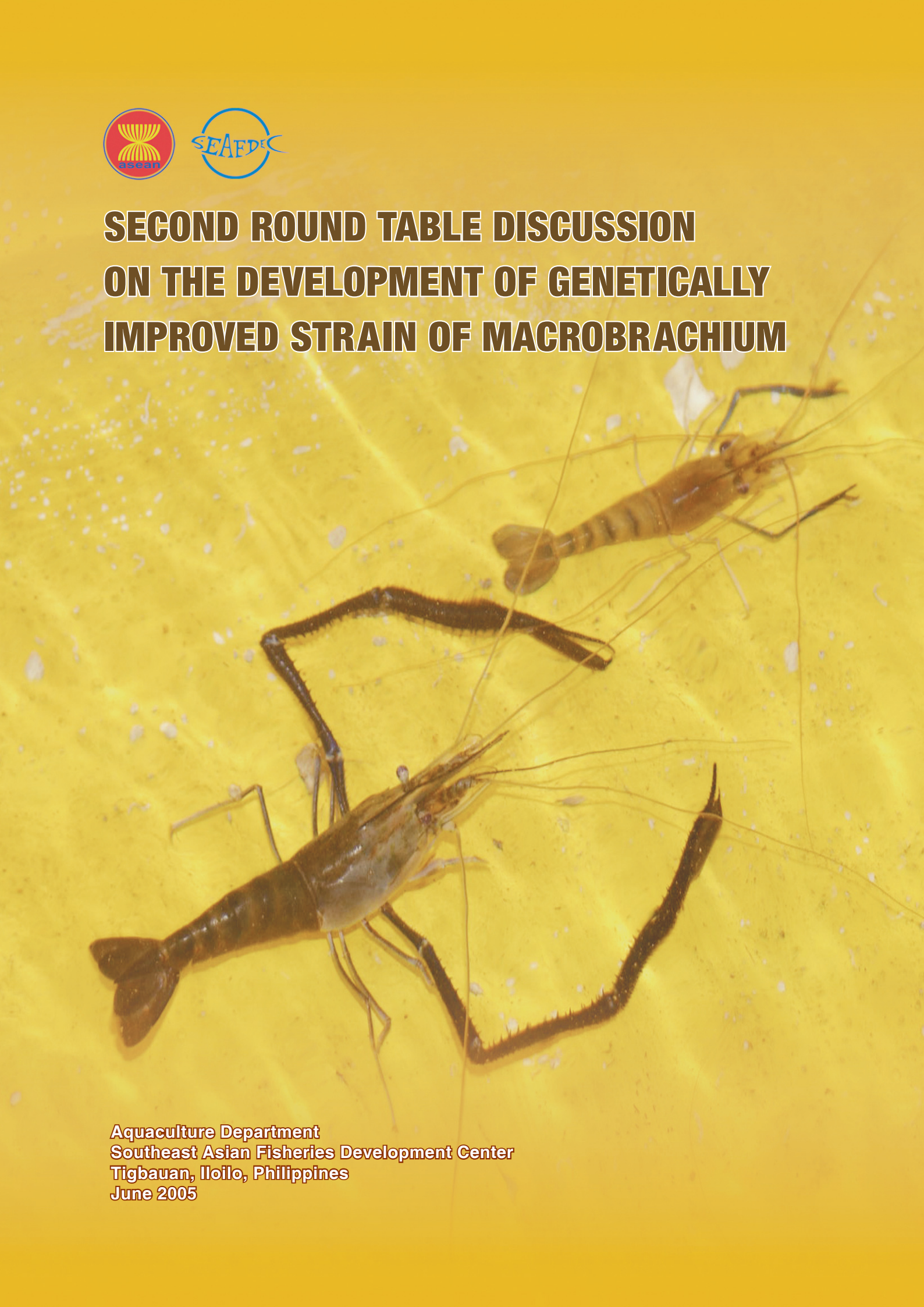
SECOND ROUND TABLE DISCUSSION ON THE DEVELOPMENT OF GENETICALLY IMPROVED STRAIN OF MACROBRACHIUM



Aquaculture Department
Southeast Asian Fisheries Development Center
Tigbauan, Iloilo, Philippines
June 2005

SECOND ROUND TABLE DISCUSSION ON THE DEVELOPMENT OF GENETICALLY IMPROVED STRAIN OF MACROBRACHIUM

A Collaborative Research under the Aquaculture Component of the
ASEAN-SEAFDEC Special Five-Year Program on
Sustainable Fisheries for Food Security in the ASEAN Region

June 2005

The Report of the Second Round Table Discussion on the Development of Genetically Improved Strain of Macrobrachium was prepared by:

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FOREWORD

The giant freshwater prawn is an economically important species but its culture is constrained by shortage of good quality seeds, possible inbreeding in domesticated broodstock and for not having developed improved strain. Through the Integrated Regional Aquaculture Program (IRAP), the collaborative research project on the Development of Genetically Improved Strain of Macrobrachium was conceived to address these concerns. IRAP is the Aquaculture Component of the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region, with funding from the Government of Japan.

The Second Round Table Discussion on the Development of Genetically Improved Strain of Macrobrachium was held in the Philippines in September 2004 to evaluate the progress of activities of the collaborative research and as a follow-up to the First Round Table Discussion, which was convened in Indonesia in 2003. During the said Round Table Discussion, delineation of responsibilities was agreed upon by the participating countries – Indonesia, the Philippines and Thailand. One of the recommendations during the First Round Table Discussion was to conduct a yearly evaluation of the project activities to enable the experts to get to know of each other's activities and come up with a consistent and improved technology on production of good quality freshwater prawn seeds that can be made available for rural aquaculture in the region.

This publication contains the progress of the collaborative research presented during the Second Round Table Discussion. I am hopeful that the collaborative efforts of the participating countries in this collaborative research will hasten the improvement of seed quality of the giant freshwater prawn in Southeast Asia. It is also hoped that through this collaboration, an improved strain of the freshwater prawn can be developed for dissemination in the region making the aquaculture of the giant freshwater prawn more profitable.

On behalf therefore of SEAFDEC, I would like to thank the Government of Japan for the financial assistance to the ASEAN-SEAFDEC Special Five-Year Program and specifically for the collaborative research project, the ASEAN Secretariat and the SEAFDEC Secretariat for their full support, the respective governments of the participating countries for providing the basic funding for the country components of the project and sharing their expertise. I also wish to commend the participants in the Round Table Discussion for their active participation and exchange of ideas during the deliberations.

ROLANDO R. PLATON, Ph.D.
Chief, SEAFDEC Aquaculture Department

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of the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries
for Food Security in the ASEAN Region

Dagupan City and Science City of Muñoz, Philippines
16-21 September 2004

Background

Adopting the Conference Resolution and Plan of Action during the ASEAN-SEAFDEC Millennium Conference held in Bangkok, Thailand in 2001, the Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region was implemented in the ASEAN region. The aquaculture component, which is the Integrated Regional Aquaculture Program (IRAP) aims to (1) assure a supply of quality seeds 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. The Program consists of two projects: (1) Aquaculture for Rural Development, and (2) Supply of Good Quality Seeds. Under the Supply of Good Quality Seeds Project, a collaborative research on the Development of Genetically Improved Strain of Macrobrachium, is being coordinated by the Aquaculture Department (AQD) of the Southeast Asian Fisheries Development Center (SEAFDEC). The participating countries of the collaborative research are Indonesia, the Philippines and Thailand.

In order to plan the detailed activities of the collaborative research and for the delineation of role and coverage of the activities in the participating countries, the First Round Table Discussion on the Development of Genetically Improved Strain of Macrobrachium was convened at the Freshwater Aquaculture Development Center, Sukabumi, West Java, Indonesia from 16 to 19 November 2003. The specific objectives of the First Round Table Discussion were to: (1) adopt common criteria for "good quality Macrobrachium seed"; (2) standardize methodology and approach in developing a genetically improved strain of Macrobrachium among the participating countries; (3) formulate definite work plan for the collaborative research project; and (4) agree on the mechanisms for sharing of results and genetic materials in Macrobrachium. The representative from Indonesia, *Dr. Ketut Sugama* was elected Chairperson of the First Round Table Discussion.

One of the recommendations during the First Round Table Discussion was to conduct an annual evaluation of the progress of activities under the collaborative research, which are being carried out by the participating countries. It was also agreed that such evaluation be discussed during the Second Round Table Discussion. The roundtable discussion will enable the participants to update each other of their activities and evaluate the status of the projects that have been conducted to draw recommendations from experiences and problems encountered during the implementation of the project. The responsibilities of the participating countries in the collaborative research as recommended during the First Roundtable Discussion held in Indonesia from 16 to 19 November 2003 are shown in Table 1.

Table 1. *Responsibilities of the participating countries in the collaborative research as recommended during the First Round Table Discussion, 16-19 November 2003.*

Reponsibilities/ Activities	Participating Countries		
	Indonesia	**Philippines**	**Thailand**
1. Selective Breeding	* Develop appropriate selective breeding protocols complementing with those of the Philippines and Thailand	* Develop appropriate selective breeding protocols complementing with those of Indonesia and Thailand (after the results of the population genetic studies)	* Develop appropriate selective breeding protocols complementing with those of the Philippines and Indonesia
	* Continue family selection combined with hybridization	* Design within family selection with rotational mating with BFAR Center in Muñoz	
		* Develop a modified mass selection with collimation and rotational mating at the BFAR Center in Dagupan	
2. Population Genetics		* Start characterization of local strains of Macrobrachium using molecular genetics techniques and morphological and morphometric measurements	
		* Study the genetic structure of the Philippine Macrobrachium which is different from Thailand, Indonesia and Borneo stocks	
3. Transfer of Macrobrachium Stock	* Adopt much greater precaution in transferring *M. rosenbergii* from Thailand and the rest of Southeast Asia to the Philippines to prevent genetic introgression of the Philippine strain	* Adopt much greater precaution in transferring *M. rosenbergii* from Thailand and the rest of Southeast Asia to the Philippines to prevent genetic introgression of Philippine strain	* Adopt much greater precaution in transferring *M. rosenbergii* from Thailand and the rest of Southeast Asia to the Philippines to prevent genetic introgression of Philippine strain
	* Observe precaution in transferring Macrobrachium stock from the Philippines to the rest of Southeast Asia	* Observe precaution in transferring Macrobrachium stock from the Philippines to the rest of Southeast Asia	* Observe precaution in transferring Macrobrachium stock from the Philippines to the rest of Southeast Asia
4. Strain Evaluation in Different Environments	* Adopt similar protocol as GIFT tilapia with caution since Macrobrachium is crustacean	* Adopt similar protocol as GIFT tilapia with caution since Macrobrachium is crustacean	* Adopt similar protocol as GIFT tilapia with caution since Macrobrachium is crustacean
5. Estimates of Heritabilities	* Design experiment to estimate heritabilities of traits under selection	* Design experiment to estimate heritabilities of traits under selection	* Design experiment to estimate heritabilities of traits under selection

Reponsibilities/ Activities	Participating Countries		
	Indonesia	Philippines	Thailand
6. Control	* Develop proper control line to measure the genetic gains after selection	* Develop proper control line to measure the genetic gains after selection	* Develop proper control line to measure the genetic gains after selection
7. Criteria for Quality Seeds	* Establish criteria for good quality seeds to guide farmers when purchasing seeds for culture	* Establish criteria for good quality seeds to guide farmers when purchasing seeds for culture	* Establish criteria for good quality seeds to guide farmers when purchasing seeds for culture
8. Specific Research Studies	* Genetic improvement of *Macrobrachium rosenbergii*	* Genetic characterization, domestication and genetic improvement of *Macrobrachium rosenbergii*	* Selective breeding programs for genetic improvement of *Macrobrachium rosenbergii*

Objectives of the Second Roundtable Discussion

The Second Round Table Discussion was convened in Dagupan City and the Science City of Muñoz, Philippines from 16 to 21 September 2004. The general objective of the 2nd Roundtable Discussion was to evaluate the activities implemented in the participating countries under the collaborative research. The specific objectives were as follows:

1. To assess the progress of activities of the collaborative research;
2. To identify problems encountered in the implementation of the research activities;
3. To develop training and information components in relation with the research activities; and
4. To recommend future course of action for the implementation of the collaborative research.

Participants in the Second Roundtable Discussion

Thirty-four participants from Indonesia, Thailand and the Philippines attended the Second Roundtable Discussion. Researchers from AQD who are involved in the collaborative research also attended the Second Round Table Discussion.

Opening of the Roundtable Discussion

SEAFDEC/AQD Chief, Dr. Rolando R. Platon, officially declared open the Second Round Table Discussion. The outgoing Chairman, Dr. Ketut Sugama of Indonesia gave a brief explanation about the project and recalled the activities during the First Round Table Discussion which was held in Sukabumi, West Java, Indonesia in September 2003. Upon the nomination by the representatives from Thailand, Mr. Westly Rosario and Dr. Melchor Tayamen of the Philippines were unanimously elected Chairman and Co-Chairman, respectively of the Second Round Table Discussion.

After a short message, the Chairman presented the list of documents for discussion. The participants adopted the provisional agenda and timetable as presented. In discussing the rationale of the activity, AQD's Mr. Wilfredo Yap said that the First Round Table Discussion enabled the participating countries to standardize their technologies and be familiar with the people involved in the country projects and their respective activities. He stressed the positive outcome of the collaboration and announced the new participant in the collaborative research from southern Philippines.

REPORT ON PROGRESS OF ACTIVITIES: PROBLEMS AND CONSTRAINTS

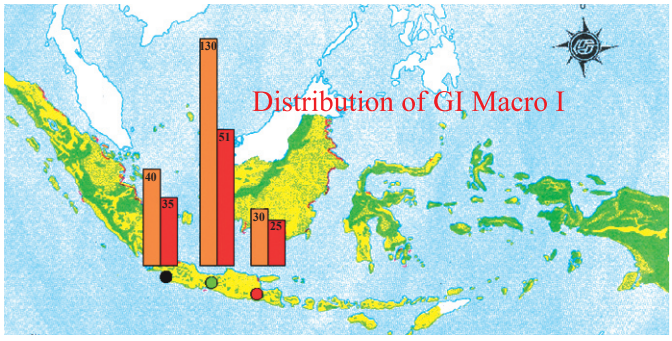
Genetic Improvement of *Macrobrachium rosenbergii* in Indonesia

Dr. Estu Nugroho, Dr. Ketut Sugana and Mr. Maskur, Directorate General of Aquaculture of Indonesia.

One way of increasing the production of freshwater prawn is through a genetic improvement program. The GI Macro seeds (Genetically Improved *Macrobrachium rosenbergii*) that Indonesia developed have been released to farmers since 2001. However, producing 50 g prawns have become difficult with survival rate of as low as 40% after 9-11 months of culture. Thus, the program to improve growth rate and increase the edible portion of the prawn was conducted.

Giant freshwater prawn is an important commodity that is successfully cultured in Indonesia. Its

Dr. Ketut Sugama (left) presented the status of the research activities in Indonesia. Dr. Estu Nugroho and Mr. Maskur discuss the distribution of GI Macro I in the country.



culture has been developed in several areas of West Java, i.e., Ciamis (Tambaksari, Pamarican and Kalipucang) and Tasikmalaya. The Indonesian Government has developed a hatchery in Jogjakarta province (Central Java), while the private sector control about seven hatcheries. In East Java, freshwater prawn is cultured in brackishwater ponds. Freshwater prawn culture has also spread to some areas of Bali Island, e.g., in Gianyar, Klungkung, Buleleng and Tabanan.

Indonesia has been recognized as the center of origin of the giant freshwater prawn because about 19 identified species are found in almost all islands of the country (Holthuis, 1980). However, this genetic resource is not yet fully used in freshwater prawn culture. Although freshwater prawn culture has been widely developed in Indonesia, some problems have been encountered, e.g., declining growth rate, disease, and the small edible portion (abdominal muscle).

In recent years, the Government of Indonesia has stressed the need to increase the production of freshwater prawn. One way to increase production is through the genetic improvement program. In 2001, the GI Macro (Genetically Improved *Macrobrachium rosenbergii*), strain of freshwater prawn has been developed and released to farmers.

The Freshwater Prawn Improvement Program of Indonesia

Recently, it has become difficult to produce 50 g female prawn and the survival rate is less than 40% after 9-11 months of grow-out culture. Since 1996, the Research Institute for Freshwater Aquaculture (RIFA) has started a genetic improvement program mainly targeted at improving the growth rate and increasing the edible portion of the prawn.

Breeding Program

RIFA believes that one way to improve freshwater prawn is through selective breeding. Artificial or mixed population was therefore formed from a number of breeders collected from the natural waters, i.e., from Tanjung air (Bekasi), Kalipucang (Ciamis) and Musi (Palembang). Subpopulation Tanjung air, collected in February 1995, had average body weight of 70 g. Individual selection was applied to this subpopulation to improve the edible portion trait. Subpopulation Kalipucang was collected in June 1996 at average weight of 72 g. Index selection was used in this population to improve growth rate and the edible portion traits. After two-step selection of the above two subpopulations, the subpopulation Musi was added (average body weight of 75 g, collected in May 1997). Family selection (24 families) was applied to the mixed population for the traits of growth rate and edible portion. The following result was obtained after the fourth generation of freshwater prawn:

No	Character	Value (mean± standard error)
1	Heritability of edible portion (h^2_{ep})	0.56 (*0.07*)
2	Heritability of body weight (h^2_{bw})	0.84 (*0.02*)
3	Inbreeding rate (F)	0.0091
4	Total length of male (cm) Total length of female (cm)	21.5 (5.55) 15.02 (3.29)
5	Percentage of carapace (male) Percentage of carapace (female)	30.45 (5.86) 32.68 (8.05)
6	Hatching rate (%)	65 – 80
7	Survival rate (% after 4 months)	46 – 53

Distribution of GI Macro Seed

GI Macro seeds have been distributed to three hatcheries in Probolinggo, East Java; Samas, Jogjakarta; and Pamarican, West Java on 24th July 2001. The seeds were grown to broodstock and the broodstock spawned to seeds for culture. Generally, the GI Macro seed did well at the start, but there were some problems after two years. First, the average body weight or size of GI Macro, varied in different locations, i.e., 130 g for male and 51 g for female in Samas, 30 g (male) and 25 g (female) in Probolinggo, and 40 g (male) and 34.5 g (female) in Pamarican. There was an indication of environmental effect on the growth rate. The growth rate differences in GI Macro was likely caused by differences in culture management. Second, the size of the edible portion gradually declined. Farmers could visually identify the GI Macro from normal based on the proportion of the body to the carapace. The GI macro mixing with other races and mating among themselves resulted in reduced response to selective breeding.

In order to solve the above problems, a mixed population of the GI Macro was again reconstructed by adding some wild population of giant freshwater prawn, following the established selection program.

Activities on the Giant Freshwater Prawn in 2004

i) Base population of GI Macro II

At least 800 breeders have been collected to perform a base population of GI Macro II. The collections originated from GI Macro I with an addition of some wild populations from Musi, Palembang, Sumatra. About 100 giant freshwater prawn, with weight of 60 to 120 g for males and 40 to 70 g for females, have been used as a genetic source through full-sibling matings that consisted of 22 families. About 1000 post-larvae (PL1) per family were collected and reared up to PL20. In this step, the survival rate of the larvae was 85-95%. Eight hundred PL20 was reared in hapa 2x2x1 m, which was placed in 200 m^2 pond for a month. The survival rate ranged from 50 to 80%, and the juveniles were 3-6 cm in length and 0.5 g to 2.2 g in weight. Three hundred of these juveniles were selected for the next breeding steps (Fig. 1).

At present, 300 hundred larvae with weight ranging from 0.5 to 2.2 g are reared in 40 m^2 in earthen pond for three months until the prawns reached the size of 20-30 g each. About 100 pairs of the best individual (in terms of growth) per family will be selected and used as breeders for the next spawning.

Apart from developing the giant freshwater prawn with the best growth this year, RIFA has also produced the second generation of GI Macro that can tolerate salinities up to 15 ppt but this strain is still under observation. Fig. 2 shows the grand parent of GI Macro while Fig. 3 shows the selection product and control farmer strain.

ii) Application of mtDNA molecular markers

Application of DNA markers has been tested to characterize a number of natural stocks of freshwater prawn since 2002. The genetic variability of freshwater prawn collected from Musi, Barito and GI Macro races were examined for polymorphism of the mitochondriaL DNA (mtDNA) markers. Six composite haplotypes were detected following digestion of **CO1** sequences with four endonucleases: Rsa I, Hae III, MboI and MspI. The average haplotype diversity was 0.603 (Table 1). Significant genetic difference was observed among the freshwater prawn populations. The biggest proportion of the major composite haplotype was due to GI Macro (originated from Citarum and Citanduy). Freshwater prawn from Musi contributed to the composite haplotype of GI Macro with frequency of 25%. Barito races have good prospects for genetic resources in the breeding program. An example of restriction patterns is shown in Figure 4.

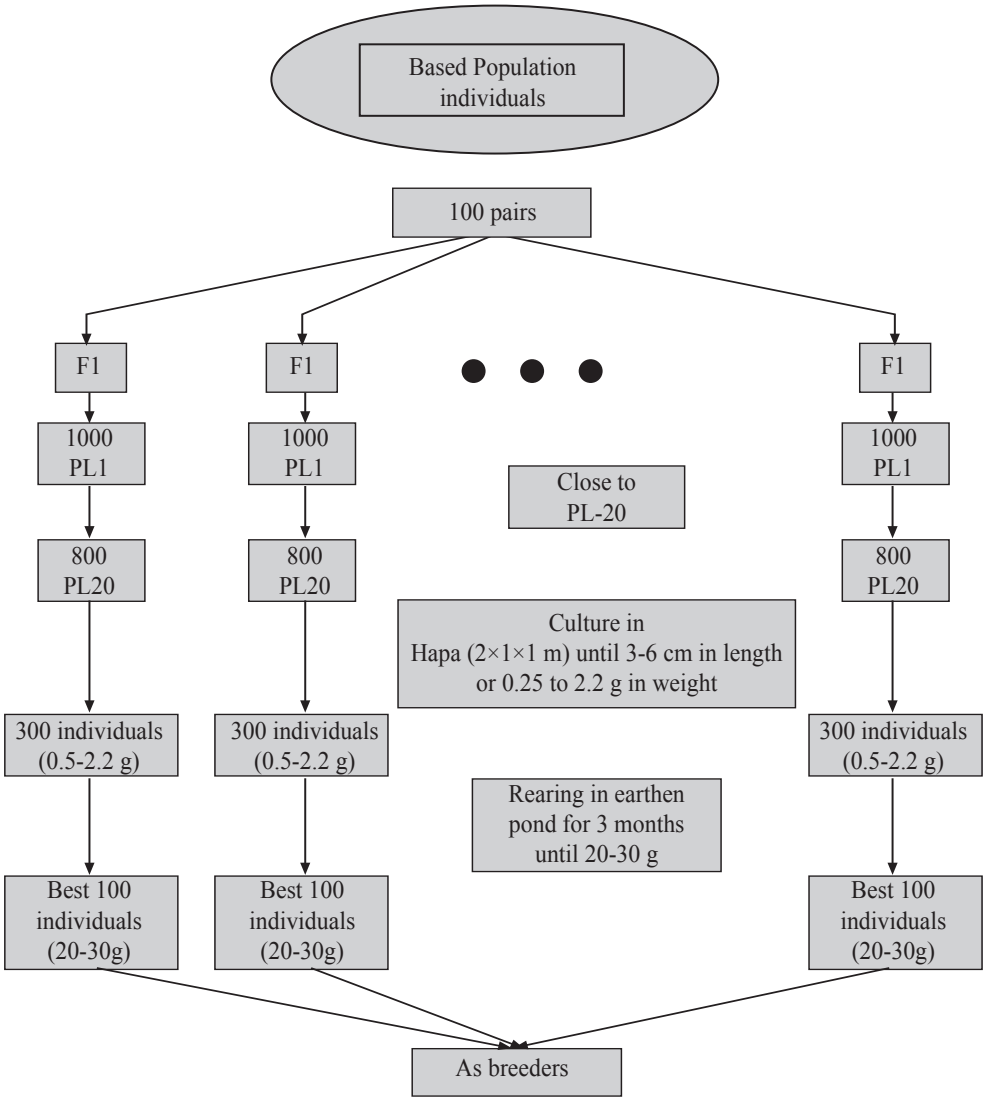


Figure 1. *Flow chart of selective breeding activity at RIFA*

Table 1. *Frequency of composite haplotype mtDNA CO1 among freshwater prawn populations with four endonucleases, Rsa I, Hae III, Mbo I and Msp I*

No	Type Composite Haplotype	Population		
		GI Macro	Musi	Barito
1	AAAA	0.375	0.071	0.647
2	ABAA	0.188	0.142	-
3	ACAA	0.250	0.642	0.353
4	ABAB	0.125	0.071	-
5	ACAB	0.062	-	-
6	ACBA	-	0.071	-
	No. of Samples	16	14	17
	No. of Haplotypes	5	5	2
	Haplotype Diversitas	0.766	0.573	0.471

iii) Application of estradiol-17β for sex reversal

The male freshwater prawn is bigger than the female. One way to produce the all-male freshwater prawn in mass scale is to use females that are genetically male, or homo gamete female. If a homo gamete female mates with a normal male, this will produce 100% male phenotype. Two treatments have been conducted under this activity, i.e., feeding and dipping. Dosage used in feeding is 30, 50 and 70 mg of hormone per kg feed, while for dipping 3, 6, 9 and 12 mg of hormone are diluted per liter. Identification of the result will be conducted in two months after treatment. Generally, giant freshwater prawn with dipping treatment has higher survival than those given feed containing hormone.

iv) Culture technology: addition of Vitamin C and E for increasing productivity of giant freshwater prawn

About 50 males weighing 50–70 g and 120 females weighing 20–40 g were kept in 2x1x1 m hapa nets at 10 individuals per hapa. The dosages were 0.05%, 0.10%, 0.15% Vit. C and 1.0%, 1.5%, 2.0% Vit. E. The larval rearing system used is a recirculating system with biofilter, ozone treatment and ultraviolet radiation. Application of this system involves supplying good quality water for larval rearing and nursery operations. PL 25-40 have been produced in this system.

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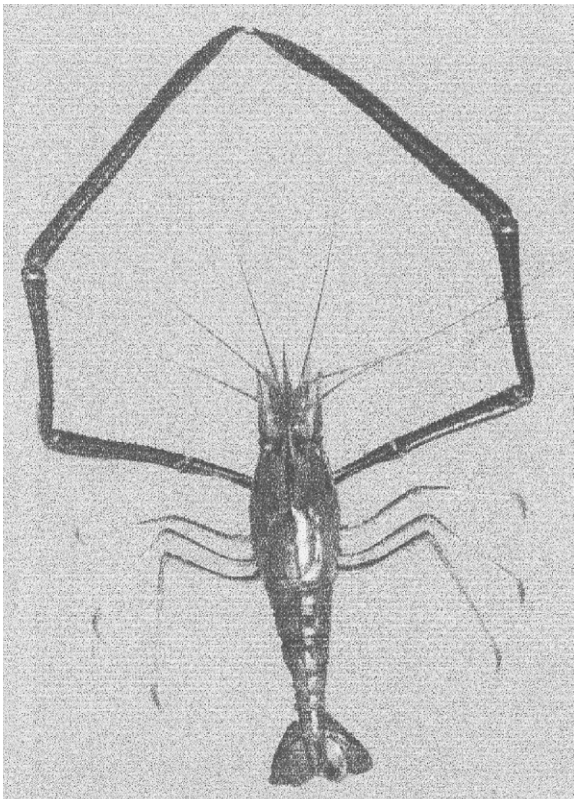


Figure 2. *Grand parent stock of freshwater prawn, GI Macro (total length 38 cm, body weight 480 g after five months)*

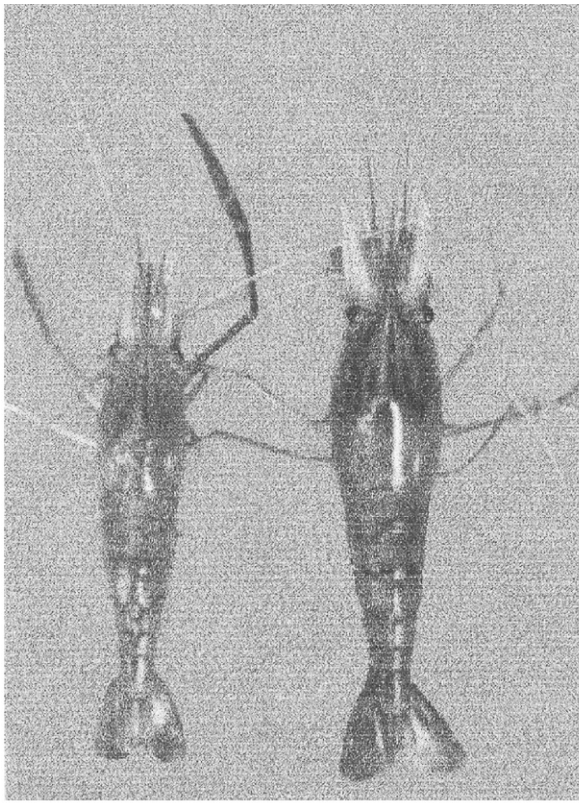


Figure 3. *Selection product (right) and control farmer strain (left) after five months rearing*

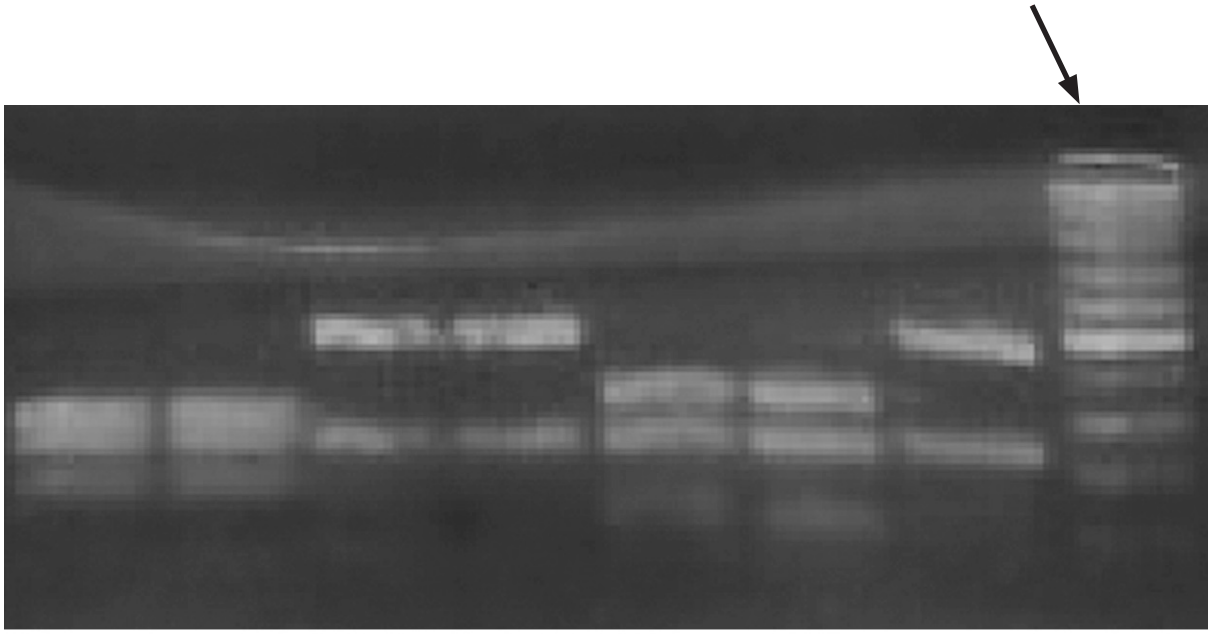


Figure 4. *Mitochondria* **CO-1** *region of freshwater prawn degenerated by Hae III endonuclease (the arrow indicate 500 bp in length)*

Mr. Westly Rosario and Dr. Melchor Tayamen answering questions raised after their presentation of their project activities in Northern Philippines

Dr. Rowena Romana-Eguia, Dr. Ma. Lourdes Aralar and Mr. Manuel Laron reporting the status of Macrobrachium studies at SEAFDEC AQD

Project on Genetic Characterization, Domestication, Genetic Improvement and Culture of *Macrobrachium rosenbergii* in the Philippines

Mr. Henry Dejarme adds humor to his presentation to the delight of the participants

Commercial production of the freshwater prawn, *Macrobrachium rosenbergii* in the Philippines is not as developed as those in Thailand and Indonesia. Although studies on *Macrobrachium* sp. (or ulang as it is locally known), started at the Binangonan Freshwater Station (BFS) of SEAFDEC/AQD in the mid 80s, research efforts were discontinued because *Macrobrachium* sp. was considered a low priority species and emphasis was given to penaeid shrimps instead.

Later studies at BFS have been revived where results have shown that although *Artemia* is still the best natural food for *M. rosenbergii*, the acceptability and potential of the lower-priced *Moina* as starter feed for the prawn has been demonstrated. Moreover, results of other studies also seemed to indicate that farming of *M. rosenbergii* in cages in lakes is a viable alternative to pond culture.

The Philippine Bureau of Fisheries and Aquatic Resources (BFAR) at its National Integrated Fisheries Technology Development Center (NIFTDC) in Dagupan City has undertaken activities on the collection of wild stocks as well as domestication and propagation of *M. rosenbergii*. From their collections, one local strain of *Macrobrachium* sp. labeled as BFAR 1 was found to have better growth performance than an earlier-found strain labeled BFAR 0. The normal juvenile rearing period of BFAR 0 is 45-50 days, whereas BFAR 1 only requires 37-40 days.

BFAR established in 2004, a Task Force for "Ulang" Aquaculture with the National Freshwater Fisheries Technology Center (NFFTC) in the Science City of Muñoz, Nueva Ecija, as the Task Force's home base. The Task Force established various programs for the revival of freshwater prawn aquaculture in the country.

Freswater prawn facilities in Northern Mindanao, Philippines: hatchery (bellow) and pond system (bottom)

Hatchery and pond culture experiments are also being conducted at the Mindanao State University at Naawan in Northern Mindanao, Philippines. Results of a number of experiments could not yet be rationalized as these are still in the early stages of implementation. However, a progress report on the activities was presented during this Round Table Discussion.

The Freshwater Prawn Research at SEAFDEC/AOD

Maria Rowena R. Romana-Equia of SEAFDEC/AQD

Research and commercial production of the freshwater prawn, *Macrobrachium* sp., in the Philippines are yet not well-developed. Although studies on *Macrobrachium* sp. (or *ulang* as it is locally known), started at the Binangonan Freshwater Station (BFS) of SEAFDEC/AQD in the mid-1980s, research efforts were discontinued soon thereafter because of: (a) inadequate technical skills; (b) problems with larval rearing and the domestication of wild stocks; and (c) the *Macrobrachium* sp. is being considered in the Philippines as a low priority species in contrast to commercially important freshwater commodities like tilapia and milkfish.

That was two decades ago and in retrospect, researches continued and the freshwater prawn in the Philippines could have been successfully domesticated and current problems concerning the limited aquaculture production of genetically depauperate non-indigenous stocks could have been resolved. With the renewed interest in the culture of alternative species like the freshwater prawn, researchers at the Binangonan Freshwater Station (BFS) started to conduct some studies on the refinement of breeding, larval rearing and culture of *Macrobrachium rosenbergii* since 2003.

The recent research studies on *Macrobrachium rosenbergii* at BFS are briefly described below:

i) Evaluation of different live food organisms as starter food for freshwater prawn larvae

This study aimed to evaluate growth, survival and post-larval production of *Macrobrachium rosenbergii* when fed different live food organisms (*Moina*, *Artemia* and a free living nematode, *Panagrellus redivivus*). Results showed that growth (measured as mean developmental stage, MDS), survival and post-larval production differed significantly among the treatments. Final body weight of *Moina*-fed larvae was higher but not significantly different ($P>0.05$) from that of *Artemia*-fed larvae. However, survival of *Moina*-fed larvae was significantly low.

Prawn larvae fed *P. redivivus* had poor survival and survived only for 8 days. Meanwhile the development of *M. rosenbergii* in this present study was faster in that 80% of the larvae reached postlarval stage after 20-25 days of rearing compared to the 34 to 36-day development period reported by Ang and Cheah (1986).

While *Artemia* is still the best natural food for *M. rosenbergii*, this study demonstrated the acceptability and potential of *Moina* as a starter feed for prawn larvae given the fact that increased body weight was observed in larvae fed *Moina*. However more work should still be undertaken to optimize the use of this and other promising alternative feeds.

ii) Farming of *Macrobrachium rosenbergii* in modular cages in Laguna de Bay

This study was conducted to determine the growth and survival of freshwater prawn in cages (2.5 x 1 x 1m^3) as affected by different stocking densities (15, 30, 60 and 90 prawns/m^2) and availability of natural food. The effect of these parameters on the population structure of different morphotypes and the degree of heterogenous individual growth (HIG) in male FW prawns was assessed. Results showed that mean sizes at harvest after 5 months of culture ranged from 14.3 g for the highest stocking density to 26.3 g for the lowest. Mean size at harvest, daily growth rate, and size class distribution were significantly influenced by stocking density with those at the lowest stocking density showing significantly better growth and overall proportion of larger prawns. Heterogeneous individual growth (HIG) was fairly evident in all treatments.

The percentage of blue-clawed males (BC-males) was not influenced by treatment but the mean weight was significantly higher in the lower stocking densities. Survival was highest in the lower stocking densities (55.3, 54.0, 52.7, and 36.9% for 15, 30, 60 and 90 prawns m^{-2}, respectively).

Feed conversion ratio (FCR) improved with decreasing stocking density ranging from 2.1 to 3. Yield per cropping increased with stocking density and ranged from 1,874 to 4,530 kg ha^{-1}.

Production values obtained in the cage cultured *M. rosenbergii* were comparable to or even higher than those reported from pond culture. Results show that the farming of *M. rosenbergii* in cages in lakes is a viable alternative to pond culture and has the potential of improve aquaculture production in lakeshore fish farming communities.

iii) Reproductive performance of various stocks and species of freshwater prawn fed high and low protein diets

This preliminary study aims to determine the reproductive efficiency of FW prawn broodstock fed high- and low-protein diets. Thus far, two *Macrobrachium* sp. (hatchery-bred *Macrobrachium rosenbergii* and wild-sourced *Macrobrachium sp.*) are being evaluated. In collaboration with the Mindanao State University (through Dr. Dejarme), more stocks shall be collected and their reproductive traits shall be compared. This strain evaluation experiment hopes to identify stocks and/or species that can later be used in improving the present hatchery stocks of *M. rosenbergii* either through crossbreeding/ hybridization and other conventional selective breeding methods.

These plans to genetically document stocks are incorporated in a general proposal entitled "Genetic characterization, domestication and improvement of *Macrobrachium rosenbergii* in the Philippines" to be pursued further under the collaboration research.

Collection of Wild Stocks, Domesticatication and Propagation of *Macrobrachium rosenbergii*
Mr. Westly R. Rosario and Editha C. Roxas of BFAR NIFTDC.

There is an expanding interest in the culture of freshwater prawn in the Philippines. This is attributed to the extensive campaign of the government, the Bureau of Fisheries and Aquatic Resources (BFAR) and some private entrepreneurs to disseminate information and seeds of the prawn nationwide.

Although freshwater aquaculture in the Philippines is still dominated by tilapia, which is an exotic fish, the profit from tilapia culture is not well appreciated except for family consumption or nutrition purposes. The freshwater prawn, an indigenous species, remains to be an important species. With freshwater prawn as an alternative species, farmers can diversify and derive higher profit from their ponds. In the Philippines, *Macrobrachium rosenbergii* stocked in 2,000 m^2 ponds may grow to about 45 g after four months and 90 to 100 g in seven months of culture (Rosario, 2002). The price of the species is five times higher than tilapia.

During the first Round Table Discussion on the Development of Genetically Improved Strain of *Macrobrachium* held at the Freshwater Aquaculture Development Center, Sukabumi, West Java, Indonesia in November 2003, the delegates from Thailand reported that the Philippine wild stocks of *Macrobrachium, M. rosenbergii rosenbergii* Philippine strain could be a better variety and therefore must be protected from contamination by non-indigenous strains. The report supports and confirms the importance of the activity of the National Integrated Fisheries and Development Center (NIFTDC) to collect live specimens of various strains of *Macrobrachium* in the country and review their performance in terms of growth and fecundity.

Geographical Distribution

In the Philippines, wild catch is available from the river tributaries and lakes in the provinces of Pangasinan, La Union, Ilocos Sur, Ilocos Norte, Cagayan, Pangasinan, Pampanga, Bulacan, Laguna, Palawan, Sorsogon, Leyte, Samar, Cotabato, Lanao, Maguindanao, Agusan and other parts of Mindanao. A survey by Agasen (2001) reported 12 species in Luzon with *Macrobrachium rosenbergii* as dominant species.

BFAR-NIFTDC collected live wild stocks of the species from Bulacan, Palawan, Bicol, and two provinces in Mindanao from Year 2002 and domesticated them at the Center. Due to limited space and manpower, the strains found to be inferior in growth performance were discarded.

Testing of Local Strains

One local strain of *Macrobrachium* (BFAR 1) collected from Mindanao was tested to have better performance than the old strain used by the Center (BFAR 0). With the BFAR 0 as benchmark, the larval rearing period of BFAR 1 is shorter by 8 to 13 days. The normal rearing period of BFAR 0 is 45 to 50 days, whereas BFAR 1 only requires 37 to 40 days. The larval rearing period is much shorter during hot months. The size of BFAR 1 larvae are bigger by 25 %. The survival rate of the larvae during rearing has improved by about 12 %. Results of field trials on growth performance are still being evaluated.

There were more than 200,000 postlarvae produced and distributed to the farmers for culture by BFAR-NIFTDC from October 2003 to present. From 100 breeders collected from the wild, the Center is now using 500 F2 and F3 breeders.

One problem encountered in the use of another local strain (BFAR 2) is the early release or detaching of eggs from the female breeders.

The basic problem encountered by BFAR-NIFTDC in the collection and use of local strains is the proper identification of species. It is therefore recommended that the collaborating countries should establish uniform guidelines and references in order to solve the problem.

Literature Cited

Rosario, W. R. 2002. Culture of Freshwater Prawn *(Macrobrachium rosenbergii) in Earthen Ponds, BFAR-NIFTDC Extension Paper, 3pp.*

Freshwater Prawn Program of BFAR

Dr. Melchor M. Tayamen of BFAR NFFTC.

The giant freshwater prawn *(Macrobrachium rosenbengii)* is one of the indigenous prawns found in many parts of the country. Locally known as *ulang*, it is a hardy species that is easily farmed. On the average, farmed *ulang* weighs from 30 to 100 grams, which translates to 10 to 25 pieces per kilo. This is very much comparable to the medium to large or jumbo sizes of brackishwater tiger shrimps or *sugpo*. In the wild, *ulang* grow as much as 500 g and sells at 300 to 350 pesos/kg ($1=₱55.50), however, the quantity harvested is limited and is dependent on its seasonality.

Despite the development of both hatchery and grow-out technologies for *ulang*, there is really no significant commercial production in the country yet, except in BFAR-operated hatcheries in Muñoz and in Dagupan. To date, the only private *ulang* hatchery is MBL Farms producing up to 150,000 PL or post-larvae per run (45 days), although there are entrepreneurs trained in Muñoz who are also operating small backyard hatcheries for prawns.

With the emerging global market on this giant freshwater prawn coupled with improved technologies, it is but imperative to speed up the development of the industry in the country. However, the industry is faced with problems and constraints that include:

- Insufficient breeders

- Insufficient supply of post-larvae or PL for stocking
- Limited market supply
- Limited funds for interested stakeholders
- Insufficient information
- Inadequate promotion of technology transfer
- Very few skilled and/or trained technicians
- Research and development of *ulang* hatchery and grow-out are still wanting new technologies

Potentials

Macrobrachium rosenbergii or *ulang* is one of several indigenous freshwater prawn species in the country. *Ulang* abounds in rivers, lakes and other tributaries in the Philippines. It is known as *udang* in the provinces of llocos, Cagayan and other parts of Northern Luzon, *kising-kising* in Pangasinan, *paje* in Palawan and Zamboanga, *padao* in Cotabato, *kalig* (big) and *urang* (small) in Leyte, *budsang* in Bicol and *ulang* in most parts of the country including Bulacan, Laguna, Zambales and others.

Considered as the world's largest, it is one of the emerging shrimp products in the global market. As a high value crop, *ulang* is exported in frozen form to the US and the European markets where its large size and comparatively lower price than the tiger shrimp makes it a popular aquatic item. When whole, it is also regarded as specialty item and are often sold live for display in aquarium tanks.

With the country's extensive inland resources about 250,000 hectares in all of the lakes, rivers and reservoirs; 106,328 hectares of freshwater swamplands and 14,531 hectares of freshwater fishponds, *ulang* grow-out has a very large potential. Although finfish such as tilapia and *bangus* dominate the farmed species in these areas, catfish, mudfish and freshwater prawn are also harvested.

Development of Technology

The culture of *ulang* was introduced in the 1970's in Asia as well as in other parts of North and South America. In the 1990s, an ASEAN-EC-Aquaculture Coordination Development Program was implemented by BFAR and one of its components is the breeding of giant freshwater prawn in glass aquaria, and later on in tanks. These pioneering studies on *ulang* production were conducted at the National Freshwater Fisheries Technology Center (NFFTC) in the Science City of Muñoz, Nueva Ecija. In the later part of 1998, BFAR had successfully mass-produced the post larvae or PL stage of this species. In 2001, the hatchery of the *ulang* was further improved and finally a protocol for its commercial hatchery was established in Muñoz and later on at the National Integrated Fisheries Technology Development Center in Bonuan, Dagupan City, Pangasinan.

Since then, BFAR continues to introduce various schemes to promote the technology to various stakeholders, e. g., conduct of trainings, dispersal programs and establishment of techno demo-sites for its culture with farmer-cooperator scheme. Various national trainers training for *ulang* hatchery and grow-out were also conducted to disseminate its potentials and opportunities. In addition, a Task Force for *ulang* promotion program was also created in the early part of 2004.

Production Trend

Production from the farming of *Macrobrachtum rosenbengii* has expanded considerably, mainly

in Asia and also in South and North America. The Food and Agriculture Organization (FAO) reported that global production of *M. rosenbergii* had risen to nearly 119,000 metric tons from only about 5,000 metric tons recorded in 1984. China, which introduced this species in 1976, contributed over 97,000 metric tons in 2000.

The FAO likewise stressed that the production statistics for this species are underestimates, because some countries have not yet clearly defined their production from more general statistical categories such as 'freshwater prawns and shrimps' or 'freshwater crustaceans'.

The same is true in the Philippines in as much as most of the *ulang* sold in the local markets are caught in the wild. There is no significant commercial farming of *ulang* in the country yet, except with MBL Farms owned by a private entrepreneur who had trained on hatchery and grow-out at the BF AR and pilot projects in small ponds in Laguna.

However, with BFAR's active promotion of *ulang* in hotels, restaurants and the public during the 1st *Ulang* Food Festival in 2003, the demand for this prawn had grown considerably. MBL Farms reported that it could no longer cope with the growing demands in Manila alone. The MBL Farm's medium scale hatchery already produces fifty thousand to one hundred fifty thousand post larvae or PLs per run.

Business Viability

The culture of *ulang* is a profitable business venture. BFAR-Muñoz techno-demo farms show a high return on investment in both *ulang* grow-out and hatchery operations. Also, based on the feasibility studies prepared by MBL Farms, the estimated ROI could range from 63 percent to 74 percent depending upon the size of the pond area. The current market price of *ulang* ranges from 300 pesos to 350 pesos depending on the size (US$1.00 = ₱55.50).

BFAR had also launched its national rice-shrimp program in 2001 to attract rice farmers into integrating *ulang* culture in their rice farms. Under this scheme, the farmer is assured of two-fold benefits: that of the same amount of rice harvest plus an additional income derived from the sale of *ulang*.

Development of Freshwater Prawn Industry in the Philippines

In spite of the development of hatchery and grow-out technologies in the freshwater prawn (locally known as *ulang*), there is really no significant commercial production of the prawn in the Philippines. This has been attributed to various constraints that include: (1) insufficient supply of breeders; (2) inadequate supply of postlarvae for stocking; (3) limited market supply; (4) limited funds for interested stakeholders; (5) lack of information; (6) limited promotion of technology; (7) inadequate skilled and/or trained technicians; (8) limited R&D on *ulang* hatchery. With the country's extensive inland resources, *ulang* aquaculture has a very large potential.

The Philippine Bureau of Fisheries and Aquatic Resources (BFAR) continues to pursue the aquaculture of *ulang* in its NFFTC in the City of Muñoz, Nueva Ecija (central Luzon), as well as disseminate the culture's potentials and opportunities. In 2004, BFAR established a Task Force with NFFTC as homebase for the promotion of *ulang* aquaculture program.

Various interventions have been programmed by the Task Force for ulang aquaculture in the Philippines. These are: (1) establishment of *ulang* hatcheries in existing EXCEL tilapia central and satellite hatcheries throughout the Philippines to produce the required prawn postlarvae; (2) establishment of hatcheries in coastal areas near the EXCEL tilapia hatcheries to increase the number of *ulang* hatcheries; (3) lease or improvement of non-performing sugpo (shrimp) hatcheris into multi-

use functions to include *ulang* seed production; (4) promotion and/or dispersal of *ulang* postlarvae throughout the country; (5) establishment of pilot techno-demo farms in collaboration with private cooperators, local government units and the academe; (6) awareness creation on the part of the fisherfolk and/or entrepreneurs on the potentials of ulang culture; (7) development of a code of conduct for sustainable ulang production; (8) refinement of the rice-prawn technology and promotion of the technology throughout the country; and (9) intensive nationwide information dissemination campaign on the economics of ulang aquaculture. With inputs coming from the IRAP collaborative research, the Philippines is assured of the sustainability of prawn aquaculture in the country.

Hatchery and Pond Culture of *Macrobrachium rosenbergii* in Northern Mindanao

Dr. Henry E. Dejarme of the MSU at Naawan.

The history and status of hatchery and grow-out culture of *Macrobrachium rosenbergii* is not based on a study nor a survey. Rather, it was derived mainly from his on-the-job experience and information gathered during visits of culture sites or shared by other workers in the culture of freshwater prawn in Northern Mindanao. Thus, the background information may not be as complete as it should be.

The history of *M. rosenbergii* hatchery operations in Northern Mindanao can be traced from minor or side activities in different locations by several institutions. Earlier attempts to produce postlarvae of *M. rosenbergii* in hatcheries by MSU faculty/researchers were conducted in the school laboratory hatchery of the College of Fisheries in MSU Marawi City and in the commercial hatchery facilities for *sugpo*, *Penaeus monodon*, at MSU Naawan.

At the MSU Marawi College of Fisheries, a faculty member led his group to initiate hatchery production of *M. rosenbergii* fry in the 1970s. The breeders were collected live from Kapay near Marawi City. Kapay is more than a thousand feet above sea level, and 30 m away from the oceanic waters of Iligan Bay. The larvae that were hatched were reared in freshwater and died after a week or so. A second and last batch of breeders collected from the same site hatched their eggs and the larvae were reared in brackishwater medium. The larvae survived a few days more than the first, yet they did not attain postlarval stage when mass mortality occurred.

In Naawan, a series of trials on the larval rearing of freshwater prawn in the late 1970s and early 1980s was part of a project that mainly included a study on the biology and ecology of the species in two prawn grounds in the municipalities of Tambulig and Siay, Zamboanga del Sur. The natural habitat of *M. rosenbergii* in Tambulig is located in the innermost portion of Panguil Bay in Northwestern Mindanao. On the other hand, the prawn habitat in Siay is in Sebuguey River that empties into Illana Bay facing the Celebes Sea.

Live berried females from the two study sites mentioned were transported to Naawan and held in wooden tanks until the eggs were hatched. The larvae were reared in brackish and green water medium and fed *Brachionus*, *Artemia*, and strained fish flesh throughout the rearing period. Unfortunately, not a single larval rearing trial was successful.

Hatchery trials to culture freshwater prawn postlarvae were also conducted by a faculty member in 1994 at the Multispecies Hatchery of the Dipolog School of Fisheries in Zamboanga del Norte. A few postlarvae were produced in about a year, but the school administration decided to discontinue this activity.

Present Status of Hatchery and Pond Culture

In March 2004, eggs of some *M. rosenbergii* breeders from the Misamis Occidental Aquamarine

Park (MOAP) in Sinacaban, Misamis Occidental were hatched at the Naawan Sugpo Hatchery. We were informed by the MOAP personnel that the breeders were actually transported as postlarvae from the BFAR-National Freshwater Fisheries Technology Center, Nueva Ecija (BFAR-NFFTC) and grown to mature stage in the earthen ponds of MOAP.

The newly hatched larvae from MOAP breeders were successfully reared to metamorphose (40,000+ pcs of postlarvae) in brackishwater medium containing *Tetraselmis*. The larvae were fed *Artemia* nauplii and strained fish flesh in the early stages until postlarvae. The larvae at the late zoeal stages and postlarvae were also given marine polychaete, *Pereneries* sp. and local earthworm. Many of the first batch of hatchery-produced prawn fry from MSU Naawan were stocked in MOAP pond (17,000 pc) and in a privately owned pond in Valencia, Bukidnon pond (16, 0000 pc). The remaining prawn fry were held in a glass aquarium for observation at MSU Naawan.

The production of prawn fry at MSU Naawan Hatchery is continuing. At present there are four batches of larvae at different stages. As of 12 September 2004, the oldest batch is 5 days old from metamorphosis (PL_5) and the youngest batch is 3 days old zoea.

There are at present, four hatchery facilities in Northern Mindanao that have continuing hatchery activities; two are established along the shoreline of Iligan Bay, the others are located in landlocked provinces (Table 1). These prawn hatcheries are producing prawn seedlings but production data are not available except for MSU Naawan and BFAR Kisolon (Table 2).

The initial breeders used in the hatchery production of *M. rosenbergii* postlarvae in BFAR Kisolon, MOAP, and MSU Naawan were postlarvae from domesticated stock at BFAR-National Freshwater Fisheries Technology Center in Nueva Ecija and grown to sexually mature stage in Northern Mindanao ponds. But an additional one hundred egg-bearing breeders were provided free of charge for MSU Naawan by *Dr. Melchor Tayamen*, the Chief of BFAR-NFFTC. These breeders were transported directly from BFAR-NFFTC on 24 July 2004.

Last 16 July 2004 three berried females from the wild prawn population in Panguil Bay were transported to MSU Naawan. Two of our current batches of larvae that are expected to metamorphose to postlarvae before the end of September were hatched from eggs of these Panguil Bay breeders. Aside from the Panguil Bay, other sources of wild stock breeders could be the Illana Bay, Mandulog River in Iligan City, Kapay in Marawi City, Macajalar Bay and Cagayan River in Cagayan de Oro City, Odiongan River in Gingoog City, and Tagoloan River in Tagoloan, Misamis Oriental.

There are many sources of prawn breeders from the wild for the other three existing hatcheries in Northern Mindanao. The MOAP hatchery in Sinacaban can tap the wild stock of giant prawns from the western side of Mindanao such as those reported in Plaridel, Misamis Occidental; and the Katipunan River in Dipolog, Zamboanga del Norte. For BFAR Kisolon Hatchery, the possible sources are the Pulangi River, Rio Grande de Mindanao and the major river tributaries of Davao Gulf such as the Tagum-Libuganon, Davao, Tuganay, Padada-Guihing, and Lasang Rivers. In the CARAGA region where the LGU Freshwater Prawn Hatchery of Prosperidad is located, the known sites of giant prawn habitats and sources of breeders are the rivers in Surigao del Norte, Surigao del Sur, along the bank of upper Agusan River in sitio of Maguinda, and the river mouth of Agusan River near Sitio Magallanes in Butuan Bay.

The culture of *M. rosenbergii* to marketable size is in the early stages of development and the culture system is confined only to small-size earthen ponds (200 m^2 - 500 m^2).

Historically, the first and only attempt to culture the giant prawn in ponds in 1980s in Northern Mindanao, was conducted by MSU Naawan using the freshwater tilapia fishpond of Buruun National

School of Fisheries in Iligan City. The few hundred seed stocks obtained from the Tambulig wild population were 7-10 cm long prawn juveniles. Fed chicken pellets, the prawns attained marketable size (30-60 g) in five months and some females were egg-bearing upon harvest.

The postlarvae from BFAR-NFFTC that were distributed for stocking in BFAR Kisolon, Bukidnon and MOAP, Sinacaban ponds were fed fish pellets. Accordingly, berried females were observed beginning on the 6th month from stocking. The prawn fry from MSU Naawan and stocked in MOAP fishpond were also fed fish pellets. In Valencia, Bukidnon, the MSU prawn fry (more than one month old) stocked in Mr. Benauro's fishpond on 6 May 2004 were fed a variety of feeds that included spoiled *balut pinoy*, rotten fish from commercial fish dealers, and farmed earth worm, African night crawlers. Last September, 41 pcs of berried females and 26 pc of mature males were collected from Mr. Benauro's pond and transported to MSU Naawan. Four of the berried females have already hatched their eggs and the larvae are being reared in 200 L glass aquaria.

Potentials for Development

The demand for prawn fry has steadily increased after its first production at MSU Naawan. The 3,000 pc of prawn postlarvae available at present are scheduled for stocking in landlocked areas of Northern Mindanao; 1,000 pc is booked for a private fishpond owner in Quezon, Bukidnon; the 2,000 pc for the DENR X livelihood program in Baongon, Cagayan de Oro City. The remaining standing orders for prawn postlarvae for September to October 2004 are as follows:

Municipal LGUs of Bukidnon 50,000 pc for demonstration earthen ponds

Mr. Benauro, Bukidnon 20,000 pc for backyard earthen ponds

Mr. Ang, Iligan City 5,000 pc for intensive culture in a concrete pond

Mr. Castillo, Cotabato 20,000 pc commercial earthen ponds

The potential for expansion of hatchery production of prawn fry in Northern Mindanao is too early to determine. But judging from the current plight of tiger shrimp culture industry and the number of fish and shrimp hatchery owners who inquire about the prospects of freshwater prawn culture, the potential could be greater than expected.

For MSU Naawan, the potential in the capacity for several folds increase in fry production will be bolstered with the transfer of management of the modern facility of the Southern Philippines Development Authority (SPDA) Hatchery to MSU Naawan. The SPDA hatchery is established inside the MSU Naawan Campus through a Memorandum of Agreement (MOA). Under this MOA the SPDA Office will turnover the facilities to MSU Naawan in October 2005.

The culture of giant prawn to marketable size in fishponds is a new aquaculture development in Northern Mindanao. It was probably partly popularized recently by the '*Palay-Ulangan*' Program of the Philippine Government. This program was targeting the vast tracts of rice fields in Northern Mindanao as new sites for raising freshwater prawns. But to date, other freshwater resources such as numerous springs, natural and manmade dams, finfish ponds that are readily convertible to prawn ponds, etc., are being eyed for prawn aquaculture. MSU Naawan has also considered the following as potential sites for expanding prawn culture activities in Northern Mindanao:

1. Privately owned idle lands close to water sources in Bukidnon, Misamis Occidental, Zamboanga, Agusan and Surigao;
2. The fishpond facilities of Iligan City National School of Fisheries in Buruun, Iligan City;
3. The springs in Linamon, Lanao del Norte through the LGU;
4. Abandoned concrete pools inside the compound of an industrial plant in Iligan City; and

5. Underutilized areas inside a privately-owned Piggery in Iligan City

Suggestions for Future R&D Activities

Many potential investors who visited MSU Naawan and expressed interest to venture into freshwater prawn culture have inquired if MSU Naawan can do something to reduce the head-body ratio of the freshwater prawn, to reduce the enormous size of the male claws, to produce all male prawn fry, and to delay the spawning of young female prawns. Their contention is that an improvement along these characteristics would make the freshwater prawn more attractive as an aquaculture species for Northern Mindanao. This inquiry is clearly suggestive of researches that are within the realm of aqua-biotechnology.

Table 1. *List of Existing Macrobrahium Hatcheries Located in Northern Mindanao*

Name of Hatchery	Agency	Location	Distance from Nearest Seawater Source
MSU Naawan Multispecies Hatchery Complex	Mindanao State University at Naawan	Naawan, Misamis Oriental	Few meters
Kisolon BFAR Freshwater Fish Hatchery	BFAR X	Kisolon Freshwater Fish Hatchery and Training Center, Bukidnon	About 60 km
Misamis Occidental Aquamarine Park(MOAP) Hatchery	Misamis Occidental Provincial Government	MOAP, Sinacaban, Misamis Occidental	Few meters
LGU Prosperidad Hatchery	Agusan del Sur Provincial Government	Prosperidad, Agusan del Sur	About 80 km

Table 2. *Some Data on the Operation of Macrobrahium Hatcheries in Northern Mindanao*

Name of Hatchery	Start of Operation	Source (s) of Breeders*	PL Production from start to present
MSU Naawan Hatchery	February 2004	MOAP Fishpond in Misamis Occidental; BFAR-National Fisheries Technology Center, Nueva Ecija; Private Fishpond in Valencia, Bukidnon; Panguil Bay	About 70,000
Kisolon BFAR Hatchery	2003	BFAR-National Fisheries Technology Center, Nueva Ecija	A few thousand postlarvae
MOAP Hatchery	2003	BFAR-National Fisheries Technology Center, Nueva Ecija (Through BFAR X)	No data
LGU Prosperidad Hatchery	2003	Agusan River	No data

Table 3: *Some Data on the Culture of Macrobrachium in Northern Mindanao Fishponds*

Pond Owner	Location of fishpond	Seedling Source/ Date of Stocking	Status
BFAR X	Kisolon Freshwater Fish Hatchery and Training Center, Bukidnon	BFAR-National Fisheries Technology Center, Nueva Ecija/ Later part of 2003	The stock has grown to sexually mature adult and is the source of breeders for hatchery operation in Bukidnon BFAR Training Center
Misamis Occidental Aquamarine Park(MOAP)	Inside the Park	BFAR-National Fisheries Technology Center, Nueva Ecija and MSU Naawan Hatchery/ Later part of 2003	The stock has grown to sexually mature adult and is the source of breeders for hatchery operation in Bukidnon BFAR Training Center
	San Fernando, Bukidnon	BFAR-National Fisheries Technology Center, Nueva Ecija/ Later part of 2003	No data
Mr. Benauro	Valencia, Bukidnon	BFAR-National Fisheries Technology Center, Nueva Ecija and MSU Naawan Hatchery/ Later part of 2003	The stock has grown to sexually mature adult and is the source of breeders for hatchery operation in Bukidnon BFAR Training Center
Mrs. Ruby Macabaya	Quezon, Bukidnon	MSU Naawan Hatchery/ September13, 2004	On-going
DENR X	Baongon, Cagayan de Oro City	MSU Naawan Hatchery/ September13, 2004	On-going


THE PHILIPPINES
South China Sea
NIFTDC
Dagupan
NFFTC
Muñoz
Philippine Sea
Cagayan de Oro City
Prosperidad
Agusan
Panguil Bay
Aquamarine Park
Misamis Occidental
Sulu Sea
MSU Naawan, Misamis Oriental
MALAYSIA
Kisolon
Bukidnon
Valencia
Bukidnon
Celebes Sea

Selective Breeding Program for Genetic Improvement of *Macrobrachium rosenbergii* in Thailand

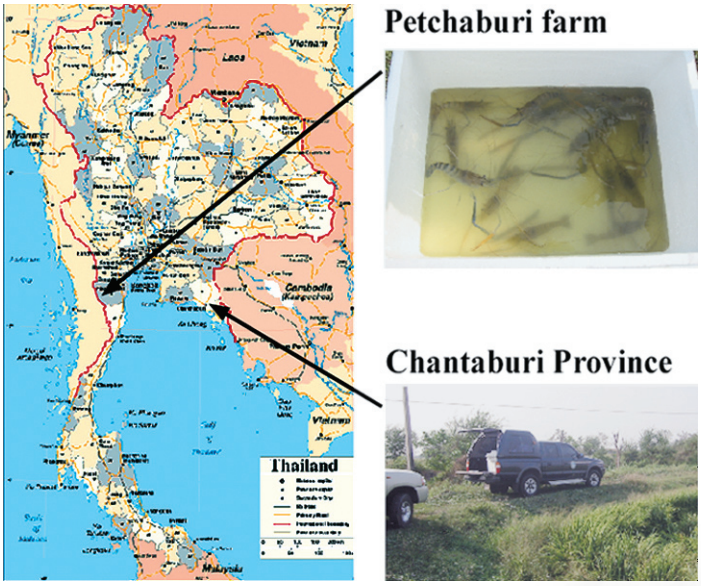
Dr. Supattra Uraiwan and Dr. Panom K. Sodsuk of the Aquatic Animal Genetic Research and Development Institute (AAGRDI) of the Department of Fisheries of Thailand.

Although the giant freshwater prawn (*Macrobrachium rosenbergii)* has been domesticated in Thailand for over decades, appropriate selective breeding program has yet to be achieved. Good quality seeds for the *Machrobrachium* aquaculture industry is therefore not regularly produced. One of the selective breeding programs on improving growth performance of the domesticated strain have been carried out at the Aquatic Animal Genetics Research and Development Institute (AAGRDI), Department of Fisheries of Thailand. AAGRDI has now developed improved and domesticated stock of *Macrobrachium rosenbergii* for two generations. Meanwhile, domesticated stocks from private hatcheries have also been acquired.

Dr. Panom Sodsuk and Dr. Supattra Uraiwan reporting the status of Macrobrachium selective breeding program in Thailand

There is, therefore, the need to develop another improved stock of this species basically from these two domesticated stocks together with a wild stock in order to improve the genetic diversity of the base population for further selective breeding program. *Macrobrachium* wild stock has been domesticated under hatchery conditions at the AAGRDI for one generation. Generally, a good base population for genetic improvement program requires high genetic variation as well as an ideally suitable stock that can be well adapted for each of different local environments. Therefore, all proper crosses of these three stocks need to be cultured in different areas of the country and then evaluated on both performance and genetic variation before selective breeding program takes place.

The preliminary genetic studies that led to appropriate selective breeding criteria have been investigated in *Macrobrachium rosenbergii* by a group of researchers (Meewan, 1991, Uraiwan *et. al*. 2002 and 2003; etc.). They estimated heritabitity on economic traits such as growth, body shape and morphotypic transformations, and they concluded that these traits have low to moderate estimated heritabilities. Therefore, the improvement of these traits can be carried out through selections. Uraiwan *et. al*. (2003) reported that the within-family selection was possible to improve growth performance of *Macrobrachium rosenbergii* with the average response rate of 5-20 % per generation. Therefore, the present selective breeding program will use the within-family selection criteria.



Molecular technology at enzyme/ protein level known as "allozyme marker" is widely accepted as a

powerful technique for studying genetic variation (Ward and Grewe, 1995), even the intra-specific population studies (Sodsuk, 1996; Sodsuk and Sodsuk, 1998a & 1998b; Sodsuk *et al*., 2001). Due to the availability of the allozyme technique, it can be basically and initially applied in the genetic variation evaluation.

Among the DNA-based molecular technologies, microsatellite marker technique has a number of suitable qualities for genetic variation evaluation including (1) high polymorphism of many allelic gene loci, (2) co-dominant alleles inheritance with the homo/ heterozygous genotype of each individual identifiable, and (3) allele frequency and heterozygosity indicating amount of genetic variation obtainable (Queller *et al*., 1993). Microsatellites display polymorphism by means of their variable numbers of tandem repeat motifs resulting in size variation, which can then be visualized by PCR with specific primers, followed by electrophoresis of the amplification product. The AAGRDI has been developing microsatellite markers for *Macrobrachium rosenbergii*, however the steps in optimizing PCR conditions for primers are needed in order that this technique could be used in addition to allozymes for evaluating genetic variation of further selective breeding program.

The objectives of Thailand's selective breeding program are:

1. To evaluate the economic traits performance and genetic variation of nine (9) crosses from three (3) stocks of *Macrobrachium rosenbergii;*
2. To improve economic traits of the best cross by suitable selection procedure;
3. To undertake PCR conditional optimization and primer test of microsatellite markers that have been developed for *Macrobrachium rosenbergii* by the AAGRDI; and
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.)

The activities of the program include:

1. Allozyme analysis

   Genetic variabilities, observed heterozygosity and number of alleles per locus, of *Macrobrachium rosenbergii* from three populations are analyzed by allozme electrophoresis.

2. Microsatellite markers

   2.1 PCR condition of microsatellite primers has been optimized considering the annealing temperature, amount of DNA template, $MgCl_2$, primer and enzyme concentration, etc.

   2.2 The primers have been tested with a number of trials of screening *Macrobrachium rosenbergii* samples from different stocks using the optimized PCR condition.

3. Selective breeding program

   3.1 Reciprocal cross of three (3) stocks of *Macrobrachium rosenbergii* (1 wild, 2 domesticated stocks) to produce nine (9) crosses

   3.2 Performance evaluation to test between nine (9) crosses under three (3) environments (4 provinces: Pathomtanee, Utradit, Chomporn and Burirum). In each environment, all crosses will be reared together in three (3) ponds. The crosses are identified by the different colors, which have been injected into the prawn muscles.

   3.3 Genetic variation will be evaluated between nine (9) crosses based on allozyme markers.

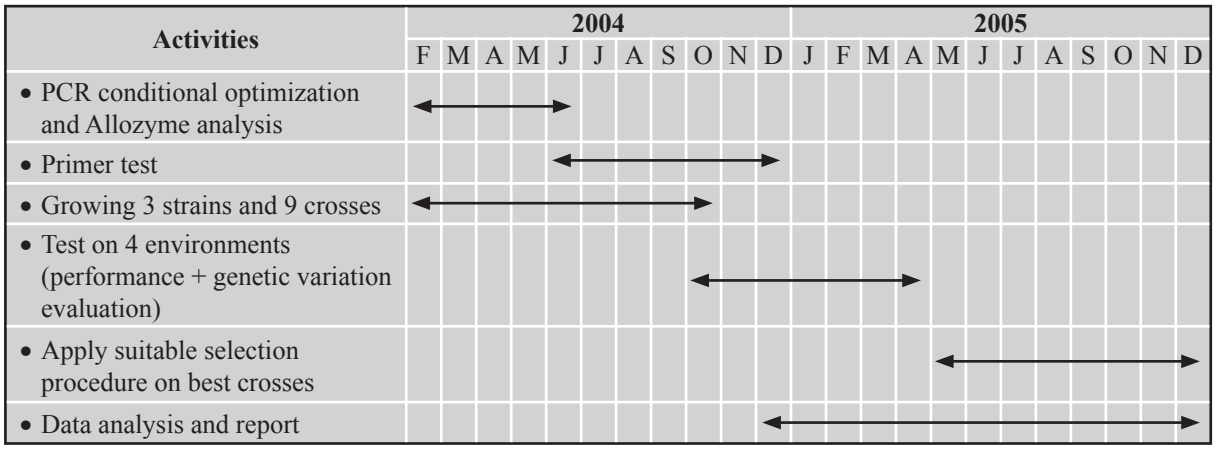
   3.4 In each environment, the best performance cross will be chosen for the selective breeding program (4 environments may not be the same cross)

3.4 The within-family selection procedure will be used to improve the economic traits of the chosen cross.

3.5 Performance and genetic variation will be evaluated in each selected generation (later on, microsatellite markers may be ready to be used additionally to allozymes).

3.6 After three (3) generations of selection, the selected lines will be evaluated under the farm conditions.

ACTIVITIES: Year 2004-2005



1. In June 2003, fifty pairs of *Macrobrachium rosenbergii* from each three stocks, namely NAGRI, Chantaburi and Petchaburi were collected to initiate the base population. Each stock has been spawned and reared separately. The offsprings of these three stocks have been reared in three 20 m^2 concrete ponds at the AAGRDI.
2. Growth performances of *Macrobrachium rosenbergii* from three stocks have been observed during August 2003 to January 2004.
3. Allozyme electrophoresis has been carried out to estimate the genetic variabilities (heterozygosity and number of alleles per locus) of the three stocks.
4. Reciprocal cross of three (3) stocks has been initiated in July 2004 to establish nine (9) cross -lines

Progress of Activities

1. The allozyme analysis on the three (3) stocks is shown in Table 1 below:

Table 1. *Observed heterozygosity and number of alleles per locus of Macrobrachium rosenbergii from three (3) stocks (NAGRI, Chantaburi and Petchaburi)*

Populations	Sample size (No. of allozyme-loci screened)	Observed heterozygosity (mean ± sd)	No. of Alleles (mean ± sd)
NAGRI	25	0.043±0.018	1.360±0.11
Chantaburi	25	0.023±0.014	1.200±0.10
Petchaburi	25	0.036±0.016	1.440±0.13

There was no difference in the genetic diversity among the three (3) stocks (p>0.05). In addition, the genetic variablities of *Macrobrachium rosenbergii* from the present study are similar to those from

the previous study of natural stocks by Sodsuk and Sodsuk (1998b). [No of alleles 1.30 (1.29-1.33), heterozygosity 0.032 (0.027-0.036)]

2. Growth comparison between the three (3) stocks illustrated a growth difference between NAGRI and the other stocks as shown in Table 2 below:

Table 2. *Mean length and weight of three stocks of Macrobrachium rosenbergii after six (6) months reared growout in concrete ponds*

Stocks	Sex	Sample	Length (mean ± sd, cm)	Weight (mean ± sd, g)
NAGRI	Male	100	13.751±0.704 [b]	31.047±6.173[b]
	Female	100	12.263±0.845 [b]	20.355±5.271[b]
		200	13.007±1.076[b]	25.701±7.842[b]
Chantaburi	Male	100	13.310±0.656[a]	29.407±12.538[a]
	Female	100	11.724±0.670[a]	17.319±6.238[a]
		200	12.517±1.034[a]	23.363±11.588[a]
Petchaburi	Male	100	13.405±0.882[a]	28.490±5.769[a]
	Female	100	11.520±1.027[a]	15.159±3.183[a]
		200	12.463±1.343[a]	21.824±8.139[a]

* The different letter illustrates statistical differences at p-value $p<0.01$

It is concluded that *Macrobrachium rosenbergii* from the NAGRI stock are larger by 4% in length and 9-15% in weight than those of the Chantaburi and Petchaburi stocks.

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Future Plan of Action

The participating countries will continue to conduct the various activities approved during the First Round Table Discussion as follows:

• *Indonesia*

- selection program to improve strain of GI Macro
- sex reversal studies
- genetic mapping

• *Thailand*

- studies on three cross lines, growth test in four environments
- growth improvement and test genetic variation
- appropriate selection procedure
- molecular activity (allozyme and DNA work)
- breeding program

• *Philippines*

- identification of stocks in collaboration with AQD and other institutions
- techno-demo with local farmers
- collection of samples (whole Philippines) and maintenance of specimens
- establishment of hatcheries (in 2005)
- characterization of stocks and species (allozyme molecular marker)

Recommendations

After series of deliberations and discussions, the participants in the Second Round Table discussion, adopted the following recommendations:

1. Use of the funds allocated for the collaborative research in 2004, shall be used for immediate activities from September 2004-March 2005. Specifically, the following activities will be conducted in the participating countries:
   1.1 Thailand: studies on allozyme and performance in genetic x environment testing
   1.2 Philippines: collection, identification and validation samples; preliminary performance evaluation of stocks
   1.3 Indonesia: multiple collection testing (3 locations) of GI Macro, identify genotype x environment interaction

2. Establish e-group among participating researchers with AQD as host, to be known as the **MBR** Discussion Group

3. Develop a logo for the Macrobrachium Group

4. Work on other activities as enumerated in future plan of action within budgetary limits

5. Prepare proposal to spin off the activity into an independent project with its own funding outside the IRAP budget. AQD should prepare the draft proposal based on responsibilities of participating countries, for comments by the countries. The deadline of the final proposal is October 2004.

6. Convene the Third Round Table Discussion on the Development of Genetically Improved Strain of Macrobrachium in Thailand in December 2005 or January 2006

DISCUSSION


Welcome Participants & Guests
SECOND ROUND TABLE DISCUSSION ON THE
ASEAN-SEAFDEC

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SEAFDEC conducts 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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- Promote and undertake aquaculture research that is relevant and appropriate for the region
- Develop human resources for the region
- Disseminate and exchange information on aquaculture

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