

Research and Training on Fish Diseases at the SEAFDEC Aquaculture Department in 2000-2004: A Review

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Abstract

This paper reviews various research and training activities on fish diseases at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC) in Iloilo, Philippines. The activities were implemented through the “Regional Fish Disease Project” of the Government of Japan Trust Fund starting in March 2000. A total of 29 research studies were conducted from 2000-2004 in the following aspects: (1) establishment and standardization of diagnostic methods; (2) biology and pathogenesis of disease pathogens; (3) disease prevention and control; (4) establishment of evaluation methods for residual chemicals in aquaculture products; and (5) epizootiology and prevention of koi herpesvirus disease. Some of these studies were conducted by scientists from the Department of Fisheries in Thailand, and from the Marine Fisheries Research Department (MFRD) of SEAFDEC in Singapore. Two sessions of hands-on training on “Important Viral Diseases of Shrimp and Marine Fish” was implemented in 2002 and 2003. Participants from the SEAFDEC member countries were funded by the project to attend the training course. The course consisted of both lecture and practical hands-on sessions. The latter focused on the use of molecular tools and other important techniques in the diagnosis of viral diseases of shrimp and marine fish. This review also provides information on publications such as proceedings, manuals, review articles, scientific papers, terminal report, annual reports, flyers, pamphlets and others as the outputs of research activities and international meetings that were organized with financial support from the project.

Introduction

Fish disease is a major constraint and threat to aquaculture production in Southeast Asia. Numerous infectious diseases have been reported from fish and shrimp cultured in this region. Currently, several new diseases have

emerged in the region. These diseases cause mass mortality of cultured species, resulting in devastating losses to the regional aquaculture production. Various chemicals including antibiotics, pesticides, disinfectants and others are often used to control fish diseases in the region. There is, however, a need to ensure that aquaculture products are safe for humans since the presence of chemical residues can negatively affect international trade of the products.

Since the year 2000, the “Regional Fish Disease Project” has been implemented at the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC) in Tigbauan, Iloilo, Philippines to address various regional fish disease problems and food safety issues through the Government of Japan (GOJ) Trust Fund. The first phase of the project entitled “Development of Fish Disease Inspection Methodologies for Artificially-Bred Seeds” started in 2000 and will end in 2004. It was initially planned to end in 2003, but was extended to 2004 because of the urgent need to study an emerging viral disease of common carp and koi (*Cyprinus carpio*) (= koi herpesvirus [KHV] disease), which was reported in Indonesia and Taiwan in 2002 and Japan in 2003. After this 5-year project, the second phase of the Regional Fish Disease Project entitled “Development of Fish Disease Surveillance System” has been proposed for another 5 years duration (2004 to 2008). The project is being conducted as one of the collaborative projects of the ASEAN-SEAFDEC Fisheries Consultative Group (FCG).

This paper reviews the activities under the Regional Fish Disease Project from 2000 to 2004, focusing on research and training conducted at the SEAFDEC Aquaculture Department.

Objectives and Activities of the Regional Fish Disease Project

The Regional Fish Disease Project supported by the GOJ Trust Fund aims to: (1) assist the health development in aquaculture in Southeast Asia; (2) promote the healthy and wholesome trading of aquaculture products in the region; and (3) develop a fish disease surveillance network in the region.

To achieve these objectives, the project conducted the following activities from 2000-2004:

1. *Research.* The specific objectives of research were to (1) develop standardized diagnostic methods for major diseases affecting economically important aquaculture species in the region; (2) develop effective prevention and control measures against microbial and parasitic diseases; (3) assess the pathogenesis of newly emerging diseases; and (4) develop monitoring methods for residual chemicals in aquaculture products.
2. *Hands-on training.* This activity was specifically aimed at developing and enhancing capability in aquatic animal health diagnosis and management of technical staff working at research centers and institutions in the region.

3. *International meetings.* These were conducted to (1) discuss the status of fish disease problems in the region, the available diagnostic methods, and prevention and control measures; (2) discuss the results of research studies conducted under the project and those generated in other regions; (3) identify and discuss aquatic animal disease issues to be solved further for sustainable aquaculture growth; and (4) discuss collaboration with other international organizations such as the Office International des Épizooties (OIE).
4. *Extension.* This activity was done to disseminate research results and technology generated by the project through (1) training courses on fish diagnosis and health management; (2) production of manuals; (3) publication of primary results in international scientific journals; (4) international meetings; and (5) sampling and field visits.

To coordinate and promote the project, two Japanese fish disease experts were dispatched to the SEAFDEC Aquaculture Department as long-term experts by the Japan International Cooperation Agency (JICA). Dr. Yasuo Inui worked as the first expert from March 2000 to March 2003, and the second expert, Dr. Kazuya Nagasawa acted as the project leader from April 2003 to date.

Research during the First Phase of the Project (2000 - 2004)

Research is the main activity component of the Regional Fish Disease Project. When the project started in 2000, it was undertaken only by scientists of the SEAFDEC Aquaculture Department. Subsequently, scientists from three research institutions under the Department of Fisheries in Thailand and those of the SEAFDEC Marine Fisheries Research Department (MFRD) in Singapore joined the project in 2001 and 2002, respectively.

During the period from 2000 to 2004, a total of 29 research studies were conducted in the following five categories:

A. Establishment and Standardization of Diagnostic Methods

In Southeast Asia, various viral diseases have been reported from cultured shrimp and fish, causing devastating losses in aquaculture production. White spot syndrome (WSS) of black tiger shrimp (*Penaeus monodon*) and viral nervous necrosis (VNN) of marine fish are well known examples of such viral diseases affecting aquaculture in the region. The research in this category was undertaken to establish and standardize diagnostic techniques, such as PCR (polymerase chain reaction) methods for viral diseases, which are applicable and practical in the region. Some research studies surveyed the distribution, occurrence and prevalence of important viral diseases. There was also a study to prevent and control VNN infection in the marine finfish hatchery.

1. Standardization of diagnostic methods for viral diseases of shrimps (SEAFDEC Aquaculture Department [AQD], 2000)

2. Standardization of PCR technique as the detection method for WSSV infection in *Penaeus monodon* (SEAFDEC/AQD, 2001)
3. Development of shrimp cell culture *in vitro* (Marine Shrimp Research and Development Center [MSRDC], Thailand, 2001-2002)
4. Standardization of diagnostic methods for monodon baculovirus (MBV) and hepatopancreatic parvovirus (HPV): Establishment of monoclonal antibodies (MAbs) against MBV and HPV (SEAFDEC/AQD, 2001-2003)
5. Epizootiology of economically important viral diseases of wild *Penaeus monodon* (SEAFDEC/AQD, 2001-2003)
6. Viral diseases of cultured marine fishes in Southeast Asia
 - 6-1. Detection and identification of viral pathogens in cultured marine finfishes in the Philippines (SEAFDEC/AQD, 2000-2003)
 - 6-2. A viral survey in diseased grouper in Thailand using virus isolation and polymerase chain reaction (PCR) technique (Aquatic Animal Health Research Institute [AAHRI], Thailand, 2001-2002)
 - 6-3. Survey of iridoviral disease in freshwater fishes in Thailand (AAHRI, Thailand, 2003)
7. Establishment of preventive measures against viral nervous necrosis (VNN) in fish broodstocks: (1) grouper, (2) milkfish, (3) red snapper, and (4) sea bass (SEAFDEC/AQD, 2001-2003)

B. Biology and Pathogenesis of Disease Pathogens

Diseases caused by protozoan and metazoan parasites often cause mass mortality of cultured fish, and, like microbial agents, the parasites are important pathogens. However, there remains limited information available on fish parasites in Southeast Asia. For example, less than 10 % of more than 2,030 species of marine and freshwater fish in the Philippines have been examined for the parasites. There are only a few studies on the parasites of cultured fish in the region. The research in this category aimed to screen economically important fish for the presence of parasites, determine diagnosis and pathology of infections, and establish prevention and control methods.

1. Parasitosis in marine and freshwater fishes: diagnosis, pathology, prevention and control of infection
 - 1-1. Screening of important parasites in economically important aquaculture fish (SEAFDEC/AQD, 2000-2003)
 - 1-2. Biology and pathology of the gill monogenean parasitic to grouper (SEAFDEC/AQD, 2000-2003)
 - 1-3. Leech infestation and its associated blood parasitic protozoans (SEAFDEC/AQD, 2000-2003)
 - 1-4. Establishment/application of prevention and control methods against parasites (SEAFDEC/AQD, 2000-2003)
2. Study on parasites of groupers in Thailand (AAHRI, Thailand, 2001-2002)

3. Screening of important parasites of freshwater fish in Thailand and neighboring countries (AAHRI, Thailand, 2003)

C. Disease Prevention and Control

Luminous vibriosis caused by *Vibrio* spp., especially *V. harveyi*, is a major bacterial disease of black tiger shrimp cultured in Southeast Asia. The research studies were intended to develop husbandry techniques, such as the use of live bacteria (probiotics) and “green water” culture system, as alternatives for chemotherapy to control vibriosis. The “green water” culture system is the finfish-integrated shrimp culture system, utilizing finfish rearing water for shrimp culture. The mechanisms on how the system works to control vibriosis were analyzed.

1. Use of bacteria as biological control agent against microbial diseases in shrimp (*Penaeus monodon*) and crab (*Scylla serrata*) hatcheries (SEAFDEC/AQD, 2000-2003)
2. Screening of probiotics as biocontrol/bioremediation in the rearing of *P. monodon*. I. Tank experiment (SEAFDEC/AQD, 2001-2003)
3. Antibacterial metabolites in the microbial and phytoplankton flora of the “green water” cultured *Penaeus monodon* (SEAFDEC/AQD, 2000-2003)
4. Investigation on the mechanism of the effect of tilapia culture water on luminous bacteria (SEAFDEC/AQD, 2001-2003)
5. Screening of *Vibrio harveyi* bacteriophage for controlling luminous disease in marine shrimp hatchery (Samutsakhon Coastal Aquaculture Development Center [SCADC], Thailand, 2001-2003)
6. Development of immunological indices for monitoring health status in *P. monodon* (SEAFDEC/AQD, 2001-2003)

D. Establishment of Evaluation Methods for Residual Chemicals in Aquaculture Products

The presence of chemical residues in aquaculture products threatens human health. To ensure safe and healthy aquaculture products, a research activity addressed the development and standardization of detection methods of residual chemicals, especially pesticides and antibiotics, in aquaculture products. The usage of antibiotics in shrimp culture was also monitored.

1. Establishment and monitoring on antimicrobial usage in shrimp aquaculture (SCADC, Thailand, 2001-2003)
2. Detection of pesticide residues in aquaculture products (SEAFDEC/AQD, 2000-2003)
3. Detection of antibiotic residues in aquaculture products (SEAFDEC/MFRD, 2002-2003)

E. Epizootiology and Prevention of Koi Herpesvirus Disease

Koi herpesvirus (KHV) disease was found in common carp and koi cultured in Indonesia and Taiwan in 2002 and Japan in 2003. The disease caused mass mortality of the fish and became a new threat to freshwater aquaculture in Southeast Asia. The research studies were conducted to elucidate various aspects of KHV infection.

1. Transmission and control of koi herpesvirus (SEAFDEC/AQD, 2004)
2. Development of PCR-based detection method and phylogenetic analysis of koi herpesvirus isolated from Asian countries (SEAFDEC/AQD, 2004)
3. Histopathology of koi herpesvirus disease (SEAFDEC/AQD, 2004)
4. Hematology of carp infected with koi herpesvirus (SEAFDEC/AQD, 2004)
5. Determination of the virucidal effects of various disinfectants on koi herpesvirus (SEAFDEC/AQD, 2004)

Hands-on Training during the First Phase of the Project (2000 - 2004)

The Seminar/Workshop on “Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques” was convened by SEAFDEC and OIE in Iloilo City, Philippines on 4-6 December 2001. One of the major recommendations of the Seminar/Workshop was to conduct the international training course on diagnosis of viral diseases, which became the basis for the “SEAFDEC Hands-on Training for Important Viral Diseases of Shrimp and Marine Fish.” The implementation of the said training course was done at the SEAFDEC Aquaculture Department in collaboration with OIE and the Network of Aquaculture Centres in Asia-Pacific (NACA).

The objective of the training course was to provide executive training on the diagnosis of viral diseases to core persons from the SEAFDEC member countries and other interested participants. These persons were expected to serve as national trainers in their respective countries. The training course consisted of the first and second phases, which were held on 6-19 November 2002 and 5-21 November 2003, respectively, at the SEAFDEC Aquaculture Department. The same set of trainees was invited to attend the two phases, but there were new participants who replaced those who were unable to come for some reasons.

A total of 12 and 11 participants attended the first and second phases of the training course, respectively. The participants came from the SEAFDEC member countries (one from each country: Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam) and other countries (China and India) although there was no participation from Brunei Darussalam in the second phase. All participants from the SEAFDEC member countries were funded by the Regional Fish Disease Project.

The first phase of the training course focused on the use of molecular tools and other important techniques in the diagnosis of viral diseases of

shrimp and marine fish, while the second phase was a continuation of the first phase, which then completed the two-year plan.

The 14-day course of the first phase consisted of lectures (11 hours or 15%) and practical activities (64 hours or 85%). Similarly, the 17-day course of the second phase was composed of 17 hours of lecture sessions (17.3%) and 81 hours practical activities (82.7%). Most of the lectures were done at the Research Division (RD) conference room. The practical activities were undertaken either at the Fish Health laboratory or in the Biotech laboratory. For the second phase, the participants brought shrimp and fish tissue samples from their respective countries for the laboratory activities.

The first phase of hands-on sessions included dissection and preservation of fish and shrimp samples, rapid detection methods for monodon baculovirus (MBV) and hepatopancreatic parvovirus (HPV) using hepatopancreas impression smears, histopathological analysis of viral diseases of shrimp and marine fish, extraction of nucleic acids (DNA and RNA) and detection of white spot syndrome virus (WSSV) and MBV in shrimp, and viral nervous necrosis virus (VNN) and iridovirus in grouper by one-step and nested polymerase chain reactions (PCR). The diagnostic techniques used were consistent with the standards set in the OIE's "Diagnostic Manual for Aquatic Animal Diseases" and the FAO's "Asia Diagnostic Guide to Aquatic Animal Diseases." Demonstrations on cell culture- and antibody-based detection methods for viruses were undertaken.

The second phase of the hands-on training consisted of preparation and preservation of tissue filtrates, cell culture passage, immune system parameters, histopathology, observation of virus using electron microscopy, detection of viruses by cell culture, antibody (Ab)-based detection of HPV and MBV, detection of MBV, WSSV, VNN and iridovirus using PCR. Demonstrations on histopathological slide preparation were also done.

In addition to these activities, all participants presented country reports describing the status of aquatic animal diseases and diagnostic capability in their respective countries and institutions.

Outputs

As the outputs of research and hands-on training activities and international meetings that were organized with financial support from the Regional Fish Disease Project, there are already publications available and many are in preparation for publication. These are listed under various categories below.

Proceedings

1. Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques (ed by Inui Y, Cruz-Lacierda ER), 2002, SEAFDEC Aquaculture Department, Iloilo. 215 p.
2. Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training (ed by Lavilla-Pitogo CR, Nagasawa K), 2004, SEAFDEC Aquaculture Department, Iloilo. 254 p.

Manuals

1. Borlongan I, Ng Poh Chuan J. 2004. Laboratory Manual of Standardized Methods for the Analysis of Pesticide and Antibiotic Residues in Aquaculture Products. SEAFDEC Aquaculture Department, Tigbauan, Iloilo. 46 p.
2. de la Peña LD, Lavilla-Pitogo CR. 2004. Control Measures against Important Viruses in Shrimp Hatchery with Emphasis on White Spot Syndrome Virus (WSSV). SEAFDEC Aquaculture Department, Iloilo. (in press).
3. de la Peña LD. 2004. Control Measures against Viral Nervous Necrosis (VNN) in Finfish Hatchery. SEAFDEC Aquaculture Department, Iloilo. (in press).
4. Lavilla-Pitogo CR, de la Peña LD. 2004. Health Management in Crab Hatchery System. SEAFDEC Aquaculture Department, Iloilo. (in press).
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2. Inui Y. 2002a. Fish disease control project of SEAFDEC Aquaculture Department. *In*: Inui Y, Cruz-Lacierda ER (eds), Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques. SEAFDEC Aquaculture Department, Iloilo, p. 181-185.
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5. Lavilla-Pitogo CR, Catedral DD, Pedrajas SDG, de la Peña L. 2002. Selection of probiotics for shrimp and crab hatcheries. *In*: Inui Y, Cruz-Lacierda ER (eds), Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques. SEAFDEC Aquaculture Department, Iloilo, p. 136-150.

6. Lio-Po GD. 2004. Summary brief: International Symposium on Koi Herpesvirus Disease. *In: Lavilla-Pitogo CR, Nagasawa K (eds), Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training. SEAFDEC Aquaculture Department, Iloilo. p. 71-73.*
7. Lio-Po GD, Cruz-Lacierda ER, de la Peña LD, Maeno Y, Inui Y. 2002. Progress and current status of diagnostic techniques for marine fish viral diseases at the SEAFDEC Aquaculture Department. *In: Inui Y, Cruz-Lacierda ER (eds), Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques. SEAFDEC Aquaculture Department, Iloilo. p. 97-106.*
8. Lio-Po GD, Leano EM, Usero RC, Guanzon Jr. NG. 2002. *Vibrio harveyi* and the “green water culture” of *Penaeus monodon*. *In: Inui Y, Cruz-Lacierda ER (eds), Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques. SEAFDEC Aquaculture Department, Iloilo. p. 172-180.*
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 8. Maeno Y, de la Peña LD, Cruz-Lacierda ER. 2003. Development of control methods of factors suppressing sustainable production of aquaculture species: experimental transmission of piscine nodavirus-induced viral nervous necrosis to the orange-spotted grouper *Epinephelus coioides*. In: Ogawa Y, Ogata GY, Maeno Y, Shimoda T, Fujioka Y, Fukuda Y (eds), Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas. JIRCAS Working Paper No. 35. Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan. p. 89-94.
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 9. Lio-Po G, Franco A. 2003. Preliminary results on the comparative effects of two commercial probiotics on total bacterial counts and on luminous *Vibrio*. Paper presented at the Philippine Society for Microbiology-Visayas Chapter 11th Annual Meeting and Regional Scientific Convention held in Cebu City, Philippines on October 23-24, 2003.
 10. Maeno Y, de la Pena L, Cruz-Lacierda ER. 2002. Susceptibility of marine fish species to piscine nodavirus from orange-spotted grouper, *Epinephelus coioides* in the Philippines. Abstracts of 5th Symposium on Diseases in Asian Aquaculture, 24-28 November 2002, Queensland, Australia, p. 46.
 11. Tendencia E. 2002. Effect of *Tilapia hornorum* on luminous bacteria *Vibrio harveyi*. Abstracts of 5th Symposium on Diseases in Asian Aquaculture, 24-28 November 2002, Queensland, Australia, p. 172.

Terminal Report

1. Recent Advances in Diagnosis and Prevention of Fish and Shrimp Diseases in Southeast Asia: Terminal Report of the Regional Fish

Disease Project on “Development of Fish Disease Inspection Methodologies for Artificially-Bred Seeds” Supported by the Government of Japan Trust Fund (ed by K Nagasawa), 2005, SEAFDEC Aquaculture Department, Iloilo. (in press).

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