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Assessment of Genetic Variability of Prawn (*Macrobrachium rosenbergii*) Post Larvae (PL) from the Broods Stocked under Different Sex Ratios

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Abstract The giant freshwater prawn (*Macrobrachium rosenbergii*) culture in Bangladesh is now at stake due to slower growth performance. The aim of the present study was to assay the impact of sex ratio on the genetic variability of larvae produced from the broods stocked under different sex ratios by analyzing Randomly Amplified Polymorphic DNA (RAPD) of *M. rosenbergii*. Broods were stocked under 3 different sex ratios viz., 1♂: 1♀, 1♂: 2♀ and 2♂: 1♀. A control population was also kept that involved the larvae production from the broods of natural source. The RAPD analysis generated 88 distinct bands of which 36 (41%) bands were polymorphic. The percentages of polymorphic loci were found to be 32%, 48%, 24% and 52% for the sex ratios 1♂: 2♀, 1♂: 1♀, 2♂: 1♀ and Control respectively. The pair wise genetic distances were 0.010, 0.007, 0.021, 0.016, 0.026 and 0.008 for 1♂: 1♀ vs. 1♂: 2♀, 1♂: 1♀ vs. Control, 1♂: 1♀ vs. 2♂: 1♀, 1♂: 2♀ vs. Control, 2♂: 1♀ vs. Control and 1♂: 2♀ vs. 2♂: 1♀ respectively. Overall gene diversity for all loci was 0.10. The unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's genetic distances clearly showed the severance of four different experimental groups into two clusters: 1♂: 1♀ and control in one and 1♂: 2♀ and 2♂: 1♀ in the other cluster. Results of the present study revealed that 1♂: 1♀ sex ratio is appropriate to establish the brood banks and for maintaining genetic variation in the offspring.

Keywords Giant freshwater prawn; Genetic variability; Sex ratios; Polymorphism

Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important crustacean species produced in inland aquaculture in many tropical and subtropical countries worldwide. The world's total farmed *M. rosenbergii* production was over 205,033 tons (market value exceeded US$ 896 million), of which 99% was produced in Asia (New, 2005). Shrimp aquaculture is one of the fastest growing economic activities in coastal areas of the Asia-Pacific region and Bangladesh is an impressive contributor in the global cultured shrimp industry; sixth largest producer in the world (Gjedrem et al., 2012). Shrimp farming in Bangladesh has been expanding since the early 1970s and reached an industrial scale followed by increasing demand for shrimp in the export market (Islam et al., 2004); there has been a very rapid global expansion of freshwater prawn farming since 1995. In early 1990s, 8306 hectare area was being used for *Macrobrachium* culture in Bangladesh using wild fry (Ahmed et al., 2008) but recently the area has been raised to 40,000 ha (Amrit and Yen, 2003). With the increasing demand for food fish and decline in capture fisheries production, aquaculture in Bangladesh is heading towards intensification. This shift from low-density to high-density culture, *i.e.*, traditional to semi-intensive or intensive culture, is consequently leading to an unprecedented rise in the demand for good quality seed (post larvae) and feeds. Bangladesh has a unique and favorable environment for prawn culture. Presently *M. rosenbergii* is commercially cultured only in the coastal districts but there is a great potential for successful prawn culture in the numerous ponds of Bangladesh. This species grows faster in suitable environmental conditions and attain marketable size within 6 months, while fin fishes require at least 1 year (Hossain and Paul, 2007). Recent problems encountered by the brackish water shrimp industry led to the boost for the culture of freshwater prawns. The culture of *M. rosenbergii* is traditionally being performed in ponds (Weimin and

Xianping, 2002; Aralar et al., 2007). The potential use of natural inland water bodies such as lakes and reservoirs for the culture of this species has been largely ignored.

At present, giant freshwater prawn culturists in Bangladesh have been facing a severe problem of slower growth performance in culture condition. Farmers claim that hatchery produced PLs do not grow as fast as wild PLs. That's why farmers are still interested on the natural seed which effects on the biodiversity. Although, at present, quite a large quantity of the required seed of the species is being collected from the nature; many hatcheries have, in recent time, been established in the private sector. The brood prawns required for the hatcheries are, in most cases, obtained from the natural harvest from the coastal rivers; there are instances of using broods from the culture farms. Hatchery production and management, inevitably warrants careful consideration for brood selection and maintenance to mitigate the occurrence of inbreeding that delays growth, feed conversion efficiency, disease resistance etc. Little attention however, has been paid to date to the genetic attributes of this species (Aflalo et al., 2006; Thanh et al., 2009). In order to reduce the pressure on the natural stocks of this species, brood banking was initiated in some coastal districts of Bangladesh. But this initiative was in vain because of improper management. As the hatchery owners buy only gravid fertilized egg carrying females for seed production, farmers tended to keep higher quantities of females in their brood banks. Farmers maintained 1♂: 4♀ or 1♂: 5♀, even 1♂: 7♀ sex ratios in the brood banks. As a result, the males were unable to fertilize the huge number of females and the seeds produced were to be of poor quality. Unfortunately, although several studies provided valuable information regarding broodstock management (Ling, 1969; Wickins and Beard, 1974; Chow, 1982; Malecha, 1983; O'Donovan et al., 1984; Chavez Justo et al., 1991; Damrong-phol et al., 1991; Daniels et al., 1992), a more fine-focused genetic approaches to improve stocks of cultured lines have not yet been widely implemented. Thus, there is the need for systematic breeding programs through different genetic means in the future to improve economically important traits in this species. Significant productivity advances have been achieved via selective breeding programs in aquaculture over the last 10 years, particularly in a number of finfish species where improvements of up to 10 to 20% per generation have been achieved (Thanh et al., 2009). The differential growth associated with different male morphotypes is another problem for the culture of this species which also warrants particular research interest. Moreover, Mather and de Bruyn (2003) reported that the productivity of *M. rosenbergii* in Taiwan and Thailand has been declined due to inbreeding depression of this species. For quality rich seed production in hatcheries, it is the imperative to develop a better brood stock with domestication where the male and female ratio should be maintained at appropriate combination. Increase of genetic variability by maintaining different sex ratios would be a plausible solution for increasing production of prawn within a short time. Increase of heterozygosity by this technique in farmed prawn, if possible, would bring a revolution in prawn aquaculture in Bangladesh creating a long lasting impact on the production increase. The objectives of this current study were: - a) to test the genetic variability of the PLs produced from different sex ratios; b) to find out the best sex ratio for the production of genetically variable and faster growing seeds of giant freshwater prawn in Bangladesh; and c) to improve the production potential of a prime aquaculture species, *M. rosenbergii* through genetic means.

1 Materials and Methods

1.1 Experimental design

The experiment was conducted in the prawn hatchery of "Fish Seed Multiplication farm (Gallamari, Khulna, Bangladesh)". Three different sex ratios were maintained *viz*., 1♂: 1♀, 1♂: 2♀ and 2♂: 1♀ respectively. A control population was also kept that involved the larvae production from the broods of natural source. Three separate ponds and in total 450 prawns (both male and female) were used in the trial. The prawns were collected from the Kocha River, Pirojpur District, Bangladesh. Mean body weight of the collected prawns ranged between 20 to 25g. This study was conducted from November 2010 to August 2011. The adult prawns were stocked at the three separated ponds for attaining maturation from November to May. Then the berried females were collected from the ponds and taken to hatchery. The larvae were reared under hatchery condition for one month up to attaining the post larvae stage (PL). Then, the larvae were brought to the

fish Biology Laboratory for DNA Extraction, PCR amplification and agarose gel electrophoresis.

1.2 Feeding of the prawns

Reproductive performance is affected by the nutrient availability of feeds supplied for culture. In order to get good response from the prawns stocked in the ponds, a good quality supplementary feed (CP 9043) was given daily. The protein content of the feed was more than 35%. Feed was applied at 3-4% of the total body weight of the stocked prawns. Feed was supplied twice a day: at evening (usually at 7 PM) and at midnight (11 PM of night).

1.3 Monitoring to check brood readiness

The prawns were checked at fortnightly interval by netting to monitor the breeding readiness. After 4 months of stocking, eggs were found in the egg chambers of female prawns. During brood stock development, the eggs were yellow in color and it changed from yellow to orange and finally became brown as the development progress within. Before hatching, the eggs became grayish black.

1.4 Brood collection for seed production

The mature berried females were collected separately from the ponds by gentle netting. At this stage, the mean body weight of the prawns ranged between 45 to 80 gm. The collected broods were soaked in 5 ppm formalin solution for 10 minutes for disinfection as well as to avoid microbial or parasitic contamination and then the broods were washed well by freshwater. The disinfected fresh broods were then transferred to the Prawn Hatchery. The ripe females were kept in the separate brood stocking tanks of the hatchery in accordance with different sex ratios by marking the tanks. Uncooked rice was fed to the broods in the brood stocking tanks.

1.5 Hatching of eggs and larvae collection

Berried females were kept in the hatching tanks with blower for getting proper oxygen. After 2-3 days, larvae were hatched out. Immediately after hatching, larvae were collected by a disinfected scoop net and the collected larvae were shifted in the larvae rearing tanks.

1.6 Feeding and rearing of the larvae

Live artemia was given to the larvae as live feed. Before supplying the artemia to the larvae rearing tank, the artemia were treated with 5 ppm formalin solution for 5 minutes and then washed with 15 ppt saline water. For the first 7 days only live feed (artemia) was given to the larvae. After 7 days, custard was given with artemia. The ingredients of custard included: powdered milk, egg, multivitamin tablet, white flour, agar powder, tetracycline tablet, vitamin-C tablet and cod liver oil tablet. Larvae were reared in 12-15 ppt water and salinity was maintained constantly at this level. Water of the larvae rearing tanks exchanged once daily. Bleaching water was used to wash the floor of the hatchery daily to avoid any type of microbial contamination.

1.7 Genomic DNA extraction from larvae

Genomic DNA of the prawn PL was extracted from tissue by using *AccuPrep*® Genomic DNA extraction kit (Bioneer, Korea) following the procedure of Chand et al., (2005). By using the DNA extraction protocol, genomic DNA was isolated from 80 different prawn post larvae (PL). There were 4 experimental groups and 20 samples were taken from each of the experimental groups. Extracted DNA was then preserved at -20 ℃ until use.

1.8 Primer selection

Five commercially available decamer primers of random sequence (Operon Technologies, Inc., Alameda, CA, USA) were selected for the RAPD analysis of giant freshwater prawn post larvae produced from different sex ratios. The 5 different primers were OPA_1, OPA_3, OPA_4, OPA_9 and OPA_{10}. Presence of bands for each primer was confirmed on the basis of intensity and resolution of the bands. Name of the selected primers, DNA sequence and G-C content have been presented in the Table 1.

Table 1 Sequence of 5 different primers used for RAPD analysis of prawn

Primer	Nucleotide Length	Sequence (5’ to 3’)	Annealing Temperature T_A (℃)
OPA1	10mer	CAG GCC CTT C	34
OPA3	10mer	AGT CAG CCA C	32
OPA4	10mer	AAT CGG GCT G	32
OPA9	10mer	GGG TAA CGC C	34
OPA10	10mer	GTG ATC GCA G	32

1.9 Determination of DNA purity and concentration

The concentration and purity of extracted DNA samples were determined from the ratio of absorbance at A_{260} and A_{280} (absorbance at 260 nm and 280 nm) using a spectrophotometer against NaOH blank cuvette. DNA sample containing cuvette was washed properly before loading next sample. Thus, a list of data for the samples for two different absorbencies was found and saved. The protocol used in this experiment was designed for a double-beam spectrophotometer. The DNA concentration and purity was determined by the following formulas:

1. Double-stranded DNA concentration (C), μg/ml = Absorbance at $A_{260} \times 50 \times 500$

2. Purity = Absorbance at A_{260} /Absorbance at A_{280}

In case of all the extracted DNA samples, A_{260}/A_{280} values were <1 which indicates satisfying purity of the extracted DNA and there was no contamination. After determining the concentrations of extracted DNA, nuclease free de-ionized sterile distilled water was added in a required volume to adjust the concentrations of all the extracted DNA samples. The adjusted DNA concentration for PCR amplification was at 20-25 ng/μL.

1.10 PCR Amplification

The PCR reactions were performed in a 20 μL reaction mixture containing 1 μL DNA sample (template DNA), 2 μL (10 pico-mole/μL) oligonucleotide primers (Bioneer, Korea), 2 μL 10X reaction buffer (Bioneer, Korea), 2 μL 10mM dNTPs mixture (Bioneer, Korea), 2 μL Taq DNA polymerase (1 unit) and 11 μL de-ionized sterile distilled water. The reaction mixtures were then placed in a DNA thermal cycler (C1000™, BIO-RAD, USA) for polymerase chain reaction. The PCR conditions for target DNA amplification were: initial extended step of de-naturation at 94℃ for 2 minutes followed by 35 cycles of de-naturation at 94℃ for 1 minute, primer annealing at 32-34℃ for 1 minute and elongation at 72℃ for 1 minute.

1.11 Agarose gel electrophoresis

After the completion of thermal cycling, 8 μL of each PCR products was analyzed electrophoretically by running through a 2% agarose gel and the amplified product size was determined by comparing with a 100 bp DNA size marker which is known as DNA Ladder (Bioneer, Korea). The 2% agarose gel constituted ethidium bromide and 1X TAE buffer. The electrophoresis apparatus (Bioneer, A-7020, Korea) 1X TAE buffer was poured to soak the agarose gel and the elcetrophoresis process was maintained at 120 V electric power for 45 minutes. The DNA Ladder provided 13 different bands of 100 to 2000 base pairs (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1600 and 2000 base pairs). The bands were observed on UV-transilluminator and photograph was taken by a Gel Cam Polaroid camera.

1.12 Data Analysis

DNA banding patterns generated by RAPD were scored as 1 for bright bands (presence of bands) and 0 for their absence of bands. POPGENE (Version 1.31) software was used to determine gene diversity (Nei, 1973), gene flow (N_m), genetic distance (D), to construct an unweighted pair group method of arithmetic mean (UPGMA) dendrogram among the populations and to perform a test of homogeneity (at 95% confidence interval) in different locus between population pairs. Tools for population genetic analyses (TFPGA; Miller, 1997) software was used to estimate the population differentiation (F_{ST}) at 5% level of significance. In this study, similarity coefficient was calculated across all possible pair wise comparison of individuals both within and among the sex ratios using the method of Lynch (1990) with the formula:

$$SI=2N_{AB}/ (N_A +N_B)$$

Where, N_{AB}= Number of fragments shared by individual A and B; N_A and N_B=Number of fragments scored for each individuals.

2 Results

A total of 80 prawn PL samples (four different experimental groups: 3 treatments for 3 different sex ratios *viz.*, 1♂: 2♀, 1♂: 1♀, 2♂: 1♀ and the control constitutes the PLs obtained from natural broods) were used for RAPD analysis with the aid of 5 different oligonucleotide primers. The primers yielded 88 distinct bands of which 36 (41%) were polymorphic. Primer OPA_3 generated the highest numbers of bands (21 bands) whereas OPA_1 and OPA_4 produced the lowest number of bands (15 bands for each). The primers OPA_9 and OPA_{10} generated 20 and 17 bands respectively. The banding patterns have been presented in the Figures 1-5.

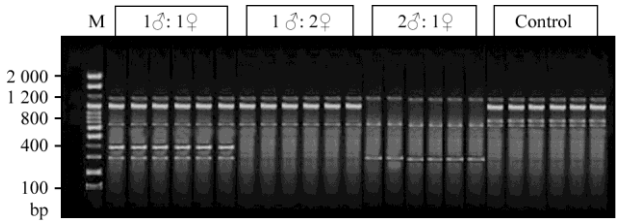

Figure 1 Randomly amplified polymorphic DNA (RAPD) profile of *Macrobrachium rosenbergii* post larvae for the primer OPA_1. M is molecular weight marker (DNA Ladder)

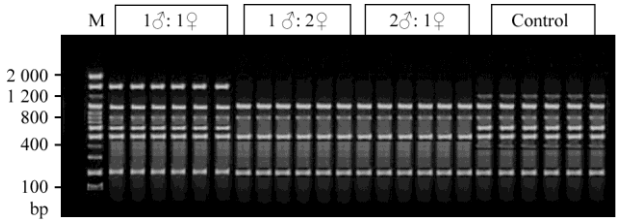

Figure 2 RAPD profile of *Macrobrachium rosenbergii* post larvae for the primer OPA_3

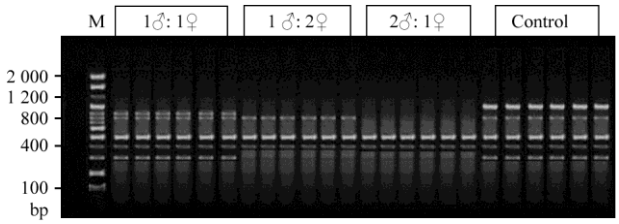

Figure 3 RAPD profiles of *M. rosenbergii* post larvae for the primer OPA_4

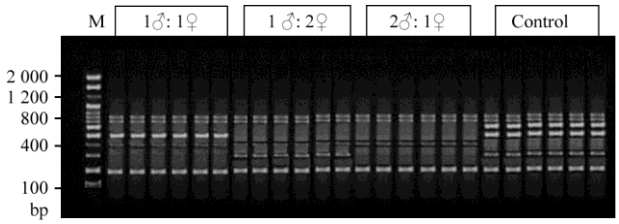

Figure 4 RAPD profiles of *M. rosenbergii* post larvae for the primer OPA_9

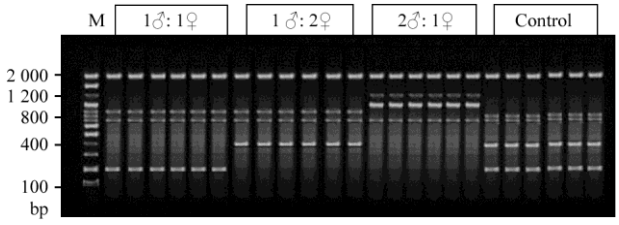

Figure 5 RAPD profiles of *M. rosenbergii* post larvae for the primer OPA_{10}

Table 2 represents the total number of bands, number of polymorphic bands and overall polymorphism proportion obtained from five different primers at different size ranges. Intra-population (within the experimental groups) genetic similarity index (SI) values for 1♂: 1♀, 1♂: 2♀, 2♂: 1♀ and control were found to be 94.1%, 96.2%, 96.8% and 93.7% respectively. Inter-population SI (S_{ij}) values for 1♂: 1♀ vs. 1♂: 2♀, 1♂: 1♀ vs. Control, 1♂: 1♀ vs. 2♂: 1♀, 1♂: 2♀ vs. Control, 2♂: 1♀ vs. Control and 1♂: 2♀ vs. 2♂: 1♀ were 91.4%, 94.7%, 92.1%, 92.5%, 91.8% and 95.6% respectively.

OPA_{10} generated the highest number (9) of polymorphic bands where as OPA_1 and OPA_3 produced the lowest number (6 for each) of polymorphic bands. Table 3 summarizes the data for all primers and experimental groups.

The highest proportion (52%) of polymorphic bands was obtained for the post larvae (PL) produced from the natural broods (control) and the lowest (24%) was obtained for the PL produced from 2♂: 1♀ sex ratio. Polymorphic band percentage of 1♂: 1♀ sex ratio (48%) was very close to that of control and comparatively lower for the sex ratio 1♂: 2♀. Overall gene diversity for all the experimental groups was found to be 0.10. The highest gene diversity value (0.10) was obtained for the control population and the lowest value (0.05) for 2♂: 1♀. Gene diversity values showed significant difference at 95% confidence interval.

The highest and lowest F_{ST} values were obtained for 2♂: 1♀ vs. Control and 1♂: 1♀ vs. Control combinations respectively (Table 4). The highest and the lowest genetic distances (Nei's genetic distance) were obtained at 0.012 for 1♂: 1♀ vs. 2♂: 1♀ and 2♂: 1♀ vs. Control combinations and 0.007 for 1♂: 1♀ vs. Control combination (Table 4). Unweighted pair group method of arithmetic mean (UPGMA) dendrogram revealed the differentiation of the experimental groups into 2 separate clusters depending on Nei's (1973) distance values (Figure 6).

3 Discussion

The results of the present study clearly indicate that healthy brood stock for successful breeding of giant freshwater prawn can be developed within the closed condition or in the ponds. The maturity and breeding cycles of *M. rosenbergii* stocked in the pond environment were similar to those of nature and similar finding was

Table 2 Number and proportion of polymorphic bands for each primer

Primer Codes	No. of Bands Scored	Total Polymorphic Bands	Overall Proportion of Polymorphism (%)	Size Range (BP)
OPA_1	15	6	40	300−1200
OPA_3	21	6	28.57	200−1600
OPA_4	15	7	46.67	300−1000
OPA_9	20	8	40	200−800
OPA_{10}	17	9	52.94	200−2000

Table 3 Number and percentages of polymorphic bands, total number of bands and gene diversity obtained from different experimental groups

Sex Ratios	1 ♂ : 2 ♀	1 ♂ : 1 ♀	2 ♂ : 1 ♀	Control
Total no. of bands	19	25	17	27
Total no. of polymorphic bands	06	12	04	14
Rate of Polymorphism (%)	32	48	24	52
Gene diversity (H)	0.06	0.09	0.05	0.10
Overall no. of polymorphic bands across the sex ratios: 22				
Overall % of polymorphic loci across the sex ratios: 41				
Overall gene diversity for all loci: 0.10				

Table 4 Population differentiation (θ or F_{ST}) at 5% level of significance, gene flow (N_m) and pair-wise genetic distance (D) values between the treatment combinations (sex ratios)

Population pairs	F_{ST} ±SD	Genetic Distance (D)
1♂: 1♀ vs. 1♂: 2♀	0.048* ±0.031 (0.021−0.102)	0.010
1♂: 1♀ vs. Control	0.019 ±0.012 (0.007−0.038)	0.007
1♂: 1♀ vs. 2♂: 1♀	0.063* ±0.038 (0.039−0.113)	0.012
1♂: 2♀ vs. Control	0.054* ±0.032 (0.032−0.109)	0.011
2♂: 1♀ vs. Control	0.071* ±0.043 (0.036−0.117)	0.012
1♂: 2♀ vs. 2♂: 1♀	0.032 ±0.024 (0.022−0.053)	0.008
All the sex ratios (across all the experimental groups/populations)		-

Note: *p<0.05 and statistically significant

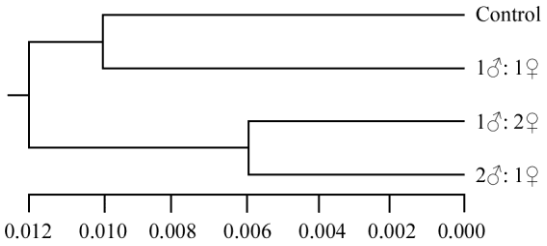

Figure 6 UPGMA dendrogram of giant fresh water prawn PLs based on Nei's (1973) genetic distance according to RAPD analysis

also obtained by Rao (1991) and Cavallo et al. (2001). They observed that the larvae hatched from the larger berried prawn's exhibit better growth and metamorphosis, resulting in a shorter larval culture period. Thus, brood banks of giant freshwater prawn can be established to get quality brood and thereby obtaining good quality seed which is a prerequisite for successful aquaculture program. Establishment of brood banks will reduce huge pressure on natural seeds.

Variations in the number of alleles have been observed in *M. rosenbergii* by different authors; e.g., 12-18 (Chand et al., 2005), 5-17 (Chareontawee et al., 2006), 4-20 (Charoentawee et al., 2007), 3-12 (Bhassu et al., 2008), 3-16 (Divu et al., 2008), 10-20 (Karaket et al., 2011) and 3-25 (Schneider et al., 2012). The number of alleles observed by Khan et al. (2014) ranged from 5 to 14 were consistent with those of Chareontawee et al. (2006) and Divu et al. (2008) but higher than reported in a wild population in India (Schneider et al., 2012). See et al., (2008) worked with 11 distinct populations of giant freshwater prawn in Malaysia and found >95% polymorphism and even 100% polymorphism among four different populations. The highest level of intra-population SI value was obtained

at 96.8% for 2♂: 1♀ population (which is almost similar to 1♂: 2♀ population at 96.2%) and the lowest SI value was 93.7% for control population (almost similar to 1♂: 1♀ at 94.1%). These results clearly indicate the lower genetic diversity in terms of gene diversity for the 1♂: 2♀ (H=0.06) and 2♂: 1♀ (H=0.05) populations. Gene diversity (H) of the control and 1♂: 1♀ populations were 0.10 and 0.09 respectively which was almost similar and statistically insignificant. However, the gene diversity values were found to be statistically significant while 1♂: 1♀ and control populations were compared with the 1♂: 2♀ and 2♂: 1♀ populations. Inter-population S_{ij} showed a very close genetic relation between 1♂: 1♀ vs. control (94.7%) and 1♂: 2♀ vs. 2♂: 1♀ (95.6%) combinations. But the S_{ij} values for other combinations were comparatively low and indicate less genetic similarity between the population pairs. See et al., (2008) obtained 50-80% inter-population genetic similarity indices among 11 different populations of giant freshwater prawn. In the present study, S_{ij} values were far higher because all the broods used were collected from the same river and then they were stocked at different sex ratios.

Statistically significant F_{ST} values were obtained for the 1♂: 1♀ vs. 1♂: 2♀, 1♂: 1♀ vs. 2♂: 1♀, 1♂: 2♀ vs. control and 2♂: 1♀ vs. control combinations but statistically insignificant values were obtained for the 1♂: 1♀ vs. control and 1♂: 2♀ vs. 2♂: 1♀ population pairs; this clearly indicates the negligible genetic variation between the later two combinations. Khan et al. (2014) reported that the F_{ST} values between the population-pairs ranged from 0.012 to 0.021 of three riverine population of *M. rosenbergii* in Bangladesh. This level of genetic differentiation between the pairs of populations is considered as low (Balloux and Lugon-Moulin, 2002). The lowest value of F_{ST} (0.012) between the Paira and the Pashur population indicated relatively closer relation between these rivers over the Naaf population.

This could be ascribed as to the selection for a single potential mate in nature and which is similar to the sex ratio 1♂: 1♀. In case of 2♂: 1♀ combination, number of male is increased in the population that lead to the occurrence of male competition as it is evident that male territory formation is common in *Macrobrachium rosenbergii*. Due to this problem, all the males were not able to take part in copulation with females and only larger dominating blue clawed males copulated with female, thereby providing reduced genetic variability for this sex ratio. The pair wise genetic distance (Nei's genetic distance, D) values revealed the highest value at 0.012 for both the 1♂: 1♀ vs. 2♂: 1♀ and 2♂: 1♀ vs. control combinations, and the lowest value (0.007) for 1♂: 1♀ vs. control combination. All of the results clearly demonstrate the very lower genetic variability (very closer genetic relation) between 1♂: 1♀ vs. control combination. See et al., (2008) observed the highest genetic distance value as 0.0653 between two populations and the lowest genetic distance value as 0.03 in case of 11 different populations of giant fresh water prawn in Malaysia. Khan et al. (2014) observed and expected heterozygosities were found to be 0.574 to 0.634 and 0.804 to 0.827, respectively are in agreement with the range reported by other authors (Chareontawee et al., 2007; Schneider et al., 2012).

Figure 6 represents the UPGMA dendrogram for the larvae produced from broods stocked under different sex ratios together with larvae produced from natural broods. The UPGMA dendrogram based on Nei's (1973) genetic distance indicated the segregation of four different experimental groups/populations of prawn into two clusters: 1♂: 1♀ and control populations in one cluster whereas 2♂: 1♀ and 1♂: 2♀ in another cluster. Results of this study clearly indicate the suitability of maintaining 1♂: 1♀ sex ratio in brood banks of giant fresh water prawn in order to produce genetically variable and high yielding post larvae from the hatcheries. Results of the present experiment further indicated that there is a clear role of sex ratio on the genetic variability of larvae of giant fresh water prawn and thus maintenance of appropriate sex ratio is a vital issue for brood stock development for this species.

4 Conclusion

In the present study, genetic variability was assessed successfully for the giant freshwater prawn post larvae produced from 3 different sex ratios and post larvae produced from the natural broods. Results obtained from this study clearly support for the maintenance of 1♂:1♀ sex ratio for brood stock development. Collection of *Macrobrachium rosenbergii* broods for hatcheries from nature has already created a sharp

decline in abundance of this species in nature. Giant freshwater prawn culture has yet a long way to go in fulfilling the target production from closed waters of Bangladesh. As the culture of this species is being suffering from the problem of lower production performance, maintenance of appropriate sex ratio (1♂: 1♀) in the brood banks with better feeding practice may be a plausible solution on a preliminary basis to get rid of this problem as it can provide genetically more variable and high yielding seeds. It is expected that the higher the male competition, the higher the chance of morphotypes formation in *M. rosenbergii* but 1♂:1♀ ratio in a breeding population is essential for its gene pool conservation and though there will be more male in the culture system ensuring greater competition, innovative measures has to be brought in the culture system to reduce male competition. Further research is needed to test the relation between sex ratios, growth and male morphotypes formation in order to solve prevailing problems of *M. rosenbergii* culture.

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