

Genetic Variation of Wild and Hatchery Populations of Indian Major Carp, Rohu (*Labeo rohita*) in Bangladesh Using Allozyme Marker

¹Rajib Sharker, ³Abdullah Al Faroque, ³Rafiqul Islam Sarder,
³Fazlul Awal Mollah and ²Shib Nath Pattadar

¹Department of Fisheries Biology and Genetics,

²Department of Aquaculture, Patuakhali Science and Technology University, Dumki, Patuakhali,

³Department of Fisheries Biology and Genetics, Bangladesh Agricultural University,
2202 Mymersing, Bangladesh

Abstract: The genetic variation of wild and hatchery populations of rohu individuals was analyzed using allozyme marker. The fish samples were collected from 3 main wilds (Halda, Jamuna and Padma) and 6 hatcheries of 3 origin (Brahmaputra and Rahim fish hatchery, Mymensingh; Rupali and Ma-fatema fish hatchery, Jessore; Bismillah and Rupali fish hatchery, Comilla) covering a wide geographical distribution of this species. The 6 enzymes (LDH, MDH, PGM, AAT, ADH GPI and G3PDH) encoded by 7 presumptive loci were screened and 4 (Mdh-1*, Gpi-1*, Gpi-2* and Pgm*) were found to be polymorphic which were interpretable in muscle with starch gel electrophoresis. The mean proportion of polymorphic loci, the mean proportion of heterozygous loci per individual, the average observed Heterozygosity (H_o) and expected Heterozygosity (H_e) were 42.85, 15.77, 0.125 and 0.184%, respectively for wild populations which were higher than the hatchery populations (33.33, 12.20, 0.075 and 0.094%, respectively). The highest gene flow value (26.725) and lowest population differentiation (0.009) found in the Halda and the Jamuna River populations indicate the close relationship among them. Based on Nei (1972)'s UPGMA dendrogram, 9 populations of rohu were divided into 2 major clusters by the genetic distance of $D = 0.092$. The cluster-1 consisted of 3 wild populations and the cluster-2 consisted of 6 hatchery populations. The present study revealed that the considerable genetic variation is maintained among the wild and hatchery populations of rohu.

Key words: Allozyme electrophoresis, genetic variation, *Labeo rohita*, hatchery population, wild population

INTRODUCTION

The Indian Major Carps (IMCs) namely catla (*Gibelion catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) are the main culture species in Bangladesh. Among IMCs rohu is the most popular Indian major carp in Bangladesh. Rohu contribute the most (23%) of total aquaculture production in the country (DoF, 2007). It is a fast growing fish in wilds and even in ponds and other closed water bodies. It has better taste and high market price.

In Bangladesh, this species is mostly available in the Padma-Brahmaputra wild system (i.e., Padma, Jamuna, Arial Kha, Kumar and Old Brahmaputra wilds) and in the Halda wild system in Chittagong. The favorite habitat of this species is the deep pools of these wilds. During monsoon, they naturally breed in the inundated wilds and flowing waters. In recent years, the natural breeding of rohu has become uncertain due to continuous

degradation of habitat caused by environmental modification and human interventions affecting spawning and feeding migration.

In the past, fish farmers used to stock naturally produced seeds for aquaculture but its supply gradually declined due to many factors such as over-exploitation, environmental pollution, blocking or changing of migration routes, degradation of breeding and feeding grounds, etc. The supply of naturally produced seeds has declined to a critically low level over the last decades and surprisingly only 1% of the total seed is reportedly caught from wilds (DoF, 2010). The rest 99% of the seeds are produced in public and private hatcheries. The hatchery produced seed of rohu is available to farmers but the quality of seed has been deteriorated to a great extent and a considerable percentage of hatchery produced seeds have shown lower growth. Of the many factors, inbreeding, genetic drift, negative selection, indiscriminate inter-specific hybridization, improper broodstock

management are mainly responsible for this genetic quality degradation (Alam *et al.*, 2002; Simonsen *et al.*, 2004, 2005; Hansen *et al.*, 2006). It is therefore, urgent to take necessary steps to minimize the earlier mentioned causes and production of quality broodstocks and its conservation could be the appropriate approach in this regard.

Genetic variation serves as an effective tool for characterization of different species or strains within a species and evaluation of changes in genetic structure of species over time. Allozyme electrophoresis is a molecular marker that is used as a well-established technique for revealing genetic variation of fish population.

MATERIALS AND METHODS

Sample collection: For allozyme electrophoresis rohu spawns were collected from 3 main wilds (Halda, Jamuna and Padma) and 6 hatchery sources of 3 regions (Brahmaputra and Rahim fish hatchery, Mymensingh District; Rupali-1 and Ma-fatema fish hatchery, Jessore District; Bismillah and Rupali-2 fish hatchery, Comilla District) covering a wide geographical distribution of this species. The spawn were reared separately in the nursery ponds of the field laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh for a period of 6 months. In order to carry out the allozyme electrophoretic study, 20 individuals taken randomly from each population were brought in the laboratory and a piece of muscle and liver tissues was taken out using scalpel and scissors in low temperature with sufficient care to avoid any contamination. Details of sources, number of specimens and date of collection are shown in Table 1.

Allozyme electrophoresis: Horizontal electrophoresis was performed using 12% hydrolyzed potato starch in citric acid 6.1 buffer system for seven enzymes (Table 2) following the method of Aebersold *et al.* (1987). Allelic variants were designated to their relative electrophoretic mobility, the most common allele was given the number a* and alternative alleles were labeled according to their rates of migration relative to that of the most common allele.

Genetic data analysis: The allele frequencies were calculated by direct count from observed genotypes. The distribution of observed genotypes was compared with the expected ones, calculated from Hardy-weinberg equilibrium using a Chi-square (χ^2) test. The analysis of chi-square (χ^2) test was performed by using POPGENE (version 1.32) (Yeh *et al.*, 1999) and G-stat (version 3.1) (Siegismund, 1995) computer program.

Table 1: Collection sites of fish samples (*L. rohita*) from different stocks in Bangladesh

Population No.	Sources of fish samples	Population name	Status of fish stocks	Date of collection (2010)
1	Halda river, Chittagong	Halda	River	July, 5
2	Jamuna river, Sirajgonj	Jamuna	River	July, 25
3	Padma river, Kustia	Padma	River	August, 15
4	Brahmaputra fish hatchery, Mymensingh	Brahmaputra	Hatchery	June, 19
5	Rahim fish hatchery, Mymensingh	Rahim	Hatchery	June, 19
6	Ma-fatema fish hatchery	Ma-fatema	Hatchery	June, 24
7	Rupali fish hatchery, Jessore	Rupali-1	Hatchery	June, 24
8	Bismillah fish hatchery, Comilla	Bismillah	Hatchery	June, 25
9	Rupali fish hatchery, Comilla	Rupali-2	Hatchery	June, 25

Table 2: Enzymes examined used for allozyme electrophoresis

Enzymes	Enzyme patterns	E.C. No.	Tissue
Lactate Dehydrogenase (LDH)	Tetramer	5.3.1.9	Muscle
Malate dehydrogenase (MDH)	Dimer	1.1.1.27	Muscle
Phosphoglucumutase (PGM)	Monomer	1.1.1.37	Muscle
Glucose-6-Phosphate Isomerase (GPI)	Dimer	5.4.2.2	Muscle
Aspartate amino Transferase (AAT)*	Dimer	2.6.1.1	Muscle
Alcohol Dehydrogenase (ADH)*	Dimer	1.1.1.1	Muscle
Glycerol 3 Phosphate Dehydrogenase (G3PDH)*	Dimer	1.1.1.8	Muscle

*Enzyme not interpretable

When the most common (major) allele existed in a frequency ≤ 0.95 at a given locus, the locus was regarded as polymorphic. The mean proportions of heterozygous loci per individual, mean proportions of polymorphic loci per population and average number of alleles per population were calculated so as to show the extent of genetic variability for each population (Lewontin and Hubby, 1966). Expected (H_e) and observed heterozygosity (H_o) were calculated after Nei (1972) with the help of POPGENE (version 1.31) (Yeh *et al.*, 1999) computer package program. The same program was also used to estimate gene flow (N_m) between populations. FSTAT program version 2.9.3 (Goudet, 2001) was used to test for differences (F_{st}) between populations. The Unweighted Pair Group Method with Averages (UPGMA) dendrogram was drawn based on Nei (1972)'s genetic distances by using TREEVIEW program (Page, 2000).

RESULTS

Alleles and genotypes: The electrophoretic patterns were found to be muscle specific for *L. rohita* in citric acid 6.1 as a buffer system and showed that the enzymes were controlled by the genes at 7 presumptive loci. A maximum of 4 alleles (*a-d) were found in pgm* locus with 7 genotypes (*aa, *ab, *ac, *bb, *bc, *cc, *cd). The 3 alleles (*a-c) were found in Gpi-1* locus with 5 genotypes (*aa,

*ab, *bb, *bc, *cc). Gpi-2* and Mdh-1* loci showed 3 genotypes (*aa, *ab, *bb) while single genotype (*aa) was found in Ldh-1*, Ldh-2* and Mdh-2* loci. On the average 2.857 genotypes were produced by 2.000 alleles at the 7 loci (Table 3).

Allele frequency: Among the 7 loci, the Jamuna population showed polymorphism in 4 loci which were *Mdh-1, *Gpi-1, -2* and Pgm. The Halda population showed polymorphism in *Mdh-1, *Gpi-1 and *Pgm loci and Brahamaputra, Rahim and Rupali-1 hatchery populations showed polymorphism in *Gpi-1, -2* and *Pgm loci. In *Mdh-1, *pgm and *Gpi-1, *Pgm loci showed polymorphism in the Padma river and Bismillah hatchery population respectively. Only one locus (*Pgm) showed polymorphism in case of Ma-Fatema and Rupali-2

hatchery. The 3 enzymes such as Aspartate Amino Transferase (AAT), Alcohol Dehydrogenase (ADH), Glycerol 3 Phosphate Dehydrogenase (G3PDH) was impossible to interpret genetically due to the expression of complex banding pattern.

Chi-square (χ^2) test: The Chi-square (χ^2) test was done in all the cases of polymorphic loci between observed and expected genotypes, based on hardy-weinberg expectation. In 14 out of 22 tests, significant deviations from expectations (HWE) were detected (Table 4). The test for fit to hardy-weinberg proportions revealed that Gpi-1* and -2* were in hardy-weinberg equilibrium in all the cases. Mdh-1* was in equilibrium in the 2 wilds (Halda and Padma). Pgm* was in equilibrium in 2 hatchery populations (Ma Fatema and Rupali-2). Among the 9 populations, only the Jamuna wild population was in disequilibrium in 2 loci (Gpi-1* and pgm*).

Inbreeding co-efficient: The inbreeding co-efficient (Fixation index, F_{is}) was measured to estimate the deviation of random mating within populations (heterozygote deficiency or excess). A negative value indicates an excess of homozygotes and a positive value an excess of heterozygotes. The excess of homozygote loci were observed in the Jamuna (Mdh-1*), the Padma

Table 3: List of the alleles and genotypes examined in *L. rohita* populations

Locus	Alleles		Genotypes	
	No.	Type	No.	Type
Ldh-1*	1	*a	1	*aa
Ldh-2*	1	*a	1	*aa
Mdh-1*	2	*a-b	3	*aa, *ab, *bb
Mdh-2*	1	*a	1	*aa
Gpi-1*	3	*a-c	5	*aa, *ab, *bb, *bc, *cc
Gpi-2*	2	*a-b	3	*aa, *ab, *bb
Pgm*	4	*a-d	7	*aa, *ab, *ac, *bb, *bc, *cc, *cd
Average	2		2.857	

Table 4: Allele frequency at 7 presumptive loci of *L. rohita* populations

Locus	Allele	Allele frequencies								
		Wild population			Hatchery population					
		1	2	3	4	5	6	7	8	9
Ldh-1*	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-2*	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-1*	*a	0.525	0.425	0.500	1.000	1.000	1.000	1.000	1.000	1.000
	*b	0.475	0.575	0.500						
P		0.018*	0.655 ^{NS}	0.000*						
χ^2		5.504	0.199	21.052						
Fis		0.498	-0.125	1.000						
Mdh-2*	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi-1*	*a	0.075								
	*b	0.925	0.950	1.000	0.875	0.950	1.000	0.900	0.950	1.000
	*c		0.050		0.125	0.050		0.100	0.050	
P		0.000*	0.000*		0.000*	0.000*		0.019*	0.000*	
χ^2		12.324	39.027		14.765	23.490		5.473	39.027	
Fis		0.639	1.000		0.771	1.000		0.444	1.000	
Gpi-2*	*a	1.000	0.950	1.000	0.925	1.000	1.000	0.950	0.900	
	*b		0.050		0.075			0.050	0.100	
P			0.000*		0.000*			0.000*	0.019*	
χ^2			39.027		12.324			39.027	5.473	
Fis			1.000		0.639			1.000	0.444	
Pgm*	*a	0.300	0.275	0.525	0.125	0.175	0.450	0.325	0.625	0.700
	*b	0.450	0.375	0.275	0.875	0.700	0.500	0.600	0.375	0.300
	*c	0.200	0.300	0.175	0.025	0.125	0.050	0.075		
	*d	0.050	0.050	0.025						
P		0.081 ^{NS}	0.469 ^{NS}	0.424 ^{NS}	0.101 ^{NS}	0.541 ^{NS}	0.000**	0.634 ^{NS}	0.507 ^{NS}	0.013*
χ^2		11.234	5.600	5.990	2.685	2.153	19.000	1.710	0.439	6.168
Fis		0.248	0.059	-0.265	0.314	-0.078	-0.834	0.054	-0.173	0.523

Statistically significant values are marked with *; P = Probability of χ^2 value; Significant level: *p<0.05; NS = Non Significant

Table 5: Genetic variabilities at 7 presumptive loci of *L. rohita* populations

Population (wild/hatchery)	Mean proportion of polymorphic loci per population (%)	Mean proportion of heterozygous loci per individual (%)	Mean No. of alleles per locus	Heterozygosity		Shannon's information index
				H _o	H _e	
1	42.86	15.69	1.714	0.114	0.190	0.307
2	57.14	21.16	1.857	0.171	0.200	0.330
3	28.57	10.46	1.571	0.092	0.163	0.254
Mean	42.85	15.77	1.714	0.125	0.184	0.297
4	42.86	15.69	1.428	0.035	0.084	0.145
5	42.86	15.69	1.571	0.071	0.119	0.205
6	14.29	5.23	1.285	0.142	0.079	0.122
7	42.86	15.69	1.571	0.085	0.117	0.198
8	42.86	15.69	1.428	0.092	0.109	0.169
9	14.29	5.23	1.142	0.028	0.061	0.087
Mean	33.33	12.20	1.404	0.075	0.094	0.154

*p = 0.95; H_o = Average heterozygosity observed; H_e = Average heterozygosity expected

(pgm*), Rahim hatchery (pgm*), Ma Fatema hatchery (pgm*), Rupali-1 hatchery (pgm*) and Bismillah hatchery (pgm*) (Table 4).

Genetic variability: The average mean number of alleles per locus was 1.714 in wild populations and 1.404 in hatchery populations. The average observed Heterozygosity (H_o) value of wild populations was ranging from 0.092 (Padma)-0.171 (Jamuna). On the other hand, among the hatchery populations the highest observed heterozygosity (0.142) was found in Rahim hatchery. The highest expected Heterozygosity (H_e) for wild populations was 0.200 in the Jamuna population and the lowest value was 0.163 in the Padma population while for the hatchery population it was found the highest (0.119) in Brahmaputra hatchery and the lowest (0.061) in Rupali-2 hatchery. The average mean value of Shannons information index for relative gene diversity was higher (0.297) in wild populations than hatchery populations (0.154). The genetic variabilities among the populations have been summarized in Table 5.

Inter population genetic structure: The Nei (1972)'s analysis of gene diversity within populations estimated the genetic differentiation (F_{ST}) and the gene flow (N_m) over all nine rohu populations were 0.196 and 1.021, respectively (Table 6). In pair-wise analysis, the population differentiation (F_{ST}) value between Brahmaputra hatchery and Rupali-2 hatchery populations was the highest (0.261) and that was the lowest (0.009) F_{ST} value between the Halda and the Jamuna wild populations. The highest F_{ST} value represents a high level of population differentiation. The gene flow value (N_m) between the Brahmaputra hatchery and Rupali-2 hatchery populations was the lowest (0.707) while that of the Halda wild and the Jamuna wild populations was the highest (26.725).

A matrix of genetic Distance (D) (Nei, 1972) was constructed based on allelic frequencies of all loci among

Table 6: Pair-wise and overall population differentiations (F_{ST}) and gene flow (N_m) in 9 *L. rohita* populations

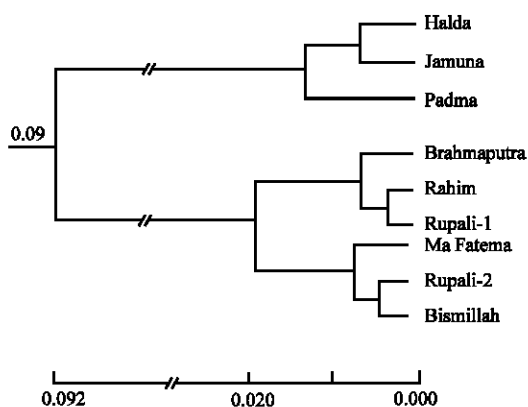
Populations	F _{ST}		N _m	
	Pair-wise	Overall	Pair-wise	Overall
Halda-Jamuna	0.009		26.725	
Halda-Padma	0.019		12.736	
Halda-Brahmaputra	0.164		1.269	
Halda-Mafatema	0.119		1.836	
Halda-Rahim	0.121		1.802	
Halda-Rupali-1	0.108		2.049	
Halda-Bismillah	0.134		1.614	
Halda-Rupali-2	0.166		1.252	
Jamuna-Padma	0.021		11.306	
Jamuna-Brahmaputra	0.210		0.936	
Jamuna-Mafatema	0.159		1.315	
Jamuna-Rahim	0.169		1.223	
Jamuna-Rupali-1	0.150		1.407	
Jamuna-Bismillah	0.172		1.199	
Jamuna-Rupali-2	0.209		0.942	
Padma-Brahmaputra	0.244		0.774	
Padma-Mafatema	0.181		1.127	
Padma-Rahim	0.146		1.452	
Padma-Rupali-1	0.150	0.196	1.410	1.021
Padma-Bismillah	0.134		1.615	
Padma-Rupali-2	0.154		1.366	
Brahmaputra-Mafatema	0.018		13.561	
Brahmaputra-Rahim	0.114		1.924	
Brahmaputra-Rupali-1	0.042		5.575	
Brahmaputra-Bismillah	0.162		1.287	
Brahmaputra-Rupali-2	0.261		0.707	
Mafatema-Rahim	0.059		3.967	
Mafatema-Rupali-1	0.012		20.218	
Mafatema-Bismillah	0.100		2.233	
Mafatema Rupali-2	0.168		1.230	
Rahim-Rupali-1	0.018		13.158	
Rahim-Bismillah	0.027		8.737	
Rahim-Rupali-2	0.051		4.595	
Rupali-1-Bismillah	0.048		4.952	

*N_m = Gene flow estimated; F_{ST} = 0.25 (1-F_{ST})/F_{ST}

9 populations ranged from 0.002-0.092. The minimum genetic distance (D = 0.002) was observed between the Bismillah and the Rupali-2 hatchery populations while the maximum value (D = 0.092) was found between the Jamuna wild and the Brahmaputra hatchery populations (Table 7). The UPGMA dendrogram (Nei, 1978) constructed from (Nei, 1972) genetic distance resulted in 2 major clusters among the 9 populations (Fig. 1). The cluster-1

Table 7: Nei (1972)'s original measures of genetic identity (above diagonal) and genetic distance (below diagonal) estimated among 9 populations of *L. rohita* based on 7 loci

Pop ID	Halda	Jamuna	Padma	Brahmaputra	Rahim	Ma Fatema	Rupali-1	Bismillah	Rupali-2
Halda	****	0.995	0.991	0.940	0.952	0.959	0.957	0.948	0.946
Jamuna	0.004	****	0.990	0.915	0.930	0.937	0.935	0.927	0.925
Padma	0.008	0.009	****	0.912	0.929	0.954	0.943	0.953	0.956
Brahmaputra	0.061	0.087	0.092	****	0.996	0.977	0.990	0.959	0.945
Rahim	0.048	0.071	0.073	0.003	****	0.986	0.996	0.972	0.961
Ma Fatema	0.041	0.064	0.046	0.022	0.013	****	0.996	0.994	0.992
Rupali-1	0.043	0.066	0.057	0.009	0.003	0.003	****	0.987	0.980
Bismillah	0.053	0.075	0.048	0.041	0.028	0.005	0.012	****	0.997
Rupali-2	0.055	0.078	0.044	0.055	0.039	0.008	0.020	0.002	****

Fig. 1: UPGMA dendrogram based on Nei (1972)'s genetic distance, summarizing the data on differentiation among 9 populations of *L. rohita*, according to the allozyme analysis

consisted with 3 wild populations and cluster-2 consisted with 6 hatchery populations and separated from each other by the genetic distance of $D = 0.087$.

DISCUSSION

The present study was attempted to identify genetic variability in the wild and hatchery populations of rohu, *L. rohita* in different regions of Bangladesh. Researchers screened 7 different enzyme system among which researchers could successfully interpret four enzymes viz. LDH, MDH, GPI and PGM and found 7 presumptive loci which are Ldh-1* and -2*, Mdh-1* and -2*, Gpi-1* and -2* and pgm*. In pgm* locus 4 alleles were found (a-d) from which allele 'd' was completely absent in hatchery populations. Absence of allele 'd' (pgm*) in hatchery populations indicates the loss of genetic variability concomitant with the bottle neck effect. The other 3 enzymes (AAT, ADH and G3PDH) did not show clear resolution might be due to buffer system, tissue and species specificity. The higher the number of alleles per locus, the higher the adaptation possibility of a population.

In the study, the mean number of alleles per locus 1.714 was higher than the reported values 1.66 from wild population of rohu (Alam *et al.*, 2002) but in case of hatchery populations it was lower than the reported value, 1.66 in hatchery population of rohu. Allozymes and morphometric analyzes were used to discriminate hilsa populations which were collected from 9 different sites within Bangladesh (Salini *et al.*, 2004). They had observed significant differences in allele frequencies and morphological variations in hilsa which may be due to the local environment. The presence of variability among populations, as well as individuals within a population is essential for their ability to survive and successfully respond to environmental changes (Ryman *et al.*, 1995). The mean number of alleles per locus as obtained in the present study among wild and hatchery populations suggests that hatchery populations maintain lower genetic variation than wild populations.

In the study for wild and hatchery populations of rohu, the mean number of polymorphic loci per population was 42.85 and 33.33%, respectively which is lower than the reported average (50%) based on allozyme studies in a hatchery population of rohu (Alam *et al.*, 2002) but higher than the value, 27.3% reported in natural and wild population of rohu (Khan *et al.*, 2006).

The average heterozygous loci in the present study were 15.77% in wild populations which was similar with the value (15%) reported by Alam *et al.* (2002) for both hatchery and wild population of rohu but higher than the value (13.33%) which was obtained by Pervej (2005) for the three populations of sharpunti (*P. sarana*) and (10%) obtained by 18 for both hatchery and natural populations of catla. In case of hatchery populations the value was 12.20% which is notably lower than the earlier mentioned reported value of Pervej (2005) and Alam *et al.* (2002).

The average H_o and H_e for wild population obtained in the present study (0.125 and 0.184, respectively) were also higher than the hatchery populations (0.075 and 0.094, respectively) which revealed lower genetic variability in hatchery populations and gene pool of the wild populations were still maintained effectively. The

average H_o and H_e for wild populations of rohu were 0.09 and 0.14, respectively and for hatchery populations of rohu the values were 0.08 and 0.11, respectively observed by Khan *et al.* (2006).

The pair-wise population differentiations (F_{ST}) was the highest (0.261) between the Brahmaputra hatchery-Rupali-2 hatchery populations with the lowest N_m value (0.707) while the lowest F_{ST} value (0.009) was found between the Halda and the Jamuna wild with highest N_m value (26.725). The overall F_{ST} value (0.196) among the nine populations of *L. rohita* as obtained in the present study is lower than that obtained for other freshwater fishes such as loach (0.774) (Khan and Arai, 2000) and freshwater Gobi (0.698) (Shimizu, 2003). However, the present F_{ST} value indicates little genetic differentiation among the populations. The degree of differentiation between populations generally reflects the amount of restriction on gene flow between them. A relatively high level of gene flow estimated in wild population pairs supported some degree of intermixing among them. The Halda wild is geographically isolated from other freshwater tidal wild originating in the hilly regions of eastern Bangladesh. Report from DoF (2012) states about the presence of 4 important natural stocks of IMCs, i.e., Brahmaputra Jamuna, Ganges-Padma, Barack-Meghna and the most importantly the Halda wild stock. Among the 4, Halda is the own stock of IMCs. So, the possibility of mixing this stock with others is very less. However, the loss of genetic variation in Halda population may be explained by its smaller size (only 35 km in length) and declining fish population resulting in decrease in the number of breeding fish at a rate of 25-30% per year by the nearby residents. The effect of geographical distance on F_{ST} and gene flow (N_m) values has been reported in stream living brown trout collected from different sections of the main stream (Carlsson *et al.*, 1999).

The genetic variabilities obtain in the present study indicate that the hatchery populations were maintaining lower genetic variation which might be due to inbreeding, genetic drift and less number of effective breeding number.

CONCLUSION

The genetic structure of rohu identified in the present study suggests developing a management and conservation plan for the species. Most of the private hatcheries produced poor quality of fry. Every year government stocks a large quantity of carp fry in the open water bodies. Massive stocking of genetically degraded stocks carp fry in natural water bodies may have serious genetic impacts on the natural stocks (Khan *et al.*, 2006). For sustainable aquaculture of these species availability

of quality seed is one of the pre-requisites. Therefore, availability of sufficient number of genetically potential broods need to be ensured for producing quality seeds through hatchery practices. Finally selective breeding needs to be done to improve the stock.

REFERENCES

- Aebersold, P.B., G.A. Winans, D.J. Teel, G.B. Milner and F.M. Utter, 1987. Manual for starch gel electrophoresis: A method for the detection of genetic variation. NOAA Technical Report NMFS61, pp: 19.
- Alam, M.A., M.S.H. Akanda, M.M.R. Khan and M.S. Alam, 2002. Comparison of genetic variability between a hatchery and a river population of rohu (*Labeo rohita*) by allozyme electrophoresis. Pak. J. Biol. Sci., 5: 959-961.
- Carlsson, J., K.H. Olsen, J. Nilsson, O. Oerli and O.B. Stabell, 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. J. Fish Biol., 55: 1290-1303.
- DoF., 2007. Fishery statistical yearbook of Bangladesh 2005-2006. Fisheries resources survey system. Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh.
- DoF., 2010. Fisheries resources survey system 2010. Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh, pp: 41.
- DoF., 2012. Fish fortnight compendium. Department of Fisheries, Ministry of Fisheries and Livestock, Government of Peoples Republic of Bangladesh, pp: 130-131.
- Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <http://www.unil.ch/izea/software/fstat.html>.
- Hansen, M.M., V. Simonsen, K.L.D. Mensberg, M.R.I. Sarder and M.S. Alam, 2006. Loss of genetic variation in hatchery-reared Indian major carp, *Catla catla*. J. Fish Biol., 69: 229-241.
- Khan, M.U.R.A. and K.A. Arai, 2000. Allozyme variation and genetic differentiation in the loach *Misgurnus anguillicaudatus*. Fish. Sci., 66: 211-222.
- Khan, M., M. Rahman, M.S. Alam and M.M.H. Bhuiyan, 2006. Allozyme variation of hatchery and river populations of rohu (*Labeo rohita*, Hamilton) in Bangladesh. Aquacult. Res., 37: 233-240.
- Lewontin, R.C. and J.L. Hubby, 1966. A molecular approach to the study of genetic Heterozygosity in natural populations. II. Amount of variation and degree of Heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics, 54: 595-609.

- Nei, M., 1972. Genetic distance between populations. *Am. Naturalist*, 106: 283-292.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583-590.
- Page, D.M.R., 2000. TREEVIEW: Tree drawing software for apple macintosh and microsoft windows. Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK., pp: 27.
- Pervej, I., 2005. Study on artificial breeding, growth and genetic variation of endangered local sarpunti, *Puntius sarana* (Hamilton) for its genetic improvement. M.S. Thesis, Department of Fisheries Biology and Genetics, Bangladesh Agricultural University.
- Ryman, N., F. Utter and L. Laikre, 1995. Protection of intraspecific biodiversity of exploited fishes. *Rev. Fish Biol. Fish.*, 5: 417-446.
- Salini, J.P., D.A. Milton, J.J. Rahman and M.G. Hussain, 2004. Allozyme and morphological variation throughout the geographic range of the Shad. Hilsa *Tenualosa ilisha*. *Fish. Res.*, 66: 53-69.
- Shimizu, T., 2003. Geographic variation of the *Japanese spinous* loach, *Cobitis takatsuensis* inferred from allozyme analysis. *Folia Biol.*, 51: 85-92.
- Siegismund, H.R., 1995. G-STAT, Version 3.1. Genetical statistical programs for analysis of population data. Botanical Institute, University of Copenhagen, Denmark.
- Simonsen, V., M.M. Hansen, M.R.I. Sarder and M.S. Alam, 2004. High level hybridization in three species of Indian major carps. *NAGA*, 27: 65-67.
- Simonsen, V., M.M. Hansen, K.L.D. Mensberg, R.I. Sarder and S. Alam, 2005. Widespread hybridization among species of Indian major carps in hatcheries, but not in the wild. *J. Fish Biol.*, 67: 794-808.
- Yeh, F.C., R. Yang and T. Boyle, 1999. A Microsoft Window Based Freeware for Population Genetic Analysis. Version 1.31, University of Alberta, Canada.