

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

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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 (A_r), the alleles number (N_a), the number of effective alleles (N_{e_a}) and observed heterozygosity (H_o). The highest mean values of N_a , N_{e_a} and A_r 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* restocking 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.

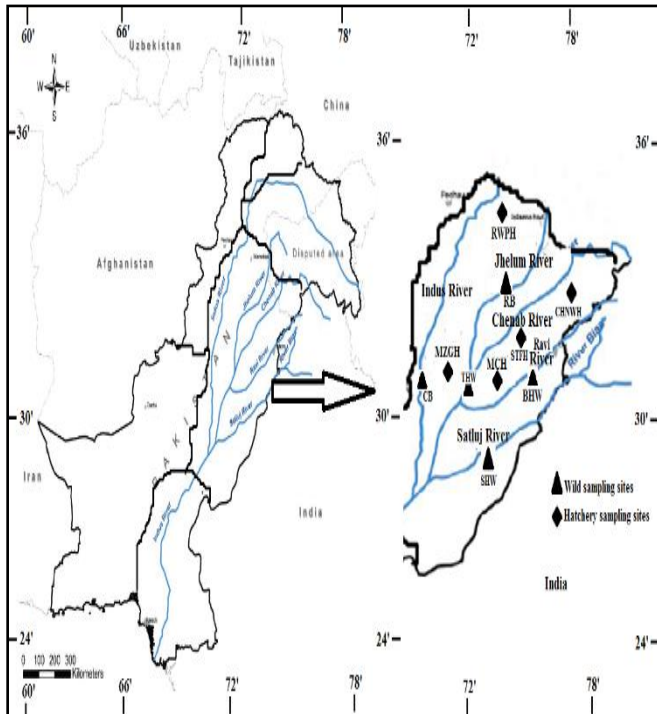


Figure 1. Map of Pakistan showing *C. catla* sampling sites.

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 20 μL reaction volume containing 50ng of template DNA, 2 μM of each primer, 0.25 μM each of the dNTPs, 1 unit of *Taq* DNA polymerase, 1.5mM MgCl_2 and 1 μL 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 (N_a), effective number of alleles (N_{e_a}), 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	<i>Cc-1</i>	(TG) ₉	F: CCTGGAACACTTTTTCTCTTGATG R: CAAGATCACACACACCACACAC	KF913007	58°C
2	<i>Cc-7</i>	(GT) ₂₁	F: CACTCTGTGCCTAGACCTCG R: CTGGAGTTTAAGCCCTGTTC	AJ294955	58°C
3	<i>Cc-9</i>	(TTATT) ₆	F: GGACCATAGGTTTGGGTTGATT R: TGACTCCAAATAGGACAAGTGG	KF913008	56°C
4	<i>Cc-10</i>	(GTTT) ₅	F: GTGAGCAGAAGAGACTG R: AGTTTTTGAACAGTGAGTG	AJ294958	60°C
5	<i>Cc-13</i>	(TG) ₁₂	F: TAGACACGGCATTAGAGACACC R: CTAGCTGCATATCACATTCTTCAC	KF913009	58°C
6	<i>Cc-15</i>	(CA) ₆	F: GGGTTGCTCTCTAAAACCTCTGG R: CTCCTTCTGCTCTCTCTGCTCT	KF913010	58°C
7	<i>Cc-19</i>	(TG) ₆	F: CATGTGTATGCTTTGTACTGTGAG R: CAATTCACCACCGATTCTTTTG	KF913011	58°C
8	<i>Cc-24</i>	(AC) ₆	F: ATTAAGGAAGAAGGCTGGAAGG R: GTGCGAAGGAAGTGACAAGAGT	KF913012	60°C
9	<i>Cc-31</i>	(GT) ₁₄	F: TGTCTAGGTGTGTTTCTCTGTGG R: GAACATGAGCGGGAAAACCTG	KF913013	60°C
10	<i>Cc-40</i>	(AG) ₆	F: TGCAATACGAAGAAGACAGTGG R: GCAAAAATACCATGCTCACAGA	KF913014	57°C
11	<i>Cc-42</i>	(TG) ₁₃	F: CTGGCCTGTATCTCGCTCTG R: TACACTTGACTAACCCGGACCT	KF913015	50°C
12	<i>Cc-46</i>	(TC) ₆	F: CTCTCCCTCTACCAGGCATTTT R: GTCAGTGTTGAAGTCTTTTCC	KF913016	59°C
13	<i>Cc-57</i>	(AG) ₁₃	F: CCACTCTTTCTTTTACTCCCCATT R: TGTAACAGCTTGTCTGGTGATAG	KF913017	54°C
14	<i>Cc-62</i>	(GA) ₇	F: TCCAACCATCCATATCAGCTAC R: TGACGACGCTATCTTCTCTCTTT	KF913018	57°C
15	<i>Cc-70</i>	(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 (N_a) 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 N_a (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 (N_{ea}) was noted from 2.462 to 3.521 and those for observed heterozygosity (H_o) 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
<i>Cc-1</i>	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
<i>Cc-7</i>	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
<i>Cc-9</i>	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
<i>Cc-10</i>	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
<i>Cc-13</i>	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
<i>Cc-15</i>	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
<i>Cc-19</i>	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
<i>Cc-24</i>	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
<i>Cc-31</i>	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
<i>Cc-40</i>	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	0.043	0	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
	167	0	0	0	0	0	0	0.04	0	0	0	0.004
<i>Cc-42</i>	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
<i>Cc-46</i>	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
<i>Cc-57</i>	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
<i>Cc-62</i>	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
<i>Cc-70</i>	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

Genetic structure of *Catla fish*

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

Source: HATCHERY

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	
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	3.000	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	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	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	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	3.000	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	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	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	0.407	0.381 ^N	0.246	0.000*	0.083 ^N	0.781 ^N	0.759	0.016	0.000*	0.000*	0.559	0.768	1.000	0.001*	-----
		NS	NS	S	NS		S	S	NS	NS			NS	NS	NS		
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	1.000 ^N	1.000 ^N	0.267 ^N	0.019 ^N	0.777 ^N	0.000*	0.000*	0.083 ^N	0.075 ^N	1.000 ^N	0.100 ^N	0.008 ^N	0.255 ^N	0.384 ^{NS}	-----
		NS	S	S	S	S	S			S	S	S	S	S	S		
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	0.018	1.000	0.200	0.138	0.386	0.342	0.000*	0.004*	0.269	0.744	0.022	0.077	0.580	0.001*	-----
		NS	NS	NS	NS	NS	NS	NS			NS	NS	NS	NS	NS		

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).

Genetic structure of *Catla fish*

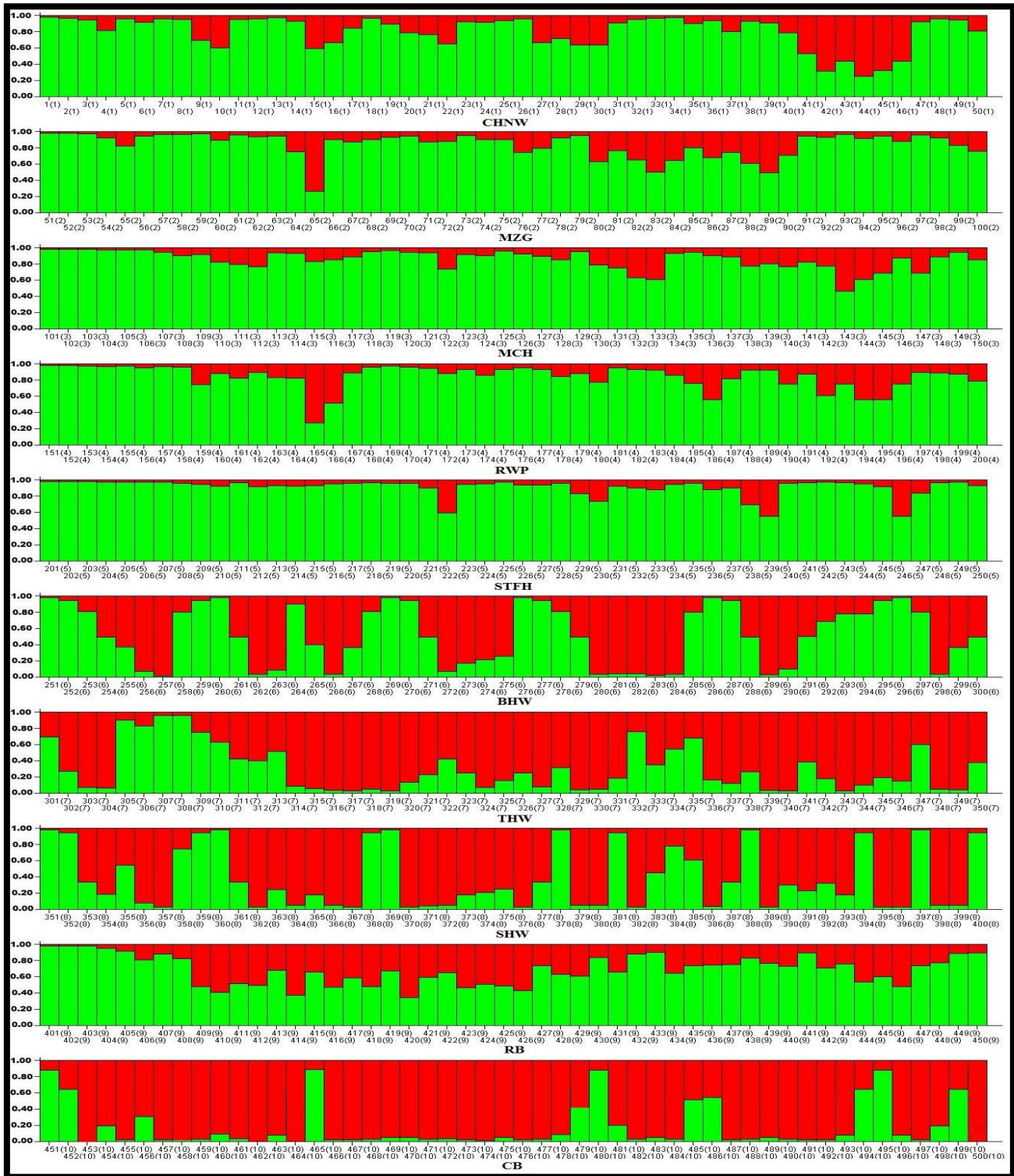


Figure 2. Genetic structuring patterns among natural and captive populations of *C. catla* as revealed by structure analysis. Each vertical column represents an individual.

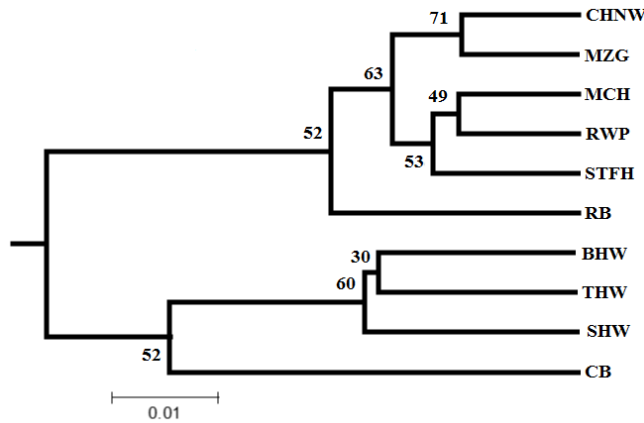


Figure 3. Genetic structuring patterns of wild and hatchery populations of *C. catla* as revealed by UPGMA dendrogram.

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 (N_a) were noted at different locus varied from 2 to 7, which are lower than the previously reported results about N_a 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 N_a 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, N_a 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 H_e and a smaller H_o (Pemberton *et al.*, 1995). The average values of $1-H_o/H_e$ 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 H_o and H_e level are not according to Saha *et al.* (2010) who reported similar levels of H_o and H_e 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 F_{ST} 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.

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