RECENT DEVELOPMENTS IN THE GENETIC IMPROVEMENT OF THE GIANT FRESHWATER PRAWN (*Macrobrachium* sp.)

AQUACULTURE DEPARTMENT
Southeast Asian Fisheries Development Center
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A Compendium of Results from the Collaborative Prawn Genetic Improvement and Seed Production Research Project under the Program on the Promotion of Sustainable Aquaculture in the ASEAN Region
(2002 - 2007)

M.R.R. Eguia and M.L.C. Aralar
Compilers

Government of Japan Trust Fund

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This publication contains results from the research project "Development of Genetically Improved Strain of *Macrobrachium*" covered by the Program on the Promotion of Sustainable Aquaculture in the ASEAN Region (previously known as Integrated Rural Aquaculture Program). It specifically includes activities from the inception of the project in 2002 up to 2005 under the Special Five-year Program on Sustainable Fisheries for Food Security in the ASEAN Region as well as research updates from 2006 to date. Project activities from 2006 to 2010 have been placed under the ASEAN-SEAFDEC Fisheries Consultative Group (FCG) collaborative mechanism with financial support from the Government of Japan Trust Fund. Dr. Koichi Okuzawa, AQD's Deputy Chief from 2005-2007, was project leader at the time the studies started until early 2007.

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For further information, please contact:

Dr. JOEBERT D. TOLEDO
Chief, SEAFDEC Aquaculture Department
Tigbauan, Iloilo 5021, Philippines
Email address: aqdchief@seafdec.org.ph

Dr. HIROSHI OGATA
Deputy Chief, SEAFDEC Aquaculture Department
Tigbauan, Iloilo 5021, Philippines
Email address: herogata@seafdec.org.ph

FOREWORD

The giant freshwater prawn, *Macrobrachium rosenbergii,* is an alternative commodity for freshwater aquaculture.

It is a high-value species and its culture could offer better profits. In the mid-80's, SEAFDEC Aquaculture Department (AQD) started research on *M. rosenbergii* but discontinued this when its freshwater aquaculture program was re-prioritized to focus on tilapia and milkfish. Now with renewed interest from SEAFDEC member-countries, AQD's Binangonan Freshwater Station revived its freshwater prawn research activities in late 2003, refining the breeding, larval rearing and culture technologies.

AQD's recent studies were part of the collaborative project on the Genetic Improvement of *Macrobrachium rosenbergii* which was implemented under the Aquaculture Component of the Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region from 2002 to 2005. This project was under the ASEAN-SEAFDEC Fisheries Consultative Group (FCG), and was conducted in Indonesia, the Philippines, and Thailand.

The project is now on its second phase (2006-2010) under the re-titled Promotion of Sustainable Aquaculture in the ASEAN Region program. The project's continuation and priorities were in response to recommendations made during the Regional Planning Meeting for the second phase of the Special Five-Year Program in February 2005. The overall aim is still to improve the genetic quality and seed production technology to produce quality seedstocks.

Previously, three roundtable discussions were held under the collaborative project. In the first discussion meeting which was held in Indonesia in 2003, formulation of the work plan was formulated and delineation of efforts was established. In 2004, the second meeting was held in the Philippines, where the progress of activities was assessed and problems encountered in the implementation of the project were identified. The third discussion was convened in Thailand in 2005 to identify constraints as well as to evaluate technologies being developed through the collaborative project.

This Compendium gathers the recent developments of the collaborative work on *M. rosenbergii.* It is organized by country since the status of research, level of technology and industry of *M. rosenbergii* differ in collaborating countries. Activities conducted by each country during the pre-project phase were included and the Compendium concludes with the results of studies.

We commend the team members and the compilers for their dedicated efforts to make freshwater prawn a profitable option for fish farmers. Their tireless promotion and development of sustainable aquaculture for food security in the ASEAN region is recognized.

JOEBERT D. TOLEDO, D. Agr.
Chief, SEAFDEC/AQD

CONTENTS

RECENT DEVELOPMENTS IN THE GENETIC IMPROVEMENT OF THE GIANT FRESHWATER PRAWN (*Macrobrachium* sp.)

Macrobrachium rosenbergii is a native species in most of the countries in Southeast Asia. Traditionally, juveniles have been reared in captivity in areas where they are caught. The early 1960's modem aquaculture of the giant freshwater prawn started with the development of its hatchery technology. At present, China is the largest producer of this species, albeit it is non native to China. In Southeast Asia, Thailand ranks as one of the world's top producer. Indonesia has also done considerable research and development efforts in promoting M *rosenbergii* for aquaculture. Production in these countries is backed by years of research which has translated to the rapid development in the culture of this species. In the Philippines, research on freshwater prawn culture started in the late 1970's but interest in this decapod crustacean was overtaken by the boom in *Penaeid* shrimps. The slump in the *Penaeid* industry coupled with the search for alternative species for freshwater culture resulted in the resurgence of interest in the giant freshwater prawn in the Philippines. The culture of the giant freshwater prawn has gained wide popularity as a high value alternative species for freshwater aquaculture.

Although the bulk of the funds for this collaborative project were provided for under the Integrated Rural Aquaculture Program (IRAP), the resources and manpower put in by the different institutions in each country involved in the project made the accomplishments of this project possible. This Compendium presents the reports of the various research institutions involved in the project. The report is organized by country and presents the status of the giant freshwater prawn industry as well as the activities conducted by each country during the pre-project phase and concludes with the results obtained from the different studies.

Project Background

The ASEAN-SEAFDEC Fisheries Consultative Group (FCG) approved the implementation by SEAFDEC Aquaculture Department of aquaculture projects which included the Integrated Regional Aquaculture Program (IRAP). The Aquaculture Component of this ASEAN-SEAFDEC Special Five-year Program on Sustainable Fisheries in the ASEAN Region, IRAP was implemented from 2003 to 2005. IRAP's two components were: (1) Aquaculture for Rural Development and (2) Supply of Good Quality Seeds. The ASEAN member countries identified priority commodities for both components of IRAP. Because of limited resources, prioritization of commodities based on commonality among ASEAN member countries was undertaken. Those countries with species of interest in common were grouped together. Under the component Supply of Good Quality Seeds, Thailand, Indonesia and the Philippines identified genetic improvement and seed production of *Macrobrachium rosenbergii* as their priority. Thus, the Collaborative Research on the Genetic Improvement and Seed Production of *Macrobrachium rosenbergii* was launched. This project aimed to:

- Lessen the period of research and hasten improvement and sustainable production of giant freshwater prawn in the ASEAN region
- To refine technology for consistent and improved production of quality giant freshwater prawn seeds for rural aquaculture in the ASEAN region in the shortest possible time
- To develop fast-growing and disease resistant strain of giant freshwater prawn for the region.

Since the status of research and the level of technology for *Macrobrachium rosenbergii* in the three countries differed, specific activities for each country differed. Indonesia focused on the genetic improvement of the species, particularly in further development of their GI Macro (Genetically Improved *Macrobrachium).* The participating institution for Indonesia was the Research Institute for Freshwater Aquaculture (RIFA) in Bogor, West Java. Thailand concentrated on selective breeding program for genetic improvement of giant freshwater prawn. Lead institution was the Aquatic Animal Genetics Research and Development Institute (AAGRDI) of the Department of Fisheries in Pathumthani. Since the Philippines is relatively new to *Macrobrachium rosenbergii* research (compared to Thailand and Indonesia), research efforts concentrated on genetic characterization, domestication, in addition to genetic improvement and culture of the species. Several agencies in the Philippines are involved: the Binangonan Freshwater Station of SEAFDEC/AQD in Rizal, the National Integrated Fisheries Technology Development Center

(NIFTDC) of the National Fisheries Research and Development Institute (NFRDI) in Pangasinan, the National Freshwater Fisheries Technology Center (NFFTC) of the Bureau of Fisheries and Aquatic Resources (BFAR) in Nueva Ecija and the Mindanao State University at Naawan (MSU-Naawan) in Misamis Oriental.

When the IRAP ended in 2005, the project continued in 2006 through funds provided for by the Government of Japan (GOJ), still through the SEAFDEC Aquaculture Department.

Country Report
INDONESIA

Giant Freshwater Prawn Culture in Indonesia[1]

Estu Nugroho and Lies Emmawati
[1]Research Institute for Freshwater Aquaculture
Laboratory of Molecular Biology
Bogor, West Java, Indonesia

Introduction

Indonesia is one of the countries in Asia with rich biodiversity, particularly in terms of the number of endemic freshwater aquatic organisms. Numerous indigenous freshwater fish species are found in Sumatera (30 spp.), Kalimantan (149 spp.), Java (12 spp.), and in Sulawesi (52 spp.) (Anonymous, 1994; Kottelat *et al.*,1993). These fauna are distributed in a total of 55 million hectares of freshwater resources consisting of lakes, dams, swamps, etc. The potential area for freshwater fish pond culture is estimated at 233,124 ha with a production of 334,085 mt/year (DGF, Indonesia. 2001), of which about 5,140 metric tons come from giant freshwater prawn culture.

Freshwater prawns are farmed in West Java, i.e., in Ciamis (Tambaksari, Pamarican and Kalipucang) and Tasikrnalaya. Government- and privately-owned commercial hatcheries are mostly found in Jogjakarta. In East Java, *Macrobrachium* culture is done in brackishwater ponds. Freshwater prawn culture has also spread to Bali Island, e.g., Gianyar, Klungkung, Buleleng and Tabanan.

Indonesia is recognized as the center of origin of freshwater prawns and to date, there are about 19 species found in its natural waters (Holthuis, 1980). Despite the advanced development of freshwater prawn culture and the availability of natural prawn populations in Indonesia, slow prawn growth rate, diseases and small edible portion remain unsolved. In recent years, the Indonesian Government through its fisheries research agency, has focused on improving the commercial production of the freshwater prawn through genetic means. Thus in 2001, a genetically improved stock of freshwater prawn has been developed and released for culture to local prawn farmers. This stock or strain has been referred to as the GI Macro or the Genetically Improved *Macrobrachium rosenbergii.*

Present Status of Freshwater Prawn Culture in Indonesia

The potential areas for giant freshwater prawn culture in Indonesia consist of paddy-ponds, freshwater- and brackishwater ponds. About 10,000 ha of the potential areas are found in Bali, 2,500 ha in West Java, 2,200 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 from the natural waters, specifically in some areas in West Java and Sumatera. This situation led to the promotion of freshwater prawn culture in Jogjakarta (Central Java) and Lamongan (East Java).

In Bali, freshwater prawn culture has been well developed since 1997 because of high market demand. Here, the estimated consumption of freshwater prawns is about 700 kg/day at US$ 4.00 to US$ 10.00/kg (before the Bali blast, 2002).

Freshwater prawns are farmed using traditional and semi-intensive systems in mono- or polyculture with common carp, tilapia, milkfish and *Puntius*. Small ponds (200 m^2) are used where postlarval fry (PL 25-40) are reared for two months. At two months, uniform-sized prawns are selected and then prawns are reared separately by size at a stocking density of 10 fry/m^2. 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

[1]*consolidated information based on presentation made during the 1st and 2nd Roundtable Discussion on the Development of Genitically Improved Strain of* Macrobrachium

demand for freshwater prawn fry, hatcheries have been developed in Jogjakarta, West Java and in Bali. The fry requirement of Gianyar-Bali farmers estimated at about 24 million/year, is partly supplied by hatcheries in Jogjakarta and East Java. Each PL 25-40 fry costs US$ 0.60-0.70. 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 has spawned interest especially among farmers with idle tiger shrimp ponds. While efforts to solve disease problems in tiger shrimp culture are being pursued, tiger shrimp production has declined, hence, freshwater prawn culture has become a viable alternative. For this purpose, a strain/stock of giant freshwater prawns with high salinity tolerance is being developed. Apart from the development of salt-tolerant prawns, efforts to formulate seed quality standards have been given due attention. Good quality prawn seedstock means fast growing, salt-tolerant postlarvae with relatively bigger edible portions.

The Freshwater Prawn Genetic Improvement Program

It is recognized that the quality of the country's freshwater prawn is genetically deteriorating. It has become difficult to produce export size (50g/pc) female prawns as the survival rate has become very low as well. To produce 50g average size male prawns, a batch's survival rate is only less than 40% in 9-11 months of culture. Since 1996, the Research Institute for Freshwater Aquaculture (RIFA formerly RIFF) implemented research 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

A selective breeding program to improve the quality of the farmed freshwater prawns by developing a synthetic prawn population using breeders from the natural populations in Tanjung Air (Bekasi), Kalipucang (Ciamis) and Musi (Palembang), has been implemented. The Tanjung Air stock (average individual body weight = 70g) was collected in February 1995. Individual selection was applied on this sub-population to improve the edible portion trait. The Kalipucang sub-population (ABW=72g) was collected in June 1996. Index selection was used in this population to improve growth rate and size of the edible portion. After a two- step selection, a synthetic population was constructed from these two sub-populations and subsequently used in crosses with the Musi sub-population (ABW=75g) collected in May 1997. Family selection (based on growth and increased edible portion) was applied to the synthetic population using 24 families. Results obtained from the fourth generation freshwater prawns are shown in Table 1.

Table 1. **Characteristics of the GI Macro after the fourth generation**

Number	Character	Value
1	Heritability of edible portion (h^2_{ep})	0.56 *(SE: 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)	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

Distribution of GI Macro Seed

GI Macro seedstock have been distributed to three hatcheries in Probolinggo, East Java; Samas, Jogjakarta; and Pamarican, West Java on 24th July 2001 (Nugroho *et al.*, 2005). The seedstock were grown to broodstock and once mature, were subsequently set for production of the next generation seedstock.

Generally, the GI Macro did well initially but problems were encountered after two years. The average body size of GI Macro varied in different locations, e.g. 130g average weight for males and 51g for females in Samas, 30g (males) and 25g (females) in Probolinggo, and 40g (males) and 34.5g (females) in Pamarican. There indications of environmental influence (e.g. culture management) on the growth rate. Apart from this, the size of the edible portion gradually declined. The farmers can visually distinguish the GI Macro from unselected stock based on the proportion of the body to the carapace. The GI Macro mixing with other stocks and mating among themselves resulted to reduced response to selective breeding.

This year, RIFA obtained the second generation of GI Macro that can tolerate salinities of up to 15 ppt but the field performance of this stock has yet to be evaluated. The GI Macro developed at RIFA are shown in Figures 1 and 2.

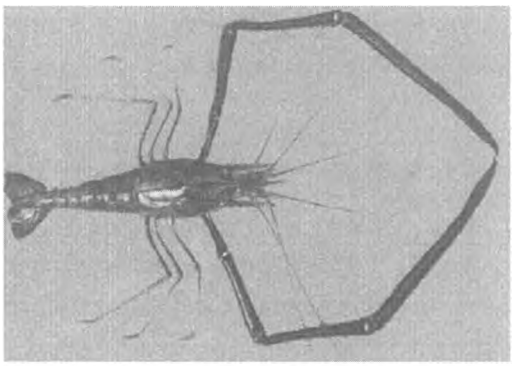

Figure 1. **Grand parent stock of fresh water prawn, GI Macro; total length: 38.0 cm and body weight: 480g**

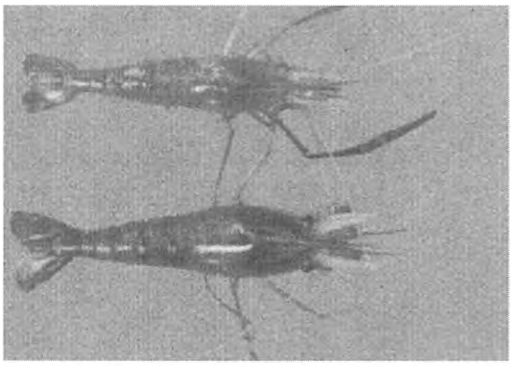

Figure 2. **Improve prawn (below) and control farmer strain (above) after five months rearing period**

Other Research Activities on the Freshwater Prawn

Application of molecular markers

DNA markers have been used to characterize a number of natural stocks of freshwater prawn collected since 2002. The genetic variability of freshwater prawns from Musi, Barito and GI Macro were examined using mitochondrial DNA cytochrome oxidase I restriction fragment length polymorphism (mtDNA CO-I RFLP) markers. Six composite haplotypes were detected following digestion of CO-I sequences with four endonucleases: Rsa I, Hae III, Mbo I and MspI. The average haplotype diversity was 0.603 (Table 2). Significant genetic differences were observed among the aforementioned freshwater prawn populations. The biggest proportion of the major composite haplotype was in the GI Macro, which came from Citatum and Citanduy. While Musi freshwater prawns contributed about 25% to the composite haplotype of GI Macro. The Barito stock is a potentially good genetic resource for future freshwater prawn breeding programs.

Application of hormone for sex reversal

Male freshwater prawns are bigger than their female counterparts. Large-scale production of all-male prawns can be done by obtaining female stocks that are can become genetically males or homogametic females. When homogametic females mate with normal males, the result is a 100% male phenotype. This research is still ongoing with initial results expected to come out before the end of 2003.

Table 2. **Frequency of composite haplotype mtDNA CO-I among freshwater prawn populations with four endonucleases, Rsa I, Hae III, Mbo I and Msp I**

Number	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	-
	Number of samples	16	14	17
	Number of haplotypes	5	5	2
	Haplotype diversity	0.766	0.573	0.471

Culture technology: closed recirculation 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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Project Proposal

Genetic Improvement of *Macrobrachium rosenbergii* in Indonesia

SOURCE OF FUNDING: Government of Japan, Government of Indonesia
DATE STARTED: January 2004

PROPONENTS {Name}:	**PARTICIPATION** {% time}
Dr. Estu Nugroho	75
Mr. Maskur	75
Mrs. Lies Emmawati	100
Mr. Agus Sasongko	100
Dr. Ketut Sugama	25

Objectives

To produce high quality seeds and develop genetically improved broodstock that can support the development of a sustainable freshwater prawn culture industry in Indonesia.

Brief Methodology

Broodstock collection

Giant freshwater prawns will be collected from South Kalimantan, South Sumatera, South Sulawesi and Bali. At least 100 pairs of prawns will be collected from each source. Microsatellite DNA and mitochondria DNA will be used to examine the freshwater prawn stocks collected from the various sites. Small plastic discs will be used as identification tags for the broodstock collected from each site.

Breeding system

Five breeding pairs from each collection site will be mated to produce full-sibs after which the broodstock will be reared in concrete tanks.

Larval rearing

Macrobrachium larvae (50-100 larvae/L) will be reared using the clear water system. Artificial feed and *Artemia* will be administered to the larvae for 35 days. Nursery rearing of the post larvae will be done in concrete tanks or earthen ponds and stocking density will be 25-50 individuals/L for 30 days of rearing. Feeding rate of about 10-15%/day will be followed at a feeding frequency of 3-4 times daily. Juveniles will be on grown in earthen ponds at a stocking density of 5-7 individuals/m^2 for five months. Feeding rate will be about 3-15%/day administered 3-4 times daily.

Selection

A combination of family and individual selection will be used to improve the growth rate and salinity tolerance of *Macrobrachium rosenbergii.* Offspring obtained from the selected and control lines will be tested in different locations in the country to determine the culture potential of the stocks.

Project Highlights

Growth of GI Macro II Strain in different locations[2]

Estu Nugroho, Iksan Khasani and Maskur
Directorate General of Aquaculture, Indonesia

Introduction

A selective breeding program was conducted to improve freshwater prawns with the use of a synthetic population formed from numerous breeders collected from Tanjung Air (Bekasi), Kalipucang (Ciamis) and Musi (Palembang). The stock from Tanjung Air was collected in February 1995 with an average body weight of 70 g/pc. Individual selection was applied to this stock to increase its edible portion. The stock from Kalipucang was collected in June 1996 with an average weight of 72g. Index selection was used in this stock to improve growth rate and edible portion. After two steps of selection, the synthetic population was constructed from these two stocks and incorporated to the stock from Musi (ABW=75g, collected in May 1997). Family selection was then applied to the synthetic population. Thus, in 2001, a new breed of freshwater prawn has been developed and released to the farmers. This strain was the GI Macro or the Genetically Improved *Macrobrachium rosenbergii* (Emmawati *et al.,* 2001). This study was conducted to evaluate the capability or performance of GI Macro in different locations/environments. Growth of the GI Macro was evaluated in three different locations : low (<10 m), moderate (150-250 m) and higher (>250 m) than the sea surface level.

Materials and Methods

Spawning activity

About 1,800 breeders (average length = 22 cm) were selected from 18 families of the GI Macro II stock. Twenty five percent of the best size (in terms of length) from each family were used as the selected line while the 25% average sized prawns were used as the control line (50 male and 50 female per family). Each family is distinguished by means of plastic tags. These tagged stocks contributed to the breeders chosen for mass spawning.

A total of 900 breeders from each line (selected and control line) with 1:1 male and female ratio were mass spawned separately in 200 m^2 concrete pond for one month. Seventy female breeders with mature eggs from the selected line and 60 female breeders with the same condition from the control line were collected for the spawning and hatching.

The spawners were treated by dipping them in 1.5 mg/l malachite green for 20 minutes before placing them into fiberglass hatching tanks. The water temperature was kept at 29-30°C using thermostat heaters. Squid was given as food at 5% of body weight and the spawners were fed thrice daily.

Larval Rearing

Larvae were collected daily using net tray and were kept in the rearing tank at 100-150 fry/liter. Larvae collected in 10 days were pooled into one batch. Clean water system with 10-12 ppt salinity was used in this process. The 1-7 day old larvae were fed *Artemia* nauplii followed by egg custard containing 55% protein and 8% fat, seven times daily. Water was changed every three days when larvae were 1-7 days old, every two days for 7-15 day old larvae and then daily until the post larval (PL) sta[illegible]

[2]*from the Report on the 2nd and 3rd Roundtable Discussion*

Post Larval Rearing

About 24,000 larvae of the selected and control line were collected and reared in different concrete tanks provided with bamboo shelters. PL 10 *M. rosenbergii* were reared until they reach 3-5 cm in size in a concrete tank in the hatchery (stocking density = 400 PL/m^2). The feed used was natural food (i.e. *Moina* and *Daphnia*), and pellets containing 38% protein at 20% of the prawns' body weight. Natural food was given once while pellets were administered four times daily.

Grow-out culture

M. rosenbergii juveniles (average size = 0.3g) were reared in *hapa* nets set in a pond at 85 juveniles/ m^2. The juveniles were cultured in three different locations: Sukamandi (low level, <10 m), Cibalagung (moderate level, 200-250 m) and Cijeruk (high level, >250 m). Pellets containing 30-33% protein were given to the prawns at 20% of the biomass, four times daily for 1-3 weeks. At 4-8 weeks, feeding rate was 15% of body weight given four times daily after which feeding rate was 10% of body weight given three times (07.00; 12.00 and 16.00). Four sampling was done twice in a month while cleaning of the *hapas* were conducted every week.

Results and Discussion

The total length, standard length, body weight and survival rates of the different *M. rosenbergii* batches are listed in Table 1.

Table 1. **Average total length (TL), standard length (SL), body weight (BW) and survival rate (SR) of *M. rosenbergii* batches**

Batch	Selected				Control			
	TL (mm)	SL (mm)	BW(g)	SR(%)	TL (mm)	SL (mm)	BW (g)	SR (%)
1	2.833	1.753	0.19	93.3	2.807	1.650	0.19	93.0
2	2.987	1.800	0.19	79.0	2.467	1.467	0.19	48.0

The results showed that after over a month of culture, the average total length and survival rate of the selected line was higher than the control line. The average total length and survival rate of GI Macro varied between groups 1 and 2. This phenomenon indicates that population of GI Macro has wide variance in terms of growth and survival. The average total length and weight of juveniles when reared for three months in *hapa* within an earthen pond is listed in Table 2.

Generally, the average total length and weight of the GI Macro selected line was better than the control line in the three different locations. The selected line of GI Macro in Sukamandi grew slower than the control line during the six-week culture period. In Cibalagung and Cijeruk, the selected line of GI Macro grew faster than the control line. The data showed that the best harvest weight is GI Macro reared in Cibalagung (7.982 + 5.991g), followed by Sukamandi (4.698 + 3.287g) and Cijeruk (4.692 + 2.011g).

Results therefore indicate that GI Macro is more suitably reared in low to moderate levels than in the high sea level area. Water temperature was 30-32°C, 28-30°C and 24-28°C in Sukamandi, Cibalagung and Cijeruk, respectively. The water sources of three different locations are: natural water source for Cijeruk, and channel water from paddy field for Cibalagung and Sukamandi. The water sources may have also influenced the growth of the GI Macro.

Table 2. **Average total length (cm) and weight (g) of GI Macro fry reared for 12 weeks in three different locations (standard deviation in brackets)**

Location	Collection	Initial	Week 2	Week 4	Week 6	Week 8	Week 12
Sukamandi	Selected	TL = 2.910 (0.527)	3.880 (0.695)	4.568 (1.416)	5.858 (1.416)	6.858 (1.459)	7.789 (1.775)
		W = 0.189 (0.089)	0.432 (0.259)	0.773 (0.569)	1.544 (1.365)	2.774 (1.889)	4.698 (3.287)
	Control	TL = 2.628 (0.742)	4.328 (0.701)	4.916 (1.142)	5.628 (1.285)	5.992 (1.299)	7.606 (1.768)
		W = 0.189 (0.089)	0.568 (0.267)	1.001 (0.864)	1.648 (1.287)	1.821 (1.272)	4.320 (3.927)
Cijeruk	Selected	TL = 2.910 (0.527)	3.976 (0.788)	5.194 (0.999)	5.944 (1.034)	6.760 (1.239)	7.990 (1.043)
		W = 0.189 (0.089)	0.514 (0.331)	1.166 (0.779)	1.782 (0.951)	2.846 (2.653)	4.692 (2.011)
	Control	TL= 2.628 (0.742)	3.724 (0.609)	4.718 (0.663)	5.162 (1.150)	5.944 (1.171)	7.224 (1.193)
		W = 0.189 (0.089)	0.394 (0.910)	0.776 (0.347)	1.134 (0.851)	1.641 (0.940)	3.579 (2.052)
Cibalagung	Selected	TL = 2.910 (0.527)	4.486 (0.870)	5.642 (0.838)	6.964 (0.961)	8.138 (1.245)	8.858 (1.859)
		W = 0.189 (0.089)	0.691 (0.357)	1.218 (0.499)	3.234 (1.333)	4.880 (2.268)	7.982 (5.891)
		TL = 2.628 (0.742)	4.196 (0.65)	5.820 (1.263)	6.600 (1.237)	7.506 (1.404)	8.556 (1.727)
		W = 0.189 (0.089)	0.545 (0.378)	1.528 (1.040)	2.384 (1.329)	3.784 (2.275)	6.648 (4.591)

Length and Weight Gain

Generally, the length gain of GI Macro reared in three different locations for three months was positive (Figure 1). However, GI Macro reared in Cijeruk only showed positive tendency during the research period, while the negative value of the length gain was observed in GI Macro reared in Sukamandi and Cibalagung. A similar result was also obtained in the weight gain of the GI Macro (Figure 2). The positive value was observed in GI Macro reared in Cijeruk, while negative value in weight gain was observed in Sukamandi and Cibalagung.

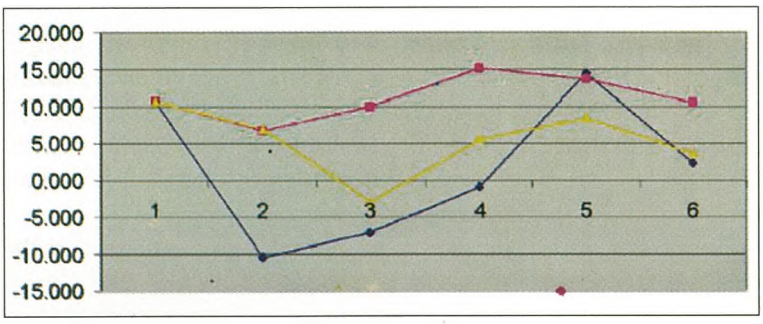

Figure 1. **Length gain of juvenile GI Macro reared in three different locations**
(Blue = Sukamandi, Pink = Cijeruk, Yellow = Cibalagung)

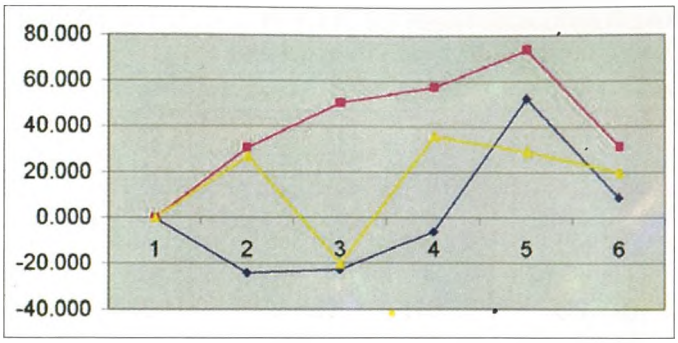

Figure 2. **Weight gain of juvenile GI Macro reared in three different locations**
(Blue = Sukamandi, Pink = Cijeruk, Yellow = Cibalagung)

This shows that the selection has a greater effect on weight gain than in length gain. Weight gain upon harvest of the GI Macro reared in Cijeruk, Cibalagung and Sukamandi were 31.09%, 20.06% and 8.750%, respectively, while the length gain at harvest of the GI Macro were 10.6%, 3.53% and 2.4% in Cijeruk, Cibalagung and Sukamandi, respectively. The highest weight and length increments of GI Macro in each location were 52% and 14% for the Sukamandi stock, 73% and 15% for Cijeruk, and 8% and 35% for Cibalagung. Nugroho *et al.* (2005) noted that a wide variation of the GI Macro was also observed genetically using DNA markers, suggesting that another selection activity should be conducted to improve their variability as a pure line.

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Collection and Evaluation of Wild and Farmed Stocks of Giant Freshwater Prawn in Indonesia[3]

Estu Nugroho[a], Sidi Asih[a], Anang Hari Kristanto[a], Sutrisno[a], Fauzan Ali[b], and Gunawan[b]

[a] Research Institute for Freshwater Aquaculture

[b] Indonesian Science Institute

Introduction

Giant freshwater prawn is an important commodity that has been cultured successfully in Indonesia. Freshwater prawn farming has been adopted in several areas of West Java, i.e. Ciamis (Tambaksari, Parigi, Rancah and Pasir Nagara) and Tasikmalaya. Some commercial hatcheries are found in Jogjakarta, a local government hatchery, and seven private hatcheries. In East Java, freshwater prawns are farmed in brackishwater ponds. Freshwater prawn culture has also spread to some areas in Bali, e.g. Gianyar, Klungkung, Buleleng and Tabanan, as well as in Riau, South Sulawesi and South Sumatera.

Inspite the development of freshwater prawn culture in Indonesia, some problems like slow growth rate, diseases and low carcass yield (small edible portion) remain unsolved. To address these problems, the Research Institute for Freshwater Aquaculture (formerly RIFF) started numerous genetic improvement programs since 1996 to improve growth rate and increase the animal's edible portion.

The genetically improved giant freshwater prawn stock named GI Macro (or Genetically Improved *Macrobrachium*), has been distributed to farmers particularly in Java. As reported in Nugroho and Emmawati (2004) and Nugroho *et al.* (2005), the performance of this stock in the different culture sites varied hence the development of a more suitable genetic base population with the use of other wild-sourced stocks in the continuous selection program was deemed necessary. An assessment of the genetic background of the wild stocks showed genetic divergence between giant freshwater prawns from western and eastern part of Indonesia (Nugroho *et al.*, 2007). A performance evaluation of giant fresh water prawn stocks will be conducted to complement the above results in order to produce high quality giant freshwater prawn seedstock.

Materials and Methods

Sample Collection

Broodstock of giant freshwater prawn were collected from Sulawesi (Makasar), Kalimantan (Pangkalanbun), Sumatera (Jambi) and Java (GI Macro-Sukabumi). Twenty eight prawns (12 males and 20 females) from Sulawesi, 45 prawns from Kalimantan (20 males and 25 females), 59 prawns from Sumatera (18 males and 41 females) and 22 prawns from Java (GI Macro, 12 males and 20 females) were brought to the Research Institute for Freshwater Aquaculture in Bogor.

Broodstock Evaluation

Broodstock of giant freshwater prawns were reared in 12 x 4 m concrete ponds and supported by paddle wheels made from fiberglass (Figure 1).The pond bottom has a substrate of gravel sand (10 cm) which serves as a medium for culturing bacteria or decomposer organisms. Water current in the pond is constant at 9-12 liter/sec.

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[3] *final report submitted March 2007*

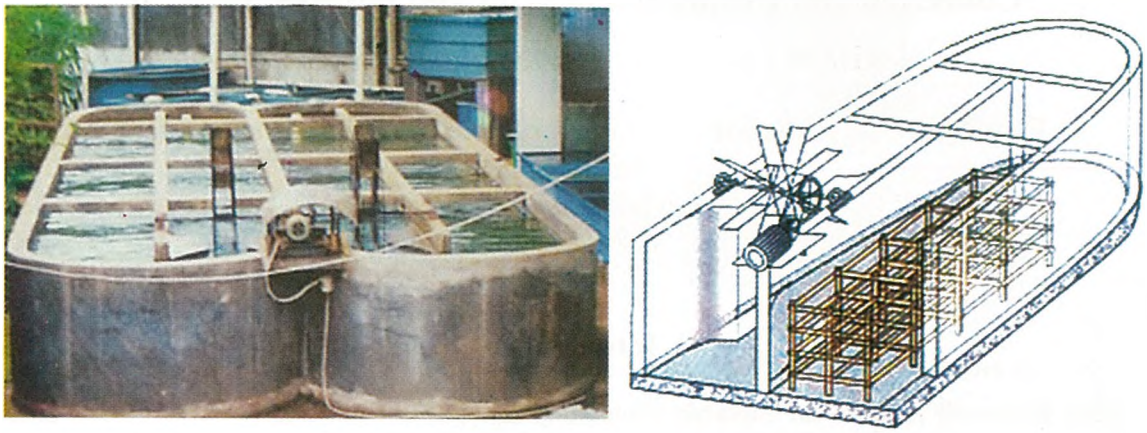

Figure 1. **A broodstock pond**

Commercial shrimp feed pellets were administered to the broodstock for two months. Breeders with fertilized eggs were taken and kept in 60 x 40 x 20 cm aquaria for hatching. The parameters recorded during this experimental period are growth rate, survival rate, fecundity and number of post larvae obtained.

The post larvae were reared in concrete ponds following standard operational procedures (SOP) for giant freshwater prawn culture at RIFA. Growth and survival rate were noted as the post larvae grew to marketable size.

Heterosis

Evaluation of heterosis was done by setting up individual mating pairs (1 female and 1 male) among giant freshwater prawn stocks tested. Breeders were taken and kept in concrete tanks for spawning. Larvae and post larvae were reared in aquaria (Figure 2), while juveniles were on-grown in concrete or earthen ponds. The rearing protocols are according to the RIFA's SOP. The parameters monitored are time of metamorphosis, survival and growth rate of larvae, post larvae and juveniles. The testing scheme to determine heterosis in intraspecific crosses is listed in Table 1.

Table 1. **Scheme for testing heterosis**

Male / Female	Sulawesi	Kalimantan	Sumatera	Java
Sulawesi	3 replicates	3 replicates	3 replicates	3 replicates
Kalimantan	3 replicates	3 replicates	3 replicates	3 replicates
Sumatera	3 replicates	3 replicates	3 replicates	3 replicates
Java	3 replicates	3 replicates	3 replicates	3 replicates

Figure 2. **A set aquarium for individual pair mating**

Selection

A base population was formed based on the result of heterosis-testing. Breeders that have good performance traits (fast growth and high survival) will be used to produce postlarvae for the selection activity. Selective breeding based on between and within family selection (selecting the upper 25% of the normal growth curve distribution) shall be undertaken.

Results and Discussion

Broodstock performance

Growth rate variation has been observed among the giant freshwater prawn stocks examined. The Kalimantan stock has the highest specific growth rate at 3.24%, followed by GI Macro (1.91%), Sulawesi (0.68%), and Sumatera (0.43%). The highest average weight was noted in the Sumatera stock at 83.6g, and followed by Sulawesi, Kalimantan and Java, at 80.2g, 48.6g and 40.2g respectively. In terms of reproductive traits, the giant freshwater prawn from Java (GI Macro) produced the highest number of eggs, i.e. 1,263 eggs/g body weight, followed by Sumatera (1258 eggs/g), Kalimantan (1,100 eggs/g) and Sulawesi (743 eggs/g). These results show that wild freshwater prawns are well-adapted and can be domesticated in hatcheries especially using modified concrete ponds.

The number of larvae produced varied from 48,000 to 55,000, while postlarval production ranged from 7,000-8,000 with a survival rate of 13.8%-16.9%. Giant freshwater prawn from Java produced the highest number of postlarvae, followed by Sulawesi, Sumatera and Kalimantan. The number of PL from each population is listed in table 2.

Table 2. **Number of larvae, postlarvae (PL), and survival rate of PL in the different populations**

Population	Number of Larvae	Number of PL	Survival Rate (%)
Sumatera	48,745 ± 11,950	7,160 ± 999	14.9 ± 0,02
Java/GI Macro	51,909 ± 6,285	8,667 ± 764	16.9 ± 0,02
Kalimantan	48,352 ± 2,590	6,591 ± 1,723	13.8 ± 0,04
Sulawesi	55,319 ± 2,311	8,179 ± 2,896	14.9 ± 0,06

D'Abramo and Brunson (1996) showed that the required time for development of larvae is about 15-40 days depending on the quality and quantity of feed, water quality and size of breeder. Giant freshwater prawn from Kalimantan and Java needed 27-28 days, while those from Sumatera and Sulawesi required 34-35 days for larval development. Furthermore, the survival rate of juveniles reared for one month was noted at 61.34% (Sumatera) to 89.30% (Java) (see Figure 3).

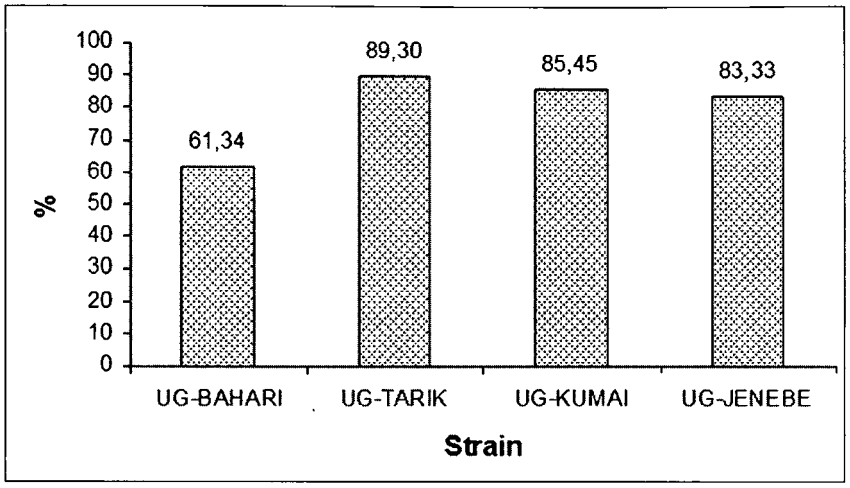

Figure 3. **Survival rate of the different freshwater prawn stocks**

Size variation is a problem in prawn culture. This variation influences the total harvest. Therefore the farmer cannot conduct total harvest, and can only sell from partial harvests because of the constraints posed by size variation. Relative size variation was also monitored. Offsprings of giant freshwater prawn from Kalimantan and Java have more homogenous sizes compared to those from Sulawesi and Sumatera. This indicates that a selection program will be more useful when breeders from Sulawesi and Sumatera stocks will be used. A comparison on the growth of juveniles reared for one month showed that the giant freshwater prawns from Kalimantan grew faster than other stocks (Figure 4).

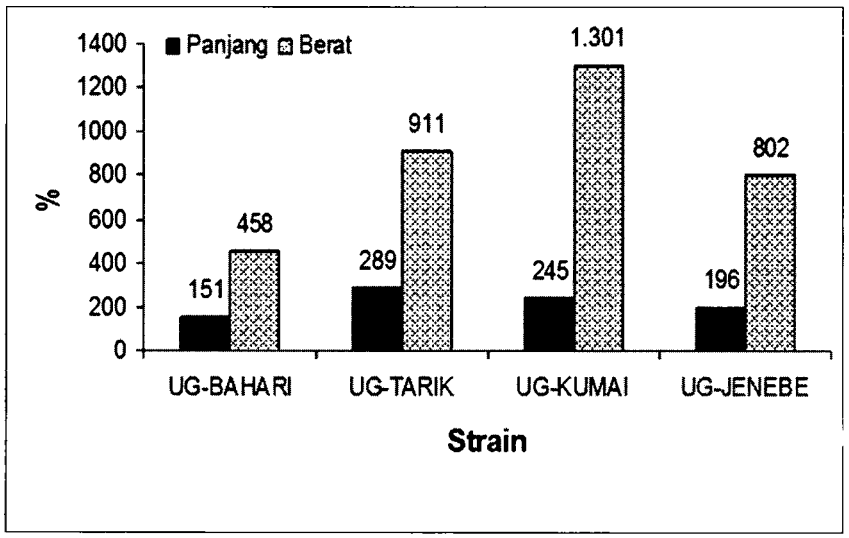

Figure 4. **Body weight (left) and body length (right) of giant freshwater stocks**

Heterosis

The effect of heterosis among giant freshwater prawn populations was measured using individual mating pairs between populations. Several pairs were mated and the larvae reared in aquaria. The number of larvae produced is listed in Table 3.

In general, the capability of male breeders to fertilize eggs from one female varies according to the source of the stock. Male freshwater prawn breeders from Sulawesi can fertilize eggs of females from all of the other test stocks, this is followed by male broodstock from Kalimantan and Sumatera which are able to fertilize eggs of females from 1-2 other populations. Meanwhile, male breeders from Java can successfully fertilize eggs of females from the Java stock only. On the other hand, the capability of females to spawn eggs

is not influenced by the source of the stock. Female broodstock from Kalimantan and Java can be mated to male broodstock from the two other populations, then followed by female breeders from Sulawesi while Sumatera female broodstock can be mated to one population. This capability to fertilize eggs depends on the size of the breeders used. Generally, male Sulawesi breeders are big therefore these have no problem when mated to female breeders from any population. Meanwhile male breeders from the Java stocks are relatively smaller than their female counterparts from the other populations therefore problems in individual pair mating occurs.

Table 3. **Body weight, body length and number of larvae produced from the intraspecific crosses**

Male / Female	Sulawesi	Kalimantan	Sumatera	Java/GI Macro
Sulawesi	WF= 50.9g LF= 17.3cm WM= LM = PL 18= 51,467 pcs PL 27= *	WF= 32.64g LF= 13.8 cm WM= LM = PL 18= 30,103 pcs PL 27= 4,170 pcs	WF= 67.24g LF= 16.0 cm WM= 193.38g LM = 22.2cm PL 18= 25,721 pcs PL 27= 7,674 pcs	WF= 30.16g LF= 13.9 cm WM= LM = PL 18= 25,721 pcs PL 27= 7,674 pcs
Kalimantan	WF= 45.33g LF= 14.2 cm WM= 34.0g LM = 16.4 cm PL 18= 20,500 pcs PL 27= 3,500 pcs	WF= 28.2g LF=14.5 cm WM= LM = PL 18= 23,726 pcs PL 27= *	NH	WF= 36.78g LF= 15.5 cm WM= 146.33g LM = 22.7 cm PL 18= 29.136 pcs PL 27=*
Sumatera	NH	WF= 33.37g LF= 14.2 cm WM= LM = PL 18= 23,421 pcs PL 27= 3,500 pcs	WF= 88.5g LF= 21.4 cm WM= LM = PL 18= 31,687 pcs PL 27= *	NH
Java/ GI Macro	NH	NH	NH	WF= 19.7g LF= 12.8 cm WM= LM = PL 18= 24,887 pcs PL 27= *

* Data unavailable to date

NH - no hatching, WF-weight of female, LF- length of female, WM - weight of male, LM - length of male

When a female breeder is bigger than the male, oftentimes mating does not ensue. This has become a limitation in freshwater prawn pair-mating therefore another option such as mating one male to 2-4 females will be tested in the next research.

The number of larvae produced varied from 20,500 pcs (♀ Sulawesi x ♂ Kalimantan cross) to 51,467 pcs (♀ Sulawesi x ♂ Sulawesi cross). Based on the preliminary data, the heterosis value based on larvae production is about -7.170. This shows that the productivity of the pure breed is better than the hybrid progenies. The pure breed Sulawesi stock gave the highest value. If data on the larval production from the pure Sulawesi was not included in analysis, then the value of heterosis will change to -2.404 or about 66% of the total heterosis value. Heterosis value of postlarval production and growth has yet to be estimated. It will require four months for the postlarvae to reach marketable size. Selection will be continued using a base population from the best performing pair from among the stocks.

Acknowledgements

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Nugroho E, AH Mulyasari, Kristanto, F Ali, Gunawan. 2007. Evaluation of Genetic Variability of Freshwater Prawn Collected from Makassar-Sulawesi, Pangkalanbun-Kalimantan, Jambi-Sumatera, Sukabumi-Java and GI Macro using mtDNA CO-I markers. Submitted to Jurnal Penelitian Akuakultur Indonesia

Genetic Characterization of GI Macro and Freshwater Prawns from Makassar-Sulawesi, Pangkalanbun-Kalimantan, Jambi-Sumatera, Sukabumi-Java using mtDNA CO-I Markers[4]

Estu Nugroho[a], Mulyasari[a], Anang Hari Kristanto[a], Fauzan Ali[b] and Gunawan[b]
[a] Research Institute for Aquaculture
[b] Indonesian Science Institute

Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a widely distributed indigeneous species in Indonesia. The giant freshwater prawn from Pangkalanbun-Kalimantan has a big head, long claws and is yellow-green in color. On the other hand, those from Kuala Tungkal-Jambi has a small head, short claws and is gold in color (Sabar and Ali, 2001).

The giant freshwater prawn is an important commodity that has been successfully farmed in Indonesia. It has been produced in several areas in West Java, i.e. Ciamis (Tambaksari, Parigi, Rancah and Pasir Nagara) and Tasikmalaya. Some commercial hatcheries (a local government hatchery, and seven private hatcheries) are found in Jogjakarta in East Java, freshwater prawns are cultured in brackishwater ponds. Freshwater prawn culture has also spread to some areas in the island of Bali, e.g. Gianyar, Klungkung, Buleleng and Tabanan, and in Riau, South Sulawesi and South Sumatera.

Several natural populations of freshwater prawns are unique to Indonesia. To date, about 19 species are found in almost all of the islands in the archipelago (Holthuis, 1980). However, this potential genetic resource has not yet been fully used in freshwater aquaculture. Moreover, inspite the fact that the freshwater prawn culture technology has been developed and adopted in Indonesia, some problems are still plaguing the industry. To solve the problems of poor growth rate, disease susceptibility and small meat yield or edible portion, the Research Institute for Freshwater Aquaculture (formerly RIFF) started numerous research programs since 1996 to improve growth rate and the size of the edible portion of cultured prawns.

The GI Macro, a selected strain of the giant freshwater prawn was developed by RIFA and has been distributed to farmers in Java. Varying results were obtained from growth trials conducted in different locations, hence another phase of selection is necessary to construct a wider and more improved base population using additional wild stocks. An assessment of the genetic background of the wild stocks is an important pre-requisite before the new selection program is initiated. Genetic variation is an important parameter to consider to enable the evaluation of individual fitness of the stock in the short term and their survival in the long term (Ferguson *et al.*, 1995). In this study, genetic variation of giant freshwater prawns collected from Sulawesi, Kalimantan, Sumatera and Java were analyzed using molecular markers.

Materials and Methods

Samples of M. rosenbergii

Twenty-seven samples of giant freshwater prawns were collected from three wild stocks (Makassar-Sulawesi, Pangkalanbun-Kalimantan, Sukabumi-Jawa), an unselected hatchery stock (Jambi-Sumatera) and the genetically improved GI Macro.

Whole DNA extraction and amplification of mtDNA CO-I region

Whole DNA extracts were obtained from pleopods of freshwater prawns using the standard phenol-chloroform procedure as follows: 5-10 mg of pleopod was placed into a 1.5 ml vial containing 500 µl DNA

lysis solution and 120 µl 0.5M EDTA (pH 8.0). Proteinase K (10µg/ml) was added onto the vial and then incubated at 37°C for 12 hours. At room temperature, chloroform (500µl) was added onto the mixture, after which the solution was kept on ice for five minutes. The solution was then centrifuged at 10,000 rpm for 10 minutes, after which the supernatant is removed and placed into a new 1.5 ml vial. Ammonium acetate (10µl) and ethanol (500µl) are then added onto the supernatant and the mixture gently vortexed. The DNA pellet was suspended by centrifugation (10 min at 10,000 rpm) and air-dried. Once dry, the pellet is re-suspended with 50-100 µl Tris-EDTA (TE) buffer and kept at 4 °C before use.

The primers used to amplify the mitochondrial DNA cytochrome oxidase I segment were LCO-1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO-2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA). This CO-I region was PCR (Polymerase Chain Reaction)- amplified using the following reaction mixture: 10 µg DNA template, 10 pmol of each primer and "pure Taq DNA ready to go" (Promega) for a total reaction volume of 25 µl. PCR cycles consist of a denaturation cycle at 95 °C for 2 min, 35 cycles for annealing (95 °C for 1 min, 45 °C for 1 min and 72 °C for 2.5 min), followed by an extension cycle at 72 °C for 10 min. The amplified mtDNA CO-I regions were restricted by seven endonucleases (Hae III, Rsa I, Mbo I, Taq I, Alu I, Sac II and Hin6 I), and separated via electrophoresis on 2-3% agarose gel in Tris-Boric-EDTA (TBE) buffer. The resulting bands were subsequently observed under a UV illuminator.

Data Analysis

mtDNA variation in the five giant freshwater prawn stocks was evaluated by examining the observed haplotypes for each type of restriction endonuclease. These endonuclease-specific haplotypes were further used in scoring the composite haplotypes which comprised the data for statistical analysis. AMOVA (Analysis of Molecular Variance) and Fst value estimations were obtained using the TFPGA (Tools for Population Genetic Analysis) program. Haplotype and genetic diversities were calculated based on the method developed by Nei and Tajima (1981) in estimating levels of genetic variation.

Result and Discussion

The mtDNA CO-I region (700-1500 bp) of giant freshwater prawns can be PCR-amplified. Four restriction endonucleases (Hae III, Rsa I, Mbo I, and Taq I) successfully cleaved the amplified DNA products. Polymorphic patterns were detected using Hae III and Rsa I, while Mbo I and Taq I gave monomorphic results (Table 1). Six and three restriction morphs were obtained using Hae III and Rsa I, respectively. An example of a restriction pattern is shown in Figure 1. The length of the mtDNA CO-I sequence of giant freshwater prawn is similar to the D-loop sequence observed in fish species such as tilapia, kingfish, yellow tail and red sea bream (Nugroho, 2001).

Table 1. **Type of enzyme and restriction site of mtDNA CO-I of giant freshwater prawn**

Number	Enzyme Type	Restriction Site	Restriction Type
1	Hae III	+	Polymorphic
2	Rsa I	+	Polymorphic
3	Mbo I	+	Monomorphic
4	Taq I	+	Monomorphic
5	Alu I	-	-
6	Sac II	-	-
7	Hin6 I	-	-

Figure 1. **Restriction pattern of mtDNA CO-I region using Hae III**

Haplotype variation levels differed according to the source of the giant freshwater prawn stocks. In general, twelve composite haplotypes can be identified based on four endonucleases that cleaved the mtDNA CO-I region. Two to five composite haplotypes were observed in most of the stocks. Giant freshwater prawns collected from Makassar-Sulawesi showed two composite haplotypes only, while those from Sukabumi-Java and GI Macro had five composite haplotypes. Haplotype diversity ranged from 0.111 (Makassar-Sulawesi) to 0.280 (Sukabumi-Java, Table 2).

Genetic variability levels (based on the number of haplotypes and haplotype diversity) in the giant freshwater prawn, differ from marine fishes which generally have haplotype numbers and diversity levels ranging from 6-17 and 0.6-0.9, respectively (Nugroho, 2001). However the variability levels are comparable to those of freshwater fishes like tilapia (Nugroho *et al.*, 2002). Relatively low genetic variation in giant freshwater prawn stocks could be due to their relatively limited capability to migrate compared to marine fishes. Low genetic variability of giant freshwater prawns indicate mainly natural selection or genetic drift in these populations.

Some stocks share similar major haplotypes. Sukabumi-Java and GI Macro possess haplotype #1; Sukabumi-Jawa, Pangkalanbun-Kalimantan, and Jambi-Sumatera, haplotype #3; GI Macro, Sukabumi-Jawa, Jambi-Sumatera and Pangkalanbun-Kalimantan, haplotype #5; while giant freshwater prawns from Makassar-Sulawesi have haplotypes #11 and #12 unique to them. Major haplotypes found common in Sukabumi-Java and GI Macro as well as Jambi-Sumatera and GI Macro can be attributed to the fact that they share common ancestries. The Sukabumi-Java stock is comprised of the first GI Macro stock that was developed from three giant freshwater prawn stocks namely, Citanduy, Citarum (West Java), and Musi (Sumatera). There is a high probability that this aforementioned GI Macro stock has interbred with the existing local population in Sukabumi-Java. Giant freshwater prawns from Pangkalanbun-Kalimantan share similar composite haplotypes with Sukabumi-Java, Jambi-Sumatera and GI Macro, however, the Kalimantan stock still possess haplotypes (#8 and #9) unique only to them. This indicates that there are ecological barriers in the migration of this stock.

Table 2. **Composite haplotype frequencies of the mtDNA CO-I region in Indonesian stocks of the giant freshwater prawn. The haplotypes resulted from the use of endonucleases Hae III, Rsa I, Mbo I, and Taq I**

No	Type of Composite Haplotype	Stock				
		Sukabumi-Java N=5	GI Macro N=5	Pangkalanbun-Kalimantan N=6	Jambi-Sumatera N=5	Makassar-Sulawesi N=6
1	BAAA	0.200	0.400	-	-	-
2	CAAA	0.200	-	-	-	-
3	ABAA	0.200	-	0.333	0.400	-
4	BBAA	0.200	-	-	-	-
5	AAAA	0.200	0.200	0.167	0.400	-
6	BCAA	-	0.200	-	-	-
7	CCAA	-	0.200	-	-	-
8	ACAA	-		0.167	-	-
9	DBAA	-	-	0.333	-	-
10	EBAA	-	-	-	0.200	-
11	FBAA	-	-	-	-	0.667
12	FAAA	-	-	-	-	0.333
	No. of Haplotypes	5	4	4	3	2
	Haplotype Diversity (h)	0.280	0.260	0.236	0.200	0.111

Statistically significant genetic differences were noted among the five giant freshwater stocks ($P< 0.05$) based on an AMOVA (Analysis of Molecular Variance). Fst pairwise comparison tests showed that the difference was observed between the giant freshwater prawns from Makassar-Sulawesi and the other four stocks. A significant difference was also detected between giant freshwater prawns from Pangkalanbun-Kalimantan and the GI Macro (Table 3).

Table 3. **Pairwise comparison of Fst**

Population	Sukabumi-Jawa	GI Macro	Banjarmasin-Kalimantan	Jambi - Sumatera	Makassar-Sulawesi
Sukabumi-Jawa	-				
GI Macro	0.528	-			
Pangkalanbun-Kalimantan	0.2376	0.0272*	-		
Jambi-Sumatera	0.6480	0.0787	0.8161	-	
Makassar-Sulawesi	0.0112*	0.0013**	0.0093**	0.0214*	-

**- significant at level, $P< 0.0$

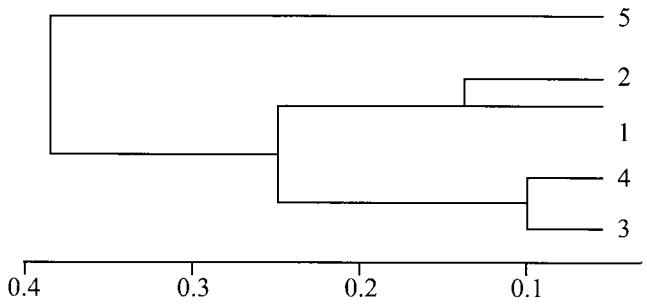
The observed differences are due to the fact that the giant freshwater prawns from Makassar-Sulawesi have different major composite haplotypes compared to the other populations. This could indicate the possibility that the stock from Makassar-Sulawesi is composed of a different freshwater prawn subspecies due to ecological barriers. De Bruyn *et al.* (2004), confirmed a biographical barrier in giant freshwater prawn populations from Tanah Genting Kra. Differences in the flora and fauna found in this area are explained in terms of the presence of the biogeographic barrier known as the Wallace line. The Wallace line theory may explain why there are differences in the stock structure of the Makassar-Sulawesi prawns (Eastern side of the Wallace line) as compared to the others (Western side).

Genetic distance (Nei and Tajima, 1981) based on restriction sites from four endonucleases is listed in Table 4. The average genetic distance among the stocks is estimated at 0.1690. The longest genetic distance was observed between Makassar-Sulawesi and GI Macro stocks, while the shortest distance is between Sukabumi-Java and GI Macro stocks.

Table 4. **Genetic distance among giant freshwater prawn stocks collected in Indonesia**

Population	Sukabumi-Jawa	GI Macro	Banjarmasin-Kalimantan	Jambi - Sumatera	Makassar-Sulawesi
Sukabumi-Java	-				
GI Macro	0.0289	-			
Pangkalanbun-Kalimantan	0.0828	0.2340	-		
Jambi-Sumatera	0.0447	0.2147	0.0131	-	
Makassar-Sulawesi	0.2325	0.3628	0.2565	0.2655	-

A dendrogram based on the genetic distance data showed three clusters founding the giant freshwater prawn stocks examined. The first group is composed of giant freshwater prawns from Sukabumi-Java and the GI Macro. Pangkalanbun-Kalimantan and Jambi-Sumatera stocks form the second cluster while the giant freshwater prawns from Makassar-Sulawesi are uniquely separated from the other groups (Figure 2). The close relationship between GI Macro and Sukabumi-Java may indicate the possibility of the occurrence of gene flow between the two stocks as the GI Macro was disseminated to the farmers in Sukabumi-Java in 2001. On the other hand, the Jambi-Sumatera and Pangkalanbun-Kalimantan stocks lumping in one cluster may be due to a correlation between biological variability and earth history (de Bruyn *et al.*, 2004).



Note: 1 = Sukabumi-Jawa
2 = GI Macro
3 = Pangkalanbun-Kalimantan
4 = Jambi-Sumatera
5 = Makassar-Sulawesi

Figure 2. **UPGMA dendrogram showing the relationship between the five giant freshwater prawn stocks from Indonesia**

The Makassar-Sulawesi giant freshwater prawns are phenotypically better than the other stocks as they are relatively bigger than the prawns from the other stocks. It has been noted that the average body weight of Makassar-Sulawesi prawns is about 83g (Ali *et al.*, unpublished). Taking into consideration the level of genetic variability of the Makassar-Sulawesi stock based on the composite haplotype, this stock is an inbred line. Inspite the fact that the Makassar-Sulawesi is inbred, considering that the stock is phenotypically larger than the others, it can be used for outcrossing. Hybridization of the Makassar-Sulawesi with the other stocks may be an effective alternative breeding program in improving the quality of giant freshwater prawn seedstocks in Indonesia.

Conclusion

Significant genetic differences among giant freshwater prawn from Makasar-Sulawesi and the other stocks were observed. The average genetic variability based on composite haplotypes is 0.217, with an average genetic distance of 0.169. Giant freshwater prawns from Makassar-Sulawesi can be used for outcrossing during the next phase of the breeding program for giant freshwater prawns in Indonesia.

Acknowledgements

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Country Report
THAILAND

Industry Status

Macrobrachium Culture Industry in Thailand[5]

Supattra Uraiwan and Panom Sodsuk
Aquatic Animal Genetics Research and Development Institute
Department of Fisheries, Thailand

Macrobrachium species in Thailand

There are numerous freshwater prawns classified under the genus *Macrobrachium* which is generally categorized under the Family *Palaemonidae* Rafinesque, 1815, sub-family *Palaemoninae* Rafinesque, 1815. In Thailand, 19 *Macrobrachium* species that thrive in either freshwater or brackishwater, have been found and identified as follows:

Species	Freshwater	Brackishwater
Macrobrachium amplimanus (Cai and Dang, 1999)	Yes	No
Macrobrachium dienbienphuense (Dang and Nguyen, 1972)	Yes	No
Macrobrachium equidens (Dana, 1825)	No	Yes
Macrobrachium eriocheirum (Dai, 1984)	Yes	No
Macrobrachium esculentum (Thallwitz, 1891)	Yes	No
Macrobrachium hirsutimanus (Tiwari, 1952)	Yes	No
Macrobrachium idae (Heller, 1862)	No	Yes
Macrobrachium lanchesteri (de Man, 1911)	Yes	No
Macrobrachium lar (Fabricius, 1793)	No	Yes
Macrobrachium latidactylus (Thallwitz, 1891)	No	Yes
Macrobrachium mirabile (Kemp, 1917)	No	Yes
Macrobrachium mieni (Dang, 19750)	Yes	No
Macrobrachium neglectus (de Man, 1905)	No	Yes
Macrobrachium niphanae (Shokita and Takeda, 1989)	Yes	No
Macrobrachium rosenbergii dacqueti (Sunier, 1925)	Yes	Yes
Macrobrachium sintangense (de Man, 1898)	Yes	Yes
Macrobrachium sirindhorn (Naiyanetr, 2001)	Yes	No
Macrobrachium yui (Holthuis, 1950)	Yes	No
Macrobrachium sp. (Cai, Naiyanetr and Ng, in press)	Yes	No

Of the nineteen species, below are the five species identified as the most economically important species for Thailand:

Macrobrachium dienbienphuense

Found in northeast of Thailand, *M. dienbienphuense* is distributed in the main rivers of Maekhong, Chi, Moon and their branches. The species has a 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.

Macrobrachium niphanae

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.

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[5]*paper presented during the 1st Roundtable Discussion on the Development of Genitically Improved Strain of* Macrobrachium, *November 2003, Indonesia*

Macrobrachium lanchesteri

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 this prawn is usually done with fishes together in one pond for additional income.

Macrobrachium sintangense

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. This prawn is also popular in the northeastern Thailand mainly because of its moderately large size which is just slightly smaller than *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, studies on its biology and life cycle should be conducted, so that appropriate aquaculture systems could be developed and the species could be introduced to the local fishfarmers.

Macrobrachium rosenbergii dacqueti

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

In the past, the distribution of *M. rosenbergii* in Thailand was reported only in the brackishwater area connected to the sea. But now it has been found in many freshwater bodies of Thailand, especially in places where large numbers of the prawn have been released by the DOF (Department of Fisheries, 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 its quality to include seeds, broodstock and products to successfully come up with the best prawn ever produced in the world.

Giant Freshwater Prawn Production, Value and Area

It is a fact that the giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important economic species in Thailand and in many Southeast Asian countries. An increasing local market demand has led to the overfishing of wild prawn stocks from natural waters every year. Hence, domestication experiments on the *Macrobrachium* have been conducted by the Department of Fisheries since 1956 to increase prawn production (Sidthimunka and Bhukaswan, 1982). This has prompted nationwide extension activities to disseminate the prawn culture technology to a number of commercial giant freshwater prawn farms. This species has now become one of the major economically important commodities in the Thai aquaculture industry.

The total prawn production in 2002 increased by 38% from 1996, with a corresponding Thai baht value increase of 89%. In terms of actual figures in 1996 and 2002, the total production were 7,200 and 10,000 metric tons, respectively, valued at 596.3 and 1,117.6 million Thai baht, respectively (Department of Fisheries and Suwannatos, 2003). The market price per kg (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).

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 devoted 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%).

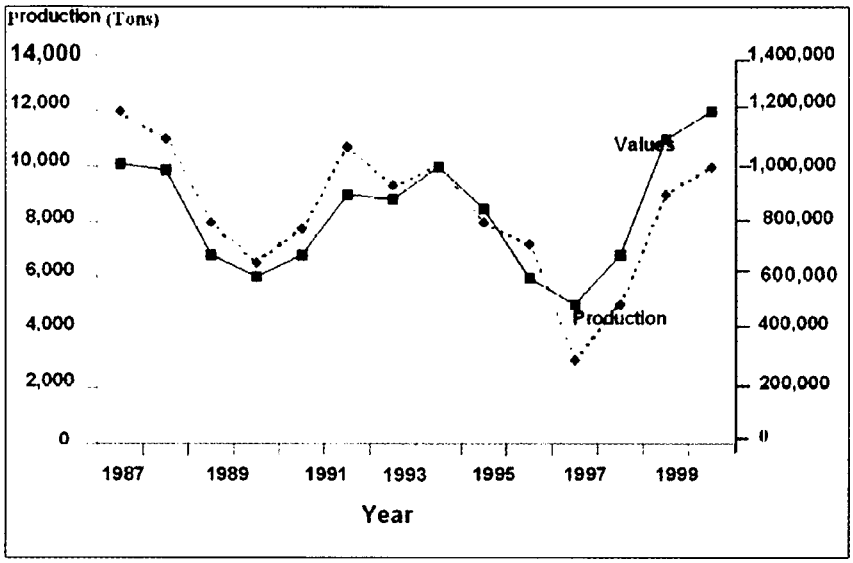

Figure 1. **Production and values of cultured *Macrobrachium rosenbergii* in 1987-2000 (Department of Fisheries, 2001)**

Commercial Hatcheries

Domestication of the giant freshwater prawns in Thailand 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 began to be developed in 1966, as early researches concentrated on larval rearing. As soon as the larval rearing practices were optimized, researches were subsequently focused on developing grow-out culture techniques.

Water quality was identified as a critical factor in larval rearing. Consequently, after several refinements in the rearing protocol, the optimum water quality conditions were established at 12 to 15 ppt for salinity, 27 to 31°C water temperature,

3-5mg/L dissolved oxygen, pH 7 to 8, and ammonia which should not exceed 0.1 mg/l (Colt and Armstrong, 1981; Suwannatos, 2003). Apart from water quality, quality seedstock production in commercial hatcheries also depend on many factors, namely: quality of parent stock, sexual maturity of female broodstock and hatchery management.

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

	Price (in Thai Baht)								
Size	1989	1990	1991	1992	1993	1994	1995	1996	1997
Large	207.42	233.92	256.0	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

Suwannatos (2003) reviewed the giant freshwater prawn rearing practices in Thailand, and pointed out four types of larval hatchery management. Each system had its own advantages and disadvantages. A number of researches have been conducted to develop these rearing systems to provide good quality seeds to the farmers and subsequently provide them the option for the type of hatchery system that will best suit their proposed site and resources.

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 closely monitor the quality of the rearing water to ensure successful rearing operations. In cases where ponds are used, the size should be between 1-3 m^2 and 1.0 m deep.

Greenwater system

This system was modified from a larval rearing scheme developed in Hawaii. A large number of quality seeds can be produced by rearing the larvae in greenwater containing the phytoplankton, *Chlorella* spp. The phytoplankton takes care of the disease problem and helps keep the water quality at an optimum pH level. 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 environmental conditions are easy to control. However, it is more costly than the other systems. Some researches that dealt with its development (Suwannatos, 2003), included the use of water treated with small weed organisms. Moreover, in this system, air circulation is optimized and the water continuously reused. Here, diseases and use of chemicals could be easily controlled. For this system, the hatchery manager should be well trained in water quality as well as on nutrition and disease management. 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 such that ecological balance can be achieved without any water change. The pond is installed with an air blower for consistent oxygen supply. Water chemical conditions are observed regularly and adjusted to suitable levels to keep the ecological system in the pond balanced.

The larvae are fed with natural zooplankton and *Artemia* nauplii. Although the system gives high juvenile survival rate, the rearing period to postlarval stage 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). Here, the hatchery manager should regularly monitor water quality and maintain the ecological balance within the pond.

Good Quality Seeds

Good quality seeds should result in high survival rate and fast growing juvenile prawn. The aforementioned hatchery systems when managed well, will be able to provide good quality seed supply to the *Macrobrachium* industry as long as 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. Sexually mature female breeders should be clean
4. Antibiotics should never be used
5. All equipment should be disinfected after every use
6. Good quality food such as *Artemia*, boiled eggs and pollock, should be used.

Grow-out Culture

Macrobrachium culture in the country is now facing a number of problems such as slow growth, lack of appropriate broodstock management schemes and diseases. To counteract these problems, commercial *Macrobrachium* farms developed an improved strain using new farming management methods. The strain has been initially introduced to the farmers, but later the private hatcheries have developed their own freshwater prawn selective breeding programs.

Macrobrachium farming in Thailand has been classified into two types: the traditional farms growing the local strain, and the *Macrobrachium* farms using the new strain. Traditional farms usually consist of small number of 0.32-0.96 ha earthen ponds stocked with prawns at 5 to 20 juveniles/m^2. The farmers prepare their own feeds. During the first month, the prawns are fed twice a day at 30-40% of the body weight. During the third month of rearing, the feeding rate is gradually reduced to 5%, and finally to 3% from the fourth month onwards. At six months, large-sized prawns are seined and sold while the small ones are left for on-growing in the culture pond. The marketable sized prawns are from 50 to 100 g/pc (Tookwinas, 2002).

Mr. Supon Sovanapreecha, owner of the Kasetsombuond Farm in Supanburi Province, has been operating his farm for three years using the new strain of *Macrobrachium* (interviewed in Thai Fisheries Gazette, 2002). The juveniles are reared in the nursing pond at 75 pc/m^2 for two months, after which these are stocked in the grow-out pond at 7-15 pc/m^2. After four and a half months, the female prawns averaging 25-33g are culled and sold. On the sixth month, the large males (100-125g) are harvested and sold. The total production of the first generation is usually about 3,750 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: 83g). Therefore, the development of a selective breeding program to improve the growth of the domesticated *Macrobrachium* strain has been recognized as an urgent concern for the freshwater prawn industry.

R&D efforts on quality improvement of *M. rosenbergii dacqueti*

Several research studies that support efforts for the improvement of the quality of the giant freshwater prawn have been implemented. Below is a summary of each of the study.

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 prawn stocks in different locations of Thailand. The study was based on the analysis of the mitochondrial DNA (mtDNA) variation restriction fragment length polymorphism. It showed significant difference in the restriction fragment length polymorphism (RFLP) patterns of mtDNA between prawn from two rivers. 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, PK 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-1*, 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 (He = 0.031 ± 0.018) for the species was found to be relatively low. Inbreeding coefficients (F-statistics), polymorphic loci differences, genetic distances between populations and dendrograms showed that all three populations were the same single population based on allozyme marker analysis.

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 six populations (Bangpakong River, Nakhorn Nayok River, Tapi River, Songkhla Lake, Kraburi River and Yaephew River) *Macrobrachium rosenbergii* de Man was carried out. The study was based on mtDNA analysis of 12S rDNA and 16S rDNA genes, that had been cut with four restriction enzymes, Alu I, Dra I, Hinf I and Tru9 I. Based on genetic distances, two major populations of the prawns were identified. 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. 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. The remaining prawns from the Kraburi and Yaephew were mixed together as another intermediate group.

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. Heritability estimates were made on the progeny from 32 full and half-sib families nested within eight sires with two dams per sire. The heritability estimates on the carapace length based on paternal, maternal and full-sib

analyses were found to be the highest 0.40 (±0.22), 0.13 (±0.07) and 0.26 (±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 (±0.04), 0.73 (±0.08) and 0.37 (±0.02) and the morphotypic transformation from small males (SM) after removing bulls were 0.21 (±0.06), 0.56 (±0.05) and 0.39 (±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 in every cage was greater than male and blue claw males at 23 weeks. The resulting heritabilities implied the possibility for trait 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 indicated that the postlarvae obtained from the Kraburi x Kraburi crosses were significantly larger than those from the Chao Phraya x Chao Phraya crosses while hybrids of the two populations did not exhibit any heterosis.

Uraiwan S, S Sumanojitraporn, 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 40th Kasetsart University Annual Conference. 632-640 pp

Sib analysis and selection procedures were designed to estimate heritability and realized heritability on growth rate of *Macrobrachium rosenbergii*. Under cage culture conditions, 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 five months old were -0.081 ± 0.014 and 0.122 ± 0.074, and 0.060 ± 0.054 and 0.030 ± 0.041, respectively.

Under pond conditions, heritabilities of length and weight of six-month old male and female prawns were 0.156 ± 0.077 and 0.142 ± 0.096, and 0.254 ± 0.080 and 0.272 ± 0.210, respectively. The realized heritabilities in the male and female prawns after one generation of within-family selection 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 six month old selected line 6 were significant ($p<0.01$) six and 12% larger by length and weight than those of the control line, respectively. Similarly, six-month old selected female prawns were significantly ($p<0.01$) 5 and 16% larger by length and weight than those of the parental line. The results showed that genetic improvement to increase growth rate of the *Macrobrachium rosenbergii* is possible.

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

A 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 to improved the growth rate of cultured prawns. The experiment consisted of two lines including a high growth selected line and a control line. 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. 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 showed that within-family selection is an efficient procedure to improve growth of the giant freshwater prawn.

Management procedures, domestication selection

Doyle RW, S Singholka, MB 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 by product of harvesting and breeding techniques, as well as correlated selection on a trait caused by artificial selection on another trait. Statistical models 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. 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 RW. 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.

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, of which each half should come from the mother (neofemale) and from the father (normal male).

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, ED Aflalo. 2003. The androgenic gland and monosex culture in prawns-biotechnological perspective. Department 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 female 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.

Future Plans and Prospects

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, e.g. nursery, grow-out culture, nutrition, diseases and genetics, were discussed. The Deparment 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 processes from the farm to the table should be established. Therefore, a code of conduct (CoC) is planned for *Macrobrachium*, similar to the one adopted for the country's marine shrimp culture industry. The planned guidelines of the CoC for *Macrobrachium* should include the following 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 agents and other chemicals
(7) effluents and solid waste management
(8) harvesting and selling
(9) social responsibility
(10) farmer association and education
(11) data collection

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Project Proposal

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

SOURCE OF EXTERNAL FUNDING: GOJ, Government of Thailand
DATE STARTED: February 2004

PROPONENTS {Name}:	PARTICIPATION {% time}
Mr. Somchart Sukawong	10
Dr. Supattra Uraiwan	30
Dr. Panom K. Sodsuk	30
Dr. Srirat Sodsuk	30
Mr. Tanan Sangkorntanakit	25
Mr. Somsak Roongtongbaisuri	25
Mr. Saravuth Jasoh	25
Mr. Wisanuporn Ratanatrivong	5

COORDINATOR:
Dr. Panom K. Sodsuk
Aquatic Animal Genetics Research and Development Institute (AGRDI)
39 Moo 1, Khlong 5, Khlong Luang,
Pathumthani 12120, Thailand

Objectives

1. To evaluate the economically important performance traits and genetic variation of nine crosses from three stocks of *Macrobrachium rosenbergii*

2. To improve the economic traits of the best cross by suitable selection procedure

3. To optimize PCR conditions for test primers 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

PCR condition of microsatellite primers will be optimized, taking into consideration the annealing temperature, amount of DNA template, $MgCl_2$, primer and enzyme concentration, etc. 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

Reciprocal crosses of three *Macrobrachium rosenbergii* stocks (one wild and two domesticated) producing nine crosses will be carried out at the two AAGRDI hatcheries (Pathumthani and Phetchaburi). The performance of the progenies from nine crosses will be evaluated under four environmental conditions

in four different provincial areas, Pathumthani, Uttaradit, Chumphon and Burirum. In each environment, all crosses will be reared together in three ponds. The individuals from each crosses are identified by color-coded dyes being injected into the prawn muscle. Simultaneous with these activities, the genetic variation of the nine crosses will be evaluated based on allozyme markers.

In each environment, the cross with the best performance will be chosen for the selective breeding program. A within-family selection procedure will be used to improve the economic traits of the chosen batch. Both performance and genetic variation will be evaluated in each selected generation. After three generations of selection, the selected lines will be evaluated under farm conditions.

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Project Highlights

Preliminary Growth Comparison of Three *Macrobrachium rosenbergii* Stocks and their Reciprocal Crosses in Four Environments[6]

Supattra Uraiwan, Panom K. Sodsuk, Wattana Lelapattara, Somsark Rungtongbaisuree, Sanga Leesanga, Tanan Sakontanakit, Wisanuporn Rattanatriwong, Kridsanupan Komanpririn, and Sriprapa Buddama
Department of Fisheries of Thailand

Introduction

Three stocks of *Macrobrachium rosenbergii* namely, "AAGRDI" (Aquatic Animal Genetic Research and Development Institute), "FARM" (Petchaburi Farm) and "WILD" stocks were used for the selective breeding program. Generally, a good base population for genetic improvement should have high genetic variability and the stock should have traits that make them adaptable in each local environment. Therefore, all possible crosses of these three stocks should be evaluated on both performance traits and genetic diversity before selective breeding takes place. Hence, the proposed genetic improvement program was divided into two parts: (1) evaluation of growth performance of the three stocks and their reciprocal crosses in four environments; and (2) the establishment of improved economic traits in the best cross using a suitable selection procedure. The four environments used were: 20 m^2 concrete pond located at the Aquatic Genetic Research and Development Research Center (AAGRDI), Pathumtani Province; and in 5 x 5 x 1.5 m^2 net cages at the three Fisheries Test and Research Centers in Chumphon, Buriram and Uttaradit Provinces. The "AAGRDI" stock was the *M. rosenbergii* selected for improved growth rate for two generations in the AAGRDI environment (Uraiwan *et al.,* 2003). The "FARM" stock originally came from a private hatchery in Petchaburi Province in 2002, which was also reared at the AAGRDI for one generation. The "WILD" stock was collected from the river in Chantaburi Province in 2002. This "WILD" stock has been domesticated under hatchery conditions at the AAGRDI for one generation. This experiment deals with the growth performance comparison and the genetic variations of these stocks conducted simultaneously by Sodsuk *et al.* (2005).

Methodology

Parent generation

In June 2003, fifty (50) pairs of *M. rosenbergii* from each of three stocks were collected to form the base population. Each stock has been spawned and reared separately, and their offspring were reared in three 20 m^2 concrete ponds at the AAGRDI. The growth of *M. rosenbergii* from the three stocks was observed from August 2003 to January 2004 (Uraiwan and Sodsuk, 2004). The results showed that the AAGRDI *M. rosenbergii* stock performed better than those of the "WILD" and "FARM" stocks at average length increment of 4% and 9-15% weight gain, respectively. In addition, allozyme electrophoresis has been carried out to estimate genetic variabilities (heterozygosity and number of alleles per locus) of the three stocks. Results showed that they were similar to *M. rosenbergii* from the natural waters {number of alleles 1.30 (1.29-1.33), heterozygosity 0.032(0.027- 0.036), Sodsuk and Sodsuk, 1998}, Uraiwan and Sodsuk (2004). There is no difference on the genetic diversity of the three (3) stocks (Sodsuk *et al.*, 2005).

Performance growth test on nine crosses

Reciprocal crosses of the three stocks were conducted from November 2004 to August 2005 to establish nine crossbred lines. The crosses are identified by the male and female parents of each cross, starting with the male parents followed by the female parents, these are as follows: AAGRDI x AAGRDI, WILD x WILD,

[6] *based on activities from late 2004-2005; title modified based on preliminary data presented during the 3rd Roundtable Discussion*

FARM x FARM, AAGRDI x FARM, FARM x AAGRDI, AAGRDI x WILD, WILD x AAGRDI, FARM x WILD and WILD x FARM. Each cross was produced from 10 pairs of *M. rosenbergii*. The hatchery and nursery phases were completed at the AAGRDI and the Petchaburi Fisheries Test and Research Center at different periods, and the post larvae were stocked for the performance growth test in four environments as indicated in following table:

Month and Year	Hatchery and nursery location	Location of performance tests
November 2004	AAGRDI	18 cages, Uttradit Fisheries test and Research center
July 2005	AAGRDI	18 cages, Buriram Fisheries Test and Research Center
August 2005	Petchaburi Fisheries Test and Research Center	18 cages, Chumphon Fisheries Test and Research Center
August 2005	AAGRDI	18 concrete ponds, AAGRDI

The nine crosses have been reared in the aforementioned environments for eight months. Standard experimental procedures such as stocking density, feeding regime and measurement schedules were adopted in all the test locations. The stocking rates of 10 and 1 prawn/m^2 were used during the 1st to the 4th month and the 5th to the 8th month of the experiment, respectively. The prawns were fed commercial shrimp pelleted feed three times a day at 3.4% of body weight. Length-weight measurements were taken monthly.

Results

Growth Comparison

Due to the difference in the stocking dates, the recorded data were *M. rosenbergii* growth (length and weight increments) at the second month for the AAGRDI and the Chumphon Fisheries Test and Research Center, and at the third and fourth months for the Buriram and Uttaradit Fisheries Test and Research Centers, respectively.

AAGRDI environment

Mean lengths and weights during the second month of the nine crosses are shown in Table 1. The cross WILD x WILD had the highest length and weight increments.These were significantly higher by 15% in length and 39% in weight than those of the AAGRDI x AAGRDI stock and by17% in length and 43% in length and weight compared to the FARM x FARM stock.

Table 1. **Mean lengths/weights and standard deviations (+sd) of nine crosses of *M. rosenbergii* reared in concrete ponds for two months at the AAGDRI environment**

Crosses (M X F)	Length (cm) + sd	Weight (g) + sd
WILD X AGGRDI	$7.516 + 0.864^{b}$	$3.905 + 1.687^{c}$
AAGRDI X WILD	$7.224 + 0.872^{c}$	$3.588 + 1.327^{g}$
AAGRDI X FARM	$7.992 + 1.102^{b}$	$4.963 + 2.306^{c}$
FARM X AAGRDI	$6.706 + 1.175^{a}$	$3.156 + 1.819^{b}$
WILD X FARM	$7.628 + 1.098^{b}$	$4.546 + 2.096^{f}$
FARM X WILD	$8.329 + 0.866^{f}$	$5.244 + 1.851^{g}$
FARM X FARM	$7.113 + 0.899^{d}$	$3.299 + 1.329^{b}$
WILD X WILD	$8.583 + 0.843^{f}$	$5.854 + 1,851^{g}$
AAGRDI X AAGRDI	$7.232 + 0.656^{a}$	$3.559 + 1.054^{g}$

The different letters illustrate significant difference at P-value<0.05

Chumphon Fisheries Test and Research Center environment

Mean length and weight data on the second month of the nine crosses are shown in Table 2. The cross WILD x AAGRDI had the highest length and weight increments. The results were significantly higher at 10 and 23% in length and weight increments than those of the AAGRDI x AAGRDI, respectively.

Buriram Fisheries Test and Research Center environment

Mean lengths and weights on the third month of the nine crosses (Table 3) showed that the cross AAGRDI x FARM had the highest increments in terms of length and weight. These were 5, 3, 4% and 24, 25 and 15% significantly higher in length and weight than the AAGRDI x AAGRDI, the FARM x FARM, and the WILD x WILD, respectively.

Table 2. **Mean lengths/weights and standard deviations (+sd) of nine crosses of *M.rosenbergii* grown in cages for two months at the Chumphon Fisheries Test and Research Center**

Crosses (M x F)	Length (cm) + sd	Weight (g)+ sd
1. WILD x AAGRDI	$8.122 + 1.074^{b}$	$4.681 + 1.870^{bc}$
2. AAGRDI x WILD	$7.676 + 1.468^{c}$	$4.036 + 2.393^{a}$
3. AAGRDI x FARM	$7.300 + 1.058^{a}$	$3.690 + 1.718^{a}$
4. FARM x AAGRDI	$7.506 + 1.357^{a}$	$4.102 + 2.364^{ac}$
5. WILD x FARM	$7.456 + 1.196^{a}$	$3.634 + 1.698^{dc}$
6. FARM x WILD	$7.210 + 1.356^{a}$	$3.502 + 1.952^{de}$
7. FARM x FARM	$7.131 + 1.165^{d}$	$3.274 + 1.797^{d}$
8. WILD x WILD	$7.736 + 1.150^{bc}$	$4.006 + 1.724^{e}$
9. AAGRDI x AAGRDI	$7.280 + 1.260^{a}$	$3.609 + 1.985^{a}$

The different letters illustrate significant difference at P-value<0.05

Table 3. **Mean lengths/weights and standard deviations (+sd.) of nine crosses of *M. rosenbergii* grown in cages for three months at the Buriram Fisheries Test and Research Center**

Crosses	Length (cm) +sd	Weight (g) + sd
1. WILD x AAGRDI	$10.430 + 1.805^{b}$	$17.220 + 6.050^{c}$
2. AAGRDI x WILD	$10.783 + 1.234^{bc}$	$16.140 + 6.571^{b}$
3. AAGRDI x FARM	$11.061 + 1.055^{b}$	$20.709 + 6.258^{c}$
4. FARM x AAGRDI	$10.447 + 1.273^{a}$	$16.710 + 6.910^{abc}$
5. WILD x FARM	$10.618 + 1.331^{ac}$	$17.740 + 5.715^{bc}$
6. FARM x WILD	$10.049 + 0.865^{d}$	$15.040 + 5.029^{de}$
7. FARM x FARM	$10.687 + 1.198^{eb}$	$15.450 + 6.162^{fbc}$
8. WILD x WILD	$10.589 + 1.126^{ae}$	$17.430 + 5.375^{a}$
9. AAGRDI x AAGRDI	$10.496 + 0.738^{a}$	$15.770 + 4.394^{a}$

The different letters illustrate significant difference at P-value<0.05

Uttaradit Fisheries Test and Research Center environment

Mean length and weight increment data on the fourth month for the nine crosses (Table 4) indicate that the cross AAGRDI x FARM had the highest length and weight increments, and these were significantly higher at 11, 12 and 13% than the AAGRDI x AAGRDI, the FARM x FARM and the WILD x WILD stocks, respectively.

Table 4. **Mean lengths/weights and standard deviations (+sd) of nine crosses of *M. rosenbergii* grown in cages for four months at the Uttaradit Fisheries Test and Research Center**

Crosses	Length (cm) + sd	Weight (g)+ sd
1. WILD x AAGRDI	12.982 + 1.094[a]	24.354 + 8.143
2. AAGRDI x WILD	12.671 + 1.287[ade]	24.449 + 6.478
3. AAGRDI x FARM	13.140 + 1.398[a]	23.977 + 8.585
4. FARM x AAGRDI	13.822 + 1.872[b]	22.083 + 7.900
5. WILD x FARM	12.500 + 1.565[ace]	22.908 + 8.379
6. FARM x WILD	12.002 + 1.217[c]	20.681 + 6.586
7. FARM x FARM	12.212 + 1.637[dc]	21.965 + 8.802
8. WILD x WILD	12.044 + 1.160[c]	22.035 + 8.375
9. AAGRDI x AAGRDI	12.267 + 1.588[ec]	20.230 + 7.567

The different letters illustrate significant difference at P-value<0.05

Heterosis

The heterosis (in length and weight) for each hybrid cross was estimated using the following formula:

$$\% \text{ heterosis} = \frac{\text{reciprocal crosses' average value} - \text{parents' average value}}{\text{parents' average value}}$$

The heterosis of all reciprocal crosses is shown in Table 5. The hybrid stocks showed positive and negative heterosis values for each environment.

Table 5. **Percent heterosis of growth (length and weight) of three reciprocal crosses of *M. rosenbergii* grown in four environments**

Environments	Reciprocal Crosses	Heterosis %	
		Length	Weight
AAGRDI	AAGRDI & Farm	1.97	18.39
	AAGRDI & Wild	6.67	23.61
	Farm & Wild	1.66	6.96
CHUMPHON	AAGRDI & Farm	2.74	13.21
	AAGRDI & Wild	4.54	14.48
	Farm & Wild	1.35	1.98
BURIRAM	AAGRDI & Farm	1.58	19.86
	AAGRDI & Wild	0.61	0.48
	Farm & Wild	2.85	0.30
UTTARADIT	AAGRDI & Farm	20.28	9.16
	AAGRDI & Wild	2.28	15.47
	Farm & Wild	1.01	3.13

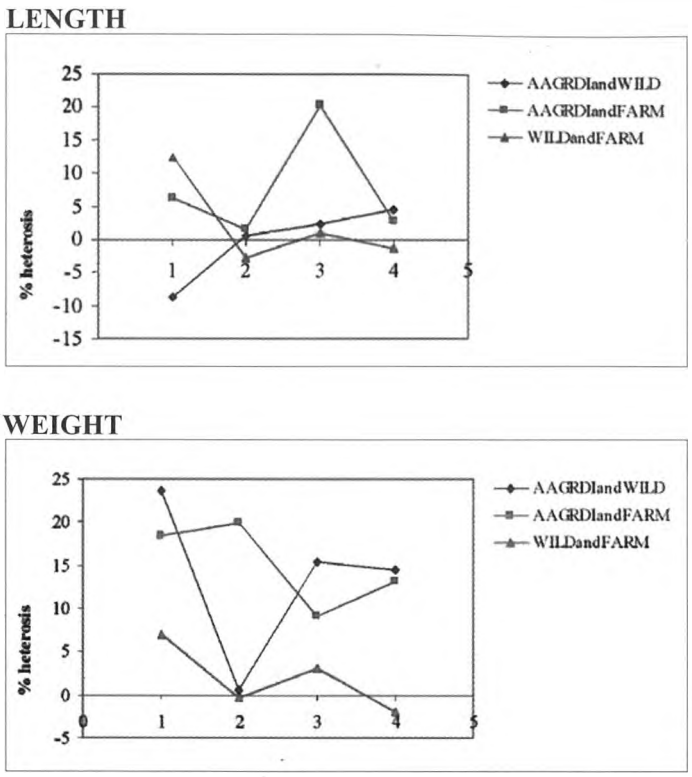

Figure 1. **Percent heterosis in length-weight of three reciprocal crosses of *M. rosenbergii* (AAGRDI and WILD, AAGRDI and FARM, and WILD and FARM) grown in four different environments:**

1= Aquatic Animal Genetics Research and Development Institute (AAGRDI)
2= Chumphon Fisheries Test and Research Center (CHUMPHON)
3= Buriram Fisheries Test and Research Center (BURIRAM)
4= Uttaradit Fisheries Test and Research Center (UTTARADIT)

Conclusions

There were differences in growth rate between three *M. rosenbergii* stocks and their hybrids. The heterosis of some crosses illustrated the possibility of improving the growth rate of *M. rosenbergii* by hybridization. However, selection within lines is necessary prior to hybridization. Likewise based on the results, the WILD x WILD cross is suitable for culture at the AAGDRI while the WILD x AAGDRI cross is suitable for culture at the Chumphon Fisheries Test Center. For the Buriram and Uttaradit Fisheries Test Centers, the AAGRDI x FARM and the FARM x AAGDRI crosses, respectively were found suitable. Finally, the differences in heterosis values in the different environments illustrated genotype-environment interaction on growth performance (Figure 1). Therefore, line performance growth test should be included at the early stages of the selective breeding program.

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Recent Updates on the Selective Breeding Program for the Genetic Improvement of *Macrobrachium rosenbergii* in Thailand[7]

Supattra Uraiwan, Panom K. Sodsuk, Somsuk Rungtongbaisuree, Wisanuporn Ratanatrivong and Sanga Leeaa-nga
Aquatic Animal Genetic Research and Development Institute (AAGRDI)
Department of Fisheries, Thailand

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

Three *Macrobrachium rosenbergii* stocks, namely, AAGRDI (Aquatic Animal Genetic Research and Development Institute), FARM (Petchaburi Farm) and WILD (Chantaburi) were used for a selective breeding program to develop a genetically improved giant freshwater prawn. For stocks to be considered as a good base population for any genetic improvement program, they generally should have high genetic variability and traits that allow them to be adaptable for each local environment. Hence, prior to conducting selective breeding, crosses of these three stocks were evaluated based on their performance and genetic diversity. The program was divided into two parts, first, the evaluation of growth performance of these three stocks and their reciprocal crosses under four common environments and second, the improvement of economic traits of the best cross through an appropriate selection procedure.

The stocks were compared in four types of environments, namely in 20 m^2 concrete ponds at the Aquatic Genetic Research and Development Research Center (AAGRDI), Pathomtanee province and in 5 x 5 x 1.5 m^2 mesh cages in three Fisheries Test and Research Centers located in Chumphon, Buriram and Uttaradit provinces. The aim of this study is to estimate the expected response to selection for growth of the intraspecific *M. rosenbergii* hybrid cross suitable for each type of culture area.

The test stocks had the following farming histories: the "AAGRDI" stock was selected for improved growth rate for two generations in the AAGRDI environment (Uraiwan *et al.*, 2003); the "FARM" stock originally came from a private hatchery in Petchaburi province in 2002, and was also reared at the AAGRDI for one generation; and the "WILD" stock was collected from a river in Chantaburi province in 2002 and has been domesticated in the AAGRDI hatchery for one generation.

Results of the one-year strain comparison program showed that there were differences in growth rate in the three *M. rosenbergii* stocks and their hybrids. (Uraiwan, *et al.*, 2005). Progenies from the nine crosses were reared in the four environments for eight months. Standard experimental procedures (stocking density, feeding regime and sampling) were used in all the rearing runs for the nine crosses. The stocking rate was 10 prawns/m^2. After eight months, results showed heterosis on the length and weight increments of these crosses at 0.65- 4.47% and 1.70-16.33%, respectively (Table 1). In the AAGRDI environment, the progenies of the WILD x AAGRDI cross showed statistically significant ($P<0.05$) growth advantage over the other crosses. In the Uttaradit Fisheries Test and Research Center and Chumphon Fisheries Test and Research Center, the progenies of the FARM x AAGRI cross were the ($P<0.05$) best growing stock while the progenies of the reciprocal cross AAGRI x FARM excelled in the Burirum Fisheries Test and Research Center.

[7] *information covers activities undertaken in 2006 based on the progress report submitted in March 2007*

Table 1. **Mean growth measurements, survival rate and the heterosis values of nine crosses of *M. rosenbergii* reared in cages for eight months in the four environments**

Environment	Cross	Performance indicators			% heterosis	
(Province)		Length (cm) + sd	Weight (g) + sd	% survival + sd	Length	Weight
Pathomtanee	1.WILD x AAGRDI	13.578+1.506^{b}	34.721+11.874^{b}	80.0+7.8	2.34	9.51
	2.AAGRDI x WILD	13.365+1.700^{b}	34.902+12.974^{b}	75.5+0.5		
	3.AAGRDI x FARM	13.124+1.938^{b}	31.827+14.174^{a}	76.5+17.7	0.65	1.70
	4.FARM x AAGRDI	13.060+1.439^{a}	30.658+10.495^{a}	82.5+5.7		
	5.WILD x FARM	13.519+1.596^{b}	34.193+11.990^{b}	66.5+19.0	1.17	6.62
	6.FARM x WILD	13.135+1.468^{a}	32.421+11.136^{c}	63.0+14.0		
	7.FARM x FARM	12.885+1.275^{d}	30.172+11.183^{a}	66.0+9.2		
	8.WILD x WILD	13.461+1.638^{b}	32.308+12.680^{c}	82.0+00		
	9.AAGRDI x AAGRDI	13.131+ 1.386^{a}	31.269+10.268^{a}	89.0+1.4		
			Mean	75.7+8.8		
Uttaradit	1.WILDx AAGRDI	15.457+2.155^{a}	51.373+27.615^{a}	80.5+7.8	2.61	8.40
	2. AAGRDI x WILD	16.400+2.566^{a}	60.975+31.569^{d}	81.0+14.8		
	3.AAGRDI x FARM	15.897+1.861^{b}	56.048+27.606^{b}	89+7.0	4.41	15.47
	4.FARM x AAGRDI	16.574+2.139^{b}	62.222+31.896^{d}	80+0.0		
	5.WILD x FARM	15.646+2.297^{b}	54.397+25.657^{b}	79+3.0	1.38	12.41
	6.FARM x WILD	15.180+2.234^{c}	50.105+28.509^{a}	76.3+7.1		
	7.FARM x FARM	15.146+2.173^{c}	45.876+24.510^{c}	78.3+0.8		
	8.WILD x WILD	15.260+1.843^{c}	47.085+19.470^{c}	84.5+7.8		
	9.AAGRDI x AAGRDI	15.935+ 2.319^{bc}	56.552+28.454^{b}	89.0+3.6		
			Mean	81.9+4.6		
Buriram	1.WILD x AAGRDI	15.400+2.280^{a}	50.695+26.38^{a}	89.4+13.0	1.18	4.57
	2.AAGRDI x WILD	16.122+2.30^{b}	58.397+29.883^{c}	86.0+0.0		
	3.AAGRDI x FARM	16.550+2.692^{bc}	64.031+25.262^{d}	80.5+3.0	4.05	16.33
	4.FARM x AAGRDI	15.726+2.313^{a}	55.057+25.962^{c}	75.5+8.9		
	5.WILD x FARM	15.944+1.931^{b}	56.443+28.355^{c}	72.0+11.5	2.58	15.29
	6.FARM x WILD	15.179+2.276^{d}	50.504+29.129^{a}	80+0.0		
	7.FARM x FARM	15.102+1.514^{d}	45.409+23.711^{b}	71.0+2.5		
	8.WILD x WILD	15.238+1.840^{c}	47.356+19.602^{b}	80+8.0		
	9AAGRDI x AAGRDI	15.917+ 2.333^{b}	56.964+28.450^{c}	80+0.0		
			Mean	79.4+6.0		
Chumphon	1.WILD x AAGRDI	12.702+1.182^{a}	23.196+9.216^{a}	69.0+0.0	3.65	12.89
	2. AAGRDI x WILD	13.102+1.070^{b}	25.602+8.067^{b}	45.5+3.0		
	3.AAGRDI x FARM	13.180+1.034^{b}	25.136+8.547^{b}	51.0+2.5	4.71	14.06
	4.FARM x AAGRDI	13.003+1.147^{b}	25.663+10.152^{b}	61.5+7.5		
	5.WILD x FARM	11.946+0.874^{c}	17.574+5.068^{c}	70.0+10.4	-3.36	13.30
	6.FARM x WILD	12.502+1.141^{a}	20.928+6.819^{a}	77.0+4.2		
	7.FARM x FARM	12.704+0.969^{a}	22.848+9.184^{a}	76.0+11.5		
	8.WILD x WILD	12.593+0.969^{a}	21.562+7.718^{a}	79.5+7.0		
	9.AAGRDI x AAGRDI	12.303+ 1.274^{a}	21.664+8.306^{a}	60.5+5.2		
			Mean	65.6+11.8		

The different letters illustrate significant difference at P-value<0.05

Meanwhile, the AAGRDI and FARM stocks were paired for spawning to produce the P_0 generation of the selection experiment in Buriram and Uttaradit Fisheries Test and Research Center. Breeding has been set up at the AAGRDI . Twenty pairs of each stocks were set up to produce 20 full-sib families. *Macrobrachium* larvae were reared separately by families until they reach the age of 45 days, after which they were transferred to the Burirum and Uttaradit Fisheries Test and Research Centers. Meanwhile the parental stocks used in the AAGRDI x FARM cross were pooled and kept for further genetic diversity analysis.

At the end of December 2006, the *Macrobrachium* stock in the Buriram Fisheries Test and Research Center were two months old. The average sizes of seven full-sib families are summarized in Table 2. There were sizes differences between families (Table 3). The estimate expected response of the *Macrobrachium* under the Buriram environment has not been estimated as the age of selection is set at four months old. Therefore, only when all then individuals in each family have reached four months shall selection for the best size within each family is conducted to produce the next generation.

Table 2. **Mean growth at two months of seven *M. rosenbergii* (AAGRDI x FARM) families reared in cages at the Buriram Genetics Test Center**

Family Number	Sex	Mean Length (cm) + sd	Mean Weight (g) + sd
1	Male	10.477+0.546	10.438+1.859
	Female	10.417+0.863	11.796+3.097
	Mixed	10.448+0.701	11.09+2.568
2	Male	9.7+0.906	9.924+0.983
	Female	10.45+2.56	11.097+3.141
	Mixed	9.852+0.946	10.89+2.93
3	Male	9.96+0.623	12.240+3.597
	Female	10.26+0.505	11.698+1.801
	Mixed	10.2+0.531	11.806+2.185
4	Male	9.756+0.72	10.036+2.792
	Female	10.014+0.801	10.693+3.277
	Mixed	9.828+0.736	10.22+2.88
5	Male	8.805+0.853	7.245+2.18
	Female	8.82+0.593	7.310+1.302
	Mixed	8.808+0.796	7.258+2.02
6	Male	8.592+0.610	6.546+1.120
	Female	8.415+0.638	6.038+1.166
	Mixed	8.5+0.618	6.282+1.15
7	Male	8.00+0.316	5.35+0.533
	Female	8.067+0.416	5.861+0.957
	Mixed	8.048+0.381	5.718+0.888

Table 3. **Analysis of variance on length-weight of seven families of *M. rosenbergii* (AAGRDI x FARM) reared for two months in cages at the Buriram Genetics Test Center**

Source	DF	Mean Square	F-Ratio	P
		LENGTH		
Families	6	21.258	43.991	0.00
Sex	1	0.194	0.401	0.528
Error	167	0.483		
		WEIGHT		
Families	6	158.105	32.166	0.00
Sex	1	4.551	0.926	0.337
Error	167	4.915		

The program plan included the application of selection on the best crosses for three Fisheries Test and Research Centers environments, namely Uttaradit, Buriram and Chumphon However, delays in the implementation have been encountered due to unusual seasonal/environmental changes such as flooding, prolonged wet and cold seasons. To deal with these problems, shelters must be provided to protect the experimental pond from rising floodwaters during the prolonged rainy season and heaters must be used in the hatchery to control the water temperature.

Genetic improvement programs require long periods for implementation to allow the attainment of set breeding goals. Therefore, selection for increased growth rate must be carried out for at least two generations. Thus far, the experiment we have conducted under the proposed program has covered mainly the P_0 generation (parent generation) of selection. Therefore, at least one generation of within-family selection on the best cross grown in the three environments, shall be pursued in 2007.

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Allozyme-based Genetic Variation in Crossbreds Produced from Three Thai *Macrobrachium rosenbergii* Stocks[8]

Panom K. Sodsuk, Supattra Uraiwan, Srirat Sodsuk
Aquatic Animal Genetics Research and Development Institute, Department of Fisheries,
Khong Ha, Khlong Luang, Pathumthani 12120, THAILAND.

Introduction

Molecular technology at enzyme/protein level known as "allozyme marker" is a widely accepted powerful technique to study genetic variation (Ward and Grewe, 1995) as well as intraspecific population studies (Sodsuk, 1996; Sodsuk and Sodsuk, 1998a & 1998b; Sodsuk *et al.*, 2001). Since the allozyme technique can be readily applied, it has become a basic tool for the evaluation of genetic variation in aquaculture stocks. This study aims to: (1) evaluate genetic variation (measured as as per locus averages of observable heterozygosities and number of alleles) of nine crosses from three *Macrobrachium rosenbergii* stocks (genetically improved AAGRDI, WILD, FARM); (2) apply polymorphic allozyme markers in the evaluation; (3) compare genetic variation among the nine crosses to determine genetic stock differences; and (4) use genetic variability and performance evaluation information in choosing the best cross for the conduct of a selective breeding program in specific farm environments.

Materials and Methods

Sample Analysis

About 40-60 individuals from both sexes of each of three stocks (the AAGRDI, wild, and private farm) and each progeny population of nine crosses were sampled. Pleopods were collected and placed in separate microtubes. The samples were kept in a -70 °C freezer prior to allozyme marker analysis. The preserved samples were electrophoretically analysed at 19-25 allozyme loci (Sodsuk *et al.*, 2005) following the protocol described by Sodsuk and Sodsuk (1998b).

Data Analysis

All allozyme data from the laboratory analyses were collected and calculated as per locus averages of heterozygosities (H) and number of alleles (NoA) for the evaluation of genetic variation. Data were analysed using BIOSYS release 1.7 of Swofford and Selander (1989). Genetic variation in the nine crosses, measured as per locus averages of heterozygosities and number of alleles (see Tables in appendix) were statistically compared following the methods of Sokal and Rohlf (1981) and Ward *et al.* (1994). This procedure was done using a statistical software known as SYSTAT of Wilkinson *et al.* (1992).

Results and Discussion

Genetic variation data (evaluated from per locus averages of heterozygosities and number of alleles) of the three stocks used in the parental crosses and all the nine crossbred stocks, are shown respectively in Tables 1 and 2. There were no significant differences among the three stocks as well as the nine crossbred stocks, based on heterozygosities and number of alleles. The heterozygosity levels and number of alleles both in the three stocks (H = 0.023-0.043, NoA = 1.20-1.44) and in the nine crosses (H = 0.010-0.042, NoA = 1.11-1.53) were similar to the natural stocks (H = 0.027-0.036, NoA = 1.29-1.33) earlier studied by Sodsuk and Sodsuk (1998b).

[8] *based on the paper presented durin the 3rd Roundtable Discussion*

Table 1. **Per locus averages of heterozygosities (H) and number of alleles (NoA) of the three initial stocks**

Stock	H	NoA
AAGRDI	0.043 (±0.018)^{A}	1.36 (±0.11)^{a}
Wild	0.023 (±0.014)^{A}	1.20 (±0.10)^{a}
Farm	0.036 (±0.016)^{A}	1.44 (±0.13)^{a}

Values in parentheses are standard errors (±S.E.)

Same superscripts in the same column means no significant differences ($p>0.05$)

Table 2. **Per locus averages of heterozygosities (H) and number of alleles (NoA) in all nine crosses**

	Cross (male x female)	H (Average ± S.E.)	A (Average ± S.E.)
T1	(Wild x AAGRDI)	0.011 (±0.008)^{A}	1.11 (±0.07)^{a}
T2	(AAGRDI x Wild)	0.042 (±0.027)^{A}	1.26 (±0.10)^{a}
T3	(AAGRDI x Farm)	0.010 (±0.007)^{A}	1.16 (±0.09)^{a}
T4	(Farm x AAGRDI)	0.016 (±0.007)^{A}	1.32 (±0.13)^{a}
T5	(Wild x Farm)	0.030 (±0.010)^{A}	1.53 (±0.14)^{a}
T6	(Farm x Wild)	0.026 (±0.013)^{A}	1.26 (±0.13)^{a}
T7	(Farm x Farm)	0.024 (±0.010)^{A}	1.37 (±0.11)^{a}
T8	(Wild x Wild)	0.018 (±0.009)^{A}	1.21 (±0.10)^{a}
T9	(AAGRDI x AAGRDI)	0.015 (±0.009)^{A}	1.16 (±0.09)^{a}

Same superscripts in the same column means no significant differences ($p>0.05$)

Table 3 shows the genetic information of the resulting heterozygosities and number of alleles, together with those resulting from growth performance (Uraiwan *et al.*, 2005). This genetically informative table is very helpful for choosing the best breeding-pair for further selection program in an appropriate area.

Conclusion

Genetic variation (measured as per locus averages of heterozygosities and the number of alleles), in the three initial stocks and all nine crosses showed no significant differences among the stocks. Genetic variability information generated by this study and the growth performance data from the study of Uraiwan *et al.* (2005), would help in choosing the best cross for selective breeding in each environment.

Table 3. **Genetic variability (expressed as heterozygosities H, number of alleles A), growth performance indicators and % heterosis of all crosses in four different areas**

Environment (months)	Mated Pair	Cross	Sodsuk (*et al.*) 2005		Uraiwan *et al.* (2005)			
					Performances		% heterosis	
			H	A	Length	Weight	Length	Weight
Uttaradit (5)	AAGRDI x Wild	T1	0.011[A]	1.11[a]	12.982	24.354	2.28	15.47
		T2	0.042[A]	1.26[a]	12.671	24.449		
	AAGRDI x Farm	T3*	0.010[A]	1.16[a]	13.140	23.977*	20.28*	9.16*
		T4*	0.016[A]	1.32[a]	13.822*	22.083		
	Wild x Farm	T5	0.030[A]	1.53[a]	12.500	22.908	1.01	3.13
		T6	0.026[A]	1.26[a]	12.002	20.681		
	Farm x Farm	T7	0.024[A]	1.37[a]	12.212	21.965	-	-
	Wild x Wild	T8	0.018[A]	1.21[a]	12.044	22.035	-	-
	AAGRDI x AAGRDI	T9	0.015[A]	1.16[a]	12.267	20.230	-	-
Buriram (4)	AAGRDI x Wild	T1	0.011[A]	1.11[a]	10.430	17.220	0.61	0.48
		T2	0.042[A]	1.26[a]	10.783	16.140		
	AAGRDI x Farm	T3*	0.010[A]	1.16[a]	11.061*	20.709*	1.58*	19.86*
		T4	0.016[A]	1.32[a]	10.447	16.710		
	Wild x Farm	T5	0.030[A]	1.53[a]	10.618	17.740	-2.85	-0.30
		T6	0.026[A]	1.26[a]	10.049	15.040		
	Farm x Farm	T7	0.024[A]	1.37[a]	10.687	15.450	-	-
	Wild x Wild	T8	0.018[A]	1.21[a]	10.589	17.430	-	-
	AAGRDI x AAGRDI	T9	0.015[A]	1.16[a]	10.496	15.770	-	-
Pathumtani (2)	AAGRDI x Wild	T1	0.011[A]	1.11[a]	7.516	3.905	-6.67	-23.61
		T2	0.042[A]	1.26[a]	7.244	3.588		
	AAGRDI x Farm	T3	0.010[A]	1.16[a]	7.922	4.963	1.97	18.39
		T4	0.016[A]	1.32[a]	6.706	3.156		
	Wild x Farm	T5	0.030[A]	1.53[a]	7.628	4.546	1.66	6.96
		T6*	0.026[A]	1.26[a]	8.329*	5.244*		
	Farm x farm	T7	0.024[A]	1.37[a]	7.113	3.299	-	-
	Wild x Wild	T8*	0.018[A]	1.21[a]	8.583*	5.854*	-	-
	AAGRDI x AAGRDI	T9	0.015[A]	1.16[a]	7.232	3.559	-	-
Chumphon (2)	AAGRDI x Wild	T1*	0.011[A]	1.11[a]	8.122*	4.681*	4.54*	14.48*
		T2	0.042[A]	1.26[a]	7.576	4.036		
	AAGRDI x Farm	T3	0.010[A]	1.16[a]	7.30	3.69	2.74	13.21
		T4	0.016[A]	1.32[a]	7.506	4.102		
	Wild x Farm	T5	0.030[A]	1.53[a]	7.456	3.634	-1.35	-1.98
		T6	0.026[A]	1.26[a]	7.210	3.502		
	Farm x Farm	T7	0.024[A]	1.37[a]	7.131	3.274	-	-
	Wild x Wild	T8	0.018[A]	1.21[a]	7.736	4.006	-	-
	AAGRDI x AAGRDI	T9	0.015[A]	1.16[a]	7.280	3.609	-	-

Asterisks (*) refer to the best crosses with good genetic attributes for rearing in specific environments

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Genetic Variation in Selected Thai *M. rosenbergii* Crossbreds Reared in Specific Environments[9]

Panom K. Sodsuk, Supattra Uraiwan, Srirat Sodsuk
Aquatic Animal Genetics Research and Development Institute, Department of Fisheries,
Khong Ha, Khlong Luang, Pathumthani 12120, Thailand

Introduction

AAGRDI has developed a domesticated and genetically improved stock of *Macrobrachium rosenbergii* for two generations. A wild stock has also been domesticated at the AAGRDI hatchery for one generation. Meanwhile, domesticated stocks from private hatcheries were also developed. Another improved stock from basically the two stocks of AAGRDI (genetically improved and the wild), together with the domesticated stock from a good private hatchery was used as base population for the selective breeding program. The improved stock therefore was developed to have a higher genetic diversity. This study is a continuation of Sodsuk *et al.* (2005)' s earlier work on the assessment of genetic diversity in crossbreds tested in different farm environments.

The objectives of this present study are: (1) to apply polymorphic allozyme markers in the evaluation of specific crossbreds identified earlier as ideal for rearing in specific environments (2) to assess/infer the genetic potential (based on genetic variability and performance trait data) and quality of the selected stocks chosen to be reared in the appropriate environment.

This study had hoped to cover a genetic assessment of selected parental stocks for each environment as listed below:

Environment	Male x Female
Uttaradit	FARM x AAGRDI
Buriram	AAGRDI x FARM
Chumphon	FARM x AAGRDI

However this report includes data mainly from the Buriram selective breeding trials.

Materials and Methods

Pleopods from about 40-60 individuals of parental and progeny stocks were sampled. Pleopods from each individual were cut and placed in separate microtubes. All pleopod samples in microtubes were preserved for further molecular analysis of allozyme markers. All preserved samples were electrophoretically analysed at 19-25 allozyme loci following a procedure already established by AAGRDI. All allozyme data from the laboratory analyses were collected and calculated as per locus averages of heterozygosities (H) and number of alleles (NoA) for genetic variation evaluation. The work was done using a software for population genetics studies (BIOSYS Release 1.7). Genetic variation, expressed as per locus averages of heterozygosities and number of alleles of the nine crosses were compared using the software SYSTAT (Wilkinson *et al.*, 1992).

Results and Discussion

The data for the Uttaradit and Chumphon have not yet been completely obtained due to unpredictable and uncontrollable effect of seasonal changes on the broodstock from these two sites. Thus far, only the data from the Buriram stocks have been completely analysed. These stocks include the AAGRDI and FARM parental stocks and their progeny (♀FARM x ♂AAGRDI) stock. Genetic variation data of the three Buriram stocks (AAGRDI, FARM and ♀FARM x ♂AAGRDI), analysed as observed heterozygosities (H) and number of alleles (A) at 21 allozyme loci are summarized in Table 1. The results from the present study show moderately higher observed heterozygosities and similar number of alleles compared to that obtained from an earlier

[9]*report submitted March 2007*

study by Sodsuk *et al.* (2005) also on three Buriram stocks (Table 2). The genetic variation data of the three Buriram stocks based on both studies confirm that the three stocks are not significantly different ($p>0.05$) from each other. Moreover, the present data also show genetic variability values close to those of natural stocks (Sodsuk and Sodsuk, 1998) (Table 3). This suggests that the ♀FARM x ♂AAGRDI progeny stock produced in Buriram is suitable and has the genetic potential for further use at the Buriram test site.

Table 1. **Observed heterozygosities (H) and number of alleles (A) of the three Buriram stocks (AAGRDI, FARM and ♀FARM x ♂AAGRDI) analysed at 21 allozyme loci (present study)**

Allozyme locus/ci	Heterozygosities (H)			Number of alleles (A)		
	AAGRDI	FARM	♀FARM x ♂AAGRDI	AAGRDI	FARM	♀FARM x ♂AAGRDI
1. *AAT-1*	0	0	0	1	1	1
2. *AAT-2*	0	0	0	1	1	1
3. *ACP*	0.333	0	0.083	2	1	2
4. *AK*	0.100	0.200	0	2	2	1
5. *ALAT*	0	0	0	1	1	1
6. *EST*	0.100	0.111	0	2	2	1
7. *ESD-1*	0	0	0	1	1	1
8. *ESD-2*	0	0	0	1	1	1
9. *GPI*	0	0	0	1	1	1
10. *MPI*	0.100	0	0.063	2	1	2
11. *PGDH*	0.100	0	0.125	2	1	3
12. *XDH*	0.100	0	0.063	2	1	2
13. *IDHP*	0.100	0.100	0.188	2	2	3
14. *G3PDH-1*	0.100	0.111	0.188	2	2	3
15. *G3PDH-2*	0	0.222	0.143	1	2	2
16. *G6PDH*	0.375	0.200	0.188	2	2	2
17. *HK*	0.100	0	0.125	2	1	2
18. *MDH-1*	0.100	0	0.150	2	1	2
19. *MDH-2*	0.100	0.100	0.375	2	2	2
20. *LDH*	0	0	0	1	1	1
21. *PGM*	0	0	0.125	1	1	2
Average (±SE)	**0.081 (±0.023)**	**0.050 (±0.017)**	**0.087 (±0.022)**	**1.57 (±0.11)**	**1.33 (±0.11)**	**1.71 (±0.16)**

Note : Values in parentheses are standard errors (±SE)

Table 2. **Observed heterozygosities (H) and number of alleles (A) of three Buriram stocks (AAGRDI, FARM and ♂AAGRDI x ♀FARM) analysed at 19-25 allozyme loci (Sodsuk *et al.*, 2005/2006)**

Allozyme locus/ci	Heterozygosities (H)			Number of alleles (A)		
	AAGRDI	FARM	♀FARM x ♂AAGRDI	AAGRDI	FARM	♀FARM x ♂AAGRDI
1. *AAT-1*	0	0	0	1	1	1
2. *AAT-2*	0.333	0.034	0.100	2	2	2
3. *ACP*	0	0	0	1	1	1
4. *AK*	0	0	0	1	1	1
5. *ALAT*	0.037	0	-	2	1	-
6. *EST*	0	0	0	1	1	1
7. *ESD*	0.080	0	0	2	2	1
8. *FBALD-1*	0	0	-	1	1	-
9. *FBALD-2*	0	0	-	1	1	-
10. *G3PDH-1*	0	0	0.05	1	1	2
11. *G3PDH-2*	0	0	-	1	1	-
12. *G6PDH*	0	0.037	0	1	2	1
13. *GPI*	0.100	0.067	0	2	3	1
14. *HK-1*	0	0	0	1	1	1
15. *HK-2*	0	0	0	1	1	1
16. *IDHP*	0.250	0.069	0	3	2	1
17. *LDH*	0	0	0	1	1	1
18. *MDH-1*	0	0	0	1	1	1
19. *MDH-2*	0.367	0.233	0	2	2	1
20. *MEP*	0.100	0.333	-	2	3	-
21. *MPI*	0	0.033	0	1	2	1
22. *PGDH*	0	0	0	1	1	1
23. *PGM*	0.100	0.103	0.083	2	2	2
24. *XDH*	0	0	0	1	1	1
25. *ODH*	0	0	-	1	1	-
Average (±SE)	**0.043 (±0.018)**	**0.036 (±0.016)**	**0.012 (±0.007)**	**1.36 (±0.11)**	**1.44 (±0.13)**	**1.16 (±0.09)**

Note : Values in parentheses are standard errors (±SE)

Table 3. **Genetic variation data, calculated for two parameters as per locus averages of heterozygosity (H) and number of alleles (A), in the three stocks from two studies (present and the Sodsuk *et al.* 2005/2006 study), in contrast with the data of natural stocks by Sodsuk and Sodsuk (1998)**

Study	Parameter	Genetic variation			
		Per locus averages of various stocks			
		AAGRDI	FARM	♂AAGRDI x ♀FARM	Natural
Present study	H	0.081 (±0.023)^{A}	0.050 (±0.017)^{A}	0.087 (±0.022)^{A}	-
	NoA	1.57 (±0.11)^{B}	1.33 (±0.11)^{B}	1.71 (±0.16)^{B}	-
Sodsuk *et al.* (2005/2006)	H	0.043 (±0.018)^{A}	0.036 (±0.016)^{A}	0.012 (±0.007)^{A}	-
	NoA	1.36 (±0.11)^{B}	1.44 (±0.13)^{B}	1.16 (±0.09)^{B}	-
Sodsuk and Sodsuk (1998)	H	-	-	-	0.027-0.036
	NoA	-	-	-	1.29-1.33

Note : Values in parentheses are standard errors (±SE)

The same alphabetic superscription in the same line indicating non-significant differences ($p>0.05$)

Although the data of Uttaradit and Chumphon stocks have yet to be obtained, this study could attain the two objectives with the available data on the Buriram stocks.

The unaccomplished parts of the study are due mainly to the prevailing uncontrollable weather conditions. These, however, could be pursued and accomplished in 2007.

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Country Report
PHILIPPINES

Giant Freshwater Prawn Farming in the Philippines[10]

Westly R. Rosario[a] and Melchor Tayamen[b]
Bureau of Fisheries and Aquatic Resources
[a]National Integrated Fisheries Technology Development Center
Dagupan City, Philippines
[b]National Freshwater Fisheries Technology Center
Science City of Muñoz, Nueva Ecija, Philippines

Introduction

Freshwater prawn thrives in inland waters like rivers, lakes, swamps, irrigation canals, estuaries and even in rivers upstream. A recent survey in Luzon Island, Philippines identified 12 species of freshwater prawn found in the island (Agasen, unpublished). The country's interest on freshwater prawn fishery started in 1914 as explained by Cowles (1914), when the freshwater prawn was recognized as one of the important fisheries during that time. In late 1976, trials were made to culture the freshwater prawn, however, the efforts were not sustained. These trials were conducted in Misamis Oriental, Mindanao between 1976-1979 by Dejarme *et al.*,with the collection of wild spawners and the subsequent rearing of *M. rosenbergii* hatchlings.

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 tapped 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 attempt failed to save the first venture of commercial *Macrobrachium rosenbergii* production in the Philippines.

Economic Importance of Freshwater Prawn

Freshwater prawn culture in the Philippines is being promoted as an alternative commodity for freshwater aquaculture, which is currently dominated by tilapia. It is a high value species and prawn culture could offer better profits. Alternate cropping or polyculture with tilapia may also result to more than 20% increase in yield (Guerrero and Guerrero, 1976).

Freshwater prawns are hardy, fast growing, able to grow in freshwater and low brackishwater conditions. The species possesses many biological advantages for commercial culture such as maturation in captivity, a relatively large size, and rapid growth rate. They feed on almost anything, e.g. terrestrial animal feeds, fish feeds, kitchen refuse, etc. Their feed conversion ratio is comparable to tilapia. Under Philippine conditions, their growth rate is high even beyond sexual maturity. They reach 45g after four months and 90 to 100g 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.

Geographic 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. It is locally known as *ulang, udang, kising-kising, paje, padao, kalig, urang* and *budsang*. Table 1 lists 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 include streams near Port Galera in Mindoro, Taytay in Palawan, Gandara in Samar, Lake Lanao in Mindanao and Jaro in Leyte.

[10]*paper presented during the 1st Roundtable Discussion*

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.

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. Preliminary investigations by BFAR-NIFTDC indicated that the species attain weights from 40 to 50g 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 shows the production of wild-caught freshwater prawn from Luzon as recorded by Agasen (unpublished). The production of *M. rosenbergii* is estimated at 0.5 to 0.75 metric tons in Pamplona River and 15-30 metric tons in the 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 the Pampanga River Delta and its tributaries as well as 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.

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

Fishing Area	Species											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Ilocos Norte												
- Bacarra/ Vintar river & tributaries		++						+		+		+
2. Cagayan												
-Cagayan River	+		++		+						+	
-Sta. Ana River	++		+		+							
-Pamplona River	++		+		+							
3. Isabela												
-Magat Dam					+	++						
4. Abra												
-Abra River & tributaries	+	++	+					+		+		+
5. La Union												
-Bararo River	+		+									
6. Pangasinan												
- Pantal River	+		+	+	+							
- Calasiao River												
-Bayambang Swamp	++		+									
7. Pampanga, Bulacan, Tarlac												
- Pampanga river delta	++		+	+					+	+		
8. Laguna												
- Laguna de Bay				+						+	+	
9. Camarines Norte												
- Lake Bato				+						+		
10. Sorsogon												
- Donsol River	+		+									
11. Palawan												
-Iwahig River	+					++				+		

1 – *M. rosenbergi* 2 – *M. lepidactylus* 3 – *M. equidens* 4 – *M. adella*
5 – *M. malcolmsonii* 6 – *M. rude* 7 – *M. mammillodatylus* 8 – *M.* sp.1 (medium sized)
9 – *M.* sp.2 10 – *M. lanchestri* 11– *Cardina* spp. 12 – *Atya mollucensis*
++ major species + minor species

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 30g, are sold at PhP250.00/kg or US$4.54/kg. Live prawns are likewise sold at PhP350.00/kg or US$ 6.36/kg. The biggest prawn from Bulacan was recorded to weigh about 500 g/pc.

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

Area/ Species	Estimated Production (in MT)	Peak Season	Fishing Gear	Market
1. Pamplona River *M. rosenbergii*	0.5 to 7.5	summertime	Spear gun	Local tourists
2. Pampanga river delta and tributaries *M. rosenbergii*	15-30	Year-round	Shrimp pot, scissors net	Export and local
3. Donsol River *M. rosenbergii*	unknown	summertime	Prawn pot	Local tourist
4. Iwahig river and tributaries *M. mammillodactylus*	unknown	Year-round	Shrimp pot, scissors net	Export and local
5. Magat Dam *M. rude*	1 to 2	Summertime	Push net	Local
6. Cagayan River *M.* spp.	3 to 5	May-December	Push net, shrimp pot, cast net	Local
7. Bacarra/ Vintar *M. lepidactylus*	35 to 40	May-December	Barricades, shrimp pot, scissors net	Export and local
8. Abra River and tributaries *M. lepidactylus*	10 to 15	May-December	Barricades, shrimp pot, scissors net	Local
9. Lake Bato *M. idella*	900 - 3600	Year-round	Push net, seine net, fish corrals	Export and local

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 the lack of available seedstock. 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 seedstock 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 ASEAN-EC-Aquaculture Coordination Development Program 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 conducted in aquaria as well as mass larval production in tanks was successful. Figure 2 shows the production of post larvae at the NFFTC. In 2001, the freshwater prawn hatchery was further improved and finally a protocol for its commercial hatchery was established in Munoz 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 establishmenmt of techno-demonstration sites for its culture using a farmer-cooperator scheme. Various national trainors' 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 (Tayamen, 2005).

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. The collection of strains will serve as the Center's genebank of the species for future genetic programs.

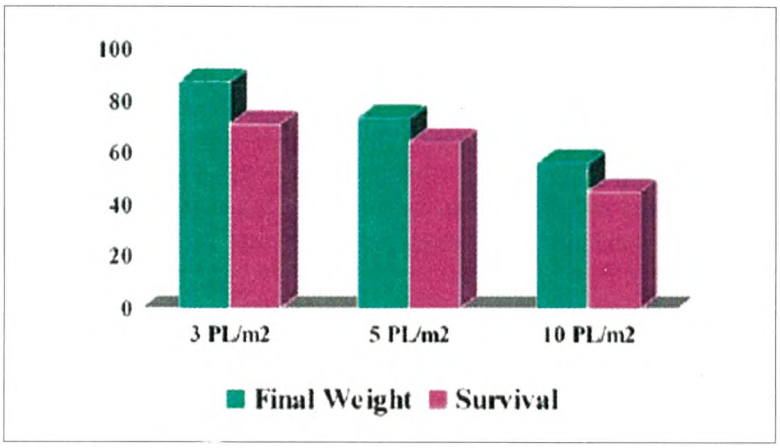
Collaboration with other institutions like SEAFDEC is encouraged particularly in larval nutrition and grow-out systems to facilitate the adoption of the species as a major aquaculture commodity by the Filipino farmers.

Freshwater Prawn Culture

The NFFTC has been conducting studies on freshwater prawn culture. Since the preliminary results (Table 3, Figure 2) 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^2 ponds after a 4-6 month culture period. A technical feasibility study based on this demonstration project is found in Annex 1.

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

Treatment	Final Weight	Survival %
3 PL/m^2	87.50	71
5 PL/m^2	73.47	64
10 PL/m^2	55.97	45



Days of Culture: 150 days

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

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 cost of seedstock production. More efforts are necessary to encourage farmers to produce 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 ensure success in the promotion of commercial *Macrobrachium rosenbergii* aquaculture in the Philippines, the following strategies and policies are recommended:

- An assessment of freshwater prawn stocks in major lakes, rivers, marshes, estuarine, reservoirs and other inland waters should be conducted to ensure adequate supply/source of broodstock for propagation purposes
- Freshwater prawn hatchery technology should be commercialized
- Environmental impact studies should be conducted in major inland waters where these species are abundant
- On-site grow-out culture demonstration through technology verification/dissemination on the monoculture or polyculture of freshwater prawns should be conducted
- Pilot testing in local government freshwater stations and collaborative projects with private prawn farmers should be pursued
- Available sites in Central Luzon especially in the *Lahar* area and other prospective areas for prawn culture should be identified
- Given the positive results of the current undertakings of BFAR with regard to freshwater prawn farming, feasibility studies should be made to encourage potential investors
- Credit financing in banks and financial institutions should be made available and in the process, promote the industry as an identified priority in the fisheries sector
- 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
- An inter-agency collaboration is necessary during the research program implementation to optimize all available resources, e.g. manpower, facilities/laboratories, equipment, financial, etc.
- Development of marketing and distribution systems for marketable sized prawns should be conducted

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 implemented as a collaborative effort among the academe, government, and international research agencies
- Development of appropriate technology for the mass production of *M. rosenbergii* post larvae
- Development of technology for grow-out culture adopting the different 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 principal stakeholders, the fisherfolk

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Other pre-project R&D efforts on *Macrobrachium* sp.

Freshwater Prawn Research at SEAFDEC/AQD

Maria Rowena R. Eguia, Maria Lourdes C. Aralar and Manuel A. Laron
SEAFDEC Aquaculture Department
Binangonan, Rizal
Philippines

The Philippines lags behind Thailand and Indonesia as far as research and commercial production of the freshwater prawn, *Macrobrachium* sp., are concerned. Although studies on *Macrobrachium* sp. (or *ulang* as it is locally known), started at the Binangonan Freshwater Station of SEAFDEC/AQD in the mid-1980's, research efforts were discontinued soon thereafter because of a) inadequate technical skills; b) problems with larval rearing and the domestication of wild stocks; and among others, c) the *Macrobrachium* sp. being considered in the Philippines as a low priority species in contrast to commercially important freshwater commodities like tilapia and milkfish. This was two decades ago and in retrospect, had researches continued, the freshwater prawn in the Philippines could have been successfully domesticated and current problems concerning 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 started to conduct some studies on the refinement of breeding, larval rearing and culture of *Macrobrachium rosenbergii* in late 2003. These studies are briefly described below:

1) Evaluation of different live food organisms as starter food for freshwater prawn larvae
(Main proponent: MA Laron)

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.

2) 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 14.3g for the highest stocking density to 26.3g 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.

* results recently published in Aquaculture Research and cited as:

Aralar MLC, EV Aralar, MA Laron, W Rosario. 2007. Culture of *Macrobrachium rosenbergii* (de Man, 1879) in experimental cages in a freshwater eutrophic lake at different stocking densities. Aquaculture Research 38: 288-294

3) Reproductive performance of various stocks and species of FW prawn fed high and low protein diets
(Main proponent: MRR Eguia)

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. 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 studies and plans to genetically document stocks have been incorporated in the general proposal entitled "Genetic characterization, domestication and improvement of *Macrobrachium rosenbergii* in the Philippines" (Sulit, 2004).

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

Henry E. Dejarme
Mindanao State University
Naawan, Northern Mindanao

The history of *M. rosenbergii* hatchery operations in Northern Mindanao can be traced from minor activities in different locations by several institutions. Earlier attempts to produce freshwater prawn postlarvae in hatcheries by Mindanao State University (MSU) faculty/researchers were conducted in the MSU-Marawi College of Fisheries (COF) and in commercial hatchery facilities for the tiger shrimp at MSU-Naawan.

Early efforts to produce freshwater prawn seedstock were done by an MSU-Marawi COF faculty member and his staff in the 1970s. Breeders were collected from Kapay, 30 m from the oceanic waters of Iligan Bay. However the group failed to rear larvae successfully to the postlarval stage. In Naawan, several larval rearing trials were conducted in the late 1970s and early 1980s as part of a project that included a study on the biology and ecology of the species in known prawn spawning grounds in Tambulig and Siay, Zamboanga del Sur. Live berried females from the two study sites were transported to Naawan and held in tanks until hatchlings were obtained. Larvae were reared in brackish and greenwater medium and fed *Brachionus*, *Artemia* and strained fish flesh. Unfortunately, not one larval rearing trial was successful.

Hatchery trials were also conducted in 1994 at the Multi-species Hatchery of the Dipolog School of Fisheries in Zamboanga del Norte. Few postlarvae were produced within one year and eventually the school discontinued the activity.

Present Status of Hatchery and Pond Culture

In 2004, eggs from some *M. rosenbergii* breeders from the Misamis Occidental Aquamarine Park (MOAP) in Sinacaban, Misamis Occidental were hatched at the Naawan Hatchery. Records have it that the original breeders came as postlarvae from BFAR-NFFTC, Nueva Ecija and grown to maturity in MOAP earthen ponds. The eggs/newly hatched larvae from MOAP were then successfully reared to postlarvae (~40,000 pcs) at the Naawan Hatchery using brackishwater medium with *Tetraselmis* and fed *Artemia* as well as fish flesh apart from the marine Polychaete (*Pereneries* sp.) and earthworm. From thence, the production of prawn fry at the MSU-Naawan Hatchery has become a continuous activity.

Presently, there are four hatchery facilities in Northern Mindanao that have continuing hatchery activities (Table 1). The founder stock used in these hatcheries came from BFAR-NFFTC in Nueva Ecija.

Table 1. **List of existing *Macrobrachium* hatcheries in Nothern Mindanao**

Name of Hatchery	Agency	Location	Distance from Nearest Seawater Source
MSU-Naawan Multispecies Hatchery	Mindanao University at Naawan	Naawan, Misamis Oriental	Few meters
Kisolan 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

Attempts to rear larvae from wild-sourced broodstock started in July 2004. Broodstock from Panguil bay were transported to MSU-Naawan. The results of the postlarval production from the hatchery-spawned Panguil Bay wild stocks shall be known by September 2004. Aside from Panguil Bay, other sources of wild stocks 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, Misamis Oriental.

There are many sources of prawn breeders from the wild for the three other existing hatcheries in Northern Mindanao. Wild stocks can be obtained from Plaridel, Misamis Occidental, Katipunan River in Dipolog, Zamboanga del Norte, Pulangi River, Rio Grande de Mindanao and major river tributaries of Davao Gulf such as Tagum-Libuganon, Davao, Tuganay, Padada-Guihing and Lasang Rivers. In the CARAGA region, known sites of freshwater prawn broodstock are the rivers in Surigao del Norte, Surigao del Sur, and Agusan River.

Culture of *M. rosenbergii* is in the early stages of development and the culture system is confined only to small-size earthen ponds (200-500 m^2). Historically, the first and only attempt to culture giant freshwater prawn in ponds in 1980s in Northern Mindanao was conducted by MSU-Naawan. The few hundred seedstock obtained from the Tambulig wild population were 7-10 cm long prawn juveniles. Fed chicken pellets, the prawns attained marketable size (30-60g) in five months and some females were berried upon harvest. At present, the BFAR-NFFTC postlarvae were distributed for stocking in BFAR Kisolon, Bukidnon and MOAP, Sinacaban ponds. These were fed fish pellets. Apart from fish pellets, other types of feed (spoiled duck egg or the locally known "balut", rotten fish, and farmed earthworm) were tried.

Potentials for Development

The demand for prawn fry has steadily increased after its first production at MSU-Naawan. The potential for expansion of hatchery production on the other hand, is too early to determine. But judging from the current plight of the tiger shrimp industry the potential could be greater than expected. The culture of giant freshwater prawn in fishponds is a new aquaculture development in Northern Mindanao. It was partly popularized recently by the rice-prawn culture (*Palay-Ulangan*) program of the Philippine Government. This program aimed to utilize the vastv tracts of ricefields in Northern Mindanao as sites for raising freshwater prawns. To date however, other freshwater resources such as springs, natural and man-made dams, finfish ponds that are readily convertible to prawn ponds are being eyed for prawn aquaculture.

Suggestions for Future R&D Activities

Many farmers express their concern over the need for R&D in prawn aquaculture. The concerns are:

1) improved head : body ratio (larger edible portion)
2) reduction of the size of the male prawn's claws
3) production of all-male fry
4) development of late-maturing female prawns

The contention of many aqua-entrepreneurs is that an improvement along these characteristics would make the freshwater prawn more attractive as an aquaculture species for Northern Mindanao. This is clearly suggestive of researches that are within the scope of aqua-biotechnology.

Project Proposal

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

AGENCIES INVOLVED: BFAR-NIFTDC, BFAR-NFFTC, SEAFDEC/AQD
SOURCE OF EXTERNAL FUNDING {If any}: GOJ, Philippine Government
DATE STARTED: January 2004

PROPONENTS {Name}:	**PARTICIPATION** {% time}
Westly R. Rosario, BFAR	30
Editha C. Roxas, BFAR	50
Melchor Tayamen, BFAR	30
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 pursue 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.

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 *M. rosenbergii* using 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; and
8. To train local farmers on proper broodstock and culture management of *M. rosenbergii.*

Brief Description of Methodology

Several known stocks of *Macrobrachium rosenbergii*, from different localities in the Philippines shall be collected and characterized genetically through morphometric and molecular marker analysis. Other indigenous *Macrobrachium* species shall likewise be collected and genetically screened to enable the development of a guide 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, refined 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.

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.

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Project Highlights

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

Maria Rowena R. Romana-Eguia[a], Maria Lourdes C. Aralar[a], Henry Dejarme[b], Westly R. Rosario[c] and Melchor M. Tayamen[d]

[a] Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC/AQD), Binangonan Freshwater Station, Binangonan, 1940 Rizal, Philippines

[b] Mindanao State University, Naawan, Northern Mindanao, Philippines

[c] Bureau of Fisheries and Aquatic Resources, National Inland Fisheries Technology Development Center, Bonuan-Binloc, Dagupan, Pangasinan, Philippines

[d] Bureau of Fisheries and Aquatic Resources, National Freshwater Fisheries Technology Center, Science City of Muñoz, Nueva Ecija, Philippines

Introduction

Extensive studies in the Philippines and in adjacent countries revealed that 528 caridean prawn species are found in this region alone (Chan, 1998). Of all the known species, the giant freshwater prawn is considered the most commercially important. Except for the Philippines, culture of the giant freshwater prawn, *Macrobrachium rosenbergii,* has already made substantial contributions to the local aquaculture production in Southeast Asia, i.e. in Thailand, Malaysia and Indonesia. However, efforts are now being made to improve the aquaculture production of *Macrobrachium rosenbergii* in the Philippines, thus, optimal methods for the culture and propagation of this high value freshwater aquaculture species are being developed by the Philippine Government fishery agencies as well as other research and academic institutions.

The Integrated Rural Aquaculture Program under the ASEAN-SEAFDEC Special Five-Year Program or what is presently known as the Program on the Promotion of Sustainable Aquaculture in the ASEAN Region enabled the Bureau of Fisheries and Aquatic Resources, the Mindanao State University and the Aquaculture Department of SEAFDEC to jointly conduct studies on the genetic characterization, domestication and improvement of *Macrobrachium rosenbergii* stocks in the Philippines in order to improve the aquaculture production of the giant freshwater prawn. Under the collaborative project which started in late 2004, specific research activities under were conducted, and an update on their results are summarized as follows:

A. Collection, domestication and propagation of wild *Macrobrachium rosenbergii* stocks

Proponents: Westly Rosario and Editha C. Roxas, BFAR-NIFTDC

During the first RoundTable Discussion held at the Freshwater Aquaculture Development Center in Sukabumi, West Java, Indonesia in November 2003, the delegates from Thailand reported that the Philippine wild stocks of *Macrobrachium*, *Macrobrachium rosenbergii rosenbergii* Philippine strain could be a better variety and therefore must be protected from contamination by non-indigenous strains. This 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.

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, unpublished) reported 12 species in Luzon with *Macrobrachium rosenbergii* as dominant species.

Collection and Domestication

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. Recently however, a Gene Bank facility for *Macrobrachium* was established by the National Integrated Fisheries Development Center (NIFTDC) in Dagupan City, Pangasinan (Rosario, 2007). This gene bank aims to conserve the country's giant freshwater prawn resource hence live specimens of the various commercially important freshwater prawn species collected in several provinces in the Philippines are being maintained in this facility.

Testing of Local Strains

One local strain of *Macrobrachium* (BFAR 1) collected by BFAR-NIFTDC 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 September 2004. From 100 breeders collected from the wild, the Center is now using 500 F_2 and F_3 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.

B. Morphometric characterization, identification and validation of *Macrobrachium* samples

Proponents: Maria Rowena R. Romana-Eguia, SEAFDEC/AQD
Henry Dejarme, MSU
Westly Rosario, BFAR-NFRDI
Edith Roxas, BFAR-NFRDI

Macrobrachium rosenbergii and other species that closely resemble the giant freshwater prawn can be caught in some of the 25 commercial fishing grounds in the Philippines (Rosario and Tayamen, 2004). Agasen (2001) identified about twelve species of freshwater prawns in a survey of river tributaries and lakes in Luzon, Philippines. An assessment of freshwater prawns in Visayan and Mindanao provinces where even larger *Macrobrachium rosenbergii* samples have been observed, has yet to be undertaken. Although studies that characterize caridean prawns have already been conducted, the exact identities of local species are often difficult to ascertain (Chan, 1998). In the Philippines, the need to validate the identity of freshwater prawn stocks, specifically *Macrobrachium rosenbergii* being collected and used by the various local research and government fishery agencies has been emphasized.

Confusion over the exact identity of both wild and hatchery-bred *M. rosenbergii* stocks stems from the fact that many of the existing hatchery stocks of the giantfreshwater prawn originated from an imported stock from Thailand which was brought in and later promoted by the Philippine Bureau of Fisheries and Aquatic Resources.

It has been shown that the Malaysian, Indonesian and Thai stocks of *Macrobrachium rosenbergii* basically of the western subspecies (*M. rosenbergii dacqueti* Sunier 1925), are different from the eastern subspecies (*M. rosenbergii rosenbergii* de Man 1895) found mainly in the Philippines (New, 2002; De Bruyn *et al.*, 2004; Chand *et al.,* 2005). This study was conducted to: (a) taxonomically validate the identity of the existing hatchery-bred and wild *Macrobrachium rosenbergii* stocks used in commercial aquaculture and *Macrobrachium* research in the Philippines; (b) identify possible sources of good quality *Macrobrachium rosenbergii rosenbergii* in the Philippines (particularly in Visayas and/or Mindanao) which can be used for domestication and selective breeding programs; and (c) characterize other indigenous *Macrobrachium* species that may have some aquaculture potential.

During the implementation of the project in September 2004, arrangements were made for Dr. Daisy Wowor and Prof. Peter Ng (noted crustacean taxonomists from the National University of Singapore and the Museum Bogoriense in Indonesia) to help validate the identity of the freshwater prawn samples collected from selected localities in the Philippines. The samples collected from each of the various sources comprised of at least three adult males, three adult females (preferably berried) and four juveniles. Individual morphometric measurements (rostral teeth, carapace length, body length, total length) were recorded and individual samples were photographed. The collected samples were initially kept for two weeks in 80% ethanol. After two weeks, the samples were individually wrapped in cheesecloth, placed in labeled plastic bags and sealed before these were sent off for validation at NUS.

Table 1 shows the number and identity of the samples collected from hatchery and wild sources in several locations in the Philippines. The Mindanao samples were procured by Prof. Henry Dejarme of the Mindanao State University.

Table 1. **Freshwater prawn samples collected for taxonomic identification**

Source	Number of samples	Identity
A. WILD		
REGION I		
Vigan, Ilocos Sur	3	*Macrobrachium lepidactyloides*
REGION II		
Buguey, Cagayan	13	*Macrobrachium latidactylus*
	4	*Macrobrachium australe*
	2	*Macrobrachium esculentum*
	8	*Macrobrachium lar*
Gonzaga, Cagayan	5	*Macrobrachium lar*
Pamplona, Cagayan	2	*Macrobrachium lar*
REGION III		
Calumpit, Bulacan	21	*Macrobrachium rosenbergii rosenbergii*
	11	*Macrobrachium rosenbergii dacqueti*
Baler, Quezon	5	*Macrobrachium lar*
Tarlac	1	*Macrobrachium idae*
	1	*Macrobrachium lanceifrons*
REGION IV		
Laguna de Bay (Binangonan, Rizal)	9	*Macrobrachium lanceifrons*
	2	*Caridina gracilirostris*
	8	*Caridina blancoi*
REGION V		
Lake Bato, Camarines Sur	10	For identification
Sorsogon	4	For identification
REGION VI		
Tangyan River (Igbaras, Iloilo)	8	*Macrobrachium australe*
	1	*Macrobrachium latidactylus*

	7	*Macrobrachium jaroense*
Leganes, Iloilo	27	*Macrobrachium rosenbergii rosenbergii*
Cairawan River (Laua-an, Antique)	4	*Macrobrachium esculentum*
	10	*Macrobrachium latidactylus*
	6	*Macrobrachium jaroense*
	2	*Macrobrachium horstii*
	1	*Macrobrachium lar*
	2	*Macrobrachium australe*
	2	*Macrobrachium lepidactyloides*
REGION VII	-	Samples yet to be collected
REGION VIII		
Divisoria, Leyte	13	*Macrobrachium latidactylus*
REGION IX		
Dapitan	1	*Macrobrachium rosenbergii*
Tambulig/ Aurora, Zamboanga del Sur	15	*Macrobrachium mamillodactylus*
(Panguil Bay)	12	*Macrobrachium equidens*
	2	*Macrobrachium rosenbergii dacqueti*
	8	*Macrobrachium rosenbergii rosenbergii*
Dinas, Zamboanga del Sur	5	*Macrobrachium rosenbergii dacqueti*
(Illana Bay)	5	*Macrobrachium rosenbergii rosenbergii*
Siay, Zamboanga Sibugay	4	*Macrobrachium rosenbergii dacqueti*
	6	*Macrobrachium rosenbergii rosenbergii*
REGION X		
Layawan, Oroquieta	5	*Macrobrachium lar*
	4	*Macrobrachium jaroense*
	1	*Macrobrachium latidactylus*
	1	*Macrobrachium equidens*
REGION XI		
Lake Apo Bukidnon	7	*Macrobrachium rosenbergii rosenbergii*
REGION XII		
Liguasan Marsh (Pikit side)	11	*Macrobrachium mamillodactylus*
	2	*Macrobrachium weberi*
	1	*Macrobrachium australe*
Pikit, North Cotabato	4	*Macrobrachium rosenbergii*
REGION XIII	3	*Macrobrachium jaroense*
Mangagoy, Surigao del Sur (Bislig Bay)	12	*Macrobrachium mamillodactylus*
	1	*Macrobrachium latidactylus*
Lake Mainit	10	*Macrobrachium lanceifrons*
Agusan River, Agusan del Sur	8	*Macrobrachium mamillodactylus*
	2	*Macrobrachium esculentum*
Bislig River	4	*Macrobrachium mamillodactylus*
ARMM		
Lake Lanao, Lanao del Sur	10	*Macrobrachium latidactylus*
B. HATCHERY		
SEAFDEC/AQD-1 (BFAR stock)	10	*Macrobrachium rosenbergii dacqueti*
SEAFDEC/AQD-2 (Leganes F_1)	60	*Macrobrachium rosenbergii rosenbergii*
SEAFDEC/AQD-3 (Zambales; orig. Calumpit)	30	Morphologically *M. r. dacqueti* but possibly mixed stock from *M. r. dacqueti* and *M.r.rosenbergii* cross
BFAR 0	5	*Macrobrachium rosenbergii dacqueti*
BFAR 1	10	*Macrobrachium rosenbergii rosenbergii*

The distinguishing characteristics of five major species which were identified from the samples are briefly described below :

1) *Macrobrachium rosenbergii* (giant river prawn)

Eastern form: *M. rosenbergii rosenbergii* (de Man, 1879)
Western form: *M rosenbergii dacqueti* (Sunier, 1925)

There are several subtle differences between these two forms or subspecies of *Macrobrachium rosenbergii.* However the main difference between them is the basal crest of the rostrum. The basal crest of the *M. rosenbergii dacqueti* is higher than that of the endemic *M. rosenbergii rosenbergii.* Apart from this feature, the body of the *M. rosenbergii dacqueti* is dark green to grayish blue with longitudinal streaks of darker and lighter color while that of the *M. rosenbergii rosenbergii* has some pattern as shown below. The giant prawn's long rostrum extends beyond the antennal scale and has 11-14 upper teeth and 8-14 lower teeth. The *M. rosenbergii's* second legs are very large, robust and of same size. In adult males, the entire second leg is densely covered with spines and sharp tubercles. The giant river prawn is the largest known *Macrobrachium* species. From the samples that were analysed, the largest adult individuals were obtained from Dinas and Tambulig, with total lengths of 23.6 cm and 23.5 cm, respectively. In *M. rosenbergii rosenbergii* all antenna are blue while in *M. rosenbergii dacqueti,* only the second antennae are blue, the rest are brown.

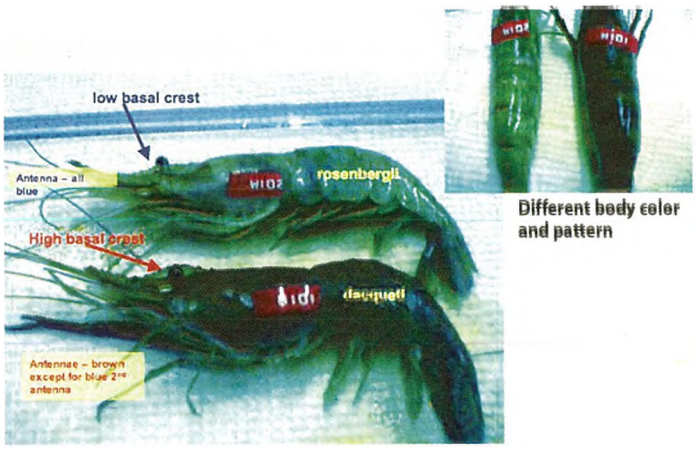

Figure 1. **Sample of *Macrobrachium rosenbergii rosenbergii* and *Macrobrachium rosenbergii daqueti***

This prawn is rarely found in pure freshwater. They normally thrive in lower parts of streams, river mouths, estuaries where the water has a higher salinity (brackishwater) as it breeds in brackish and seawater (Chan, 1998). From the samples analysed, 13 individuals from Tambulig were *M. equidens* (2.2 cm average carapace length, 9 cm total length, 7.2 cm body length, and 9 upper and 5 lower rostral teeth). The rostrum of the *M. equidens* almost always exceeds the distal end of the antennal scale. The large second legs are marbled like tortoise shell.

3) *Macrobrachium mamillodactylus* (knobtooth prawn)

The distinct feature of this species is the shape of the rostrum. The tip of the rostrum does not reach the distal end of antennal scale. The large second legs are longitudinally striped. The fingers of the second legs are not covered by soft short hair, but instead have rows of tubercles along the cutting edges. Samples of *M. mamillodactylus* were obtained from Tambulig and Mangagoy in Mindanao. The average measurements of the samples were: 3.6 cm carapace length, 12.1 cm total length and 10 cm body length. The rostrum has 11-13 upper teeth and 3-5 lower teeth.

4) *Macrobrachium latidactylus* (scissor river prawn)

This species is found mainly in estuarine and inshore marine waters. Adults are commonly found in tidal freshwater but larval development is in sea or brackishwater. One of the distinguishing features of this species is the unequal size of the large second leg even in young specimens. *M. latidactylus* samples were obtained from Mangagoy and Lake Lanao. The largest sample was obtained from Mangagoy (2.8 cm carapace length, 7.1 cm body length and 8.1cm total length).

5) *Macrobrachium lanceifrons* (Philippine river prawn)

This species is locally known as *hipon tagunton*. It is one of the commercially important prawn species in Laguna de Bay as it is used for human consumption and for duck food. The tip of the rostrum of *M. lanceifrons* is slightly curved upwards in full grown individuals but straight in the young. The second pair of walking legs or chelipeds is equal in length in young specimens but unequal in fully grown individuals. Fully grown males are best distinguished from fully grown females by the length and shape of the second leg or cheliped. In the male, this is longer and is provided with felted hairs on the mobile finger. Samples of *M. lanceifrons* were identified from the collection obtained in Lake Mainit. The Lake Mainit samples had a 1.5 cm average carapace length, 4.3 cm. body length and 5.3cm total length.

Thus far, we have identified and taxonomically validated 14 *Macrobrachium* species (*M. australe*, *M. equidens*, *M. esculentum*, *M. horstii*, *M. idae*, *M. jaroense*, *M. lanceifrons*, *M. lar*, *M. latidactylus*, *M. lepidactyloides*, *M. mamillodactylus*, *M. rosenbergii rosenbergii*, *M. rosenbergii dacqueti*, *M. weberi*) found mostly in Visayas and Mindanao. To complete the list, samples of four species confirmed in earlier reports as found in the following sites: Cebu, Mindoro, Camarines Sur, Surigao del Norte and Samar. These species are *Macrobrachium nipponense* (non-indigenous species brought into the Philippines and stocked in Camarines Sur), *Macrobrachium placidulum*, *Macrobrachium scabriculum* and *Macrobrachium latimanus* shall be collected. Once completed, we soon hope to finish the draft of the scientific manuscript and field identification guide based on the data generated from this study.

From some of the collected samples, observations on the breeding behavior and distribution in specific habitats/microhabitats will be noted in order to determine their exact nature (that is whether they are found naturally in the collection areas as wild stocks or as accidental/intentional introductions from hatchery populations).

Samples especially of *M. rosenbergii dacqueti* and *M. rosenbergii rosenbergii* are being processed for genetic marker analysis (mtDNA sequence and hopefully msDNA analysis) at the SEAFDEC-based Aquaculture Biotechnology Laboratory. Samples of these two subspecies will be analysed to determine the level of intraspecific variation among the populations and their phylogenetic relationships as many of these stocks are now found mixed in several commercial fishing grounds in the Philippines.

C. Genetic characterization of commercially important Philippine stocks of freshwater prawn, *Macrobrachium* sp., using DNA markers

Proponents: Maria Rowena R. Eguia, SEAFDEC/AQD
Henry Dejarme, Mindanao State University

The main objectives of this study are: a) to genetically characterize existing hatchery-bred and wild *Macrobrachium rosenbergii* stocks using mtDNA-RFLP markers; and b) to determine and compare the genetic diversity of the various prawn stocks that could serve as baseline data for biodiversity conservation or for a genetic program that will enhance growth and other economically important traits in the *Macrobrachium* sp.

Pleopod and muscle tissue samples were taken from several freshwater prawn stocks obtained from various locations. Methods to extract DNA and PCR-amplify mitochondrial DNA cytochrome oxidase I (CO-I) from wild and hatchery samples of *M. rosenbergii* have been optimized. mtDNA CO-I has been successfully PCR-amplified using Carini and Hughes (2004) protocol as follows: 5 min at 94°C; 35 cycles of – 30 sec denaturation at 94°C, 30 sec annealing at 55°C, 45 sec extension at 72°C; 7 min at 72°C. Processing of samples for genetic variability analysis is 70% completed. Preliminary results show distinct genetic differences between hatchery and wild stocks based on restriction morphs obtained after digestion with restriction enzymes: *Hae III, Rsa I, Msp I, EcoRI and Mbo*. However parameters estimating genetic variation can only be computed after all the samples have been analysed.

Conditions for mtDNA sequencing using primers flanking the mtDNA CO-I region are now developed and have been optimized. Thus far mtDNA CO-I from one hatchery stock (n=3 individuals, all *M. rosenbergii dacqueti* from Thailand) and three wild stocks: Leganes (n=5 individuals), Pampanga (n=3 individuals) and Zambales (n=5 individuals) have been sequenced. The wild stocks Leganes and Pampanga are taxonomically *M. rosenbergii rosenbergii* while the Zambales stock's identity has yet to be confirmed as either *M. rosenbergii dacqueti* or an interspecific hybrid based on the comparison of the sequence analysis. Work on mtDNA sequencing using representative samples from each stock shall be continued with the sequencing of *M. rosenbergii rosenbergii and M. mamillodactylus* (the outgroup) samples from Mindanao.

D. Evaluation of growth performance of two strains of *M. rosenbergii* in cages in Laguna de Bay

Proponent: Maria Lourdes C. Aralar, SEAFDEC/AQD

Macrobrachium rosenbergii from two separate stocks (CAL-progenies of the native strain from Calumpit, Bulacan; and BFAR-progenies of the strain from BFAR, originally from Thailand) were reared in net cages in Laguna de Bay at 15 prawns m^2. Two runs have been conducted for five months, the first run was from October 2004 to March 2005 and the second run was from April to September 2005. In the first run, CAL showed significantly better specific growth rate (SGR) than BFAR (4.6 vs 3.9%) but no differences in final weight, yield, and feed conversion ratio (FCR). Although CAL showed slightly better survival than BFAR, the difference was not significant (74.3 vs 69.1%). For the second run, survival (80.4 vs 61.1%), SGR (2.9 vs 2.6%), and FCR (2.1 vs 2.7) were significantly better in CAL than BFAR. Like the first run, there were no significant differences in the final weight of the two strains (24.0 vs 24.3g). Figures 1, 2 and 3 show the weight, SGR and survival trends in both BFAR and CAL stocks during the two runs.

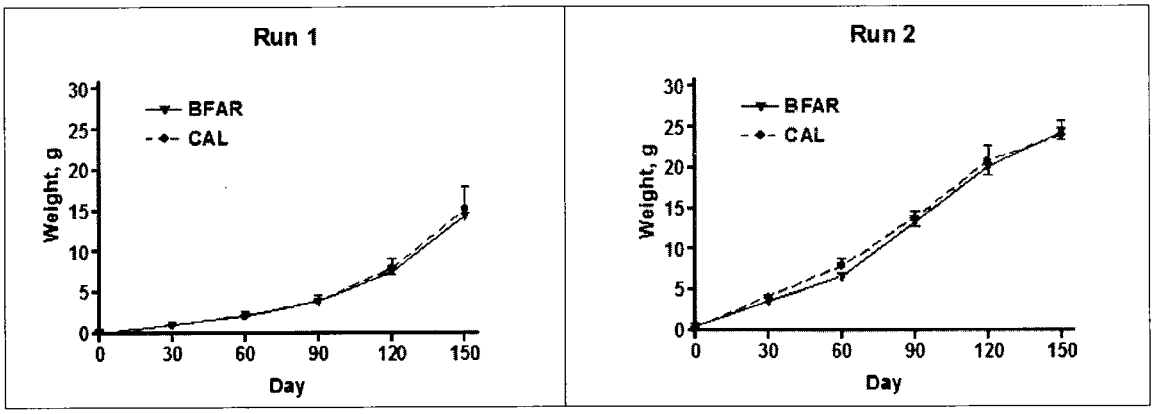

Figure 2. **Graphs showing the increase in weight of the stocks, BFAR and CAL during the two experimental runs**

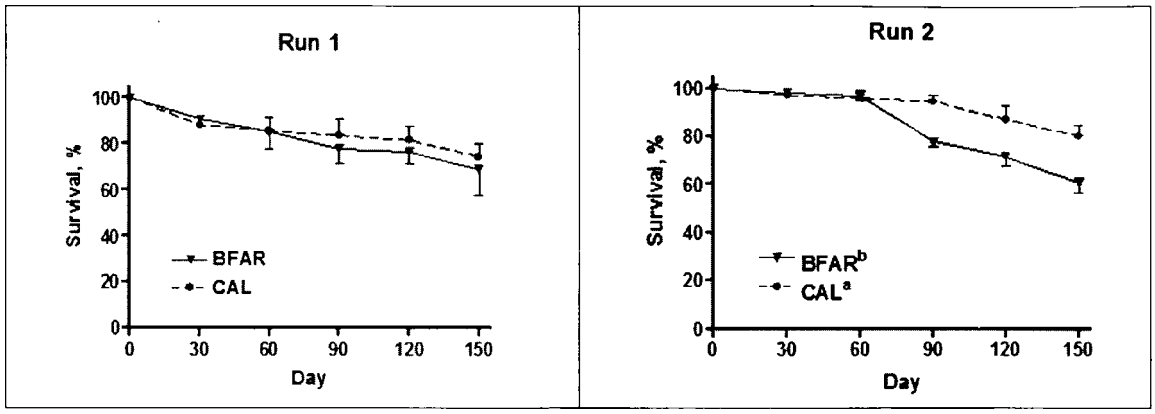

Figure 3. **Graphs showing the percentage survival of the stocks, BFAR and CAL during the two experimental runs**

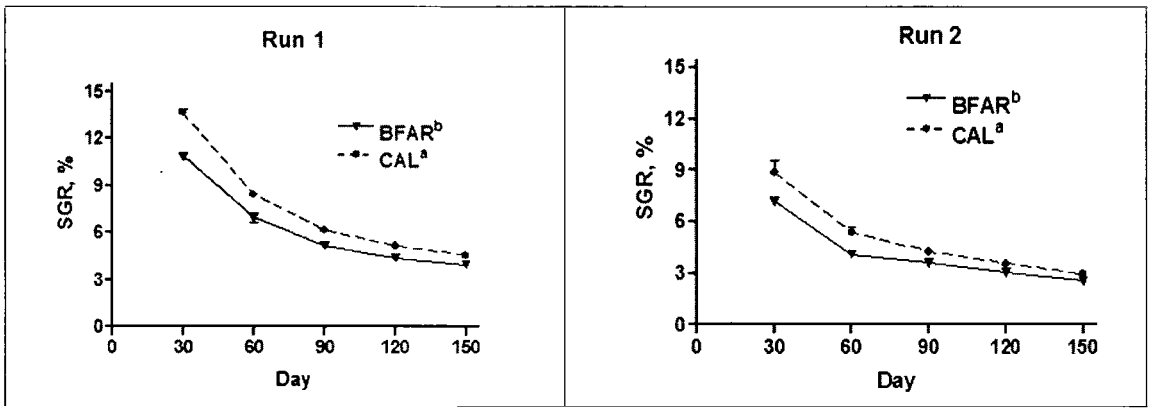

Figure 4. **Graphs showing the specific growth rate (SGR %) of the stocks, BFAR and CAL during the two experimental runs**

Meanwhile, post larvae of *M. rosenbergii* obtained from broodstock from Calumpit (in Bulacan) were stocked in commercial sized (7 x 7 x 1 m) cages in the lake in February of this year. Treatments included various feed types (shrimp feed, tilapia feed, catfish feed); mesh size of cages (*hapa* vs. b-net); and shelter (with or without). After two months of culture in the net cages, results show that feed type, and presence or absence of net shelters had no significant effect on growth and survival of *M. rosenbergii*. Mesh size affected survival, with higher rates observed in those reared in *hapa* net (91%) compared with those in b-net cages (78%). Larger prawns were observed in the *hapa* cages (3.4g) compared to those in b-net cages (2.7g), although the difference was not statistically significant.

E. Performance of juvenile *Macrobrachium rosenbergii rosenbergii* and *Macrobrachium rosenbergii dacqueti* cultured in *hapa* net cages at three stocking densities

Proponent: Henry Dejarme, Mindanao State University

This study aims to determine the suitable densities for *M. rosenbergii rosenbergii* and *M. rosenbergii dacqueti* in *hapa* nets in a nursery facility. The juveniles used were progenies of wild *M. rosenbergii rosenbergii* from Leyte and that of the domesticated *M. rosenbergii dacqueti* stock. The stocking densities used in the rearing trials were 25, 35 and 50 juveniles per square meter and the trial lasted for three months. The prawns were fed commercial shrimp pellets at 10% body weight from

August 24 to November 5, 2005 or 73 days. Every two weeks, prawn samples were taken for growth measurements (weight) and survival rates were noted.

Results showed that *M. rosenbergii dacqueti* is a fast-growing sub-species than *M. rosenbergii rosenbergii* (Figure 4). Discrepancies in size between the two subspecies were more prominent in the final weight measurements on the 73rd day of culture. The final result is consistent with the sluggish weight increases recorded every two weeks for the native subspecies. On survival however, *M. rosenbergii dacqueti* had a lower survival rate. This is attributed to the mass mortality observed on the 47th day of culture. Barring the occurrence of mass mortality which could have been prevented, it can be stated although inconclusively, that while the type of subspecies significantly influenced survival and growth rates in this study, the densities did not. However confirmatory trials must be conducted to verify the results of this study.

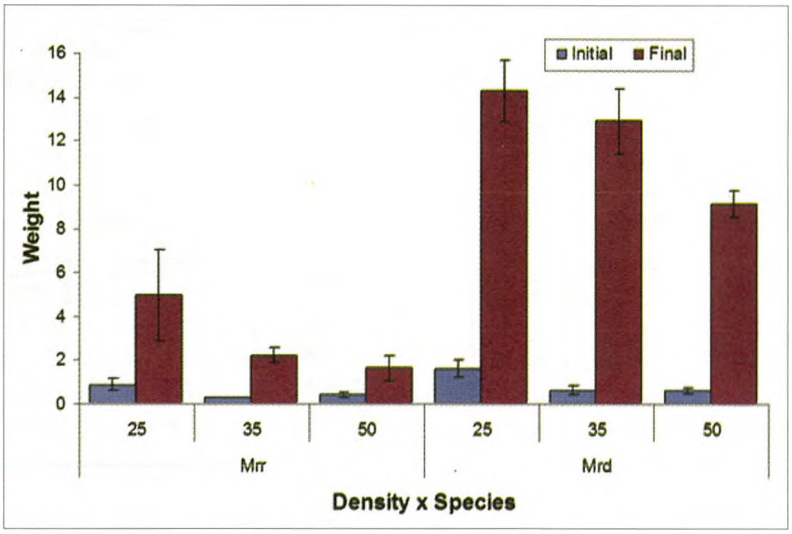

Figure 5. **Initial and final weight of *Macrobrachium rosenbergii dacqueti* and *M. r. rosenbergii***

F. Culture of juvenile *Macrobrachium rosenbergii dacqueti* in *hapa* net cages at four stocking densities

Proponent: Henry E. Dejarme, Mindanao State University

The traditional practice in places where *M. rosenbergii* are grown to marketable size in earthen ponds is direct seeding with hatchery-reared post larvae (PL). However, an increasing number of grow-out farmers prefer to stock larger juveniles (average weight = 2.0g) that have been reared from PL for two months in nursery facilities because, among others reasons, early mortalities will have already occurred before the seed stocks are transferred to grow-out facilities. Nurseries can be stocked at much higher densities than grow-out enclosures but optimum density has not been clearly ascertained. This study was conducted to determine the suitable densities for the production of large juvenile prawns of *M. rosenbergii dacqueti* Sunier 1925 in 1 x 1 x 1 m *hapa* nets. The densities 50 juveniles, 100, 200, and 300 per sq. m were tested in RCBD experiment at the concrete reflecting pool at MSU-Naawan. Two batches of hatchery-produced juveniles were used and cultured separately in Modules 1 and 2. The juveniles were fed commercial shrimp pellets for two months (64 days) and sampled for survival and growth in terms of weight, length every two weeks. The highest average weight gain and body length were obtained from stocking density of 50 pieces per sq. m. both for Module 1 (2.07g, 2.07 mm) and Module 2 (2.4g, 2.4 mm) (Figure 5). Survival was highest at stocking density of 200 juveniles per sq. m (Figure 6).

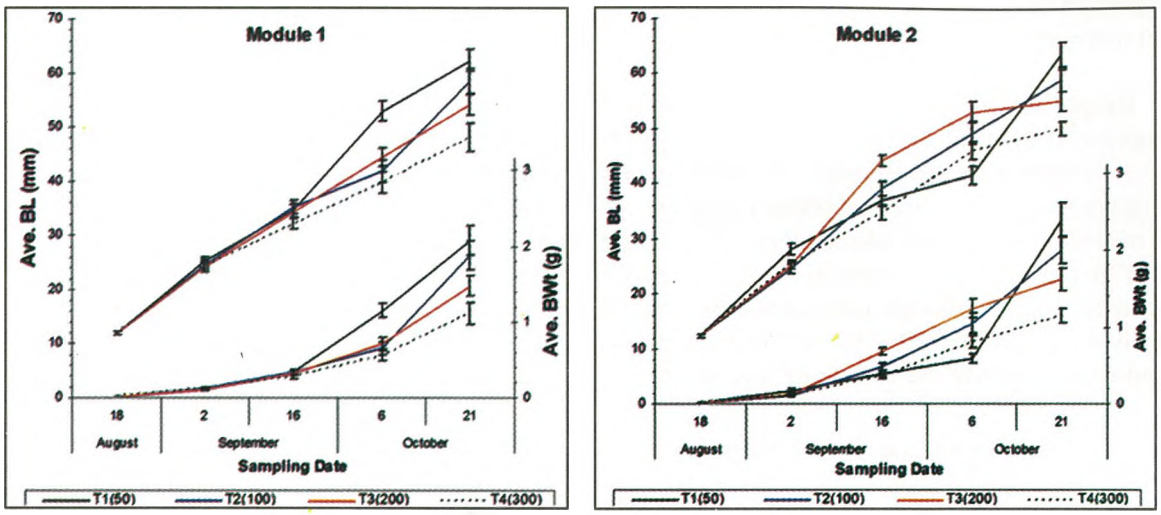

Figure 6. **Prawn juveniles in Modules 1 and 2**

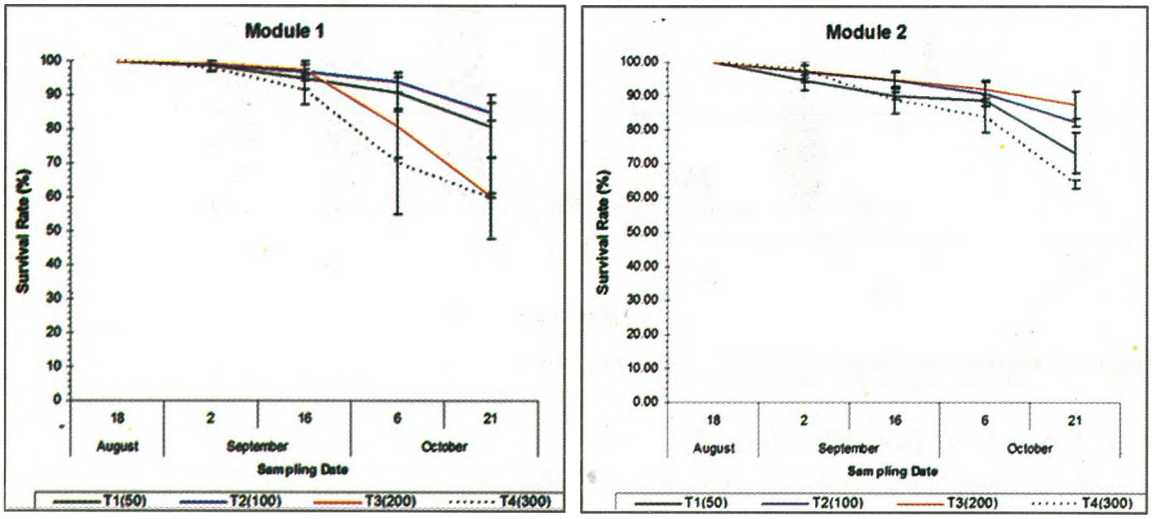

Figure 7. **Survival curves of experimental prawn juveniles in Modules 1 and 2**

A well recognized characteristic of *Macrobrachium* species is that individual prawns grow distinctly at different rates. Such growth feature was observed in the present nursery study. This phenomenon is also known as heterogeneous individual growth (HIG). Studies have demonstrated that some exceptionally fast-growing individuals may become up to 15 times larger than the population mode within 60 days after metamorphosis. Jumpers became obvious within two weeks after metamorphosis. Slow-growing prawns (laggards) only become apparent later, about five weeks following metamorphosis.

In a nursery study, however, survival is more important than growth because the interest is to produce the most number of larger juveniles per sq. m for subsequent stocking in grow-out ponds. In the present study, the results suggest 200 prawn juveniles per sq. m is a suitable initial stocking density in nursery culture. Yet the results are not definitive because the survival at lower densities (50 and 100 pieces per sq. m) was unexpectedly lower. No matter the reasons for this least outcome, the results still suggest that high survival can be attained at 200 prawn juveniles per sq. m stocking rate. If 200 is the most desirable initial stocking density in the nursery, then a big nursery cage or a number of smaller cages with a total of 570 sq. m area is needed to stock a hectare of grow-out prawn pond at the usual stocking rate of 10 juvenile prawns per sq. m.

F. Reproductive efficiency of two *M. rosenbergii* stocks at different protein levels

Proponent: Maria Rowena R. Eguia, SEAFDEC/AQD

Spawning sets (1 male : 5 females) of four-month old *Macrobrachium rosenbergii* from a hatchery stock (BFAR strain, *M. rosenbergii rosenbergii*) and a wild stock (Calumpit strain, possibly *M. rosenbergii rosenbergii x M. r. dacqueti* F_1s) were placed in replicate 2 x 2 x 1 m outdoor concrete tanks in April 2005. Stocks were fed using the following treatments: Treatment A: low protein (commercial fish feed pellets) at 2% of the prawn biomass; Treatment B: high protein (prawn feed pellets) at 2% of the prawn biomass and Treatment C: low protein (commercial fish feed pellets), given *ad libitum*. The reproductive efficiencies of the stocks were compared. Six months after stocking, preliminary observation showed that the BFAR stock fed low protein diet (fish feed pellets) *ad libitum* spawned more frequently (average number of spawning episodes=15.7) than prawns given fixed amounts of fish feed (10.7) and prawn feed (6.3). Calumpit stocks spawned less frequently at 9 (Treatment C), 8.7 (Treatment A) and 6 (Treatment B) spawning episodes. The average number of hatchlings produced per gram body weight of the female prawn broodstock was highest in the BFAR stocks at 669.7 (for treatment C), 665.28 (for treatment B) and 567.2 (for treatment A). The same ranking was observed in the Calumpit stock at 598.4, 532.7 and 438.7, respectively.

A similar experiment was also set up in lake-based netcages using five-month old prawns to determine if the reproductive efficiency of the two strains is influenced by the type of spawning system. Results showed differences in the reproductive efficiency of the two stocks especially in terms of the average number of hatchlings per gram female body weight. BFAR stocks fed low protein fish feed *ad libitum* had the highest number of hatchlings at 648/g body weight followed by those fed fish feed at 2% prawn biomass (583/g) and the high protein prawn feed pellets (578/g). On the other hand, Calumpit stocks fed low protein fish feed at 2% prawn biomass had the most number of hatchlings per gram female body weight (823/g), followed by low protein fishfeed administered *ad libitum* (741/g) and finally high protein prawn feed (609/g).

H. Promotion of freshwater prawn farming technology

Proponent: Melchor Tayamen, BFAR NFFTC

The Philippine Bureau of Fisheries and Aquatic Resources (BFAR) continues to pursue the aquaculture of *ulang* at the NFFTC in Munoz, Nueva Ecija (Central Luzon) as well as disseminate the potential and opportunities that freshwater prawn farming can offer. In 2004, BFAR established a Task Force with NFFTC as homebase for the promotion of the freshwater prawn 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 freshwater prawn hatcheries; (3) lease, improvement or conversion of unproductive shrimp hatcheries into multi-species hatcheries that will include freshwater prawn hatchery 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 potential 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.

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RECOMMENDATIONS FOR FUTURE RESEARCH AND OTHER ACTIVITIES

PROPOSED PROGRAM OF ACTIVITIES
GENETIC IMPROVEMENT AND SEED PRODUCTION OF *MACROBRACHIUM*

Priority Activities	Duration	Responsible Country/Agency
Genetic Improvement of *M. rosenbergii*		
• Research		
○ Strains of *M. rosenbergii* with better seed production traits and grow-out characteristics	(target dates)	Thailand, Indonesia, Philippines
Indonesia: (1) Collection of wild stock from Sulawesi to construct a base population GI Macro II, and another potential populations such as Kalimantan (2006) (2) Evaluation and characterization of GI Macro II, Sulawesi and Kalimantan using molecular marker (2006) (3) Selective breeding program on the synthetic population (2007-2009)	2007	
Philippines: (1) Continue activities on procurement of good broodstock, performance evaluation, improve hatchery & nursery operations (2006) (2) Domestication and selective breeding (2006-2010) (3) Training/ capacity building (2006-2010) (4) Continue stocking density investigation on 5-10 day old PL (post larvae) at much higher densities for both species and for other indigenous species (2006)	After 2010	
Thailand: (1) Appropriate selective breeding program to improve growth of *Macrobrachium rosenbergii* in different parts of Thailand (2) Use of allozyme marker to detect genetic variation in *Macrobrachium rosenbergii* together with growth performance in selective breeding program	2009	
○ Genetically characterized strains of *M. rosenbergii* of member countries/characterized *M. rosenbergii* stocks in SE Asia (population variation in each country: country-to-country requirements, except Thailand and Indonesia) with Thailand and Indonesia; Member countries	2008-2010	AQD in consultation
• Human Capacity Building		
○ Manual on grow-out of *M. rosenbergii* in ponds and lake based cages	End 2006	Thailand, Indonesia, Philippines
○ Popular publication on genetic improvement of *M. rosenbergii*	End of 2008	Thailand, Indonesia
○ Manual on protocols for the genetic characterization of *M. rosenbergii*	2007	Thailand, Indonesia, Philippines
○ Training for technical persons and extension officers on hatchery and grow-out of *M. rosenbergii*	mid 2006	
○ Training for fish farmers at core countries	Late 2006	Thailand, Indonesia, Philippines

○ Farm demonstration for recipient countries	Late 2007	Lao PDR, Cambodia, Myanmar, Brunei Darussalam
○ On-site training for fish farmers of recipient countries	Late 2008 or early 2009	Lao PDR, Cambodia, Myanmar, Brunei Darussalam
○ Training on genetic characterization of *M. rosenbergii*	2007	AQD, core countries

ANNEX

COST AND RETURN ANALYSIS OF FRESHWATER PRAWN CULTURE TECHNO-DEMO IN CAUAYAN, ISABELA, PHILIPPINES (see other pages)

Pond Area	0.05 ha (500 m^2)
Culture Period	4-6 months
No. of croppings	2 croppings/year
Stocking rate	5 pc/m^2
PL Requirement	2,500 pcs
PL cost	P 2.50/pc
Survival rate	75%
No.of stock	1,875 pcs
Size of harvest	25 pc/kg (ABW 40g)
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 @ 20.00 kg
Fertilizer/chemical required	P 1,500.00
Capital cost for 500 m^2	
Cost of land (500 m^2)	P 20,000.00
Construction cost farm implement	2,000.00 1,500.00 P 23,500.00
Production cost per cropping (2 croppings/year)	
Post-harvest at 5 pc/m^2 (2,500 pcs @ P 2.50) Feeds (150 kg @ P 20.00/kg) Fertilizer/chemicals Labor Travel/shipment Cost	P 6,250.00 3,000.00 1,500.00 600.00 1,500.00 P12,850.00
Depreciation/Year	
Construction Cost Farm implements	P 400.00 500.00 P 900.00
Sales	
500 m^2 x 5pc PL/m^2 x 2 croppings 5,000 pcs x 75% recovery @ 25 pcs/kg	5,000 less 25% monthly 150 kg x P 350.00 kg P 52,500.00

Total Project Cost	
Capital Cost Working Capital	P 23,500.00 12,850.00 P 36, 350.00
Net income before tax Sales - 52,500 - (25,700 + 900) = 52,500 - 26,600	= P 25,900.00
Net Income after tax Net income = P 25,900.00 – 15% provision	= P 22,015.00
Cash Payable Period 36,350.00 22,015.00	ROI = 22,015.00 x 100% = 60% 36,350.00

From the Output of the Planning Workshop for the Special Five-Year Program, Bangkok, Thailand, 29 November – 2 December 2005

PROJECT TEAM MEMBERS

INDONESIA	
Dr. Estu Nugroho Director Research Institute for Freshwater Aquaculture Jl Sempur No.1, Bogor, West Java, Indonesia Tel: (62-251)313200 Fax: (62-251)327890 E-mail: estunugro@yahoo.co.uk	**Mr. Maskur** Head Sukabumi Freshwater Aquaculture Development Center Ministry of Marine Affairs and Fisheries Jl Selabintana No. 17, Sukabumi 43114, West Java, Indonesia Tel: (62-266)225211, 225240; Fax: (62-266)221762 Email: bbats@telkom.net
THAILAND	
Mr. Wattana Leelapattara Director Aquatic Animal Genetics Research and Development Institute (AAGRDI) Department of Fisheries Khling-ha, Khlong Luang, Pathomthani 12120 Thailand Tel: (66-2) 577 5058 to 60 Fax: (66-2)577 5062 E-mail: wattan@fisheries.go.th	**Dr. Supattra Uraiwan** Head, Marine and Coastal Fish Biotechnology Research Group Aquatic Animal Genetics research and Development Institute (AAGRDI) Department of Fisheries Khlong-ha, Khlong Luang, Pathomthani 12120 Thailand Tel: (66-2)577 5058 to 60 Fax: (66-2)5775062 Email: supatra@fisheries.go.th supathrau@yahoo.com
Dr. Panom K. Sodsuk Head Inland Fish Biotechnology Research Group Aquatic Animal Genetics Research and Development Institute (AAGRDI) Department of Fisheries Khlong-ha, Khlong Luang, Pathumthani, Thailand Tel: (66-2) 577 5058 to 60 Fax: (66-2)577 5062 Email: panoms@fisheries.go.th panomks@yahoo.com	**Dr. Srirat Sodsuk** Aquatic Animal Genetics Research and Development Institute (AAGRDI) Department of Fisheries Khlong-ha, Khlong Luang, Pathumthani, Thailand Tel: (66-2) 577 5058 to 60 Fax: (66-2)577 5062 Email: srirats@fisheries.go.th sriratsodsuk@yahoo.com
Wisanuporn Rattanariwong Chief Uttaradit Fisheries Test and Research Center Umphur Tron, Uttaradit, Thailand Tel: (66-55) 491002; (66-1) 688 8766 Fax: (66-55) 49534 Email: wisanuporn@thai.com	**Dr. Sanga Leesanga** Director, Chumphon Fisheries Test and Research Center Maung, Chumphon 86100 Thailand Tel: 0-7750-3358 Fax: 0-7750-3358 Email: leesanga@hotmail.com
Kridsanupan Komanpirin Fisheries Biologist 12/35 M 8T Thungka Maung District Chumphon Province 86100, Thailand Tel: (66-77) 503358 Fax: (66-77) 503358 Email: cpngenetics@thaimail.com	**Buddama Sriprapa** Fisheries Biologist Petchaburi Test and Research Center T. Lamphukbae A. Banlam P. Petchaburi, Thailand Tel: (66-32) 478210 Fax: (66-32) 478211 Email: phgre_dof@yahoo.com

PHILIPPINES	
Dr. Westly Rosario Director National Integrated Fisheries Technology Development Center Bureau of Fisheries and Aquatic Resources Bonuan Binloc, Dagupan City, Philippines Tel: (63-75) 523 5412 Fax: (63-75) 523 0385 Email: westlyrosario@yahoo.com	**Ms. Editha C. Roxas** Aquaculturist National Integrated Fisheries Technology Development Center Bureau of Fisheries and Aquatic Resources Bonuan Binloc, Dagupan City, Philippines Tel: (63-75) 523 5412 Fax: (63-75) 523 0385 Email: ecroxas05@yahoo.com
Dir. Melchor M. Tayamen Chief, National Freshwater Fisheries Technology Center Bureau of Fisheries and Aquatic Resources CLSU Compound, Science City of Muñoz Nueva Ecija, Philippines Tel: (63-44)456 0671 Fax: (63-44)4560671 Email: nfftc@mozcom.com	**Prof. Henry E. Dejarme** Faculty/Researcher Mindanao State University at Naawan Naawan 9023, Misamis Oriental, Philippines Tel/Fax: (638822)720552 E-mail: hed_seth@yahoo.com
Dr. Maria Rowena R. Romana-Eguia Scientist SEAFDEC Aquaculture Department Binangonan Freshwater Station Binangonan, 1940 Rizal Philippines Tel/Fax: (63-2)6523244; (63-2) 2893687 E-mail: mreguia@seafdec.org.ph	**Dr. Maria Lourdes C. Aralar** Scientist Binangonan Freshwater Station SEAFDEC Aquaculture Department Binangonan, 1940 Rizal Philippines Tel: (63-2)652 0527; (63-2)289 3687 E-mail: mlcaralar@seafdec.org.ph