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Loss of Genetic Polymorphism in Hatchery Produced *Cirrhinus mrigala* as Revealed by Microsatellite Loci Analyses

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Key words
Genetic structure, Bottleneck, Molecular markers, Conservation genetics, Hatchery populations

ABSTRACT

Preservation of genetic variations is critical to maintain the evolutionary potential and fitness of fish populations. The purpose of the present study was to assess the genetic structure of eight different hatchery populations of *Cirrhinus mrigala* based on microsatellite loci. The numbers of alleles extended from 2 to 5 with the average values varied from 3.166 - 3.833. The average observed heterozygosity values varied from 0.478-0.549. The average values of inbreeding coefficient (F_{IS}) varied from 0.175 to 0.261. Significant deviation from Hardy-Weinberg equilibrium was found in 14 out of 96 tests. The pairwise values of population differentiation ranged from 0.0055 to 0.0334. Analysis of molecular variance (AMOVA) revealed a significant genetic structuring between the hatchery populations. The UPGMA dendrogram divided the populations into two main clusters. Bottleneck was observed for all the hatchery populations. The findings of the present study would be helpful for defining effective management units in order to maintain the genetic integrity of commercially important freshwater fish species.

INTRODUCTION

The aquaculture practices are often responsible for reducing the genetic diversity of fish populations possibly due to inbreeding, genetic drift and founder effect (Ellergern and Galtier, 2016). This leads to the reduced fitness and adaptability to ecological changes which eventually limits the genetic potential for artificial selection (Dudgeon *et al*., 2006). Genetic diversity is directly related to sustain the biological potential, developmental stability and to increases the survival chances of both wild and cultured populations in changing environments (Rowe *et al*., 2017). Recognizing the importance of genetic diversity within a species is necessary for their management. Therefore, understanding the existing genetic status of fish populations will be helpful in making the sustainable management decisions for better conservation and restoration of fish genetic resources (Melis *et al*., 2018).

The quality of hatchery produced seeds may compromise the effectiveness of stocking programs mainly coupled with limited broodstock (Loukovitis *et al*., 2014). The introduction of poor quality seeds to natural waters is possibly disturbing the genetic integrity of wild populations regarding their fitness and productivity. Moreover, the lack of technical knowledge among the hatchery operators causes hybridization, negative selection and genetic introgression. All these factors collectively result in loss of fecundity, viability, resistance against diseases and resilience against environmental stressors which may lead to extinction of local fish populations (Booy *et al*., 2000; Hedrick and Fredrickson, 2010). Furthermore, a genetically different hatchery population will result an abrupt change in the genetic structure of the wild populations. Therefore, current information over the genetic diversity and population structure of hatchery-reared populations is direly required before the implementation of any conservation plan.

With the rapid expansion of aquaculture, the information about the gene pool of individual candidate species has become crucial for successful breeding programs. As, this could prove beneficial to elucidate the genetic differences among natural populations, assessing

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genetic variation within captive stocks and to ascertain the genetic impact of aquaculture on wild populations for the upholding of sustainable aquaculture. The artificial propagation of economically important fish species is based upon the maintenance of the brood stock captured from the natural water systems. Since the beginning of 21st century, natural resources have become more vulnerable due to overexploitation and the supply of seed from the natural resources tend to be declined (Chen *et al*., 2010). Therefore, to meet the demand of expanding aquaculture in Pakistan, about 99% of fish seed is artificially produced in hatcheries (Khan *et al*., 2008). Although hatcheries are considered an important source for the supply of seed to restock the natural water systems but there is a serious concern among the fisheries stakeholders about the performance of hatchery produced seed in terms of commercial production. It is generally observed that the brooders are not often exchanged in hatcheries for generations, which results in the reduction of genetic diversity and output of hatchery stocks (Hasanat *et al*., 2014). The breeding programs need to consider the genetic integrity of species in question for successful and sustainable aquaculture at a time (Subasinghe *et al*., 2009).

The fish Mrigal, *Cirrhinus mrigala* is indigenous to the freshwater systems of the Indian subcontinent including countries like Pakistan, India, Bangladesh and Nepal. This species is amongst the top 20 freshwater fish species being cultured for aquaculture purposes in Asian countries (FAO, 2009). Microsatellite DNA markers being versatile, highly polymorphic and having high mutation rates are the most edifying markers in studies related to fish genetics. Moreover, with the advantage of easy and low cost detection by PCR, they have become the markers of choice for a wide range of application in conservation, population genetics and evolutionary biology (Tripathy, 2018). The purpose of the present research work was to report the genetic status of hatchery produced *C. mrigala* by using microsatellite markers.

MATERIALS AND METHODS

Collection of samples and DNA extraction

A total of 280 specimens of *Cirrhinus mrigala* were collected from eight selected hatcheries of Punjab province, Pakistan. At the sampling sites, the fish individuals were identified based on their key morphological features (Mirza and Sharif, 1996). The fish specimens were collected from the districts of Lahore (LHR), Gujranwala (CHW), Rawalpindi (RWLP), Faisalabad (FSD), Sargodha (SGD), Bahawalpur (BHWP), Mianchannu (MNCH) and Muzaffargarh (MZG). In order to avoid any mixing of specimens from various sources, the collected individuals were placed in marked zip lock bags and transported to the laboratory for further analysis by keeping them in crushed ice box.

In the laboratory, the total DNA was extracted from dorsal muscle tissues by opting the methodology (phenol/chloroform) of Sambrook and Russel (2001). The quality and quantity of the isolated DNA was assessed through agarose gel electrophoresis (0.8%) and NanoDrop (260 nm), respectively.

Microsatellite loci amplification and visualization

Twelve pairs of primers by Lal *et al*. (2004) (*MFW1, MFW2* and *MFW17*) and Das *et al*. (2005) (*Lr1, Lr3, Lr6, Lr10, Lr12, Lr20, Lr21, Lr23* and *Lr24*) were cross amplified in the respective fish. The PCR amplification of microsatellite loci was done in gradient thermal cycler (Multigene Optimax, Labnet USA) in a 20 µL reaction volume, having 50 ng of template DNA, 2 µM of each primer, 1 µL of 10 X reaction buffer, 0.25 µM of each dNTPs, 1 unit of *Taq polymerase* and 1.5 mM of $MgCl_2$. The cycles were as follows: initial denaturation was directed at 94ºC for 3 min followed by 35 cycles of 94ºC for 30 s, annealing at various temperatures (according to the respective primer) for 30 s and the final extension was carried out at 72ºC for 5 min.

The vertical gel electrophoresis was conducted on 8% non-denaturing polyacrylamide gel to separate the amplified PCR products. After the completion of electrophoresis, the silver staining protocol was followed for the visualization of bands (Sanguinetti *et al*., 1994).

Analysis of microsatellite data

The probability of scoring error (large alleles, null alleles and stuttering bands) in the genotypic data was analyzed through Micro-Checker Version 2.2.1 (Oosterhout *et al*., 2004). POPGENE Version 1.31 (Yeh *et al*., 1999) was used to estimate various indices of genetic diversity viz., allele numbers (*Na*), effective number of allele (*Nae*), observed heterozygosity (H_o), expected heterozygosity (H_e) and deviation from *HWE*. For adjusting the significance for Hardy-Weinberg equilibrium, sequential Bonferroni correction was applied (Rice, 1989).

The allele frequency, allelic richness (*Ar*) and inbreeding coefficient (F_{IS}) was calculated by using the program FSTAT Version 2.9.3.2 (Goudet, 2002). Among the populations, the genetic differentiation (F_{ST}) was assessed by following Weir and Cockerham's 1984. ARLEQUIN Version 3.1 was used to check the hierarchal partition of genetic diversity through AMOVA (Excoffier *et al*., 2005). The software TFPGA Version 1.3 (Miller, 1997) was used to construct the UPGMA dendrogram based on Nei's (1972) unbiased distance. To detect whether the populations have undergone a recent genetic bottleneck, sign and Wilcoxon test was used under three different mutation models (infinite allele model, two-phase model and stepwise-mutation model) following the Bottleneck program Version 1.2.02 (Piry *et al*., 1999).

The population genetic structure was evaluated by using Structure Version 2.3.2 (Pritchard *et al*., 2000; Falush *et al.*, 2003). Nine autonomous runs were directed for each K value and Structure Harvestor (Earl and Vonholdt, 2012) was employed to specify the number of genetic clusters as described by Evanno *et al*. (2005).

RESULTS

Genetic diversity

All the parameters of genetic diversity examined in this study have been presented in Table I. The microsatellite data analysis with Micro-checker revealed no scoring errors at any loci. The size of alleles ranged between 144-252 bp. All the loci were proved to be polymorphic. The number of alleles (*Na*) in the hatchery populations of *C. mrigala* varied from 2.000 to 5.000 at each locus with average values ranged from 3.166 to 3.833. The average values of *Nae* varied from 2.899 to 3.371. The heterozygosity level was observed low to moderate. The average values of *Ho* ranged from 0.478 in MNCH to 0.549 in LHR hatchery population. The minimum and maximum average values of expected heterozygosity were measured in BHWP (*He*=0.645) and LHR (*He*=0.691) populations, respectively. Positive average values of inbreeding coefficient (F_{IS}) were examined in all the studied hatchery stocks of *C. mrigala* and varied from minimum 0.175 to maximum 0.261. For *HWE*, 14 out of 96 test were found significant at $p<0.05$.

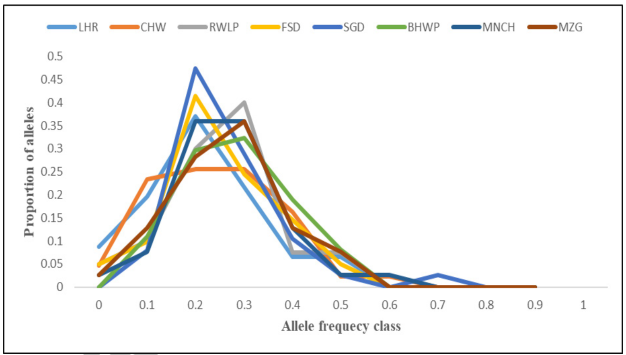

Fig. 1. Proportion of alleles showing genetic bottleneck in hatchery populations of *C. mrigala.*

Table I. Various parameters of genetic diversity for *C. mrigala* populations based on microsatellite markers.

Populations/ parameters	Loci												
	Lr1	**Lr3**	**Lr6**	**Lr10**	**Lr12**	**Lr20**	**Lr21**	**Lr23**	**Lr24**	**MFW1**	**MFW2**	**MFW17**	**Average**
LHR													
Na	2	5	3	5	4	4	5	3	5	2	4	4	3.833
Ar	2	5	3	5	4	4	5	3	5	2	3.971	4	3.830
Nae	2	4.495	2.829	3.99	3.673	3.751	4.131	2.941	4.231	1.985	2.941	3.485	3.371
Ho	0.485	0.514	0.571	0.657	0.514	0.542	0.6	0.657	0.6	0.514	0.457	0.485	0.549
He	0.507	0.788	0.655	0.76	0.738	0.744	0.768	0.669	0.774	0.503	0.669	0.723	0.691
F_{IS}	0.043	0.334	0.13	0.137	0.307	0.273	0.222	0.019	0.208	-0.022	0.32	0.332	0.191
PHWE	0.798 ^{NS}	0.005 ^{NS}	0.014 ^{NS}	0.045 ^{NS}	0.003 ^{NS}	0.027 ^{NS}	0.036 ^{NS}	0.303 ^{NS}	0.003 ^{NS}	0.897 ^{NS}	0.052 ^{NS}	0.000*	--
CHW													
Na	2	5	3	4	4	3	4	3	4	2	5	4	3.583
Ar	2	5	3	4	4	3	4	3	4	2	5	4	3.583
Nae	1.998	4.117	2.725	3.485	2.927	2.855	3.555	2.852	3.729	1.876	3.996	3.618	3.144
Ho	0.4	0.457	0.542	0.628	0.485	0.514	0.571	0.571	0.6	0.457	0.542	0.485	0.521
He	0.506	0.768	0.642	0.723	0.667	0.659	0.729	0.658	0.742	0.473	0.76	0.734	0.671
F_{IS}	0.213	0.392	0.157	0.133	0.276	0.222	0.219	0.134	0.175	0.035	0.289	0.342	0.215
PHWE	0.204 ^{NS}	0.000*	0.600 ^{NS}	0.041 ^{NS}	0.027 ^{NS}	0.061 ^{NS}	0.002 ^{NS}	0.591 ^{NS}	0.206 ^{NS}	0.833 ^{NS}	0.000*	0.000*	--
RWLP													
Na	2	3	3	4	4	4	4	2	4	2	4	4	3.333
Ar	2	3	3	4	4	4	4	2	4	2	4	4	3.333

Table continued on next page.............

Populations/ parameters	Loci											
	Lr1	**Lr3**	**Lr6**	**Lr10**	**Lr12**	**Lr20**	**Lr21**	**Lr23**	**Lr24**	**MFW1**	**MFW2**	**MFW17**	**Average**
Nae	1.993	2.976	2.976	3.775	3.695	3.101	3.656	1.993	3.964	1.993	3.684	3.324	3.094
Ho	0.428	0.457	0.514	0.457	0.485	0.457	0.6	0.542	0.514	0.485	0.457	0.542	0.495
He	0.505	0.673	0.673	0.745	0.74	0.687	0.737	0.505	0.758	0.505	0.739	0.709	0.664
F_{IS}	0.132	0.325	0.239	0.39	0.327	0.338	0.188	-0.075	0.325	0.04	0.385	0.237	0.237
PHWE	0.360 ^{NS}	0.028 ^{NS}	0.034 ^{NS}	0.002 ^{NS}	0.002 ^{NS}	0.002 ^{NS}	0.007 ^{NS}	0.657 ^{NS}	0.003 ^{NS}	0.813 ^{NS}	0.000*	0.007 ^{NS}	--
FSD													
Na	2	4	3	4	4	5	3	3	3	2	4	4	3.416
Ar	2	3.971	3	4	4	5	3	3	3	2	4	4	3.414
Nae	1.974	2.948	2.885	3.678	3.882	4.596	2.852	2.882	2.689	1.96	3.723	3.678	3.145
Ho	0.428	0.571	0.514	0.514	0.542	0.514	0.6	0.4	0.657	0.4	0.657	0.628	0.535
He	0.5	0.67	0.662	0.738	0.753	0.793	0.658	0.662	0.637	0.496	0.742	0.738	0.670
F_{IS}	0.146	0.149	0.204	0.307	0.282	0.335	0.09	0.4	-0.032	0.179	0.116	0.125	0.191
PHWE	0.387 ^{NS}	0.364 ^{NS}	0.076 ^{NS}	0.002 ^{NS}	0.018 ^{NS}	0.005 ^{NS}	0.818 ^{NS}	0.007 ^{NS}	0.749 ^{NS}	0.241 ^{NS}	0.202 ^{NS}	0.000*	--
SGD													
Na	2	3	3	4	3	4	3	3	4	2	4	3	3.166
Ar	2	3	3	4	3	4	3	3	4	2	4	3	3.166
Nae	1.581	2.92	2.913	3.786	2.845	3.775	2.78	2.885	3.97	1.942	3.763	2.948	3.009
Ho	0.371	0.6	0.6	0.485	0.542	0.457	0.571	0.485	0.6	0.485	0.542	0.571	0.525
He	0.373	0.667	0.666	0.746	0.658	0.745	0.649	0.662	0.759	0.492	0.744	0.67	0.652
F_{IS}	0.005	0.102	0.08	0.353	0.177	0.39	0.103	0.27	0.212	-0.007	0.274	0.149	0.175
PHWE	0.978 ^{NS}	0.618 ^{NS}	0.314 ^{NS}	0.016 ^{NS}	0.119 ^{NS}	0.008 ^{NS}	0.029 ^{NS}	0.148 ^{NS}	0.026 ^{NS}	0.935 ^{NS}	0.003 ^{NS}	0.007 ^{NS}	--
BHWP													
Na	2	4	2	3	4	4	4	3	3	2	3	3	3.083
Ar	2	4	2	3	4	4	4	3	3	2	3	3	3.083
Nae	1.942	3.798	1.998	2.962	3.895	3.279	3.324	2.716	2.98	1.993	2.906	2.998	2.899
Ho	0.485	0.542	0.514	0.542	0.542	0.628	0.428	0.342	0.4	0.485	0.6	0.542	0.504
He	0.492	0.747	0.506	0.672	0.754	0.705	0.709	0.641	0.674	0.505	0.665	0.676	0.645
F_{IS}	0.014	0.255	-0.015	0.195	0.283	0.11	0.399	0.469	0.41	0.04	0.1	0.2	0.205
PHWE	0.935 ^{NS}	0.001*	0.929 ^{NS}	0.331 ^{NS}	0.075 ^{NS}	0.222 ^{NS}	0.000*	0.001*	0.002 ^{NS}	0.813 ^{NS}	0.498 ^{NS}	0.318 ^{NS}	--
MNCH													
Na	2	4	3	4	4	4	3	3	4	2	3	3	3.250
Ar	2	4	3	4	4	4	3	3	4	2	3	3	3.250
Nae	1.876	3.763	2.909	3.169	3.751	3.729	2.966	2.909	3.786	1.942	2.991	2.755	3.045
Ho	0.457	0.542	0.457	0.514	0.4	0.4	0.457	0.542	0.571	0.485	0.514	0.4	0.478
He	0.473	0.744	0.665	0.694	0.744	0.742	0.672	0.665	0.746	0.492	0.675	0.646	0.663
F_{IS}	0.035	0.25	0.317	0.235	0.466	0.465	0.323	0.187	0.237	-0.007	0.241	0.385	0.261
PHWE	0.833 ^{NS}	0.100 ^{NS}	0.015 ^{NS}	0.031 ^{NS}	0.000*	0.000*	0.056 ^{NS}	0.021 ^{NS}	0.255 ^{NS}	0.935 ^{NS}	0.065 ^{NS}	0.005 ^{NS}	--
MZG													
Na	2	3	3	4	4	3	3	3	4	2	4	4	3.250
Ar	2	3	3	4	4	3	3	3	4	2	4	3.971	3.247
Nae	1.974	2.998	2.812	3.673	3.495	2.765	2.845	2.614	3.751	1.96	3.618	3.024	2.960
Ho	0.428	0.485	0.457	0.514	0.428	0.514	0.571	0.485	0.514	0.457	0.485	0.542	0.490
He	0.5	0.676	0.653	0.738	0.724	0.647	0.658	0.626	0.744	0.496	0.734	0.679	0.656
F_{IS}	0.146	0.285	0.304	0.292	0.412	0.208	0.133	0.193	0.312	0.081	0.342	0.203	0.242
PHWE	0.387 ^{NS}	0.065 ^{NS}	0.103 ^{NS}	0.087 ^{NS}	0.004*	0.063 ^{NS}	0.534 ^{NS}	0.110 ^{NS}	0.002*	0.631 ^{NS}	0.004*	0.299 ^{NS}	--

Table II. Pairwise genetic differentiation (below diagonal) and genetic distance (above diagonal) among the hatchery populations of *C. mrigala.*

Populations	LHR	CHW	RWLP	FSD	SGD	BHWP	MNCH	MZG
LHR	--	0.0528	0.0562	0.0630	0.0728	0.0743	0.0652	0.0904
CHW	0.0073*	--	0.0901	0.0783	0.0716	0.0678	0.0836	0.0606
RWLP	0.0092*	0.0253*	--	0.0586	0.0854	0.1018	0.0761	0.0830
FSD	0.0121*	0.0199*	0.0112*	--	0.0527	0.0776	0.0541	0.0618
SGD	0.0181*	0.0184*	0.0251*	0.0096*	--	0.0932	0.0477*	0.0561
BHWP	0.0196*	0.0173*	0.0334*	0.0221*	0.0308*	--	0.0793	0.0860
MNCH	0.0132*	0.0224*	0.0194*	0.0090*	0.0069*	0.0229*	--	0.0460*
MZG	0.0252*	0.0126*	0.0234*	0.0134*	0.0117*	0.0268*	0.0055*	--

*Significant at $p<0.05$

Genetic structure

Among the hatchery populations of *C. mrigala*, the maximum value of population differentiation (0.0334) was calculated between the RWLP and BHWP population pair while the minimum (0.0055) between the MNCH-MZG population pair. The values of genetic distance were observed ranging from 0.0460 to 0.1018 (Table II). The analysis of molecular variance (AMOVA) revealed 66.95% variations within the individuals of hatchery populations of *C. mrigala*. Whereas, minor variation 4.11% was evident among the populations and 28.94% variation was found among the individuals within populations (Table III). A recent genetic bottleneck was found in all the populations after applying the tests under different mutation models (IAM, TPM and SMM) (Fig. 1). The UPGMA dendrogram was constructed to check the genetic relationship between the populations which resulted in two main clusters (A and B). The first cluster (A) was divided into two subclusters, including MNCH, MZG, SGD and FSD in one subcluster while RWLP in the other. Cluster B was also divided into two subclusters having the LHR and CHW populations in first subcluster while BHWP alone in the other subcluster (Fig. 2). Structure analysis also revealed two distinct clusters over 9 independent runs for each K value. The mean estimated log likelihood value was observed maximum for K=2 suggested the probability of belonging to two populations which was denoted by different colors of the columns (Fig. 3).

Table III. Hierarchical partition among different populations of *C. mrigala* by AMOVA.

Source of variation	Degree of freedom	Sum of square	Vari-ance	percentage variation
Among populations	7	284.600	0.40522	4.11
Among individuals within populations	272	3343.371	2.84947	28.94
Within individuals	280	1846.000	6.59286	66.95

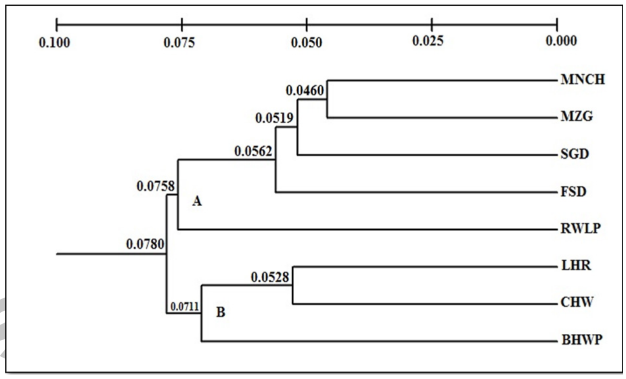

Fig. 2. UPGMA clustering pattern between the *C. mrigala* populations.

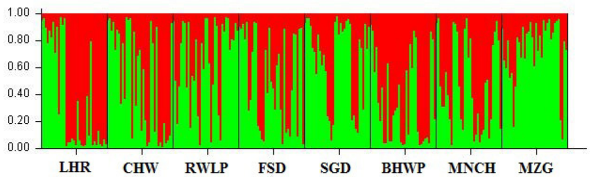

Fig. 3. Structure analysis for *C. mrigala* populations.

DISCUSSION

In the present study, the average values of *Na* and *Ar* in hatchery populations of *C. mrigala* ranged from 3.166 to 3.833 and 3.083 to 3.830, respectively. The highest value of *Na* and *Ar* were observed in LHR hatchery and the lowest in BHWP hatchery population. The values of *Nae* were observed lower than the observed number of alleles (*Na*) which indicated that the frequencies of all alleles are not equal. In hatchery populations, the allelic loss is more conspicuous due to limited number of brooders which may lead to genetic drift. Allelic loss is more critical than the altered allele frequencies because the latter may change over time but once the alleles are lost they cannot be recovered again. Thus, in hatchery propagation the genetic factors must be considered to maintain a high level of genetic variation (Danish and Singh, 2017a).

A low-to-moderate level of heterozygosity with average values ranged between 0.478 to 0.549 was found in all the populations. The maximum value was observed in LHR and minimum in MNCH population. The lower heterozygosity in hatchery populations of freshwater fish species is also confirmed by other authors (Danish and Singh, 2017b; Ahmadi *et al*., 2018). Low level of genetic variability can negatively affect the viability of individuals within a species and is a serious concern for the conservation of biodiversity (Allendorf *et al*., 2012). The average values of expected heterozygosity were found higher than the observed heterozygosity and varied between 0.645 - 0.691. The inbreeding and negative selection during hatchery breeding programs might be responsible for the low level of heterozygosity in the domestic populations (Li *et al*., 2016). Low effective size of populations in hatcheries makes them more prone to extinction as compared to the wild populations. So, for sustainable management of hatchery stocks the sufficient number of census population relative to their *Ne* is necessary to maintain (Hare *et al*., 2011).

Both positive and negative values of F_{IS} were observed at all the microsatellite loci in the *C. mrigala* populations. On average the hatchery populations showed positive values and are considered as inbred. The positive values of F_{IS} indicated the excess of homozygotes and deficiency of heterozygotes due to nonrandom mating (Nosova *et al*., 2019). It is well known that inbreeding and genetic drift are the two main factors with strong influence on small and isolated populations, resulting in the loss of genetic variations which may limit the probability of existence of a population (Frankham *et al*., 2010). For *HWE*, 14 among 96 tests were significantly deviated which are considered as heterozygote deficient. The deficiency of heterozygotes is primarily triggered by the small number of brooders, inbreeding depression and improper domestication practices prevailing in hatcheries (An *et al*., 2011). Consistent results were also observed in *Cyprinus carpio* by Bixheku *et al*. (2019).

The microsatellite analysis revealed low level of population differentiation in hatchery populations with the average values ranging from 0.0055 to 0.0334 in MNCH-MZG and RWLP-BHWP population pairs, respectively. The inadequate knowledge regarding the genetic issues and the conventional mixing of the gene pools by hatchery operators might be the reason of low level of genetic differentiation among the hatchery populations. Similar conclusions were drawn by Nazish *et al*. (2018) for *Hypophthalmicthys molitrix*. A recent genetic bottleneck was found in the studied populations. Generally, the limited allele numbers as a result of allelic loss are a sign of genetic bottleneck in hatcheries due to the small effective population size (Norris *et al*., 1999). It is required to maintain adequate number of brooders to avoid such kind of serious bottleneck in hatcheries.

AMOVA is an appropriate mean to determine the level of genetic similarity and differentiation among populations and also allows the examination of hierarchal partitioning of genetic variations in various populations (Grassi *et al*., 2004). The inferences of the present study revealed a significant genetic structuring among the hatchery populations. Li *et al*. (2017) also found the same inferences in wild and captive populations of *Hemibarbus maculates*. Among the hatchery populations, the UPGMA dendrogram showed two main clusters. The first cluster included MNCH, MZG, SGD, FSD and RWLP while the second cluster comprised of LHR, CHW and BHWP populations. The clustering pattern of the hatchery populations followed their geographical proximities to some extent. The populations in the same cluster despite their large geographical distance could be related to the traditional hatchery management practices, exchange of brooders and the common origin of the individuals (Haque and Hoq, 2016). Furthermore, the microsatellite data analysis by the STRUCTURE analysis also suggested the presence of two discrete genetic clusters.

CONCLUSIONS

The present study provides useful insight to the genetic status of the *C. mrigala* and would serve as a base line information for the efficient monitoring of the impact of climatic and anthropogenic factors on natural populations. The reduced genetic variability in hatchery populations indicated the signs of genetic erosion that has been occurring in these stocks obviously due to inbreeding and genetic drift. The hatchery operators need to consider the genetic aspects during breeding programs for the production of good quality seed to ensure the sustainability of the sector. Furthermore, the genetic structure of *C. mrigala* as identified in this study suggests the need for the development of management and conservation plans for restoring the genetic integrity of this species.

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Ethical statement and IRB approval

The study the Institutional Biosafety and Bioethics Committee of University of Agriculture, Faisalabad (D. No. 3617/ORIC, dated: 19/06/2023) and was carried out by following all the guidelines of National Biosafety 2005, Punjab Biosafety Rules 2014 and Punjab Animal Health Act 2019.

Statement of conflict of interest

The authors have declared no conflict of interest.

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