

Fish Disease Control Project of SEAFDEC Aquaculture Department

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INTRODUCTION

In the past 10 years, aquaculture production in Southeast Asia has grown rapidly and contributed to worldwide food supply. However, a number of infectious diseases have emerged, with the rapid and uncontrolled development of aquaculture, threatening the sustainability of aquaculture in the region. Moreover, the widespread use of chemicals including antibiotics to control these diseases can pose a danger to consumers' health and become an obstacle for trading of the cultured produce. It is, thus, urgent to establish effective control measures against infectious diseases, as well as monitor methods for chemical use.

Realizing these needs, the Aquaculture Department of Southeast Asian Fisheries Development Center (SEAFDEC AQD) with the support of the Japanese Government Trust Fund, initiated the Regional Fish Disease Control Project titled "Development of Fish Disease Inspection Methodologies for Artificially Bred Seeds" from 2000-2003.

The Project consists of the following activities:

1. Research to
 - 1.1 develop standardized diagnostic methods for the region;
 - 1.2 develop disease control husbandry techniques; and
 - 1.3 develop a monitoring method for residual chemicals in aquaculture products.
2. International workshop for the harmonization and standardization of diagnostic method as well as disease control husbandry methods;
3. Hands-on training on diagnostic methods for important diseases in the region; and
4. Development of a disease control network in the region.

RESEARCH AND DEVELOPMENT

At the start of the Project in 2000, research and development activities were conducted solely by SEAFDEC AQD. In order to make the Project more efficient and more relevant to the

region, three agencies of the Department of Fisheries-Thailand were invited to collaborate starting 2001. These are: the Aquatic Animal Health Research Institute (AAHRI), the Marine Shrimp Research and Development Center (MSRDC), and the Samutsakhon Coastal Aquaculture Development Center (SCADC).

The main research activities are as follows:

1. Establishment and standardization of diagnostic methods

Recent pandemics of viral diseases in shrimp as represented by white spot syndrome virus (WSSV) have produced catastrophic economic damage to aquaculture. Similar viral disease impact is also expected for marine fish aquaculture, which has been rapidly growing in the region. The research in this section aims to establish and standardize diagnostic methods for viral diseases, which are practical and applicable in the region. The section also contains some survey on the distribution of important viral diseases in the region.

Shrimp viruses

One study aimed to standardize polymerase chain reaction (PCR) technique as the detection method for WSSV. The study has already refined the extraction methods of DNA and primers to be used. Thus, the technique is ready for the proposed training in the Project. Two studies are tackling the development of new diagnostic methods: one is addressing the production of monoclonal antibody for hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV); another is establishing a shrimp cell culture system.

A survey is ongoing on the distribution of important viral diseases in wild *Penaeus monodon*. The survey is important in identifying populations of wild shrimp that are free of systemic viruses, since broodstock development of *P. monodon* solely relies on wild stock.

Marine fish viruses

First, we aim to establish virus detection in marine fish at SEAFDEC AQD. Through this Project, the cell lines SBK-2, GF, EPC, FHM, SSN-1, SHS and WSS₂C₁ have been made available. The PCR and immuno-cytochemistry methods for several important viral diseases are also available. These techniques will be utilized for the proposed training under this Project. Using these techniques, viral examination of eggs, larvae and broodstock of various fishes have started. As an output of this activity, we demonstrated by histopathology, PCR and cell culture method using SSN-1 that the mass mortality of grouper larvae observed at SEAFDEC AQD was caused by viral nervous necrosis (VNN). This is the first confirmative identification of VNN in the Philippines. Taking into consideration the impact of VNN, we also began establishing a model system to prevent and control VNN in the hatchery.

In Thailand, a similar survey on viral diseases in grouper is ongoing focusing, on iridovirus and nodavirus, the most devastating viruses in marine fish.

2. Biology and pathogenesis of disease agents

Parasitic infestation has been known to cause mass mortalities in many marine and freshwater fishes. Effective control of parasites necessitates the knowledge of their life cycles, good health management of culture systems of the host fish, and the availability of treatment methods. Thus, a series of studies was conducted in the Philippines and Thailand to screen economically important fishes for the presence of parasites, determine diagnosis and pathology of infections and host-parasite relationships, and establish methods of prevention and control.

Screening for parasites was done in grouper (*Epinephelus coioides*), red snapper (*Lutjanus argentimaculatus*), rabbitfish (*Siganus guttatus*) and catfish (*Clarias macrocephalus*). The most common ectoparasites observed among these fishes were the trichodinids, skin and gill flukes, digeneans, copepods and leech while nematodes were the most common endoparasites. Parallel screenings conducted in 2001 in the Philippines and Thailand on grouper indicated similar parasite fauna.

Healthy grouper fingerlings experimentally exposed to the gill fluke *Pseudorhabdosynochus* sp. resulted in 100% mortality within 72 h of exposure at 5,000 monogeneans/10 fish.

Since heavy gill fluke infestation was found to have caused high level of mortality in grouper, preventive measures were developed. Among the treatments tested, the 1 h bath of 200 ppm H₂O₂ effectively removed the gill parasite.

3. Disease prevention and control

Luminescent vibriosis has long been a major constraint in shrimp culture in the region. Research in this section aims to develop husbandry techniques such as the use of probiotics and the “green water” culture system as alternative strategies for the control and prevention of shrimp vibriosis.

One study assessed the use of live bacteria (probiotics) as a biological control agent against the serious disease luminescent vibriosis and its pathogen, *Vibrio harveyi* in shrimp and crab aquaculture. Tests were conducted to measure growth suppression of the pathogen by competition with antagonistic bacteria in mixed cultures and manipulate gut bacterial flora by introducing benign bacteria through live or inert feeds, or through the water.

Potential probiotic bacteria were isolated from healthy hatchery-reared crustacean larvae and their environments. From the 80 bacterial isolates tested in one-on-one competition experiments, 10 strains were found to suppress the growth of *V. harveyi* within 24 h. One isolate from *Chlorella* sp. culture is considered a promising probiotic for crustacean hatcheries since the bacterium is not pathogenic to crab and shrimp larvae, can associate closely with the larvae and dominate their associated bacterial flora, can readily be incorporated into live *Brachionus plicatilis*, and can easily be cultured in liquid media.

Another study will evaluate probiotics using a new, experimental method. The method has been refined and ready for evaluation. The candidate probiotics selected in this study as well as commercial probiotics will be evaluated.

The “green water” technique, which transfers rearing water from tilapia culture ponds to shrimp ponds, is also one of the several modifications in shrimp culture to prevent bacterial and viral diseases. Bacterial, fungal and algal isolates associated with the “green water” culture of *P. monodon* were screened for inhibitory metabolites against *V. harveyi*. The results indicated that extracellular and intracellular metabolites of some of the bacteria, fungi and phytoplankton of the “green water” system inhibit the growth of *V. harveyi*.

In a preliminary tank experiment, the existence of tilapia reduced the concentration of *V. harveyi*. This tank experiment is expected to clarify the mechanism of “green water” (or finfish integration) shrimp culture system.

Another study addresses the possible utilization of bacteriophage of *Vibrio* for controlling vibriosis. Samples have been obtained from shrimp hatchery and grow-out farms.

4. Establishment of methods to evaluate residual chemicals in aquaculture products

The presence of chemical residues in aquaculture food products threatens human health. To answer the need for risk-free, nontoxic food, research in this area aims to develop monitoring methods for chemicals in aquaculture products and publication of manuals for the safe and efficient use of chemicals in aquaculture.

Detection of pesticide and antibiotic residues in aquaculture products underwent its first phase of activities. Various extraction techniques for some aquaculture products (shrimps, milkfish, grouper, sea bass, siganid, catfish, tilapia and seaweed) were standardized. The recovery, efficiency, detection limits and reproducibility of methods for 18 pesticides were established.

To collect basic data for guidelines and strategies for antimicrobial usage, a survey on drug resistant-bacteria in shrimp farms in Thailand was conducted. A total of 360 bacterial isolates were collected from 13 places. Among these, 289 were identified as *Vibrio* spp. and 25 as unidentified luminous bacteria. Examination of drug resistance of these bacteria showed that out of 169 isolates, 39 were resistant to oxytetracycline, 6 to oxolinic acid and 2 to chloramphenicol.

COLLABORATION FOR DISEASE CONTROL IN THE REGION

Possible networking schemes for disease control in the region and collaborative schemes for regional diagnosis are shown as Figures 1 and 2. SEAFDEC AQD will collaborate with other international organizations such as OIE, FAO and NACA, focusing on development and dissemination of disease diagnosis and prevention and control methods and on network formation in research activities.

TRAINING

During the *S,minorFWorHshop on Disease Control in Fish and Shrimp Aquaculture in Southeast Asia: Diagnosis and Husbandry Technique*, prioritization of the diseases and diagnostic methods in the region, as well as a training program was discussed. Based on this output, the Project plans to conduct a training course in late 2002 on *Diagnosis of Important Viral Diseases of Shrimp and Marine Fish* in the region.

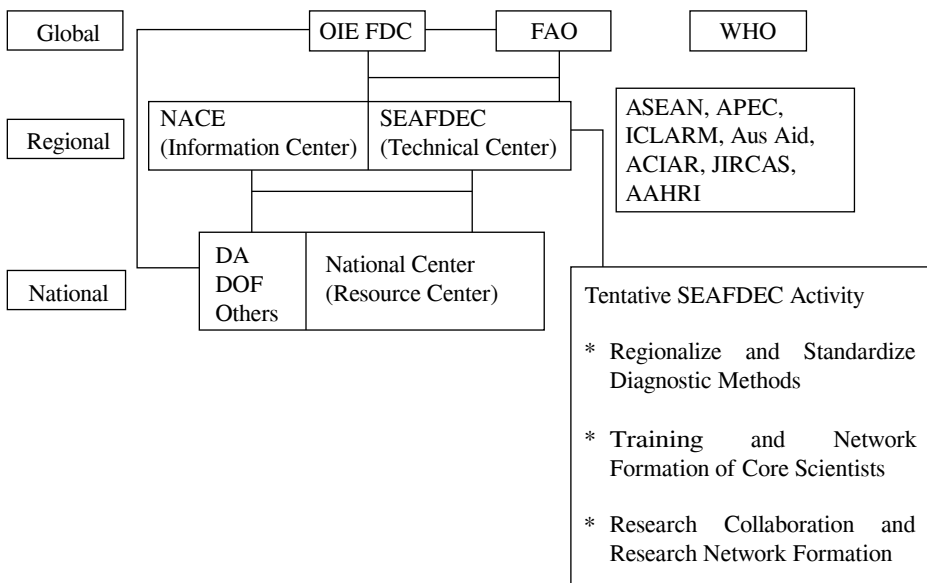


Figure 1. A possible regional network for fish control

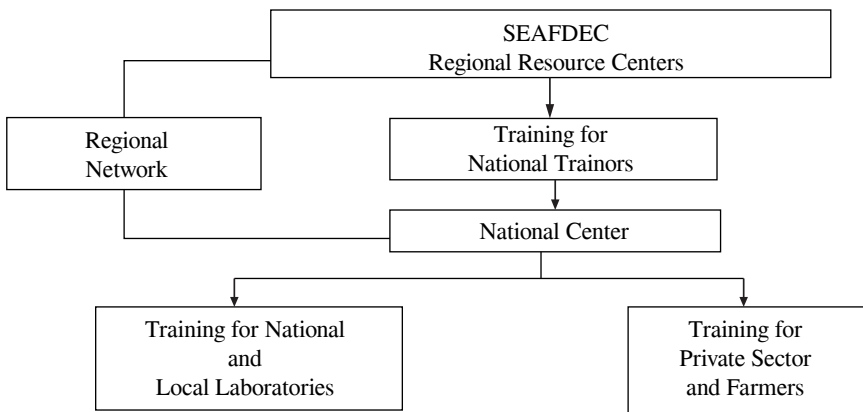


Figure 2. Collaboration scheme for regional diagnosis