swimming in circles and in some cases splastic lateral flexure of the body. Chew-Lim *et al.* (1998) isolated a nodavirus comparable to the striped jack nervous necrosis virus (SJNNV). Nodavirus was collected on *E. tauvina* juvenile samples (2-4 cm). It caused high mortalities during the period from 1986 to 1991 in Singapore. In Japan, Fukuda *et al.* (1996) reported mortalities of sevenband grouper *E. septemfasciatus*, with body weights ranging from 170 g to 1850 g during the summer season (July to October) in 1993-1994 when water temperature was between 25°C to 28°C. Affected fish exhibited upside down swimming, inflation of the swim bladder and degeneration of nervous tissues. The disease was diagnosed as VNN. Sohn and Park (1998) reported VNN among sevenband grouper in Korea. The disease occurred during the summer season, with mortalities reaching more than 80% within a few weeks. Clinical signs included anorexia, dark coloration, spiral swimming behaviour and vertebral deformity. In Thailand, 'paralytic syndrome' caused by VNN was reported by Danayadol *et al.* (1995) among 2.5 to 15.0 cm sized *E. malabaricus*, with mortality reaching 100% in small fish and less than 20% in large fish. Clinical signs included lethargy, dark body coloration, loss of appetite and corkscrew swimming.

The second viral disease, 'sleepy grouper disease', caused by Iridovirus was first reported by Chua *et al.* (1994). It occurred in early grow-out and market sized *E. tauvina* in Singapore in 1992. In Taiwan, an outbreak occurred in 1995 among *Epinephelus* spp. Chou *et al.* (1998) identified the causative agent as Grouper Iridovirus of Taiwan (TGIV). In 1999, Kasornchandra (1999) and Khongpradit *et al.* (1999) reported two types of Iridoviruses, 'GIV-1' and 'GIV-2' or blister disease, which affected unspecified grouper fry and fingerlings and *E. coioides*, respectively.

Other diseases of viral origin include the following:

- red grouper reovirus disease, which occurred in red grouper (*P. maculatus*) imported from Indonesia for culture in Singapore (Chew-Lim *et al.*, 1992)
- the golden-eye disease (astro-like virus) which caused 90% mortality among *E. tauvina* in Sumatra (see Arthur and Ogawa 1996)
- lymphocystis disease virus of *E. fuscoguttatus* in Malaysia (Oseko *et al.* 1999), and herpes virus of *E. awoara* juveniles in China (Chen 1996)

Table 2. Viral Infection of Grouper Cultured in Asia

Disease Pathogen	Host Stage Affected	Signs and Behaviour Other Characteristics	Country	Reference
<b>NODAVIRUSES</b>				
Similar to VNN	<i>E. akaara</i> larval juvenile	Larval stage: 7-8 mm TL (14 day old) Heavy mortality at 9-10 mm and continued until 20 mm Juveniles: 2.6-3.9 mm Rearing temperature for both cases: 25-27°C 80% mortality Listless swimming near surface of water, abrupt whirling and sinking to bottom	Japan	Mori <i>et al.</i> 1991
VNN	<i>E. moara</i>	Hatchery mass mortality Retina and brain of diseased fish were necrotic and vacuoles formed after degeneration of dead cells Viral particles with diameter of 28 nm densely present in the cytoplasm of neuron cells in these tissues.	Japan	Nakai <i>et al.</i> 1994 ; Muroga 1995
Nodavirus 'Spinning grouper disease'	<i>E. taurina</i>	2 weeks in 1991 among fry Heaviest mortalities 7-9 days after onset of clinical signs Loss of equilibrium, uncoordinated and weak swimming movements, swimming in circles and in some cases splayed lateral flexure of the body No visible external or internal lesion	Singapore (similar disease also reported in this paper from Malaysia and Philippines)	Chua <i>et al.</i> 1995
Paralytic syndrome VNN	<i>E. malabaricus</i>	2.5 to 15.0 cm in body length Lethargy, dark body coloration, loss of appetite, corkscrew swimming Water temperature: 28 to 30°C Mortality in small sized fish (2.5 to 7.5 cm) reached 100%, while large fish (over 15 cm) less than 20% Vacuolation and virus-like particles observed in brain and eyes	Thailand	Danayadol <i>et al.</i> 1995
VNN	<i>E. septemfasciatus</i> (Sevenband grouper) 170 to 1850 g	Summer season (July to October 1993/1994) Water temperature: 25-28 °C Upside down swimming behaviour, inflation of swimbladder, degeneration of nervous tissues	Japan	Fukuda <i>et al.</i> 1996
Nodavirus Viral Nervous Necrosis (VNN)	<i>E. septemfasciatus</i> (Sevenband grouper) Adults and larval stages	During summer season Mortality over 80% within a few weeks Anorexia, dark coloration, spiral swimming behaviour and vertebral deformity	Korea RO	Sohn and Park 1998
Nodavirus comparable to Striped Jack Nervous Necrosis Virus (SINN)	<i>E. taurina</i> juveniles	1986 to 1991 samples High mortalities in 2-4 cm juveniles	Singapore	Chew-Lim <i>et al.</i> 1998

Table 2. Viral Infection of Grouper Cultured in Asia (continued)

Disease Pathogen	Host Stage Affected	Signs and Behaviour Other Characteristics	Country	Reference
<b>IRIDO VIRUSES</b>				
Iridovirus 'Sleepy Grouper Disease'	<i>E. tauvina</i> Early grow out and market-sized	April to August 1992 50% mortality among early grow-out (100-200 g) and market-sized (2-4 kg) Affected fish generally lacked external lesion, however, were extremely lethargic and did not respond to physical stimulation, inappetence solitary and either hung at the water surface or remained at the net bottom Deaths occurred at night time or early hours of the morning Since 1992	Singapore	Chua <i>et al.</i> 1994
Iridovirus Grouper Iridovirus of Taiwan (TGIIV)	<i>Epinephelus</i> sp.	Outbreak in 1995, 60% mortality Anemia, Swimming in circles	Chinese Taipei	Chou <i>et al.</i> 1998
Iridovirus 'GIV-1'	Unspecified fry and fingerling of grouper	Reduced feed consumption, lethargy and appearance of darkening on the fish body especially on the postanal end including fin Moribund fish float up to the water surface, sink down to bottom and die 30-50% of affected fish die Icosahedral virus whose size ranges from 140-160 nm in diameter	Thailand	Kasornchandra 1999
Iridovirus 'GIV-2' 'Blister Disease'	<i>E. coioides</i>	Appearance of whitish blisters on the body and fins Highly localised severe inflammation of the epidermal and dermal layer Dermis was necrotized, containing exudation and hemorrhagic infiltration at the area of intact layer Presence of icosahedral to round-shaped virions with a diameter of 180-200 nm in infected liver, spleen, kidney and lesions	Thailand	Khongpradit <i>et al.</i> 1999
<b>REO VIRUS</b>				
Red grouper Reovirus	<i>Plectropomus maculatus</i>	Inappetence and lethargy, followed by death 2-3 days later Virus isolated from spleen: ribonucleic acid, ether resistant, and able to tolerate heat treatment up to 56°C. Electron microscopy showed a reovirus, diameter of 67-72 nm, double-membrane capsid and no envelope Similar to grass carp virus	Imported from Indonesia for culture in Singapore	Chew-Lim <i>et al.</i> 1992
<b>ASTRO-LIKE VIRUS</b>				
Golden eye disease (astro-like virus)	<i>E. tauvina</i>	90% mortality Anemia leading to suffocation of affected fish	Sumatra	cited in Arthur and Ogawa 1996 as personal communication with Leigh Owens (James Cook University)
<b>LYMPHO CYSTITIS</b>				
Lymphocystis	<i>E. fuscoguttatus</i> Juveniles (6.3-21.9 g BW)	May 1999 Many nodules on snout, lower jaws, fins and skin Abnormally dark colored patches of skin with numerous nodules superimposed with cream-colored particles	Malaysia	Oseko <i>et al.</i> 1999
<b>HERPES VIRUS</b>				
Herpes virus	<i>E. awoara</i> Juveniles	Infected fish become paralyzed and exhausted and die at bottom of cage	China	Chen 1996

It appears that there are several viruses affecting several species of cultured grouper:

- (a) nodavirus – Viral Nervous Necrosis or VNN
- (b) iridovirus – GIV-1, GIV-2 and TGIV
- (c) lymphocystis
- (d) Herpes virus
- (e) golden eye disease (astro-like virus), and
- (f) red grouper reovirus disease (see Table 2)

### Bacterial Diseases

A range of bacterial diseases has also been reported, including *Vibrio* spp., *Pseudomonas* sp., *Pasteurella piscicida* and *Flexibacter* sp. (Table 3). Bacteriosis caused by *Pseudomonas* sp. among *E. tauvina* cultured in Malaysia was first reported by Nash *et al.* (1987), where all age groups were affected during an outbreak in November/December to February/March in 1982-1986. Mortalities ranged from 20% to 60%. Affected fish showed extensive haemorrhagic erosions and ulcerations of the skin, fins and tail. Vibriosis was reported among *E. suillus* (= *E. coioides*) broodstock in cages and tanks in the Philippines (Lavilla-Pitogo *et al.* 1992), *E. tauvina* in Kuwait (Saeed 1995), *E. malabaricus* in Thailand (Chinabut 1996) and *E. malabaricus* and *Epinephelus* sp in Malaysia (Wong and Leong 1990, Palanisamy 1999). Another bacterial infection, 'red boil disease' caused by *Flexibacter* sp. among *E. malabaricus* in Thailand was reported by Danayadol *et al.* 1996. In Japan, pasteurellosis caused by *Pasteurella piscicida* is a problem among juvenile red spotted grouper in hatcheries (Muroga 1995).

**Table 3.** Bacterial Diseases of Grouper Cultured in Asia

Disease Bacteria Isolated	Species Stage Affected	Signs Behaviour Other characteristics	Country	Reference
Bacteriosis <i>Pseudomonas</i> sp.	<i>E. tauvina</i>	During northeast monsoon season (November/December to February/March)1982-1986 20-60% mortalities in raft cage cultured grouper All age groups affected Extensive haemorrhagic erosions and ulcerations of the skin, fins and tail	Malaysia	Nash <i>et al.</i> 1987
<i>Pasteurella piscicida</i>	<i>E. akaara</i>	Juveniles in hatcheries	Japan	Muroga 1995
'Red boil disease' <i>Flexibacter</i> sp.	<i>E. malabaricus</i>		Thailand	Danayadol <i>et al.</i> 1996
<i>Vibrio</i> spp.	<i>E. salmoides</i>	Swimming near water surface, loss of balance and anorexia Dark body color with external haemorrhagic lesions on body Fin and tail rot common,, eye lens sometimes turned opaque	Malaysia	Ong 1988
	<i>E. malabaricus</i>		Malaysia	Wong and Leong 1990
	<i>E. suillus</i>	Broodstock in cages and tanks Fish also harbored significant numbers of monogenean parasites causing gill lesions	Philippines	Lavilla-Pitogo <i>et al.</i> 1992
	<i>Epinephelus</i> sp.		Malaysia	Palanisamy <i>et al.</i> 1999
<i>Vibrio harveyi</i>	<i>E. tauvina</i>		Kuwait	Saeed 1995
<i>Vibrio parahaemolyticus</i>	<i>E. malabaricus</i>		Thailand	Chinabut 1996

### *Parasitic Diseases*

A large number of parasites (Table 4) was reported; some cause significant problems for grouper aquaculture. The parasites (protozoans, myxozoans, microsporans, monogeneans, trematodes, crustaceans, nematodes, cestodes, acanthocephalans and hirudineans) include external (skin and gills) and internal parasites. Parasite fauna of healthy and diseased *E. malabaricus* in Malaysia, Thailand and Philippines was most extensively studied by Leong and Wong (1988 and 1990). Infection with *Cryptocaryon irritans*, monogenean infection by *Benedenia* sp., *N. girellae*, *Diplectanum* and *P. epinepheli* and leech infection seem to cause serious problems to grouper. *N. girellae* has been well studied and information on its life-cycle, reproduction, immunity, susceptible hosts and treatment is available (see Bondad-Reantaso *et al.*, 1995a, 1995b and others cited in this report). One characteristic of this monogenean is low host specificity, which is rare for monogeneans. It has spread to at least twelve other cultured species in Japan including three species of grouper. Benedeniid monogeneans are particularly dangerous in net cage culture systems, where their eggs entangle the net meshing with elongated appendages, which makes re-infection much easier for the parasites (Ogawa, 1996). *Cryptocaryon irritans* is also a well-studied parasite. There is information on its life-cycle, pathology treatment and other biological characteristics such as temperature and salinity tolerance (Jee *et al.* 1997, Yuasa *et al.* 1999).

There were several reports indicating mixed parasitic/bacterial infection. These include reports of Lavilla-Pitogo *et al.* (1992), who reported that fish suffering from vibriosis also harbored a significant number of monogenean parasites causing gill lesions, and the results of the survey conducted by Chua *et al.* 1993.

Studies on the parasite fauna of groupers are useful in making import risk assessments and should be encouraged.

### *Other Diseases*

Other diseases (Table 5) are associated with import mortalities, handling and transportation mortalities and include diseases of undiagnosed (white spots, mortalities without clinical signs) or unknown aetiology (swim bladder syndrome, pop-eye or exophthalmia).

Among these diseases, the swim bladder syndrome has been extensively studied by Hua *et al.* (1994), who postulated that the syndrome closely resembled lipid peroxidase intoxication occurring on fish fed with putrefied trash fish or pelleted diets containing rancid lipid components. They indicated that although a virus was not isolated, their research was not sufficient to rule out the possibility of virus infection. This syndrome has been observed among *E. coioides*, *E. tauvina* and *E. akaara* in Singapore, Malaysia and China (Chong and Chao 1986, Leong 1994, Hua *et al.* 1994).



Table 4. Parasites reported from grouper cultured in Asia.

Parasite Species	Host	Country	Reference
<b>PROTOZOA</b>			
<i>Brooklynella</i> sp.	<i>Epinephelus</i> sp.	China	Chen 1996
<i>Cryptobia</i> sp.	<i>Epinephelus</i> sp.	China	Chen 1996
<i>Cryptocaryon irritans</i>	<i>E. bontoides</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. coioides</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. malabaricus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
		Thailand	Chinabut 1996, Ruangpan and Tubkaew 1993, Danayadol 1999; Leong and Wong 1990
	Malaysia	Leong and Wong 1988; Leong and Wong 1990	
	<i>Epinephelus</i> sp.	China	Chen 1996
	<i>C. altivelis</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1988; Yusa <i>et al.</i> 1999
<i>E. tauvina</i>	Kuwait	Rasheed 1989	
<i>Riboscyphidia</i> sp.	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999b
<i>Scyphidia</i> sp.	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<i>Trichodina</i> sp.	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999b
	<i>E. bontoides</i>	Indonesia	Koesharyani <i>et al.</i> 1999a
	<i>E. malabaricus</i>	Thailand	Chinabut 1996; Leong and Wong 1990
Malaysia		Leong and Wong 1988, 1990	
<i>Vorticella</i> sp.	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999b
<b>MYXOZOA</b>			
<i>Myxosoma</i> sp.	<i>Epinephelus</i> sp.	China	Chen 1996
<i>Ceratomyxa</i> sp.	<i>Epinephelus</i> sp.	China	Chen 1996
<i>Sphaerospora</i> sp.	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<b>MICROSPORA</b>			
<i>Pleistophora</i> sp.	<i>Epinephelus</i> sp.	China	Chen 1996
<b>MONOGENEA</b>			
<i>Benedenia</i> sp.	<i>E. malabaricus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
		Thailand	Chinabut 1996
	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<i>E. tauvina</i>	Kuwait	Al-Marzouq and Al-Rifae 1994	
	Singapore	Chua <i>et al.</i> 1993	
<i>B. epinepheli</i>	<i>E. akaara</i>	Japan	Ogawa <i>et al.</i> 1995b
	<i>E. moara</i>	Japan	Ogawa <i>et al.</i> 1995b
	<i>E. septemfasciatus</i>	Japan	Ogawa <i>et al.</i> 1995b
<i>Cycloplectanum epinepheli</i>	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<i>Dactylogyrus</i> sp.	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999b
<i>Dactylogyrus</i> spp.	<i>E. malabaricus</i>	Thailand	Ruangpan and Tubkaew 1993, Danayadol 1999
<i>Gyrodactylus</i> sp.	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<i>Haliotrema</i> sp.	<i>C. altivelis</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. akaara</i>	Japan	Ogawa <i>et al.</i> 1995a
	<i>E. bontoides</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. cyanopodus</i>	Japan	Ogawa <i>et al.</i> 1995b
	<i>E. coioides</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. malabaricus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>P. leopardus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<i>Megacotyloides epinepheli</i>	<i>E. malabaricus</i> (wild)	Malaysia	Leong and Wong 1988, 1990
<i>Megacotyloides</i> sp.	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<i>Tareenia</i> sp.	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<i>Diplectanum</i> sp.	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<i>Pseudorhabdosynochus</i> sp.	<i>C. altivelis</i>	Indonesia	Koesharyani <i>et al.</i> 1999a
	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<i>Pseudorhabdosynochus epinepheli</i>	<i>E. malabaricus</i>	Malaysia	Leong and Wong 1988, 1990
		Thailand	Leong and Wong 1990
		Philippines	Leong and Wong 1990
F. Dactylogyridae	<i>E. coioides</i>	Philippines	Eraza-Pagador 1999
F. Diplectanidae	<i>E. coioides</i>	Philippines	Eraza-Pagador 1999
<b>TREMATODA</b>			
<i>Allopodocotyle</i> sp.	<i>E. tauvina</i>	Malaysia	Kolandasamy and Shaharom-Harrison 1999
<i>Allopodocotyle serrani</i>	<i>E. malabaricus</i> (wild)	Malaysia	Leong and Wong 1988
<i>Cardicola</i> sp.	<i>E. malabaricus</i>	Thailand	Chinabut 1996, Leong and Wong 1990
		Malaysia	Leong and Wong 1988
<i>Ectenurus</i> sp.		Malaysia	Leong and Wong 1988

Table 4. Parasites reported from grouper cultured in Asia (continued).

Parasite Species	Host	Country	Reference
<i>Gonapodasmius</i> sp.	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<i>Helicometrina nimia</i>	<i>E. malabaricus</i>	Malaysia	Leong and Wong 1990
<i>Lecithochirium neopacificum</i>	<i>E. malabaricus</i>	Thailand	Chinabut 1996
		Malaysia	Leong and Wong 1988
		Philippines	Leong and Wong 1990
<i>Pearsonellum</i> sp.	<i>Epinephelus</i> spp.	Myanmar	SiSi Hla Bu 1999
<i>Prosorhynchus pacificus</i>	<i>E. malabaricus</i>	Thailand	Chinabut 1996, Leong and Wong 1990
		Malaysia	Leong and Wong 1988, 1990
		Philippines	Leong and Wong 1990
<i>Prosorhynchus</i> sp. C	<i>E. tauvina</i>	Malaysia	Kolandasamy and Shaharom-Harrison 1999
<i>Prosorhynchus</i> sp.	<i>Epinephelus</i> spp.	Myanmar	SiSi Hla Bu 1999
	<i>E. malabaricus</i> (wild)	Malaysia	Leong and Wong 1988
<i>Pseudopicoeloides</i> sp.		Thailand	Chinabut 1996
<i>Pseudometadena celebesensis</i>	<i>E. malabaricus</i>	Malaysia	Leong and Wong 1988
<i>Stephanostomum</i> sp.		Malaysia	Leong and Wong 1988
F. Didymozidae	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999a
	<i>Epinephelus</i> spp.	Myanmar	Si Si Hla Bu 1999
<b>CRUSTACEA</b>			
<i>Caligus</i> sp.	<i>C. altivelis</i>	Indonesia	Koesharyani <i>et al.</i> 1999a
	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<i>Ergasilus borneoensis</i>	<i>E. malabaricus</i>	Malaysia	Leong and Wong 1990
<i>Gnathia</i> sp.	<i>E. malabaricus</i> (wild)	Thailand	Chinabut 1996
<i>Lepeoptheirus</i> sp.	<i>E. coioides</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. malabaricus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>E. fuscoguttatus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
	<i>P. leopardus</i>	Indonesia	Koesharyani <i>et al.</i> 1999a, 1998
<i>Thebius</i> sp.	<i>E. malabaricus</i>	Thailand	Chinabut 1996
<b>NEMATODA</b>			
<i>Contracaecum</i> sp.	<i>E. malabaricus</i>	Malaysia	Leong and Wong 1988
<i>Echinocephalus</i> sp.	<i>E. malabaricus</i> (wild)	Malaysia	Leong and Wong 1988
<i>Raphidascaaris</i> sp. (larva)	<i>E. malabaricus</i>	Thailand	Chinabut 1996, Leong and Wong 1990
		Malaysia	Leong and Wong 1990
		Philippines	Leong and Wong 1990
2 species (unspecified)	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999b
<b>CESTODA</b>			
Tetraphyllidae	<i>E. malabaricus</i>	Thailand	Chinabut 1996
		Malaysia	Leong and Wong 1988
Cestoda gen sp. (metacestode)	<i>E. malabaricus</i>	Thailand	Leong and Wong 1990
		Philippines	Leong and Wong 1990
<b>ACANTHOCEPHALA</b>			
<i>Acanthocephalus</i> sp.	<i>E. malabaricus</i>	Malaysia	Leong and Wong 1988, 1990
		Thailand	Leong and Wong 1990
<b>HIRUDINEA</b>			
Unidentified species	<i>E. coioides</i>	Philippines	Cruz-Lacierda <i>et al.</i> 1999a
	<i>E. malabaricus</i>	Thailand	Chinabut 1996
		Malaysia	Leong and Wong 1988
	<i>Epinephelus</i> sp.	Philippines	Somga <i>et al.</i> 2001

Table 5. Grouper diseases of undiagnosed or unknown origin.

Characteristics	Species Affected	Country	Reference
Swim Bladder Syndrome Over-inflation of the swim bladder Loss of buoyancy control: <ul style="list-style-type: none"> <li>Fish swimming in a head-down position near the surface</li> <li>Fish swimming at the surface with their backs exposed</li> <li>Fish swimming intermittently and erratically on their sides</li> <li>Fish swimming upside down with visibly distended abdomens</li> </ul> Widespread over a short period Less than 10% of fish affected No evidence of pathogen involvement	<i>E. coioides</i> 30 g to in excess of 10 kg fish	Singapore	Chong and Chao 1986
Swim Bladder Syndrome: inability to deflate hyperinflated swim bladder, lack external, internal lesions, death due to secondary causes (sunburn, Vibriosis), < 1 week course, 1-10% mortality rates, all year, worst in August/November CPE agent isolated from 2 outbreaks; possible viral agent affecting CNS	<i>E. tauvina</i> >200 g fish	Singapore	Chua <i>et al.</i> 1993
Swimbladder disease: Occurs within a short period of time particularly during intermonsoonal period	<i>E. tauvina</i>	Malaysia	Leong 1994
Grouper's Syndrome – also called Distensive disease, whirling disease, swim-bladder distention and swim-bladder syndrome Pathological features: General focal inflammation of the gills, gall bladder, swimbladder, kidney, GIT, heart, brain, spinal cord and ovary Oedematous change of the liver, kidney, spinal cord and stomach wall	<i>E. akaara</i> <i>E. chlorrostigma</i>	China	Hua <i>et al.</i> 1994
“Bent Body Syndrome”: spasticity of axial musculature, inability to maintain equilibrium; lack external and internal lesions, death due to secondary causes, 1-2 weeks course, 10-60% mortality, June-July and October- November; CPE agent from one fish; possible viral agent affecting CNS	<i>E. tauvina</i> All sizes, mainly 50-300 g fish	Singapore	Chua <i>et al.</i> 1993
Popeye (Exophthalmos)	<i>E. coioides</i>	Singapore	Chong and Chao 1986
“Red Ulcer Disease”: Haemorrhagic ulcerations, tail/fin rot, 1 week course, 20-40% mortality rate, occurs all year round; parasitic and bacterial components on laboratory examination	<i>E. tauvina</i> All sizes affected	Singapore	Chua <i>et al.</i> 1993
White spots, eroded caudal fin, fin and tail rot Common during first 2 months of culture period Mortality may reach 70%	<i>Epinephelus</i> spp.	Philippines	Somga <i>et al.</i> 2001
Ulceration, red spots with tail and fin rot Common during grow-out stage Chronic and mortality may range from 10-35%	<i>Epinephelus</i> spp.	Philippines	Somga <i>et al.</i> 2001
Bulging eyeball, eye cataract Grow-out stage, mortalities less than 5%	<i>Epinephelus</i> spp.	Philippines	Somga <i>et al.</i> 2001
Ruptured gallbladder Acute with no external sign January and February	<i>Epinephelus</i> spp.	Philippines	Somga <i>et al.</i> 2001
Mortalities without gross signs Nursery and grow-out stages Acute and mortalities may reach 100%	<i>Epinephelus</i> spp.	Philippines	Somga <i>et al.</i> 2001
Mortalities without external/internal lesions Outbreak in 1990, 40% mortality	Red Grouper – <i>Plectropomus</i> spp. 100-200 g fish	Singapore	Chua <i>et al.</i> 1993

### Health Management Options for Grouper and other Marine Finfish Diseases

Information on effective health management strategies for grouper and other marine finfish diseases is provided in a number of reports, workshop proceedings and scientific literature. The most noteworthy sources include:

- Common Diseases of Marine Foodfish, Fisheries Handbook No. 2 by YC Chong and TM Chao, Primary Production Department of Singapore (1986)
- Parasites and diseases of cultured marine finfishes in Southeast Asia by Leong Tak Seng of the Universiti Sains Malaysia (1994)
- Aquaculture Health Management Strategies for Marine Fishes, Proceedings of a Workshop in Honolulu, Hawaii, October 9-13, 1995 by KL Main and C Rosenfeld of The Oceanic Institute (1996), and
- various scientific publications from Australia, China PR, Korea, Indonesia, Japan, Singapore, Malaysia, Philippines, Thailand and Chinese Taipei

The information presented in this section is based on the above sources. Additional health management options may be applicable or can be further explored for achieving better health in grouper aquaculture. These include:

- vaccination
- diagnostics and research
- epidemiological approach to disease control
- disease surveillance, monitoring and reporting
- responsible movement of live aquatic animals, and
- generic approaches to health management such as good farming practices, stress management, sanitation and hygienic measures, food and trash fish feed management, prevention of exotic diseases; management of infectious diseases and careful use of chemotherapeutants

### ***Generic Approaches to Health Management***

The objective of health management is to attain optimal production levels that will generate the highest possible profit to farmers, provide a stable supply of aquatic animal products and contribute to the national economy. This can be achieved through a programme that will prevent the occurrence of diseases, and if diseases do occur, reduce their incidence and severity, limit their spread, and prevent recurrence.

- *Good Farming Practices*

A health management programme has several requirements and must cover all levels of aquaculture activity. At the production level, the requirements for a healthy environment include strong healthy seed and juveniles, proper nutrition, appropriate waste management, optimal water quality, and regular monitoring.

At the farm site level, good record keeping is essential. It should cover all aspects of farm operation. Farmers should be trained to understand the importance and value of such information in determining the course or nature of a disease outbreak, providing accurate and rapid diagnosis, and enabling sensible management decisions for intervention and control. Record keeping is crucial to aquaculture and can go a long way in supporting effective health and productivity management efforts. A good farm profile should contain the following information:

- Treatment administered
- Clinical signs (behaviour, appearance)
- Farm lay-out (inflow, outflow, connection of ponds)
- Animals cultured (species, numbers, origin, age classes)
- Yields (per pond, per cage, per farm, normal survival rates)
- Nutrition (live food, manufactured food, sources, feeding practices)
- Management practices (continuous stocking, closed operation, stocking densities)
- Mortality data (affected sites, cages, ponds along with approximate percentages and numbers), and
- Unusual events (abnormal weather changes, mortality above average, yield below average, land-use activity, run-off, spills, abnormal growth, spawning events)

In addition to regular record keeping, there should be a continuous monitoring and updating of information (new animals on farm, change of feed, new ponds connected, new farm upstream).

- *Stress Management*

Stress is defined as the sum of biological reactions to any adverse stimulus, physical, mental or emotional, internal or external, that tends to disturb the organism's homeostasis. Should these compensating reactions be inadequate or inappropriate, they may lead to disorders (Dorland's Illustrated Medical Dictionary 1988). It is a condition that exists in practical fish farming. Sources of fish stress include overcrowding, poor water quality, fluctuating temperature, poor nutrition, bad management, careless handling, inappropriate transportation methods and procedures, adaptation to new environment; and stress of capture and handling in case of wild caught fish, and associated stress due to chemotherapy.

Chong and Chao (1986) reported that import mortalities are the most important cause of losses to local fish farmers in Singapore and noted a number of stress effects in newly imported fish. The effects included shock, physiological

failure, off-feed, cannibalism and increased susceptibility to pathogens. They recommended some preventive measures as indicated in Box 2 below:

<b>Box 2</b> (from Chong and Chao 1986).	
<b><u>Stress Effects</u></b>	<b><u>Recommended Preventative Measures</u></b>
<ul style="list-style-type: none"> <li>• Shock</li> <li>• Physiological failure</li> <li>• Off-feed</li> </ul>	<ul style="list-style-type: none"> <li>Appropriate packing techniques</li> <li>Proper acclimatization</li> <li>Stimulating feeding activity by using natural food in the beginning and including a few farmed conditioned fry/fingerling with the newly arrived stock to help familiarization with feeding regime</li> </ul>
<ul style="list-style-type: none"> <li>• Cannibalism</li> </ul>	<ul style="list-style-type: none"> <li>Separation of fish according to size; lowering the density and frequent feeding</li> </ul>
<ul style="list-style-type: none"> <li>• Increased susceptibility to pathogens</li> </ul>	<ul style="list-style-type: none"> <li>Sanitation of imported fry and fingerlings in order to:               <ul style="list-style-type: none"> <li>Reduce the parasite and bacterial load</li> <li>Seal open wound by antiseptic treatment</li> <li>Prevention of attack by pathogens in the new environment</li> </ul> </li> </ul>

In order to avoid mortalities due to opportunistic pathogens, handling and transportation stress and to ensure better survival of newly arrived stocks, Shariff and Arulampalam (1996) recommended the establishment of on-shore nursery facilities, which will give farmers the flexibility to introduce the fingerlings into the marine cages at the appropriate time after acclimatization.

A contingency for disease prevention is the prophylactic use of drugs, chemicals and biological treatments. As there will always be infectious agents in the environment, every effort should be made to prevent the progression of infection to a disease. Prophylactic procedures are useful during handling and transportation (seining, handling, shipping) particularly because fish are most vulnerable to injury, trauma or physiological stress during this time. Once the protective mucous layer of fish, scale or skin are damaged, fish become susceptible to air-borne, pathogenic bacteria, and other bacterial, fungal and parasitic infection. Some recommended prophylactic measures include:

- simple dip treatments with formalin (for ectoparasitic infections) or antibiotics (for injuries due to handling) before and after transportation, to ensure eradication of parasites and stress related to bacterial pathogens (Chong and Chao 1986)
- transportation methods to avoid stress: reducing temperature fluctuations, using appropriate packing densities (not to exceed 129 g biomass per liter of water for a 12 hrs period between packing and release); and use of anaesthesia. Anaesthesia can facilitate handling, loading and transportation by inducing quiescence (inactiveness), which in turn causes a reduction in excretion of ammonia and carbon dioxide in oxygen consumption, thus making pH values and quality of the transport water relatively fairly constant (Shariff and Arulampalam 1996).

Careful handling of animals is recommended including the use of non-abrasive materials and tools (knotless netting for scoop nets and cages). Fish should not be fed before handling (Chong and Chao 1986).

Maintaining a balanced environment through good farming management practices, avoiding stressful conditions, and practicing sanitary and hygienic measures (listed below) can minimize the occurrence of opportunistic pathogens.

- *Sanitation Measures and Hygienic Practices*

Adherence to strict hygiene practices and sanitation standards can maintain fish health at production facilities and minimize diseases caused by infectious agents and preventing their spread via personnel and equipment. Some sanitation and hygiene practices include disinfection of hatchery equipment such as tanks, nets, boots and vehicles) and water supply (UV irradiation, ozone and micro-filtration, using good quality well water, sterilization of waste water from broodstock rearing tanks) (Sako, 1996). Farm level sanitary processes such as healing open wounds with antiseptics and antibiotics (acriflavine, nitrofurazone, formalin) and transshipment sanitization (addition of chemicals such as antibiotics and anaesthesia to facilitate transportation: 100 ppm formalin bath for one hour followed by 30 ppm nitrofurazone for four hours) had also been recommended (Chong and Chao 1986).

Cleaning and changing of net cages should be a regular activity. This practice reduced the incidence of monogenean infection in Japanese mariculture, where the long egg filaments produced by these parasites entangled in the net meshing and continued their life cycle. During the early years of mariculture in Japan (1970s and 1980s), the application of organic tin coating on the net meshing to prevent the growth of fouling organisms suppressed the propagation of monogenean infection through some unknown mechanism. When the use of the chemical was banned in 1996 because of fear of its accumulation in the host fish and some environmental concerns, the monogenean infection recurred (Ogawa 1996).

Bio-fouling is another consistent problem in cage culture systems. It decreases water exchange, reduces the supply of dissolved oxygen and removal of wastes products. The waste products may act as a reservoir for pathogenic microorganisms. Australia is currently developing anti-fouling polymers and coatings that release biodegradable anti-fouling compounds. Field trials suggested that the anti-fouling polymers prevented fouling for 260 days (Hodson *et al.* 1999). This new development can be further explored for use in grouper aquaculture.

- *Food and Trash Fish Feed Management*

There have been reports that trash fish can be a source of infection (Yashiro *et al.* 1999), and that some diseases involving opportunistic pathogens result from deficient nutrition. Leong and Wong (1988) reported that the trematodes, cestodes, nematodes and acanthocephalans recovered from cultured grouper were most probably transmitted through trash fish. Proper nutrition is a vital component of any culture activity; it is essential to the health of animals. High quality trash fish in sufficient quantity with vitamin and mineral supplementation where possible is recommended (Leong 1994).

In Japan, food management is realized by (a) bath treatment of live food with nitrofurans derivatives, sodium nifurstyrenate to decrease the number of bacteria and (b) immunostimulant supplementation (beta-carotene) (Sako 1996).

Other recommended management practices for food and trash fish feed include keeping chilled trash fish for only up to three days; sorting to remove crabs, shrimp or unsuitable materials by washing with seawater using a pressurized water jet; and removal of uneaten or wasted trash fish from the system.

- *Prevention of Entry of Exotic Diseases and Risk Assessment*

There are a number of other generic approaches to disease prevention, which may include stock movement controls, destruction of clinically sick animals, emergency harvest of apparently healthy animals, following prior to stocking, sanitary measures (disinfection) and others. Although disinfection protocols and movement control can decrease the spread of disease by personnel, equipment and farmed animals, the agent may still remain in the water system. In most instances, by the time a diagnosis is confirmed, the agent would have been in the system for some time. The fate of any agent in the natural environment under natural conditions remains largely unknown. Once an agent has gained access to a water system, prevention of spread becomes very difficult. Prevention of entry of an infectious agent to the system is the preferred option. As long as the disease agent is absent from the system, a disease will not occur. Therefore, for exotic diseases the risks of introduction must be identified, assessed and managed.

Import Risk Analysis (IRA) is a transparent and science-based process of assessing disease risks associated with the importation of aquatic animals and their products (genetic material, feed stuff, biological products, pathological material). This process should be implemented in aquatic animal importation.

IRA is a fairly new concept in the region. Australia and New Zealand are perhaps the only countries conducting risk assessments for aquatic animal health imports. The Australian Quarantine and Inspection Service or AQIS recently published two documents on IRA: (a) The AQIS Import Risk Analysis Process and (b) Import Risk Analysis on Live Ornamental Finfish. They provide useful information in making IRA and describes the procedures being followed by the Australian government for importing plants, animals and their products.

- *Management of Infectious Diseases*

Management of viral diseases requires a basic understanding of concepts regarding viruses. Most viruses are transmitted vertically (by eggs and fluids during spawning) and horizontally (host to host). Diagnosis of viral infection requires culture cell lines, electron microscopy and trained specialists. There are no therapeutic treatments for viral diseases. Viral infection, when it occurs, causes high mortalities, and may spread quickly if not contained. Therefore, an ideal approach would be prevention and management.

When an infection of viral origin exists, the following generic measures are recommended: destruction of infected stocks, emergency harvest, detection of carriers and finding ways to eliminate or limit the access of the carriers into the system.

In Japan, VNN infection of striped jack is controlled by the following prophylactic measures (Nakai *et al.* 1995):

- Suppression of virus multiplication in spawners by reducing stress factors
- Elimination of spawners and eggs positive for viral infection detected by PCR technique
- Disinfection of eggs and equipment with appropriate chemicals
- Rearing batches of larvae and juveniles in separate tanks supplied with disinfected water, and
- Separate rearing of larval and juveniles from broodstock to prevent water-borne infection caused by viruses shed from infected broodstock.

Most bacterial infections are associated with stress. The agents (*Vibrio* spp.) are naturally present in the aquatic environment. Maintaining the highest possible quality of environment by applying proper chemotherapeutants (baths for external infections and medications for systemic infections), sanitation, disinfection, and prophylactic treatment during transportation and handling prevent the progression of an infection to a disease and can minimise the occurrence of stress-related bacterial infection.

Management of a parasitic infection depends on whether the infection is external or internal. A number of external parasites respond well to chemotherapy as indicated in Table 6. Other internal and tissue dwelling parasitic infections are much more difficult to control. There is no known treatment for internal parasites such as myxosporeans. Parasitic infections are threatening when present in large numbers. This condition is usually worsened by poor environment and bad management such as poor water quality high, stocking densities, deficient nutrition, inappropriate waste management. Some of these parasites (myxosporeans and other flatworms) have complex life cycles. Information on their life cycles is required in order to determine appropriate control measures such as prevention of entry, chemotherapy, breaking the life cycle, eliminating or limiting access of other hosts involved in the life cycle. In addition, control of parasitic infection in open-water marine systems (such as nets and cages) is more difficult than control of infections in a freshwater system which is more controlled (Ogawa 1996).

- *Careful Use of Chemotherapeutants*

Chemotherapy is widely used to control infectious parasitic, bacterial and fungal diseases. It has been used for control of parasitic and bacterial diseases of grouper as indicated in Table 6. However, experience demonstrates that there are problems in chemical treatment of fish diseases; in some cases the treatments can be harmful because of associated stress.

In Singapore, Chong and Chao (1986) reported some cases of drug overdose leading to fish death and other detrimental side effects. Formalin overdose results in severe gill damage. Ulcerative dermatitis develops with repeated treatment with nitrofurazone leading to fish death. Potassium permanganate use in marine conditions resulted in rapid reduction of MnO<sub>4</sub><sup>-</sup> to manganese oxide which is toxic to fish.

Chemotherapeutants are only effective against some pathogen groups. Rather than providing a solution to health problems, they became palliative measures. Application of chemotherapeutants has created problems with toxicity, resistance, residues and possibly some public health and environmental consequences. Their efficacy under certain aquatic conditions (open water systems) remains questionable, and they can be costly. The use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antibiotics and other therapeutic drugs. These companies are now coming under increased scrutiny. Other constraints to the use of chemotherapy include the lack of pharmacokinetic data on drugs used, lack of standard procedures on use, safety issues, low number of licensed products, cost and time involved in registration and licensure requirements, and existing legislation, which ranges from very restrictive regulations to no regulation at all (OIE 1992).

Chemotherapy has certain value in preventing and controlling diseases in aquatic animals. It should be used in a judicious manner and the cost-benefit should be evaluated. Efforts should also be made to develop alternative methods of controlling diseases.

- *Aquatic Systems Health Management*

The aquatic environment is a complex system which obscures the distinction between health, sub-optimal performance and disease. During epizootics, it is often difficult to determine the cause. It is usually the result of a series of linked events which can be a combination of environmental factors, the health condition of stocks, the presence of an infectious agent or poor husbandry and management practices. A clear understanding of the relationship between the host, pathogen and environment is required. When addressing aquatic animal health problems, the environment with all biological processes should be considered a single entity.

Although in some cases disease control measures can be developed without information about the pathogens, knowledge of the disease organism, its natural history and impact on hosts greatly assists in the development of appropriate and cost-effective control procedures. The risks and impacts of a disease vary according to the prevailing conditions, therefore, the intervention for mitigating the problem may be different. An ideal option would be to use an aquatic systems health management approach. This approach involves (a) taking into consideration the environment, the host and the pathogen, (b) determining options (cull, treat, quarantine, disinfect) that are available for a specific disease situation, (c) performing a cost-benefit analysis and (d) proceeding with an appropriate health plan.

### ***Diagnostics and Research***

Conventional methods for pathogen screening and disease diagnosis include tissue culture and electron microscopy for viruses, isolation and serology for bacteria, necropsy using light microscopy for parasites, and histopathology for understanding the mechanism of disease. Considerable progress has been made in the development of immunoassays and DNA-based diagnostic methods such as fluorescent antibody tests (FAT), enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), *in situ* hybridization (ISH), dot blot hybridization (DBH) and polymerase chain reaction (PCR) amplification techniques.

An expert consultation on “DNA-based Molecular Diagnostic Techniques: Research Needs for Standardization and Validation of the Detection of Aquatic Animal Pathogens and Diseases” was jointly organized by ACIAR, CSIRO, DFID, FAO and NACA and held in February 1999 in Bangkok, Thailand. The experts recognized the advantages of using DNA-based methods for pathogen detection. These technologies offer rapid results with potentially high sensitivity, specificity, and repeatability at relatively low cost. They have been adopted in shrimp culture, where the conventional histological procedures lack specificity and culture-based methods are not applicable. The consultation evaluated the use of DNA-based methods for important diseases and recommended development of a PCR based technology for viral encephalopathy and retinopathy or VER (Walker and Subasinghe 2000).

In Japan, PCR is used for detecting VNN infection in spawners and eggs of striped jack. In Korea, it is used for confirming VNN infection of sevenband grouper. Other countries such as Thailand, Chinese Taipei and Singapore use tissue culture methods and electron microscopy for diagnosis of viral infection. Rapid detection and diagnosis are required for important diseases such as those currently affecting grouper. Early detection of disease is important so that rational decisions for management, intervention or control can be made.

Research is fundamental to the development of health management programmes. It assists in understanding the disease mechanism and development of appropriate and cost-effective control procedures. Reference laboratories and collaborating centres are critically important to successful implementation of an aquatic animal health programme. Besides providing confirmatory diagnosis and facilitating research, the reference laboratories and collaborating centres standardize, validate and assist in the quality control of development and research programs.

Diagnostics and research should provide services to farmers and should be aimed at assisting farmers in solving aquatic animal problems and increasing farm productivity. To achieve this, the linkage between diagnosticians, farmers and researchers should be improved and strengthened.

### ***Vaccination***

Inducing and building resistance to diseases is another approach to controlling fish diseases. A wide range of methods exists including immunostimulants, adjuvants and vaccine carriers.

In developed countries, vaccination offers a good alternative to disease prevention and control. In Norway, bacterial diseases (vibriosis, coldwater vibriosis and furunculosis) are controlled through vaccination. In Japan, efforts are being undertaken to develop appropriate vaccines for major viral and bacterial diseases of finfishes. Commercial



Table 6. Chemotherapeutants and other treatments used for bacterial and parasitic diseases of grouper in the Asia-Pacific.

Pathogen	Host Species	Treatment	Reference
<i>Cryptocaryon irritans</i>	<i>Cromileptes altivelis</i>	Transfer of infected fish to a <i>Cryptocaryon</i> -free tank to keep fish free from trophont infection; followed by another transfer 3-4 days when trophonts would have been shedded from the fish Varied success using the following treatment: Formalin at 200 ppm for ½ to 1 hr, depending on fish's tolerance Formalin at 100 ppm + acriflavine 10 ppm for 1 hr Formalin 25 ppm + malachite green 0.15 ppm for 12 hrs Nitrofurazone at 30 ppm for 12 hrs Malachite green at 0.5 ppm for ½ hr Methylene blue 0.1 ppm for ½ hr 100% freshwater for 1 hr (seabass and estuarine grouper only) Other recommended measures: • Isolate sick fish from healthy fish • Remove dead fish and terminally sick fish from net cages and destroy • Treat as soon as symptoms develop	Yuasa <i>et al.</i> 1999
	Estuarine or greasy grouper	0.10-0.15 ppm malachite green mixed with 2.5 ppm formalin effective when used at an early stage	Chong and Chao 1986
Monogenean infection (e.g. <i>N. girellae</i> , <i>E. epinepheli</i> , <i>Benedenia</i> sp.)	<i>E. malabaricus</i>		Damayadol 1999
	<i>Cromileptes altivelis</i>	Bath treatment with 150 ppm hydrogen peroxide for 30 minutes for 7 consecutive days	Koesharyani <i>et al.</i> 1999b
<i>Dactylogyrus</i> spp.	<i>E. tauvina</i>	Freshwater bath treatment for 15 minutes effective for dislodging parasites from gills and skin of broodstock	Al-Marzouq and Al-Rifae 1994
	<i>E. malabaricus</i>	250 ppm formalin for 30 min for 3 days or continuous bath in 0.3 ppm Dipterex for 3 days	Damayadol 1999, Ruangan and Tubkaew 1993
<i>Diplectanum</i> sp. or other gill parasites	Unspecified marine fish	Formalin at 200 ppm for ½ to 1 hr with strong aeration, for 3 days Formalin at 25 ppm + malachite green at 0.15 ppm – overnight bath Acriflavine at 10 ppm bath for 1 hr or 100 ppm dip for 1 minute Dipterex at 20 ppm for 1 hr 100% freshwater for 1 hr (seabass and estuarine grouper only)	Chong and Chao 1986
	<i>Pseudohabdosynchus</i> - like infection	250 ppm formalin for 1 hr or 2 hr freshwater treatment	Cruz-Lacierda <i>et al.</i> 1999b
Marine leech	<i>E. coioides</i> (spawner and juveniles)	Careful manual removal using wet cloth; 50 ppm formalin bath treatment for 1 hr with ample aeration for 3 consecutive days	Cruz-Lacierda <i>et al.</i> 1999b
	Vibriosis <i>Vibrio</i> sp.	Antibiotic treatment: Oxytetracycline: at 0.5 g per feed for 7 days Sulphonamides or potentiated sulphonamides: at 0.5 active ingredients per kg feed for 7 days Chloramphenicol: at 0.2 g per kg feed for 4 days If fish are not eating, bath treatment: Nitrofurazone: at 15 ppm for at least 4 hrs Sulfonamides at 50 ppm active ingredients for at least 4 hrs Oxytetracycline administered by intramuscular injection for 5 days at a dose of 25 mg/kg body weight (for broodstock)	Chong and Chao 1986
	<i>E. stillus</i>		Lavilla-Pitogo <i>et al.</i> 1992

vaccines are available for red sea bream iridovirus (injection type), ayu and salmonid *Vibrio anguillarum* (immersion type), yellowtail *Lactococcus graviae* (*Enterococcus serioricida*) (feeding type), and they have been reported to be effective. The iridovirus vaccine is currently used only for red sea bream, but efforts are being made for the development of iridovirus vaccine for other fish species. It is expected that more vaccines will be available within the next five years (Ogawa, K, University of Tokyo, pers. comm.).

Leong and Fryer (1993) enumerated the required attributes and characteristics of an acceptable vaccine. These are:

- Long term protection
- Safe to the vaccinated animal
- Inexpensive to produce, license and cost effective
- Protection against all serotypic variants of the pathogen
- Protection at the time when the animal is most susceptible to the disease
- Adequate immunoprotection from the target disease under intensive rearing conditions found at commercial hatcheries and farms, and
- Easy administration, preferably orally or by immersion, and their application does not unduly disrupt the normal management scheme.

Vaccines are specific for certain diseases. Their use requires considerable research on the target disease, involves careful planning, efficacy and cost evaluation. Vaccination against any disease is one of the fish health management options for disease prevention but it does not mean total exclusion of a particular disease.

Plumb (1995) evaluated the potential use of vaccines in Asian aquaculture and reported that there were only three vaccines used during that time: a multivalent vibrio (*Vibrio* spp.) preparation for shrimp, a *V. anguillarum* vaccine for fish, and, occasionally, a *Yersinia ruckeri* vaccine used for trout. Efforts on vaccine development in the Asia-Pacific region is very much at the experimental stage but Plumb (1995) indicated that there was great interest in their development and future use.

It is uncertain whether vaccination can offer protection against grouper diseases in Asian aquaculture as it has for other important diseases in developed countries. Vaccination needs to be evaluated in terms of efficacy, marketability and cost-benefit.

### ***Epidemiological Approach to Disease Control***

The concepts of epidemiology for use in aquatic animal disease control has recently been introduced in the region. Epidemiology uses population as the unit of study where a bi-directional approach (downward approach from the animal to organ, tissues, cell, molecule; and upward approach from the animal to pond, farm, province, country) to disease investigation provides insights into understanding the disease processes and allows the development of possible control strategies. Available studies on shrimp diseases in the Mekong Delta (Turnbull *et al.* 1999), Australia and Indonesia (Callinan *et al.* 1999) and EUS (Khan and Lilley 2001) using the concepts of epidemiology have led to the identification of risk factors, their impact and interventions that are practically applicable. Although there are limitations on using epidemiology for aquatic animal pathogens (its inability to detect abnormal host carriers of significant pathogens especially those with low or unknown host-specificity), the approach shows good potential for improving health management and developing appropriate control measures.

### ***Disease Surveillance, Monitoring and Reporting***

Reporting aquatic animal disease is one of the major components of the FAO-NACA TCP/RAS/6714 in the Asia-Pacific region. The "Asia-Pacific Quarterly Aquatic Animal Disease Report" is produced in cooperation with the Office International des Epizooties (OIE) or World Animal Health Organization (Representation for Asia-Pacific) and contains a list of diseases including those listed by OIE and other diseases, which are deemed important to the region. The reporting system commenced in July 1998.

VER is listed under the category 'Other Significant Diseases'. This category refers to diseases of current or potential international significance in aquaculture, which have not been included in the list of diseases notifiable to the OIE because of their importance, geographical distribution, current knowledge or lack of approved diagnostic methods (OIE, 1997). VER has several names, such as 'seabass viral encephalitis', 'viral nervous necrosis' or VNN, 'striped

jack viral nervous necrosis' of SJVNN, 'fish viral encephalitis'. The status of this disease has been recently reviewed by Rodgers and Furones (1998), who have indicated that the causal agent is a member of the Nodavirus family, comprising a new group of strains different from the insect viruses (Nishizawa 1996).

In Australia, this virus is present on Australian barramundi, and reported in nine of fifteen months since the reporting system began (third quarter of 1998). The disease was also reported in Japan during July and September 1998, and in Taipei China in August 1998 and May 1999. Singapore and Thailand did not report this disease during the period but the disease is known to occur in these countries (in Singapore, the last major outbreak was reported in Nov-Dec. of 1997 among seabass fry). In the Philippines, the disease is suspected but not confirmed (NACA/FAO 1999a, NACA/FAO 1999b).

National disease surveillance and reporting systems to support regional and international disease reporting obligations are effective strategies for control and prevention of trans-boundary diseases. They have proven to be highly effective in terrestrial livestock disease control programs. In aquatic animal diseases, however, there are various factors that can make surveillance and reporting a more difficult endeavor. Aquaculture production involves a large number of species, a wide range of culture systems and management practices, and a variety of diseases (some with low or unknown host specificity and many with non-specific symptoms). Disease reports comprise the building blocks of an aquatic animal health information system that is invaluable for verification and validation of disease information generated at the country level, which in turn depicts the disease situation at the regional level. This information is required for instituting control and eradication programmes and effective early warning mechanisms to reduce the impact of new or exotic diseases.

Countries actively involved in the international trade of live groupers are urged to continuously participate in this reporting system, and strive to improve their reporting capabilities by using a higher level of surveillance. Countries with sound infrastructure and a demonstrated record of containing and controlling disease outbreaks will have a significant trade advantage. Surveillance, monitoring and reporting systems will serve as a value added label to aquaculture and fisheries products, because it will reflect the country's demonstrated ability of providing documented valid information on the health, origin and quality of their traded commodities.

### ***Responsible Movement of Live Aquatic Animals***

Trans-boundary aquatic animal diseases receive a lot of attention because of the high mortality and significant losses they can cause to national economies, their unpredicted and widespread nature, the speed of spreading among susceptible population, and the constant threat they pose to the livelihood of aqua-farmers. Experience with EUS and the viral diseases of shrimp demonstrated that trans-boundary aquatic animal diseases cross national or administrative borders, therefore, they need to be addressed on a regional basis through cooperation between countries and through harmonization of approaches. Effective cooperation at all levels is required.

Another major component of the FAO-NACA TCP/RAS/6714 is the development of "Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals" (FAO/NACA 2000). This document was developed through a consultative review process by representatives from twenty-one Asian governments, scientists and experts on aquatic animal health, as well as representatives from various national, regional and international agencies and organizations. It contains a set of Guiding Principles on movement of living aquatic animals within and across national boundaries and proposes practical and effective strategies to minimize the risks of introduction, spread and establishment of trans-boundary aquatic animal diseases. The document contains technical guidelines on a number of health issues including :

- zoning
- disease diagnosis
- import risk analysis
- contingency planning
- implementation strategies
- regional capacity building
- pathogens to be considered
- disease surveillance and reporting
- national strategies and policy frameworks, and
- health certification and quarantine measures

Lessons learned from the disastrous viral epizootics experienced in shrimp aquaculture indicate that countries should be more cautious in the international movement of aquatic animals. This is directly applicable to the increasing trade in live groupers. The technical guidelines will assist in making realistic assessment of risks associated with the movement of live grouper. They will guide governments in making careful decisions that will allow movement of live grouper in a responsible and safe way, which does not interfere unreasonably with international trade.

***Update on Regional Research Programme on Grouper Viruses***

During the last several years, there has been a growing concern over the increasing number of diseases and other health problems of marine finfish (including groupers) experienced by countries in the region. A number of regional workshops recognized the need to give more attention to grouper health management in order to sustain the development of grouper aquaculture:

Regional Workshop on Sustainable Aquaculture of Grouper and Coral Reef Fishes  
*December 1996, Sabah*

FAO-NACA-OIE, Development of Technical Guidelines on Quarantine, Health Certification and Information Systems for the Responsible Movement of Live Aquatic Animals under the FAO-NACA TCP/RAS/6714  
*February 1999, Bangkok*

FAO-NACA-DFID-GOB Asia Regional Scoping Workshop on Primary Aquatic Animal Health Care in Rural, Small-Scale Aquaculture Development  
*September 1999, Dhaka*

The scientific presentations during the recently concluded 4<sup>th</sup> Symposium on Diseases in Asian Aquaculture (November 1999, Philippines) reflected the scarcity of information and research on grouper health. This lack of information makes it difficult to conduct risk assessments for the responsible movement of live groupers and justifies the need for more concerted efforts to address such concerns.

In view of the above, the Fisheries Working Group (FWG) of the Asia Pacific Economic Cooperation (APEC) has approved support for a one year project. The project would initiate a survey on the impact of grouper viral diseases and involve a workshop that will provide a platform for discussing current knowledge on grouper health and diagnostic techniques for viral diseases. The project will enable development of a regional programme to support identified research needs, particularly on grouper viral transmission, vaccine development and strategies to reduce the risks of introduction and transfer of pathogens that may be associated with the increasing international trade of groupers. The project will also involve review of options for subsequent funding. FWG 02/2000 has the following objectives:

- Update current knowledge on grouper health, particularly viral diseases, their impact, including standard and rapid techniques for viral disease diagnosis
- Develop a regional programme on grouper health that will assist in reducing losses due to grouper diseases, initially by identifying research needs that will address:
  - a) Development of suitable cell lines for grouper viral isolation
  - b) Development of techniques for grouper viral identification and diagnosis
  - c) Development of protocols for grouper viral disease induction and investigation on modes of virus transmission in grouper
  - d) Prevention and control of viral diseases (viral nervous necrosis – VNN, and iridovirus infection) of grouper culture at hatchery stage
- Develop strategies to minimize the risks of pathogen transfer through responsible movement of live grouper
- Identify funding mechanisms that will support the implementation of the regional programme on grouper health
- Strengthen the network of aquatic animal health scientists working on grouper and other marine fish diseases in the APEC region

Based on these objectives, three major tasks identified in the Request for Proposal for the implementation of this project are:

- Task 1: Organize a survey on impacts of grouper viral and other health problems
- Task 2: Organize a workshop “Development of a Regional Research Program on Grouper Virus Transmission and Vaccine Development”
- Task 3: Prepare a report containing the proceedings of the workshop, a synthesis of the grouper viral disease impact survey and economy reviews, regional framework on research for grouper viral disease and other health problems, strategies for responsible movement of live groupers, and options for subsequent funding.

The surveys and workshop were carried out in consultation with the Aquatic Animal Health Research Institute (AAHRI) of the Department of Fisheries of Thailand and the Network of Aquaculture Centres in the Asia-Pacific (NACA). AAHRI is overseeing the implementation of this project, and NACA is cooperating with APEC in the overall coordination of the Asia-Pacific grouper aquaculture programme.

### **Conclusions**

Health management is an important tool for the prevention of disease in aquaculture and supporting the sustainability of aquaculture production.

This review indicated that the number of diseases affecting grouper has increased steadily with expansion and intensification of grouper aquaculture and trade. Some of the diseases, particularly VER or VNN, and some parasites (*N. girellae* and *C. irritans*) have been described in detail. Other viral infections and diseases of undiagnosed status or unknown aetiology have been reported.

Viral diseases appear to be the most significant diseases that can impact grouper aquaculture. Development of early detection methods should receive priority. These methods are important in making decisions for management, intervention or control.

There is information on farm-level health management techniques in Japan, Singapore, Malaysia, and Thailand. This information can be used for grouper aquaculture.

Risks and impact of a disease vary according to prevailing conditions, therefore, the intervention methods for mitigating the problem may be different. An aquatic systems health management approach is recommended. This approach includes taking into consideration the environment, the host and the pathogen, determining options (cull, treat, quarantine, disinfect) that are available for a specific disease situation, conducting a cost-benefit analysis and proceeding with a good health plan.

Chemotherapy is widely used. It includes a range of chemicals and antiseptic, antibiotics, anti-bacterial treatments and other treatments such as freshwater bath treatments. The development of vaccines is underway, particularly in Japan. Efforts should be made to develop alternative methods for controlling grouper diseases.

Apart from the existing knowledge on infectious diseases affecting grouper, there is very little information on the impact of grouper diseases. More information should be gathered because this is important in prioritizing diseases. The APEC funded project on evaluating the impact of grouper diseases and establishing a regional research framework on grouper health is a well-timed initiative. The framework will hopefully provide solutions to important disease problems in grouper aquaculture and contribute to its sustainability.

As the industry is currently dependent on introduced stock, a more precautionary approach regarding the movement of groupers should be a consideration at the national, regional and international level. The expanding trade in live grouper increases the risks of moving pathogens that may come along with the movement of host fish. There are a number of instruments such as codes of practice, agreements, and technical guidelines which are aimed at addressing important issues concerning trans-boundary transfer of aquatic animal pathogens.

The success of implementing such codes depend on national programmes that complement and support such agreements through policies, legislation and regulations that protect and sustain aquatic animal production. The trans-boundary nature and commonality of grouper diseases, the need to harmonize approaches to disease detection, and the need to make efficient use of limited resources make a strong case for effective cooperation at the regional and international level.

Continuous support to transparent disease reporting and dynamic exchange of information and technologies is recommended. The FAO-NACA TCP/RAS 6714 provides a solid platform for strengthening collaboration within the Asia-Pacific region towards providing solutions to aquatic animal diseases in Asian aquaculture.

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