

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.512.019>

## Deterioration of Genetic Diversity: Concern in Hatchery Populations of Catla (*Catla catla*, Hamilton 1822: Cypriniformes, Cyprinidae) of Sylhet District in Bangladesh

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### ABSTRACT

Haors, are important source of freshwater fish (both production and diversities) population in Bangladesh which is mostly found in greater Sylhet district. Based on this fisheries diversity a large number of fish hatcheries have developed here for commercial aquaculture in this area but the genetic statuses in terms of effective breeding number, heterozygosity, genetic distance among population groups etc. of these hatcheries remain unknown. Therefore, to know the genetic status of these fish hatcheries the present study was carried out. This study reveals the genetic variation of hatchery populations of *Catlacatla* from three major hatcheries in Sylhet district named Mahbub Fish Hatchery, Shurma Gate Fish Hatchery, and Nabigonj Fish Hatchery in contrast to Padma river population as a wild population. The study was based on analysis of six microsatellite loci. The average number of alleles was ranged from 5.00 in the Shurma Gate Fish Hatchery to 6.33 in the Mahbub Fish Hatchery. Deviation from Hardy-Weinberg equilibrium was observed depending on the locus. Significant dwindle of heterozygosity was observed among the populations provided an indication that populations were may be under stress due to inbreeding , low number of effective individuals ( $N_e$ ) and collection of brood from nearby hatcheries. The unweighted pair group method with averages (UPGMA) dendrogram based on genetic distance resulted in two clusters: the Padma river population was alone in one cluster whereas the rest of the populations made another cluster.

### Keywords

Catla catla,  
PCR,  
Population  
structure,  
Genetic variation,  
microsatellite.

### Article Info

Accepted:  
08 November 2016  
Available Online:  
10 December 2016

### Introduction

Haors, which are bowl-shaped depressions between the natural levees of a river subject to monsoon flooding every year comprising an area of about 8000 km and dispersed mostly the eastern region of Bangladesh (Khan *et al.*, 1994) in the districts of

Sunamgonj, Sylhet, Moulavibazar, Hobigonj, Netrokona & Kishoreganj (Chakraborty, 2006) is one of the important source of freshwater fish population and production (Salauddin and Islam, 2011). In the year 2012-13 Major carp production was

about 21.39% of the total inland fish production and among them only catla fish (*Catla catla*, second most commercially important freshwater fish in Bangladesh) contribution was about 8.03% (FRSS, 2014)) but due to environmental modifications through siltation, dam construction and other anthropogenic activities, the opportunity of riverine fish to feed, navigate, and migrate and spawn has been constrained in the last two decades. Moreover, indiscriminate catching the brood fish during the breeding time (April- May) by different fishing methods is claimed to contribute in the reduction of the carp hatchling production at the rate of 25-30 percent per year especially in the Halda river (major river for natural seed production of freshwater fish), as a consequence the supply of fry from natural sources is decreasing day by day.

To overcome fish seed scarcity, a large number of hatcheries have been established which, at present, fulfill 99% of the total fish seed demand of the country (DoF, 2003). It is indeed during aquaculture practices and release of fry into natural water bodies like Haors also contribute to the species degradation, as inbreeding and hybridization are common practices in the hatcheries. Furthermore genetic characterization within and among populations is essential for an evolutionary interpretation or interaction and for the management of endangered or commercially important taxa because more recently, studies employing allozymes (Simonsen *et al.*, 2005) and microsatellite DNA markers (Alam & Islam, 2005) have revealed low levels of variation in wild and hatchery catla population from Bangladesh provide us an indication about species genetic diversity degradation of this population. Thus investigation of the genetic status of released fry of catla population of Haor collected from different hatcheries in

Sylhet district is more important to know the genetic quality of this species to find out a suitable management strategy.

## **Materials and Methods**

### **Collection of samples and isolation of genomic DNA**

Sample was collected from four major hatcheries in Sylhet district named Mahbub Fish Hatchery, Shurma Gate Fish Hatchery, Nabigonj Fish Hatchery and Padma river population from DuivaiSonaliMatshow Hatchery, Faisal Agro Fisheries from Rajshahi district respectively (figure: 1). Sample was randomly collected from 30 pair breeders which were used to produce the fish that were analysed. Approximately 500 mg of the caudal fin tissues was clipped from each individual. The fin clips were individually cut into small pieces with scissors and ground with a tissue homogenizer in 1.5 ml micro centrifuge tubes. The genomic DNA was isolated following Proteinase-K digestion, phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v) extraction and ethanol precipitation method.

### **Amplification of microsatellite loci**

The following tetranucleotide repeat loci developed by Naish&Skibinski (Naish&Skibinski1998) *Ccat A12*, *Ccat G1*,*Ccat G2*,*Ccat C3* and *Cc6*, *Cc7*, *Cc8*,and *Cc9* dinucleotide repeat locideveloped by McConnell *et al.*, (McConnell *et al.*, 2001), were used for polymerase chain reaction (PCR) amplification of microsatellite markers in this study to produce scorable amplification products. The annealing temperatures were 58<sup>0</sup>C for *Ccat A12*, *Ccat G1*, *CcatG2*,*Ccat C3*, and *Cc9*, 52<sup>0</sup>C for *Cc6*.The PCR was performed in a 12 µl reaction volume containing 30 ng of template DNA, 0.24mM of each primer,

0.9mM of each dNTP, 1.8unit of Taq DNA polymerase (GENE Pvt. Ltd., Bangalore, India) and 12 unit of Taq DNA polymerase and 10x reaction buffer containing 1.2mM MgCl. Thermal profile was consisted of 3 min initial denaturation at 94°C followed by 35 cycles, each of 30sec at 94°C, 30 sec at the annealing temperature and 1min at 72°C ending with an additional 5 min at 72°C for final elongation. An oil-free thermal cycler (Master Cycler Gradient, Eppendorf Germany) was used for conducting the polymerase chain reaction.

### **Electrophoretic separation and visualization of PCR products**

Three microliters (µl) from each of the PCR-products were used for electrophoresed on 6% denaturing polyacrylamide gels containing 19: 1 acrylamide: bisacrylamide and 7 M urea. Electrophoresis was conducted using the SequiGen GT sequencing gel electrophoresis system (BIO-RAD Laboratories, Hercules, CA). A pre-run of the gel for 30 min at 120W was followed by a final run at 60W and 50°C upon loading of denatured PCR products for a specified period of time depending on the size of amplified DNA fragment (usually 1 hour for 100 bp). A molecular weight marker DNA (100 bp DNA ladder, New England Biolabs Inc. Beverly, MA) was loaded on either side of the gel. After completion of electrophoresis, the DNA fragments were visualized essentially following the Promega (Madison, WI) silver staining protocol.

### **Scoring and analysis of microsatellite data**

The bands representing particular alleles at the microsatellite loci were scored manually. The size of each allele was estimated using the software DNA-FRAG, version 3.03

(Nash 1991). A genotypic data matrix was constructed for all loci. Fit of genotype data to Hardy–Weinberg proportions was estimated using the software POPGENE (version 1.31) (Yeh *et al.*, 1999) with 1000 simulated samples. The software GSTAT (Siegismund1995) was used for estimating allele frequencies and for applying the homogeneity test between populations. The significance of differences between F values was tested using the software FSTAT, version 2.9.3.2 (Goudet, 2001). The software GDA (Lewis and Zaykin, 2001) was used for estimating the genetic distance computed by Nei (Nei, 1972), and the dendrogram was constructed and drawn using the software TREEVIEW (Page 1996). Finally, locus by locus AMOVA analysis considering groups and populations as sources of variation was assessed by ARLEQUIN 3.1 software (Excoffier *et al.*, 2005) package.

## **Results and Discussion**

### **Genetic variation in different populations**

All of the screened six microsatellite loci were found to be polymorphic ( $P_{95}$ ). The locus *Ccat C3* had the highest number of alleles (9) followed by lowest in *Cc9* that is (3). The average number of alleles was highest in the population Mahbub Fish Hatchery (5.8333) and Shurma Gate Fish Hatchery had the least number of average alleles (5.000). The observed average heterozygosity ( $H_o$ ) was highest in the population Padma river population (0.6611) followed by Shurma Gate Fish Hatchery, Mahbub Fish Hatchery and Nabigonj Fish Hatchery that was (0.6057); (0.6056); (0.5889) in a descending order (Table 1).

The allelic frequencies of all the loci in all the populations are shown in the Table 2. The Sizes of alleles ranged from 124 to

149bp for the locus *Ccat A12*, 124 to 134 bp for the locus *Ccat G1*, 130 to 150bp for the locus *Ccat C3*, 215 to 231bp for the locus *Cc9*, 391 to 483 bp for the locus *Ccat G2* and 180 to 210bp for the locus *Cc6*. Shurma Gate Fish Hatchery populations had more null alleles (9) than other populations (Table 2). The alleles *Ccat A12*<sub>124, 129</sub> and *Cc9*<sub>231</sub> were found only (i.e. private allele) in Padma river population on the contrary *CcatC3*<sub>145</sub> and *Cc6*<sub>180</sub> found in Mahbub Fish Hatchery population.

PA= private allele, bp= base pair

### **Deviation from Hardy-Weinberg proportion**

In 14 of 24 tests, significant deviations from the Hardy-Weinberg expectations were detected. The test for fit to Hardy-Weinberg proportions revealed that, at locus *Ccat C3*, *Ccat G2* all the populations were found to have deviation from Hardy-Weinberg whereas opposite was true for locus *Cc9*. But at Populations Mahbub Fish Hatchery all the locus were found deviated from Hardy-Weinberg expectations except the locus *Ccat A12* (Table1).

### **Inter-population genetic structure**

The population differentiation ( $F_{ST}$ ) between the Padma river population and the Nabigonj Fish Hatchery population was the highest (0.0580) and significant among the population pair, while the  $F_{ST}$  metric between the Mahbub Fish Hatchery and Nabigonj Fish Hatchery population (-0.0044) was the lowest and non-significant. The estimated gene flow ( $N_m$ ) value between the Mahbub Fish Hatchery and the Nabigonj Fish Hatchery population across all the studied loci was the highest, while the  $N_m$  value between the Mahbub Fish Hatchery and the Padma river population was the lowest (Table 4).

Results of pair-wise comparisons among samples of different populations of catla using homogeneity tests were done (Table 3). Of the 36 tests, 16 were found to be significant and among all the population pairs were non homogeneous at locus *Cc9*, and *Cc3* (except between Shurma Gate Fish Hatchery and Nabigonj Fish Hatchery pairs of population). Genetic distance value between the Nabigonj Fish Hatchery and Padma river population populations was the highest (0.1924) while that of the Nabigonj Fish Hatchery and Mahbub Fish hatchery was the lowest (0.0322) [Table 4]. The UPGMA dendrogram (Figure: 2) based on Nei's (Nei, 1972) genetic distance resulted in two major clusters: the Padma river population population alone was in one cluster, and the remaining three populations in the other cluster.

Locus by locus AMOVA analysis which was performed (Table 5) considering groups and populations as sources of variation. Percentages of variation of the Number of alleles ( $F_{ST}$ ) among groups, among populations within group's and within population were estimated. The highest percentage of variation (85.96%) was found in the within population component. Components among groups and among populations within groups showed low and similar magnitudes (3.32-13.72%).

Statistically significant values are marked with asterisks. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and NS= Non-significant. This study showed that genetic characterization of *Catla catla* populations by microsatellite variability is a reliable tool for understanding the inter- and intra-relationships among the investigated populations, and could be of great benefit in breeding strategies. To compare populations originating from distant regions, a low number of microsatellites (4-6) are

sufficient to obtain robust results (Kohlmann *et al.*, 2005). As the genetic structure of population always shows changing in their pattern to see this, the level of genetic differentiation (i.e.,  $F_{ST}$ ), number of alleles, heterozygosity, sample sizes and the number of loci used in the present simulation study are within the range that is encountered in this empirical study.

**Genetic variation**

The result of  $H_o$  was not consistent with the result of average number of alleles. The population Mahbub Fish Hatchery had the highest  $H_o$  (0.6949) and average number of alleles (5.8333). Thus genetic variability was higher in this population than others.

**Table.1** Allelic and genetic variation at four microsatellite loci in three populations of *Catla catla* (N=No.of alleles,  $H_o$  =heterozygosity observed,  $H_e$  =heterozygosity expected)

Microsatellite loci	Parameters	Mahbub Fish Hatchery	Padma river population	Shurma Gate Fish Hatchery	Nabigonj Fish Hatchery
<i>Ccat A12</i>	$N$	6	6	6	6
	$N_e$	3.7344	3.7657	3.1690	3.8136
	$H_o$	0.6000	0.5667	0.6333	0.6333
	$H_e$	0.7446	0.7469	0.6960	0.7503
	$1-H_o/H_e$	0.1941	0.2412	0.0900	0.1559
	H-W test	28.702983 (15) **	39.460073(15) **	15.972852(15) <sup>ns</sup>	23.168542 (15) <sup>ns</sup>
<i>Ccat G1</i>	$N$	4	4	4	4
	$N_e$	2.2032	1.9088	2.8986	2.2086
	$H_o$	0.4667	0.6000	0.5667	0.5333
	$H_e$	0.5554	0.4842	0.6661	0.5565
	$1-H_o/H_e$	0.1597	-0.2391	0.1492	0.0416
	H-W test	60.193470 (6) ***	5.153310(6) <sup>ns</sup>	7.470287 (6) <sup>ns</sup>	60.195402 (6)***
<i>Ccat C3</i>	$N$	9	8	6	8
	$N_e$	6.1644	5.6075	5.0139	6.0000
	$H_o$	0.5554	0.4842	0.6667	0.7000
	$H_e$	0.8520	0.8356	0.8141	0.8475
	$1-H_o/H_e$	0.3481	0.4205	0.1810	0.1740
	H-W test	119.590852(36)***	48.759744(21)*	47.439924(15)***	110.45908 (28)***
<i>Cc9</i>	$N$	3	4	3	3
	$N_e$	1.7375	2.1609	1.8614	1.8018
	$H_o$	0.4000	0.7000	0.4667	0.8475
	$H_e$	0.4316	0.5463	0.4706	0.4525
	$1-H_o/H_e$	0.0732	-0.2813	0.2872	-0.8729
	H-W test	4.083345 (3) <sup>ns</sup>	11.802793(6) <sup>ns</sup>	3.812712 (3) <sup>ns</sup>	4.620303 (3) <sup>ns</sup>
<i>Ccat G2</i>	$N$	7	7	6	7
	$N_e$	5.2023	4.8128	4.2553	5.4545
	$H_o$	0.7333	0.7333	0.7000	0.7667
	$H_e$	0.8215	0.8056	0.7780	0.8305
	$1-H_o/H_e$	0.1073	0.0897	0.1002	0.0768
	H-W test	61.047727 (21)***	56.290486(21)***	54.520118 (15)***	60.799684 (21)***
<i>Cc6</i>	$N$	6	5	5	5
	$N_e$	4.7493	4.6512	2.4931	2.9364
	$H_o$	0.7000	0.6333	0.6000	0.5333
	$H_e$	0.8028	0.7983	0.6090	0.6706
	$1-H_o/H_e$	0.1280	0.2066	0.0147	0.2047
	H-W test	38.534371 (15)***	21.301479(10) *	8.876974(10) <sup>ns</sup>	11.818089 (10) <sup>ns</sup>
Average $H_o$ over loci		0.6056	0.6611	0.6057	0.5889
Average $H_e$ over loci		0.7013	0.7028	0.6723	0.6847
Average number of alleles		5.8333	5.6667	5.0000	5.5000
Polymorphism( $P_{95}$ )		1.0	1.0	1.0	1.0

Statistically significant values are marked with asterisks NS=not significant, \*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$

**Table.2** Frequency of alleles at three microsatellite loci in different populations of two strains of *Catla catla*.

Locus	Allele size(bp)	Padma river population	Mahbub Fish Hatchery	ShurmaGate Fish Hatchery	Nabigonj Fish Hatchery
<i>Ccat A12</i>	124	0.417 <sup>PA</sup>	0.000	0.000	0.000
	129	0.083 <sup>PA</sup>	0.000	0.000	0.000
	133	0.000	0.150	0.050	0.150
	136	0.000	0.050	0.067	0.050
	138	0.083	0.083	0.050	0.083
	141	0.150	0.417	0.467	0.417
	145	0.233	0.267	0.267	0.267
	149	0.033	0.033	0.100	0.033
<i>Ccat G1</i>	124	0.700	0.517	0.550	0.517
	129	0.083	0.033	0.183	0.033
	131	0.067	0.017	0.117	0.017
	134	0.150	0.433	0.150	0.433
<i>Ccat C3</i>	130	0.133	0.217	0.133	0.217
	134	0.067	0.150	0.200	0.150
	137	0.133	0.167	0.117	0.167
	138	0.300	0.200	0.300	0.200
	140	0.167	0.133	0.133	0.150
	143	0.050	0.067	0.000	0.067
	145	0.000	0.017 <sup>PA</sup>	0.000	0.000
	146	0.133	0.017	0.117	0.017
	150	0.017	0.033	0.000	0.033
<i>Cc9</i>	215	0.633	0.767	0.750	0.767
	221	0.167	0.133	0.150	0.133
	225	0.183	0.100	0.100	0.100
	231	0.017 <sup>PA</sup>	0.000	0.000	0.000
<i>Ccat G2</i>	391	0.000	0.083	0.000	0.083
	403	0.017	0.167	0.000	0.167
	411	0.100	0.150	0.050	0.150
	428	0.333	0.250	0.383	0.250
	433	0.100	0.233	0.200	0.233
	456	0.217	0.033	0.200	0.033
	464	0.150	0.083	0.117	0.083
	483	0.083	0.000	0.050	0.000
<i>Cc6</i>	180	0.000	0.017 <sup>PA</sup>	0.000	0.000
	183	0.117	0.200	0.017	0.033
	196	0.267	0.250	0.283	0.183
	200	0.200	0.233	0.133	0.267
	206	0.167	0.100	0.017	0.033
	210	0.250	0.200	0.550	0.483
Number of null alleles		5	4	9	6

**Table.3** Homogeneity between the samples of *Catla catla* (X2 values followed by degrees of freedom in parentheses)

Loci	Stocks	Padma river population	Shurma Gate Fish Hatchery	Nabigonj Fish Hatchery
Ccat A12	MahbubFish Hatchery	49.981916 (7)***	9.478641 (5) <sup>ns</sup>	2.539229 (5) <sup>ns</sup>
Ccat G1		14.504225 (3)**	19.560538(3)***	0.140394 (3) <sup>ns</sup>
Ccat C3		10.389523 (8) <sup>ns</sup>	13.576686 (8) <sup>ns</sup>	1.665655 (8) <sup>ns</sup>
Cc9		2.909613 (3) <sup>ns</sup>	0.171054 (2) <sup>ns</sup>	0.088417 (2) <sup>ns</sup>
Ccat G2		32.223559 (7)***	32.379379 (7)***	0.726640 (6) <sup>ns</sup>
Cc6		2.750598 (5) <sup>ns</sup>	22.297384 (5) ***	15.487831 (5)**
Ccat A12	Padma river population	-	48.058574(7)***	47.183773 (7)***
Ccat G1		-	4.364597 (3) <sup>ns</sup>	16.775591 (3)***
Ccat C3		-	10.177120 (7) <sup>ns</sup>	9.257540 (7) <sup>ns</sup>
Cc9		-	2.136508 (3) <sup>ns</sup>	2.197531 (3) <sup>ns</sup>
Ccat G2		-	5.035060 (6) <sup>ns</sup>	31.539683 (7)***
Cc6		-	20.344487 (4)***	15.200812 (4) **
Ccat A12	Shurma Gate Fish Hatchery	-	-	7.214555 (5) <sup>ns</sup>
Ccat G1		-	-	21.142286 (3)***
Ccat C3		-	-	14.053718 (7)*
Cc9		-	-	0.059384 (2) <sup>ns</sup>
Ccat G2		-	-	31.215584 (7)***
Cc6		-	-	4.877112 (4) <sup>ns</sup>

Statistically significant values are marked with asterisks. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 and NS= Non-significant

**Table.4** MultilocusFST (above diagonal), Nei genetic distance (1972) and Nm(below diagonal) values between pairs of three populations of *Catla catla* across six microsatellite Loci

Population	Mahbub Fish Hatchery	Padma river population	Shurma Gate Fish Hatchery	Nabigonj Fish Hatchery
Mahbub Fish Hatchery	****	0.0444**	0.0341**	-0.0044 <sup>NS</sup>
Padma river population	0.1599(7.5705)	****	0.0424**	0.0580**
Shurma Gate Fish Hatchery	0.1205(9.0948)	0.1415(7.8777)	0.0000	0.0221**
Government Fish Hatchery	0.0322(33.4523)	0.1924(6.1415)	0.0880(11.8707)	****

**Table.5** Analysis of molecular variance (AMOVA) of 6 microsatellite loci in the hatchery and Padma river populations of *Catlacatla*

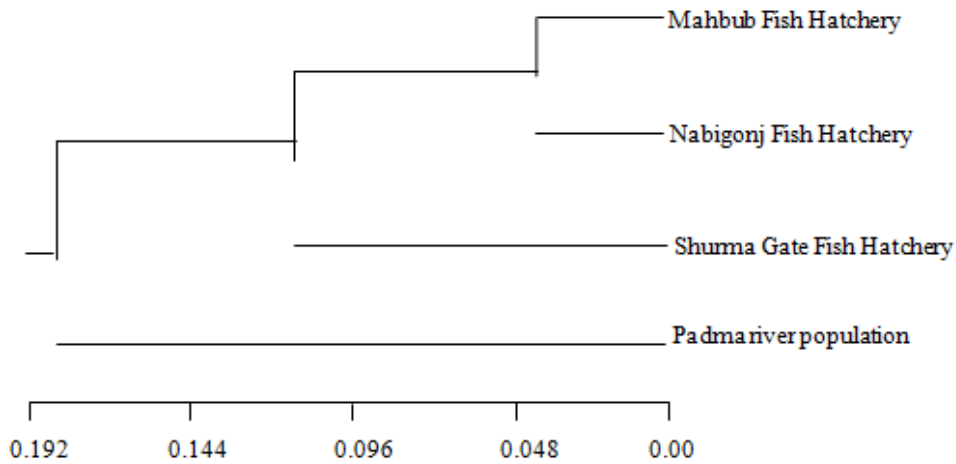
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Indices
Among each population groups	3	19.767	0.07138 <sub>va</sub>	3.32	FIS :0.11084
Among individuals within groups	116	267.500	0.23010 <sub>vb</sub>	10.72	FST :0.03324
Within all populations	120	221.500	1.84583 <sub>vc</sub>	85.96	FIT :0.14040
Total	239	508.767	2.14731		

Significance level= P<0.05

Fig.1 Map of Bangladesh showing the spots from where the samples were collected.



Fig.2 UPGMA dendrogram based on the genetic distance computed by Nei (1972) between *C. catla* populations, according to microsatellite DNA analysis



The average observed heterozygosities ( $H_o$ ) was lower than expected heterozygosities ( $H_e$ ) of all the four populations, this means that all the populations have some losses of heterozygosity reported in hatchery populations by Alam and Islam (2005) in species *Catla catla*, Jewel *et al.*, (2006) in

*Cyprinus carpio*, Hansen *et al.*, (2000), Was and Wenne (2002) in *Salmotrutta*, Tessier *et al.*, (1997) in *Salmosalar*; this discrepancy between heterozygosity is likely because alleles with low frequency contribute little to the overall heterozygosity, as reflected by the asymptotic relationships between



expected heterozygosity and effective number of alleles for a given effective population size  $N_e$  (Tessier *et al.*, 1997).

### **Hardy-Weinberg departure**

The test for Hardy-Weinberg equilibrium revealed departures with an overall tendency to heterozygote deficiency. A deficit in Hardy-Weinberg equilibrium has been reported Alam and Islam (2005) in hatchery populations of *Catla catla*, Jewel *et al.*, (2006) *Cyprinus carpio*. This departure of Hardy-Weinberg equilibrium can generally be explained through inbreeding and genetic drift is very common in a hatchery population which reduce the genetic variability Alam and Islam (2005), different selection pressures performed by hatchery owners that result in inbreeding and out breeding depression which is unavoidable in hatchery condition together with presence of null alleles [alleles that are not amplified because of the absence of a matching primer sequence which may lead production of homozygotes reported by Paetkau and Strobeck (1995) that may lead misinterpretation in the deviation of Hardy-Weinberg equilibrium in the natural populations reported by Ferguson (1995), the Wahlund effect (This occurs when two or more genetically differentiated populations are inadvertently sampled as a single population).

### **Inter-population genetic structure**

Pair wise  $F_{st}$  values were used to detect the genetic distance between the pairs of populations. The  $F_{st}$  value was highest between the population Padma river population and Government Fish Hatchery. Nei's genetic distance value was also highest (0.1924) between these populations. This represents high level of genetic differentiation among the populations

possibly caused by random genetic drift. The lowest  $F_{st}$  value (0.0322) between population Mahbub Fish Hatchery and Nabigonj Fish Hatchery indicates these two populations are genetically very similar to each other which may be due to brood fish exchange among this fish hatcheries reported by Islam and Alam (2004), Rahman *et al.*, (2009) in *Catla catla*, Hulak *et al.*, (2010) in *Cyprinus carpio* that is a common practice in most of the fish hatcheries in Bangladesh (AMOVA indicated that there is only 3% of the variation among each population groups) but reason of slight high genetic distance among different populations group was unknown.

In conclusion, in this work we have demonstrated that genetic diversity is still declining trend of catla populations which is supported by the AMOVA table i.e. existing variation within all populations is 85.96 %. Management factors such as the high exchange of brood fish between hatcheries could be the major causes affecting breeding in catla fish population subdivision. In such cases, additional constraints, such as the minimum levels of contribution of each population should be included in the conservation strategy. To maintain private alleles, which present at low in number in such small populations to be dispersed for avoiding an increase of the inbreeding rate across population. The systematic use of molecular markers particularly microsatellite can facilitate the comprehensive management of this population and should be combined with breeding schemes to improve economic traits for avoiding the deterioration of the production.

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**How to cite this article:**

Nazmul Haque and Tahmina Hoq. 2016. Deterioration of Genetic Diversity: Concern in Hatchery Populations of Catla (*Catla catla*, Hamilton 1822: Cypriniformes, Cyprinidae) of Sylhet District in Bangladesh. *Int.J.Curr.Microbiol.App.Sci*. 5(12): 171-181.

doi: <http://dx.doi.org/10.20546/ijcmas.2016.512.019>