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Allozyme variation of hatchery and river populations of rohu (*Labeo rohita*, Hamilton) in Bangladesh

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Abstract

The genetic variations of rohu (*Labeo rohita*, Hamilton) sampled from five hatchery populations (Arabpur, Brahmaputra, Comilla, Kishorganj and Natore) and three major river populations (the Halda, the Jamuna and the Padma) were analysed by allozyme electrophoresis. Ten enzymes encoded by 11 loci were screened, and six were polymorphic. Alleles at three loci (*Est-1**, *Gpi-1** and *Gpi-2**) proved variable for hatchery and river populations, and the *Mdh-2** locus exhibited heterozygous genotypes for river populations only. Polymorphic loci per population (27.3 ± 5.3%), heterozygous loci per individual (15.5 ± 1.2%) and relative gene diversity (0.27 ± 0.08) in river populations were higher than those for hatchery populations (25.5 ± 1.8%, 10.7 ± 1.6% and 0.25 ± 0.01 respectively). Also, the observed heterozygosity (*Ho*) and expected heterozygosity (*He*) (0.09 ± 0.03 and 0.14 ± 0.04 respectively) in river populations were higher than those in hatchery populations (0.08 ± 0.01 and 0.11 ± 0.01 respectively). The lower levels of genetic variability in hatchery populations suggested the occurrence of inbreeding and/or genetic drift. The pairwise population differentiation (F_{ST}) values showed a lower level of genetic differentiation between hatchery and river population pairs. The unweighted pair-group method with arithmetic mean dendrogram of Nei's genetic distances showed a relationship between the genetic distance and geographic distance. The populations were clustered into three groups: the Padma in one group, the Halda in second group and the Jamuna, including five hatcheries, in the third group. Highly diversified rohu individuals were observed in the Padma and Halda Rivers, whereas less genetically variable individuals were found in the Jamuna River and five hatcheries. These findings can be useful for rohu hatchery propagation to enhance the sustainable aquaculture production.

Keywords: allozyme variation, rohu (*Labeo rohita*, Hamilton), polymorphic loci, Bangladesh

Introduction

Aquaculture contributes nearly 40% of total fish production in Bangladesh (Hussain & Mazid 2001). Aquaculture production in Bangladesh includes polyculture of Indian major carps such as Rohu (*Labeo rohita*), Catla (*Catla catla*, Hamilton) and Mrigal (*Cirrhinus mrigala*, Hamilton). Among them, rohu has the highest market price and constitutes nearly 23% of the fish produced through aquaculture (Alam 2001). There has been a considerable decrease in the population size of many economically important species, including major carps, in all Bangladesh rivers (Das & Barat 1990).

Nearly 1000 hatcheries are presently producing fry in Bangladesh, but hatcheries lack basic knowledge of broodstock management. Consequently, the quality of broodstock is reported to have deteriorated (Hussain & Mazid 2001). The sources or origin of rohu produced in hatcheries are not known. This is complicated by the common practice of releasing artificially produced fry into rivers, lakes and reservoirs.

The inbreeding and species purity of broodstock used for fry production and aquaculture are questionable in almost all hatcheries in Bangladesh. Most hatcheries rear their own broodstock and rarely recruit new individuals from natural sources or exchange parental fishes between farms. Each hatch-

ery can be considered an isolated and genetically closed unit (Eknath & Doyle 1990). The hatchery populations, therefore, can be considered as isolated sub-populations of the original ancestral population in older hatcheries, which are isolated and, likely, inbred lines of the same population. Commercial hatcheries often use sperm from closely related species, particularly in the case when there is shortage of conspecific sperm. Hatchery stocks of the three Indian major carps (*C. catla, L. rohita* and *C. mrigala*) were analysed using allozyme markers and were found to have a high incidence of hybridization (Simonsen, Hansen, Sarder & Alam 2004).

Introgressed hybrid carps are common in hatchery-produced stocks (Hussain & Mazid 2001). When the hatchery-derived mixed-species broods are released into the river stock, it may become further introgressed. Disruption of co adapted gene pools by introduction of hybrids can occur. Introduction of hatchery stocks into river stocks may lead to damage to the genetic integrity of locally adapted natural stocks.

Allozyme electrophoresis is an important technique for the genetic assessment of both wild and hatchery populations (Lewontin 1974; Kimura 1983; Nei & Koehn 1983; Ryman & Utter 1987). Allozyme studies have not been undertaken to identify genetic variation in hatchery and river populations of rohu in Bangladesh. The objective of the present study is to compare the genetic variation and differentiation with the hatchery and river rohu populations in Bangladesh using allozyme electrophoresis analysis.

Materials and methods

Fish specimens

Rohu (*L. rohita*) samples were collected from eight populations held in five hatchery and three river sources (Table 1 and Fig. 1). The hatchery samples were collected randomly from a large number of individuals (within the age range of 3–4 months). Muscle and liver samples were taken from each individual and stored at − 21 °C until electrophoretic analysis.

Electrophoresis

Horizontal starch gel electrophoresis and histochemical-staining techniques were used according to Shaw and Prasad (1970). The enzymes analysed and enzyme commission (EC) numbers are shown in Table 2. Amine-citrate buffers (CA 6.1 and 7.0) (Clayton

Table 1 Populations of rohu (*Labeo rohita,* Hamilton) analysed in the present study

Population no.	Localities: Hatchery/river*	No. of individuals
1	Fish hatchery (Arabpur, Jessore) **[Arabpur]**	30
2	Fish hatchery (Brahmaputra, Mymensingh) [**Brahmaputra**]†	30
3	Fish hatchery (Gangalia, Comilla) **[Comilla]**	30
4	Fish hatchery (Sadar, Kishorganj) [**Kishorganj**]†	30
5	Fish hatchery (Sadar, Natore) **[Natore]**	30
6	Halda River (Hathajari, Chittagong) **[Halda]**	28
7	Jamuna River (Bahadurabad Ghat, Jamalpur) **[Jamuna]**	28
8	Padma River (Pansha, Rajbari) **[Padma]**	28

*Collection of samples with the district and Sadar area; items in bold show the code name of each population.
†The distance between Brahmaputra and Kishorganj hatchery is about 30 km while the distance among other six locations is not less than 300 km.

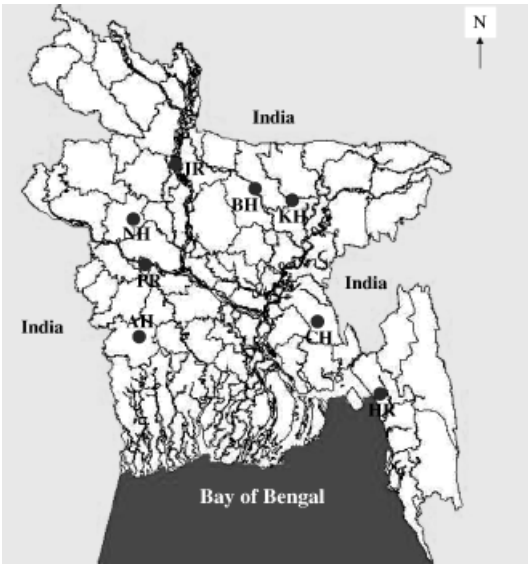

Figure 1 Map showing the collection sites of rohu (*Labeo rohita*) populations. The populations are referred as (1) Arabpur hatchery (AH), (2) Brahmaputra hatchery (BH), (3) Comilla hatchery (CH), (4) Kishorganj hatchery (KH), (5) Natore hatchery (NH), (6) Halda River (HR), (7) Jamuna River (JR) and (8) Padma River (PR).

Table 2 Ten enzymes analysed in rohu samples

Enzyme (abbreviation)	Enzyme structure	EC number	Tissue
Aspartate aminotranferase (AAT)*	Dimer	2.6.1.1	M
Alcohol dehydrogenase (ADH)*	Dimer	1.1.1.1	M\L
Esterase (EST)	Monomer	3.1.1	M\L
Glyceroldehyde-3-phosphate dehydrogenase (G3PDH)†	Dimer	1.1.1.8	M
Glucose-6-phosphate isomerase (GPI)	Dimer	5.3.1.9	M\L
Glucose-6-phosphate dehydrogenase (G6PDH)†	Dimer	1.1.1.49	M
Isocitrate dehydrogenase (IDHP)*	Dimer	1.1.1.42	M
Lactate dehydrogenase (LDH)*	Tetramer	1.1.1.27	M
Malate dehydrogenase (MDH)	Dimer	1.1.1.37	M
Phosphoglucomutase (PGM)	Monomer	5.4.2.2	M

*Enzymes showing monomorphism.
†Enzyme uninterpretable because of complex banding.
M, muscle; L, liver; EC, enzyme commission.

& Tretaik, 1972) were used in allozyme electrophoresis. Gel slices (1 mm) were histochemically stained for different enzyme activities as described by Aebersold, Winans, Milner and Utter (1987).

Genetic data analysis

Genotypes were inferred directly from observed phenotypes. Hardy–Weinberg equilibrium of genotype frequencies was tested using a χ^2 test. When the most common allele existed in a frequency less than 0.95 at a given locus, this locus was regarded as polymorphic. The analysis of allozyme data were performed using POPGENE (version 1.32) (Yeh, Yang & Boyle 1999), G-STAT (version 3.1) (Siegismund 1995) and TREEVIEW (Roderick 2000) computer program packages. Using POPGENE program, the mean proportion of heterozygous loci per individual, the mean proportion of polymorphic loci per population and the average number of alleles per locus were calculated to quantify genetic variability for each population (Lewontin & Hubby 1966; Lewontin 1974). Expected heterozygosity (*He*) and observed heterozygosity (*Ho*) were examined according to Nei and Roychoudhury (1973). The inbreeding coefficient (*Fis*) was measured to estimate the deviation from random mating within populations (heterozygote deficiency or excess) (Wright 1978). A negative value indicates an excess of homozygotes and a positive value indicates an excess of heterozygotes. The relative gene diversity of each population was measured by Shannon's information index (Lewontin 1972). The state of being free from the effects of selection (natural/anthropological) of each population was measured by the Ewens–Watterson test for neutrality (Manly 1985) using 1000 simulated samples.

Based on Nei's genetic distance (*D*) (Nei 1972), a dendrogram was constructed using the unweighted pair group method of arithmetic average (UPGMA). The topology was tested by a bootstrap analysis with 1000 pseudoreplicate trees. Genetic differentiation between pairs of populations was analysed by calculating pairwise F_{ST} values and testing their significance by permuting individuals between populations using the program ARLEQUIN 2.0 (Schneider, Roessli & Excoffier 2000).

Results

In the samples examined, no mixing of rohu with Catla (*C. catla*) or Mrigal (*C. mrigala*) was found by visual examination, but abnormalities were observed based on external morphology such as curved and cylindrical body shape, and enlargement of the head and the abdomen.

Allele frequencies were calculated directly from inferred genotypes at 11 loci from eight localities (Table 3). Among them, five loci (*Aat-1**, *Adh-1**, *Idhp-1**, *Ldh-1** and *Ldh-2**) were invariant (data not shown) and six loci (*Est-1**, *Gpi-1**, *Gpi-2**, *Mdh-1**, *Mdh-2** and *Pgm**) were polymorphic (Table 3). The enzyme esterase (EST) exhibited two loci (*Est-1** and *Est-2**). The *Est-1** locus was variable, but the *Est-2** locus was not interpretable because of complex banding pattern.

The enzyme glycerol-3-phosphate dehydrogenase (G3PDH) and glucose-6-phosphate dehydrogenase (G6PDH) were dimeric at least in two loci of each (*G3pdh-1** and *G3pdh-2**, *G6pdh-1** and *G6pdh-2** respectively). The alleles at these four loci were not considered here because of faint enzyme activity.

Hardy–Weinberg equilibrium was indicated by good agreement between observed and expected genotype frequencies at specific loci in specific populations. Significant differences ($P < 0.05$) in genotype frequencies at *Est-1**, *Mdh-1** and *Pgm** loci occurred between hatchery and river populations. The excess of homozygous genotypes was observed in Arabpur (*Est-1** and *Mdh-1**), Kishorganj (*Est-1** and *Mdh-1**) and Natore (*Est-1**) hatchery populations, and in Halda (*Mdh-1**) and Jamuna (*Mdh-1** and *Mdh-2**) river populations (Table 3).

The mean percent of polymorphic loci per population was somewhat higher (27.3 ± 5.3%) in river

Table 3 Allele frequencies at six polymorphic loci for hatchery and river rohu samples

		Allele frequencies							
		Hatchery population					River population		
Locus	Allele	1	2	3	4	5	6	7	8
*Est-1**	**a*	0.283	0.133	0.267	0.283	0.333	0.446	0.607	0.214
	**b*	0.700	0.617	0.683	0.717	0.650	0.482	0.393	0.786
	**c*	0.017	0.250	0.050	–	0.017	0.072	–	–
P		0.911	0.001*	0.037*	0.237	0.191	0.004*	0.000	0.360
Ho		0.467	0.200	0.267	0.500	0.600	0.321	0.143	0.286
He		0.437	0.549	0.467	0.413	0.474	0.573	0.486	0.343
Fis		−0.087	0.629	0.412	−0.231	−0.287	0.429	0.701	0.152
*Gpi-1**	**a*	1.000	1.000	0.967	1.000	1.000	0.643	1.000	1.000
	**b*	–	–	0.017	–	–	0.357	–	–
	**c*	–	–	0.016	–	–	–	–	–
P		–	–	0.000	–	–	0.202	–	–
Ho		–	–	0.033	–	–	0.357	–	–
He		–	–	0.066	–	–	0.468	–	–
Fis		–	–	0.487	–	–	0.222	–	–
*Gpi-2**	**a*	1.000	1.000	0.967	1.000	1.000	0.625	1.000	0.929
	**b*	–	–	0.033	–	–	0.375	–	0.071
P		–	–	0.000	–	–	0.000	–	0.000
Ho		–	–	0.000	–	–	0.036	–	0.000
He		–	–	0.066	–	–	0.477	–	0.135
Fis		–	–	1.000	–	–	0.924	–	1.000
*Mdh-1**	**a*	0.883	1.000	0.850	0.867	0.550	0.589	0.982	1.000
	**b*	0.117	–	0.150	0.133	0.450	0.411	0.018	–
P		0.506	–	0.039*	0.434	0.003*	0.005*	0.000	–
Ho		0.233	–	0.167	0.267	0.233	0.750	0.036	–
He		0.210	–	0.259	0.235	0.503	0.493	0.036	–
Fis		−0.013	–	0.345	−0.154	0.529	−0.549	−0.018	–
*Mdh-2**	**a*	1.000	1.000	1.000	1.000	1.000	1.000	0.982	0.036
	**b*	–	–	–	–	–	–	0.018	0.964
P		–	–	–	–	–	–	0.000	0.000
Ho		–						0.036	0.000
He		–	–	–	–	–	–	0.036	0.070
Fis		–	–	–	–	–	–	−0.018	1.000
*Pgm**	**a*	0.667	0.350	0.367	0.367	0.417	0.732	0.518	0.411
	**b*	0.333	0.550	0.633	0.550	0.517	0.268	0.482	0.589
	**c*	–	0.100	–	0.083	0.066	–	–	–
P		0.002*	0.002*	0.100	0.789	0.003*	0.000	0.006*	0.274
Ho		0.200	0.500	0.333	0.500	0.333	0.036	0.250	0.393
He		0.452	0.575	0.472	0.566	0.564	0.399	0.508	0.493
Fis		0.550	0.115	0.282	0.101	0.399	0.909	0.499	0.188
Average *Ho*	$n = 11$	0.082	0.064	0.045	0.115	0.106	0.136	0.042	0.062
SE		0.047	0.047	0.027	0.062	0.060	0.073	0.024	0.042
Average *He*	$n = 11$	0.100	0.102	0.121	0.110	0.140	0.219	0.097	0.095
SE		0.055	0.069	0.057	0.061	0.073	0.077	0.060	0.051

*Significance level: $P < 0.05$; *n*, number of total loci examined.

Ho, average heterozygosity observed; *He*, average heterozygosity expected; *Fis*, within-population deviation from random mating; *P*, Probability of χ^2 value.

than (25.5 ± 1.8%) in hatchery populations. The mean percent of heterozygous loci per individual was also higher on average in river (15.5 ± 1.2%) than in hatchery (10.7 ± 1.6%) populations. However, the mean number of alleles per locus was higher (1.5 ± 0.1) in hatchery than in river (1.4 ± 0.1) populations (Table 4). The *Ho* and *He* (0.09 ± 0.03 and 0.14 ± 0.04 respectively) in river populations were higher than those in hatchery populations (0.08 ± 0.01 and 0.11 ± 0.01 respectively). The mean

Table 4 Genetic variabilities at 11 loci for hatchery and river rohu samples

	Population (hatchery/river)	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	Neutrality
					Ho	*He*		
Hatchery	1	27.3	10.0	1.4	0.08	0.10	0.21	0.74
	2	18.2	6.7	1.4	0.06	0.10	0.23	0.64
	3	27.3	16.7	1.6	0.05	0.12	0.27	0.73
	4	27.3	10.0	1.4	0.12	0.11	0.24	0.74
	5	27.3	10.0	1.5	0.11	0.14	0.29	0.69
	Mean ± SE	25.5 ± 1.8	10.7 ± 1.6	1.5 ± 0.1	0.08 ± 0.01	0.11 ± 0.01	0.25 ± 0.01	0.71 ± 0.02
River	6	36.4	17.9	1.5	0.14	0.22	0.43	0.76
	7	18.2	14.3	1.4	0.04	0.10	0.19	0.78
	8	27.3	14.3	1.4	0.06	0.10	0.20	0.79
	Mean ± SE	27.3 ± 5.3	15.5 ± 1.2	1.4 ± 0.1	0.09 ± 0.03	0.14 ± 0.04	0.27 ± 0.08	0.78 ± 0.01

Ho, average heterozygosity observed; *He,* average heterozygosity expected.

Table 5 F_{ST} values with the homogeneity test (above diagonal) and Nei's genetic distances, *D* (below diagonal) among rohu population pairs

	1	2	3	4	5	6	7	8
1		0.028*	0.029*	0.031*	0.029*	0.045*	0.018*	0.071*
2	0.018		0.031*	0.027*	0.026*	0.065*	0.022*	0.069*
3	0.012	0.008		0.014*	0.016*	0.062**	0.017*	0.067*
4	0.009	0.008	0.0001		0.016*	0.066**	0.021*	0.057*
5	0.023	0.037	0.014	0.014		0.034**	0.027*	0.084**
6	0.058	0.098	0.072	0.076	0.059		0.035**	0.095**
7	0.018	0.027	0.020	0.018	0.038	0.073		0.072*
8	0.158	0.153	0.150	0.149	0.187	0.263	0.165	

*$P < 0.05$.
**$P < 0.01$.

values of Shannon's information index for relative gene diversity and neutrality for the effects of natural selection were higher (0.27 ± 0.08 and 0.78 ± 0.01 respectively) in river populations than in hatchery populations (0.25 ± 0.01 and 0.71 ± 0.02 respectively) (Table 4).

The genetic distance (*D*) ([21]Nei 1972) varied from 0.001 to 0.263 among eight samples (five hatcheries and three rivers). The minimum *D*-value (0.001) was observed between the Comilla and Kishorganj hatcheries. The highest value (0.263) was observed between Padma and Halda populations (Table 5). The UPGMA dendrogram based on Nei's (1972) genetic distance indicated that the eight populations of rohu were clustered into three groups: the Padma River population alone belonged to one group separated from other seven populations by the genetic distance $D = 0.263$. The Halda River population constituted second group, separated from the other six populations by the genetic distance $D = 0.112$. The third group was little differentiated and included the Jamuna River and five hatchery populations (Fig. 2).

The F_{ST} value between the Padma and the Jamuna population was the highest, and that between the Comilla and Kishorganj population was the lowest among all population pairs (Table 5). The cluster patterns described above the Padma, the Halda and the other six populations were obtained on the consensus tree with 1000 bootstrap support. The pairwise comparisons of the variance (nodal value) for the Padma River vs. the Halda River was higher (99.5%) than those for all other between-population comparisons (Halda–Natore: 96.5%; Halda–Jamuna: 81.5%; Halda–Arabpur: 81.4%; Jamuna–Brahmaputra: 64.3%; Brahmaputra–Comilla: 36.7%; Comilla–Kishorganj: 22.3%). The Jamuna River population was closer to hatchery populations with a nodal value of 64% (Table 5).

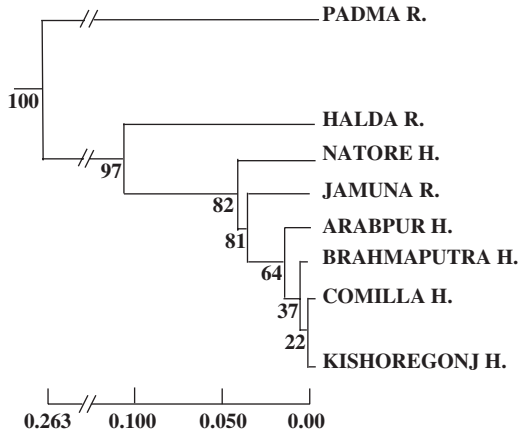

Figure 2 Unweighted pair-group method with arithmetic mean dendrogram of Nei's (1972) genetic distance, *D* and nodal values among eight rohu (*Labeo rohita*) populations.

Discussion

Although several attempts have been made to study genetic variation of hatchery and river rohu (*L. rohita*) population in Bangladesh using protein and RAPD markers (Alam, Akanda, Khan & Alam 2002; Islam & Alam 2004), the population structure remained inconclusive because of use of a single unknown source of hatchery stock as the sample. The present study was the first attempt to determine the genetic variation among five hatcheries (private and government) and three major river populations of rohu using allozyme markers in Bangladesh.

Variations of two enzyme loci, EST (*Est-1**) and GPI (*Gpi-1** and *Gpi-2**), differentiated hatchery and river populations. In hatchery samples, the allele frequency **a* was predominant and **b* was dominant in the *Est-1** locus, whereas both the alleles were dominant in the river samples. The GPI loci differentiated the river samples by the presence of polymorphic loci that was absent in hatchery samples. However, the effective number of broodstock was apparently reduced in the hatchery samples; the frequencies of common alleles at a given locus were higher in hatchery than in river populations (Table 3). Overall, a lower genetic variability was found within the hatchery populations, and the lowest was observed in Kishorganj hatchery, which concomitantly had small numbers of effective breeders. Egenolf (1996) also observed a lower genetic variability in hatchery stocks of rohu (*L. rohita*) in Bangladesh using seven polymorphic allozyme loci. Using RAPD markers, the low levels of intra-specific genetic variation in hatchery stocks of rohu (*L. rohita*) in Bangladesh (Islam & Alam 2004) and major carps in India (Barman, Barat, Yadav, Banerjee, Meher, Reddy & Jana 2003) were observed. A number of studies have clearly demonstrated a significant loss of genetic diversity following hatchery culture of fish species, e.g., *Salmo salar* L. (Cross & King 1983; Verspoor 1988); crustaceans, e. g., *Panaeus japonicus* (Sbordoni, De Matthaeis, Cobolli-Sbordoni, La Rossa & Mattoccia 1986); molluscs, e.g., *Crassostrea gigas* (Hedgecock & Sly 1990), *Mercenaria mercenaria* (Dillon & Manzi 1987) and *Haliotis iris* (Smith & Conroy 1992).

In the present study, the negative values of within-population deviation from random mating (*Fis*) indicated high homozygosity levels in hatchery samples, which were interpreted as being a result of inbreeding (Table 3). Lower levels of relative gene diversity (Shanon's information index) and neutrality in hatchery populations indicate that limited numbers of individuals might have frequently been chosen for artificial propagation (Table 4). As a result, lower growth rate, deformities and lower reproductive performance were observed in broodstock used for hatchery production compared with wild seed (Khan 2004).

The proportion of polymorphic loci in the Padma and the Halda populations (30% and 50% respectively) is relatively high. The Jamuna samples were collected from Bahadurabad Ghat, Jamalpur, where fish dealers mixed hatchery stock with river stock to obtain a higher market price. This might have the effect of sharing and resulting introgression (Mr. Abdullah, pers. comm.). The overall lower levels of genetic variability of the Jamuna River than other two rivers suggested the availability of hatchery seed in the Jamuna River population. The lower growth performance of the Jamuna stocks compared with Halda and Padma Rivers in communal pond stocking also suggested the loss of genetic variability (Shah, Sarder and Biswas 2002; Khan 2004).

In the present study, the F_{ST} value among the river populations ranging from 0.017 to 0.095 indicated the high genetic differentiation, which was also observed in the same rivers' rohu sample using RAPD analysis (0.035–0.097) (Islam & Alam 2004). The higher genetic distance between the Padma and the Halda ($D = 0.263$) or between the Halda and the other six populations ($D = 0.112$) may be explained by the geographical isolation between each pair (Table 5).

The Padma River (120 km long and 4–8 km wide) naturally originates from the lower Ganges. It enters Bangladesh at Nawabganj district and runs to confluence with the Jamuna at Goalanda Ghat. The diversified ecosystem and tributaries maintained the wild broodstock without mixing (Fig. 1). The Halda is a geographically isolated freshwater tidal river originating in the hilly region in Bangladesh. It was reported that the Halda is the only natural spawning ground of major carps within the freshwater tidal zone (Azadi, Kibria, Jahangir & Akhteruzzaman 2003). As a result, there is no probability of mixing with other stocks. However, massive broodfish collection during breeding season may affect the Halda. Recently, it has been reported that in the Halda River, the percentage of rohu fry among major carps (rohu, catla and mrigal) has been reduced from 12% to 1% between 2002 and 2004 (Khan 2004). The low levels of genetic distance between the Jamuna River and five hatchery populations are a consequence of the hatchery populations having been founded by individuals collected from that river.

In Bangladesh, with the recent rapid expansion of fish culture, farmers have come to depend upon private and public hatcheries for fish fry. Generally, private and public hatchery operators produce poor-quality carp fry and presumably, large quantities of such inferior carp fry are being stocked in open waterbodies under the government's massive carp fry stocking program (Alam *et al.* 2002). Mass stocking of genetically poor-quality stocks in natural waterbodies may have serious genetic impacts on the natural stocks. In that case, the genetic diversity of broodstock of hatchery owners needs to be increased through collection of broods from different natural stocks, and through proper brood management for selective breeding.

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