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

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Bangkok, Thailand
3-4 December 2005

The Round Table Discussion is one of the major activities of the collaborative project on Genetic Improvement and Seed Production of *Macrobrachium rosenbergii* under the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region: Aquaculture Component.

The participating countries of the collaborative project:

Indonesia 

Thailand 

Philippines 

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Cover photo: AAGRDI, Thailand

From the Office of the Chief

The collaborative project on Genetic Improvement and Seed Production of *Macrobrachium rosenbergii* is a major component of the research activity under SDII-2: Supply of Good Quality Seeds of the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region: Aquaculture Component.

Under this collaborative project, closely related activities are conducted in each participating country (Indonesia, Thailand, the Philippines), in order to improve the genetic characteristics and seed production technology for quality eggs of *M. rosenbergii* necessary for culture.

Three round table discussions were held under the collaborative project. In the first discussion in Sukabumi, Indonesia in 2003, the definite work plan was formulated and the delineation of efforts was established. The second discussion in Dagupan City and the Science City of Muñoz in the Philippines in 2004 assessed the progress of activities and identified the problems encountered in the implementation of the collaborative project.

This time, the third round table discussion was convened in Bangkok, Thailand in December 2005 in order to identify problems and constraints as well as evaluate technologies being developed through the collaborative project.

As the year 2005 is also the final year of the implementation of the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region: Aquaculture Component vis-à-vis the collaborative project, it was therefore deemed necessary to hold the third round table discussion in order to plan the future course of action of the research activity.

However, in the ensuing SEAFDEC discussions on the ASEAN-SEAFDEC Special Five-Year Program on Sustainable Fisheries for Food Security in the ASEAN Region, AQD has been assured of the continued implementation of the collaborative project under Phase II of the Special Five-Year Program. This is because the economic importance of *M. rosenbergii* for food security in the ASEAN region has been well recognized.

Therefore, as Chief of the SEAFDEC Aquaculture Department (AQD), allow me to thank the collaborating agencies and institutions of the collaborative project for their continued efforts and cooperation, specifically: the Aquatic Animal Genetics Research and Development Institute of the Department of Fisheries of Thailand; the Research Institute for Freshwater Aquaculture, and the Sukabumi Freshwater Aquaculture Development Center of the Directorate General of Aquaculture of Indonesia; the National Freshwater Fisheries Technology Center and the National Integrated Fisheries Technology Development Center of the Bureau of Fisheries and Aquatic Resources in the Philippines; and the Mindanao State University at Naawan (Philippines) for collaborating with AQD's Binangonan Freshwater Station.

Recognizing that there is more to be done as far as research on the genetic improvement of *M. rosenbergii* is concerned, AQD has recommended for the extension of the collaborative project from 2006 to 2010. Because of the significant accomplishment of the project, we have been assured of its continuation. Let us therefore continue our dedicated efforts in this collaborative project in order to attain our objectives.

ROLANDO R. PLATON, Ph.D.
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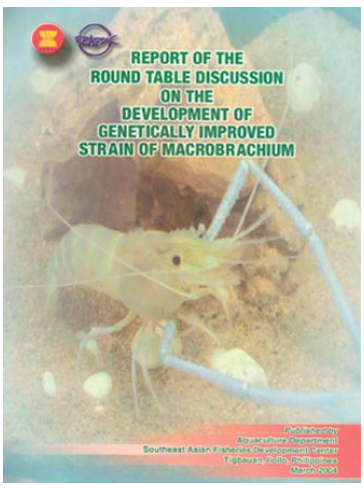
January 2006

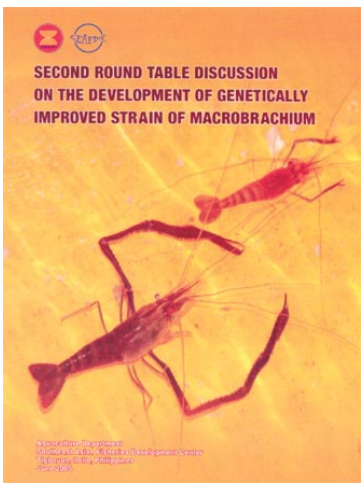
THE THIRD ROUND TABLE DISCUSSION ON THE DEVELOPMENT OF GENETICALLY IMPROVED STRAIN OF MACROBRACHIUM
Bangkok, Thailand
3-4 December 2005

INTRODUCTION

During the Seminar-Workshop on the Integrated Regional Aquaculture Program (IRAP) as the Aquaculture Component of the Special Five-Year Program, held in Thailand in September 2002, a common species with the required technology was identified as priority by three countries. The giant freshwater prawn, *Macrobrachium rosenbergii* and the genetic improvement and seed production of the giant freshwater prawn, were identified as the priority common species and the required technology, respectively. Thus, the collaborative project on Genetic Improvement and Seed Production of *Macrobrachium rosenbergii* has been conducted as part of the activities of IRAP from 2003 to 2005 with Indonesia, Thailand and the Philippines as the participating countries. The collaborative project was intended to optimize resources in these countries while working towards the improvement of the genetic characteristics and seed production technology of *M. rosenbergii*.

Including this meeting, three Roundtable Discussions have been conducted under this Collaborative Project.





Convened in Sukabumi, West Java, Indonesia in November 2003, the first discussion came up with the following recommendations: (a) adoption of common criteria for "good quality Macrobrachium seed"; (b) standardization of methodologies in the development of a genetically improved Macrobrachium strain; (c) formulation of a definite work plan for the collaborative project; and (d) development of mechanisms for the dissemination of research results and use of genetic materials on Macrobrachium.

During the First Roundtable Discussion, it was agreed that the participating countries work on specific activities to avoid duplication of efforts. Thus, Indonesia concentrated on the Evaluation of the growth rate of GI (Genetically Improved) Macro II strain in different locations while Thailand's work is on the selective breeding for genetic improvement of *M. rosenbergii*. The Philippines has been tasked to study the morphometric characterization and performance evaluation of Macrobrachium stocks and closely related species in the Philippines.

The Second Roundtable Discussion was conducted in Dagupan City and the Science City of Muñoz in the Philippines in September 2004 to: (a) assess the progress of activities of the collaborative project; (b) identify problems encountered in the implementation of the planned activities; (c) develop training and information components in relation to the planned activities; and (d) recommend future course of action for the implementation of the collaborative project.

Report of the First Discussion (top) was printed in 2004, and the Second Discussion Report (below) was printed in 2005

In order to continue assessing the progress and status of the activities in the participating countries, the Third Roundtable Discussion was held in Bangkok, Thailand from 3 to 4 December 2005 in order to:

1. assess the progress of activities under the collaborative project;
2. identify problems and concerns;
3. evaluate technologies through the collaborative project; and
4. recommend future course of action for the collaborative project under the second phase of the Special Five-Year Program.

THE THIRD ROUND TABLE DISCUSSION

Rationale

Since the recommendations during the 2003 Roundtable Discussion included an annual evaluation of the progress of activities on the status of the projects and an assessment of the problems in the implementation of the activities, the Third Roundtable Discussion was therefore necessary and this was convened in Bangkok, Thailand from 3 to 4 December 2005.

Under the collaborative project are the following three major activities being implemented in the participating countries:

1. **Indonesia**: Genetic improvement of *Macrobrachium rosenbergii* with emphasis on the assessment of the performance of the genetically improved (GI) Macro;
2. **Thailand**: Selective breeding program for genetic improvement of the giant freshwater prawn; and
3. **Philippines**: Morphometric characterization and performance evaluation of Macrobrachium stocks and closely related species in the Philippines.

The status of these activities needs to be assessed in order to develop a technology for consistent and improved production of quality freshwater prawn seedstock that will be disseminated to countries in the region.

The Third Round Table Discussion Participants

Objectives

The general objective of the Third Round Table Discussion was to evaluate the activities implemented in the participating countries under the collaborative project. The specific objectives were to:

1. assess the progress of activities under the collaborative project;
2. identify problems and concerns;
3. evaluate technologies through the collaborative project; and
4. recommend future course of action.

Participants

The Roundtable Discussion was attended by 32 participants representing the participating countries of the collaborative project (Indonesia, Thailand and the Philippines) as well as the SEAFDEC Secretariat and the SEAFDEC Training and Aquaculture Departments.

Discussion

SEAFDEC Secretary-General *Dr. Siri Ekmaharaj* after giving his welcome remarks officially opened the Third Round Table Discussion. AQD Chief *Dr. Rolando Platon* initially chaired the session and presented a brief summary of the activities of the collaborative project. He informed the participants that the program has been conducted since 2003 under the Aquaculture Component of the ASEAN-SEAFDEC Special Five Year Program on Sustainable Fisheries for Food Security in the ASEAN Region. Dr. Platon continued by requesting the participants to introduce themselves and the agencies/institutions they were representing.

SEAFDEC Secretary-General Dr. Siri Ekmaharaj (left), incoming Discussion Chairman Dr. Wattana Leelepat (center) and AQD Chief Dr. Rolando Platon (right) during the Opening of the Third Round Table Discussion

Outgoing Discussion Chairman Dr. Melchor Tayamen at the Opening of the Discussion

The outgoing Chairman *Dr. Melchor Tayamen* of the Philippines briefed the participants about the progress of the collaborative project. He then asked the participants to choose the Chairman for the Discussion. Nominated by the representative from Indonesia and seconded by the representative from the Philippines, the representative from Thailand, *Dr. Wattana Leelepat* was elected Chairman of the Third Round Table Discussion. The incoming Chairman discussed the agenda for the proposed two-day Discussion and encouraged the participants to rationally evaluate the progress of the collaborative project in order to come up with the desired plan of action.

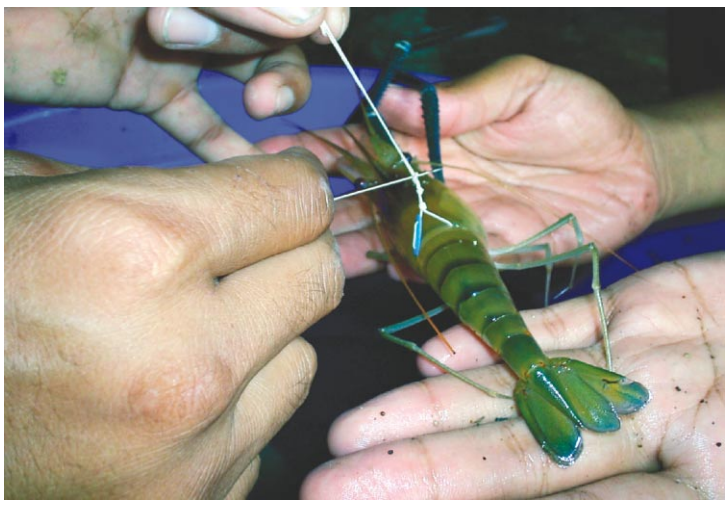
PROGRESS OF ACTIVITIES UNDER THE COLLABORATIVE PROJECT: SUMMARY

Evaluation of the Growth Rate of GI Macro II Strain in Different Locations (Indonesia)

Assisted by *Mr. Maskur* as his co-worker, *Dr. Estu Nugroho* reported on the final results of their study which was conducted in Indonesia. In the study, selective breeding program has been conducted to improve the freshwater prawn from synthetic population gathered from numerous breeders collected from the waters of Tanjung Air (Bekasi) collected in February 1995, Kalipucang (Ciamis) collected in June 1996, and Musi (Palembang) collected in May 1997. Index selection was applied to these subpopulations to improve the edible portion trait of the freshwater prawn.

The synthetic population was constructed from the two subpopulations that were then added to the subpopulation from Musi. Family selection was applied to the synthetic population. In 2001, GI Macro or the Genetically Improved *Macrobrachium rosenbergii* has been developed and released to the farmers. Based on the farmers' feedback, the sustainability of GI Macro culture was evaluated in different locations and verification studies were conducted to improve the growth rate. From the 1800 selected breeders taken from 18 families of GI-macro II, 25% were used as a selected line while the 25% residual to average size was used as the control line (50 male and 50 female per each family).

Mr. Maskur (left) and Dr. Estu Nugroho (right) presenting the results of their study in Indonesia

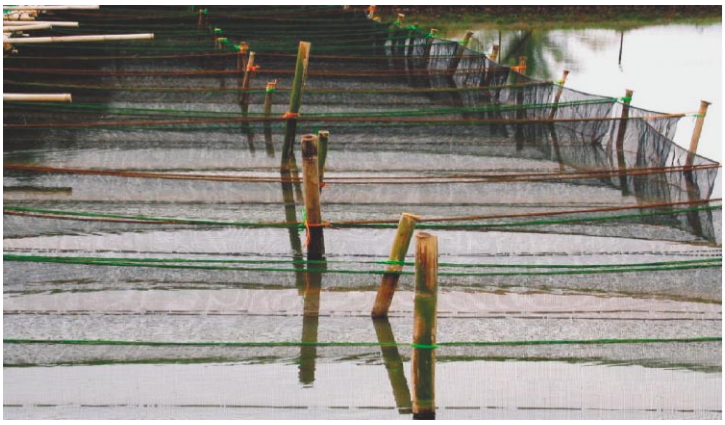


Plastic tagging of freshwater prawn breeders

Each family, differentiated by plastic tags, comprised about 50 breeders selected for the 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. Mature eggs (chocolate color) from seventy female breeders (selected line) and from 60 female breeders with same condition from the control line were collected for hatching.

About 24 thousand larvae from the selected and control lines have been collected and reared in different concrete tank provided with bamboo shelters. The resulting PL10 larvae were reared up to 3-5 cm in a concrete tank at 400 PL/m^2 and fed four times daily with natural feed (i.e., *Moina* and *Daphnia*), and pellet feed containing 38% protein at 20% of body weight. *M. rosenbergii* juveniles at 0.3 g ave weight were reared in hapa in the pond at 85 juveniles/m^2 in three different locations (Sukamandi, Cibalagung and Cijeruk).

The results indicated that the average total length and survival rate of selected line after over one month culture was higher than the control. The average total length and survival rate of GI Macro varied between batches 1 and 2, indicating that the population of GI Macro has wide variance. The average total length and weight of GI Macro from the selected line was better than the control in three different locations. Even the selected line of GI Macro in Sukamandi grew slower than the control in the first six weeks. In Cibalagung and Cijeruk, the selected line of GI Macro grew faster than the control after 12 weeks of rearing. The best harvest weight was the GI Macro reared in Cibalagung and followed by Sukamandi and Cijeruk. This showed that rearing GI Macro is more suitable in low to moderate level than in high sea level area.



Rearing of freshwater prawn juveniles in hapa nets in one of the three different locations

It should be noted that the water sources of the three locations are: natural water in Cijeruk, channel water from paddy field in Cibalagung as well as in Sukamandi, which might have also influenced the growth rate of the GI Macro.

The length gain of the GI Macro reared in three different locations for three months was positive. However, GI Macro reared in Cijeruk only showed positive tendency during the research period, while the negative value of length gain was still observed in GI Macro reared in Sukamandi and Cibalagung. A similar result was also obtained in the weight gain of the GI Macro. The positive value of weight gain was observed in GI Macro reared in Cijeruk, while a negative value was observed in Sukamandi and Cibalagung. The selection activity has more effect on weight gain than in the length gain. The highest weight and length gain of the GI Macro in each location was 52% and 14% (Sukamandi) after 8 weeks, 73% and 15% (Cijeruk) after 8 and 6 weeks, and 35% and 8% (Cibalagung) after 6 and 8 weeks. The wide variance of GI macro weight and length was also observed genetically using DNA Markers. Thus, another selection activity should be conducted to improve their variability as a pure line. The heritability and response selection will also be done in the next phase of the study.

Selective breeding program for genetic improvement of M. rosenbergii (Thailand)

Growth comparison of three M. rosenbergii stocks and their reciprocal crosses grown in four environments

Dr. Supattra Uraiwan presenting the results of one study conducted in Thailand

In her report on the growth comparison of three *M. rosenbergii* stocks, *Dr. Supattra Uraiwan* of the Aquatic Animal Genetics Research and Development Institute (AAGRDI) of Thailand explained that three stocks (AAGRDI, FARM (Petchaburi Farm) and WILD) of *Macrobrachium rosenbergii* were used for AAGRDI's selective breeding program. The crosses of these three stocks were evaluated in terms of performances and genetic variations before selective breeding program took place. AAGRDI's selective breeding program has two main parts: (1) evaluation of growth performance of the three stocks and their reciprocal crosses grown under four environments, and (2) improvement of the economic traits of the best cross adopting a suitable selection procedure.

In the preparation of the parent generation, fifty pairs of *M. rosenbergii* from each three stocks were collected for the base population. The stocks were spawned and their respective larvae were reared separately in three 20 m^2 concrete ponds. The growth performances of *M. rosenbergii* from three stocks were observed from August 2003 to January 2004.

The results illustrated that the *M. rosenbergii* of the "AAGRDI" stock performed better than the "WILD" and "FARM" stocks at an average of 4% and 9-15% in lengths and weights, respectively. In addition, allozyme electrophoresis has been carried out to estimate genetic variabilities (heterozygosity and number of alleles per locus) of the three stocks.

The results further illustrated that the genetic variabilities of *M. rosenbergii* from the present study are similar to those of *M. rosenbergii* from the natural waters {No of alleles 1.30 (1.29-1.33), heterozygosity 0.032(0.027-0.036)}. It was also observed that there was no difference on genetic diversity between these three stocks.

The AAGRDI stock (left), FARM stock (center) and WILD stock (right) used in the study

The reciprocal cross of the three stocks was conducted from November 2004 to August 2005 to establish nine cross–lines. The crosses indicated by male and female parents of each cross, starting with the male parents followed by the female parents, are as follows: AAGRDI x AAGRDI, WILD x WILD, 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 male and female *M. rosenbergii*. The hatching and nursing period took place at the AAGRDI and the Petchaburi Fisheries Test and Research Center in different periods while the post larvae were transferred for the performance growth test in four environments (AAGRDI, Chumphon Fisheries Test and Research Center, Buriram Fisheries Test and Research Center, and Uttaradit Fisheries Test and Research Center).

The nine crosses were reared under the above environments for 8 months. Similar experiment procedures such as stocking density, feeding regime and measurement schedules were used in all treatments. The stocking rate of 10 and 1 prawn/m^2 was used from the 1st to the 4th month and the 5th to the 8th month, respectively. The prawns were fed with commercial pellet shrimp feed three times a day at 3.4% of body weight. Because of the difference in starting time, the growth in lengths and weights of *M. rosenbergii* were monitored after the 2nd month for the AAGRDI and the Chumphon Fisheries Test and Research Center, and after the 3rd and 4th month for the Buriram and Uttaradit Fisheries Test and Research Centers, respectively.

In the AAGRDI environment, the cross WILD x WILD gained the highest in lengths and weights. It was significantly higher in lengths and weights than those of the AAGRDI x AAGRDI and the FARM x FARM, respectively. At the Chumphon Fisheries Test and Research Center environment, the cross WILD x AAGRDI had the highest in lengths and weights. It was significantly higher in lengths and weights than those of the AAGRDI x AAGRDI. At the Buriram Fisheries Test and Research Center environment, the cross AAGRDI x FARM gained the highest in lengths, significantly higher than those of the AAGRDI x AAGRDI, the FARM x FARM and the WILD x WILD.

At the Uttaradit Fisheries Test and Research Center environment, the cross AAGRDI x FARM had the highest in lengths and weights, significantly higher than those of the AAGRDI x AAGRDI, the FARM x FARM and the WILD x WILD.

AAGRDI facilities (left), and below are the facilities at Buriram Fisheries Test and Research Center (left), Chumphon Fisheries Test and Research Center (center) and Uttaradit Fisheries Test and Research Center (right) which were used in the study

Allozyme marker based comparison on genetic variation among M. rosenbergii *populations produced from a cross-breeding system of three different stocks in Thailand*

According to Thailand's *Dr. Panom Sodsuk*, a selective breeding program on the giant freshwater prawn including studies on improving the growth performance on the domesticated strain was also carried out at the Aquatic Animal Genetics Research and Development Institute (AAGRDI) of the Department of Fisheries of Thailand.

Dr. Sodsuk added that AAGRDI has already developed a domesticated and genetically improved stock of *Macrobrachium rosenbergii* for two generations. A wild stock has also been domesticated under the hatchery condition of the AAGRDI for one generation.

Meanwhile, domesticated stocks from private hatcheries were also developed. But there is still a need to develop another improved stock of the species basically from the two stocks of AAGRDI, the genetically improved and the wild, together with the domesticated stock from a good private hatchery so that the new created stock, which will be used as base population for further selective breeding program, would have been developed with higher genetic diversity.

Dr. Panom Sodsuk reporting on the progress of the allozyme marker study on the freshwater prawn conducted in Thailand

Specifically, the study aims to: (1) evaluate genetic variation (in terms of genetic variabilities as per locus averages of observable heterozygosities and number of alleles) of nine crosses from the above three mentioned stocks (the genetically improved by AAGRDI, the wild, and the private farm) of *Macrobrachium rosenbergii*; (2) apply polymorphism system of allozyme markers in the evaluation; (3) compare the evaluated genetic variation among the nine crosses to see differences; and (4) Use the informations of genetic variation evaluated, together with the performances, to choose the best cross for further selective breeding program in appropriate area. About 40-60 individuals of both sexes of each of the three stocks (AAGRDI, WILD and FARM) and each progeny population of nine crosses were sampled. Pleopods from each individual were cut and collected 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 particular software for population genetics studies (BIOSYS release 1.7.) The genetic variations, as per locus averages of heterozygosities and number of alleles of the nine crosses were compared using the software SYSTAT. Results indicated that the amounts of genetic variation, evaluated as per locus averages of heterozygosities and number of alleles, of the three initial stocks and all nine crosses showed no significant differences among the three initial stocks, as well as the nine crosses, both by heterozygosities and number of alleles. The appearance of heterozygosities and number of alleles both in the three initial stocks and in the nine crosses were close to those of the natural stocks studied before at the AAGRDI. Based on the resulting heterozygosities and number of alleles from the study, certain best crosses could be chosen for appropriate culture area.

Representatives from Chumphon Fisheries Test and Research Center, Buriram Fisheries Test and Research Center, and Uttaradit Fisheries Test and Research Center who helped implement the project in Thailand

Morphometric Characterization and Performance Evaluation of Different Macrobrachium stocks and closely related species in the Philippines (Philippines)

The Philippine Bureau of Fisheries and Aquatic Resources (BFAR), the Mindanao State University (MSU) and AQD's Binangonan Freshwater Station jointly conducted preliminary studies on the genetic characterization, domestication and improvement of *Macrobrachium rosenbergii* stocks in the Philippines in order to improve the aquaculture production of the freshwater prawn. A study on collection, identification and validation of Macrobrachium samples was conducted by BFAR and AQD.

In addition, two studies on the evaluation of growth performance of two strains of *M. rosenbergii* in lake environment: reproductive efficiency of two *M. rosenbergii* stocks at different protein levels, and preliminary observations on the juvenile performance of *Macrobrachium rosenbergii rosenbergii* and *M. rosenbergii dacqueti* in hapa net cages at different stocking densities were conducted by AQD. A study on *Macrobrachium rosenbergii* and other indigenous species in Mindanao and Visayan islands (Philippines) was conducted by MSU.

Collection, Identification and Validation of Macrobrachium Samples

Twelve species of *Macrobrachium rosenbergii* and other species that closely resemble the giant freshwater prawn have been caught from 25 commercial fishing grounds in the Philippines. The identities of local species, wild or hatchery-bred were validated. The western subspecies of Malaysian, Indonesian and Thai stocks of *Macrobrachium rosenbergii* and eastern subspecies found mainly in the Philippines were differentiated. The study was conducted in order 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.

The team from the Philippines who are involved in the project

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

*Macrobrachium rosenbergii (*giant river prawn*)*
Eastern form*: M. rosenbergii rosenbergii* (De Man, 1879)
Western form: *M. rosenbergii dacqueti* (Sunier, 1925)

AQD's *Dr. Ma. Rowena Eguia* reported that 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*. 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.

AQD's Dr. Rowena Eguia reporting on the morphometric characteristics of various Macrobrachium strains in the Philippines

Dr. Eguia added that the second legs of *M. rosenbergii* 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 and from the samples analyzed, the largest adult individuals were obtained from Dinas and Tambulig (in Mindanao in the Philippines), with total lengths of 23.6 cm. and 23.5 cm, respectively.

The basal crest of the wild-sourced M. rosenbergii rosenbergii (bottom) is higher than that of the hatchery-bred M rosenbergii dacqueti (top).

Macrobrachium equidens
(Rough river prawn or estuarine prawn)

This prawn (right) is rarely found in pure freshwater, as they normally thrive in lower parts of streams, river mouths, estuaries where the water has a higher salinity (brackishwater) and observed to breed in brackish and sea water. From the samples analyzed, 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.

Macrobrachium equidens sample from Tambulig in Mindanao

Macrobrachium mamillodactylus (knobtooth prawn)

M. mamillodactylus from Tambulig (in Mindanao, the Philippines)

The distinct feature of this species (left) 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 and the fingers of the second legs are not covered by soft short hair but 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.

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 occurs in the sea or brackishwater environment. This species has unequal size of the large second leg even in young specimens. *M. latidactylus* samples were obtained from Mangagoy and Lake Lanao (in Mindanao). The largest sample was obtained from Mangagoy (2.8 cm carapace length, 7.1 cm body length and 8.1 cm total length).

M. latidactylus samples from Mangagoy (left) and Lake Lanao (right)

Macrobrachium lanceifrons (Philippine river prawn)

Samples of M. lanceifrons from Lake Mainit

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.

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.


BFAR's Dr. Melchor Tayamen reporting on the collection of freshwater prawn species in Luzon area

Samples of *M. lanceifrons* were identified from the collection obtained in Lake Mainit (in Southern Mindanao, Philippines). The Lake Mainit samples had a 1.5 cm average carapace length, 4.3 cm. body length and 5.3 cm total length. More samples should be collected and observations on the breeding behavior and distribution in specific habitats/microhabitats should be done in order to determine the exact nature (that is whether they are found naturally in the collection areas as wild stocks or as accidental or intentional introductions from hatchery populations). Samples especially of *M. rosenbergii dacqueti and M. rosenbergii rosenbergii* will be processed for genetic marker analysis in 2006. Samples of these two subspecies will be collected from various populations to determine the level of intraspecific variation among the populations and their phylogenetic relationships.

Evaluation of Growth Performance of Two Strains of M. rosenbergii *in Cages in Laguna de Bay*

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 a stocking density of 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 from April to September 2005.

In the first run, CAL showed significantly better specific growth rate (SGR) than BFAR 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.


Culture of prawn in Laguna de Bay for evaluation of growth performance

For the second run, survival, SGR, and FCR were significantly better in CAL than BFAR. Like the first run, there were no significant differences in the final weight of the two strains.

Reproductive Efficiency of Two M. rosenbergii *Stocks at Different Protein Levels*

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 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*.

AQD's Dr. Ma. Lourdes Aralar reporting on the results of freshwater prawn studies conducted in Lake Laguna de Bay

Six months after stocking, preliminary observation showed that the BFAR stock fed low protein diet (fish feed pellets) *ad libitum* spawned more frequently than prawns given fixed amounts of fish feed and prawn feed. Calumpit stocks spawned less frequently: 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.

In a similar experiment set up in lake-based net cages using five-month old prawns to determine if the reproductive efficiency of the two strains is influenced by the type of spawning system, the survival of post-larvae produced from the spawns was low in both the BFAR and Calumpit stocks. The highest postlarval survival rate was achieved in one batch of spawn produced in an earlier trial run conducted in November 2004 (these F_1s were reared further to become parents of the breeders used in the on-going experiment).

Macrobrachium rosenbergii *and Other Indigenous Macrobrachium species in Mindanao and Visayan Islands*

Specimens of adult and juvenile Macrobrachium were collected from six known prawn grounds in different parts of Mindanao (Philippines). The description of the specimens formed as basis for its taxonomic identification, and the growth and survival of *Macrobrachium rosenbergii* was determined using various culture systems and compared the performance of wild and hatchery-bred prawn fry.

The prawns in hapa nets were fed commercial shrimp pellets at 10% body weight. The results of the study showed that *M. rosenbergii dacqueti* is a fast growing subspecies compared to *M. rosenbergii rosenbergii*. The observation was evident even before the experiment was started.

The discrepancies in size between the two species were even prominent in the final weights. On survival however, *M. rosenbergii dacqueti* had lower rate upon termination of the study. This is attributed to the mass mortality observed when plankton bloom collapsed.

Dr. Henry Dejarme of MSU reporting on the results of the prawn study conducted in Mindanao

Apart from continuing these aforementioned research activities, more studies related to the domestication (refinement of nursery and grow-out technologies) and performance assessment of wild freshwater prawn stocks/species will be done in the next phase of the project before any efforts to genetically improve growth and survival in existing hatchery stocks can be undertaken.

RECOMMENDATIONS

During the Discussion, the participants presented their recommendations for the collaborative project, which were later adopted:

1. SEAFDEC-ASEAN Member Countries should be cautious in introducing *M. rosenbergii* species in their respective countries;
2. More countries should be encouraged to participate in the collaborative project;
3. A Proposal should be developed by AQD in consultation with the participating countries for external funding; and
4. The foregoing plan of action initiated by the participating countries should be adopted for the collaborative program.

RESOLUTION

The participants also submitted a Resolution for the SEAFDEC to consider. The following Resolution was adopted during the Discussion.

1. That cooperation between the three countries now involved in the program, Indonesia, Philippines and Thailand be further strengthened through improved communication.
2. That AQD initiate a proposal in consultation with the three countries involved for submission to donor agencies for possible funding.
3. That SEAFDEC AQD encourage the active participation of other member countries.
4. That all SEAFDEC member countries be reminded of the risks of bringing in Macrobrachium stock from another country due to possibility of disease transfer and contamination of local genetic resources even if the species is native to most of the SEAFDEC member countries.

FUTURE PLAN OF ACTION

In support of the output of the Planning Workshop for the Special Five-Year Program (Aquaculture Component): 2006-2010, which was convened by AQD in Bangkok, Thailand from 30 November to 2 December 2005, the activities under the Genetic Improvement of *M. rosenbergii* identified during the Planning Workshop were prioritized further during the Round Table Discussion to form part of the major activities to be carried out in the collaborative project for 2006-2010. Thus, the Proposed Program of Activities for the Collaborative Project on Genetic Improvement and Seed Production of Macrobrachium shall include research on:

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)

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)

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

The genetically characterized strains of *M. rosenbergii* of member countries and the characterized *M. rosenbergii* stocks in Southeast Asia (*population variation in each country: country-to-country requirements, except Thailand and Indonesia*), will also be pursued.

For Human Capacity Building, the following have been proposed for 2006 to 2010:

1. Manual on grow-out of *M. rosenbergii* in ponds and lake based cages
2. Popular publication on genetic improvement of *M. rosenbergii*
3. Manual on protocols for the genetic characterization of *M. rosenbergii*
3. Training for technical persons and extension officers on hatchery and grow-out of *M. rosenbergii*
4. Training for fish farmers at core countries
5. Farm demonstration for recipient countries
6. On-site training for fish farmers of recipient countries
7. Training on genetic characterization of *M. rosenbergii* for research officers

FIELD TRIP

Arranged by the representatives from Thailand, the Field Trip was conducted on 4 December 2005. The areas visited were: the AAGRDI Center in Petchaburi, the Petchaburi Fisheries Test and Research Center, Private Hatchery of Marine Snail (Maculated Ivory Wheik, *Babylonia areolata*) in Petchaburi, and Private Hatchery and Culture Farm of *M. rosenbergii* in Nakhonpathom and Ratchaburi Provinces.

The participants at the Petchaburi Fisheries Test and Research Center (left and below), with the Head of the Center Mr. Tanan Sanggontanagit (above right) briefing the participants on the activities of the Center

The participants (above) at the Artemia facilities in Petchburi (Artemia is an important natural food for the prawn larvae)

The participants in Petchburi (above) and in a privately-operated Macrobrachium farm (below) in Nakhonpathom Province, Thailand

EVALUATION OF GROWTH RATE OF GI Macro II STRAIN IN DIFFERENT LOCATIONS

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INTRODUCTION

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

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

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

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

The subpopulation from Kalipucang was collected in June 1996 with an average weight of 72 g. Index selection was used in this population to improve the growth rate and edible portion trait. After two steps selection, the synthetic population was constructed from these two subpopulations and added to the subpopulation from Musi (ave body weight of 75 g collected in May 1997). Family selection was then applied to the synthetic population.

Thus, in 2001, a certain race 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). Based on the feedback from farmers, and in order to evaluate the capability of GI Macro in different locations, this study intended to know the GI Macro growth rate in three different locations i.e. low (<10m), moderate (150-250m) and high (>250) of sea surface level.

MATERIALS AND METHODS

Spawning activity

About 1800 breeders with total length size of up to 22 cm have been selected from 18 families of GI-macro II stock population. Twenty five percent of the best size in terms of length of each family was used as the selected line while the 25% residual to average size was used as the control line (50 male and 50 female per family). Each family is differentiated by means of a plastic tagging and contributed to about 50 breeders selected in the 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 1 month. Seventy female breeders with mature eggs (chocolate color) from the selected line and 60 female breeders with the same condition from the control line have been collected for the spawning and hatching.

Table 1. Characteristics of GI Macro after fourth generation

No	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) Total length of female (cm)	21.53 (5.45) 15.02 (3.19)
5	Percentage of carapace (male) Percentage of carapace (female)	30.45 (5.86) 32.68 (8.05)
6	Hatching rate (%)	65.27 – 80.0
7	Survival rate (% per 4 months)	46.3 – 53.1

Breeders were treated by dipping them in 1.5 mg/l malachite green for 20 min before placing them into hatching fiberglass tank. The water temperature was kept at 29-30°C using thermostat heaters. Squid was given as food at 5% of body weight and feeding frequency was three (3) times daily.

Larval Rearing

Larvae were collected using net tray daily and kept into 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. *Artemia* naupli was given to the 1-7 days old larvae followed by egg custard containing 55% protein and 8% fat 7 times daily. Water was changed every three days when larvae was 1-7 days old, every two days for larvae 7-15 days old and then daily up to post larvae (PL).

Post Larval Rearing

About 24 thousand larvae of the selected and control line were collected and reared in different concrete tanks provided with bamboo shelters. PL-10 of *M. rosenbergii* was reared up to 3-5 cm in a concrete tank in the hatchery at 400 PL/m^2. Feed used was natural food (i.e. *Moina* and *Daphnia*), and pellet containing 38% protein at 20% of body weight. The natural food was given once while pellet was given 4 times daily.

Grow-out culture

M. rosenbergii juveniles, ave size 0.3 g, was reared in a hapa nets placed in the 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).

Pellet containing 30-33% protein was used for 1-3 weeks at 20% of body weight 4 times daily (07.00; 10.00; 13.00 and 17.00). At 4-8 weeks, feeding rate was 15% of body weight given 4 times daily after which feeding rate was 10 of body weight given three times (07.00; 12.00 and 16.00). Sampling was done twice in a month while cleaning of the hapa was conducted every week.

RESULTS AND DISCUSSION

Total length, standard length, body weight and survival rate of *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

	Selected				Control			
Batch	TL (mm)	SL (mm)	BW (gram)	SR (%)	TL (mm)	SL (mm)	BW (gram)	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 over a month 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 indicated that population of GI Macro has wide variance in terms of growth and survival. The average total length and weight of juveniles during rearing for three months in a net placed at an earthen pond is listed in Table 2.

Table 2. Average total lenght (cm) and weight (g) of GI Macro fry during rearing for 3 months in three different loacations (standard deviation in bracket)

Location	Collection	Initiated	2 week	4 week	6week	8 week	12 week
Sukamandi	Selected	TL=2.910 (0.527)	3.880 (0.695)	4.568 (1.416)	5.858 (1.459)	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)
	Control	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)

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 weeks culture period. In Cibalagung and Cijeruk, the selected line of GI Macro grew faster than the control line during the 12 weeks rearing period. It is recognized that the best harvest weight is GI Macro reared in Cibalagung (7.982 $\pm$ 5.991 g), followed by Sukamandi (4.698 $\pm$ 3.287g) and Cijeruk (4.692 $\pm$ 2.011 g).

This phenomenon showed that GI Macro is more suitable reared in low to moderate level than in high sea level area. Water temperature was 30-32ºC, 28-30°C and 24-28ºC 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.

Length and Weight Gain

Generally, the length gain of GI Macro reared in three different locations for three months was positive (Fig. 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 (Fig. 2). The positive value was observed in GI Macro reared in Cijeruk, while negative value in weight gain was observed in Sukamandi and Cibalagung.

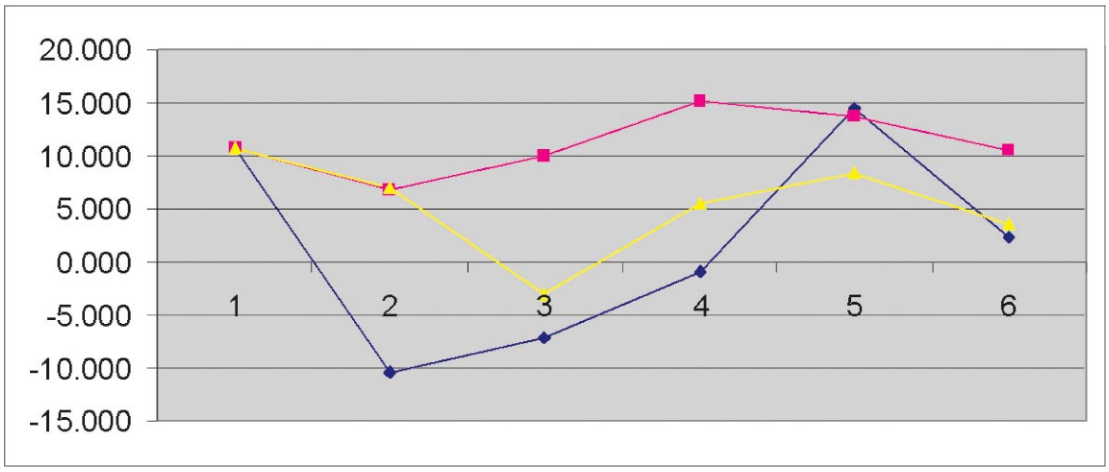

Fig. 1 Length gain of juvenile GI Macro reared in three different locations
(Blue = Sukamandi, Pink = Cijeruk, Yellow = Cibalagung)

This shows that the selection activity has more affect on weight gain than in length gain. The harvest weight gain of the GI Macro reared in Cijeruk, Cibalagung and Sukamandi were 31.09%, 20.06% and 8.750%, respectively. While the harvest length gain of the GI Macro was 10.6%, 3.53% and 2.4% in Cijeruk, Cibalagung and Sukamandi, respectively. The highest weight and length gain of GI Macro in each location was 52% and 14% (Sukamandi) in 8 week period, 73% and 15% (Cijeruk) in 8 weeks and 6 weeks, and 35% and 8% (Cibalagung) in 6 week and 8 week period. Nugroho et.al (2005) had indicated 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.

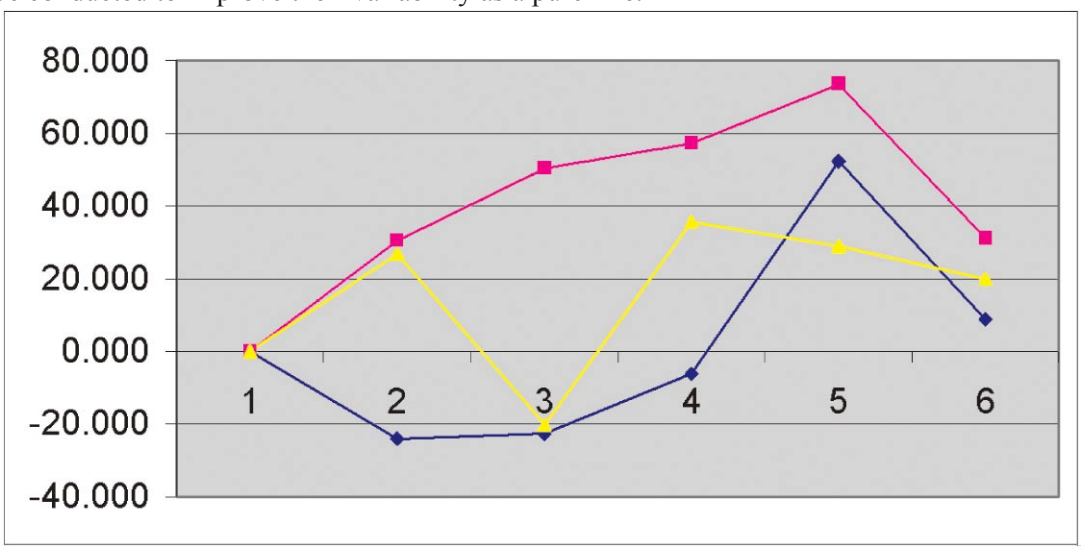

Fig. 2 Weight gain of juvenile GI Macro reared in three different locations
(Blue = Sukamandi, Pink = Cijeruk, Yellow = Cibalagung)

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SELECTIVE BREEDING PROGRAM FOR GENETIC IMPROVEMENT OF *MACROBRACHIUM ROSENBERGII* IN THAILAND

I. Growth comparison of three *Macrobrachium rosenbergii* stocks and their reciprocal crosses grown in four environments

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INTRODUCTION

Three stocks of *Macrobrachium rosenbergii* including "AAGRDI" (Aquatic Animal Genetic Research and Development Institute), "FARM" (Petchaburi Farm) and "WILD" stocks were used for the selective breeding program. Generally, the good base population for genetic improvement program required high genetic variations as well as suitable stock that can be adapted for each local environment. Therefore, all proper crosses of these three stocks need to be evaluated on both performances and genetic variations before selective breeding program takes place. Thus, the program was divided into two parts: (1) evaluation of growth performance of the three stocks and their reciprocal crosses grown in four environments; and (2) establishment of improved economic traits of the best cross by 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 5x5x1.5 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* that has been selected for improving growth rate of 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 pairs of *M. rosenbergii* from each three stocks were collected to initiate the base population. Each stock has been spawned and reared separately, the offspring of which were reared in three 20 m^2 concrete ponds at the AAGRDI. The growth performances of *M. rosenbergii* from the three stocks were observed from August 2003 to January 2004 (Uraiwan and Sodsuk, 2004). The results illustrated that the *M. rosenbergii* of the "AAGRDI" stock performed better than those of the "WILD" and "FARM" stocks at averages of 4% and 9-15% in lengths and weights, respectively. In addition, allozyme electrophoresis has been carried out to estimate genetic variabilities (heterozygosity and number of alleles per locus) of three stocks.were similar to those of *M. rosenbergii* from the natural waters {No 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 genetic diversity among the three (3) stocks (Sodsuk *et al*., 2005).

Performance growth test on nine crosses

Reciprocal crosses of the 3 stocks were conducted from November 2004 to August 2005 to establish 9 cross–lines. The crosses identified by the male and female parents of each cross, starting with the male parents followed by the female parents, are as follows: AAGRDI x AAGRDI, WILD x WILD, 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 male and female *M. rosenbergii*. The hatching and nursing period took place at the AAGRDI and the Petchaburi Fisheries Test and Research Center in different periods, and the post larvae were stocked for the performance growth test in four environments as indicated in following table:

Month and Year	Place of hatching and nursing	Places and environments of cross-line performance growth test
November 2004	AAGRDI	18 cages at the Uttaradit Fisheries Test and Research Center
July 2005	AAGRDI	18 cages at the Buriram Fisheries Test and Research Center
August 2005	Petchaburi Fisheries Test and Research Center	18 cages at Chumphon Fisheries Test and Research Center
August 2005	AAGRDI	18 concrete ponds at the AAGRDI

The 9 crosses have been reared in the abovementioned environments for eight months. Similar experimental procedures such as stocking density, feeding regime and measurement schedules were used in all cases. The stocking rate of 10 and 1 prawn/m^2 was used during the 1st to the 4th month and the 5th to the 8th month of the experiment, respectively. The prawns were fed with commercial pellet shrimp 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 starting time, the monitored were the growth in lengths and weights of *M. rosenbergii* at the 2nd month for the AAGRDI and the Chumphon Fisheries Test and Research Center, and the 3rd and the 4th month for the Buriram and Uttaradit Fisheries Test and Research Centers, respectively.

1.1. The AAGRDI environment

Mean lengths and weights duringt the 2nd month of the 9 crosses are shown in Table 1. The cross WILD x WILD gained the highest in lengths and weights. It was significantly higher 15, 17 and 39, 43 % in lengths and weights than those of the AAGRDI x AAGRDI and the FARM x FARM, respectively.

Table 1. Mean lengths/weights and standard deviations (+sd.) of 9 crosses of M.rosenbergii grown in concrete ponds for two months at the AAGDRI environment

Crosses	Length (cm.) ± sd.	Weight (g.)± sd.
1. WILD x AAGRDI	7.516 ± 0.864^{b}	3.905 ± 1.687^{e}
2. AAGRDI x WILD	7.244 ± 0.872^{c}	3.588 ± 1.327^{g}
3. AAGRDI x FARM	7.922 ± 1.102^{b}	4.963 ± 2.306^{c}
4. FARM x AAGRDI	6.706 ± 1.175^{e}	3.156 ± 1.819^{b}
5. WILD x FARM	7.628 ± 1.098^{b}	4.546 ± 2.096^{f}
6. FARM x WILD	8.329 ± 0.866^{f}	5.244 ± 1.851^{i}
7. FARM x FARM	7.113 ± 0.899^{d}	3.299 ± 1.329^{h}
8. WILD x WILD	8.583 ± 0.843^{f}	5.854 ± 1.851^{a}
9. AAGRDI x AAGRDI	7.232 ± 0.656^{a}	3.559 ± 1.054^{g}

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

1.2. The Chumphon Fisheries Test and Research Center environment

Mean lengths and weights on the 2nd month of the 9 crosses are shown in Table 2. The cross WILD x AAGRDI had the highest lengths and weights. It was significantly higher at 10 and 23% in lengths and weights than those of the AAGRDI x AAGRDI, respectively.

1.3. The Buriram Fisheries Test and Research Center environment

Mean lengths and weights on the 3rd month of the 9 crosses (Table 3) showed that the cross AAGRDI x FARM gained the highest in lengths and weights, which was 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 9 crosses of M.rosenbergii grown in cages for two months at the Chumphon Fisheries Test and Research Center environment

Crosses	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 9 crosses of *M. rosenbergii* grown in cages for three months at the Buriram Fisheries Test and Research Center environment

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

1.4. The Uttaradit Fisheries Test and Research Center environment

Mean lengths and weights on the 4th month of the 9 crosses (Table 4) indicated that the cross AAGRDI x FARM had the highest lengths and weights, which was significantly higher at 11, 12 and 13% than the AAGRDI x AAGRDI, the FARM x FARM and the WILD x WILD, respectively.

Table 4. Mean lengths/weights and standard deviations (+sd.) of 9 crosses of M.rosenbergii grown in cages for four months at the Uttaradit Fisheries Test and Research Center environment

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^{a}

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

Heterosis

The heterosis in lengths and weights for each hybrid cross-line was estimated using the following formula:

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

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

2.1 The AAGRDI environment

2.1.1 The hybrid AAGRDI and FARM was significantly different from the AAGRDI and the FARM stocks (heterosis= 1.97% in length and 18.39% in weight).

2.1.2 The hybrid AAGRDI and WILD was significantly different from the AAGRDI and the WILD stocks (heterosis= -6.67% in length and -23.61% in weight).

2.1.3. The hybrid WILD and FARM was significantly different from the FARM stock (heterosis= 1.66% in length and 6.96% in weight).

2.2. The Chumphon Fisheries Test and Research Center environment

2.2.1 The hybrid AAGRDI and FARM was significantly different from the FARM stock (heterosis= 2.74% in length and 13.21% in weight).

2.2.2 The hybrid AAGRDI and WILD was significantly different from the AAGRDI stock (heterosis= 4.54% in length and 14.48 % in weight).

2.2.3 The hybrid WILD and FARM was significantly different from the FARM stock (heterosis= -1.35% in length and -1.98% in weight).

2.3. The Buriram Fisheries Test and Research Center environment

2.3.1 The hybrid AAGRDI and FARM was significantly different from the AAGRDI stock (heterosis= 1.58% in length and 19.86% in weight).

2.3.2 The hybrid AAGRDI and WILD was significantly different from the AAGRDI stock (heterosis= 0.61% in length and 0.48 % in weight).

2.3.3. The hybrid WILD and FARM was significantly different from the FARM stock (heterosis= -2.85% in length and -0.30% in weight).

2.4. The Uttaradit Fisheries Test and Research Center environment

2.4.1 The hybrid AAGRDI and FARM was significantly different from the AAGRDI stock (heterosis= 20.28% in length and 9.16% in weight).

2.4.2 The hybrid AAGRDI and WILD was significantly different from the AAGRDI stock (heterosis= 2.28% in length and 15.47 % in weight).

2.4.3. The hybrid WILD and FARM was significantly different from the FARM stock (heterosis= 1.01% in length and 3.13% in weight).

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
	Farn&Wild	1.66	6.96
CHUMPHON	AAGRDI&Farm	2.74	13.21
	AAGRDI&Wild	4.54	14.48
	Farn&Wild	-1.35	-1.98
BURIRAM	AAGRDI&Farm	1.58	19.86
	AAGRDI&Wild	0.61	0.48
	Farn&Wild	-2.85	-0.30
UTTARADIT	AAGRDI&Farm	20.28	9.16
	AAGRDI&Wild	2.28	15.47
	Farn&Wild	1.01	3.13

CONCLUSION

1. There were differences in growth rate between three *M. rosenbergii* stocks and their hybrids.
2. The heterosis of some crosses illustrated the possibility of improving the growth rate of *M. rosenbergii* by hybridization. However, the selection within lines is needed before the hybridization.
3. Based on the results, the WILD x WILD cross is suitable for culture at the AAGDRI. Whereas, 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.
4. The differences in heterosis values in different environments illustrated the genotype- environment interaction on growth performance. (Figure 1.) Therefore, line performance growth test should be included at the early stage of the selective breeding program.

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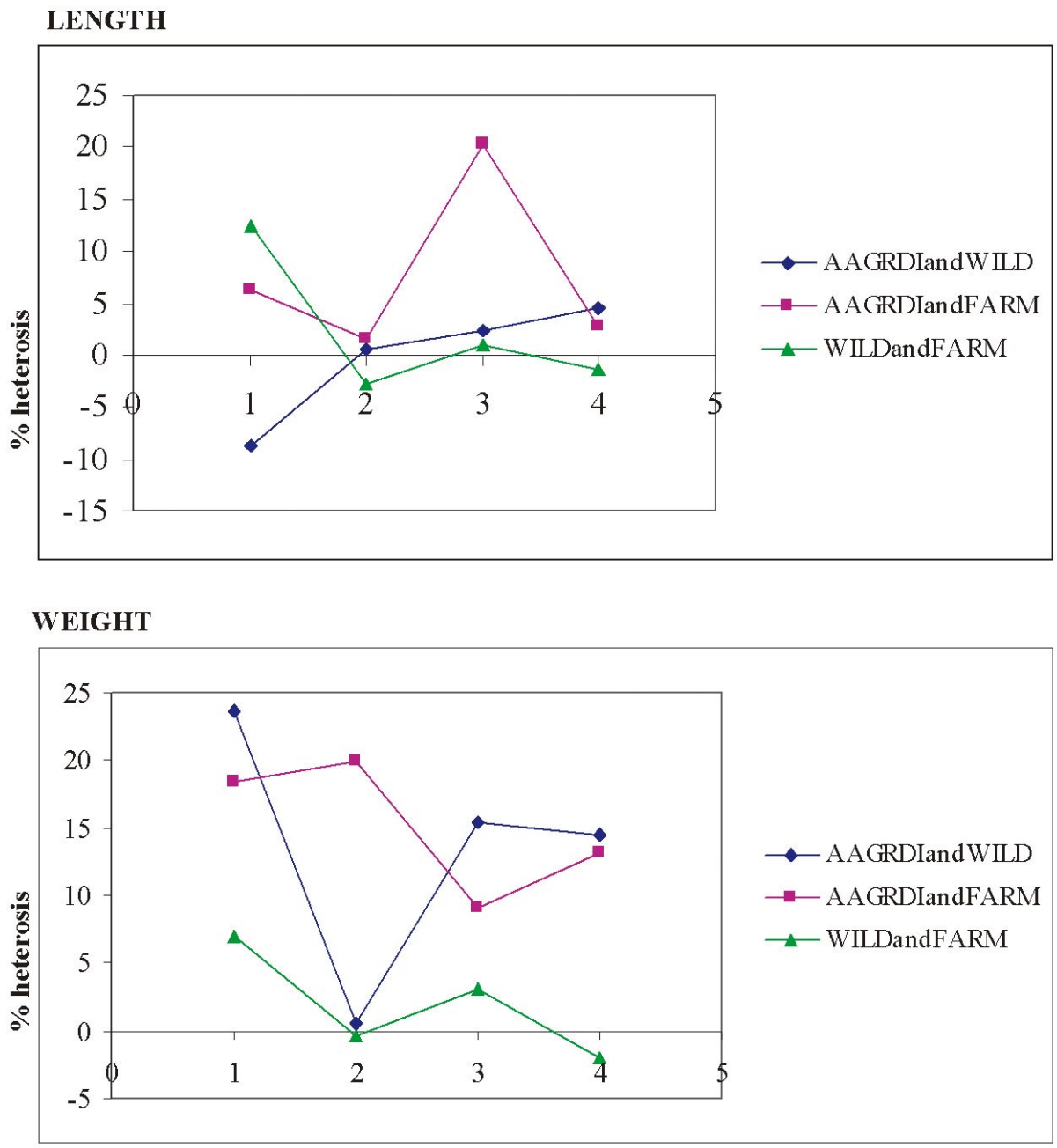

Fig. 1 Percent heterosis in length -weight of 3 reciprocal crosses of *M.rosenbergii* (AAGRDI and WILD, AAGRDI and FARM, and WILD and FARM) grown in 4 different environments:

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)

ALLOZYME MARKER BASED COMPARISON ON GENETIC VARIATION AMONG *MACROBRACHIUM ROSENBERGII* POPULATIONS PRODUCED FROM A CROSS-BREEDING SYSTEM OF THREE DIFFERENT STOCKS IN THAILAND

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INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* has been domesticated in Thailand for decades, but appropriate selective breeding program of this species has not yet been achieved. Thus, a good quality seed for the *Machrobrachium* industry is therefore not regularly produced. A selective breeding program which includes improvement of growth performance on the domesticated strain was carried out at the Aquatic Animal Genetics Research and Development Institute (AAGRDI), Department of Fisheries. The institute has now developed a domesticated and genetically improved stock of *Macrobrachium rosenbergii* for 2 generations.

A wild stock was also domesticated under hatchery conditions at AAGRDI for 1 generation. Meanwhile, domesticated stocks from private hatcheries have also been sourced. There is therefore a need to develop another improved stock of the species basically from the two stocks of AAGRDI, the genetically improved and the wild, together with the domesticated stock from a good private hatchery. This is because the new created stock, which will be used as base population for further selective breeding program, should be developed with higher genetic diversity.

Generally, a good base population for a genetic improvement program requires high genetic variation as well as an ideally suitable stock that can be well adapted for different local environments. Therefore, all proper crosses of these three stocks should be cultured in different areas of the country and their performance and genetic variation evaluated before a selective breeding program will have taken place.

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 intra-specific population studies (Sodsuk, 1996; Sodsuk and Sodsuk, 1998a & 1998b; Sodsuk *et al*., 2001). Due to the availability of the allozyme technique, it is basically and initially applied for the genetic variation evaluation.

The objectives of this research were to: (1) evaluate genetic variation (in terms of genetic variabilities as per locus averages of observable heterozygosities and number of alleles) of nine crosses from the above three mentioned stocks (the genetically improved by AAGRDI, the wild, and the private farm) of *Macrobrachium rosenbergii*; (2) apply polymorphism system of allozyme markers in the evaluation; (3) compare the evaluated genetic variation among the nine crosses for differences; and (4) use of the information on genetic variation evaluated, together with the performances, to choose the best cross for further selective breeding program in an appropriate area.

MATERIALS AND METHODS

Sample Analysis

About 40-60 individuals of both sexes of each of the three stocks (the AAGRDI, wild, and private farm) and each progeny population of 9 crosses were sampled. Pleopods from each individual were cut and collected in separate microtubes. All pleopod samples in microtubes were preserved at -70 ºC in deep freezer for further molecular analysis of allozyme markers. The preserved samples were electrophoretically analysed at 19-25 allozyme loci (see Tables in appendix) following the procedure already studied before in *Macrobrachium rosenbergii* 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 genetic variation evaluation. The work made use of particular software for population genetics studies known as BIOSYS release 1.7 of Swofford and Selander (1989).

The genetic variations, as per locus averages of heterozygosities and number of alleles, of 9 crosses (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 statistical software known as SYSTAT of Wilkinson *et al*. (1992).

RESULTS AND DISCUSSION

The genetic variation, evaluated as per locus averages of heterozygosities and number of alleles, of three initial stocks and all 9 crosses, is shown respectively in Tables 1 and 2. There were no significant differences among the three initial stocks as well as the 9 cross, both by heterozygosities and number of alleles. The appearance of heterozygosities and number of alleles both in the three initial stocks (H = 0.023 – 0.043, NoA = 1.20 – 1.44) and in the 9 crosses (H = 0.010 – 0.042, NoA = 1.11 – 1.53) were close to those of the natural stocks (H = 0.027 – 0.036, NoA = 1.29 – 1.33) studied before by Sodsuk and Sodsuk (1998b).

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) of all 9 crosses

	Cross (male x female)	H (Average ±S.E.)	NoA (Average ±S.E.)
T_1	(Wild x AAGRDI)	0.011 (±0.008)^{A}	1.11 (±0.07)^{a}
T_2^1	(AAGRDI x Wild)	0.042 (±0.027)^{A}	1.26 (±0.10)^{a}
T_3^2	(AAGRDI x Farm)	0.010 (±0.007)^{A}	1.16 (±0.09)^{a}
T_4^3	(Farm x AAGRDI)	0.016 (±0.007)^{A}	1.32 (±0.13)^{a}
T_5^4	(Wild x Farm)	0.030 (±0.010)^{A}	1.53 (±0.14)^{a}
T_6^5	(Farm x Wild)	0.026 (±0.013)^{A}	1.26 (±0.13)^{a}
T_7^6	(Farm x Farm)	0.024 (±0.010)^{A}	1.37 (±0.11)^{a}
T_8^7	(Wild x Wild)	0.018 (±0.009)^{A}	1.21 (±0.10)^{a}
T_9^8	(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

1. Amounts of genetic variation, as per locus averages of heterozygosities and the number of alleles, of three initial stocks and all 9 crosses were evaluated and statistically compared but non-significant differences were obtained among the stocks.

2. Heterozygosities and number of alleles obtained from this study together with growth performances from the study of Uraiwan *et al*. (2005), would help in choosing the best cross for an appropriate area.

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Table 3. Genetic informations by heterozygositiies (H), number of alleles (NoA), growth performances and % heterosis of all crosses in four different areas

Environment (months)	Mate pair	Crosses	Sodsuk *et al.* (2005)		Uraiwan *et al.* (2005)			
					Per formances		% heterosis	
			H	NoA	Length	Weigth	Length	Weigth
Uttaradit (5)	AAGRDI & Wild	T_1	0.011[A]	1.11[a]	12.982	24.354	2.28	15.47
		T_2	0.042[A]	1.26[a]	12.671	24.449		
	AAGRDI & Farm	T_3*	0.010[A]	1.16[a]	13.140	23.977*	20.28*	9.16*
		T_4*	0.016[A]	1.32[a]	13.822*	22.083		
	Wild & Farm	T_5	0.030[A]	1.53[a]	12.500	22.908	1.01	3.13
		T_6	0.026[A]	1.26[a]	12.002	20.681		
	Farm & Farm	T_7	0.024[A]	1.37[a]	12.212	21.965	-	-
	Wild & Wild	T_8	0.018[A]	1.21[a]	12.044	22.035	-	-
	AAGRDI & AAGRDI	T_9	0.015[A]	1.16[a]	12.267	20.230	-	-
Buriram (4)	AAGRDI & Wild	T_1	0.011[A]	1.11[a]	10.430	17.220	0.61	0.48
		T_2	0.042[A]	1.26[a]	10.783	16.140		
	AAGRDI & Farm	T_3*	0.010[A]	1.16[a]	11.061*	20.709*	1.58*	19.86*
		T_4	0.016[A]	1.32[a]	10.447	16.710		
	Wild & Farm	T_5	0.030[A]	1.53[a]	10.618	17.740	-2.85	-0.30
		T_6	0.026[A]	1.26[a]	10.049	15.040		
	Farm & Farm	T_7	0.024[A]	1.37[a]	10.687	15.450	-	-
	Wild & Wild	T_8	0.018[A]	1.21[a]	10.589	17.430	-	-
	AAGRDI & AAGRDI	T_9	0.015[A]	1.16[a]	10.496	15.770	-	-
Pathumthani (2)	AAGRDI & Wild	T_1	0.011[A]	1.11[a]	7.516	3.905	-6.67	-23.61
		T_2	0.042[A]	1.26[a]	7.244	3.588		
	AAGRDI & Farm	T_3	0.010[A]	1.16[a]	7.922	4.963	1.97	18.39
		T_4	0.016[A]	1.32[a]	6.706	3.156		
	Wild & Farm	T_5	0.030[A]	1.53[a]	7.628	4.546	1.66	6.96
		T_6*	0.026[A]	1.26[a]	8.329*	5.244*		
	Farm & Farm	T_7	0.024[A]	1.37[a]	7.113	3.299	-	-
	Wild & Wild	T_8*	0.018[A]	1.21[a]	8.583*	5.854*	-	-
	AAGRDI & AAGRDI	T_9	0.015[A]	1.16[a]	7.232	3.559	-	-
Chumphon (2)	AAGRDI & Wild	T_1*	0.011[A]	1.11[a]	8.122*	4.681*	4.54*	14.48*
		T_2	0.042[A]	1.26[a]	7.576	4.036		
	AAGRDI & Farm	T_3	0.010[A]	1.16[a]	7.30	3.69	2.74	13.21
		T_4	0.016[A]	1.32[a]	7.506	4.102		
	Wild & Farm	T_5	0.030[A]	1.53[a]	7.456	3.634	-1.35	-1.98
		T_6	0.026[A]	1.26[a]	7.210	3.502		
	Farm & Farm	T_7	0.024[A]	1.37[a]	7.131	3.274	-	-
	Wild & Wild	T_8	0.018[A]	1.21[a]	7.736	4.006	-	-
	AAGRDI & AAGRDI	T_9	0.015[A]	1.16[a]	7.280	3.609	-	-

Asterisks (*) identify the best crosses with the best genetic informations to be chosen in appropriate areas.

APPENDIX

Appendix Table 1. Observable heterozygosities (H) and number of alleles (NoA) of the three stocks (the AAGRDI, wild and private farm)

Allozyme locus/ci	Heterozygosities (H)			Number of alleles (NoA)		
	AAGRDI	Wild	Private farm	AAGRDI	Wild	Private farm
1. AAT-1	0	0	0	1	1	1
2. AAT-2	0.033	0.050	0.034	2	2	2
3. ACP-1	0	0	0	1	1	1
4. ACP-2	0	0	0	1	1	1
5. ALAT	0.037	0	0	2	1	1
6. EST	0	0	0	1	1	1
7. ESD	0.080	0	0	2	1	2
8. FBALD-1	0	0	0	1	1	1
9. FBALD-2	0	0	0	1	1	1
10. G3PDH-1	0	0	0	1	1	1
11. G3PDH-2	0	0	0	1	1	1
12. G6PDH	0	0	0.037	1	1	2
13. GPI	0.100	0.050	0.067	2	2	3
14. HK-1	0	0	0	1	1	1
15. HK-2	0	0	0	1	1	1
16. IDHP	0.250	0.316	0.069	3	3	2
17. LDH	0	0	0	1	1	1
18. MDH-1	0	0	0	1	1	1
19. MDH-2	0.367	0	0.233	2	1	2
20. MEP	0.100	0.158	0.333	2	2	3
21. MPI	0	0	0.033	1	1	2
22. PGDH	0	0	0	1	1	1
23. PGM	0.100	0	0.103	2	1	2
24. XDH	0	0	0	1	1	1
25. ODH	0	0	0	1	1	1
Average (±S.E.)	0.043 (±0.018)	0.023 (±0.014)	0.036 (±0.016)	1.36 (±0.11)	1.20 (±0.10)	1.44 (±0.13)

Appendix Table 2. Observable heterozygosities (H) of each progeny population of 9 crosses

Allozyme Locus/ci	Heterozygosities								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
1. AAT-1	0	0.067	0	0	0	0	0	0	0.111
2. AAT-2	0	0	0.100	0	0	0	0	0	0
3. ACP	0	0	0	0	0	0	0	0	0
4. AK	0	0	0	0	0.100	0	0.053	0	0
5. EST	0	0	0	0	0	0	0	0	0
6. ESD-1	0	0	0	0.050	0	0	0	0	0
7. ESD-2	0	0	0	0	0	0	0.053	0	0
8. GPI	0	0	0	0	0	0	0	0	0
9. MPI	0.060	0	0	0	0.050	0	0.150	0.060	0
10. PGDH	0	0	0	0	0	0	0	0	0
11. XDH	0	0	0	0	0	0	0	0	0
12. IDHP	0	0	0	0	0.050	0	0.100	0	0.067
13. G3PDH	0	0.500	0	0.050	0.118	0.200	0	0.133	0
14. G6PDH	0	0	0	0	0	0	0	0	0
15. HK	0	0	0	0	0.105	0.050	0	0	0
16. MDH-1	0	0.118	0	0.056	0	0	0	0	0
17. MDH-2	0.143	0.059	0	0.100	0.105	0.150	0.050	0.067	0.111
18. LDH	0	0	0	0	0	0	0	0	0
19. PGM	0	0.059	0.083	0.056	0.050	0.100	0.050	0.067	0
Average (±S.E.)	0.011 (±0.008)	0.042 (±0.027)	0.010 (±0.007)	0.011 (±0.008)	0.030 (±0.010)	0.026 (±0.013)	0.024 (±0.010)	0.018 (±0.009)	0.015 (±0.009)

Appendix Table 3. Number of alleles (NoA) of each progeny population of 9 crosses

Allozyme Locus/ci	Number of alleles								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
1. AAT-1	1	2	1	1	1	1	1	1	2
2. AAT-2	1	1	2	1	1	1	1	1	1
3. ACP	1	1	1	1	1	1	1	1	1
4. AK	1	1	1	1	2	1	2	1	1
5. EST	1	1	1	1	1	1	1	1	1
6. ESD-1	1	1	1	2	2	1	1	1	1
7. ESD-2	1	1	1	1	1	1	2	1	1
8. GPI	1	1	1	1	1	1	1	1	1
9. MPI	2	1	1	1	2	1	2	2	1
10. PGDH	1	1	1	1	1	1	1	1	1
11. XDH	1	1	1	1	1	1	1	1	1
12. IDHP	1	1	1	1	2	1	2	1	2
13. G3PDH	1	2	2	3	3	3	2	2	1
14. G6PDH	1	1	1	1	1	1	1	1	1
15. HK	1	1	1	1	2	2	1	1	1
16. MDH-1	1	2	1	2	2	1	1	1	1
17. MDH-2	2	2	1	2	2	2	2	2	2
18. LDH	1	1	1	1	1	1	1	1	1
19. PGM	1	2	2	2	2	2	2	2	1
Average (±S.E.)	1.11 (±0.07)	1.26 (±0.10)	1.16 (±0.09)	1.32 (±0.13)	1.53 (±0.14)	1.26 (±0.13)	1.37 (±0.11)	1.21 (±0.10)	1.16 (±0.09)

MORPHOMETRIC CHARACTERIZATION AND PERFORMANCE EVALUATION OF DIFFERENT *MACROBRACHIUM ROSENBERGII* STRAINS AND OTHER COMMERCIALLY IMPORTANT FRESHWATER PRAWNS IN THE PHILIPPINES

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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 ASEAN-SEAFDEC Special Five-Year Program (Aquaculture Component) 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, specific research activities under "Morphometric characterization and performance evaluation of different *Macrobrachium rosenbergii* strains and other commercially important freshwater prawns in the Philippines" were conducted, and their preliminary results are summarized as follows:

A. Collection, Identification and Validation of *Macrobrachium* Samples

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 giant freshwater prawn originated from an imported stock from Thailand which was brought in and later promoted by the Philippine Bureau of Fisheries and Aquatic Resources for commercial aquaculture. Studies have 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 or NUS) 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 3 adult males, 3 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 Bulacan and selected sites in Mindanao. 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		
Calumpit, Bulacan (Luzon)	11	*Macrobrachium rosenbergii dacqueti*
	9	*Macrobrachium rosenbergii rosenbergii*
Dinas (ZDSMindanao)	5	*Macrobrachium rosenbergii dacqueti*
	5	*Macrobrachium rosenbergii rosenbergii*
Siay (ZDS Mindanao)	4	*Macrobrachium rosenbergii dacqueti*
	6	*Macrobrachium rosenbergii rosenbergii*
Tambulig (Mindanao)	11	*Macrobrachium mamillodactylus*
(Panguil Bay	12	*Macrobrachium equidens*
	2	*Macrobrachium rosenbergii dacqueti*
	8	*Macrobrachium rosenbergii rosenbergii*
Mangagoy (Mindanao)	12	*Macrobrachium mamillodactylus*
	1	*Macrobrachium latidactylus*
Lake Lanao (LDN/ LDSMindanao)	10	*Macrobrachium latidactylus*
Lake Mainit (SDN Mindanao)	10	*Macrobrachium lanceifrons*
B. Hatchery		
SEAFDEC/AQD	10	*Macrobrachium rosenbergii dacqueti*
BFAR 0	5	*Macrobrachium rosenbergii dacqueti*
BFAR 1	10	*Macrobrachium rosenbergii dacqueti*

The distinguishing characteristics of each species which were identified from the samples collected 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 body color and pattern of the wild-sourced M. rosenbergii rosenbergii (left) is distinctly different from the M. rosenbergii dacqueti (right)

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

Macrobrachium equidens (Rough river prawn or estuarine prawn)
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, as shown below). 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.

2) *Macrobrachium mamillodactylus* (knobtooth prawn)
The distinct feature of this species is the shape of the rostrum (below). 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, 12.1cm. total length and 10cm body length. The rostrum has 11-13 upper teeth and 3-5 lower teeth.

M. mamillodactylus from Tambulig

3) *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 (Figures A and B below). *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).

A.

B.

M. latidactylus sample from (A) Mangagoy and(B) Lake Lanao

4) *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.

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 1.5cm average carapace length, 4.3 cm. body length and 5.3cm total length.

Samples of M. lanceifrons from Lake Mainit (southern Mindanao)

More samples will be collected and 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* shall be processed for genetic marker analysis (mt DNA sequence and msDNA analysis) at the SEAFDEC-based Aquaculture Biotechnology Laboratory in early 2006. Samples of these two subspecies will be collected from various populations and 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.

A. Evaluation of Growth Performance of Two Strains of *M rosenbergii* in Cages in Laguna de Bay

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 a stocking density of 15 prawns/m^2.

Two runs have been conducted for five months, the first run from October 2004 to March 2005 and the second 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 in 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.

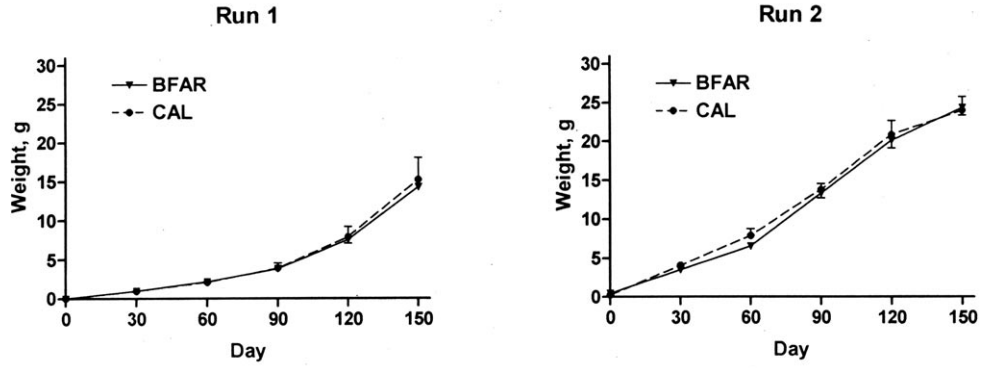

Fig. 1 Graphs showing the increase in weight of the stocks, BFAR and CAL during the two experimental runs

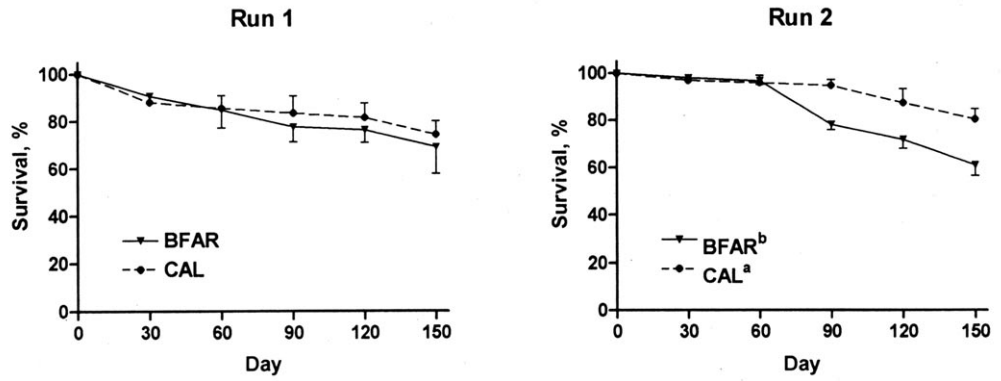

Fig. 2 Graphs showing the percentage survival of the stocks, BFAR and CAL during the two experimental runs

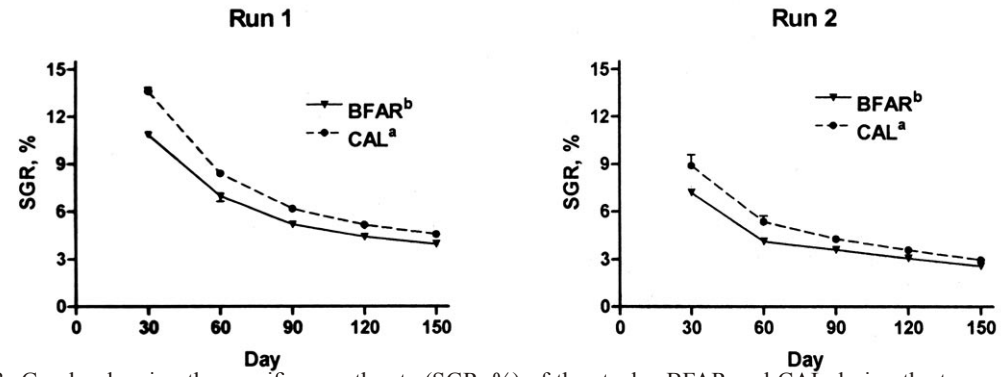

Fig. 3 Graphs showing the specific growth rate (SGR, %) of the stocks, BFAR and CAL during the two experimental runs

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

Spawning sets (1male: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 2x2x1 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. Last month, a similar experiment was 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.

The survival of postlarvae produced from the spawns obtained in this run was quite low (0.17% to 3.54%) for both BFAR and Calumpit stocks. The highest postlarval survival rate of 66.4% was achieved for one batch of spawn produced in an earlier trial run conducted in November 2004 (these F_1s were reared further to become parents of the breeders used in the on-going experiment). To improve the survival of larvae from both the BFAR and Calumpit stocks, refinements in the larval rearing method will soon be made by *Engr. Emiliano V. Aralar* and *Mr. Manuel A. Laron* who attended a month-long training course on Freshwater Prawn Hatchery Operations in Suratthani, Thailand.

D. *Macrobrachium rosenbergii* and other Indigenous *Macrobrachium* species in Mindanao and Visayan Island

The present study was conducted in pursuit of the general objectives of ASEAN-SEAFDEC AQD's Special Five-Year Program on Sustainable Fisheries in the ASEAN Region and the specific objectives of the collaborative project on the Genetic Improvement and Seed Production of the giant freshwater prawn, *Macrobrachium rosenbergii*.

The activities were focused on the survey and specimen collection of local stock of *Macrobrachium rosenbergii* and other indigenous Macrobrachium species in Mindanao. For reason of proximity some part of Visayan Islands were also considered as collection sites. The specimens were ethanol-preserved for description and measurements at MSU Naawan, preliminary taxonomic identification at SEAFDEC Binangonan and taxonomic verification with the assistance of taxonomists.

From September to December 2004 up to the current year we surveyed known prawn grounds and collected specimens of adult and juvenile Macrobrachium specimens from in Lake Lanao, Lanao del Sur; Tambulig and Aurora, Zamboanga del Sur in the upper tidal reaches of Panguil Bay; Dinas, Zamboanga del Sur in the riverine and estuarine areas in Illana Bay; in dendritic rivers connecting Sebuguey Bay in Siay, Zamboanga Sebugay; in Mangagoy River, Bislig Bay, Surigao del Sur, and in Lake Mainit, Caraga Region. The collected specimens were recorded in terms of place and date of collection, sex, rostral teeth, carapace length, total length, and body weight (Appendix Tables A-D). A number of preserved specimens were sent to the National University of Singapore (NUS) for morphometric characterization and taxonomic identification. Five species were identified by NUS taxonomists, namely: *Macrobrachium rosenbergii rosenbergii*, *M. equidens*, *M. mammillodactylus*, *M. lanceifrons*, and *M. latidactylus* (Table 1). The sources of Macrobrachium specimens used in the study are Lake Mainit, Panguil Bay, and Illana Bay (see Appendix Tables).

Table 1. Macrobrachium species in Mindanao

Collection Date	Collection Site	# species	Species
11/29/04	Panguil Bay	10	*M. r. rosenbergii*
		13	*M. equidens*
		10	*M. mammillodactylus*
12/11/04	Lake Maiinit	10	*M. lanceifrons*
12/15/04	Sebuguey Bay	10	*M. r. rosenbergii*
12/15/04	Illana Bay	10	*M. r. rosenbergii*
02/06/05	Bislig Bay	12	*M. mammillodactylus*
		5	*M. latidactylus*
09/02/04	Lake Lanao	10	*M. latidactylus*

Collection of specimen in other parts of Mindanao (Agusan River, Misamis Oriental, Davao provinces, Rio Grande de Mindanao in Cotabato, Zamboanga peninsula, and Sulu-Jolo area) will be conducted for ethanol preservation and subsequent taxonomic identification. Collection of live specimen from these areas and from previous collection sites will also be done for future laboratory cross breeding activities at MSU Naawan and SEAFDEC Binangonan.

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Appendix Table A. Macrobrachium from Lake Mainit (collection date 11/12/04)

Sample #	Sex	Rostral Teeth	Remarks	CL (mm)	TBL (mm)	BW (g)
1	M	9\3		17	55	5.09
2	M	9\4		27	62	5.62
3	M	9\4		18	55	4.79
4	M	Broken Rostrum		18	66	4.9
5	M	9\3		17	52	4
6	M	9\4		16	56	4.76
7	M	8\4		18	58	4.94
8	M	10\4		18	57	4.84
9	M	9\4		18	56	5.01
10	M	9\4		16	60	5.73
11	M	8\3		21	64	6.59
12	M	9\3		17	59	5.8
13	M	9\3		14	51	3.33
14	M	9\4		19	59	4.62
15	F	10\4		12	41	1.32
16	F	10\3		11	41	1.28
17	F	10\4		12	46	1.99
18	F	8\4		12	43	1.71
19	F	8\3	Berried Brown	14	46	2.16
20	F	10\4		10	26	0.88
21	F	7\3		13	48	2.07
22	F	8\3		10	37	1.03
23	F	9\3		10	41	1.58
24	F	8\3	Berried Black	9	39	1.18
25	F	9\3		12	42	1.6
26	F	8\3	Berried	11	39	1.28
27	F	9\4		10	36	1.29
28	F	8\3		10	37	0.98

Appendix Table B. Macrobrachium of Illana Bay (collected date 11/12/04)

Sample #	Sex	Rostral Teeth	Remarks	CL (mm)	TBL (mm)	BW (g)
56	M	14\11		66.5	176.5	161.44
57	F	3+10\10		56	160	85.81
58	M	3+9\9		67	170.5	148.08
59	M	12\9		69	186	160.21
60	M	11\10		72	186	175.23
61	F	14\11		48	143	70.78
62	F	Broken rostrum Newly molted		47	133	54.98 57.49
63	F	14\11		45	135	83.34
64	F	12\10		54	146	56.22
65	F	12\10		44	133	53.29
66	F	12\10	Berried brown	42	138	45.8
67	F	12\10	Berried orange	40	118	65.63
68	F	13\11		46	138	124.17
69	M	13\10		64	167	45.16
70	F	13\11		42	123	27.2
71	J	12\10	Newly molted	32	102	43.89
72	F	12\11		42	121	36.96
73	F	11\10		41	119	22.21
74	J	11\10		31	106	62.84
75	F	12\11		47	138	70.73
76	F	13\11	Berried orange	46	137	45.13
77	F	13\11		41	120	33.77
78	F	12\10		38	112	44.92
79		13\11		45	123	31.88
80		12\11		37	110	

Appendix Table C. Macrobrachium from Panguil Bay (collected date 11/12/04)

Sample #	Sex	Rostral Teeth	Remarks	CL (mm)	TBL (mm)	BW (g)
1	M	1+11\4		35	107	26.37
2	M	1+10\5		36	102	23.25
3	M	1+11\4		38	113	32.49
4	M	1+10\4		39	114	37.49
5	M	1+10\4		33.5	100	22.26
6	M	1+11\4		39	104	34.58
7	F	1+10\4		37	105	27.88
8	F	1+9\3		35	101	24.06
9	F	1+10\4		37	110	28.24
10	F	1+11\4		30.5	96	20.08
11	F	3+9\9	Brown eggs	47.5	144	79.17
12	F	3+11\11		52	147	82.03
13	F	3+10\11	Brown eggs	44	130.5	58.86
14	M	4+9\11		70.5	181.5	152.97
15	F	3+9\10	Orange eggs	45	134	61.37
16	F	2+10\9	Orange eggs	47	138	67.44
17	F	3+11\10	Gray eggs	45.5	136	69.25
18	M	3+10\10		63.5	167.5	122.31
19	F	3+9\11	Orange eggs	42.5	124.5	45.14
20	M	2+10\10		54	147	84.13
21	J	2+10\11		29.5	91.5	16.09
22	M	3+10\10		30	87	17.06
23	F	3+10\10		32	99.5	21.53
24	F	4+10\10		25.5	84	12.32
25	M	2+10\10		32	98	20.64
26	M	3+9\10		30.5	95.5	17.36
27	M	3+10\9		27.5	85	14.35
28	F	3+10\7		28	91	16.43
29	F	2+9\10		28	87	13.94
30	F	11\4	Black eggs	24.5	75	10.71
31	F	2+8\4		25.5	77.5	12.22
32	F	2+8\5		24	75	8.54
33	J	3+8\5		23	71	8.85
34	F	3+9\6		24.5	74	10.56
35	F	3+9\5		21.5	87.5	6.95
36	F	10\5		24	74	10.26
37	J	9\5		23	87	8.06
38	M	13\4		29	88	15.51
39	J	11\5		23	71	9.42
40	F	9\4		25	76	9.55
41	J	2+7\5		22	71	7.99
42	F	9\5		24	76.5	10.21
43	F	9\6		23.5	72	9.31
44	F	10\4		24.5	77	9.94
45	J	9\4		23.4	66.5	7.06
46	F	10\4	Yellow eggs	25.5	76	12.15
47	J	6\4		23	69	8.69
48	F	12\5		26	80	12.42
49	F	12\4	Yellow eggs	25	78	12.03
50	F	11\3		28	83	11.53
51	J	12\4		22	74	9.2
52	F	11\4		25	77	11.27
53	F	8\3	Brown eggs	23	77.5	10.58
54	F	10\4		26	82	12.76
55	F	11\3		27	81	11.53

Appendix Table D. Macrobrachium from Panguil Bay (collected date 11/12/04)

Sample #	Sex	Rostral Teeth	Remarks	CL (mm)	TBL (mm)	BW (g)
81	M	Broken	Newly molted	77	207	210
82	M	13\10		72	193	231.5
83	M	11\12		70	189	169.7
84	M	13\10		60	168	133.45
85	F	10\10		55	159	104.62
86	F	13\11		57	156	94.17
87	F	14\9		49	147	73.46
88	M	14\12		69	189	178.02
89	F	12\9		51	141	74.29
90	F	12\10		39.5	120.5	41.36
91	F	12\10		39	123	45.8
92	F	12\10		42	125	49.48
93	F	13\10	Orange egg	42	136	63.25
94	F	14\10	Orange egg/ Newly molted	42	126	53.18
95	J	12\9	Newly molted	44	127	48.27
96	J	12\10		39	112	32.52
97	F	14\9		42	130	52.53
98	F	13\11	Newly molted	40	120	41.4
99	J	12\10		34	92	21.84
100	J	10\10		32	100	23.35
101	J	13\10		27	82	13.01
102	J	12\10		27	85	14.8
103	J	12\11		26	79	12.05
104	J		Newly molted/ Broken	27	76	11.75
105	J	12\11		25	79	11.21
106	J	11\9	Newly molted	35	97	25.45
107	J	Broken	Newly molted	35	103	26.99
108	J	12\9		28	88	15.23
109	J	11\10		32	95	20.56
110	F	12\9		45	132	55.14
111	J	13\10		33	98	21.96
112	J	11\10		36	104	28.41
113	J	12\10		29	90	17.03
114	J	11\11		24	81	11.81
115	J	11\8	Newly molted	27	87	16.09
116	J	11\9		26	83	12.78
117	J	11\10		32	98	21.21
118	J	13\12		32	97	19.24
119	J	12\9		27	81	11.96
120	J	11\8		28	88	16.35
121	J	12\10		31	93	19.08
122	J	12\10		30	96	21.02
123	J	12\9		25	79	8.82
124	J	11\11		27	83	13.05
125	J	11\9		28	81	12.69
126	J	11\9		19	87	14.31
127	F	12\9		40	122	44.51
128	F	11\11		40	123	47.24
129	F		Newly molted	40	118	36.27
130	F	10\7	Newly molted/ head separated	39	110	31.66
131	F	11\10		40	117	40.2
132	F	12\10		38	112	37.57
133	F	12\10		39	115	S

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PROPOSED PROGRAM OF ACTIVITIES

COLLABORATIVE PROJECT ON GENETIC IMPROVEMENT AND SEED PRODUCTION OF MACROBRACHIUM

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

Prioritized during the Third Roundtable Discussion on the Development of Genetically-Improved Strain of Macrobrachium
Bangkok, Thailand, 3-4 December 2005

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 Kkalimantan (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*)**	**2008-2010**	**AQD (coordinating) in consultation with Thailand and Indonesia; Member countries**
• **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* for research officers**	**2007**	**AQD, core countries**