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# Assessment of genetic diversity in wild and hatchery populations of mrigal *Cirrhinus cirrhosus* (Hamilton-Buchanan) using allozyme markers

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**ABSTRACT**
Allozyme markers were applied to elucidate the genetic structure of three rivers (viz. the Halda, the Jamuna and the Padma) and three hatchery (Brahmaputra, Raipur and Sonali) populations of mrigal (*Cirrhinus cirrhosus)*. Electrophoretic analysis of twelve enzymes revealed a total of 20 loci of which four were polymorphic ($P_{95}$). Compared to the hatchery populations, the average observed and expected heterozygosity were higher and the inbreeding coefficients were lower in the river populations. Significant deviations from Hardy-Weinberg expectation were observed in 16.67% cases. The population pair-wise $F_{ST}$ values were low (0.0095-0.0719) to moderate but mostly significant. The number of families and effective population sizes were higher in the river group compared to the hatchery group. Since the level of inbreeding in the hatchery population is high, it is advisable to make periodic renewal of the hatchery broodstocks with fish from relatively better river sources.

**Keywords:** Genetic variation; Bottleneck; *Cirrhinus cirrhosus*; Inbreeding; Population differentiation.

## 1. Introduction
Polyculture of the Indian major carps, which includes catla (*Catla catla)*, rohu *(Labeo rohita)* and mrigal (*Cirrhinus cirrhosus)* is a common practice in Bangladesh. Annual pond catch of the three Indian major carps is about 52 percent of the total pond production [1] of the country. Among the three species, though the growth rate of mrigal is relatively slow, as a bottom feeder, it constitutes an important combination of carp polyculture with the surface feeder *Catla catla* and column feeder *Labeo rohita*. In the pond culture practices of Bangladesh, mrigal alone contributes 12.45 percent to the total pond production [1]. *C. cirrhosus*, a member of the family Cyprinidae, is naturally available in Bangladesh, Myanmar, Nepal, India and Pakistan. Besides these countries, *C. cirrhosus* has been introduced to Bhutan, The Philippines, former USSR, Japan, Sri Lanka, Laos, Malaysia, Thailand, Vietnam and Mauritius [2]. The world aquaculture production of *C. cirrhosus* was 378622 MT in 2010, which ranked 24$^{th}$ among all aquaculture species [3].

The increasing demand of carp seeds due to the rapid expansion of aquaculture practices promoted establishment of as many as 983 carp hatcheries across the country [4]. Thus major dependence on nature for the supply of seeds of Indian major carps has been replaced by hatchery produced seeds which is a good sign for aquaculture industry as a whole. However, there is concern among the stakeholders that the hatchery-produced seeds now a days are not performing well as it did in the early years of hatchery production in the 1980s apparently due to poor broodstock management, inbreeding, negative selection and inter-specific and inter-generic hybridizations [5-7]. Inbreeding in the hatchery stocks is apprehended because most hatchery owners rear their own brood stock from the leftover fingerlings and generally do not recruit brood from natural sources nor exchange breeders between farms. When such breeding practice is maintained for successive generations, it is expected that the genetic variation and performance of the hatchery stocks will be reduced. All these events has led to an increased demand for the river spawn among the fish farmers and spawn/naturally fertilized eggs are collected from the rivers, particularly from the river Halda every year without assessing the impact of the indiscriminate collection. The scope of fish spawning in the two big rivers, the

Padma and the Jamuna, are also at stake due to massive destruction of habitat resulting from siltation.

Proper knowledge of the population genetic structure of any species is not only important from ecological and biological point of view, but is also valuable for management perspectives of both captive and wild stocks. Some information is available on the population genetic structure of *Catla catla* and *Labeo rohita* [6, 8-13]. However, though an integral component of carp polyculture, little is known about the genetic structure of *C. Cirrhosus* of populations in Bangladesh.

As a codominant marker, allozyme electrophoresis is a useful technique for studying genetic variability within and among populations of fishes [14, 15]. Using allozyme markers, we have investigated the patterns of genetic variation within and among three major river and three representative hatchery stocks of *C. cirrhosus* in Bangladesh and report a comparison between the river and hatchery populations.

## 2. Materials and Methods

### 2.1 Sample collection

Spawn samples of *C. cirrhosus* were collected from three rivers namely the Halda, the Jamuna and the Padma and reared in three separate earthen nursery ponds at Bangladesh Agricultural University, Mymensingh campus until they attained sizes of ~7 cm. For samples of hatchery origin, three hatcheries were selected from three regions of the country. These were Brahmaputra Hatchery (BH) of Mymensingh (Central region), Raipur Government Hatchery (RH), Luxmipur (Eastern region) and Sonali Hatchery (SH), Jessore (South-West region). Fingerlings of approximately same size (~7 cm) were collected in live condition directly from the hatcheries. Muscle tissues collected from each individual were preserved at -20 $^0$C until used for allozyme electrophoresis.

### 2.2 Allozyme electrophoresis

For genotyping fishes at allozyme loci, we used standard horizontal starch gel electrophoresis method as recommended by Shaw and Prasad [16] and described in the NOAA Technical Report [17].

### 2.3 Statistical analysis of genetic data

The genotype of individual fish at the allozyme loci was determined and used for estimating genetic variation, population and family structure and differentiation parameters in the populations. Allele frequencies were calculated directly from the observed genotypes. When the most common (major) allele existed in a frequency less than or equal to 0.95 at a given locus, the locus was regarded as polymorphic. For each population sample, the number of alleles, observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated using the software ARLEQUIN version 3.5 [18]. The allelic richness and $F_{IS}$ were estimated using the program FSTAT 2.9.3.2 [19]. For comparing heterozygosities observed among the populations, we conducted analysis of variance (ANOVA) test using the software XLSTAT 2013. Conformity to the Hardy-Weinberg expectation (HWE) was tested by the exact p values calculated by a Markov chain randomization methods implemented by the software ARLEQUIN 3.5 [18] with the following parameters: forecasted chain length-1000000; number of dememorization steps- 100000). The inbreeding coefficient ($F_{IS}$) within each population at each locus and the population pairwise $F_{ST}$ values were calculated using the software FSTAT 2.9.3.2 [19]. For multiple comparisons, the global significance level of 0.05 was subjected to sequential Bonferroni correction [20] Nei's [21] genetic distance was estimated using the software GenAlex 6.5 [22] and used for constructing unweighted pair-group method with averages (UPGMA) dendrogram by the software MEGA 5.0 [23]. For assigning individuals to the source populations, we applied a direct method of Paetkau *et al*. [24] and a simulation-exclusion method of Cornuet *et al*. [25] both implemented by the software GENECLASS 2.0 [26]. We reconstructed full-sib families nested within half-sib families using the genotype of 381 offspring collected from six different populations using the software COLONY 1.2 [27]. The family reconstruction was performed without accounting for any allelic dropouts and other genotyping errors with the assumption that only one parent, the male, was polygamous. Effective population size (*Ne*) was estimated by the full likelihood method assuming random mating using the software COLONY 2.0 [28]. We examined the evidence for genetic bottlenecks in each population by assessing excess of heterozygosity under the infinite allele model (IAM) using the software BOTTLENECK version 1.2.02 [29].

## 3. Results

### 3.1 Within population genetic variation and conformity to Hardy-Weinberg expectation

The electrophoretic patterns of the twelve enzymes from muscle tissue revealed 20 presumptive loci. The average numbers of alleles per locus were 1.30 in the HR population and 1.25 in all other five populations. Allele frequencies calculated from the observed genotypes of 381 individuals sampled from six populations confirmed four loci (*Est-1**, *G3pdh-2**, *G6pdh-1** and *Gpi-2**) (20%) as polymorphic ($P_{95}$) in all the six populations (Table 1). Among these four loci, three alleles were detected at *Est-1** and *G3pdh-2** and two alleles were detected at loci *G6pdh-1** and *Gpi-1**. The allelic richness (*Ar*) of four polymorphic loci in the BH population was 2.50 and that for the other five populations was 2.25. The observed heterozygosity ($H_o$) ranged from 0.3595 (BH) to 0.4541 (JR) and the expected heterozygosity (*He*) ranged from 0.4087 (BH) to 0.4943 (HR). The mean *Ho*(0.4508±0.0025) and *He* (0.4731±0.011) values of the three river populations were higher than the mean *Ho* (0.3625±0.0029) and *He* (0.4180±0.004) values of the three hatchery populations (Table 2). However, ANOVA revealed no significant difference between the mean heterozygosities of the river and hatchery groups (P>0.05). Excess of heterozygote was observed at loci *G3pdh-2** inthe Halda, Jamuna, and the Padma river and the Raipur and Sonali hatchery populations. Excess of heterozygote was also observed at locus *Est-1** in the Brahmaputra hatchery population and at locus *Gpi-1** in the Halda and Padma river populations (Table 2). The $F_{IS}$value (0.690) at locus *G6pdh1** in the Halda river population indicated significant deficit in heterozygote (P<0.0.0083) (after Bonferroni corrections; initial k=6). The mean $F_{IS}$ value of the hatchery group was 0.133 and that of the river group was 0.046 (Table 2). The exact test for nonconformity to Hardy-Weinberg expectation revealed significant deviations in four out of 24 tests: the locus *G3pdh2** in the Halda, Jamuna and the Padma population and *G6pdh1** in the Halda population (Table 2).

**Table 1:** Allele frequency at four polymorphic loci out of the 20 presumptive loci of *C. cirrhosus*. N is the number of individuals genotyped.

| Loci | Allele | HR (N=60) | JR (N=60) | PR (N=90) | BH (N=57) | RH (N=57) | SH (N=57) |
|---|---|---|---|---|---|---|---|
| *Est-1** | **a* | 0.450 | 0.550 | 0.611 | 0.614 | 0.421 | 0.526 |
| | **b* | 0.550 | 0.450 | 0.389 | 0.386 | 0.570 | 0.474 |
| | **c* | 0.000 | 0.000 | 0.000 | 0.000 | 0.009 | 0.000 |
| *G3pdh-2** | **a* | 0.250 | 0.258 | 0.244 | 0.044 | 0.167 | 0.035 |
| | **b* | 0.575 | 0.708 | 0.678 | 0.912 | 0.763 | 0.842 |
| | **c* | 0.175 | 0.033 | 0.078 | 0.044 | 0.070 | 0.123 |
| *G6pdh-1** | **a* | 0.608 | 0.467 | 0.694 | 0.570 | 0.684 | 0.728 |
| | **b* | 0.392 | 0.533 | 0.306 | 0.430 | 0.316 | 0.272 |
| *Gpi-1** | **a* | 0.292 | 0.317 | 0.333 | 0.439 | 0.246 | 0.535 |
| | **b* | 0.708 | 0.683 | 0.667 | 0.561 | 0.754 | 0.465 |
| Mean no. of alleles | | 1.30 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Polymorphic loci ($P_{95}$) | | 20% | 20% | 20% | 20% | 20% | 20% |

### 3.2 Inter-population genetic structure

The population pair-wise $F_{ST}$ values ranged from 0.0095 (HR-RH) to 0.0719 (JR-SH). Among the 15 population pair-wise $F_{ST}$s, nine were found to be significant that include: HR-JR, HR-BH, HR-SH, JR-BH, JR-RH, JR-SH, PR-BH, PR-SH and RH-SH (Table 3). The genetic distance [21] between the population pairs ranged from 0.014 (HR-RH) to 0.071 (JR-SH). The population clustering constructed by the UPGMA method divided the six mrigal populations into two clusters. The three rivers along with the Raipur hatchery populations belonged to one cluster and the Brahmaputra and the Sonali hatchery population belonged to the second cluster (Figure 1).

**Table 2:** Genetic variability parameters at four polymorphic in three river and three hatchery populations of *C. cirrhosus* allozyme loci. *Ar*: allelic richness; *Ho*: heterozygosity observed; *He*: heterozygosity expected; $F_{IS}$: inbreeding coefficient; HWEP: probability for Hardy-Weinberg expectation exact test

| Locus | Parameters | Population | | | | | |
|---|---|---|---|---|---|---|---|
| | | HR | JR | PR | BH | RH | SH |
| *Est-1** | *Ar* | 2.000 | 2.000 | 2.000 | 3.000 | 2.000 | 2.150 |
| | *Ho* | 0.4000 | 0.4000 | 0.3555 | 0.5263 | 0.4386 | 0.4561 |
| | *He* | 0.4991 | 0.4991 | 0.4779 | 0.4781 | 0.5019 | 0.5030 |
| | $F_{IS}$ | +0.200 | +0.200 | +0.257 | -0.102 | +0.127 | +0.094 |
| | $P(F_{IS}>0.0)$ | 0.1146 | 0.0646 | 0.0188 | 0.8708 | 0.2188 | 0.3625 |
| | HWEP | 0.1921 | 0.1888 | 0.0249 | 0.5767 | 0.4842 | 0.5943 |
| *G3pdh-2** | *Ar* | 3.000 | 3.000 | 3.000 | 3.000 | 3.000 | 3.000 |
| | *Ho* | 0.7500 | 0.5833 | 0.6444 | 0.1578 | 0.4035 | 0.3157 |
| | *He* | 0.5811 | 0.4340 | 0.4774 | 0.1653 | 0.3882 | 0.2769 |
| | $F_{IS}$ | -0.294 | -0.348 | -0.352 | +0.045 | -0.04 | -0.142 |
| | $P(F_{IS}>0.0)$ | 1.0000 | 1.0000 | 1.0000 | 0.3542 | 0.7042 | 1.0000 |
| | HWEP | 0.0001* | 0.0084* | 0.0004* | 0.3512 | 0.6589 | 0.7679 |
| *G6pdh-1** | *Ar* | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 |
| | *Ho* | 0.1500 | 0.5000 | 0.3444 | 0.3684 | 0.2807 | 0.2982 |
| | *He* | 0.4805 | 0.5019 | 0.4267 | 0.4944 | 0.4359 | 0.3994 |
| | $F_{IS}$ | +0.690 | +0.004 | +0.194 | +0.257 | +0.358 | +0.255 |
| | $P(F_{IS}>0.0)$ | 0.0021* | 0.5813 | 0.0667 | 0.0438 | 0.0104 | 0.0625 |
| | HWEP | 0.0000* | 1.0000 | 0.0835 | 0.0639 | 0.0118 | 0.0886 |
| *GPi-1** | *Ar* | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 |
| | *Ho* | 0.4833 | 0.3333 | 0.4666 | 0.3859 | 0.3508 | 0.3684 |
| | *He* | 0.4166 | 0.4364 | 0.4469 | 0.49682 | 0.3738 | 0.5019 |
| | $F_{IS}$ | -0.162 | +0.238 | -0.044 | +0.225 | +0.062 | +0.268 |
| | $P(F_{IS}>0.0)$ | 0.9354 | 0.0625 | 0.7354 | 0.0854 | 0.4708 | 0.0458 |
| | HWEP | 0.3437 | 0.0785 | 0.8125 | 0.10913 | 0.7226 | 0.0621 |
| Mean over all loci | | | | | | | |
| | *Ho* | 0.4458 | 0.4541 | 0.4527 | 0.3595 | 0.3684 | 0.3596 |
| | SE | 0.123 | 0.055 | 0.069 | 0.076 | 0.034 | 0.035 |
| | *He* | 0.4943 | 0.4678 | 0.4572 | 0.4087 | 0.4250 | 0.4203 |
| | SE | 0.034 | 0.019 | 0.012 | 0.081 | 0.029 | 0.053 |
| | $F_{IS}$ | 0.099 | 0.030 | 0.010 | 0.121 | 0.134 | 0.146 |
| | (SE) | 0.0625 | 0.3354 | 0.4208 | 0.0417 | 0.0438 | 0.0292 |
| Mean (±SE) of river and hatchery groups | | | | | | | |
| | *Ho* | | *He* | | $F_{IS}$ | | |
| River | 0.4508±0.0025 | | 0.4731±0.011 | | 0.046±0.04669 | | |
| Hatchery | 0.3625±0.0029 | | 0.4180±0.004 | | 0.133±0.1124 | | |

### 3.3 Assignment of individuals into populations and into families

The direct method of Paetkau *et al*. [24] correctly assigned 25% (HR) to 44% (SH) of the individual fish to their source population; overall correct assignment was 34.64% (132/381) (Table 4). The method of Cornuet *et al*. [25] performed with 10000 simulated individuals correctly assigned 29.4% of the individuals. The other 249 individuals that could not be correctly assigned to particular populations were identified as migrant among the population pairs. The number of first generation migrant ranged from 10 (4%) (HR-BH) to 25 (10%) (HR-PR and BH-SH). Table 5 shows the number of full-sib and half-sib families and the effective population size estimated from the real data of the offspring of the six populations. The number of full-sib families ranged from 16 (HR) to 22 (PR) and the number of half- sib families was six each for the three river and the RH populations and five each for the BH and SH populations. The effective population size of the PR population estimated by full likelihood method assuming random mating was found to be the highest (18) and that of the SH population was found to be the lowest (14) (Table 5). For a comparison between the hatchery and river groups, we conducted a sibship reconstruction test combining individuals of all three hatchery under the hatchery group and individuals of all three rivers under the river group. We detected a total of 27 full-sib families nested under seven half-sib families in the hatchery group and 41 full-sib families nested under nine half-sib families in the river group (Table 6).

**Table 3:** Population pair-wise $F_{ST}$ values (above-diagonal) and the corresponding p-values (below diagonal).

| | HR | JR | PR | BH | RH | SH |
|---|---|---|---|---|---|---|
| HR | 0.0000 | 0.0166 | 0.0163 | 0.0619 | 0.0095 | 0.0623 |
| JR | 0.0033* | 0.0000 | 0.0235 | 0.0316 | 0.0313 | 0.0719 |
| PR | 0.0100 | 0.00667 | 0.0000 | 0.0339 | 0.0196 | 0.0390 |
| BH | 0.0033* | 0.0033* | 0.0033* | 0.0000 | 0.0503 | 0.0184 |
| RH | 0.0733 | 0.0033* | 0.0166 | 0.0033 | 0.0000 | 0.0516 |
| SH | 0.0033* | 0.0033* | 0.0033* | 0.03000 | 0.0033* | 0.0000 |

### 3.4 Evidence for recent genetic bottleneck

The tests of mutation-drift equilibrium for detecting recent genetic bottlenecks, presence of many individuals originating from very few individuals due to sharp decline in population size in the recent past generations, revealed signs of bottleneck in *C. cirrhosus* populations. The bottleneck test was performed under the infinite allele model (IAM) and results obtained by the sign test, standard difference test (SDT) and the mode-shift test confirmed excess of heterozygote in some cases. Sign test detected bottlenecks in all three river populations and SDT detected bottlenecks in all six populations ($P<0.05$); shifted mode in allele frequencies (the mode shift test) was detected in all three river (HR, JR, PR) and the SH samples indicating bottleneck in these populations. Wilcoxon rank test could not detect bottleneck in any of the six populations but the probability ($P=0.031$) was very close to the critical value (one tail $P=0.025$) in the three river and RH populations (Table 7).

## 4. Discussion

### 4.1 Genetic variation within population

We have analyzed the distribution and pattern of genetic variation in natural and hatchery populations of *C. cirrhosus*, an important major carp species, using allozyme markers. We evaluated the within-population genetic variation on the bases of allelic richness, observed and expected heterozygosity and inbreeding coefficient ($F_{IS}$). The proportion of polymorphic loci obtained in the present study was lower than those reported in the Indian river populations of *C. cirrhosus* (29.2%) [30], Bangladesh river and hatchery stocks of *Labeo rohita* (27%) [12] and Indian stocks of *Labeo calbasu* (35%) [31] but within the range for bony fish as reported by Nevo *et al*. [32]. Rana *et al*. [33] reported 13-46% allozyme loci to be polymorphic in the river and hatchery populations of *Catla catla*. The average number of alleles per locus (1.25 - 1.30) as obtained in the present study in *C. cirrhosus* stocks is similar to that of river samples and lower to that of hatchery samples of *C. cirrhosus* reported by Simonsen *et al*. [6]. Simonsen *et al*. [6] however confirmed that the higher number of alleles in the hatchery samples of *C. cirrhosus* was due to introgression from other carp species through hybridization. The observed and expected heterozygosity, obtained in the *C. cirrhosus* populations were within the range reported by other authors [30, 31]. Since the allelic richness was similar in all the river and hatchery populations and the mean heterozygosity of the river populations were higher than those of the hatchery populations, we applied an ANOVA only for the heterozygosity data but no significant difference was observed between the mean heterozygosity of the river and the hatchery groups ($P>0.05$). The mean $F_{IS}$ value for the hatchery populations was almost three times of that of the river populations indicating a high level of inbreeding in the hatchery populations that needs to be taken care of. For the four polymorphic allozyme loci, tested in the analysis, all three river and one hatchery (Raipur) were found to show significant nonconformity to Hardy-Weinberg expectation in one locus each due to excess of heterozygosity. In addition to these four cases, we have observed an excess of heterozygosity in several other cases of loci-population combinations that might be the reflection of recent genetic bottlenecks. To confirm if the populations experienced a sharp decline in recent past generations, we performed sign test, the standard difference test, Wilcoxon's test and mode shift tests under IAM. Three of the four tests involving heterozygosity excess and allelefrequency distribution suggested genetic signature of population bottleneck in the river populations of mrigal in Bangladesh. However, the results of the bottleneck test should be treated with caution as it is based only on four polymorphic allozyme loci and the Wilcoxon test could not detect excess of heterozygosity in any of the population, though the probability (0.0312) was very close to one tail critical p value of 0.0125. Applying allozyme markers Singh *et al*. [31] detected genetic bottlenecks in five river populations of *Labeo calbasu* in India.

### 4.2 Population structuring

We obtained $F_{ST}$ values ranging from 0.0095 to 0.0719 among different population pairs of *C. cirrhosus* which are slightly higher to those (-0.00285 to 0.0302) of the river populations of mrigal in India [30]. Khan *et al*. [12] reported $F_{ST}$ values ranging from 0.014 to 0.099) in *Labeo rohita*. However, 60% of the population pair-wise $F_{ST}$s were found to be significant in the present study. In addition to the significance test, the ranges are also mentioned for a better understanding. Wright [34] categorized

**Table 4:** Assignment of individuals into populations by direct and Bayesian simulated methods. Percentage of assignment to a correct particular correct population of origin is in parenthesis (upper panel). Estimates of mgrants between different population pairs (Lower panel, below diagonal)

| Source | Correct assignment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Direct real assigned number of individuals (%) | | | | | | | Simulation |
| | HR | JR | PR | BH | RH | SH | Overall | Over all |
| | 15 (25.00) | 24 (40.00) | 29 (32.22) | 22 (38.59) | 17 (29.82) | 25 (43.85) | 132 (34.64) | 29.40% |
| Number of individuals detected as migrant among population pairs (below diagonal) | | | | | | | | |
| HR | - | | | | | | | |
| JR | 18 | - | | | | | | |
| PR | 25 | 23 | - | | | | | |
| BH | 10 | 11 | 13 | - | | | | |
| RH | 16 | 14 | 16 | 21 | - | | | |
| SH | 12 | 9 | 19 | 25 | 17 | - | | |

**Table 5:** Number of full-sib (FS) and half-sib (HS) families identified in six studied populations separately (upper panel) and combined into river and hatchery groups (lower panel).

| Population | No. of half-sib family | No. of full-sib family | *Ne* | 95% Confidence Interval for *Ne* |
|---|---|---|---|---|
| HR | 6 | 16 | 16 | 9-34 |
| JR | 6 | 19 | 16 | 9-34 |
| PR | 6 | 22 | 18 | 10-35 |
| BH | 5 | 19 | 15 | 8-31 |
| RH | 6 | 20 | 16 | 9-32 |
| SH | 5 | 19 | 14 | 7-30 |

the level of population differentiation on the bases of the $F_{ST}$ values as low (<0.05), moderate (0.05 to 0.15), great (0.15 to 0.25) and very great (>0.25). According to this classification out of the 15 pair-wise $F_{ST}$ estimates, four pairs (HR-BH, HR-SH, JR-SH and RH-SH) belong to moderate differentiation and the other 11 pairs belong to the low differentiation categories. The $F_{ST}$ values among the river-river populations were lower than those among the hatchery-hatchery and the hatchery-river population pairs (Table 4). Hansen *et al.* [11] reported higher $F_{ST}$ values between the hatchery-hatchery populations compared to the river-river populations of *Catla catla*.

### 4.3 Assignment of individuals into populations and families

We performed two types of assignment tests- assigning individuals into their source population and assigning individuals into siblings. We have detected seven half-sib families in the hatchery group and nine half-sib families in the river group. This trend is similar to that of Hansen *et al*. [11] who detected seven half-sib families in the river samples and five half-sib families in the hatchery samples of *Catla catla*. It is to be noted that similar to the other rivers populations, the number of half-sib families are six inthe Padma population, though the number of offspring genotyped from the Padma was 90 while those from the Halda and the Jamuna were 60 each. The efficiency of the methods for assigning individualsinto populations depends on the amount of genetic differentiation exists among the stocks and the sample size and the number of loci analyzed [25, 26]. We obtained a very low score in assigning individuals into their source populations that might be due to the low level of population differentiation ($F_{ST}$<0.0719) and involvement of a few polymorphic allozyme loci. The low level of population differentiation may also be explained by the fact that fish

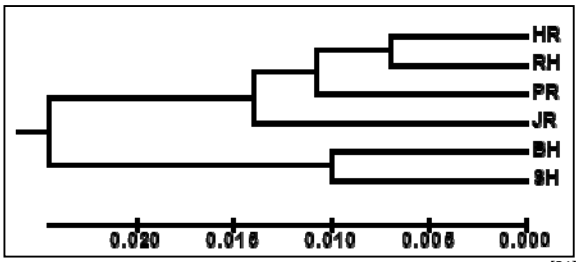


**Fig 1:** UPGMA dendrogram based on genetic distance [21]. HR: Halda river; JR: Jamuna river; PR: Padma river; BH: Brahmaputra hatchery; RH: Raipur

seeds produced in Jessore and Mymensingh (two large hubs) and the Raipur hatchery (largest fish breeding complex in the Eastern region) as well as spawn collected from the Halda River are transported to all over the country. Therefore, day by day, the genetic differentiations among the fish stocks in Bangladesh have been reduced. It has clearly been elucidated by the assignment test where only approximately one-third of the individuals could be correctly assigned to their source population and the rest two-thirds of the individuals were detected as migrant among the populations (Table 4). The salient findings of the present study are reduced polymorphism, evidence for recent bottlenecks and lack of sharp population structuring.

Construction of barrage and excess withdrawal of water from the upstream rivers in India created environmental problems and habitat destruction in the two major river systems: the Ganges- leading to the river Padma and its tributaries and the Brahmaputra- leading to the river Jamuna and its tributaries in

Bangladesh. On the other hand, indiscriminate collection of fertilized eggs and spawn as well as year round catching of adult fish from the river Halda [35] are threatening equilibrium between the catch and recruitment in this important breeding ground of the major carps including *C. cirrhosus*. As have been demonstrated in our previous studies involving other major carp species such as *Catla catla* [10] and *Labeo rohita* [13], the genetic diversity in the major carp species in Bangladesh are lower compared to their Indian counterparts supporting the claim for habitat degradation and over exploitation in the wild populations and inbreeding in the hatchery populations.

**Table 6:** Number of full-sib (FS) and half-sib (HS) families identified in combined samples of three hatcheries (hatchery group) and three rivers (river group).

| Hatchery Group | | | River Group | | |
|---|---|---|---|---|---|
| **Half-sib family** | **Full-sib family** | **No. of individuals** | **Half-sib family** | **Full-sib family** | **No. of individuals** |
| HS-1 | FS-1 | 2 | HS-1 | FS-1 | 1 |
| | FS-2 | 3 | | FS-2 | 7 |
| | FS-3 | 3 | | FS-3 | 15 |
| | FS-4 | 3 | | FS-4 | 3 |
| | FS-5 | 2 | HS-2 | FS-1 | 7 |
| | FS-6 | 2 | | FS-2 | 10 |
| HS-2 | FS-1 | 8 | | FS-3 | 5 |
| | FS-2 | 2 | | FS-4 | 5 |
| | FS-3 | 6 | | FS-5 | 5 |
| HS-3 | FS-1 | 5 | | FS-6 | 7 |
| | FS-2 | 7 | HS-3 | FS-1 | 7 |
| | FS-3 | 9 | | FS-2 | 2 |
| | FS-4 | 6 | | FS-3 | 8 |
| HS-4 | FS-1 | 7 | | FS-4 | 4 |
| | FS-2 | 3 | | FS-5 | 2 |
| HS-5 | FS-1 | 4 | | FS-6 | 5 |
| | FS-2 | 14 | | FS-7 | 3 |
| | FS-3 | 3 | | FS-8 | 3 |
| | FS-4 | 6 | HS-4 | FS-1 | 3 |
| | FS-5 | 3 | | FS-2 | 6 |
| | FS-6 | 2 | | FS-3 | 7 |
| | FS-7 | 6 | | FS-4 | 3 |
| | FS-8 | 3 | HS-5 | FS-1 | 6 |
| HS-6 | FS-1 | 17 | | FS-2 | 8 |
| | FS-2 | 8 | | FS-3 | 2 |
| | FS-3 | 5 | | FS-4 | 1 |
| | FS-4 | 8 | | FS-5 | 2 |
| HS-7 | FS-1 | 2 | HS-6 | FS-1 | 6 |
| | FS-2 | 9 | | FS-2 | 4 |
| | FS-3 | 4 | | FS-3 | 5 |
| | FS-4 | 8 | HS-7 | FS-1 | 8 |
| | FS-5 | 1 | | FS-2 | 7 |
| | | | | FS-3 | 2 |
| | | | HS-8 | FS-1 | 5 |
| | | | | FS-2 | 3 |
| | | | | FS-3 | 8 |
| | | | | FS-4 | 3 |
| | | | HS-9 | FS-1 | 8 |
| | | | | FS-2 | 6 |
| | | | | FS-3 | 6 |
| | | | | FS-4 | 1 |

**Table 7:** Results of bottleneck test. The values indicate probability under the hypotheses of mutation drift equilibrium. P<0.05 indicates evidence of recent bottlenecks. Mode shift normal indicates L-shaped allele frequency distribution, meaning no occurrence of recent bottlenecks and shifted indicate signs of genetic bottlenecks

| **Population** | **Infinite allele model (IAM)** | | | **Mode shift** |
|---|---|---|---|---|
| | **Sign Test** | **Standard Different Test (SDT)** | **Wilcoxon Tests One tail Prob. for Heterozygosity excess** | |
| HR | 0.033* | 0.0006* | 0.031 | Shifted |
| JR | 0.030* | 0.0011* | 0.031 | Shifted |
| PR | 0.026* | 0.0008* | 0.031 | Shifted |
| BH | 0.213 | 0.0115 | 0.062 | Normal |
| RH | 0.040 | 0.0231* | 0.031 | Normal |
| SH | 0.207 | 0.0069* | 0.062 | Shifted |

## 5. Conclusion

In conclusion, we have analyzed different genetic and ecological parameters such as genetic variability, population differentiation and family and effective population size of the Bangladesh stocks of *C. cirrhosus*, all have important implications for sustainable utilization of genetic resources of this important carp species. We however, recommend using more polymorphic markers such as microsatellite, for fine scale genetic characterization of this species to provide information on genetic variation, population differentiation and effective population size of *C. cirrhosus*.

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## 7. Reference

1. FRSS. Fishery statistical yearbook of Bangladesh (2009-2010). Fisheries Resource Survey System (FRSS), Department of Fisheries, Bangladesh, 2012, 42.
2. Reddy PVGK. Genetic resources of Indian major carps. FAO Fisheries Technical Paper. No. 387. FAO, Rome, Italy, 1999, 76.
3. FAO. FAO Yearbook, Fishery and Aquaculture Statistics 2010. Food and Agriculture Organization of the United Nations, Rome, Italy, 2012; 78.
4. DoF. Fish Week Compendium 2010, Department of Fisheries (DoF), Bangladesh, 2013, 144.
5. Eknath AE, Doyle RW. Effective population size and rate of inbreeding in aquaculture of Indian major carps. Aquaculture 1990; 85:293-305.
6. Simonsen V, Hansen MM, Mensberg KLD, Sarder MRI, Alam MS. Widespread hybridization among species of Indan major carps in hatcheries, but not in the wild. Journal of Fish Biology 2005; 67:794-808.
7. Bondad-Reantaso MG. Assessment of freshwater fish seed resources for sustainable aquaculture. FAO Fisheries Technical Paper No. 501. FAO, Rome, Italy. 2007, 105-128.
8. Alam MA, Akanda MSH, Khan MMR, Alam MS. Comparison of genetic variability between a hatchery and a river population of rohu (*L. rohita*) by allozyme electrophoresis. Pakistan Journal of Biological Sciences 2002; 5(9):959-961.
9. Islam MS, Alam MS. Randomly amplified polymorphic DNA analysis of four different populations of the Indian major carp *L. rohita* (Hamilton). Journal of Applied Ichthyology 2004; 20:407-416.
10. Alam MS, Islam MS. Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. Aquaculture 2005; 246:151-160.
11. Hansen MM, Simonsen V, Mensberg KLD, Sarder MRI, Alam MS. Loss of genetic variation in hatchery-reared Indian major carp, *Catla catla*. Journal of Fish Biology 2006; 69(Suppl B):229-241.
12. Khan MMR, Alam MS, Bhuiyan MMH. Allozyme variation of hatchery and river populations of rohu (*Labeo rohita*, Hamilton) in Bangladesh. Aquaculture Research 2006; 37:233-240.
13. Kimura M. The Neutral Theory of Molecular Evolution. Cambridge University Press. Cambridge 1983, 367.
14. Alam MS, Jahan M, Hossain MM, Islam MS. Population genetic structure of three major river populations of Rohu, *Labeo rohita* (Cyprinidae: Cypriniformes) using microsatellite DNA Markers. Genes and Genomics 2009; 31(1):43-51.
15. Ryman N, Utter F. Population genetics and fishery management. University of Washington Press, Seattle 1987; 420.
16. Shaw CR, Prasad R. Starch gel electrophoresis of enzymes: a compilation of recipes. Biochemical Genetics 1970; 4:297-320.
17. Aebersold PB, Winans GA, Teel DJ, Milner GB, Utter FM. Manual for starch gel electrophoresis: A method for the detection of genetic variation. NOAA Technical Report. NMFS 61, US Department of Commerce, 1987, 19.
18. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 2110; 10:564-567.
19. Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available: http://www2.unil.ch/popgene/softwares/fstat.htm; February 2002.
20. Rice WR. Analysing tables of statistical tests. Evolution 1989; 43:223-225.
21. Nei M. Genetic distance between populations. American Naturalists 1972; 106:283-292.
22. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics 2012; 28:2537-2539.
23. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary GeneticsAnalysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 2011; 28:2731-2739.
24. Paetkau D, Calvert W, Stirling Iand Strobeck C. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 1995; 4:347-354.
25. Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 1999; 153:1989-2000.
26. Piry S, Alapetite A, Cornuet JM, Paetkau D, Bauhouin L, Estoup A. GENECLASS2 a software for genetic assignment and first generation migrant detection. Journal of Heredity 2004; 95:536-539.
27. Wang J. Sibship reconstruction from genetic data with typing errors. Genetics 2004; 166:1963-1979.
28. Wang J, Santure AW. Parentage and sibship inference from multilocus genotype data under polygamy. Genetics 2009; 181:1579-1594.
29. Cornuet JM, Luikart G. Description and power analysis of two tests for detecting recent population bottlenecks

from allele frequency data. Genetics 1996; 144:2001-2014.
30. Chauhan T, Lal KK, Mohindra V, Sigh RK, Punia P, Gopalkrishnan A *et al*. Evaluating genetic differentiation in wild populations of the Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882): Evidence from allozyme and microsatellite markers. Aquaculture 2007; 269:135-149.
31. Singh RK, Lal KK, Mohindra V, Punia P, Sah RS, Mishra AK *et al*. Assessing genetic differentiation in geographic populations of *Labeo calbasu* using allozyme markers. Biochemical Genetics 2010; 48(9-10):760-78.
32. Nevo E. Genetic variation in natural populations: pattern and theory. Theoretical Population Biology 1978; 13:121-177.
33. Rana RS, Bhat KV, Lakhanpal S, Lakra WS. Comparative genetic diversity in natural and hatchery populations of Indian major carps (*C. catla* and *L. rohita*). Asian-Australasian Journal of Animal Sciences 2004; 17(9):1153-1328.
34. Wright S. Evolution and the genetics of populations, Vol. 4, Viability within and among natural populations. University of Chicago Press, Chicago, 1978, 590.
35. Azadi MA, Arshad-ul-Alam M. Spawn fishery of major carps (*Catla catla, Labeo rohita*, *Cirrhinus cirrhosus* and *Labeo calbasu*) of the river Halda. Book of Abstract. Fifth Fisheries Conference and Research Fair 2012. Dhaka, Bangladesh.