



**REPORT OF THE  
ROUND TABLE DISCUSSION  
ON THE  
DEVELOPMENT OF  
GENETICALLY IMPROVED  
STRAIN OF MACROBRACHIUM**



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The Round Table Discussion on the Development of Genetically Improved Strain of *Macrobrachium* is part of the Collaborative Research on Genetic Improvement and Seed Production of *Macrobrachium rosenbergii* under the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region: Aquaculture Component.

The participating countries in the Collaborative Research are:

Indonesia



Philippines



Thailand



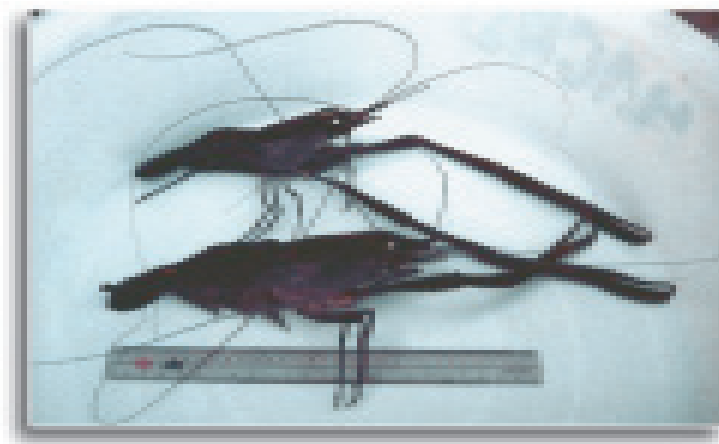
This publication documents the outcome of the Round Table Discussion including the proposals submitted by the participating countries for implementation under the Collaborative Research.



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**Freshwater Aquaculture Development Center  
Sukabumi, West Java, Indonesia  
16-19 November 2003**





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*From the Office of the Chief of SEAFDEC Aquaculture Department*

Recognizing that the culture of the giant freshwater prawn in the ASEAN region is constrained by shortage of good quality seeds, lack of domesticated broodstock with selected strains, and absence of appropriate culture system; that freshwater prawn is becoming an economically important species for aquaculture in the region; and that some countries have already developed technologies on its culture in various scales of development, the Integrated Regional Aquaculture Program (IRAP) as the Aquaculture Component of the ASEAN-SEAFDEC Special Five-Year Program has pooled the expertise and technologies in the ASEAN Region on freshwater prawn aquaculture, and decided to conduct a collaborative research on the genetic improvement and seed production of the giant freshwater prawn, *Macrobrachium rosenbergii*.

The collective effort of Thailand, Indonesia and the Philippines in collaborating for the conduct of this research is expected to lessen the period of research and hasten the improvement and sustainable production of the giant freshwater prawn in the ASEAN region. It is the goal of the collaborative research to come up with technology for consistent and improved production of quality freshwater prawn seeds that could be made available for rural aquaculture in the region, in the shortest possible time. Through this collaborative effort, a fast growing and disease resistant strain of the freshwater prawn could also be established for dissemination in this region.

On behalf therefore, of the ASEAN and SEAFDEC and as the Program Co-Manager for AQD of the Special Five-Year Program, I wish to thank the Fisheries Agency of the Government of Japan for providing the Special Funds for the implementation of IRAP and specifically this collaborative research; the ASEAN Secretariat and the SEAFDEC Secretariat for the full support accorded to this activity; the Governments of Thailand, Indonesia, and the Philippines for providing the expertise and the existing technologies on freshwater prawn aquaculture; and the scientists and researchers involved in the collaborative research and the support staff for their efforts.

We are indeed grateful that this collaborative research was able to convene its first Round Table Discussion in November 2003 to delineate responsibilities. For their efforts, I also wish to commend the participants in the Round Table Discussion for their involvement in the deliberations. This Report documents the country papers presented, the recommendations and the research proposals that would serve as basis for the conduct of the collaborative research in respective countries, the results of which will be disseminated to the other countries in the region for the improvement of the freshwater prawn aquaculture in the ASEAN region.

  
**ROLANDO R. PLATON, Ph.D.**  
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# **REPORT OF THE ROUND TABLE DISCUSSION ON THE DEVELOPMENT OF GENETICALLY IMPROVED STRAIN OF MACROBRACHIUM**

**Freshwater Aquaculture Development Center  
Sukabumi, West Java, Indonesia  
16-19 November 2003**

## **INTRODUCTION**

As a follow-up of the outcome of the ASEAN-SEAFDEC Conference on Sustainable Fisheries in the Third Millennium: Fish for the People in Bangkok, Thailand in November 2001, SEAFDEC implements the ASEAN-SEAFDEC Special Five-year Program on the Contribution of Sustainable Fisheries to Food Security in the ASEAN Region. The Aquaculture Component of this Special Five-Year Program is the Integrated Regional Aquaculture Program (IRAP) with AQD as the Lead Department.

IRAP has two components, SDII-1: Aquaculture for Rural Development; and SDII-2: Supply of Good Quality Seeds. Vietnam has been identified as the ASEAN Lead Country for SDII-1, while Indonesia is the ASEAN Lead Country for SDII-2. The ASEAN-SEAFDEC member countries are the participating and beneficiary countries of the Program.

IRAP aims to: (1) assure a supply of quality seed stocks of various aquatic commodities; (2) promote environment-friendly aquaculture; and (3) assure that the development of aquaculture will benefit the rural populace through consultation, demonstration and dissemination of specific aquaculture technologies. IRAP also intends to launch an information dissemination campaign to make the people of the region aware of the tremendous potential that aquaculture can offer to produce low-cost protein and generate livelihood opportunities for the rural poor.

SDII-1 (Aquaculture for Rural Development) is expected to come up with appropriate responsible aquaculture technologies that will help alleviate poverty and ensure food security for peoples in the rural areas of the ASEAN region. Specifically, SDII-1 aims to: (1) verify aquaculture technologies for appropriate species in various ecosystems; and (2) disseminate the aquaculture technologies to small-scale fish farmers.

SDII-2 (Supply of Good Quality Seeds) is expected to come up with appropriate and responsible seed production technologies in support of the aquaculture and stock enhancement programs in the ASEAN region, and eventually contribute to food security and sustainable development of the fisheries sector in the region. The specific objectives of SDII-2 are to: (1) verify and demonstrate appropriate seed production technologies of target species; (2) verify on-farm broodstock management techniques; and (3) disseminate seed production technology to fish farmers.

Under IRAP technologies developed by some institutions in the region especially on hatchery rearing may be refined for adoption by certain countries. Since a common problem in aquaculture is feeds and feeding management, some studies on environment-friendly and low-cost feed formulations are also conducted. Testing of low-cost feeds based on formulations already developed by AQD is done in conjunction with the activities on pilot demonstration and verification.



Moreover, during the IRAP Seminar-Workshop held in Bangkok, Thailand in September 2002, three countries proposed to conduct genetic improvement of *M. rosenbergii*. Thus, it was decided that the resources and expertise from these countries would be pooled to develop a collaborative research on the genetic improvement and seed production of *M. rosenbergii*.

Such common species with the required technology, identified by three countries as part of their activities for pilot demonstration and verification under the project on Supply of Good Quality Seeds of the IRAP, would be the subject of a collaborative research. Based on the required technology identified, the collaborative research on the Genetic Improvement and Seed Production of *Macrobrachium rosenbergii* would be conducted as part of IRAP with Indonesia, Philippines and Thailand as the participating countries.

In order to optimize resources, the collaborative research work would be conducted on *M. rosenbergii* with closely related activities in these countries. For the planning of the detailed activities of the collaborative research as well as for the delineation of the role and coverage of each participating country, the round table discussion was convened at the Freshwater Aquaculture Development Center of the Balai Budidaya Air Tawar (BBAT) in Sukabumi, West Java, Indonesia from 17 to 19 November 2003.

## OBJECTIVES

The objectives of the Round Table Discussion were:

1. To adopt common criteria for “good quality *Macrobrachium* seed”;
2. To standardize methodology and approach in developing a genetically improved strain of *Macrobrachium* among the participating countries;
3. To formulate definite work plan for the remaining two years or so of the project; and
4. To agree on mechanics for the sharing of results and genetic materials in *Macrobrachium*.

## AGENDA

The agenda adopted for the Round Table Discussion were:

1. Election of Chairperson of the Round Table Discussion
2. Criteria for Good Quality *Macrobrachium*
3. Work Plan (including specific activities for each participating country)
4. Time Table and Persons Involved
5. Adoption of Recommendations and Output of the Discussion
6. Other Matters

## ROUND TABLE DISCUSSION PARTICIPANTS

The Round Table Discussion was participated in by representatives from the three collaborating countries: Indonesia, the Philippines, and Thailand. Observers from the Directorate General of Aquaculture based in Jakarta, Indonesia as well as technical staff of the BBAT-Sukabumi also attended the Round Table Discussion. The representative from Indonesia, *Dr. Ketut Sugama*, was elected Chairperson of the Round Table Discussion.





## COUNTRY PAPERS

Country representatives from Indonesia, the Philippines and Thailand presented country papers that contained the following information:

- Brief status of industry: quantity and value of production, area devoted to *Macrobrachium*, typical stocking density, yields and average body weight (ABW) at harvest, comparison with past results if any even if anecdotal, future plan and prospects,
- *Macrobrachium* seed quality in commercial hatcheries
- Present country definition of good quality seed
- Strains or Families of *Macrobrachium* that have been identified in the country
- Status of existing R & D effort on seed quality improvement.



Venue of the November 2003 Round Table Discussion on Genetically Improved Strain of *Macrobrachium*: Freshwater Aquaculture Development Center, BBAT, Sukabumi, West Java, Indonesia





## MACROBRACHIUM AQUACULTURE IN PARTICIPATING COUNTRIES



### Indonesia

The giant freshwater prawn (*Macrobrachium rosenbergii*) is cultured in Indonesia mainly in the island of Bali as well as in East and West Java, where more than 50,000 ha is used for its culture. The culture system is traditional and semi-intensive either mono- or polyculture with common carp, tilapia, milkfish and *Puntius*. Prawn production using the monoculture system averaged at 600 kg/ha/year and 300/ha/year for the polyculture. The giant freshwater prawn is cultured in freshwater ponds or paddy-cum-ponds, except in East Java where the prawn is cultured in brackishwater ponds.



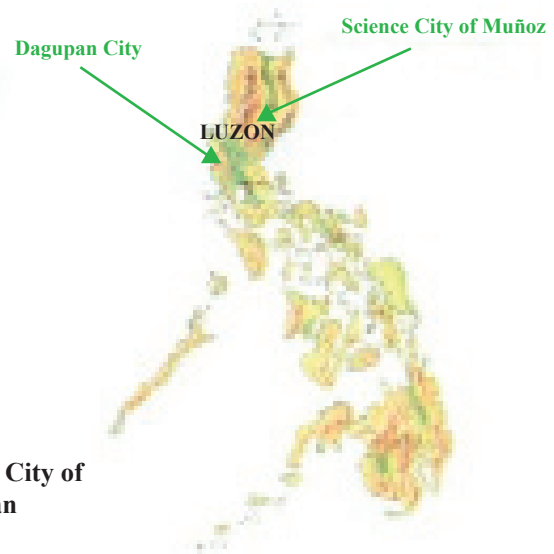
**Freshwater prawn pond in Sukabumi, Indonesia (left) visited by Round Table Discussion Participants (right)**

The Directorate General of Aquaculture through its facilities at the Research Institute for Freshwater Aquaculture has been conducting studies to improve the quality of the freshwater prawn, which was observed to be genetically deteriorating. A selective prawn breeding program has been implemented in order to improve production. This activity led to the development of an improved strain identified as the GI Macro or Genetically-Improved *Macrobrachium*. The culture of this improved prawn strain is now being adopted by some fish farmers in selected areas of the country.

However, there is still a need to further improve the production of the freshwater prawn and to develop a strain with high tolerance for salinity. The latter is aimed at culturing prawn in shrimp ponds that have not been used since the devastation of the country's shrimp culture industry due to shrimp disease problems. The culture of freshwater prawn is identified as Indonesia's alternative to the shrimp culture industry.

### Philippines

Culture of the giant freshwater prawn in the Philippines actually started in the early 1900s when it was then considered an important industry of the country. However, its culture was not sustained until the 80s when it was revived but again eventually abandoned due to certain product diversification.



**Map of the Philippines (right) showing the Science City of Muñoz, Nueva Ecija and Dagupan City, Pangasinan**



In early 2000, the Bureau of Fisheries and Aquatic Resources (BFAR) through its National Freshwater Fisheries Technology Center (NFFTC) in Muñoz, Nueva Ecija and the National Integrated Fisheries Technology Development Center (NIFTDC) in Dagupan City embarked on a semi-commercial production of the giant freshwater prawn, *Macrobrachium rosenbergii*.

The NFFTC and NIFTDC are conducting research studies that would lead to the production of quality prawn seeds for distribution to fish farmers in the country. These two centers are now commercializing a sustainable freshwater prawn aquaculture throughout the Philippines.

At present, the freshwater prawn culture industry in the Philippines is still in its development stage. In order to hasten the adoption of the culture technology by the fish farmers, it is important that production of the prawn on commercial scale be verified. Efforts are now being done by BFAR to convince the fish farmers to adopt the species as an aquaculture commodity with economic importance.

### **Thailand**

The giant freshwater prawn is one of the most important economic aquaculture species in Thailand. Its culture is well-developed with production steadily increasing during the past five years at about 10,000 mt/year. Through the Department of Fisheries (DOF), rearing systems have been developed producing good quality seeds for the farmers. This means that the seeds being distributed have high survival rate and are fast growing.

However, a number of problems have beset the giant freshwater prawn culture industry in Thailand. These include: slow growth, lack of appropriate broodstock management, and the occurrence of some diseases.

Thailand is also establishing good production procedures for the prawn from the farm to the table. It is now developing the Code of Conduct similar to that developed for the marine shrimp aquaculture. Thailand has also embarked on a number of R&D activities aimed at improving the quality of the prawn. These activities include selective breeding programs and the development of biotechnological approaches to genetic improvement.

## **RECOMMENDATIONS**

In the ensuing discussions, the participants in the Round Table Discussion agreed on the specific activities that would be implemented in the participating countries for the improvement of the giant freshwater prawn. Specifically, the following recommendations were agreed upon:

### **Selective Breeding**

- Each country will develop its own appropriate selective breeding protocols, without duplicating but instead complementing each other.
- Thailand shall continue within family selection but would also develop mass selection procedures for farmers.
- Indonesia to continue family selection combined with hybridization
- Philippines to design a within family selection with rotational mating at the BFAR-NFFTC in the Science City of Muñoz, while a modified mass selection with collimation and rotational mating will be developed at the BFAR-NIFTDC in Dagupan City. Selective breeding protocols would be designed after the completion of the population genetic studies.



### **Population Genetics**

- Philippines should start the characterization of local strains of *Macrobrachium* using molecular genetic techniques and morphological and morphometric measurements, in view of the very significant findings of other researchers (Mather, personal communication; Sudsok, personal communications) that the subspecies *Macrobrachium rosenbergii* is found only in the Philippines with possible affiliation with Australia.
- The Philippine *Macrobrachium rosenbergii* is different from that of Thailand, Indonesia and Borneo. Thus, it is urgent to study the genetic structure of this very important resources.

### **Transfer of Macrobrachium Stock**

- Due to the fact that the Philippine stock of *Macrobrachium* is genetically different from that of the rest of Southeast Asia, there is a need to adopt much greater precaution in the transfer of *Macrobrachium rosenbergii* from Thailand and the rest of Southeast Asia to the Philippines in order to avoid genetic introgression of the Philippine strain of *Macrobrachium*.
- The same precaution should also be observed in the transfer of *Macrobrachium* stock from the Philippines to the rest of Southeast Asia.

### **Strain Evaluation in Different Environments**

- Adopt similar protocol as the GIFT tilapia but with caution because *Macrobrachium* is a crustacean and is very different from tilapia.

### **Estimates of Heritabilities**

- There is an urgent need to design a proper experiment to estimate heritabilities of traits under selection.

### **Control**

- It is important to develop a proper control line in order to really measure the genetic gains after selection.

### **Criteria for Quality Seeds**

- As a short-term output while genetic improvement is ongoing, it is essential to establish criteria for good quality seeds in order to guide farmers when purchasing seeds. In this regard, the short paper by Dr. Melchor Tayamen shall serve as a guide.

### **Deadline for Detailed Proposal**

- Deadline for the submission of detailed proposals by participating countries is on 15 December 2003.

### **Venue of Next Round Table Discussion**

- The proposed venue for the next Round Table Discussion is the Philippines tentatively before or after ISTA VI, which will be held in the Philippines in September 2004.



## FIELD TRIP

In order to be able to obtain a first hand information on the status of the giant freshwater prawn aquaculture in Indonesia, field trips were conducted for the Round Table Discussion participants to the Pelabuhan Ratu Macrobrachium Hatchery of the Directorate General of Aquaculture in West Java, the *M. rosenbergii* ponds also in West Java, and the Fresh Fish Market in Sukabumi.



The Government Macrobrachium Hatchery (above) in Pelabuhan Ratu, West Java, Indonesia



Freshwater prawn-fish polyculture pond (left) and prawn monoculture pond (right) in Indonesia



Freshwater fish trading (above) at the Fresh Fish Market in Sukabumi, West Java, Indonesia



## WORK PLAN

The work plan of the collaborative research on the genetic improvement of *M. rosenbergii* for each participating country to undertake as contained in the proposals submitted by the country representatives and agreed upon during the Round Table Discussion, is summarized as follows:

### ***Indonesia: Production of high quality seeds and broodstock***

- Broodstock collection
- Characterization using molecular starter
- Development of a sustainable tagging system
- Development of breeding and larval rearing
- Development of nursery for post-larvae and grow-out for juveniles
- Development of selection techniques and conduct of multiple location testing

*The activities in Indonesia will be conducted in Sukabumi and Sukamandi, West Java, Indonesia.*

### ***Philippines: Genetic characterization, domestication and genetic improvement***

- Determining the genetic diversity of wild and farmed stocks
- Developing a sound broodstock management and selective breeding methods
- Production of quality *M. rosenbergii* seedstock for rural aquaculture

*The activities in the Philippines will be conducted at BFAR-NFFTC, BFAR-NIFTDC and at SEAFDEC Aquaculture Department's Binangonan Freshwater Station.*

### ***Thailand: Selective breeding program for genetic improvement***

- Evaluation of the economic traits performance and genetic variation of the various crosses of *M. rosenbergii*
- Improvement of the economic traits of the best cross
- Conduct of PCR conditional optimization and primer testing of microsatellite markers
- Application of polymorphism system of the molecular markers

*The activities in Thailand will be conducted at the Aquatic Animal Genetics Research and Development Institute in Pathum Thani.*

The abovementioned work plan is derived from the summary proposals submitted by the representatives from each participating country, which are also included in the later part of this Report.



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## COUNTRY PAPERS



Representatives from the participating countries in the collaborative research on the Genetic Improvement and Seed Production of *Macrobrachium rosenbergii*, presented country papers on Macrobrachium aquaculture in their respective countries.

Specifically, the following country papers were presented:

- ◆ **Giant Freshwater Prawn Culture in Indonesia**  
*Estu Nugroho and Lies Emmawati*  
Research Institute for Freshwater Aquaculture  
Bogor, West Java, Indonesia
- ◆ **Culture of Giant Freshwater Prawn: Philippines**  
*Westly Rosario and Melchor Tayamen*  
Bureau of Fisheries and Aquatic Resources  
Philippines
- ◆ **The Macrobrachium Culture Industry in Thailand**  
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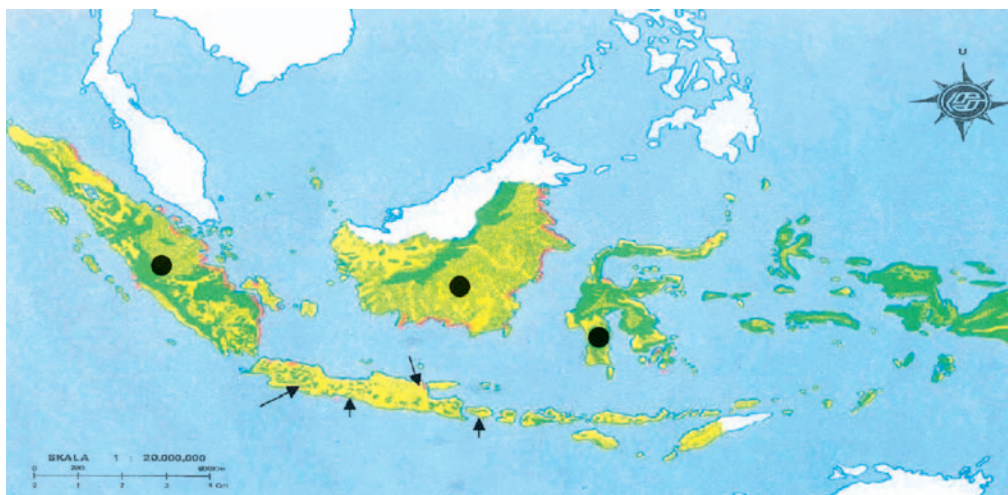
# GIANT FRESHWATER PRAWN CULTURE IN INDONESIA



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## INTRODUCTION

Indonesia is one of the countries that have high levels of biological diversity in terms of freshwater fishes, the country's rich biological resources, characterized by a high level of endemism. About 30 endemic species of freshwater fishes are found in Sumatera, 149 species in Kalimantan, 12 species in Java, and 52 species in Sulawesi (Anonym, 1994; Kottelat et al., 1993). The country's total freshwater area is 55 million ha consisting of lakes, dams, swamps and other water bodies. The potential area for freshwater pond fish culture is estimated at 233,124 ha with a production of 334,085 mt/year (DGF, Indonesia, 2001), of which about 5140 mt comprises the giant freshwater prawn.



**Distribution of the giant freshwater prawn in Indonesia**

Recently, the giant freshwater prawn has been considered an important commodity that is successfully cultured in Indonesia. Freshwater prawn culture has been developed in several areas of West Java, i.e., in Ciamis (Tambaksari, Pamarican and Kalipucang) and Tasikmalaya. Commercial hatcheries are mostly found in Jogjakarta area with the Government operating one hatchery while the private sector operates at least seven hatcheries. In East Java, freshwater prawn culture is conducted in brackish water ponds. The development of the freshwater prawn culture has also spread to Bali Island, e.g., in Gianyar, Klungkung, Buleleng and Tabanan.

Freshwater prawn population in Indonesia is unique and its geographical distribution is in almost all islands. Indonesia is recognized as the center of origin from freshwater prawn because of about 19 species still existing until now (Holthuis, 1980). However, the potential genetic resource is not yet fully utilized in freshwater prawn culture. Further, despite the advanced development of freshwater prawn culture in Indonesia, some problems have been found, e.g., declining growth rate, diseases and the edible portion getting smaller. In recent years, the Government of Indonesia stressed its focus on the increased production of the freshwater prawn. One of the ways being promoted to achieve increased production is through a genetic improvement program. Thus, in 2001, a certain race of



freshwater prawn has been released and developed for culture by the country's fish farmers. This strain is the GI Macro or the Genetically Improved *Macrobrachium rosenbergii*.

## **PRESENT STATUS OF FRESHWATER PRAWN CULTURE IN INDONESIA**

The potential area for giant freshwater prawn culture consists of freshwater ponds, paddy-ponds and brackishwater ponds. About 10,000 ha of the potential areas are found in Bali, 2500 ha in West Java, 2200 ha in Central Java, and 21,000 ha in East Java.

Since 1990 there has been an indication of a decreasing production of giant freshwater prawn caught from the natural waters. This phenomenon happens in some areas in West Java and Sumatera. This situation led to the promotion of freshwater prawn culture from Jogjakarta (Central Java) and in Lamongan (East Java). In Bali Island, freshwater prawn culture is well developed since 1997 because of its increasing demand. The estimated consumption of freshwater prawn is about 700 kg/day at the price of US\$ 4.00 to US\$10.00/kg (before the Bali blast).

Freshwater prawn is cultured in Indonesia using the traditional and semi intensive systems in mono- or polyculture with common carp, tilapia, milkfish and Puntius. The ponds used are relatively small about 200 m<sup>2</sup>/pond. Recently, freshwater prawn culture involves rearing the fry (PL 25-40) for two months, followed by the selection of uniform sized prawn and then rearing the prawn separately by size at a stocking density of 10 fry/m<sup>2</sup>. Production using this scheme is about 300 kg/year (polyculture) and 600 kg/year (from monoculture) with an average size of 30 g/pc.

In order to meet the demand for freshwater prawn fry, hatcheries have been developed in Jogjakarta, West Java and in Bali. The fry needed by farmers in Gianyar-Bali estimated at about 24 million/year, is partly supplied from hatcheries in Jogjakarta and East Java. The price of fry is US\$0.60-0.70/pc for PL 25-40. The production capacity of hatcheries in Bali is about 7 million fry/year, about 300,000 fry/year in West Java, and 11 million fry/year in Jogjakarta.

Freshwater prawn culture in Indonesia is doing very well utilizing the shrimp ponds that have not been used because of the shrimp culture devastation due to the shrimp virus problems. Efforts to recover the tiger shrimp production have not gone far, thus, freshwater prawn culture has become an alternative industry especially in shrimp ponds that are no longer used. For this purpose, a strain of freshwater prawn with high tolerance for salinity is being developed. Further, standardization of seed quality produced from hatcheries is required to guarantee good quality seeds that are distributed to farmers. The country's criteria of good quality seed include fast growing, high tolerance for salinity, and bigger edible portion.

## **IMPROVEMENT OF THE FRESHWATER PRAWN PROGRAM**

It is recognized that the quality of the county's freshwater prawn is genetically deteriorating. As experienced recently, it has been difficult to produce female prawn at a size of 50 g/pc (export size) because the survival rate is very low. Moreover, to produce male prawn until the size of 50 g/pc, the survival rate is only less than 40% in 9-11 months of culture. Since 1996, the Research Institute for Freshwater Aquaculture (RIFA formerly RIFF) has implemented a number of programs with the main objective of improving the growth rate and increasing the edible portion of the prawn. The improvement program includes the following activities:



### Breeding Program

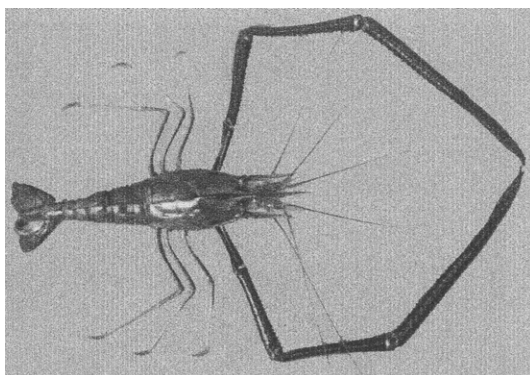
Selective breeding program has been conducted to improve the freshwater prawn from synthetic population gathered from numerous breeders collected from the natural waters of Tanjung Air (Bekasi), Kalipucang (Ciamis) and Musi (Palembang). Subpopulation from Tanjung Air was collected in February 1995 with an average body weight of 70 g/pc. Individual selection is applied to this subpopulation to improve the edible portion trait.

The subpopulation from Kalipucang was collected in June 1996 with an average weight of 72 g/pc. Index selection was used in this population to improve the growth rate and edible portion traits. After two steps selection, the synthetic population was constructed from these two subpopulations and added to the subpopulation from Musi (average body weight of 75 g/pc collected in May 1997). Family selection was applied to the synthetic population on the traits of growth rate and edible portion (24 families). The result obtained after the fourth generation of freshwater prawn is shown in Table 1.

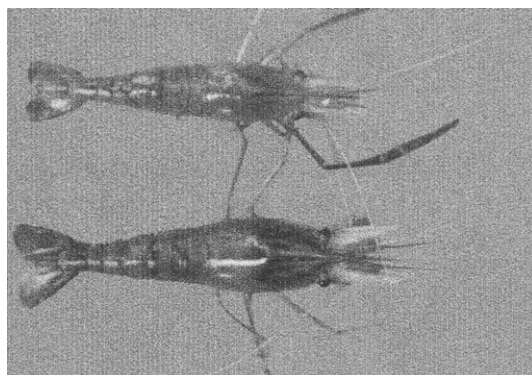
**Table 1. Results after the fourth generation of freshwater prawn developed at RIFA**

No.	Character	Value
1	Heritability of edible portion ( $h^2_{ep}$ )	0.56 ( <i>SE: 0.07</i> )
2	Heritability of body weight ( $h^2_{bw}$ )	0.84 ( <i>0.02</i> )
3	Inbreeding rate (F)	0.0091
4	Total length of male (cm)	21.53 (5.45)
	Total length of female (cm)	15.02 (3.19)
5	Percentage of carapace (male)	30.45 (5.86)
	Percentage of carapace (female)	32.68 (8.05)
6	Hatching rate (%)	65.27-80.0
7	Survival rate (% per 4 months)	46.3-53.1

This year, RIFA obtained the second generation of GI Macro that has salinity tolerance of up to 15 ppt but this strain is still under observation. The GI Macro developed at RIFA are shown in Figures 1 and 2.



**Figure 1. Grandparent stock of freshwater prawn, GI Macro; total length: 38.0 cm and body weight: 480 g**



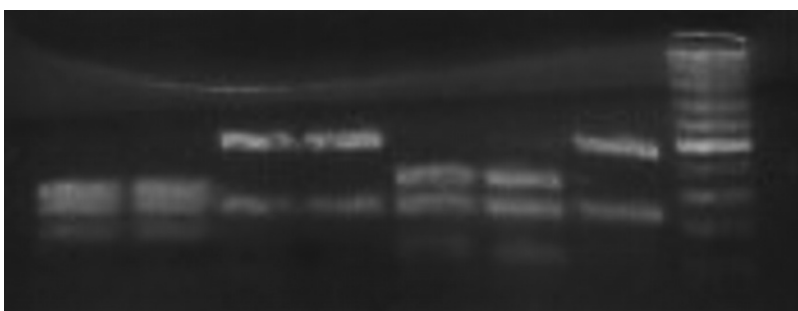
**Figure 2. Improved prawn (below) and control-farmer strain (above) after 5 months rearing period**



## **Other Research Activities on the Freshwater Prawn**

### **1. Application of molecular marker**

The application of DNA markers has been tested to characterize a number of natural stocks of freshwater prawn collected since 2002. The genetic variability of freshwater prawn collected from Musi, Barito and GI Macro races were examined using polymorphism of the mitochondria DNA (MtDNA) markers. Six composite haplotypes were detected following digestion of CO-1 sequences with four endonucleases: Rsa I, Hae III, MhoI and MspI. The average haplotype diversity was 0.603. Significant genetic difference was observed among freshwater prawn populations. The biggest proportion of the major composite haplotype was in the GI Macro, which came from Citatum and Citanduy. While freshwater prawn of Musi has contributed to the composite haplotype of GI Macro with frequency of 25%, Barito races have good prospects as genetic resource for breeding programs. An example of the restriction patterns is shown in Figure 3.



**Figure 3. Mitochondria CO-1 region of freshwater prawn digested by Hae III endonuclease (the arrow indicated 500 bp in length)**

### **2. Application of hormone for sex reversal**

Male freshwater prawns are bigger in size than the female ones. An alternative to produce all male freshwater prawn in mass scale is done by obtaining female stock that are genetically male or homogamete female. When the homogamete female mates the normal male, the result is a 100% male phenotype. This research is still ongoing with initial results expected to come out before the end of 2003.

### **3. Culture technology: closed re-circulation system for larval rearing and nursery**

The larval rearing system used is re-circulation with biofilter, ozone addition and UV radiation. This system is intended to supply good quality of water for larval rearing and nursery. The PL 25-40 produced using this system, are now being cultured in ponds.

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# CULTURE OF GIANT FRESHWATER PRAWN: PHILIPPINES



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## INTRODUCTION

Freshwater prawn thrives in inland bodies of water like rivers, lakes, swamps, irrigation canals, estuaries and even in rivers upstream. More than 100 species were reported to exist worldwide. A recent survey in Luzon Island, Philippines identified 12 species of freshwater prawn found in the island.

The country's interest on freshwater prawn fishery started in 1914 as explained by Cowles (1914), when he identified that freshwater prawn was one of the important fisheries during that time. Likewise, he discussed the geographical distribution of the species, the value of fishery and biology.

In late 1976, trials were conducted to culture the freshwater prawn, however, the studies were never sustained. For these trials, the collection of wild spawners and larval rearing of *M. rosenbergii* was conducted in Misamis Oriental, Mindanao between 1976-1979 by Dejarme et al. Post larval stage was attained but larval rearing lasted only for 39 days. There were more attempts in the past to adopt the technology for the propagation of the species but the efforts never progressed beyond research at institution level.

In 1981, a local banker-industrialist established a 100-hectare commercial *Macrobrachium* farm in Sta. Rosa, Nueva Ecija and a hatchery in Bulacan. Services of experts from Israel were utilized for the project. Marketable prawns were sold live in Metro Manila utilizing in-house retail outlets. After a few years, the company diversified their operations to include tilapia culture. However, even the diversification failed to save the first commercial production venture of *Macrobrachium rosenbergii* in the Philippines.

## ECONOMIC IMPORTANCE OF FRESHWATER PRAWN

The culture of freshwater prawn in the Philippines is intended to diversify the commodities used for freshwater aquaculture, which is currently dominated by tilapia. It is a high value species and its culture could offer better profit. Alternate cropping or polyculture with tilapia may also result to more than 20% increase in yield (Guerrero and Guerrero, 1976).

Freshwater prawns are hardy and fast growing, being able to grow in freshwater and low brackishwater conditions. The species possesses many biological advantages for commercial culture including attaining maturation in captivity, a relatively large size, and rapid growth rate. They feed on anything, such as terrestrial animal feeds, any fish feeds, kitchen refuse, etc.

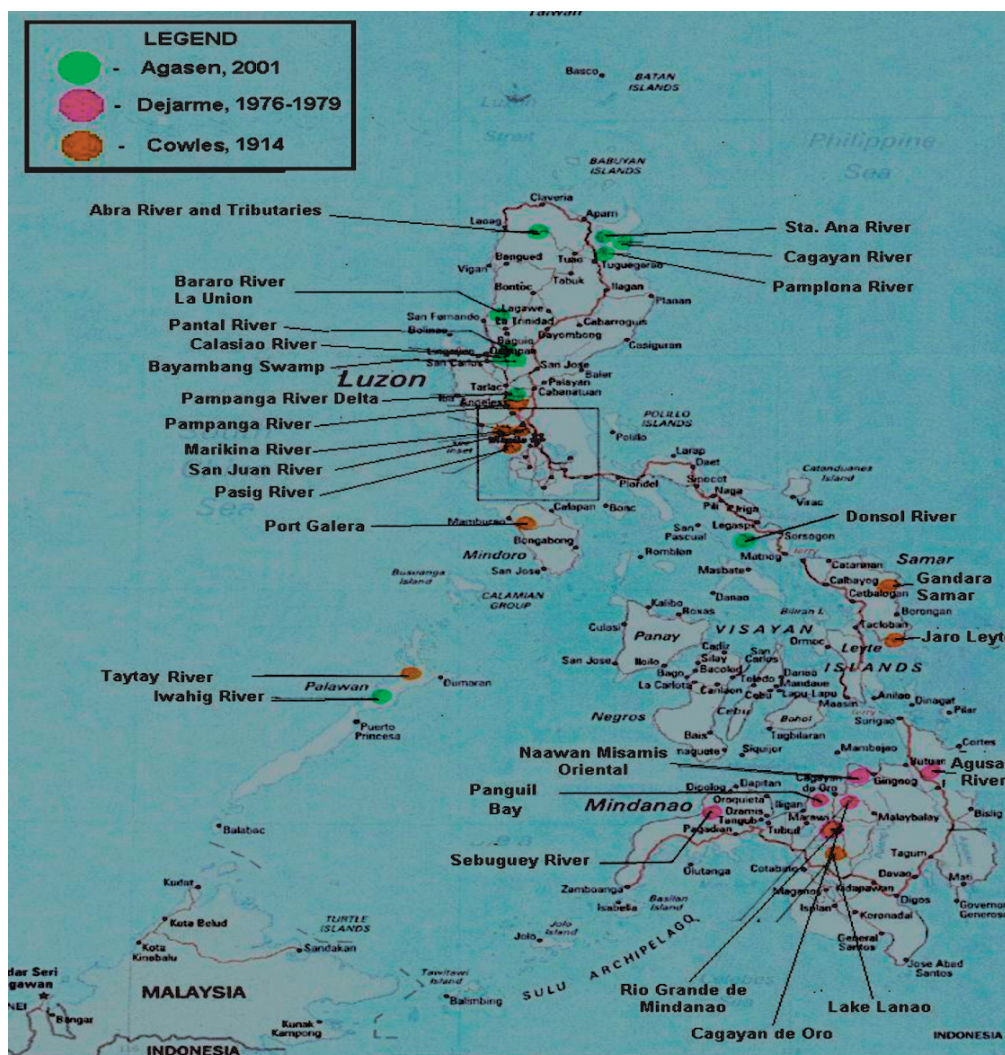
Their feed conversion ratio is comparable to tilapia. In Philippine condition, their rate of growth is high even after attaining sexual maturity. They reach a weight of about 45 g after four months and 90 to 100 g after seven months of culture in earthen ponds (Rosario, 2002). The current market price of *M. rosenbergii* is more than PhP250.00/kg in Central Luzon.





## GEOGRAPHICAL DISTRIBUTION

The species is endemic in the Philippines, where wild catch is available from river tributaries and lakes in the provinces of Ilocos, Cagayan, Pangasinan, Pampanga, Bulacan, Laguna, Palawan, Bicol region, Leyte, Samar, Cotabato, Lanao, Maguindanao, Agusan and some parts of Mindanao (Figure 1). It is locally known as ulang, udang, kising-kising, paje, padao, kalig, urang and budsang. Table 1 indicates the freshwater prawn species in Luzon, Philippines, while the estimated production, peak season, fishing gear used and market of freshwater prawns in major fishing grounds in the Philippines are shown in Table 2.



Cowles (1914) reported that the Palaemons were collected from the rivers in Luzon Island namely, Marikina, San Juan, Pasig River near Manila and Pampanga River. Other sources included streams near Port Galera in Mindoro, Taytay in Palawan, Gandara in Samar, Lake Lanao in Mindanao and Jaro in Leyte.

A study conducted by Dejarme et.al. from 1976 to 1979 reported a collection of *Macrobrachium rosenbergii* in Naawan, Misamis Oriental. The species were mostly found in the upper tidal reaches of Agusan River, Cagayan de Oro River, Rio Grande de Mindanao, Sebuguey River and Panguil Bay.



Table 1. Freshwater prawn species caught in different fishing grounds in Luzon, Philippines (Agasen, 2001)

Fishing Area	Species											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Ilocos Norte												
-Bacsal Vintar		11						1		1		1
-River & Tributaries												
2. Cagayan												
-Cagayan River	1		11		1						1	
-Sta. Ana River	11		1		1							
-Pampora River	11		1		1							
3. Ibabai												
-Atagal Dam					1	11						
4. Abra												
-Abra River and Tributaries	1	11	1					1		1		1
5. La Union												
-Bacelo River	1		1									
6. Pangasinan												
-Pantol River	1		1	1	1							
-Cabrera River												
-Bayambang Swamp	11		1									
7. Pampanga, Subic, Tarlac												
-Pampanga (via Dalis)	11		1	1					1	1		
8. Laguna												
-Laguna de Bay				1						1	1	
9. Camarines Norte												
-Lakas Bato				1						1		
10. Sorsogon												
-Dinaloa River	1		1							1		
11. Palawan												
-Iwahig River	1						11			1		

- |                            |                            |                               |                                  |
|----------------------------|----------------------------|-------------------------------|----------------------------------|
| 1 - <i>M. rosenbergii</i>  | 2 - <i>M. lepidactylus</i> | 3 - <i>M. equidens</i>        | 4 - <i>M. adella</i>             |
| 5 - <i>M. malcolmsonii</i> | 6 - <i>M. rude</i>         | 7 - <i>M. mamillodactylus</i> | 8 - <i>M. sp1</i> (medium sized) |
| 9 - <i>M. sp2</i>          | 10 - <i>M. lanchestri</i>  | 11 - <i>Cardina spp.</i>      | 12 - <i>Atya mollucensis</i>     |
| ++ major species           | + minor species            |                               |                                  |



In 2001, Agasen (2001) reported nine commercial fishing grounds of freshwater prawns in Luzon, Philippines namely: Cagayan, Sta. Ana, Pamplona rivers in Cagayan province; Abra River and its tributaries; Bararo River in La Union; Pantal and Calasiao Rivers, and Bayambang swamps in Pangasinan; Pampanga River Delta in Pampanga; Donsol River in Sorsogon; and, Iwahig River in Palawan (Table 1). The survey identified 12 species of freshwater prawns in Luzon. Along with four other species, *M. rosenbergii* was a dominant species. The other four species caught in commercial quantity throughout the year were *M. lepidactylus*, *M. idella*, *M. rude* and *M. mammillodactylus*. The species found to have aquaculture potential were *M. rude*, *M. mammillodactylus* and *M. malcolmsonii*.

### STATUS OF PRODUCTION

There are no available data on aquaculture production of freshwater prawn because it is only recently that commercial hatcheries for *Macrobrachium rosenbergii* have been established. Investigations by BFAR-NIFTDC indicated that the species attain weights from 40 to 50 g in four to five months of culture. After six to seven months of culture in earthen ponds, they may grow to a size larger than 90 g/pc (Rosario and Roxas, 2000; Rosario 2002). More information on production is yet to be collected from researchers and from established Farmer Pilot Projects.

**Table 2. The estimated production, peak season, fishing gear used and market of freshwater prawns in major fishing grounds (E.V. Agasen, 2001)**

Area/Species	Estimated Production (in MT)	Peak Season	Fishing Gear	Market
1. Pamplona River <i>M. rosenbergii</i>	0.5 to 0.75	summer time	Spear Gun	Local tourist
2. Pampanga river delta and tributaries <i>M. rosenbergii</i>	15-30	Year-round	Shrimp pot, scissors net	Export and Local
3. Donsol River <i>M. rosenbergii</i>	Unknown	summer time	Prawn pot	Local tourist
4. Iwahig river and tributaries <i>M. mammillodactylus</i> <i>M. rosenbergii</i>	Unknown	Year-round	Shrimp pot, scissors net	Export and Local
5. Magat Dam <i>M. rude</i>	1 to 2	summer time	Push net	Local
6. Cagayan River <i>M. spp</i>	3 to 5	May-December	Push net, shrimp pot, cast net	Local
7. Bacarra/Vintar River and Tributaries <i>M. lepidactylus</i>	35 to 40	May-December	Barricades, shrimp pot, scissors net	Export and Local
8. Abra river and tributaries <i>M. lepidactylus</i>	10 to 15	May-December	Barricades, shrimp pot, scissors net	Local
9. Lake Bato <i>M. idella</i>	900-3,600	Year-round	Push net, seine net, fish corrals	Export and Local



Table 2 shows the production of freshwater prawn collected from the wild in Luzon as recorded by Agasen (2001). The production of *M. rosenbergii* is estimated at 0.5 to 0.75 mt in Pamplona River and 15-30 mt in Pampanga River Delta. *M. rosenbergii* is likewise caught in Iwahig River and its tributaries and Donsol River but the catch was not quantified.

*M. rosenbergii* can be found during summertime in Pamplona River and Donsol River while it is found year-round in Pampanga River Delta and its tributaries and in Iwahig River and its tributaries. They are commonly caught using spear gun, shrimp pot, and scissors net. The prawns are sold to local tourists or exported abroad.

## MARKET

Freshwater prawns are usually sold from the place of origin, and any excess is sold to local markets. In areas where wild stocks abound like in Bulacan, freshwater prawn with an average weight of 30 g, are sold at PhP250.00/kg or \$4.54/kg. Live prawns are likewise sold at P350.00/ kg or US \$6.36/kg. The biggest prawn from Bulacan was recorded to weigh about 500 g/pc.

## COMMERCIAL HATCHERIES AND SEED QUALITY

While freshwater prawn is a major commodity in other countries, the prospect of culturing *M. rosenbergii* in the Philippines was hampered by unavailability of seeds. It was not until 2001 when the Philippine Government, through BFAR-NIFTDC in Dagupan City and BFAR-NFFTC in Muñoz City, embarked on a semi-commercial production of *M. rosenbergii*.

At present, these two Aquaculture Technology Research Centers are dispersing freshwater prawn seeds throughout the country. Specifically, the Centers accomplished the following developments:

### ***BFAR-National Freshwater Fisheries Technology Center (NFFTC) in Muñoz, Nueva Ecija***

In 1992, *M. rosenbergii* was imported from Thailand by BFAR and trials were conducted to breed the species. This was during the implementation of the AADCP in the Philippines. It was during the AADCP that collection of Philippine founder stocks was conducted in the upper Pampanga River system, Bulacan; Chico River in Bugalla, Pangasinan; and Cavinti, Laguna. In 1998, breeding trials in aquaria succeeded by mass production in tanks, were successful. Figure 2 shows the production of post larvae at the NFFTC. Currently, BFAR-NFFTC operates a *Macrobrachium* hatchery and actively conducts farmers training in many parts of the country.

### ***BFAR-National Integrated Fisheries Technology Development Center (NIFTDC)***

Studies on hatchery management at the NIFTDC started during the second quarter of 1999. The commercial protocol that entail lower production cost but with higher survival rate was developed in 2001. More than 903,000 PL 18 and juveniles were produced and dispersed to the different regions of the country.

Different strains of *M. rosenbergii* are being collected, bred and evaluated for growth performance. Other information on the culture of the strains are considered. The collection of strains will serve as the Center's gene-bank of the species for future genetic program.

Collaboration with other institutions like SEAFDEC is encouraged particularly in larval nutrition and grow-out systems to facilitate the adoption of the species as a major aquaculture commodity by the Filipino farmers.



## FRESHWATER PRAWN CULTURE

The NFFTC has been conducting studies on freshwater prawn culture. Since results (Table 3, Figure 2) of such studies have been promising, the technology developed has been packaged and disseminated to the fish farmers.

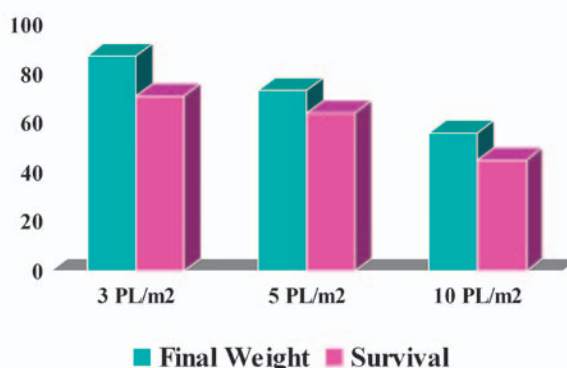
BFAR through the NFFTC has promoted the establishment of Techno-Demonstration Projects involving: (1) small-scale backyard ponds; (2) integrated prawn-rice culture; and (3) grow-out culture with tilapia in fishponds.

One of the Techno-Demo Projects in Cauayan, Isabela produced 150 kg in 500 m<sup>2</sup> ponds after a 4-6 months culture period. The cost and return analysis for this Techno-Demo site is shown in Table 4.

Table 3. Average growth of freshwater prawn culture at BFAR-NFFTC

Treatment	Final Weight (g)	Survival (%)
3 PL/m <sup>2</sup>	87.50	71
5 PL/m <sup>2</sup>	73.47	64
10 PL/m <sup>2</sup>	55.97	45

Figure 2. Final weight of freshwater prawn cultured at BFAR-NFFTC



Days of Culture: 150 days

## POTENTIALS FOR DEVELOPMENT

The culture of freshwater prawn in the Philippines is still in its infancy stage. Much is yet to be done to lower the production cost of seeds. More effort is necessary to attract and convince farmers to adopt the species as an aquaculture commodity. The market for the species is yet to be established for *Macrobrachium* farming to evolve into an industry similar to that of tilapia and milkfish.

## RECOMMENDATIONS

- To produce *Macrobrachium rosenbergii* in commercial scale, the following strategies and policies are recommended.
  - Stock assessment of all major lakes, rivers, marshes, estuarine, reservoirs and other inland waters should be conducted to ascertain adequacy of supply including broodstock for propagation purposes



- The current program of BFAR serves as a good start for investment opportunities, hence feasibility study should be undertaken to attract potential investors
- Environmental impact studies in major inland water areas where these species are in abundance should be conducted
- Credit financing in banks and financial institutions should be made available, through representations to the Board of Investment to promote the industry as an identified priority in the fisheries sector
- Freshwater prawn hatchery technology should be commercialized
- On-site grow-out culture demonstration through technology verification/dissemination on the monoculture or polyculture schemes for the freshwater prawn should be conducted
- Available sites in Central Luzon especially in the Lahar area and other prospective freshwater areas should be identified
- Pilot testing at local government freshwater stations for grow-out demonstration and also private fish farmer's project should be pursued
- A national master plan for freshwater prawn aquaculture should be formulated and designed to identify sources of supply (abundance and deficit) and necessitate definite market linkages so that benefits shall accrue to producers and consumers
- Since lack of data on the culture of *Macrobrachium* species in the country led to difficulty for both domestic and international markets especially in assessing the local supply and demand, linkage with other government agencies and international organizations should be strengthened in order to gather continuing data on the *Macrobrachium* outlook
- An inter-agency collaboration is necessary during the initial stages of the program implementation to assess all resources, e.g. manpower, facilities/laboratories, equipment, financial, etc.
- Since prawn farming requires developed aquaculture support services, training, research, extension, infrastructure facilities, and development of marketing and distribution systems should be pursued

#### **FUTURE PLANS FOR *Macrobrachium rosenbergii* AQUACULTURE IN THE PHILIPPINES**

- ⊕ Development of the NFFTC and NIFTDC as the National Centers for the production of quality broodstock and post larvae of freshwater prawn
- ⊕ Improvement of the quality of *Macrobrachium rosenbergii* through crustacean genetic research
- ⊕ Development of appropriate technology for the mass production of *M. rosenbergii* post larvae
- ⊕ Development of technology for grow-out culture adopting the different aquaculture farming systems
- ⊕ Distribution/dispersal of quality post larvae for grow-out culture in various areas of the country
- ⊕ Dissemination of freshwater prawn technology to new entrepreneurs and the stakeholders – the fisherfolk



**Table 4. Cost and Return Analysis of Freshwater Prawn Culture Techno-Demo in Cauayan, Isabela**

Pond Area	0.05 ha (500 m <sup>2</sup> )
Culture Period	4-6 months
No. of croppings	2 croppings/year
Stocking rate	5 pc/m <sup>2</sup>
PL Requirement	2,500 pc
PL cost	P 2.50/pc
Survival rate	75%
No. of stock	1,875 pc
Size of harvest	25 pc/kg (ABW 40 g)
Production	150 kg
Price/kg	P 350.00
Total sale	P 26,250.00
Feed required	P 3,000.00
FCR	1.5
Total feeds required	150 kg
Cost of feed	P 3,000.00 @ P 20.00 kg
Fertilizer/chemical required	P 1,500.00
Capital cost for 500 m <sup>2</sup>	
Cost of land (500 m <sup>2</sup> )	P 20,000.00
Construction cost	2,000.00
Farm implement	<u>1,500.00</u>
	P 23,500.00
Production cost per cropping (2 croppings/year)	
Post-harvest at 5 pc/m <sup>2</sup> (2,500 pc @ P 2.50)	P 6,250.00
Feeds (150 kg @ P 20.00/kg)	3,000.00
Fertilizer/chemicals	1,500.00
Labor	600.00
Travel/Shipment Cost	<u>1,500.00</u>
	P 12,850.00
Depreciation/Year	
Construction cost	P 400.00
Farm implements	<u>500.00</u>
	P 900.00



<b>Sales</b>	
500 m <sup>2</sup> x 5 pc PL/m <sup>2</sup> X 2 croppings 5,000 pc x 75% recovery @ 25 pc/kg	5,000 less 25% mortality <u>150 kg x P 350.00/kg</u> P 52,500.00
<b>Total Project Cost</b>	
Capital Cost	P 23,500.00
Working Capital	<u>12,850.00</u>
	P 36,350.00
<b>Net Income Before Tax</b>	
Sales – 52,500 – (25,700 + 900) = 52,500 – 26,600	= P 25,900.00
<b>Net Income After Tax</b>	
Net Income = P 25,900.00 – 15% provision	= P 22,015.00
<b>Cash Payable Period</b>	
$\frac{36,350.00}{22,015.00}$	ROI = $\frac{22,015.00}{36,350.00} \times 100\% = 60\%$



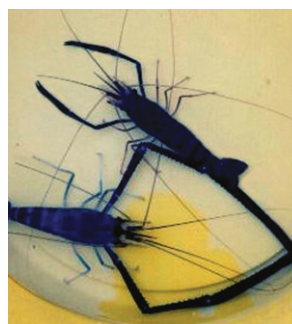
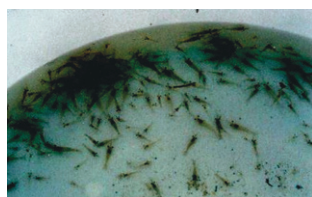
In earthen ponds, freshwater prawns can grow from 40 to 50 g in 4-5 mo and more than 90 g in 6-7 mo



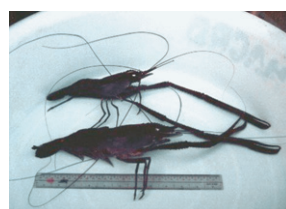
Freshwater prawns are usually sold in farms while the excess are sold in local markets







A semi-commercial production of *M. rosenbergii* promoted by the BFAR-NFFTC in Muñoz, Nueva Ecija, and BFAR-NIFTDC in Dagupan City. These two centers disperse prawn seeds to farmers all over the Philippines.



At BFAR-NIFTDC, various strains of *M. rosenbergii* are bred and evaluated for growth performance.

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**STATUS OF *Macrobrachium rosenbergii* INDUSTRY**

**Production, Value and Area**

Giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important economic species in Thailand as well as in many Southeast Asian countries. It is highly demanded as food for the Thais that lead to its gradual over-catching from the natural waters every year. Thus, domestication experiments on the *Macrobrachium* have been conducted by the Department of Fisheries since 1956 to increase prawn production (Sidthimunka and Bhukaswan, 1982). This resulted in the nationwide extension of its culture technology to a number of commercial giant freshwater prawn culture companies. Now this species has become one of the economic commodities in the country's aquaculture industry. Recently, statistics report showed that the total prawn culture in 2002 increased by 38% from the year 1996, while the total value in the same year increased by 89% from the year 1996. The statistics also showed that in 1996 and 2002, the total production were 7200 and 10,000 mt, respectively, valued at 596.3 and 1,117.6 million Thai Baht, respectively (Department of Fisheries and Suwannatos, 2003). The market price per kg of freshwater prawn, which varies according to the prawn sizes, has been increasing since 1989 (Table 1). In 1997, the large, medium and small sizes increased by 76%, 123% and 81% from those in year 1989, respectively (Table 1).

**Table 1. Price of cultured giant freshwater prawn between 1989-1997 (Department of Fisheries, 2001)**

Size	Price (In Thai Baht)								
	1989	1990	1991	1992	1993	1994	1995	1996	1997
Large	207.42	233.92	256.01	272.92	278.25	302.83	290.08	325.58	365.92
Medium	93.15	129.17	132.63	124.17	33.24	163.47	170.67	186.17	207.33
Small	49.97	62.51	63.08	66.34	65.62	69.65	72.83	71.03	90.83

The freshwater prawn annual value and the annual production have increased during the past four years as shown in Figure 1. Most of the culture areas are located in the central part of the country. The total area of giant freshwater prawn culture in 2000 was 3926.9 ha, 69 % of which are in six provinces, namely: Ratchaburi (56%), Nakornpathom (13%), Supanburi (18%), Ayuthaya (3%), Karnjanaburi (6%), and Chachanksoa (3%).

**Commercial Hatcheries**

Domestication of the giant freshwater prawns in Thailand was started in 1966 at the Songkhla and Bangkhen Fisheries Stations while small commercial hatcheries started to operate in 1973 (New, 1982 and Suwannatos, 2003). Giant freshwater prawn hatchery practices have been developed since 1966, when the early researches concentrated on larval rearing until it became a routine, then later on the grow-out culture. Since water quality condition became a critical factor in larval rearing, the optimum levels of this factor were established including salinity from 12 to 15 ppt, temperature from 27 to 31°C, dissolved oxygen from 3 to 5 mg/l, pH from 7 to 8, and ammonia which should not be more than 0.1 mg/l (Colt and Armstrong, 1981 and Suwannatos, 2003). Seed quality in commercial hatcheries also depended on many factors, namely: water quality, parent stock, female parent maturity condition and hatchery management.

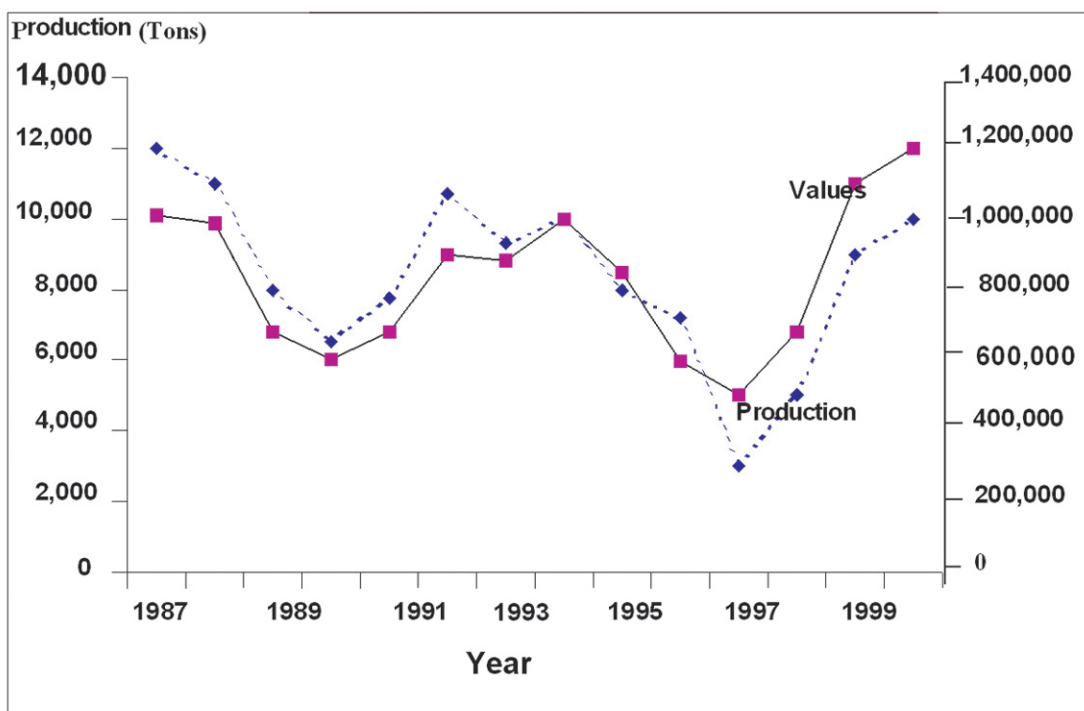


Figure 1. Production and values of cultured *Macrobrachium rosenbergii* in 1987-2000 (Department of Fisheries, 2001)



A typical small-scale freshwater prawn hatchery in Thailand

Suwannatos (2003) reviewed the giant freshwater prawn rearing practices in Thailand, and he concluded that there were four types of larval hatchery management. Each system had different advantages and disadvantages to the farmers. However, a number of researches have been conducted to develop these rearing systems to provide good quality seeds to the farmers. Thus, the farmers have the choice on the type of system they would adopt based on their experience and the hatchery conditions.

### *Clear water system*

Simple and less expensive, this system could be applied in a small hatchery such as a backyard hatchery because there is no requirement for ponds. However, the hatchery manager should monitor closely the water quality to make sure that these are above the optimum levels. The pond size should be between 1-3 m<sup>2</sup> and 1.0 m deep.



### ***Greenwater system***

This system was modified from a larval rearing system developed in Hawaii. A large number of quality seeds can be produced using this system by rearing the larvae in greenwater with the phytoplankton, *Chorella* spp. The phytoplankton controls the water quality at an optimum pH and also the disease problem. However, this system requires a number of ponds and the rearing period is longer (more than 30 days) compared with that of the other systems.

### ***Recirculation system***

This system is preferred for hatcheries that are far from the sea because it requires less seawater, and the environmental conditions are easy to control. However, it is more costly than the other systems. Some researches that dealt with the development of this system (Suwannatos, 2003), included the use of water treated with small weed organisms and air circulation, with the water to be continuously reused. Therefore, diseases and use of chemicals could be easily controlled. For this system, the hatchery manager should be well trained on water quality analysis as well as on nutrition and disease control. Rearing period is also longer (more than 30 days) compared with that of the clear water system.

### ***Earthen pond system***

A group of biologists at the Phetchaburi Coastal Aquaculture Station, Department of Fisheries developed a technology for rearing the larvae of giant freshwater prawn in earthen ponds (Tunsutapanich *et.al.*, 1994). In this system, water quality in the earthen pond is maintained to have ecological balance without any water change. The pond is installed with air blower for consistent oxygen supply. Water chemical conditions are observed regularly and adjusted to suitable levels for the balanced ecological system in the pond.

The larvae are fed with natural zooplankton and with *Artemia* nauplii and adult. Although the system gives high survival rate of juveniles, its rearing period is longer than that of the other systems. The total production in this system is 5.1 million juveniles per 0.16 ha with an average survival rate of 88% (Tunsutapanich *et. al.*, 1994). For this system, the hatchery manager should observe the water quality regularly and maintain the ecological balance and food chain within the pond.

## **GOOD QUALITY SEEDS**

Good quality seeds should result in high survival rate and fast growing juvenile prawn. The above hatchery systems would be able to provide good quality seed supply to the *Macrobrachium* industry provided the following measures are followed:

1. Suitable stocking density for larval rearing should be 20-40 post larvae/l
2. Temperature during the rearing period should range from 28 to 30°C
3. Female parents should be clean with good maturity condition
4. Antibiotics should never be used
5. All equipment should be disinfected every after use
6. Good quality food such as *Artemia*, boiled eggs and Pollock, should be used



Larval rearing of giant freshwater prawn in earthen ponds developed at the Phetchaburi Coastal Aquaculture Station, Department of Fisheries, Thailand

## GROW-OUT CULTURE

*Macrobrachium* culture in the country is now facing a number of problems such as slow growth, lack of appropriate broodstock management and disease occurrence. To counteract these problems, commercial *Macrobrachium* farms developed a new improved strain with new farming management. The strain has been initially introduced to the farmers, and the private hatcheries have developed their own selective breeding programs for such strain.

*Macrobrachium* farming in Thailand has been classified into two types: the traditional farms growing the local strain, and the *Macrobrachium* farms using a new strain. Traditional farms usually consist of small number of earthen ponds 0.32 –0.96 ha adopting a stocking density of 5 to 20 juveniles/m<sup>2</sup>. The farmers prepare their own feeds. During the first month, the prawns are fed twice a day at a feeding rate of 30–40% of the body weight. During the third month of rearing, the feeding rate is gradually reduced to 5%, and in the following month, to 3%. In the sixth month, large size prawns are seined out for sale while the small ones are left in the culture pond. The market size prawns should be between 50 to 100 g/pc (Tookwinas, 2002).

One farm which uses the new strain of *Macrobrachium* is the Kasetsombuond Farm in Supanburi Province. The owner, Mr. Supon Sovanapreecha, has been operating this farm using the new strain of *Macrobrachium* for three years (interviewed in Thai Fisheries Gazette, 2002). The juveniles are reared in the nursing pond at the stocking density of 75 pc/m<sup>2</sup> for two months, after which these are stocked in the grow-out pond at 7–15 pc/m<sup>2</sup>. After four and a half months, the female prawns with an average size of 25–33 g are culled for sale. On the sixth month, the large males (100–125 g) are harvested and sold. The total production of the first generation is usually about 3750 kg/ha with a sex ratio of 80% males to 20% females. However, production usually decreases by 10% in the second and third generations (male size: 83 g). Therefore, selective breeding program to improve the growth of the *Macrobrachium* domesticated strain has been recognized as an urgent concern for the freshwater prawn industry.



## FUTURE PLAN AND PROSPECTS

Recently, the National Thailand Research Fund in cooperation with the Department of Fisheries conducted a workshop on the “Participation of increased effort in *Macrobrachium* industry” on 20 August 2003. The main objective of the workshop was to discuss the aspects of increased efficiency in producing *Macrobrachium rosenbergii* as a new premium aquaculture commodity. Problems and plans on the different aspects were discussed including, nursery, grow-out culture, nutrition, diseases and genetics. The Department of Fisheries proposed to develop a selective breeding program to improve the economic traits of the *Macrobrachium rosenbergii* cultured in Thailand. In addition, researches to develop a practical farm management protocol for each part of the country were also planned. As *Macrobrachium rosenbergii* is now becoming a premium aquaculture commodity, good production process from the farm to the table should be established. Therefore, the code of conduct (CoC) is planned to be developed similar to that of the country’s marine shrimp culture. The planned guidelines of the CoC for *Macrobrachium* should include the following 11 aspects (Tookwinas et.al., 2002 and Tookwinas, 2002):

- (1) suitable site selection
- (2) general pond management
- (3) stocking density
- (4) feed management
- (5) prawn health management
- (6) therapeutic agent and other chemicals
- (7) effluents and solid waste management
- (8) harvesting and selling
- (9) social responsibility
- (10) farmer association and education
- (11) data collection.

## MACROBRACHIUM SPECIES THAT HAVE BEEN IDENTIFIED IN THAILAND

All *Macrobrachium* species are classified in the Family Palaemonidae Rafinesque, 1815, Subfamily Palaemoninae Rafinesque, 1815. The following nineteen *Macrobrachium* species have been found and identified in Thailand :

Species	Freshwater	Brackishwater
<i>Macrobrachium amplimanus</i> (Cai and Dang, 1999)	Yes	No
<i>Macrobrachium dienbienphuense</i> (Dang and Nguyen, 1972)	Yes	No
<i>Macrobrachium equidens</i> (Dana, 1825)	No	Yes
<i>Macrobrachium eriocheirum</i> (Dai, 1984)	Yes	No
<i>Macrobrachium esculentum</i> (Thallwitz, 1891)	Yes	No
<i>Macrobrachium hirsutimanus</i> (Tiwari, 1952)	Yes	No
<i>Macrobrachium idae</i> (Heller, 1862)	No	Yes
<i>Macrobrachium lanchesteri</i> (De Man, 1911)	Yes	No
<i>Macrobrachium lar</i> (Fabricius, 1793)	No	Yes
<i>Macrobrachium latidactylus</i> (Thallwitz, 1891)	No	Yes
<i>Macrobrachium mirabile</i> (Kemp, 1917)	No	Yes
<i>Macrobrachium mieni</i> (Dang, 19750)	Yes	No
<i>Macrobrachium neglectus</i> (De Man, 1905)	No	Yes
<i>Macrobrachium niphanae</i> (Shokita and Takeda, 19890)	Yes	No
<i>Macrobrachium rosenbergii dacqueti</i> (Sunier, 1925)	Yes	Yes
<i>Macrobrachium sintangense</i> (De Man, 1898)	Yes	Yes
<i>Macrobrachium sirindhorn</i> (Naiyanetr, 2001)	Yes	No
<i>Macrobrachium yui</i> (Holthuis, 1950)	Yes	No
<i>Macrobrachium</i> sp. (Cai, Naiyanetr and Ng, in press)	Yes	No



Of the nineteen species, the following five species are the most economically important species for Thailand:

**1. *Macrobrachium dienbienphuense* (Fig. 2 & 3)**

*M. dienbienphuense* has been found in northeast of Thailand, distributed in the main rivers of Maekhong, Chi, Moon and their branches. The species has moderate size, the biggest of which is about 6-7 cm. The prawns are being sold in the markets along the Maekhong and Moon rivers, particularly in Ubon Ratchathani Province. There is no developed culture technology for this species yet, may be because the prawn can still be captured in good quantities throughout the year from the natural waters.

**2. *Macrobrachium niphanae* (Fig. 4 & 5)**

*M. niphanae* has attractive characteristics with red brown spots lining around each of all the five pairs of walking legs. This prawn could pass as an ornamental aquatic species of economic importance. The species could be a subject for study for its biological details in order that an appropriate aquaculture system could be developed. This species is not well known to some people because it is found only in waterfalls and streams and very rarely in other watercourses and rivers.

**3. *Macrobrachium lanchesteri* (Fig. 6 & 7)**

*M. lanchesteri* is very similar to *M. niphanae* in terms of size and morphology. The species is found in almost every inland waterbody and is very often found together with *M. niphanae*. Because of its abundance, *M. lanchesteri* has become important to the local people in the rural areas. Many people like to eat the prawn even though its size is very small. In some provinces in northeast Thailand, culture of the prawn is usually done with fishes together in the one pond to earn more money.

The following table compares the morphological characteristics of *M. niphanae* and *M. lanchesteri*.

Morphological characteristics	<i>M. niphanae</i>	<i>M. lanchesteri</i>
1. Rostral teeth	2-3 (7-13) / 2-3	1 (7-10) / 3-4
2. Five pairs of walking leg	With red brown spots lining around each one of all walking legs	Clear, without red brown spot
3. Rostrum	Longer than antennular peduncle but shorter than scaphocerite	Slender and same length as antennular peduncle
4. Position of hepatic spine	Between the first and second ones of the upper rostral teeth	Behind the first one of the upper rostral teeth
5. The second pair of walking legs	With little short hair appearing on the carpus and merus	With little short hair appearing only on the fingers of mature males

**4. *Macrobrachium sintangense* (Fig. 8 & 9)**

*M. sintangense* is a species morphologically similar to *M. rosenbergii*. Its size is about the same as the immature *M. rosenbergii*. The biggest size of *M. sintangense* is about 8-9 cm. The shrimp is also popular with the people in the northeastern Thailand mainly because of its moderately large size which is just slightly smaller to *M. rosenbergii*. People in some areas call this prawn “Kung Kam Kram” which is the Thai term for *M. rosenbergii*. The prawn is sold in many provinces along the Maekhong and Moon rivers. Due to the relative economic importance of the species, study of its biology and life cycle should be conducted, so that appropriate aquaculture system could be developed and the species could be introduced to the local fish-farmers.

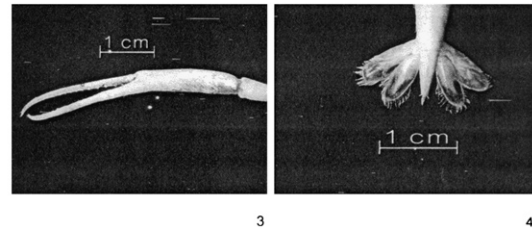
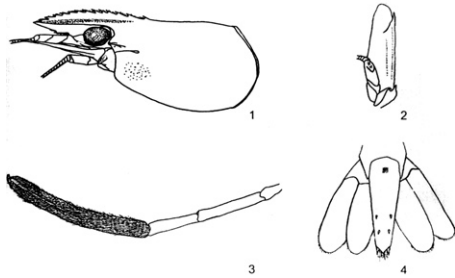
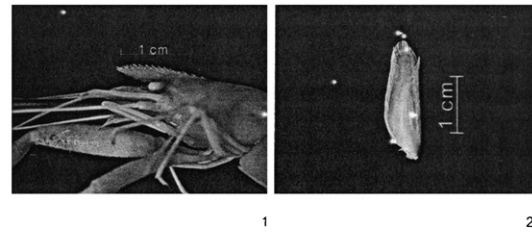
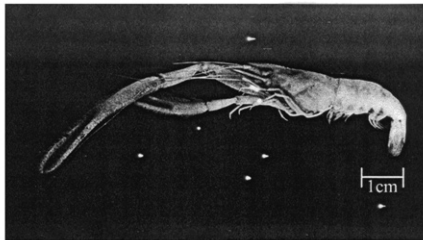


Figure 2. *Macrobrachium dienbienphuense* (Dang and Nguyen, 1972:)  
 1. Carapace and rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod

Figure 3. Morphological characters of *M. dienbienphuense*  
 1. Rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod

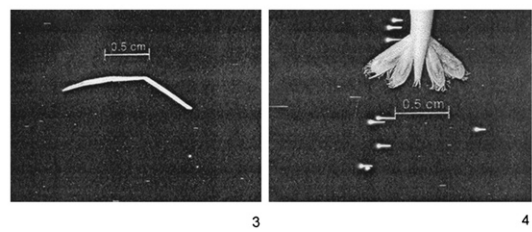
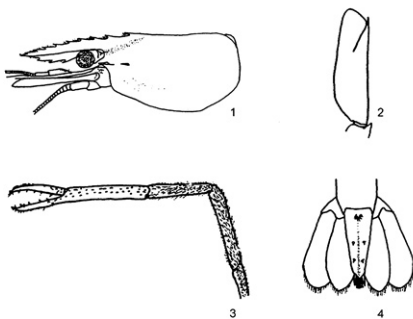
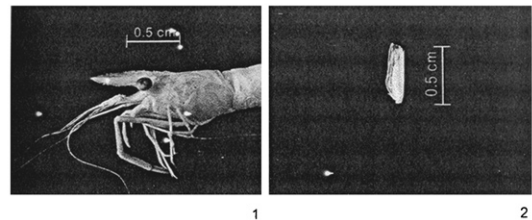
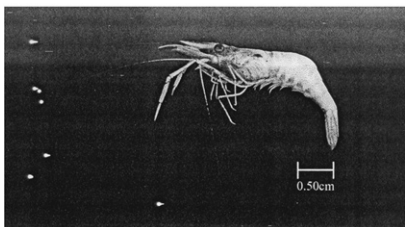


Figure 4. *Macrobrachium niphanæ* (Shokita and Takeda, 1989)  
 1. Carapace and rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod

Figure 5. Morphological characters of *M. niphanæ*  
 1. Rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod



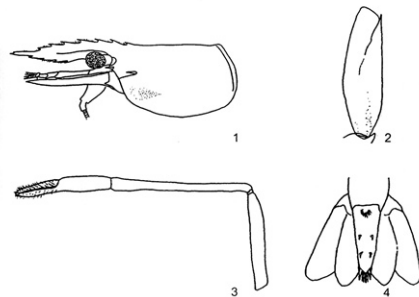
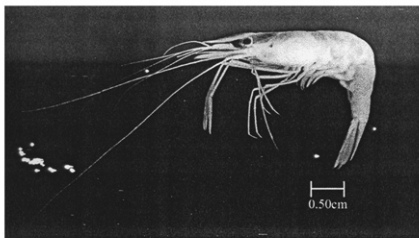
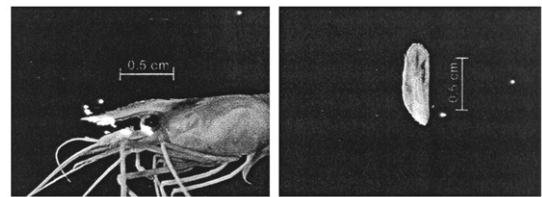
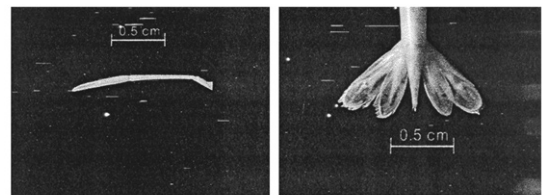


Figure 6. *Macrobrachium lanchesteri* (DeMan, 1911)  
 1. Carapace and rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod



1 2



3 4

Figure 7. Morphological characters of *M. lanchesteri*  
 1. Rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod

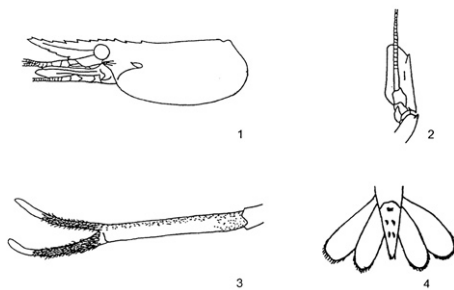
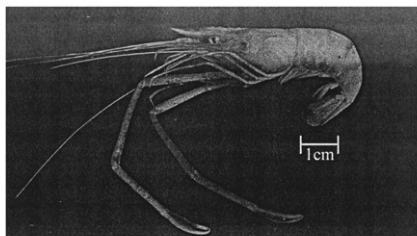
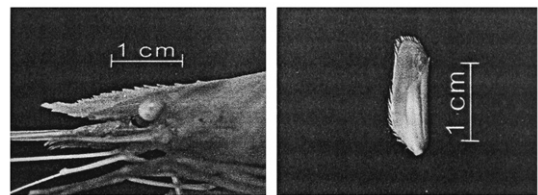
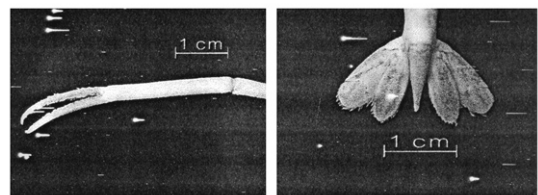


Figure 8. *Macrobrachium sintangense* (DeMan, 1898)  
 1. Carapace and rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod



1 2



3 4

Figure 9. Morphological characters of *M. sintangense*  
 1. Rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod



### 5. *Macrobrachium rosenbergii dacqueti* (Fig. 10 & 11)

*M. rosenbergii* has been famously known as the “giant freshwater prawn” since 1958. It comprises two subspecies identified by the differences in some morphological characteristics and in particular their geographical distribution. One subspecies found in the Papuasias area between Papua New Guinea and Australia, and the areas around the Philippines, is *M. rosenbergii rosenbergii* (De Man, 1879). The other species, distributed in the Indo-west Pacific from Indonesia to India including Thailand, is *M. rosenbergii dacqueti* (Sunier, 1925).

The following table compares the different characteristics of *M. rosenbergii dacqueti* and *M. rosenbergii rosenbergii*:

Characteristics	<i>M. rosenbergii dacqueti</i>	<i>M. rosenbergii rosenbergii</i>
1. Rostrum	The part behind eye is convex and longer than scaphocerite	The part behind eye is straight and as long as or shorter than scaphocerite
2. The second pair of walking legs	With large spines scattering, the end of the spines being straight	With small spines scattering, the end of the spines being horn-like
3. Distribution	India, Burma, Thailand, Malaysia and Indonesia	Papuasia and the Philippines

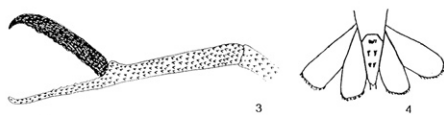
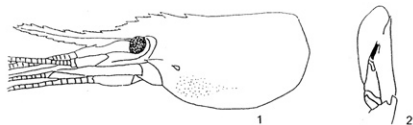
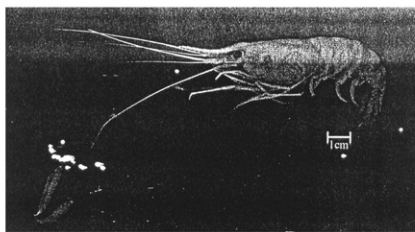


Figure 10. *Macrobrachium rosenbergii dacqueti* (Sunier, 1925)  
 1. Carapace and rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod

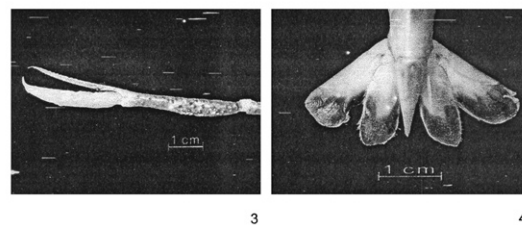
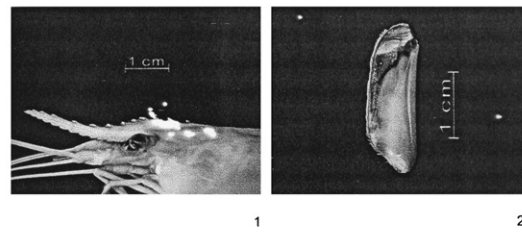


Figure 11. Morphological characters of *M. rosenbergii dacqueti*  
 1. Rostrum, 2. Scaphocerite  
 3. The 2<sup>nd</sup> right walking-leg, 4. Telson and uropod



In the past, the distribution of *M. rosenbergii* in Thailand was reported only in the brackishwater connected to the sea. But now it has been found in many freshwater bodies of Thailand, especially in places where large amount of the prawn have been released by the DOF (Department of Fisheries of Thailand) over the past decades. The species is highly economically important, particularly the big ones which are very expensive and popular as premium food. Many farms in Thailand, particularly in the central area, have done breeding and culture activities for this species as an industry for quite some time. However, the DOF of Thailand is still trying to improve the prawn quality including seeds, broodstock and products to come up with the best prawn ever produced in the world.

## **R & D EFFORTS ON QUALITY IMPROVEMENT OF *M. rosenbergii dacqueti***

The following gives a summary of the R&D efforts done in Thailand on *M. rosenbergii*:

### **(1) CYTOGENETIC INFORMATION STUDY**

Wanitchanon, A. and J. Phetsiri. 2000. A chromosome study of *Macrobrachium rosenbergii* from Thailand. Research Report, Taksin University, Songkhla, Thailand

A chromosome study was conducted on the freshwater prawn, *Macrobrachium rosenbergii* found in Thailand. Fifty specimens of male and female, average 17.00 cm, had a second pair of periopods removed. The specimens were kept in a small aquarium for one week to induce the regeneration blastema. Spreads of metaphase chromosome were prepared from the regenerating tissue. Chromosome number was counted based on 50 cells. The study found that the diploid chromosome number was 118. The karyotype consisted of 45 metacentric and submetacentric pairs and 14 telocentric and acrocentric pairs.

### **(2) GENETIC DIFFERENTIATION AND POPULATION STRUCTURE STUDIES**

Yaitavorn, P. 1989. Mitochondrial DNA variation in giant freshwater prawn (*Macrobrachium rosenbergii* de Man). M.Sc. Thesis, Chulalongkorn Univ., Bangkok, Thailand

A study on genetic variation in natural population of giant freshwater prawn *Macrobrachium rosenbergii* de Man was undertaken to identify the races of the prawn in different locations of Thailand. The study was based on the analysis of the mitochondrial DNA (mtDNA) variation. The mtDNA was isolated from the hepatopancreas of prawns and cut with restriction enzyme, *Sau3A* I, to generate DNA fragments ranging from 0.2 to 2.0 kb. These DNA fragments were cloned into vector pUC12 at *BamH* I site and transformed into *E. coli* strain JM 107. After colony hybridization and Southern blot hybridization, fifty-one recombinant clones were obtained. These clones were further selected for strongly hybridized signal with  $\alpha$ -<sup>32</sup>P dATP labelled mtDNA, and used as probes to analyze mtDNA variation in prawns. The recombinant DNA No.1, which inserted a fragment of about 1.1 kb. and had restriction endonuclease sites for *Rsa* I, *Hha* I, *Hae* III and *BstU* I, was able to distinguish the prawns from the two geograhic populations. It showed significant difference in restriction fragment length polymorphism (RFLP) pattern of mtDNA between prawn from two rivers, Kraburi in the south and Bangpakong in the central, by Southern blot hybridization with the recombinant DNA No.1. The prawns from Bangpakong showed strong discrete band at 1.1 kb. but those from Kraburi showed the band at about 0.7 kb. By using this clone, the RFLP patterns of Bangpakong River and that from Kung Kam Thong Farm, both of which are in central Thailand, were found similar.

**Sodsuk, S. and P.K. Sodsuk. 1998. Genetic diversity of giant freshwater prawn from three locations of Thailand. Technical Paper No. 18/1998. National Aquaculture Genetics Research Institute, Department of Fisheries, Thailand**

Giant freshwater prawn (*Macrobrachium rosenbergii* de Man) from three locations of Thailand (Chachoengsao, Surat Thani and Songkhla) was identified using allozyme electrophoresis. A total of 24 enzyme loci were detected



from pleopod, muscle and hepatopancreas. Three loci, *GPI\**, *MPI\** and *PGM-I\**, were found to be polymorphic ( $P_{0.95} = 0.125$ ). No significant differences from Hardy-Weinberg equilibrium were observed within any single population. Mean expected heterozygosity ( $H_e = 0.031 \pm 0.018$ ) for the species was found to be relatively low. A number of genetic differences evaluated, inbreeding coefficients (*F*-statistics), polymorphic loci differences within each pair of populations and among all populations, genetic distances between populations and dendrograms constructed using genetic distances, showed that all three populations studied were the same single population based on the technique at allozyme level.

**Vanavichit, A. et al. (pers. comm.) Agricultural Genetic Engineering and Biotechnology Center, Kasetsart Univ. Research and Development Institute, Kamphaengsaen Campus, Nakhornpathom, Thailand**

Genetic differentiation among populations of *Macrobrachium rosenbergii* de Man was carried out. The prawn specimens from six localities used in the study were 68 individuals from Bangpakong River, 83 individuals from Nakhorn Nayok River, 55 individuals from Tapi River, 41 individuals from the upper part of Songkhla Lake, 69 individuals from Kraburi River (a border river between Thailand and Myanmar) and 116 individuals from Yaephew River in Myanmar. The study was based on mtDNA analysis focusing the 12S rDNA and 16S rDNA genes, that had been cut with four restriction enzymes, *Alu* I, *Dra* I, *Hinf* I and *Tru*9 I. Based on genetic distances resulting from the study, two major populations of the prawns were obtained. One major population consisted of prawns from all water-bodies in Thailand, the Bangpakong, Nakhorn Nayok, Tapi and Songkhla, and another 63.46% of the prawns from Kraburi together. The other major population included 65.46% of the prawns from the Yaephew in Myanmar. Genetic distance between the two major populations was 0.931. Further to this, the remaining prawns from the Kraburi and Yaephew were mixed together as another intermediate group.

### (3) SELECTIVE BREEDING PROGRAMS

**Meewan, M. 1991. Morphological inheritability and growth of giant freshwater prawns. M.Sc. Thesis, Asian Institute of Technology (AIT), Thailand**

An experiment to estimate the heritability of growth in relation to morphotypic transformation among full and half sib families of the freshwater prawn, *Macrobrachium rosenbergii* was undertaken. Observations on prawn growth and morphotypic transformation were made over a period of 31 weeks, during which the juvenile prawns were kept in hapa enclosure and the adults in cages. The hapas and cages were suspended in an earthen pond where the water quality was maintained homogeneously under suitable conditions. The heritability estimates were made on the progeny from 32 full and half sib families nested within eight sires with two dams per sire. The broad sense heritability was estimated for carapace length, body length, claw length and total body weight. The heritability estimates on the carapace length based on paternal, maternal and fullsib analyses were found to be the highest 0.40 ( $\pm 0.22$ ), 0.13 ( $\pm 0.07$ ) and 0.26 ( $\pm 0.11$ ) at 23 weeks, respectively.

The heritability on morphotypic transformation at 31 weeks from orange claw males (OC) to blue claw males (BC) were 0 ( $\pm 0.04$ ), 0.73 ( $\pm 0.08$ ) and 0.37 ( $\pm 0.02$ ) and the morphotypic transformation from small males (SM) after removing bulls were 0.21 ( $\pm 0.06$ ), 0.56 ( $\pm 0.05$ ) and 0.39 ( $\pm 0.03$ ) for paternal, maternal and fullsib analyses, respectively. The survival rate in the cage culture of initial stock to 23 weeks ranged from 55% to 96%. The number of females presented in every cage was greater than male and blue claw males dominated among three male morphotypes at 23 weeks. The resulting heritabilities implied the possibility for traits improvement.

**Rattikansukha, C. 1993. Intraspecific hybridization in *Macrobrachium rosenbergii* de Man. M.Sc. Thesis, Chulalongkorn Univ., Bangkok, Thailand**

An intraspecific hybridization of two *Macrobrachium rosenbergii* populations, using the reciprocal crosses of prawns from Kraburi and Chao Phraya Rivers was carried out. Results of the study indicated that the postlarvae obtained from the Kraburi parents presented body length significantly larger than body length of those obtained from the Chao Phraya parents, while hybrids of the two populations were not presenting heterosis. The result implied some differences between the economic traits of different populations or strains of this species. Thus, further study on the strain selection and improvement should be carried out.

**Uraiwan, S., Sumanojitraporn, S. and K. Ampolsak. 2002. Genetic improvement to increase growth rate of giant freshwater prawn (*Macrobrachium rosenbergii* de Man) : heritability estimates and within-family selection. The Proceedings of 40<sup>th</sup> Kasetsart University Annual Conference. pp. 632-640**



Sib analysis and selection procedures were respectively designed to estimate heritability and realized heritability on growth rate of *Macrobrachium rosenbergii*. The first procedure was carried out in cages with mixed sex rearing at the Ayuthaya Freshwater Fisheries Center, Ayuthaya Province between 1991 to 1992, and in concrete ponds with separate sex rearing at the Aquatic Animal Genetics Research and Development Institute, Pathumthani Province between 1996 to 1997. Under the cage culture condition, heritabilities were estimated from 16 full-sib and 8 half-sib families using nested analysis of variance model. Heritabilities of length and weight of male and female prawns 5 months old were  $-0.081 \pm 0.014$  and  $0.122 \pm 0.074$ , and  $0.060 \pm 0.054$  and  $0.030 \pm 0.041$ , respectively.

Under pond conditions, 17 full-sib families were designed. Heritabilities of length and weight of male and female prawns 6 months old were  $0.156 \pm 0.077$  and  $0.142 \pm 0.096$ , and  $0.254 \pm 0.080$  and  $0.272 \pm 0.210$ , respectively. The realized heritabilities were estimated after one generation of within-family selection, the male and female prawns at 6 months old, these were 0.331 and 0.058, and 0.745 and 0.395, respectively. After one generation of selection for large size, female prawns of the selected line 6 months old were significant ( $p \leq 0.01$ ) 6 and 12% larger by length and weight than those of the control line, respectively. Similarly, female prawns at 6 months old of the selected line were significant ( $p \leq 0.01$ ) 5 and 16% larger by length and weight than those of the parental line, respectively. The results illustrated that genetic improvement to increase growth rate of the *Macrobrachium rosenbergii* is possible.

**Uraivan, S., Sumanojitraporn, S., Ampolsak, K. and S. Jeenmik. 2003. Response to within-family selection on growth rate of freshwater prawn (*Macrobrachium rosenbergii* de Man). The Seminar on Fisheries 2003. 7-9<sup>th</sup> July 2003, at the Department of Fisheries, Thailand**

Selective breeding program to improve growth rate of giant freshwater prawn (*Macrobrachium rosenbergii*) was carried out at the Aquatic Animal Genetics Research and Development Institute from 1998 to 2000. Within family selection procedure was applied on the growth rate of cultured prawns. The experiment consisted of two lines including a high growth selected line and a control line. The selection responses were estimated after one generation of selection. Female prawns of the selected line at 20 weeks old were ( $p < 0.01$ ) 4 (12%) and 5 (20%) significantly larger by length and weight than those of the control line and their parent generation, respectively.

Similarly, male prawns of the selected line at 20 weeks old were ( $p < 0.01$ ) 5 (18%) and 7 (14%) significantly larger by length and weight than those of the control line and their parent generation, respectively. The estimated realized heritability at one generation of selection was moderate. The average heritability in length and weight at 20 weeks old were 0.38 and 0.22, respectively. The results of this experiment illustrated that the within-family selection is the efficient procedure to improve growth of the giant freshwater prawn, and this method is being recommended for broodstock management.

#### (4) MANAGEMENT PROCEDURE AND SELECTIVE EFFECTS CONTROL

**Doyle, R.W., Singholka, S. and M.B. New. 1983. "Indirect selection" for genetic change: a quantitative analysis illustrated with *Macrobrachium rosenbergii*. *Aquaculture*, 30 : 237-247**

The term "indirect selection" means selection, which is exerted on a trait by methods other than deliberate artificial selection for the trait itself. It includes selection, which may be an incidental byproduct of harvesting and breeding techniques, as well as correlated selection on a trait caused by artificial selection on another trait.

Expressions are derived for calculating the intensity of indirect selection in aquaculture environments. The calculations are illustrated with growth-rate data on *M. rosenbergii* from prawn ponds in Thailand, and with computer-generated data, which simulate measurements made during a multiple mark-recapture experiment. Indirect selection for growth is probably negligible in Thai prawn farms and hatcheries at present, but small changes in management practice could exert strong indirect selection on growth rate. One very effective change is by collecting the broodstock as early in the production cycle as possible. Control of indirect selection may be useful for the genetic improvement of aquaculture stock, especially in developing countries and other situations where an intensive artificial selection program is not economically or biologically desirable. Like all selection programs, the probability of success is critically dependent on the genetics of the traits being selected.

**Doyle, R.W. 1983. An approach to the quantitative analysis of domestication selection in aquaculture. *Aquaculture*, 33 : 167-185**



Domestication selection is defined as natural selection on traits, which affect survival and reproduction in a human-controlled (domestic) environment. By altering various aspects of the environment, domestication selection can be made either to augment or to oppose artificial selection on traits of commercial importance. An example has been shown on the effects of selection on growth in *Macrobrachium rosenbergii* associated with variable development rate and age-at-harvest. It is concluded that management procedures can have strong selective effects and that genetic changes (for good or ill) may be expected to occur rapidly if the obvious genetic conditions are met.

## (5) BIOTECHNOLOGICAL APPROACHES TO GENETIC IMPROVEMENT

**Vanavichit, A. et al. (pers. comm.) Agricultural Genetic Engineering and Biotechnology Center, Kasetsart Univ. Research and Development Institute, Kamphaengsaen Campus, Nakhornpathom, Thailand**

All-male production is one method for the aquaculture stock of *Macrobrachium rosenbergii* to be more effective, because normal male prawns (zz) grow much better than the normal females (zw) especially in the first six months of culture. The method for all-male production includes sex-chromosome manipulation based on sex reversal to female using female synthetic hormone. The procedure also needs development of molecular or DNA marker which is a sex-linked marker to be used for selecting the right neofemale prawn, carrying the male zz-chromosomes, to mate with a normal male, also carrying the zz-chromosomes. Consequently, all progenies obtained should be all-male with zz-chromosomes, half of which should be from the mother (neofemale) and father (normal male) each.

### 1. Sex reversal to female using synthetic hormone

The sex reversal in *Macrobrachium rosenbergii* to female using female synthetic hormone, 17 $\beta$ -estradiol, is based on two treatments: oral administration and immersion in water containing the hormone. Giving hormone begins with the fifteen-days-postlarvae at 0, 50, 100 and 150 mg of 17 $\beta$ -estradiol/g of food for oral administration treatment, and 0, 50, 100, and 150 mg of 17 $\beta$ -estradiol/l of water for the immersion treatment.

Both treatments were for 15, 30 and 45 days. Results in the oral administration treatment indicated that (1) the sex-ratios obtained from every experimental group were not significantly different from the normal 1:1; (2) abnormal males were found in every experimental group; and (3) the best survival rate of 90% was in the group fed with the 100 mg/g for 30 days. Results of the immersion treatment illustrated that the higher dosage and duration of immersion undertaken, the higher female sex ratio is effectively obtained. The highest percent of female, 81.63 was obtained from the group immersed in 150 mg/l for 45 days. The sex ratio of this group was significantly different from the normal 1:1, presenting a 70% survival rate.

### 2. Sex-linked marker development

Searching for sex-linked marker in *Macrobrachium rosenbergii* was based on AFLP (Amplified Fragments Length Polymorphism) technique with the use of 7 primer pairs for PCR-I and 92 primer pairs for PCR-II. Results indicated that only 6 pairs were found to have polymorphic bands between male and female prawns. These DNA bands were sequenced and the DNA pieces being male and female sex-specific were found. Primers to be used for amplifying these pieces of DNA were designed from the sequences at both ends of each DNA piece. The designed primers were tested with prawn samples from male and female populations. The results indicated that the primers were still not good enough to identify the different sex properly and still could not be used for fieldwork.

**Klinbu-nga, S. (pers. comm.). Marine Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), based in Chulalongkorn University campus Bangkok, Thailand**

Klinbu-nga (pers. comm.) has also been searching for sex-specific DNA markers in *Macrobrachium rosenbergii* using AFLP technique. He claimed that five male-specific markers and four female-specific markers have been found. Further to this, he has been doing more advanced research by examining the expression of genes at terminal ends of the vas deferens and oviducts of male and female prawns, respectively using the RAP-PCR technique. Markers expressing specifically the small claw males (340 bp) and the females (415 bp) have been found. All of the DNA markers found have been cloned and will be tested for the sex-specificity.

**Sagi, A. and E.D. Aflalo. 2003. The androgenic gland and monosex culture in prawns – biotechnological perspective. Dept. of Life Sciences and the Institute for Applied Biosciences, Ben Gurion Univ. of the**



### Negev, Beer Sheva, Israel.

Males of the freshwater prawn, *Macrobrachium rosenbergii*, grow faster and reach a larger size at harvest compared to females, making the culture of monosex all-male population advantageous. Sexual differentiation in crustaceans is regulated by the androgenic gland (AG) found to be exerting morphological, anatomical, physiological and behavioral effects. The AG plays a pivotal role in the regulation of male differentiation and in the inhibition of female differentiation. In *M. rosenbergii*, complete sex reversal was achieved by AG removal in immature males, resulting in female differentiation, including the development of ovaries, oviducts and female gonopores. Similarly, AG implantation into immature females leads to the development of testes, sperm ducts and male gonopores. *M. rosenbergii* that had undergone sex reversal proved to be capable of mating with normal specimens and producing progenies. Early attempts to culture all male populations through manual segregation were reported from Israel and recently from India and other countries. The production of monosex prawn



Harvest and transport of freshwater prawn fry (right) for grow-out culture in earthen ponds (left) following the Code of Conduct Guidelines established by Thailand

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Tunsutapanich, A., Sanggontangit, T. and Phiudam, G., 1994. New Technology used for seed producing and nursing giant prawn (*Macrobrachium rosenbergii* de Man) larvae in earthen pond. Proceeding on Annual Fisheries Conference 1994. p. 49-54.

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## RESEARCH PROJECT PROPOSALS



As agreed during the Round Table Discussion, each country will develop its own appropriate selective breeding protocols and other related studies. In this connection, project research proposals were submitted and reviewed during the Round Table Discussion taking into consideration resources available and making sure that there will be delineation of efforts.

Thus, the following research activities will be conducted in respective participating countries:

- 1. Genetic improvement of *Macrobrachium rosenbergii* – Indonesia**
- 2. Genetic characterization, domestication and genetic improvement of *Macrobrachium rosenbergii* - Philippines**
- 3. Selective breeding program for genetic improvement of *Macrobrachium rosenbergii* – Thailand**

The Summary Proposals of each research activity are included in the following pages of this Report.







## SUMMARY PROPOSAL



**ACTIVITY TITLE :** Genetic Improvement of *Macrobrachium rosenbergii*

**COUNTRY :** Indonesia

**STATION :** Sukabumi - Sukamandi

**SOURCE OF EXTERNAL FUNDING** {If any}: GOI

**DURATION:** In Months {indicate total months}: 24 months

**DATE STARTED:** January 2004

**EXPECTED DATE OF COMPLETION:** December 2005

<b>PROPOSERS</b> {Name}:	<b>PARTICIPATION</b> {% time}
1. Dr. Estu Nugroho	75
2. Mr. Maskur	75
3. Mrs. Lies Emmawati	100
4. Mr. Agus Sasongko	100
5. Dr. Ketut Sugama	25

### **RATIONALE**

Freshwater prawn population in Indonesia is unique and the geographical distribution of about 19 species covers almost all the islands of Indonesia, thus Indonesia has been recognized as the center of origin of the freshwater prawn. However the potential genetic resource is not yet utilized in the country's freshwater prawn culture industry. Despite the development of the freshwater prawn culture in Indonesia, some problems have been found, e.g. declining growth rate, low survival rate, low resistance to diseases and small edible portion. An alternative means to solve these problems is to produce good quality seeds through the application of selective breeding.

### **OBJECTIVES**

The main objective of this activity is to produce high quality seeds and develop a stock candidate breeders to support the development of a sustainable freshwater prawn culture industry.

### **BRIEF METHODOLOGY**

1. Broodstock collection

Giant freshwater prawns will be collected from South Kalimantan, South Sumatera, South Sulawesi and Bali. Collection from the wild would be taken for at least 100 pairs of prawns from each source.

2. Characterization using molecular marker

Microsatellite DNA and mitochondria DNA will be used to examine the freshwater prawn stocks collected from the various sites.

3. Tagging system

Plate of plastic will be used for tagging to identify the broodstock collected from each site.

4. Breeding system

The breeding system would be developed for full-sibs type for at least five pairs from



each collection site. After which the brood stock will be reared in concrete tanks.

5. Larval rearing

Larval rearing of *Macrobrachium* would make use of the clear water system at a density of 50 – 100 larvae/l. Artificial feed and Artemia will be used in the larval rearing lasting for 35 days.

6. Nursery of the post larvae

Nursery of the post larvae would make use of concrete tanks or earthen ponds and stocking of post larvae will be 25 – 50 individuals/l for 30 days rearing. Feeding rate of about 10 –15 %/day will be adopted at feeding frequency of 3 – 4 times daily.

7. Grow-out culture of fingerlings

Grow-out culture of fingerlings will be done in earthen ponds at a stocking density of 5 – 7 individuals/m<sup>2</sup> for 5 months culture. Feeding rate will be about 3 – 15 %/day and a feeding frequency of 3 – 4 times daily.

8. Selection

Combination of family and individual selection will be used to improve the growth rate and salinity tolerance of *Macrobrachium rosenbergii*.

9. Multiple location testing

Offspring obtained from the selection and control lines will be tested in different locations of the country to evaluate the culture potentials of the stocks.

**KEY REFERENCE:**

World fish, SEAFDEC, BFAR, INGA, INFIGRAD, IDRC

**SCHEDULE OF ACTIVITIES: YEAR 2004**

Activity	J	F	M	A	M	J	J	A	S	O	N	D
1. Preparation	■											
2. Evaluation			■									
3. Selection				■								
4. Hybridization					■							
5. Multi location testing								■				
6. Mass Production								■				



## SUMMARY PROPOSAL



**ACTIVITY TITLE:** Genetic characterization, domestication and genetic improvement of *Macrobrachium rosenbergii*

**COUNTRY:** Philippines

**STATION(S):** BFAR-NIFTDC, BFAR-NFFTC, SEAFDEC/AQD

**SOURCE OF EXTERNAL FUNDING** {If any}: ASEAN-SEAFDEC Special Five-Year Program, Philippine Government

**DURATION:** In Months {indicate total months}: 24 mos. (Phase I), 36 mos. (Phase II)

**DATE STARTED:** January 2004

**EXPECTED DATE OF COMPLETION:** December 2005 (Phase I)

**PROPONENTS** {Name}:

**PARTICIPATION** {% time}

Westly R. Rosario	BFAR	30%
Editha C. Roxas	BFAR	50%
Melchor Tayamen	BFAR	30%
Jodecel Danting	BFAR	50%
Maria Rowena R. Eguia	SEAFDEC/AQD	50%
Maria Lourdes C. Aralar	SEAFDEC/AQD	50%
Manuel A. Laron	SEAFDEC/AQD	50%

### RATIONALE

The giant freshwater prawn (*Macrobrachium rosenbergii*) is native to tropical countries in South and Southeast Asia, parts of Oceania and the Pacific. It has great potential as a species for rural aquaculture as demonstrated by Thailand and Indonesia. Recent findings show that the Philippine stock of *Macrobrachium rosenbergii*, basically an eastern subspecies (*M. rosenbergii rosenbergii* De Man 1895), is different from the eastern subspecies (*M. rosenbergii dacqueti* Sunier 1925) found in India, Thailand, Malaysia and some parts of Indonesia (New, 2002). Hence, there is an urgent need to develop molecular genetic markers to identify and characterize the different subspecies and/or stocks available in the country. There is also a need to continue ongoing studies on morphometric characterization and domestication of local stocks and the refinement of nursery and grow-out technologies, before any efforts to improve growth and survival in existing culture stocks through genetic modification, can be undertaken.

### GENERAL OBJECTIVES

1. To determine the genetic diversity of wild and farmed stocks of freshwater prawns,
2. To develop sound broodstock management and selective breeding methods for the maintenance and/or improvement of the genetic quality of farmed *Macrobrachium rosenbergii* stocks in the Philippines; and
3. To produce quality *M. rosenbergii* seedstock for rural aquaculture.



## SPECIFIC OBJECTIVES

1. To identify and differentiate *Macrobrachium rosenbergii* from other indigenous *Macrobrachium* species through morphometric and molecular marker methods;
2. To characterize the different local stocks of *Macrobrachium rosenbergii* using allozyme and advanced DNA-based molecular markers;
3. To refine existing breeding and husbandry techniques for the successful domestication of wild *M. rosenbergii* stocks;
4. To develop viable low-input schemes in the production of quality *M. rosenbergii* seedstock;
5. To evaluate economically important performance traits in the different local *M. rosenbergii* stocks;
6. To formulate and adopt, when necessary, appropriate selective breeding methods for the genetic improvement of local *M. rosenbergii* stocks;
7. To develop local experts in freshwater prawn research and farming through training, information exchange and research collaboration with other international organizations and academic institutions (e.g. Tohoku University in Japan and/or Queensland University of Technology in Australia); and
8. To train local farmers on proper broodstock and culture management of *Macrobrachium rosenbergii*

## BRIEF DESCRIPTION OF METHODOLOGY

### Research Component

#### Phase I (2004-2005)

Several known stocks of *Macrobrachium rosenbergii*, from different localities in the Philippines shall be collected and characterized genetically through morphometric and molecular marker analysis (allozyme and microsatellites). Other indigenous *Macrobrachium* species shall likewise be collected and genetically screened to enable the development of an index that will be used to identify and differentiate *M. rosenbergii* from other native *Macrobrachium* species. After determining interspecies and interstock differences based on morphological traits and genetic markers, at least three *Macrobrachium rosenbergii* stocks belonging to genetically diverse populations will be used for breeding and domestication studies.

Existing techniques for breeding, larval rearing and grow-out of these local stocks shall be developed and standardized for use in subsequent stock comparison work. The local stocks and possibly one imported domesticated stock (e.g. from Thailand) will be compared for growth and survival in different culture environments (tank, cage and ponds). Simultaneous studies will also be conducted to assess their reproductive efficiencies. A genetic improvement program on Philippine *Macrobrachium rosenbergii* stocks shall be undertaken if the results of the performance evaluation prove that local stocks are genetically deteriorated and/or inferior compared to the imported Asian farmed stock.

#### Phase II (2006 onwards)

From the earlier stock comparison runs, a component analysis on the variation in culture traits shall



have been performed and the extent by which genotype, environment and/ or both influence growth (and other traits) shall have been noted. From thence we shall determine which domesticated stock (or stocks) is genetically diverse and has the potential for superior culture performance. Several selective breeding schemes shall be tried to further improve growth, survival and reproductive performance of Philippine *Macrobrachium rosenbergii* stocks especially if a large percentage of the phenotypic variation is due to genetic differences and if the selected trait(s) is highly heritable.

Since the thrust is to promote rural aquaculture development, the selective breeding methods that will be tried should be simple enough for the farmers to adopt. A simple mass selection method and a within-family selection scheme with rotational line crossing will be tried to improve performance traits in the Philippine *Macrobrachium rosenbergii* stock. On the other hand, if the stock comparison studies show that non-genetic factors greatly influence culture performance, then the development of optimum breeding and husbandry methods shall be given more emphasis.

## **Training Component**

### Phase I (2004-2005)

The recently completed Aquaculture Biotechnology Research Facility (Biotech Lab) established at the SEAFDEC/AQD premises in Tigbauan, Iloilo, Philippines is for the primary use of the Philippine Department of Agriculture-Bureau of Fisheries and Aquatic Resources. This facility has a Molecular Endocrinology and Genetics (MEG) Laboratory where DNA-based population genetics studies can be conducted. To date however, some equipment and chemicals have yet to be purchased for the facility to become fully operational. Genetic screening of the local *Macrobrachium* stocks can be performed in this laboratory if funds for this project can be allocated for the purchase of the necessary chemicals (primers, chemicals for DNA extraction and electrophoresis etc.) and laboratory equipment (e.g. vertical slab electrophoretic apparatus; refrigerated microcentrifuge, etc).

While waiting for the Biotech Lab-MEG laboratory to become fully operational, it is proposed that at least one staff from BFAR will be trained in a population genetics laboratory in Japan (e.g. Tohoku University) where he/she can start doing some sequence analysis on a Philippine *M. rosenbergii* stock. The preliminary research cum training shall cover the development of microsatellite primers for use in subsequent genetic characterization studies. The skills training will be for a month, preferably during the first quarter of 2004 and the work shall be continued in the Biotech-MEG Laboratory in Iloilo.

Apart from the training in Japan, a week-long training workshop on molecular genetic techniques shall be held in mid-2004 at the MEG Laboratory in Iloilo. BFAR and AQD staff will participate in this training course, which will be conducted by a Japanese visiting scientist from Tohoku University and MRR Eguia of SEAFDEC/AQD. It is important that the visiting scientist will assist the Philippine counterpart in ensuring that the population genetics laboratory of the Biotech Lab-MEG Laboratory is fully functional and that the protocol for laboratory analyses are standardized. From thence, analysis of all the collected samples shall be conducted at the Aquabiotech-MEG laboratory. Subsequent training sessions specifically on statistical analysis using population genetics software shall be conducted as well.

### Phase II (2006 onwards)

In preparation for the development of a selective breeding program for the Philippine *Macrobrachium rosenbergii*, a training workshop on the freshwater prawn broodstock management and quantitative genetics will be held and participated in by BFAR and AQD staff.



## DETAILED METHODOLOGY

### Phase I (2004-2005)

#### **Study I: Survey and collection of stocks of *Macrobrachium rosenbergii* and other indigenous *Macrobrachium* species**

Stocks of *M. rosenbergii* and other *Macrobrachium* species shall be collected from sites where these indigenous freshwater prawns are found to be abundant. Based on earlier surveys, the giant freshwater prawn is found in at least seven regions (specifically in 18 provinces) in the Philippines (BFAR et al., 2003). Some members of the project team shall either travel to some of these collection sites or coordinate with BFAR regional offices to be able to obtain at least 100 pc. of freshwater prawn (*Macrobrachium* sp.) from each source. These samples will be brought to BFAR-NIFTC in Dagupan, Pangasinan where all collected Philippine wild stocks of *Macrobrachium* sp. will be kept. BFAR-NIFTC shall start domesticating and breeding some of these stocks for the proposed stock comparison studies. BFAR-NIFTC shall publish an updated record of all the possible sources of wild giant freshwater prawns in the Philippines.

#### **Study II: Development of standard morphometric and morphologic indices for the identification of indigenous *Macrobrachium* sp.**

BFAR-NIFTC shall serve as a repository of all collected live *Macrobrachium* sp. samples. They shall document samples from each stock by recording specific morphological and meristic parameters at the different growth stages (egg, larva, post-larvae and adult) that can be used to identify stocks or species. The morphometric data that will be obtained in adult *Macrobrachium* samples will be similar to those measured in *Penaeid* sp. (Goswami et al 1986; Lester and Pante, 1992) but with slight modifications.

#### **Study III: Development of molecular genetic markers for Philippine wild stocks of *Macrobrachium* sp.**

##### A. Allozyme variation study

Tissues (eye, muscle, hepatopancreas, pleopods etc.) samples shall be taken from some of the *Macrobrachium* sp. individuals for preliminary (enzyme-tissue specificity) analysis of allozyme markers. Once specific tissues are identified to exhibit good resolution for polymorphic enzyme loci, these shall be subsequently used in the biochemical screening of at least 30 samples from each stock. Allozyme variation in individuals belonging to the different stocks and species shall be analyzed to determine the genetic structure of existing wild populations of *Macrobrachium* sp. and to identify markers that can be used to distinguish the different species and stocks of *Macrobrachium* sp.

##### B. Microsatellite marker analysis

Preliminary DNA sequencing shall be done (in collaboration with a Japanese/ Australian university) on some *Macrobrachium* samples to identify and characterize microsatellite loci in wild *Macrobrachium* sp. collected from the Philippines. Primers shall be designed and optimum PCR conditions shall be determined for use in amplifying DNA for subsequent microsatellite variation work. Pleopods from at least 30 individuals per stock of wild *Macrobrachium* sp. shall be collected and stored in ethanol for microsatellite marker analysis. About 5-8 microsatellite loci identified to be polymorphic shall be used to characterize stocks and species collected from the different localities.

As in the allozyme study, the genetic structure and genetic variability of the various stocks and species shall be determined and microsatellite markers unique to each species/ stocks shall be identified. Molecular markers, particularly highly variable microsatellites,



are used in monitoring genetic changes in wild and farmed stocks and are important in the conservation and sustainable management of any aquaculture resource (O Connell and Wright, 1997).

#### **Study IV: Development and refinement of procedures for domestication (breeding, larval rearing and grow-out) of wild *M rosenbergii* stocks**

##### **A. Refinement of breeding and larval rearing techniques for *Macrobrachium rosenbergii* - Evaluation of different live food organisms as starter food for freshwater prawn larvae**

This study aims to evaluate the potential of different live food organisms as substitute for *Artemia* in rearing *M. rosenbergii* larvae. In experiment 1, five different live foods (*Moina*, *Brachionus sp.*, *Panagrellus redivivus*, *Ceriodaphnia* and *Artemia*) will be evaluated. In experiment 2, the two most promising substitute for *Artemia* will be fed to *M rosenbergii* larvae at three feeding levels and frequencies. This is to optimize the use of live food. The larval rearing period will end after 34 - 36 days when 80% of the population attains the postlarval stage (Ang and Cheah, 1986). The proximate composition and fatty acid content of the feed samples will be determined before the feeding experiment. Other studies that will develop and promote the use of low-input seed production and larval rearing schemes will also be pursued.

##### **B. Farming of *Macrobrachium rosenbergii* in modular cages in Laguna de Bay**

Since the methods for FW prawn culture in tanks and ponds (Rosario, 2001) have been established, this study aims to adopt with appropriate modifications, the existing technology for the culture of *M. rosenbergii* in ponds to a freshwater lake. Specifically this study, which shall be conducted at SEAFDEC/AQD-BFS aims to determine the growth, survival of this prawn as affected by different stocking densities, varying surface area of artificial substrate, seasonal variation in natural food availability and in polyculture with other freshwater fishes (Nile tilapia, bighead carp). The effect of these parameters on the population structure of different morphotypes as well as the degree of heterogenous individual growth (HIG) in male FW prawns will be assessed (Ranjeet and Kurup, 2002). Finally, the viability of a commercial lake-based culture system for *M rosenbergii* will be evaluated.

#### **Study V: *Stock Evaluation***

A preliminary study will be conducted by W. Rosario and his staff at BFAR-NIFTDC to evaluate the reproductive performance of at least three genetically diverse stocks of Philippine *M rosenbergii* and an imported stock from Thailand. Larval growth and survival shall likewise be assessed using the same stocks in experiments to be conducted by MA Laron of SEAFDEC/AQD and W. Rosario. The quality of the larvae used for these stock comparison studies will be evaluated based on the condition indices developed by Tayamen and Brown (1999). Growth and survival from juvenile to marketable size will be compared simultaneously in ponds and cages using the same *M rosenbergii* stocks. Pond-based studies will be implemented in BFAR-NFFTC under the supervision of M. Tayamen while cage-based studies shall be conducted at the Binangonan Freshwater Station of SEAFDEC/AQD by MLC Aralar.

#### ***Phase II (2006 onwards)***

The breeding goals and methodologies for the second phase (selective breeding component) can be described more clearly and in detail once baseline data from Phase I (esp. on the stock comparison work) become



available. Since the program will end in December 2006, preliminary selective breeding work that has been started in 2006 shall be continued for at least until one generation of selection has been achieved.

## EXPECTED OUTPUT

### Research Component

From the three-year project, baseline data on the genetic structure of wild and farmed *M. rosenbergii* stocks and other indigenous *Macrobrachium* species shall be generated. This information will serve not only as a guide for fishfarmers in identifying the various commercially important giant freshwater prawn stocks and species, it will also become an important reference for the efficient and sustainable management of this valuable aquaculture resource. Philippine-based technologies on *Macrobrachium* culture is not as advanced as those of Thailand and Indonesia, hence through this project, broodstock and culture management methods in ponds and lake-based cages shall have been refined and optimized to suit local conditions using indigenous freshwater prawn stocks which have the potential for superior growth performance. Although genetic improvement and selection work would still have to be continued beyond the three-year program, the fishfarmers will be able to start producing quality seeds by adopting sound breeding and culture practices developed from the results of the domestication and stock performance evaluation studies.

### Training and Extension Component

Through the ASEAN-SEAFDEC IRAP program, more local researchers would have been trained on freshwater prawn farming and genetics research. Technology demonstration workshops will be conducted and extension manuals will be written for the fishfarmers as part of the plan to disseminate the technologies developed from the project.

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## SUMMARY PROPOSAL



**ACTIVITY TITLE:** Selective breeding program for genetic improvement of *Macrobrachium rosenbergii*

**COUNTRY:** Thailand

**STATION(S):** Aquatic Animal Genetics Research and Development Institute (AAGRDI)

**SOURCE OF EXTERNAL FUNDING** {If any}: ASEAN-SEAFDEC Special Five-Year Program, Government of Thailand

**DURATION:** In Months {indicate total months}: At least 24 months

**DATE STARTED:** February 2004

**EXPECTED DATE OF COMPLETION:** December 2005

PROPONENT {Name}: 1. Mr. Somchart Sukawong (Director of AAGRDI) 2. Dr. Supattra Uraivan and staff 3. Dr. Panom K. Sodsuk and staff 4. Dr. Srirat Sodsuk and staff 5. Mr. Tanan Sangkorntanakit and staff 6. Mr. Somsak Roongtongbaisuri and staff 7. Mr. Saravuth Jasoh and staff 8. Mr. Wisanuporn Ratanatrivong and staff	PARTICIPATION {% time} 10 30 30 30 25 25 25 25
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**COORDINATOR:** Dr. Panom K. Sodsuk  
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### OBJECTIVES

1. To evaluate the economic traits performance and genetic variation of 9 crosses from 3 stocks of *Macrobrachium rosenbergii*.
2. To improve the economic traits of the best cross by suitable selection procedure.
3. To do PCR conditional optimization and primer test of microsatellite markers that have been developed for *Macrobrachium rosenbergii* by the AAGRDI.
4. To apply polymorphism system of molecular markers (allozymes and/or microsatellites) in the genetic variation evaluation.  
(Allozyme markers will be basically and initially used, and microsatellites may be additionally applied later for further selective breeding program.)



## BRIEF METHODOLOGY

### 1. Microsatellite markers

- 1.1 PCR condition of microsatellite primers will be optimized taking into consideration the annealing temperature, amount of DNA template, MgCl<sub>2</sub>, primer and enzyme concentration, etc.
- 1.2 The primers will be tested through a number of trials for the screening of the *Macrobrachium rosenbergii* samples from different stocks using the optimized PCR condition.

### 2. Selective breeding program

- 2.1 Reciprocal crosses of 3 stocks of *Macrobrachium rosenbergii* (1 wild and 2 domesticated stocks) producing 9 crosses will be carried out at the two AAGRDI hatcheries (Pathumthani and Phetchaburi).
- 2.2 Performance will be evaluated between the 9 crosses under 4 environmental conditions in four different provincial areas, Pathumthani, Uttaradit, Chumphon and Burirum. In each environment, all crosses will be reared together in 3 ponds. The crosses are identified by different colors being injected into the prawn muscle.
- 2.3 Genetic variation will be evaluated between the 9 crosses based on allozyme markers.
- 2.4 In each environment, the cross with the best performance will be chosen for the selective breeding program (4 environments may not be the same cross)
- 2.5 The within-family selection procedure will be used to improve the economic traits of the chosen cross.
- 2.6 Performance and genetic variation will be evaluated in each selected generation. (Later, microsatellite markers may be used to the allozymes)
- 2.7 After 3 generations of selection, the selected lines will be evaluated under farm conditions.

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**T**he Southeast Asian Fisheries Development Center (SEAFDEC), a regional treaty organization based in Bangkok, Thailand was established in December 1967 to promote fisheries development in the region. Its member countries are Japan, Malaysia, the Philippines, Singapore, Thailand, Brunei Darussalam, the Socialist Republic of Vietnam, Union of Myanmar, Indonesia and Cambodia. The Council of Directors who represents SEAFDEC Member Countries is the policy-making body of the organization.

SEAFDEC does research on appropriate fisheries technologies, trains fisheries and aquaculture technicians, and disseminates fisheries and aquaculture technologies. Four departments were established to pursue these objectives

The Training Department (TD) in Samut Prakan, Thailand (1967) for marine capture fisheries training

The Marine Fisheries Research Department (MFRD) in Singapore (1967) for fishery post-harvest technology

The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development

The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of marine fishery resources in the exclusive economic zones (EEZs) of SEAFDEC Member Countries

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Develop human resources for the region

Disseminate and exchange information on aquaculture

The Aquaculture Department in the Philippines maintains four stations: in Iloilo Province, the Tigbauan Main Station and the Dumangas Brackishwater Station; in Guimaras, the Igang Marine Substation; and in Rizal, the Binangonan Freshwater Substation.



Tigbauan Main Station Dumangas Brackishwater Station



Igang Marine Substation



Binangonan Freshwater Station

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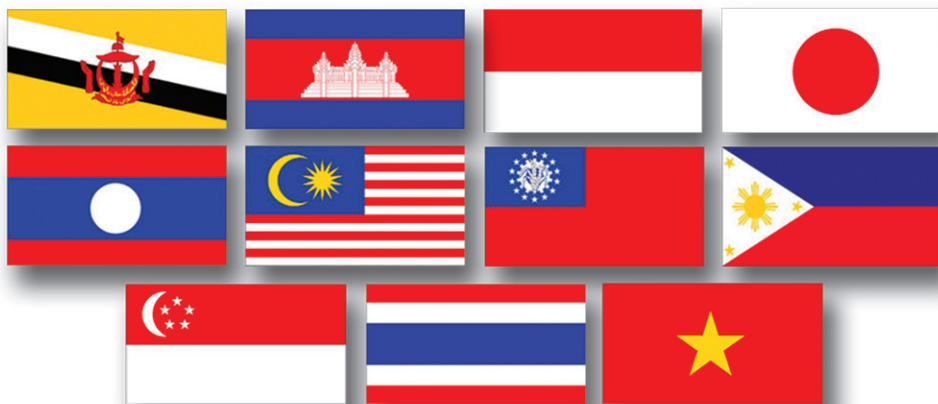
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