Pak. J. Agri. Sci., Vol. 55(4), 929-939; 2018
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/18.7237
http://www.pakjas.com.pk

PATTERNS OF GENETIC VARIABILITY IN NATURAL AND HATCHERY POPULATIONS OF *Catla catla* BASED ON MICROSATELLITE DNA MARKERS

Tanveer Ahmed[1,*] and Khalid Abbas[1]

[1]Aquaculture Biotechnology Laboratory, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad-38040, Pakistan
[*]Corresponding author's e-mail: tanvirahmeduaf@gmail.com

The patterns of genetic variability and genetic differentiation among five wild and five hatchery populations of *Catla catla* were assessed using 15 microsatellite markers in a total of 500 individuals (50 individuals per population). In both wild and hatchery populations of *C. catla,* the level of genetic diversity was observed low-to-moderate in terms of an average allelic richness (*Ar*), the alleles number (*Na*), the number of effective alleles (*Nea*) and observed heterozygosity (*Ho*). The highest mean values of *Na*, *Nea* and *Ar* were found in the wild populations in comparison to hatchery populations. On the average base, the values of inbreeding coefficient (F_{IS}) in hatchery populations were found high. Total 13 out of 150 tests were found to deviate from *HWE* significantly at $p<0.05$. The pairwise estimates of F_{ST} revealed limited genetic differentiation between hatchery but low-to-moderate among wild populations. Most of the variation was found within individuals by applying AMOVA. Analysis of genetic relatedness among all the populations was estimated by constructing UPGMA dendrogram that showed two main clusters. The outcomes of the study would be helpful in resolving the genetic issues relating to *C. catla* re-stocking plans and broodstock management practices.
Keywords: Catla carp, genetic diversity, genetic structure, SSR markers

INTRODUCTION

The Catla carp (*Catla catla*) is an important freshwater fish species and native to the riverine systems of Pakistan, Bangladesh, India, and Myanmar. It is a popular fishery target due to its high market demand (FAO, 2014). In Pakistan, it also contributes to the country's aquaculture production significantly. However, during captive management, its gene pool is likely subject to various changes.

Describing the genetic diversity and genetic structure of fishery stocks after few generation is imperative for conservation, management, stock identification, stock improvement, stock augmentation and selective breeding programs to maintain diverse gene pool (Ciftci and Okumus, 2002). Genetic variability is necessary for long-term survival of populations. However, due to human interventions (e.g. overfishing, pollution, alteration of hydrological regimes, fisheries induced selection, inbreeding etc.), genetic variation can become reduced and even lost (Ciftci and Okumus, 2002; Abbas *et al.*, 2010). The genetic structuring of populations is subjected to temporal changes. The pattern and magnitude of these changes depend on both natural causes as well as on intensity of human interventions (Ciftci and Okumus, 2002).

Numerous environmental and biological processes can impact upon the genetic structure of aquatic species. Biological processes include e.g. homing or kin groups associations which increase spatial genetic structuring by reducing the gene flow among populations. From an environmental point of view, the most attention has perhaps received by geographical distance separating populations (Rousset, 2000).

Awareness about genetic issues in relation to artificial breeding is low in Pakistan. Various private and public fish hatcheries attempt to prevent the potential genetic degradation of endemic and cultured species but due to lack of technical knowledge and awareness about genetic status of fish species; negative selection, widespread inbreeding depression and genetic introgression by hybridizations has continued to occur (Evans *et al.*, 2004).

Various molecular markers are helpful in elucidating genetic variability and differentiation within and among fisheries stocks, respectively (Yue *et al.*, 2009). Microsatellite markers are popular tools in population genetics because of their high variability and the relative ease with which they can be developed for any species (Chistiakov *et al.*, 2006).

The aim of this study was to assess the levels of genetic diversity and differentiation among ten Catla populations in Pakistan. In particular, our aim was to test whether genetic variability within hatchery populations was reduced compared to wild population, and whether there was evidence for inbreeding and significant differentiation between different hatchery populations.

MATERIALS AND METHODS

***Fish sampling*:** A total of 500 individuals of *Catla catla* were collected from five hatcheries and five riverine sites from the Province of Punjab, Pakistan. The hatcheries included Fish Seed Hatchery Chennawan (CHNW), Muzaffargarh (MZG), Mian Channu (MCH), Rawalpindi (RWP) and Satiana (STFH), whereas the natural populations were sampled with gill nets from Balloki Headworks (BHW) located on River Ravi, Trimmu Headworks (THW) located on River Chenab, Sulemanki Headworks (SHW) located on River Sutlej, Rasul Barrage (RB) located on River Jhelum and Chashma Barrage (CB) located on River Indus of Punjab, Pakistan (Fig. 1). The collected individuals were kept on ice and immediately transported to the laboratory where they were frozen and stored at -20°C. The populations were named after the initial letters of the sampling localities. The collected individuals were identified and confirmed to be *C. catla* using the key features.

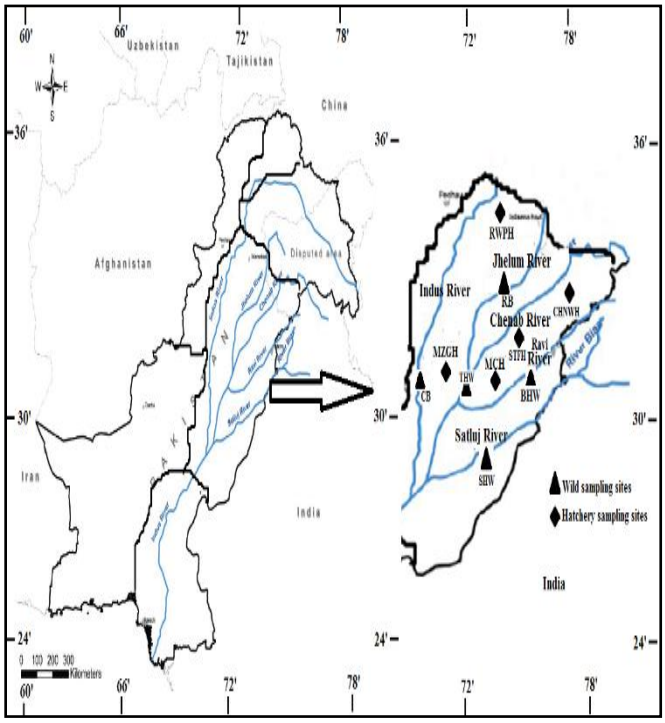

Figure 1. Map of Pakistan showing *C. catla* sampling sites.

***DNA isolation and quantification*:** From frozen muscle tissues, genomic DNA was isolated using the proteinase-K and standard phenol/chloroform DNA isolation methods of Yue and Orban (2005) with slight modifications. The quality of the isolated DNA was assessed through 0.8% agarose gel electrophoresis incorporated in TAE buffer with bromophenol blue loading dye. The concentration of DNA samples was tested using a UV spectrophotometer at 260 nm.

***Amplification of microsatellite loci*:** The targeted microsatellite loci were amplified by gradient thermal cycler using fifteen primer pairs (i.e. *Cc-1, Cc-7, Cc-9, Cc-10, Cc-13, Cc-15, Cc-19, Cc-24, Cc-31, Cc-40, Cc-42, Cc-46, Cc-57, Cc-62* and *Cc-70*) reported by McConnell *et al.* (2001) and Sahu *et al.* (2014). The microsatellite primer characteristics are given in Table 1. The PCR amplifications were performed in a 20µL reaction volume containing 50ng of template DNA, 2µM of each primer, 0.25µM each of the dNTPs, 1 unit of *Taq* DNA polymerase, 1.5mM $MgCl_2$ and 1µL 10X reaction buffer using gradient thermal cycler. The microsatellite loci were amplified one by one at standard thermal cycler conditions. The temperature profile consisted of 3 minutes initial denaturation at 94°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at the respective annealing temperature (Table 1), and ending with elongation for 5 min at 72°C.

***Microsatellite analyses*:** The PCR products were separated on vertical 5% non-denaturing polyacrylamide gels containing acrylamide:bis-acrylamide (19:1) and visualized with the silver-staining protocol of Sanguinetti *et al.* (1994). After gel imaging in the Gel documentation system (UVCI, Major Science, USA), the allelic bands were scored manually and compared with the mixed range DNA ladder (Thermo Fisher Scientific, USA) to determine the size of allelic bands.

***Data analyses*:** The microsatellite data set was analysed for detecting probable genotyping errors (i.e. null-alleles, large allele dropout and stuttering bands) with the software MICRO-CHECKER 2.2.1 (Oosterhout *et al.*, 2004). To describe the genetic characteristics of a populations, allele frequencies, number of alleles (*Na*), effective number of alleles (*Nea*), allelic richness (A_r), observed (H_o) and expected (H_e) heterozygosity, as well as inbreeding coefficient (F_{IS}) were estimated with FSTAT Version 2.9.3.2 (Goudet, 2002). For each locus, the random walk Markov-chain algorithm in ARLEQUIN software (Excoffier *et al.*, 2005) was employed to test for deviations from *HWE*.

Genetic divergence among populations was assessed with F_{ST} (Weir and Cockerham's, 1984). By analysis of molecular variance (AMOVA), the hierarchical partition of genetic diversity was assessed using ARLEQUIN, Ver. 2.000 (Excoffier *et al.*, 2005). Genetic structuring was also investigated with Principal Component Analysis (PCA) in R Software Ver. 3.4.0. Using software TFPGA Version 1.3 (Miller, 1997), UPGMA dendrogram based on Nei's (1972) unbiased distance was analyzed.

Population structuring was assessed also with the software STRUCTURE 2.3.2 (Pritchard *et al*., 2000; Falush *et al*., 2003), using a burn-in length of 50,000 and 100,000 MCMC (Monte-Carlo Markov Chain) iterations. Five independent runs were conducted for each k-value and number of genetic clusters were determined according to Evanno *et al*. (2005) with STRUCTURE HARVESTER (Earl and Vonholdt, 2012).

Table 1. Characteristics of the microsatellite loci as reported by McConnell *et al.* (2001) and Sahu *et al.* (2014).

Sr. No	Locus	Repeat unit	Primer sequence (5'-3')	GenBank Accession no.	T_a (°C)
1	*Cc-1*	(TG)9	F: CCTGGAACACTTTTTCTCTTGATG R: CAAGATCACACACACCACACAC	KF913007	58°C
2	*Cc-7*	(GT)21	F: CACTCTGTGCCTAGACCTCG R: CTGGAGTTTAAGCCCTGTTC	AJ294955	58°C
3	*Cc-9*	(TTATT)6	F: GGACCATAGGTTTGGGTTGATT R: TGACTCCAAATAGGACAAGTGG	KF913008	56°C
4	*Cc-10*	(GTTT)5	F: GTGAGCAGAAGAGACTG R: AGTTTTTGAACAGTGAGTG	AJ294958	60°C
5	*Cc-13*	(TG)12	F: TAGACACGGCATTAGAGACACC R: CTAGCTGCATATCACATTCTTCAC	KF913009	58°C
6	*Cc-15*	(CA)6	F: GGGTTGCTCTCTAAAACTCTGG R: CTCCTTCTGCTCTCTCTGCTCT	KF913010	58°C
7	*Cc-19*	(TG)6	F: CATGTGTATGCTTTGTACTGTGAG R: CAATTCACCACCGATTCTTTTG	KF913011	58°C
8	*Cc-24*	(AC)6	F: ATTAAGGAAGAAGGCTGGAAGG R: GTGCGAAGGAAGTGACAAGAGT	KF913012	60°C
9	*Cc-31*	(GT)14	F: TGTCTAGGTGTGTTTCTCTGTGG R: GAACATGAGCGGGAAAACTG	KF913013	60°C
10	*Cc-40*	(AG)6	F: TGCAATACGAAGAAGACAGTGG R: GCAAAAATACCATGCTCACAGA	KF913014	57°C
11	*Cc-42*	(TG)13	F: CTGGCCTGTATCTCGCTCTG R: TACACTTGACTAACCCGGACCT	KF913015	50°C
12	*Cc-46*	(TC)6	F: CTCTCCCTCTACCAGGCATTTT R: GTCAGGTGTTGAAGCTCTTTCC	KF913016	59°C
13	*Cc-57*	(AG)13	F: CCACTCTTTCTTTTACTCCCCATT R: TGTAACAGCTTGTCTGGTGATAG	KF913017	54°C
14	*Cc-62*	(GA)7	F: TCCAACCATCCATATCAGCTAC R: TGACGACGCTATCTTCTCTCTTT	KF913018	57°C
15	*Cc-70*	(TG)6	F: CGCTCAGGTTACCCAGCATT R: CACACACACACGCAACAGATAC	KF913019	58°C

Where F– forward, R – reverse, T_a - primer specific annealing temperature

RESULTS

All the screened microsatellite loci were found to be variable in all the examined populations. Depending on the screened microsatellite locus and examined fish population, the patterns of genetic variability varied. The average allele frequency and allele size was observed ranged from 0.001 to 0.4995 and 65 to 209 base pairs, respectively (Appendix 1).

***Genetic diversity*:** There was no evidence of scoring errors related to large allele dropout, stutter bands or presence of null alleles at any locus.

The number of alleles (*Na*) at each locus was noted ranged from 2 to 7, with mean values varied from 2.867 to 4.4667. The highest mean value of *Na* (4.4667) was observed in a wild population (THW) and the minimum (2.867) in the captive STFH population. The range of average values for an effective number of alleles (*Nea*) was noted from 2.462 to 3.521 and those for observed heterozygosity (*Ho*) from 0.472 to 0.693. Average expected heterozygosity (H_E) was measured ranged from 0.582 to 0.704. Highest H_e value was observed in the wild population captured from THW, and the lowest in the captive population collected from STFH. The mean inbreeding coefficient (F_{IS}) ranged from -0.045 to 0.204, with the highest F_{IS} value observed in a hatchery population (CHNW) and the lowest in a wild population (CB). Out of 150 tests, 13 tests were found to deviate from *HWE* significantly at $p<0.05$ in this study (Table 2).

***Population genetic structure*:** Among pairs of populations, the values of genetic differentiation and unbiased genetic distance indicated considerable difference in magnitude at $_P<0.05$. The pairwise estimates of F_{ST} indicated low to moderate level of genetic differentiation among the wild and hatchery populations. The pairwise F_{ST} estimates ranged from 0.0009 to 0.1239. The highest level of differentiation was found 0.1239 between populations of STFH and CB, while the lowest (0.0009) between MZG and MCH populations (Table 3).

Appendix 1. Allele frequency and size (bp) observed at each locus in natural and captive populations of *C. catla*.

Locus	Allele size (bp)	CHNW	MZG	MCH	RWP	STFH	BHW	THW	SHW	RB	CB	Average
Cc-1	130	0.344	0.33	0.309	0.34	0.372	0.276	0.25	0.449	0.163	0.5	0.3333
	138	0.422	0.436	0.468	0.436	0.457	0.459	0.32	0.347	0.49	0.27	0.4105
	142	0.189	0.191	0.223	0.17	0.17	0.184	0.23	0.173	0.153	0.18	0.1863
	146	0.044	0.043	0	0.032	0	0.061	0.09	0.031	0.194	0.03	0.0525
	148	0	0	0	0.021	0	0.02	0.11	0	0	0.02	0.0171
Cc-7	141	0.365	0.287	0.383	0.372	0.467	0.24	0.224	0.32	0.15	0.41	0.3218
	145	0.458	0.394	0.383	0.34	0.402	0.38	0.286	0.46	0.52	0.3	0.3923
	147	0.125	0.181	0.234	0.17	0.13	0.29	0.245	0.2	0.21	0.25	0.2035
	153	0.042	0.138	0	0.074	0	0.07	0.163	0.02	0.08	0.01	0.0597
	155	0.01	0	0	0.043	0	0.02	0.061	0	0.04	0.03	0.0204
	161	0	0	0	0	0	0	0.02	0	0	0	0.002
Cc-9	101	0.372	0.281	0.456	0.622	0.489	0.378	0.265	0.276	0.18	0.306	0.3625
	106	0.436	0.385	0.4	0.357	0.413	0.48	0.316	0.571	0.5	0.347	0.4205
	111	0.138	0.271	0.144	0.02	0.098	0.122	0.255	0.153	0.25	0.245	0.1696
	116	0.053	0.063	0	0	0	0.02	0.163	0	0.07	0.102	0.0471
Cc-10	65	0.646	0.469	0.583	0.596	0.606	0.53	0.439	0.245	0.37	0.06	0.4544
	69	0.354	0.531	0.417	0.404	0.394	0.47	0.5	0.755	0.5	0.67	0.4995
	73	0	0	0	0	0	0	0.061	0	0.13	0.27	0.0461
Cc-13	132	0.286	0.33	0.33	0.385	0.468	0.42	0.204	0.37	0.316	0.082	0.3191
	144	0.531	0.426	0.415	0.448	0.436	0.17	0.49	0.42	0.469	0.735	0.454
	156	0.184	0.245	0.255	0.167	0.096	0.27	0.214	0.16	0.194	0.143	0.1928
	158	0	0	0	0	0	0.14	0.092	0.05	0.02	0.041	0.0343
Cc-15	155	0.448	0.457	0.367	0.378	0.574	0.43	0.39	0.28	0.23	0.21	0.3764
	163	0.552	0.543	0.5	0.551	0.426	0.39	0.41	0.28	0.51	0.57	0.4732
	167	0	0	0.133	0.071	0	0.18	0.2	0.44	0.26	0.22	0.1504
Cc-19	143	0.337	0.245	0.378	0.427	0.565	0.276	0.16	0.16	0.23	0.22	0.2998
	147	0.469	0.468	0.408	0.438	0.435	0.398	0.45	0.5	0.24	0.24	0.4046
	155	0.194	0.223	0.214	0.135	0	0.235	0.28	0.25	0.33	0.33	0.2191
	161	0	0.064	0	0	0	0.092	0.11	0.09	0.2	0.21	0.0766
Cc-24	124	0.298	0.309	0.315	0.394	0.435	0.32	0.184	0.21	0.2	0.19	0.2855
	128	0.266	0.468	0.446	0.479	0.413	0.27	0.398	0.34	0.29	0.39	0.376
	134	0.202	0.223	0.196	0.128	0.152	0.18	0.337	0.31	0.27	0.26	0.2258
	138	0.191	0	0.043	0	0	0.18	0.071	0.14	0.21	0.12	0.0955
	140	0.043	0	0	0	0	0.05	0.01	0	0.03	0.04	0.0173
Cc-31	148	0.394	0.383	0.287	0.323	0.287	0.296	0.21	0.17	0.14	0.2	0.269
	152	0.479	0.457	0.394	0.479	0.394	0.296	0.25	0.27	0.21	0.25	0.3479
	160	0.128	0.16	0.213	0.198	0.181	0.224	0.42	0.39	0.35	0.34	0.2604
	166	0	0	0.106	0	0.138	0.112	0.08	0.12	0.08	0.15	0.0786
	168	0	0	0	0	0	0.061	0.04	0.05	0.22	0.06	0.0431
	170	0	0	0	0	0	0.01	0	0	0	0	0.001
Cc-40	151	0.383	0.383	0.378	0.298	0.396	0.26	0.1	0.07	0.1	0.19	0.2558
	153	0.479	0.457	0.418	0.266	0.365	0.22	0.24	0.25	0.31	0.26	0.3265
	155	0.138	0.16	0.122	0.202	0.208	0.28	0.29	0.39	0.24	0.22	0.225
	157	0	0	0.082	0.191	0.031	0.07	0.14	0.22	0.08	0.22	0.1034
	161	0	0	0	0.043	0	0.14	0.15	0.07	0.14	0.09	0.0633
	165	0	0	0	0	0	0.03	0.04	0	0.13	0.02	0.022
	167	0	0	0	0	0	0	0.04	0	0	0	0.004
Cc-42	128	0.323	0.367	0.427	0.394	0.435	0.245	0.14	0.09	0.15	0.07	0.2641
	136	0.479	0.541	0.552	0.479	0.413	0.357	0.41	0.39	0.26	0.17	0.4051
	140	0.198	0.092	0.021	0.128	0.152	0.194	0.33	0.46	0.24	0.32	0.2135
	144	0	0	0	0	0	0.051	0.11	0.03	0.11	0.15	0.0451
	146	0	0	0	0	0	0.153	0.01	0.03	0.24	0.29	0.0723
Cc-46	121	0.344	0.354	0.245	0.319	0.385	0.327	0.214	0.16	0.19	0	0.2538
	127	0.427	0.469	0.468	0.351	0.448	0.306	0.214	0.38	0.29	0.48	0.3833
	129	0.208	0.177	0.223	0.245	0.167	0.194	0.357	0.36	0.3	0.31	0.2541
	131	0.021	0	0.064	0.085	0	0.173	0.214	0.1	0.22	0.16	0.1037
	135	0	0	0	0	0	0	0	0	0	0.05	0.005
Cc-57	136	0.311	0.378	0.394	0.383	0.372	0.41	0.15	0.25	0.194	0	0.2842
	142	0.444	0.418	0.479	0.457	0.426	0.21	0.23	0.4	0.347	0.38	0.3791
	146	0.2	0.122	0.128	0.16	0.202	0.27	0.36	0.24	0.163	0.34	0.2185
	152	0.044	0.082	0	0	0	0.05	0.18	0.11	0.255	0.18	0.0901
	158	0	0	0	0	0	0.06	0.08	0	0.041	0.09	0.0271
	160	0	0	0	0	0	0	0	0	0	0.01	0.001
Cc-62	189	0.383	0.351	0.354	0.337	0.354	0.27	0.21	0.3	0.45	0.01	0.3019
	195	0.394	0.436	0.375	0.469	0.469	0.48	0.36	0.45	0.2	0.47	0.4103
	199	0.213	0.181	0.219	0.194	0.177	0.17	0.34	0.21	0.18	0.35	0.2234
	207	0.011	0.032	0.052	0	0	0.08	0.09	0.04	0.17	0.17	0.0645
Cc-70	160	0.378	0.635	0.522	0.448	0.489	0.24	0.24	0.41	0.29	0.11	0.3762
	172	0.551	0.365	0.478	0.552	0.511	0.41	0.41	0.25	0.31	0.38	0.4217
	184	0.071	0	0	0	0	0.35	0.35	0.34	0.4	0.49	0.2001
	186	0	0	0	0	0	0	0	0	0	0.02	0.002

Table 2. Genetic diversity at different microsatellite loci in natural and captive populations of *C. catla*.

Populations	Parameters	Loci															Average
		Cc-1	*Cc-7*	*Cc-9*	*Cc-10*	*Cc-13*	*Cc-15*	*Cc-19*	*Cc-24*	*Cc-31*	*Cc-40*	*Cc-42*	*Cc-46*	*Cc-57*	*Cc-62*	*Cc-70*	
Source: HATCHERY																	
CHNW	*Na*	4.000	5.000	4.000	2.000	3.000	2.000	3.000	5.000	3.000	3.000	3.000	4.000	4.000	4.000	3.000	3.4667
	Ar	4.000	4.937	4.000	2.000	3.000	2.000	3.000	5.000	3.000	3.000	3.000	3.997	4.000	3.957	3.000	3.4594
	Nea	2.916	2.764	2.812	1.814	2.546	1.993	2.701	4.029	2.489	2.527	2.701	2.895	2.955	2.830	2.229	2.6800
	Ho	0.400	0.640	0.480	0.280	0.540	0.420	0.520	0.520	0.420	0.420	0.560	0.500	0.440	0.460	0.480	0.4720
	He	0.657	0.638	0.644	0.449	0.607	0.498	0.630	0.752	0.598	0.604	0.630	0.655	0.662	0.647	0.551	0.6148
	1-Ho/He	0.391	-0.003	0.255	0.376	0.111	0.157	0.174	0.308	0.298	0.305	0.111	0.236	0.335	0.289	0.129	0.2315
	FIS	0.342	-0.032	0.224	0.372	0.097	0.126	0.166	0.283	0.265	0.271	0.080	0.216	0.274	0.261	0.118	0.2042
	PHWE	0.072 NS	0.771 S	1.000 S	0.013 S	1.000 S	0.558 S	0.778 S	0.075 S	0.241 S	0.241 S	1.000 S	0.773 S	0.230 S	1.000 S	1.000 NS	------
MZG	*Na*	4.000	4.000	4.000	2.000	3.000	2.000	4.000	3.000	3.000	3.000	3.000	3.000	4.000	4.000	2.000	3.2000
	Ar	4.000	4.000	4.000	2.000	3.000	2.000	4.000	3.000	3.000	3.000	3.000	3.000	4.000	4.000	2.000	3.2000
	Nea	2.950	3.429	3.287	1.999	2.859	1.999	3.073	2.776	2.606	2.606	2.307	2.661	2.933	2.857	1.835	2.6785
	Ho	0.740	0.660	0.560	0.340	0.520	0.460	0.520	0.480	0.540	0.540	0.500	0.580	0.460	0.560	0.300	0.5173
	He	0.661	0.708	0.696	0.500	0.650	0.500	0.675	0.640	0.616	0.616	0.567	0.624	0.659	0.650	0.455	0.6144
	1-Ho/He	-0.12	0.068	0.195	0.320	0.200	0.080	0.229	0.250	0.124	0.124	0.118	0.071	0.302	0.138	0.341	0.1626
	FIS	-0.18	0.023	0.171	0.299	0.160	0.025	0.181	0.207	0.082	0.082	0.106	0.041	0.299	0.098	0.335	0.1287
	PHWE	0.007 NS	0.229 S	1.000 S	0.465 S	0.550 S	1.000 S	1.000 S	1.000 S	0.771 S	0.771 S	1.000 S	0.561 S	0.392 S	0.767 S	0.029 NS	-----
MCH	*Na*	3.000	3.000	3.000	2.000	3.000	3.000	3.000	4.000	4.000	4.000	3.000	4.000	3.000	4.000	2.000	3.2000
	Ar	3.000	3.000	3.000	2.000	3.000	3.000	3.000	4.000	4.000	4.000	2.997	4.000	3.000	4.000	2.000	3.1998
	Nea	2.776	2.822	2.459	1.923	2.885	2.491	2.807	2.950	3.381	2.933	2.067	3.073	2.489	3.119	1.972	2.6765
	Ho	0.480	0.520	0.500	0.360	0.540	0.500	0.440	0.740	0.520	0.460	0.460	0.520	0.420	0.480	0.400	0.4893
	He	0.640	0.646	0.593	0.480	0.653	0.599	0.644	0.661	0.704	0.659	0.516	0.675	0.598	0.679	0.493	0.6160
	1-Ho/He	0.250	0.195	0.157	0.250	0.174	0.165	0.317	-0.120	0.262	0.302	0.109	0.229	0.298	0.293	0.188	0.2046
	FIS	0.207	0.162	0.103	0.239	0.132	0.156	0.313	-0.205	0.227	0.299	0.075	0.181	0.265	0.278	0.140	0.1715
	PHWE	1.000 NS	0.219 NS	0.369 NS	0.137 NS	0.763 NS	1.000 NS	0.767 NS	0.003*	0.358 NS	0.392 NS	0.779 NS	1.000 NS	0.241 NS	0.537 NS	0.386 NS	-------
RWP	*Na*	5.000	5.000	3.000	2.000	3.000	3.000	3.000	3.000	3.000	5.000	3.000	4.000	3.000	3.000	2.000	3.3333
	Ar	4.999	5.000	2.994	2.000	3.000	3.000	3.000	3.000	3.000	5.000	3.000	4.000	3.000	3.000	2.000	3.3329
	Nea	2.946	3.305	1.924	1.891	2.641	2.229	2.527	2.489	2.701	4.029	2.489	3.358	2.606	2.701	1.993	2.6552
	Ho	0.600	0.660	0.420	0.480	0.620	0.480	0.580	0.420	0.560	0.520	0.420	0.620	0.540	0.520	0.420	0.5240
	He	0.661	0.697	0.480	0.471	0.621	0.551	0.604	0.598	0.630	0.752	0.598	0.702	0.616	0.630	0.498	0.6074
	1-Ho/He	0.092	0.054	0.125	-0.019	0.002	0.129	0.040	0.298	0.111	0.308	0.298	0.117	0.124	0.174	0.157	0.1341
	FIS	0.049	0.021	0.126	-0.049	-0.026	0.118	0.017	0.265	0.080	0.283	0.265	0.079	0.082	0.166	0.126	0.1068
	PHWE	1.000 NS	0.126 NS	0.231 NS	0.770 NS	0.158 NS	1.000 NS	0.254 NS	0.241 NS	1.000 NS	0.075 NS	0.241 NS	0.754 NS	0.771 NS	0.778 NS	0.558 NS	-------
STFH	*Na*	3.000	3.000	3.000	2.000	3.000	2.000	2.000	3.000	4.000	4.000	3.000	3.000	3.000	3.000	2.000	2.8667
	Ar	3.000	3.000	3.000	2.000	3.000	2.000	2.000	3.000	4.000	4.000	3.000	3.000	3.000	3.000	2.000	2.8667
	Nea	2.641	2.431	2.307	1.873	2.346	1.923	1.923	2.536	3.429	2.943	2.536	2.641	2.746	2.661	1.997	2.4622
	Ho	0.560	0.480	0.520	0.340	0.360	0.440	0.360	0.560	0.660	0.620	0.560	0.620	0.500	0.580	0.440	0.5067
	He	0.621	0.589	0.567	0.466	0.574	0.480	0.480	0.606	0.708	0.660	0.606	0.621	0.636	0.624	0.499	0.5825
	1-Ho/He	0.099	0.185	0.082	0.271	0.373	0.083	0.250	0.075	0.068	0.061	0.075	0.002	0.214	0.071	0.119	0.1351
	FIS	0.055	0.145	0.037	0.252	0.351	0.053	0.214	0.025	0.023	0.041	0.025	-0.026	0.179	0.041	0.074	0.0993
	PHWE	1.000 NS	0.248 NS	0.563 NS	0.125 NS	0.008 NS	0.770 NS	0.228 NS	0.547 NS	0.229 NS	0.139 NS	0.547 NS	0.158 NS	0.380 NS	0.561 NS	0.770 NS	-------
Source: WILD																	
BHW	*Na*	5.000	5.000	4.000	2.000	4.000	3.000	4.000	5.000	5.000	5.000	5.000	3.000	4.000	4.000	4.000	4.133
	Ar	5.000	5.000	4.000	2.000	4.000	3.000	4.000	5.000	4.960	5.000	5.000	3.000	4.000	4.000	4.000	4.131
	Nea	3.990	2.904	3.071	1.987	3.316	2.707	2.752	2.904	2.554	3.385	3.765	2.700	2.933	3.159	3.133	3.017
	Ho	0.580	0.680	0.540	0.480	0.500	0.660	0.380	0.680	0.460	0.480	0.600	0.580	0.560	0.480	0.640	0.553
	He	0.749	0.656	0.674	0.497	0.698	0.631	0.637	0.656	0.608	0.705	0.734	0.630	0.659	0.683	0.681	0.660
	1-Ho/He	0.226	-0.037	0.199	0.034	0.284	-0.047	0.403	-0.037	0.244	0.319	0.183	0.079	0.150	0.298	0.060	0.157
	FIS	0.236	-0.027	0.209	0.027	0.293	-0.037	0.403	-0.027	0.253	0.328	0.193	0.089	0.145	0.307	0.070	0.164
	PHWE	0.205 NS	1.000 S	0.019 S	1.000 S	0.563 S	0.385 S	0.0004*	0.774 S	0.266 S	0.001 S	0.0007 NS	1.000 S	0.551 S	0.0004*	0.076 NS	------
THW	*Na*	5.000	6.000	4.000	3.000	4.000	3.000	4.000	5.000	5.000	7.000	5.000	4.000	5.000	4.000	3.000	4.4667
	Ar	5.000	6.000	4.000	3.000	4.000	3.000	4.000	5.000	5.000	7.000	4.980	4.000	5.000	4.000	3.000	4.4653
	Nea	4.202	4.480	3.808	2.241	3.021	2.776	3.139	3.268	3.436	5.076	3.238	3.794	4.102	3.363	2.872	3.5210
	Ho	0.700	0.680	0.600	0.400	0.660	0.660	0.580	0.840	0.520	0.820	0.800	0.700	0.640	0.760	0.580	0.6627
	He	0.762	0.777	0.737	0.554	0.669	0.640	0.681	0.694	0.709	0.803	0.691	0.736	0.756	0.703	0.652	0.7043
	1-Ho/He	0.081	0.125	0.186	0.278	0.013	-0.032	0.149	-0.210	0.267	-0.021	-0.157	0.049	0.154	-0.082	0.110	0.0607
	FIS	0.091	0.117	0.180	0.272	-0.004	-0.021	0.159	-0.234	0.276	-0.011	-0.148	0.038	0.164	-0.072	0.120	0.0618
	PHWE	0.567 NS	0.022 S	0.051 S	1.000 S	0.010 NS	0.361 S	0.026 S	0.183 S	0.002 S	0.100 S	0.022 S	0.741 S	0.025 S	0.000*	0.003 NS	-------

SHW	*Na*	4.000	4.000	3.000	2.000	4.000	3.000	4.000	4.000	5.000	5.000	5.000	4.000	4.000	4.000	3.000	3.8667
	Ar	4.000	4.000	3.000	2.000	4.000	3.000	4.000	4.000	5.000	5.000	5.000	4.000	4.000	4.000	3.000	3.8667
	Nea	2.801	2.822	2.380	1.626	2.929	2.854	2.889	3.631	3.693	3.666	2.677	3.230	3.424	2.957	2.889	2.9644
	Ho	0.700	0.620	0.560	0.440	0.820	0.640	0.600	0.580	0.720	0.960	0.420	0.620	0.560	0.640	0.480	0.6240
	He	0.643	0.646	0.580	0.385	0.659	0.650	0.654	0.725	0.729	0.727	0.626	0.690	0.708	0.662	0.654	0.6491
	1-Ho/He	-0.09	0.040	0.034	-0.143	-0.245	0.015	0.082	0.200	0.013	-0.320	0.330	0.102	0.209	0.033	0.266	0.0350
	FIS	-0.09	0.050	0.015	-0.204	-0.236	0.025	0.092	0.209	0.023	-0.311	0.338	0.112	0.218	0.043	0.275	0.0370
	PHWE	0.774 NS	0.407 NS	0.381 S	0.246 NS	0.000*	0.083 S	0.781 S	0.759 NS	0.016 NS	0.000*	0.000*	0.559 NS	0.768 NS	1.000 NS	0.001*	-------
RB	*Na*	5.000	5.000	4.000	2.000	4.000	3.000	4.000	5.000	6.000	6.000	5.000	4.000	5.000	4.000	3.000	4.333
	Ar	5.000	5.000	4.000	2.000	4.000	3.000	4.000	5.000	6.000	6.000	5.000	4.000	5.000	4.000	3.000	4.333
	Nea	3.096	3.432	2.579	1.993	3.358	2.627	3.365	4.122	4.092	4.550	3.981	3.696	3.434	2.953	2.872	3.343
	Ho	0.580	0.680	0.540	0.420	0.560	0.680	0.960	0.680	0.760	0.860	0.620	0.720	0.580	0.760	0.460	0.657
	He	0.677	0.709	0.612	0.498	0.702	0.619	0.703	0.757	0.756	0.780	0.749	0.729	0.709	0.661	0.652	0.688
	1-Ho/He	0.143	0.040	0.118	0.157	0.203	-0.098	-0.366	0.102	-0.006	-0.102	0.172	0.013	0.182	-0.149	0.294	0.047
	FIS	0.134	0.050	0.110	0.167	0.212	-0.088	-0.386	0.112	-0.013	-0.092	0.165	0.007	0.191	-0.139	0.303	0.049
	PHWE	1.000 NS	1.000 S	1.000 S	0.267 S	0.019 S	0.777 S	0.000*	0.000*	0.083 S	0.075 S	1.000 S	0.100 S	0.008 S	0.255 S	0.384 NS	-------
CB	*Na*	5.000	5.000	4.000	3.000	4.000	3.000	4.000	5.000	5.000	6.000	5.000	4.000	5.000	4.000	4.000	4.400
	Ar	5.000	4.980	4.000	3.000	4.000	3.000	4.000	5.000	5.000	6.000	5.000	4.000	4.980	3.980	4.000	4.396
	Nea	2.804	3.110	3.501	1.903	1.820	2.498	3.861	3.679	4.095	4.785	4.119	2.820	3.327	2.685	2.519	3.168
	Ho	0.620	0.800	0.820	0.540	0.480	0.520	0.820	0.880	0.960	0.680	0.680	0.860	0.840	0.480	0.420	0.693
	He	0.643	0.678	0.714	0.475	0.450	0.600	0.741	0.728	0.756	0.791	0.757	0.645	0.699	0.628	0.603	0.661
	1-Ho/He	0.036	-0.179	-0.148	-0.138	-0.066	0.133	-0.107	-0.208	-0.270	0.140	0.102	-0.333	-0.201	0.235	0.303	-0.047
	FIS	0.046	-0.169	-0.159	-0.128	-0.125	0.117	-0.097	-0.199	-0.261	0.150	0.112	-0.323	-0.191	0.245	0.310	-0.045
	PHWE	0.781 NS	0.018 NS	1.000 NS	0.200 NS	0.138 NS	0.386 NS	0.342 NS	0.000*	0.004*	0.269 NS	0.744 NS	0.022 NS	0.077 NS	0.580 NS	0.001*	-----

Table 3. Pairwise population differentiation (F_{ST}) (above diagonal) and Nei's Unbiased Genetic distance (below diagonal) between populations of *C. catla*.

Populations	CHNW	MZG	MCH	RWP	STFH	BHW	THW	SHW	RB	CB
CHNW	-	0.0075*	0.0041*	0.0093*	0.0102*	0.0318*	0.0504	0.0669	0.0587	0.0985
MZG	0.0327*	-	0.0009*	0.0161*	0.0182*	0.0389*	0.0536	0.063	0.0626	0.1049
MCH	0.0276*	0.0209*	-	0.0025*	0.0067*	0.0306*	0.0544	0.0627	0.0618	0.1023
RWP	0.0353*	0.0459*	0.0241*	-	0.0039*	0.0317*	0.056	0.0683	0.0742	0.1064
STFH	0.0346*	0.0470*	0.0288*	0.0235	-	0.0437*	0.0744	0.0805	0.0845	0.1239
BHW	0.0830	0.0975	0.0805	0.0796	0.0981	-	0.0242	0.0337*	0.0272*	0.0706
THW	0.1281	0.1338	0.1386	0.1377	0.1730	0.0861	-	0.0243*	0.02*	0.0378*
SHW	0.1557	0.1449	0.1462	0.1551	0.1739	0.0954	0.0741	-	0.0307*	0.0507
RB	0.0428*	0.0479*	0.0435*	0.0439*	0.0479*	0.0662	0.1187	0.1431	-	0.0502
CB	0.2399	0.2554	0.2513	0.2564	0.2914	0.1985	0.1106	0.1289	0.2239	-

*Significant at $p \leq 0.05$

Table 4. Analysis of molecular variance (AMOVA) table for *C. catla* populations.

Source of variance	df	MSS	Variance	% Variation
Between populations	9	38.1267	0.3291	6.1758
Between samples within Populations	490	5.2151	0.2152	4.0379
Within individuals	500	4.7847	4.7848	89.786

The results were very similar when Nei's unbiased genetic distances were analysed (Table 3) for genetic differentiation and genetic distance values for population pairs.

Analysis of AMOVA specified that 6.1758% differentiation was contributed due to variation between populations of *C. catla* while 89.786% was due to differentiation within individuals (Table 4).

STRUCTURE HARVESTER admixture model inferences showed consistent results obtained across the 10 autonomous runs. Highest estimated log-likelihood mean value and delta-k value was noted for K = 2 (Fig. 2).

Genetic relatedness among the populations was further investigated by constructing UPGMA dendrogram tree. Two major clusters or clades were observed which predict that the populations in both clusters had shown a close relationship (Fig. 3). All the populations in the first cluster were hatchery (CHNW, MZG, MCH, STFH and RWP) as well as one wild population (RB) while the second cluster included all the wild populations (BHW, THW, SHW and CB).

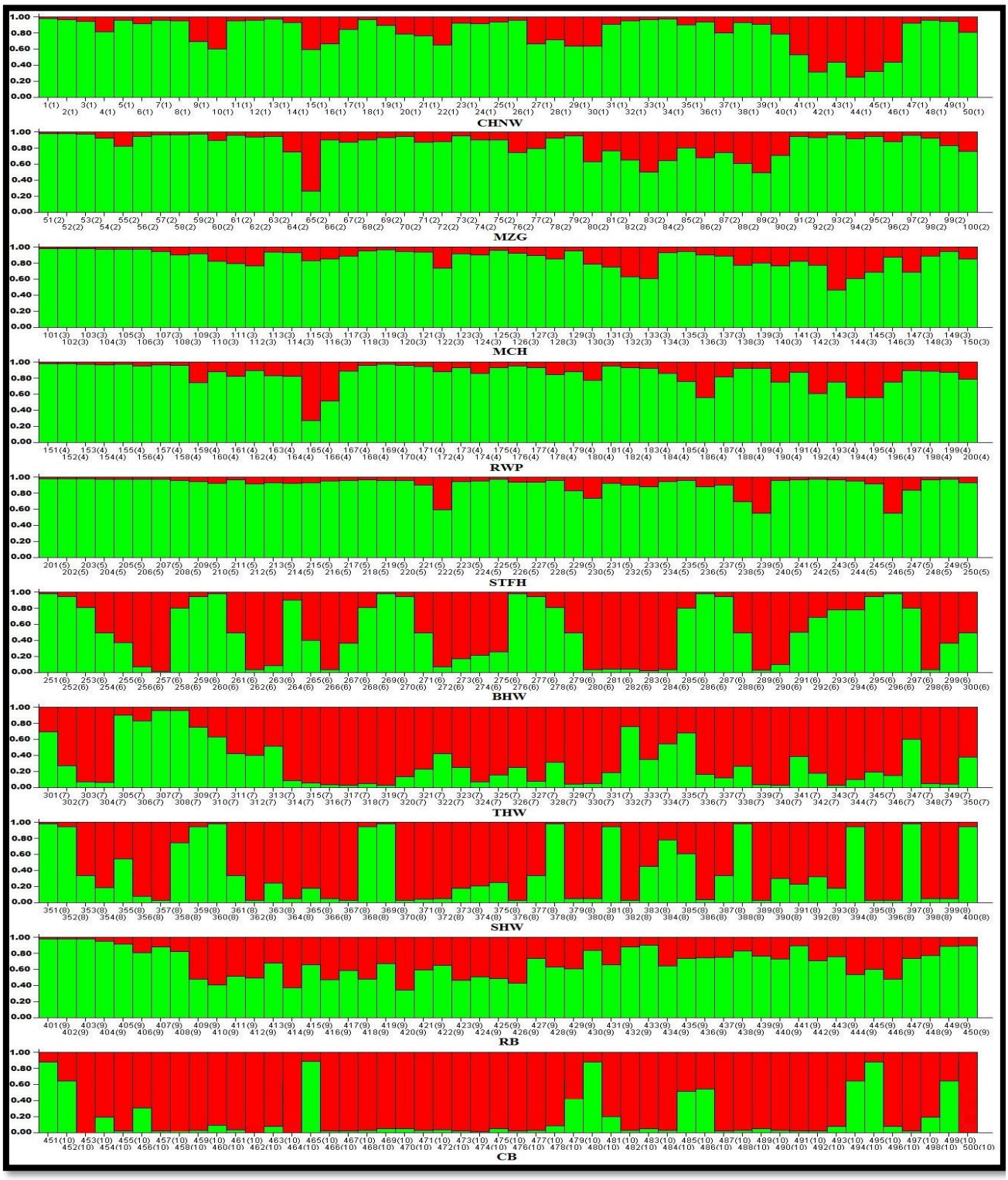

Figure 2. Genetic structuring patterns among natural and captive populations of *C. catla* as revealed by structure analysis. Each vertical column represents an individual.

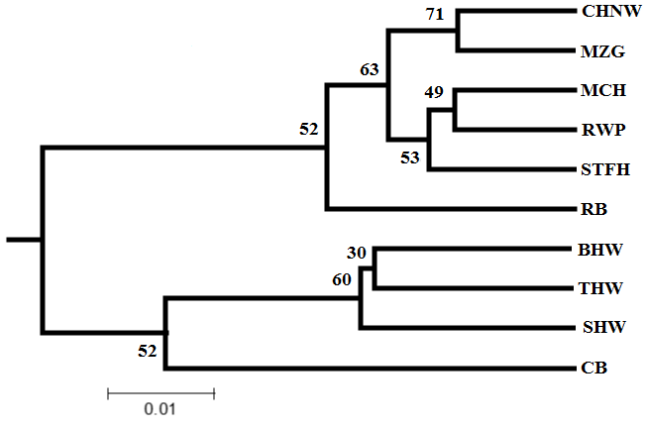

Figure 3. Genetic structuring patterns of wild and hatchery populations of *C. catla* as revealed by UPGMA dendrogram.

DISCUSSION

In Pakistan, Catla carp (*Catla catla*) is an endemic freshwater fish species and contributes to the country aquaculture production significantly. However, during captive management its gene pool is subject to various changes. Though few studies on genetic structure of this fish species are available in literature (McConnell *et al.*, 2001; Rana *et al.*, 2004; Alam and Islam, 2005; Islam *et al.*, 2005; Basak *et al.*, 2014; Sahu *et al.*, 2014; Masih *et al.*, 2014); however, the present study is the first attempt to reveal the genetic structure of natural and hatchery populations of *C. catla* in Pakistan.

In Pakistan, Catla fish seed is available mainly from public and private hatcheries for culture purpose. Different hatchery management differs somewhat from elsewhere in whole Pakistan. Public hatcheries are involved in the wild stock enhancement of a targeted quantity of Catla seed into public waters as a part of the restocking programs. The quality of hatchery produced seeds not analyzed before their release in rivers and associated flood plains. Mostly their performance in the natural water is poor because the huge water body and many other factors compared to captive conditions

***Genetic diversity*:** Maintaining the prominent levels of genetic diversity is essential for the evolutionary potential of both natural and captive populations, as standing genetic diversity provides the raw material which natural selection can act upon when environment change happens. Furthermore, individuals with low heterozygosity are usually inferior of many economically vital features like disease resistance, growth and fertility (Beardmore *et al.*, 1997).

In the present study, the level of genetic diversity noted in all the examined wild and captive populations of *C. catla* were low-to-moderate. All the genetic measures indicated that the wild population possessed higher genetic diversity whereas genetic diversity was lower in hatchery populations. The number of alleles (*Na*) were noted at different locus varied from 2 to 7, which are lower than the previously reported results about *Na* for freshwater fish species by Sahoo *et al.* (2014). The highest mean value of number of alleles, allelic richness and effective number of alleles were observed in the wild fish population captured from THW and minimum in hatchery population collected from STFH. For genetic variation, allelic diversity and heterozygosity are important but the *Na* is dependent on the effective population size much more than that of heterozygosity (Nei *et al.*, 1972). Therefore, to estimate of genetic diversity in a population for selection, conservation, and enhancement programs, *Na* is suitable (Diz and Presa, 2009). In all the examined wild and hatchery populations of *C. catla*, the values of effective alleles were noted less in comparison to number of alleles suggesting that the alleles were lost. Related results about low to moderate level of genetic diversity were reported by Barroso *et al.* (2005) in wild and captive populations of *Brycon opalinus* and Rahman *et al.*, (2009) in wild captured and hatchery-reared *C. catla*. The wild population showed higher heterozygosity whereas the values in hatchery populations were lower. The average values of observed heterozygosity (H_o) and expected heterozygosity (H_e) ranged from 0.472 to 0.693 and from 0.582 to 0.704. The values of H_e were higher in comparison to H_o. The presence of either homozygosity at a locus or population under bottleneck condition may be the reasons of excess *He* and a smaller *Ho* (Pemberton *et al.*, 1995). The average values of 1-*Ho/He* and inbreeding coefficient (F_{IS}) were positive in most of the examined populations but one wild population (CB) showed negative mean value that indicated heterozygosity excess in this population. Equivalent conclusions were stated by Sultana *et al.* (2015) who detected low-to-moderate levels of heterozygosity in domestic *Labeo rohita* populations. The findings of the present study about *Ho* and *He* level are not according to Saha *et al.* (2010) who reported similar levels of *Ho* and *He* in riverine and hatchery populations of *Labeo calbasu*.

Similarly, the average values of inbreeding coefficient (F_{IS}) were positive and higher in captive populations, suggesting a loss of genetic diversity in hatcheries. Fish population captured from CB showed negative value of F_{IS}. Positive F_{IS} values confirm the homozygosity excess in a population (Abbas *et al.*, 2010). Higher F_{IS} in captive stocks could affect negatively on spawning rate, hatching and survival rate of fry, feed conversion and growth performance (Wang *et al.*, 2002; Ala-Honkola *et al.*, 2009) Related inferences were reported by Brown *et al.* (2005) for commercially important Gilthead seabream (*Sparus aurata*) but Perez-Enriquez *et al.*, (1999) observed a low level of inbreeding coefficient in *Pagrus major*.

After correction of significance levels for 150 simultaneous tests ($p<0.05$), only thirteen instances revealed significant departure from *HWE*. A significant departure from *HWE* was

caused by several possible factors such as assortative mating, Wahlund effects, or inbreeding. Related results about deviated from *HWE* were reported by Zhuo *et al.* (2012) in *Channa argus*; in wild and hatchery stocks of Bream (*Abramis brama orientalis*) by Hosseinnia *et al.* (2014) but no deviation from *HWE* was observed in *Salmo saler* by Ribeiro *et al.* (2008).

***Population genetic structure*:** The pairwise estimates of F_{ST} indicated limited to moderate level of genetic differentiation among the wild and hatchery populations of *C. catla*. The highest level of genetic differentiation was found among population pairs of STFH-CB that specified the dissimilar genetic origin while the lowest between the populations of MFG and MCH that indicated the similar genetic origin of these populations. The value of *Fst* from 0 to 0.05 indicates a low level of genetic differentiation (Wright, 1987). Among pairs of populations, the unbiased genetic distance indicated considerable variation ($P<0.05$) in magnitude. The highest genetic distance was measured among STFH-CB while, minimum between the populations of MZG-MCH. A small value of genetic distance suggests both populations have the similar genetic background. Comparable results were reported about genetic structure by Singh *et al.,* (2012) in natural and hatchery populations of *L. calbasu* and Yousefian *et al.* (2011) for *C. carpio.* The findings of the present study about low genetic differentiation are dissimilar to the findings reported by Pedreschi *et al.* (2013) and O'Donnell *et al.* (2014).

AMOVA indicated most of the variation lies within individuals while low variation percentage exist between samples within populations. Similar genetic variation pattern was observed in other freshwater fishes by Chaturvedi *et al.* (2011) and Gopalakrishnan *et al.* (2009).

In this study, two major clusters or clades were observed among both the wild captured and hatchery captive populations by constructing UPGMA dendrogram tree which predict that the populations in both clusters had shown a close relationship. All the populations in the first cluster were hatchery (CHNW, MZG, MCH, STFH and RWP) as well as one wild population (RB) while the second cluster included all the wild populations (BHW, THW, SHW and CB).

***Conclusion*:** The findings of this study provided baseline information about genetic status of *C. catla* in hatchery and wild populations. It is a useful starting-point for further studies to evaluate genetic relationships among *C. catla* populations. Additionally, the assessment of genetic variation in wild and captive populations of *C. catla* could be effective for planning future breeding programs and maintaining a diverse gene pool. However, further studies involving genetic analysis with more markers and population samples covering different wild and hatchery sources throughout Pakistan still needs to be performed to formulate a good management policy.

***Acknowledgements*:** Authors would like to acknowledge Higher Education Commission (HEC), Pakistan on providing research grant for this study under the project No. 20-2074/NRPU R & D. Author would also like to acknowledge Juha Merila (Academy Professor, Ecological Genetics Research Unit, University of Helsinki, Finland) and Paolo Momigliano for their assistance in writing this manuscript.

REFERENCES

Abbas, K., X. Zhou, Y. Li, Z. Gao and W. Wang. 2010. Microsatellite diversity and population genetic structure of Yellowcheek, *Elopichthys bambusa* (Cyprinidae) in the Yangtze River. Biochem. System. Ecol. 38**:**806-812.

Alam, M.S. and M.S. Islam. 2005. Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. Aquaculture 246:151-160.

Ala-Honkola, O., A. Uddstrom, B. Diaz Pauli and K. Lindstrom. 2009. Strong inbreeding depression in Male mating behavior in a poeciliid fish. J. Evol. Biol. 22:1396-1406.

Barroso, R.M., A.W.S. Hilsdorf, H.L.M. Moreirac, P.H. Cabellod and Y.M. Traub-Csekod. 2005. Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae) using microsatellites. Aquaculture 247: 51-65.

Basak, A., A. Ullah, M.N. Islam and M.S. Alam. 2014. Genetic characterization of brood bank stocks of *Catla catla* (hamilton) (Cyprinidae:Cypriniformes) collected from three different rivers of Bangladesh. J. Anim. Plant Sci. 24:1786-1794.

Beardmore, A.L., C.G. Mair and C.G. Lewis. 1997. Biodiversity in aquatic systems in relation to aquaculture. Aquacult. Res. 28:829-839.

Bondad-Reantaso M.G. 2007. Assessment of freshwater fish seed resources for sustainable aquaculture. FAO Fisheries technical paper, Rome; pp.381-385.

Brown, R.C., J.A. Woolliamsb and B.J. McAndrewa. 2005. Factors influencing effective population size in commercial populations of gilthead seabream, *Sparus aurata*. Aquaculture 247:219-225.

Chaturvedi, A., V. Mohindra, R.K. Singh, K.K. Lal, P. Punia, R. Bhaskar, A. Mandal, L. Narain and W.S. Lakra. 2011. Population genetic structure and phylogeography of cyprinid fish, *Labeo dero* (Hamilton, 1822) inferred from allozyme and microsatellite DNA marker analysis. Mol. Biol. Rep. 38:3513-3529.

Chistiakov D.A., B. Hellemans and F.A.M. Volckaert. 2006. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. Aquaculture 255:1-29.

Ciftci, Y. and I. Okumuu. 2002. Fish population genetics and applications of molecular markers to fisheries and aquaculture: basic principles of fish population genetics. Turk. J. Fish. Aquat. Sci. 2:145-155.

Diz, P.A. and P. Presa. 2009. The genetic diversity pattern of *Mytilus alloprovincialis* in Galician Rías (NW Iberian estuaries). Aquaculture 287:278-285.

Earl, D.A. and B.M. Vonholdt. 2012. STRUCTURE HARVESTER: a website and programme for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4:359-361.

Evanno, G., S. Regnaut and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611-2620.

Evans, B., J. Bartlett, N. Sweijd, P. Cook and N.G. Elliott. 2004. Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). Aquaculture 233:109-127.

Excoffier, L., G. Laval and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolut. Bioinformat. 1:47-50.

Falush, D., M. Stephens and J.K. Pritchard. 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. Genetics 164:1567-1587.

FAO. 2014. Cultured Aquatic Species Information Program, *Catla catla* (Hamilton, 1822). Food and Agriculture Organization of the United Nations, Fisheries and Aquaculture Department, Rome, Italy; p.11.

Gopalakrishnan, A., K.K. Musammilu, V.S. Basheer, L. John, K.G. Padmakumar, K.K. Lal, V. Mohindra, P. Punia, K. Dinesh, H. Manjebrayakath, A.G. Ponniah and W.S. Lakra. 2009. Low genetic differentiation in the populations of the Malabar carp *Labeo dussumieri* as revealed by allozymes, microsatellites and RAPD. Asian Fish. Sci. 22:359-391.

Goudet, J., 2002. FSTAT Version 2.9.3.2. A programme to estimate and test gene diversities and fixation indices. Institute of Ecology, University of Lausanne, Switzerland.

Hosseinnia, Z., A. Shabany and H. Kolangi-Miandare. 2014. Comparison of genetic variation of wild and farmed Bream (*Abramis brama orientalis*; berg, 1905) using microsatellite markers. Mol. Biol. Res. Commun. 3:187-195.

Islam, M.S., A.S.I. Ahmed, M.S. Azam and M.S. Alam. 2005. Genetic analysis of three river populations of *Catla catla* (Hamilton) using randomly amplified polymorphic DNA markers. Asian-Aust. J. Anim. Sci. 4:453-458.

Masih, P., R.K. Luhariya, R. Das, A. Gupta, V. Mohindra, R.K. Singh, R. Srivastava, U.K. Chauhan, J.K. Jena and K.K. Lal. 2014. Cross-priming of microsatellite loci in subfamily Cyprininae (Family Cyprinidae): their utility in finding markers for population genetic analysis in three Indian major carps. Mol. Biol. Rep. 41:5187-5197.

McConnell, S.K.J., J. Leamon, D.O.F. Skibinski and G.C. Mair. 2001. Microsatellite markers from the Indian Major Carp species, *Catla catla*. Mol. Ecol. Notes 3:115-116.

Miller, M.P. 1997. Tools for Population Genetic Analyses (TFPGA) V l.3: A Windows Program for the Analysis of Allozyme and Molecular Genetic Data. Flagstaff: Northern

Arizona, University.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

O'Donnell, T.P., M. R. Denson and T.L. Darden. 2014. Genetic population structure of spotted seatrout *Cynoscion nebulosus* along the south-eastern U.S.A. J. Fish Biol. 85**:**374-393.

Oosterhout, C.V., W.F. Hutchinson, D.P.M. Wills and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535-538.

Pedreschi D, M. Kelly-Quinn, J. Caffrey, M. Grady and S. Mariani. 2013. Genetic structure of pike (*Esox lucius*) reveals a complex and previously unrecognized colonization history of Ireland. J. Biogeogr. 41:548-560.

Pemberton, J.M., J. Slate, D.R. Bancroft and J.R. Barrett. 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol. Ecol. 4:249-252.

Perez-Enriquez, R., M. Takagi and N. Taniguchi. 1999. Genetic variability and pedigree tracing of a hatchery–reared stock of red sea bream *Pagrus major* used for stock enhancement, based on microsatellite DNA markers. Aquaculture 173:413-423.

Pritchard, J.K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multi locus genotype data. Genetics 155:945-959.

Rahman, S. M. Z., M. R. Khan, S. Islam and S. Alam. 2009. Genetic variation of wild and hatchery populations of the catla Indian major carp (*Catla catla* Hamilton 1822: Cypriniformes, Cyprinidae) revealed by RAPD markers. Genet. Mol. Biol. 32:197-201.

Rana, R.S., K.V. Bhat, S. Lakhanpal and W.S. Lakra. 2004. Comparative genetic diversity in natural and hatchery populations of Indian Major Carps (*Catla catla* and *Labeo rohita*). Asian-Aust. J. Anim. Sci. 17:1197-1203.

Ribeiro, A., P. Moran and A. Caballero. 2008. Genetic diversity and effective size of the Atlantic salmon *Salmo salar* L. inhabiting the River Eo (Spain) following a stock collapse. J. Fish Biol. 72:1933-1944.

Rousset, F. 2000. Genetic differentiation between individuals. J. Evol. Biol. 13:58-62.

Saha, D., N.S. Akter, M.N. Islam, M.A.R. Hossain and S. Alam. 2010. Bottleneck in the endangered kalibaus, *Labeo calbasu* (*cyprinidae: cypriniformes*) populations in Bangladesh revealed by microsatellite DNA marker analysis. Gene Genom. 32:47-53.

Sahoo, L., B.P. Sahu, S.P. Das, K. Subrat, Swain, D. Bej, A. Patel, P. Jayasankar and P. Das. 2014. Limited genetic differentiation in *Labeo rohita* (Hamilton 1822) populations as revealed by microsatellite markers. Biochem. Systemat. Ecol. 57:427-431.

Sahu, B.P., L. Sahoo, C. G. Joshi, P. Mohanty, J. K. Sundaray, P. Jayasankar and P. Das. 2014. Isolation and characterization of polymorphic microsatellite loci in Indian major carp, *Catla catla* using next–generation sequencing platform. Biochem. Systemat. Ecol. 57:357-362.

Sanguinetti, C.J., E.D. Neto and A.J.G. Simpson. 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17:915-919.

Singh, R.K., K.K. Lal, V. Mohindra, P. Punia, R.S. Sah, R. Kumar, A. Gupta, R. Das, W.S. Lakra and S. Ayyappan. 2012. Genetic diversity of Indian Major Carp, *Labeo calbasu* (Hamilton, 1822) populations inferred from microsatellite loci. Biochem. Syst. Ecol. 44:307-316.

Sultana, F., K. Abbas, Z. Xiaoyun, S. Abdullah, I. Qadeer and R. Hussnain. 2015. Microsatellite markers reveal genetic degradation in hatchery stocks of *Labeo rohita*. Pak. J. Agri. Sci. 52:775-781.

Wang, S.Z., J.J. Hard and F. Utter. 2002. Salmonid inbreeding: a review. Rev. Fish Biol. Fish. 11:301-319.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F statistics for the analysis of population structure. Evolution 38:1358-1370.

Wright, S. 1978. Evolution and the genetics of population's variability within and among natural populations, 2nd Ed. University of Chicago Press, Chicago.

Yousefian, M. 2011. Genetic variations of common carp (*Cyprinus carpio* L.) in South-eastern part of Caspian Sea using five microsatellite loci. World J. Zool. 6:56-60.

Yue, G.H. and L. Orban. 2005. A simple and affordable method for high-throughput DNA extraction from animal tissues for polymerase chain reaction. Electrophoresis 26:3081-3083.

Yue, G.H., Z.Y. Zhu, L.C. Lo, C.M. Wang, G. Lin, F. Feng, H.Y. Panga, J. Lia, P. Gonga, M. Liua, J. Tanb, R. Choub, H. Limb and L. Orbanc. 2009. Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. Aquaculture 293:22-28.

Zhuo, X., R. Liang, Y. Chen, G. Huang, D. Yu and J. Zou. 2012. Genetic characterization of northern snakehead (*Channa argus*) populations in China using microsatellite markers. Biochem. Systemat. Ecol. 43:25-31.