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Genetic quality deterioration in captive developed broodstocks of *Macrobrachium rosenbergii* revealed by allozyme electrophoresis

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Abstract. Broodstocks were produced in captive condition (Project developed broodstock, PB) by rearing river origin juveniles of *M. rosenbergii* and their performance was compared with Halda river origin broodstock (HB) and Gher developed broodstock (GB). Berried females from all the broodstocks were bred in a hatchery and their F_1 progenies (F_1HB, F_1PB and F_1GB) were produced. Genetic variation of the three broodstocks and their F_1 progenies of *M. rosenbergii* were assessed by allozyme electrophoresis. Genetic variability was higher in two wild origin broodstocks (HB and PB) than others. Genetic variability reduced in all F_1 progenies. The values of pairwise F_{st} (0.0355) and genetic distance (0.047) between two wild origin brood-stocks (HB and PB) were lowest indicating their close relationship which was also evident through dendrogram. Separation of their F_1 progenies (F_1HB and F_1PB) by forming another sub-cluster in dendrogram supports the occurrence of genetic change in F_1 generation. Decline of effective breeding number (N_e) in F_1HB and F_1GB as compared to their parents indicated occurrence of inbreeding in F_1 generation. The present study revealed that hatchery managers should not use captive origin broodstocks year after year rather broods should be produced in every year by rearing wild PL or juveniles with appropriate brood management techniques.

Keywords: Genetic variation, F_1 progenies, *Macrobrachium rosenbergii*

Introduction

Farming of giant freshwater prawn (*Macrobrachium rosenbergii*) (here in after referred as 'prawn') plays an important role in the economy of Bangladesh. In 2016-17 fiscal year, Bangladesh produced approximately 52,544 mt of freshwater prawn of which approximately 6,627 mt was exported (DoF 2017). It is a popular species to the farmers having potential to grow in freshwater ponds and reservoirs across the country. So, many freshwater prawn hatcheries have been established in the country for producing post-larvae (PL). Prawn hatcheries usually collect ready to spawn berried females for producing PL. Though prawn hatchery managers prefer wild berried females collected from rivers, the supply of them is rather irregular and uncertain. Ahmed (2008) reported that approximately 85% of total required broods came from prawn farms like *ghers* (traditional water bodies transformed for extensive prawn farming by making dykes around rice fields) and ponds; and only 15% obtained from wild sources. Hence, hatcheries are compelled to use captive origin broods sourced from *gher* or ponds, though some hatchery operators prefer captive origin broods due to low price. Inbreeding of prawn was reported in Thailand which is believed to be resulted from broodstock commonly being sourced from grow-out ponds rather than from wild leading to the accumulation of high level of inbreeding over time (New 2000, Mather and de Bruyn 2003).

Depletion in prawn production due to inbreeding in hatchery stocks has also been reported in some other countries like Taiwan, China, USA etc. (Weimin and Xianping 2002, Mather and de Bruyn 2003, Mather 2009, Schneider *et al*. 2013). So, prawn hatchery managers should not use brood stocks originated from grow-out ponds or '*ghers*' for production of PL for consecutive generations. They may use wild broods but mass collection of wild broods from nature may result reduction in effective breeding number, causing genetic drift (small size of breeding population). Reduction in wild population of prawn in Bangladesh has already been reported by Khan *et al*. (2014) which is a great concern for prawn industry of the country. It is, therefore, necessary to conserve natural stocks of prawn. Hatchery managers need to maintain their own broodstock for steady production of PL and the quality of broodstocks should be maintained as like wild ones. As wild stocks can provide an immediate resource for addressing genetic diversity, prawn hatcheries may collect wild PL or juveniles in sufficient numbers and maintain them in ponds with proper feeding and water management for brood production. In addition, genetic status of these wild origin-pond reared broodstocks and their progenies should be studied and compared with available broods (wild and captive origin broods) before making any suggestion for hatcheries. Therefore, the present study was designed to assess genetic variation of the wild and captive origin brood stocks of *M. rosenbergii* and any quality degradation in F_1 generation.

Materials and Methods

Source of experimental prawn: The study was carried out under an adaptive research project on quality broodstock development of prawn, *M. rosenbergii* in captive pond condition in Noakhali region, the south-east part of Bangladesh. The wild origin-captive reared broodstock of freshwater prawn was developed by rearing wild juveniles collected from the Bulla river (a local river of Noakhali region connected to the Meghna river system) in the ponds of a private farm known as Southern Agro Fisheries and Allied Ltd. (SAFAL) and designated as 'Project developed brood- stock' (PB). The juveniles (PB) were stocked in two earthen ponds (600 m^2 each) of the farm divided into six equal sized compartments (180 m^2 each) with bamboo made bana (fences made with bamboo strips and nylon thread) set with nylon net and fixed with bamboo poles. Each compartment was stocked with 180 juveniles (one juvenile per square meter) maintaining male-female ration at 1:1 (average length, 14.75 ± 0.05 cm and weight, 35.60 ± 0.00 g) and reared up to maturation by providing a formulated feed (approximately 32% protein) at the ration of 3-5% per day. River water was added to the ponds during full moon and dark moon from the Bulla river to renew the water of each compartment as well as to maintain the water depth at desired level. Wild broodstocks of freshwater prawn were collected from the Halda river, designated as 'Halda broodstock' (HB) and captive origin broodstocks were collected from a *gher* of Bagerhat district, designated as 'Gher broodstock' (GB). They were kept at Upakul Freshwater Prawn Hatchery (UFWPH), Noakhali for breeding. Berried females of PB were collected from the SAFAL farm and transported to the same hatchery for breeding. Newly Hatched F_1 nauplii of three broodstocks (HB, PB and GB) designated as F_1HB, F_1PB and F_1GB, respectively were reared at UFWPH for production of PL and they were reared in separate earthen ponds of the SAFAL farm up to maturity stage.

Allozyme electrophoresis: For genetic analysis, horizontal starch gel electrophoresis method as recommended by Shaw and Prasad (1970) was used. Muscle sample (5-10 g) of thirty individuals of each of three broodstocks and their respective F_1 progenies were carefully collected and stored at -20°C. For electrophoresis amine-citrate buffer (CA 6.1 and CA 7.0) system (Clayton and Tretiak 1972) was used. After electrophoresis, the gel slices (about 1 mm thickness) were histochemically stained for different enzyme activities as described by Aebersold *et al*. (1987). Ten enzymes were analyzed for the study: Lactate dehydrogenase (LDH), Malate dehydrogenase (MDH), Phosphoglucomutase (PGM), Glucose-6-phosphate Isomerase ((GPI), Esterase (EST), Glyceraldehyde-3-phosphate dehydrogenase (G3PDH), Isocitrate dehydrogenase (IDH), Aspartate aminotransferase (AAT), Alcohol dehydrogenase (ADH), and Glucose-6-phosphate dehydrogenase (G6PDH). After staining the gel, the loci were numbered consecutively and the electrophoretic bands corresponding to multiple alleles at each locus were alphabetically named as *a, *b etc. in the order of detection according to the migration rates toward anode.

Genetic data analysis: The genotype of individual prawn at all allozyme loci were determined and used for estimating genetic variation as well as population structure and differentiation parameters in the populations. Allele frequencies were calculated by direct count of the proportion of different alleles. Number of alleles at each locus were calculated in FSTAT version 2.9.3 (Goudet 2002). Observed direct count heterozygosity (H_o) and unbiased expected heterozygosity (H_e) (Nei, 1978) were computed using TFPGA version 1.3 (Miller, 1997). Deviations from Hardy-Weinberg equilibrium (F_{is}) within samples were quantified by Weir and Cockerham's (1984) *f* using FSTAT version 2.9.3 (Goudet 2002). To verify the reason behind population differentiation, locus by locus molecular analysis of variance (AMOVA) was computed in ARLEQUIN Version 3.11 (Excoffier *et al*. 2006); populations were grouped as- i) broodstocks (HB, PB and GB) and progenies (F_1HB, F_1PB and F_1GB); ii) wild (HB and PB) or captive (GB, F_1HB, F_1PB and F_1GB) origin and iii) Halda (HB and F_1HB), Project developed (PB and F_1PB) and Gher (GB and F_1GB) origin based on the name of three broodstocks. To evaluate the extent of population differentiation, pairwise F_{st} (Weir and Cockerham 1984) between populations was calculated in FSTAT and an exact test of pairwise genotypic differentiation was done in GENEPOP version 4.0 (Rousset 2008). Genetic distances and relationships among populations were assessed by calculating Cavalli-Sforza and Edwards (1967) chord distance D_C in POPULATION 1.2.28 software (Langella, http://www.cnrs-gif.fr/pge/). The bootstrap values for each tree was calculated by 1000 bootstrap resampling across loci. A phylogenetic tree topology based on the distance measure was constructed by the neighbor joining method (Saitou and Nei 1987). These procedures were performed in POPULATION 1.2.28 software (Langella, http://www.cnrs-gif.fr/pge/). The tree was visualized using Tree View 1.6.6 (Page 1996) (website: http://taxonomy.zoology.gla.ac.uk/rod/rod.html). Effective population size (N_e) was estimated using the program LDNe v.1.31 (Waples and Do 2008) (lowest allele frequency used: 0.01). Finally, the evidence for a genetic bottleneck was evaluated using software BOTTLENECK version 1.2.02 (Piry *et al*. 1999) assuming an infinite allele mutation (IAM) model or a stepwise mutation model (SMM).

Results

Alleles, genotypes and allele frequency: Among the ten enzymes used, three (ADH, AAT and G6PDH) produced either very fade or ambiguous banding pattern in most cases which were not interpretable and not considered for further analysis. The electrophoretic patterns showed that rest seven enzymes were controlled by the genes at nine presumptive loci, three of them were monomorphic (*Ldh**, *Gpi**, *Est-2**) and six of them were polymorphic at P_{99} (*Mdh-1**, *Mdh-2**, *Pgm**, *Est-1**, *G3pdh** & *Idh**) (Table I). Both number of alleles and gene diversity were higher in parental broods than their F_1 progenies. Private alleles were found only in broodstocks, in PB at the locus *Mdh-2** (frequency: 0.033) and in GB at the locus *Pgm** (frequency: 0.017) (Table I).

Table I. Allele frequency at six polymorphic loci of three broodstocks and their F_1 progenies of *M. rosenbergii*

Enzymes	Locus	Allele	HB	PB	GB	F_1HB	F_1PB	F_1GB
Malate dehydrogenase	*Mdh-1**	**a*	0.083	0.067	0.067	-	0.167	0.150
		**b*	0.917	0.933	0.933	1.000	0.833	0.850
	*Mdh-2**	**a*	1.000	0.967	1.000	1.000	1.000	1.000
		**b*	-	0.033	-	-	-	-
Phosphoglucomutase	*Pgm**	**a*	1.000	1.000	0.983	1.000	1.000	1.000
		**b*	-	-	0.017	-	-	-
Esterase	*Est-1**	**a*	0.233	0.317	0.117	0.300	0.183	-
		**b*	0.767	0.683	0.883	0.700	0.817	1.000
Glyceraldehyde-3-phosphate dehydrogenase	*G3pdh**	**a*	0.167	0.383	0.100	-	-	-
		**b*	0.833	0.617	0.900	1.000	1.000	1.000
Isocitrate dehydrogenase	*Idh**	**a*	0.800	0.900	0.283	0.533	0.650	0.350
		**b*	0.200	0.100	0.717	0.467	0.350	0.650

Genetic diversity: None of the three broodstocks and their F_1 progenies deviated significantly ($p > 0.05$) from Hardy-Weinberg expectations (data not shown). The mean proportion of polymorphic loci per population (%), Observed heterozygosity (*Ho*) and Expected heterozygosity (*He*) in the broodstocks (51.852 ± 6.416, 0.126 ± 0.013 and 0.125 ± 0.019) were higher than those values of the progenies (25.926 ± 6.415, 0.089 ± 0.019 and 0.100 ± 0.019, respectively) (data not shown). Allelic pattern of three broodstocks and their F_1 progenies is shown in Fig. 1. Among the three broodstocks, total number of alleles was higher in PB (14) and GB (14) than that of HB (13). Among the three F_1 progenies, total number of alleles in F_1HB (11) and F_1GB (11) was lower than that of F_1PB (12). Among the broodstocks, mean number of alleles per locus was higher in PB (1.556) and GB (1.556) than that of HB (1.444) which reduced in F_1 progenies (Fig. 1). Mean number of effective alleles per locus was highest in PB (1.232) and lowest in F_1GB (1.131). Heterozygosity was highest in PB whereas lowest in F_1GB. Level of heterozygosity reduced in F_1 progenies compared to the respective broodstocks. Among all the populations, highest genetic variability was observed in PB and lowest in F_1GB (Fig. 1).

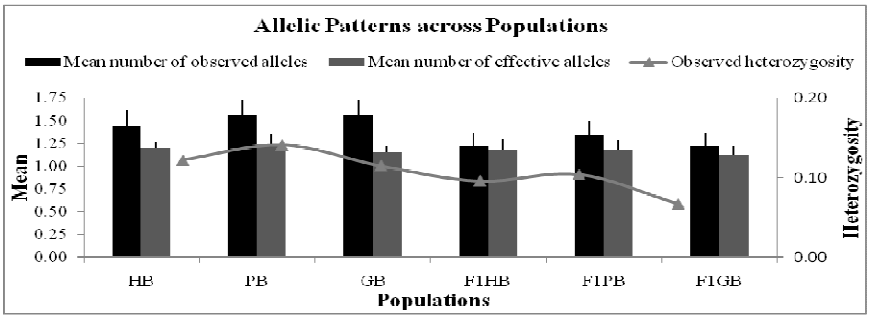

Fig. 1. Allelic pattern and heterozygosity of three broodstocks and their F_1 progenies of *M. rosenbergii*.

Population differentiation and relationship: The exact test P-values for genotypic differentiation and pair-wise population differentiation (F_{st}) (Weir and Cockerham 1984) values were significant ($p < 0.05$) in all cases except between HB and PB (Table II). Among the three broodstocks, estimate of pair-wise F_{st} between two wild origin broodstocks, HB and PB (0.0355) was lowest and not significant ($p > 0.05$) indicated close relation between them (Table II). The highest value of pair-wise F_{st} (0.3482) was observed between PB and F_1GB; and the lowest value of pair-wise F_{st} (0.0240) was observed between GB and F_1GB (Table II). Results of locus by locus AMOVA test, as presented in Table III, indicated that population differentiation occurred between populations due to their origin- whether they were of wild or culture origin (F_{CT}: 0.155; $P = 0.047$). Factorial correspondence analysis (Fig. 2) also supports the results of AMOVA indicating that differentiation between populations occurred due to origin- wild or captive. Factorial correspondence analysis exhibited that GB was isolated from two wild origin broodstocks by forming a cluster with captive origin three F_1 progenies indicating GB was not of wild origin. Separation of F_1 progeny from their parental population as shown in factorial correspondence analysis (Fig. 2) revealed the occurrence of genetic changes in F_1 generation. Two captive origin F_1 progenies (F_1HB and F_1PB) of wild origin broodstocks (HB and PB) isolated from their parents and also isolated from the captive origin broodstock (GB) and its F_1 progeny (F_1GB) (Fig. 2).

Table II. Exact test *P*-values (Fisher's method) for pairwise genotypic differentiation as implemented in GENEPOP Version 4.1.4 (above diagonal) and pairwise F_{st} (Weir and Cockerham 1984) of three broodstocks and their F_1 progenies (below diagonal) of *M. rosenbergii*

Populations		Brood stocks			F_1 progenies		
		HB	PB	GB	F_1HB	F_1PB	F1GB
Brood stocks	HB	000	0.134^{NS}	0.000***	0.000***	0.005**	0.000***
	PB	0.0355 ^{NS}	000	0.000***	0.000***	0.000***	0.000***
	GB	0.2042*	0.2995*	000	0.000***	0.000***	0.011*
F_1 progenies	F_1HB	0.0822*	0.1936	0.0910*	000	0.004**	0.000***
	F_1PB	0.0357*	0.1563*	0.1234*	0.0358*	000	0.000***
	F_1GB	0.2274*	0.3482*	0.0240*	0.1361*	0.1076*	000

Markov chain parameters: dememorization: 10,000; number of batches: 100; iterations per batch: 10,000. NS: Not significant ($p > 0.05$); *Significant at $p < 0.05$. **Significant at $p < 0.01$. ***Significant at $p < 0.001$.

Table III. Locus by locus AMOVA results over six polymorphic loci with populations clustered in three ways

Sources of variation	Populations clustered into two groups: broodstocks and progenies			Populations clustered into three groups: Halda, Project developed and *Gher*			Populations clustered into two groups: wild and captive origin		
	Sum of squares	Variance components	Variation (%)	Sum of squares	Variance components	Variation (%)	Sum of squares	Variance components	Variation (%)
Between groups	6.700	0.005 (Va)	0.750	18.739	0.046 (Va)	7.563	18.843	0.100 (Va)	15.450
Between populations within groups	23.556	0.089 (Vb)	14.820	11.517	0.055 (Vb)	9.041	11.413	0.039 (Vb)	5.978
Between individuals within populations	92.767	0.025 (Vc)	4.140	92.767	0.025 (Vc)	4.086	92.767	0.025 (Vc)	3.850
Within individuals	87.000	0.483 (Vd)	80.290	87.000	0.483 (Vd)	79.310	87.000	0.483 (Vd)	74.722
Total	210.023	0.602	100	210.023	0.609	100	210.023	0.647	100
Fixation indices:	F_{CT}: 0.007 F_{SC}: 0.149 F_{IS}:0.049 F_{IT}:0.197	P = 0.306 P = 0.000*** P = 0.178 P = 0.000***		F_{CT}: 0.076 F_{SC}: 0.098 F_{IS}:0.049 F_{IT}:0.207	P = 0.334 P = 0.000*** P = 0.161 P = 0.000***		F_{CT}:0.155 F_{SC}:0.071 F_{IS}:0.049 F_{IT}:0.253	P = 0.047* P = 0.000*** P = 0.155 P = 0.001**	

*Statistically significant at 5% nominal level. **Statistically significant at 0.01% nominal level. ***Statistically significant at 0.001% nominal level. Significant tests were performed with 1023 permutations.

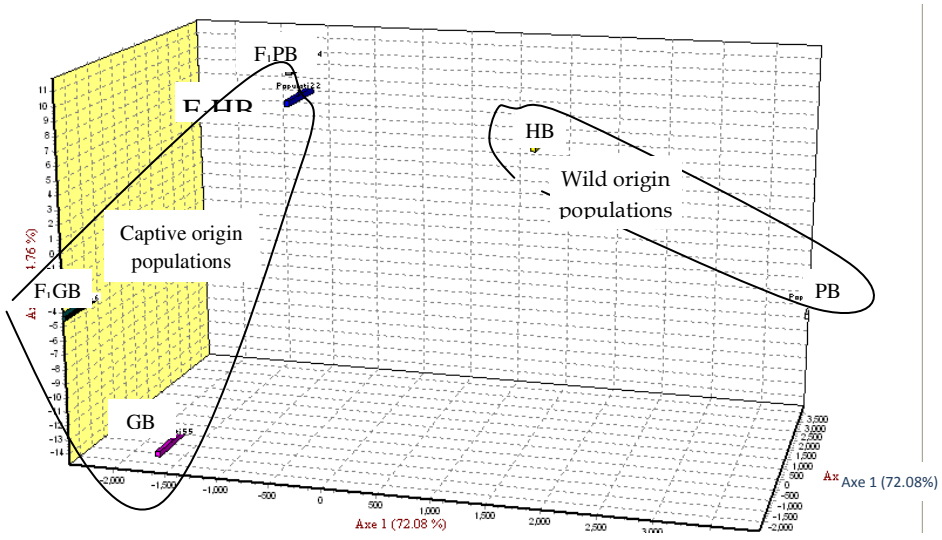

Fig. 2. Factorial correspondence analysis, using 9 loci, exhibiting two distinct population clusters grouped into wild and cultured populations of *M. rosenbergii*.

***Genetic distance*:** Cavalli-Sforza and Edwards (1967) chord genetic distance (D_C) among three broodstocks and their F_1 progenies are shown in Table IV. Among all the three parental broodstocks and their F_1 progenies, D_C value between HB and PB was the smallest (0.047) indicating their close relationship. The largest distance was observed between PB and F_1GB (0.154). The neighbor joining tree generated from the D_C values (Fig. 3) illustrated a clear division between wild and captive origin broodstocks and their F_1 progenies. All six populations were separated by two clusters. Two wild origin populations (HB and PB) formed a cluster and F_1 progenies of them (F_1HB and F_1PB), being of captive origin, formed another sub-cluster. Captive origin broodstocks, GB and its F_1 progeny, F_1GB were separated from other populations and formed another sub-cluster.

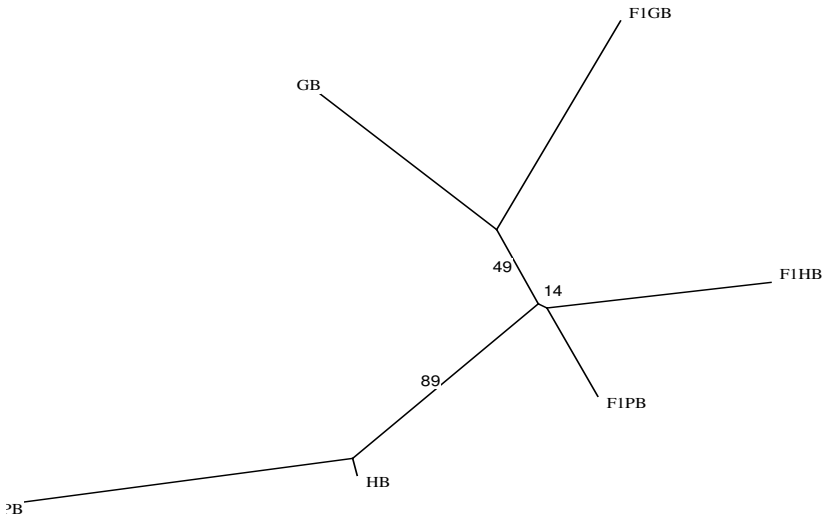

Fig. 3. Neighbor joining tree showing the genetic distance among three broodstocks and their F_1 progenies of *M. rosenbergii*. The numbers refer to the percentage bootstrap values from 1,000 replications of resampled loci.

Table IV. Cavalli-Sforza & Edwards (1967) chord genetic distance (D_C) (above diagonal) of three brood stocks and their F_1 progenies of *M. rosenbergii*

Populations		Brood stocks			F_1 progenies		
		HB	PB	GB	F_1HB	F_1PB	F_1GB
Brood stocks	HB	000	0.047	0.068	0.076	0.055	0.105
	PB		000	0.112	0.109	0.103	0.154
	GB			000	0.085	0.076	0.071
F_1 progenies	F_1HB				000	0.048	0.081
	F_1PB					000	0.054
	F_1GB						000

***Effective population size*:** Estimates of effective population size, N_e, for the both parental broodstocks and their F_1 progenies are presented in Table V. As highest two alleles were found at any of the loci studied, estimates of N_e were determined based on diallelic correlations between pairs of loci. Estimate of N_e appeared to be highest (18.4) in HB. Estimates of N_e in F_1HB (10.1) and F_1GB (10.2) were lower than their parental population. N_e in both PB and F_1PB was low compared to the actual number of broodstocks used and very similar. N_e estimates in all 6 populations with 95% CI were very wide ranging from 0.2 to ∞ (Table V).

Table V. Estimates of effective population size (N_e) in three broodstocks and their progenies of *M. rosenbergii* (Diallelic)

Parameters	HB	F_1HB	PB	F_1PB	GB	F_1GB
S	30	30	30	30	30	30
R^2	0.01524	0.00020	0.06598	0.06561	0.05427	0.00065
N_e	18.4	10.1	10.2	10.3	15.9	10.2
95% CI	5.8-∞	0.9-∞	1.0-∞	0.2-∞	1.3-∞	0.2-∞

Note: S denotes the harmonic mean of sample sizes; R^2 denotes the arithmetic mean of squared correlations between alleles at all pairs of loci. N_e denotes the effective number of breeders in one spawning season. Diallelic refers to N_e estimates in which the most common allele at a locus was kept while all other alleles were pooled into one composite allele.

***Bottleneck analysis*:** Results of the tests for population bottlenecks of three parental broodstocks and their F_1 progenies are presented in Table VI. When an IAM model was assumed, gene diversity excess ($H_e > H_{eq}$) has been demonstrated for all populations (Table VI). However, number of loci with heterozygosity deficiency was zero for all three F_1 progenies and all the loci of them were with heterozygosity excess. But, none of the population exhibited significant ($p > 0.05$) outcomes in either 'sign test' or 'Wilcoxon sign rank test' when an IAM model or SMM model was assumed (Table VI).

Table VI. Results of tests for recent bottlenecks, using the approach and the computer program

Model	HB	F_1HB	PB	F_1PB	GB	F_1GB
Mean H_e	0.282	0.467	0.259	0.350	0.193	0.361
Mutation Model: IAM						
Mean H_{eq}	0.207	0.201	0.204	0.207	0.207	0.200
Expected number loci with heterozygosity excess	1.650	0.830	2.130	1.290	2.080	0.830
No. of loci with heterozygosity deficiency	1.000	0.000	3.000	0.000	3.000	0.000
No. of loci with heterozygosity excess	3.000	2.000	2.000	3.000	2.000	2.000
P (Sign test)	0.194	0.173	0.637	0.080	0.655	0.171
P (Wilcoxon test)	0.125	0.250	0.813	0.125	0.813	0.250
Mutation Model: SMM						
Mean H_{eq}	0.250	0.254	0.252	0.253	0.247	0.253
Expected number loci with heterozygosity excess	1.910	0.980	2.420	1.450	2.370	0.950
No. of loci with heterozygosity deficiency	1.000	0.000	3.000	0.000	3.000	0.000
No. of loci with heterozygosity excess	3.000	2.000	2.000	3.000	2.000	2.000
P (Sign test)	0.280	0.242	0.529	0.112	0.221	0.244
P (Wilcoxon test)	0.313	0.250	1.000	0.125	0.219	0.250

Note: IAM denotes tests assuming an infinitive allele mutation model and SMM denotes tests assuming a stepwise mutation model. 'No. of loci with heterozygosity excess' denotes the number of loci that have higher heterozygosity values than would be expected among the polymrophic loci of a population at mutation-drift equilibrium.

Discussion

***Genetic diversity*:** Among the three parental broodstocks, HB and PB were of river origin while GB was sourced from a traditional grow-out farm, gher, i.e., captive origin. However, number of polymorphic loci in GB (5) was higher than HB (4) and a private allele was also found in GB (*Pgm**) (Table I). In fact, prawn farmers in Bangladesh stock their *ghers* or ponds with juveniles batch by batch at different dates purchased from local fry traders who collect juveniles from different nurseries. The nurseries are stocked with PL collected from different hatcheries. Thus, juveniles developed through rearing of PL produced in different hatcheries may be stocked in the same *gher* or pond which might be the cause of greater number of polymorphic loci in GB than HB. However, mean number of effective alleles per locus and observed heterozygosity of GB was the lowest among three parental broodstocks (Fig. 1). Schneider *et al*. (2013) reported that genetic diversity of a culture stock of prawn was higher than those of wild stock of India. The Indian wild population did not possess any private allele but Indian-cultured population exhibited highest contributions to the total diversity with the highest number of private alleles. They assumed that it was happened due to cross-breeding between different stocks of cultured prawns in India. On the other hand, the possible causes of less genetic divergence in Indian wild population of prawn as indicated by them were over-exploitation of wild broodstock and/or PL as well as environmental changes. Such over-exploitation of wild stock of prawn is also common in Bangladesh, that was evident in some other riverine populations of Bangladesh (Khan *et al*. 2014), might be the cause of lower number of polymorphic loci in HB.

In the present study, loss of alleles in three F_1 prawn progenies relative to respective broodstocks was occurred. Mean proportion of polymorphic loci also decreased in F_1 progenies. Private alleles possessed by the parental population was absent in respective F_1 progeny. Smith and Conroy (1992) reported loss of private alleles at allozyme loci in F_1 progeny of hatchery stocks of abalone, *Haliotis iris*. Allelic loss in F_1 progenies in the present study might be due to genetic alteration caused by genetic drift as a consequence of captive culture. This is agreed with the findings of Schneider *et al*. (2013) who reported loss of genetic diversity in cultured stocks of freshwater prawn. Loss of alleles also occurred in F_1 generation of abalone (*H. rubra* and *H. midae*) produced in hatchery (Evans *et al*. 2004).

***Population differentiation and relatedness*:** AMOVA results (Table III), factorial correspondence analysis (Fig. 2) and the neighbor joining tree (Fig. 3) topology demonstrated a clear division between the wild (HB, PB) and captive origin populations (GB, F_1HB, F_1PB, F_1GB). Differentiation at allozyme loci was reported between hatchery produced F_1 generations and their wild stocks of abalone, *H. iris* (Smith and Conroy, 1992), and multiple generations of hatchery and wild stocks of brown trout, *Salmo trutta* (Ryman and Ståhl 1980). Though HB and PB were collected from two different rivers of Bangladesh, the Halda and the Bulla respectively, both rivers are connected through the common estuary of the Bay of Bengal where mixing of freshwater prawn larvae and PL of different river origin takes place. After completion of larval development, PL migrates from the estuary to the rivers, thus gene flow between different riverine populations may occur on regular basis. That is why estimate of population differentiation between HB and PB was lowest among three broodstocks and was not significant ($p > 0.05$). The lowest value of genetic distance (D_c) between HB and PB (0.047) (Table IV) among all broodstocks and their F_1 progenies of prawn also demonstrated their closeness. Mulley and Latter (1980) reported that genetic differentiation was progressively less for those species of Penaeid prawns whose life cycles appeared to provide greater opportunities for mixing of populations. The significant difference in pair-wise F_{st} values between HB and GB (0.2042), and PB and GB (0.2995); AMOVA test, factorial correspondence analysis and the neighbor joining tree (Fig. 3) clearly demonstrated GB was of captive origin. On the other hand, significant difference in value of pair-wise F_{st} was observed between wild broodstocks and their captive reared F_1 progenies, together with factorial correspondence analysis and the neighbor joining tree indicates genetic alteration in F_1 generation. Such F_1 progenies originating from wild populations with less genetic variability should not be used as broodstocks in hatcheries.

***Effective population size (N_e)*:** As genetic variability of three parental broodstocks declined within one generation as observed in F_1 progenies, it was necessary to investigate the occurrence of inbreeding. Generally, inbreeding in a random mating population will not occur within one generation. However, it may happen to some extent in populations under culture condition. Estimates of N_e in all parental populations were lower than the actual number of females used in the hatchery possibly due to small sample size or varying contribution of different prawn broods as was evident in Penaeid shrimp (Sbordoni *et al*. 1986, Dixon *et al*. 2008) and Barramundi, *Lates calcarifer* (Frost *et al*. 2006). However, estimates of N_e in HB and GB were higher than those of their respective progenies indicating occurrence of inbreeding. The estimate of N_e in PB was low indicating the occurrence of genetic drift during domestication which was supported by Dixon *et al*. (2008) who observed significant decline of the estimate of N_e in culture populations of *P. monodon* due to large drop in diversity during domestication event.

***Bottleneck test*:** A reduction in the effective population size following a population bottleneck is correlated with a decrease in heterozygosity and a loss of rare alleles (Wright 1931). Nei *et al.* (1975) presumed that during bottleneck allelic diversity would decrease faster than heterozygosity. In the present study, allelic diversity reduced in all three F_1 progenies in relation to their parental populations. Rare alleles observed in parental broodstocks were also disappeared in respective F_1 progenies. In all F_1 progenies, gene diversity excess ($H_e > H_{eq}$) had been demonstrated and number of loci with heterozygosity deficiency was zero when an IAM model was assumed indicated that they had undergone a bottleneck (Table VI). However, none of them exhibited significant outcome in either 'sign test' or 'Wilcoxon sign rank test' when an IAM or SMM model was assumed. Detection of genetic variation is somewhat problematic in some taxonomic groups which exhibit remarkably low levels of natural variation (Mather, 2009) and Decapod crustaceans belong to such groups (Hedgecock *et al.* 1982). Nevertheless, the present study clearly demonstrated the genetic changes in F_1 progenies.

***Managerial considerations*:** Genetic variation of a stock is important for commercial traits such as growth rate, body size etc.; and fitness traits such as disease resistance, survival rate etc. (Vuorinen 1984). The loss of genetic variation in F_1 generation may occur in cultured populations due to genetic drift resulting homozygosity at most of the loci in subsequent generations. Such homozygosity might have negative influence on traits like growth, reproduction, disease resistance etc. through detrimental effect of recessive genes (Bierne *et al.* 2000). In the present study, the loss of genetic variation in F_1 generations is already evident. So, production of PL from such F_1 broodstocks might accelerate the rate of inbreeding resulting depletion in production performance traits. So, freshwater prawn hatchery managers should produce their own broodstocks in their ponds by rearing wild PL/juveniles collected from the rivers and replace them every year with a new batch of prawn broodstocks which are developed by collecting wild PL or juveniles and rearing them in the same way. In addition, brood banks of prawn need to be established for seed production maintaining of genetic variation following appropriate management measures incorporating genetic principles. As the sex ratio at which the broods are maintained has an important role on genetic variation of the progenies (Islam *et al.* 2014), sex ratio of freshwater prawn should be maintained at 1:1 during broodstock maintenance either in hatcheries or brood banks.

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