

Opening of the Roundtable Discussion

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

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

REPORT ON PROGRESS OF ACTIVITIES: PROBLEMS AND CONSTRAINTS

Genetic Improvement of *Macrobrachium rosenbergii* in Indonesia

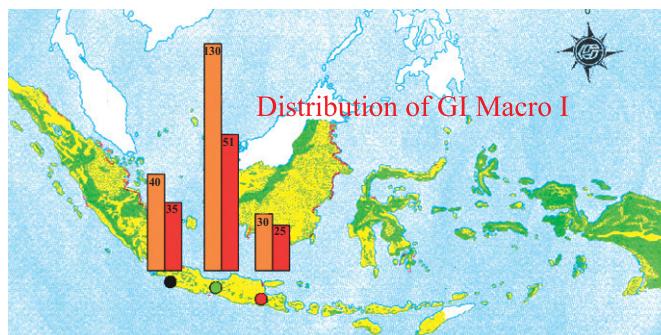
Dr. Estu Nugroho, Dr. Ketut Sugana and Mr. Maskur, Directorate General of Aquaculture of Indonesia.

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

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



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



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

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

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

The Freshwater Prawn Improvement Program of Indonesia

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

Breeding Program

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

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



Distribution of GI Macro Seed

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

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

Activities on the Giant Freshwater Prawn in 2004

i) Base population of GI Macro II

At least 800 breeders have been collected to perform a base population of GI Macro II. The collections originated from GI Macro I with an addition of some wild populations from Musi, Palembang, Sumatra. About 100 giant freshwater prawn, with weight of 60 to 120 g for males and 40 to 70 g for females, have been used as a genetic source through full-sibling matings that consisted of 22 families. About 1000 post-larvae (PL1) per family were collected and reared up to PL20. In this step, the survival rate of the larvae was 85-95%. Eight hundred PL20 was reared in hapa 2x2x1 m, which was placed in 200 m² pond for a month. The survival rate ranged from 50 to 80%, and the juveniles were 3-6 cm in length and 0.5 g to 2.2 g in weight. Three hundred of these juveniles were selected for the next breeding steps (Fig. 1).

At present, 300 hundred larvae with weight ranging from 0.5 to 2.2 g are reared in 40 m² in earthen pond for three months until the prawns reached the size of 20-30 g each. About 100 pairs of the best individual (in terms of growth) per family will be selected and used as breeders for the next spawning.

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

ii) Application of mtDNA molecular markers

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

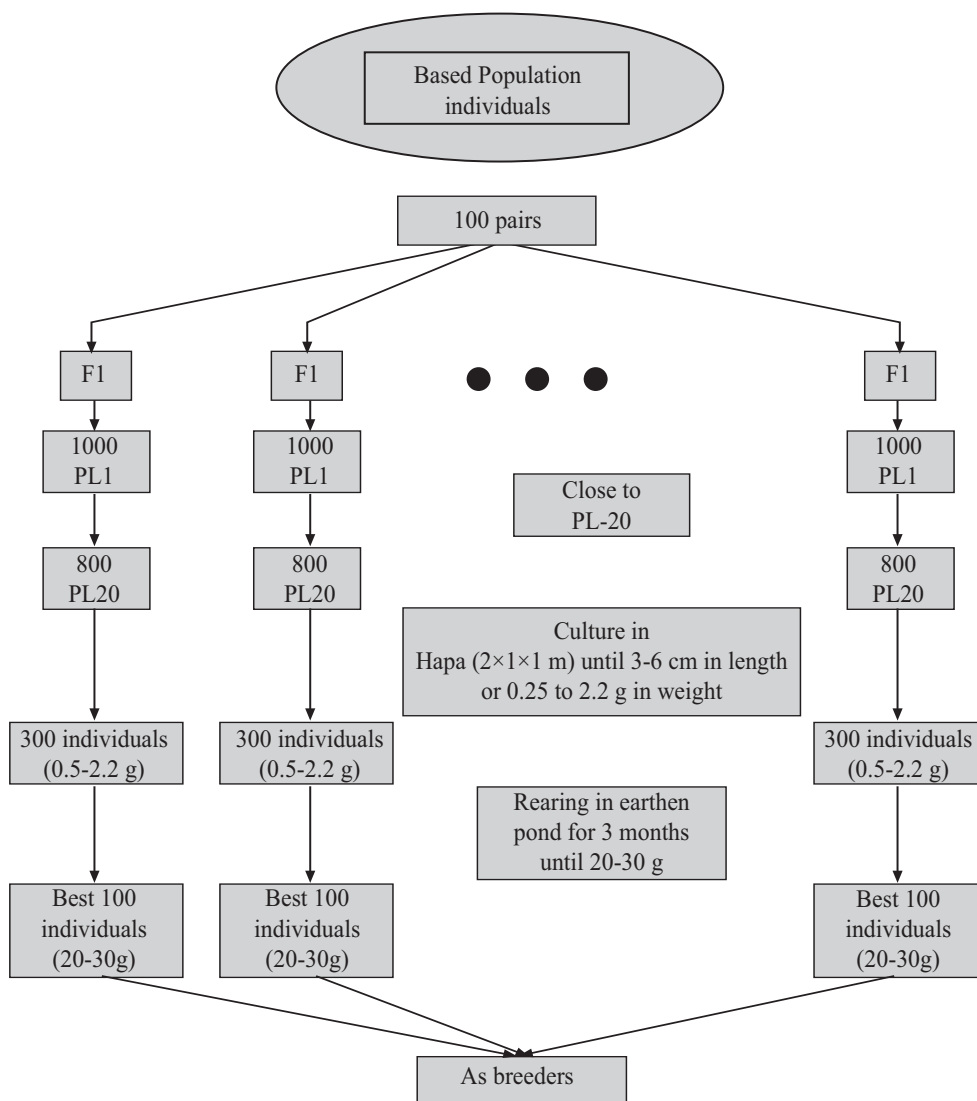


Figure 1. Flow chart of selective breeding activity at RIFA

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

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



iii) Application of estradiol-17 β for sex reversal

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

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

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

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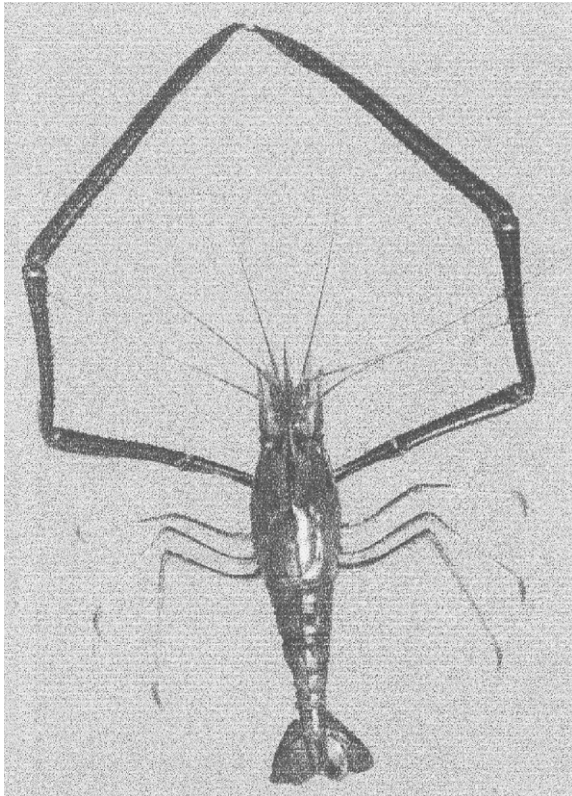


Figure 2. Grand parent stock of freshwater prawn, GI Macro (total length 38 cm, body weight 480 g after five months)

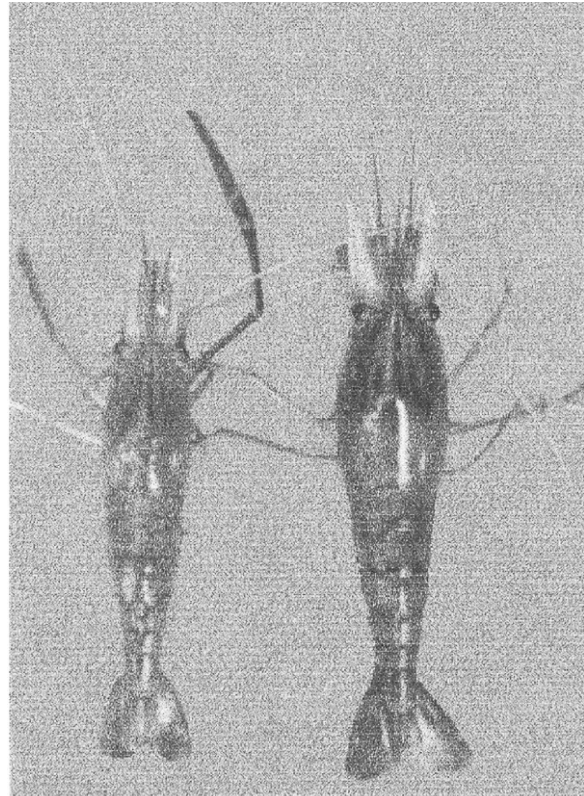


Figure 3. Selection product (right) and control farmer strain (left) after five months rearing

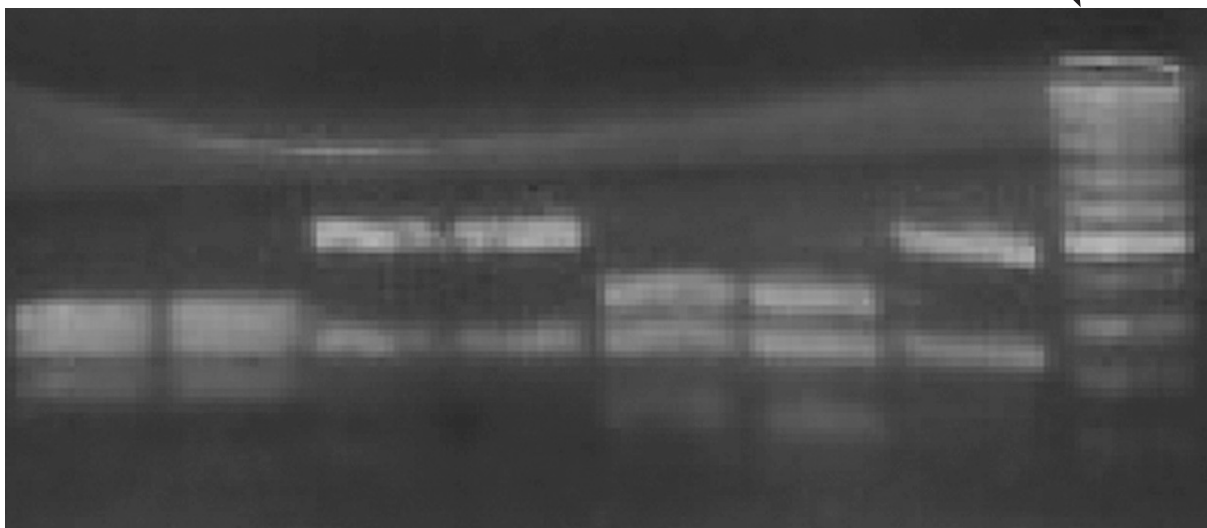


Figure 4. Mitochondria **CO-1** region of freshwater prawn degenerated by Hae III endonuclease (the arrow indicate 500 bp in length)

