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Population genetic of *Petroleuciscus esfahani* (Teleostei: Cyprinidae) in Zayandeh Rood River, Iran

Neda Shojaee [1], Salar Dorafshan [1]*, Nourollah Mirghaffari [1] and Majid Talebi [2]
[1] Department of Natural Resources, Isfahan University of Technology, Isfahan, 84156-83111, Iran
[2] Department of Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Abstract
Population genetics of *Petroleuciscus esfahani* in Zayandeh Rood River, Iran were analysed using 120 samples of adult fish from four stations of the river Cheshmeh Dimeh (CHD), Khersoonak (KH), Chamgordan (CH) and Safaeye Bridge (SB) and 5 pairs of microsatellite primers. All loci showed polymorphism. A total number of 54 alleles were recorded across loci ranging from 6 at CnaB-030 to 17 at Ca3. The mean number of alleles per populations ranged from 9.6 in CHD to 8.6 in others. Mean observed heterozygosity at the five loci detected ranged from 0.92 to 1.00 which showed high level of genetic diversity in each population. Deviation from Hardy-Weinberg equilibrium was obvious in most combinations (locus × population), mainly due to heterozygosity excess. The lowest and highest genetic distances were calculated between CHD-KH and CHD-CH populations, respectively. The results showed low but significant F_{ST} values between each pair of the populations. This investigation represented at least four separate populations of *P. esfahani* in the river which showed the effects of river landscape fragmentation on population genetic structure of *P. esfahani*.
Key words: Microsatellite, *Petroleuciscus esfahani*, Population, Zayandeh Rood River

Introduction

All around the world, civilization has arisen near to the constant high quality water resources. In the Middle East, the role of freshwater resources such as rivers has been very critical for human civilization because of the historical low availability of water. The Zayandeh Rood River as the only main surface water in the central part of Iran has different ecological, social and economical key roles. In recent decades, several anthropogenic activities such as dam and weir construction, water pollution, introduction of non-native fish species such as common carp, *Cyprinus carpio* or semi-natural phenomenon like several years of drought and climate changes have significantly altered its ecological structure. The Zayandeh Rood dam was built in 1971 is one of the highest dam in the Middle East (about 100 m height) which was built to provide hydroelectric power as well as constant water supply for agriculture (more than 100000 ha) for many years. Indeed, several non-continual weirs have been made in the river mainly in downstream to regulate water level and water direction mainly for agriculture purposes. It is well-documented that

* Corresponding Author: sdorafshan@cc.iut.ac.ir

human activities such as water pollution (Nadig *et al.*, 1997) and dam or weir construction (Hansen *et al.*, 2014). However, the ecological response of the aquatics is very species-specific dependent on dispersal ability (body size), effective population size, and intensity of fragmentation (Blanchet *et al.*, 2010).

The Zayandeh Rood chub, *Petroleuciscus esfahani* is a native species in the Zayandeh Rood and Karoon (Tigris) basins in Iran (Coad and Bogutskaya, 2010). The species has wide distribution in the river both in up and down stream regions. The average body size of the fish is about 120 mm and it mainly feed on aquatic invertebrates and insects (Keivany *et al.*, in press). Despite of the importance of the Zayandeh Rood chub with respect to ecological view, the effects of different man-made or natural destructive phenomenon on the population structure of the species is unknown. The understanding of genetic diversity is one of the most important steps toward stable ecological management of fish stocks (Ward and Grewe, 1995).

Several approaches such as allozyme and DNA markers have been used for population structure studies. Among which, short simple tandem repeat (SSR or microsatellite) received much more attention during last decade mainly, because of high level of polymorphisms, co-dominant nature, uniform spreading all over the genome, reproducibility as well as neutral modality (Okumuş and Ciftci, 2003; Liu and Cordes, 2004). It has several practical applications in fisheries and aquaculture management such as population genetic structure analysis (Rezaei *et al.*, 2010; Ghasabshiran *et al.*, 2013), stock, strain or individual identification, gene mapping and parentage and pedigree analysis (Beaumont and Hoare, 2003; Liu, 2007).

Currently, there is no information available about genetic structure of Zayandeh Rood chub in different regions (up and downstream) of the River. So, the purpose of this research was to study the population genetic structure of the species as a native and common fish species in the one of the most important river ecosystem in the central part of Iran. The results of this study can help managers to find out more about the impact of the different anthropogenic activities on the river ecosystem as a whole.

Materials and Methods

Sample collection

One hundred and twenty (120) individuals of *Petroleuciscus esfahani* were collected from four stations in October 2011 including Cheshmeh Dimeh (CHD), Khersoonak (KH), Chamgordan (CH) and Safaeye Bridge (SB) (Figure 1), the two first stations as an upstream location, located before Zayandeh Rood Dam and the others as a downstream station after the dam. All stations located in Isfahan province except for CHD which is located in Charmahal va Bakhtiari province (Figure 1). For each sample, 2-3 g dorsal fin tissue was collected and conserved in absolute ethanol for subsequent DNA extraction and amplification.

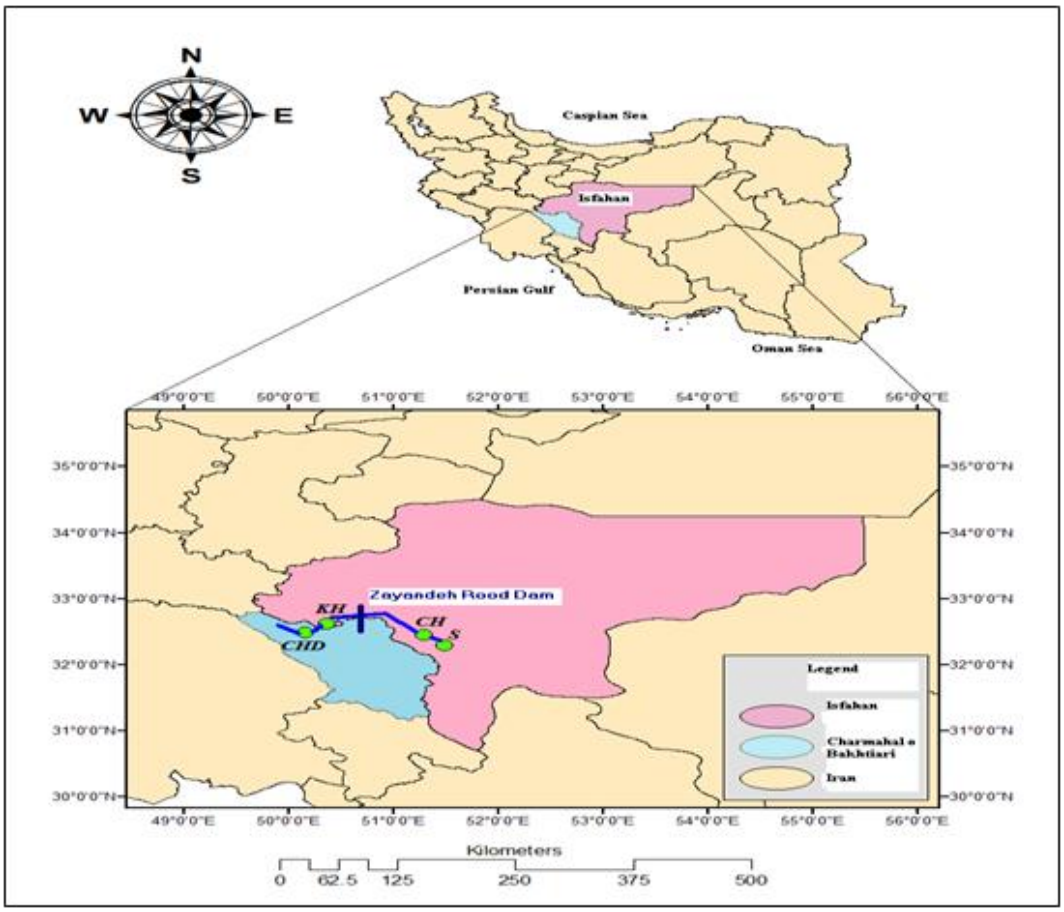

Figure 1. Sampling stations for *Petroleuciscus esfahani* along with Zayandeh Rood River. CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

DNA Extraction

Total genomic DNA was extracted from dorsal fin tissue following the Bio Basic Kit of Genet Bio Company (Canada) based on manufacturers manual provided in the kit. The quality and concentration of DNA were assessed using 1% agarose gel electrophoresis and spectrophotometry. Finally, DNA was stored at -20˚C until use.

PCR Profiles and Primer Sequences

Five pairs of microsatellite primers (CtoF-172, Bl1-2b-CnaB-030, LleA-071 and Ca3) were selected over 41 microsatellite loci designed for European Cyprinids (Dubut *et al.*, 2010; Table 1). These loci have been selected based on the PIC (polymorphic information content, number of observed alleles in different cyprinid species such as *Telestes souffia* and *Telestes muticellus* (Dubut *et al*., 2009a), *Leuciscus leuciscus* (Dubut *et al*., 2009b), *Alburnus alburnus* (Dubut *et al.*, 2010) as well as *Alburnus mossulensis* (Shafee *et al.*, 2013).

The polymerase chain reaction (PCR) conditions, especially annealing temperatures, were optimized for the microsatellite loci as a necessity to produce scorable amplification products. Polymerase chain reaction was performed in a 10 µL reaction volume containing 100-150 ng of template DNA, 10 pmol of each primer, 200 µM each of the dNTPs, 1 U of *Taq* DNA polymerase (Cinagene, Tehran, Iran), 1.5 mM $MgCl_2$ and 1X PCR buffer. Thermal cycling condition for five loci were: an initial denaturation at 94ºC for 2 minutes

followed by 35 cycles of 30 second at 94ºC, Annealing temperature 56ºC for all loci except Ca3 which annealed at 58 ºC for 30 s, 30 s at 72 ºC and the final extension of 72 ºC for 5 min. PCR products were separated on a polyacrylamide 12% gel and then stained by silver nitrate method (May *et al.*, 1997). Alleles were sized using an allelic ladder each gel contained (100 bp, Fermentase, Germany) to assist in consistent scoring of alleles.

Table 1. Characteristics of *Petroleuciscus esfahani* microsatellite loci used at present study* (*- Annealing temperature was 56ºC for all loci except Ca3 which annealed at 58 ºC)

Locus	Primer sequence 5'3'	GenBank Accession	Motif	Predicted Size
BL1-2b	F:TTTGCACTAGTAACGAGCATCA R:CAGCACAGTTTCTCCATCCA	FJ468347	$(TG)_{12}$	140-170
LleA-071	F:GTCTTAGATTGTGTAGCGGG R:ACTTCAGTTACTAAGAGATTAGTGA	FJ601719	(CA)6T(AC)10	340-350
CnaB-030	F:ACGAATGAGAAGCTCGTG R:TCGTCATGCAGTTCATCCT	GU254028	(AC)6	120-130
CtoF-172	F:ACCAAGGTGAAAGCCTGTAA R:GGACACGATGACAACGG	GU254034	(GT)13N14(TG)3	110-124
Ca3	F:GGACAGTGAGGGACGCAGAC R:TCTAGCCCCCAAATTTTACGG	AF277575	(TAGA)14	150-200

DNA Analysis

Effective number of alleles (Ne), gene flow (Nm), genetic distance and allelic frequency were determined as number of alleles per locus (A) and heterozygosity (H) directly from microsatellite phenotypes using GENEPOP version 3.2 (Raymond and Rousset, 1995). To test for deviation from Hardy-Weinberg Equilibrium (HWE) comparisons were made between observed heterozygosity (Ho), and expected heterozygosity (He) using exact tests as implemented by Power Marker version 3.0 (Liu and Muse, 2005) and GENEPOP 1.2 (Raymond and Rousset, 1995). This software employed the Markov chain method to estimate the probability of significant deviation from HWE. Genetic differences between populations were evaluated by calculating pairwise F_{ST} values and testing their significance by boot strapping analysis (1000 replicates) using ARLEQUIN 3.1 (Schneider *et al.*, 2000). This program was also used to partition variation within and between populations using by analysis of molecular variance AMOVA version 4 procedures (Excoffier and Schneider, 2005). Genetic differentiations among four populations (KH, CHD, SB and BA) were also evaluated using ARLEQUIN 3.1 (Schneider *et al.*, 2000).

Results

Within population variation

All five loci were polymorphic and variable in all populations (Table 2). A total of 54 alleles ranging in size from 103 to 350 bp were found over the five loci. The number of alleles ranged from 6 in CnaB-030 to 17 in Ca3 locus. Within populations, the lowest mean number of alleles per locus (8.6) was observed in three populations including KH, SB and CH. While the highest mean number of alleles per locus (9.6) was found in CHD population. Average observed heterozygosity was calculated from 0.92 in CH to 1.00 in KH population (Table 2).

Table 2. Genetic variabilities of five microsatellite loci in four populations of *Petroleuciscus esfahani* in Zayandeh Rood River in Iran. N. Sample size; Ho. Observed heterozygosity; He. Expected heterozygosity; No. Observed allele; Ne. Effective allele; PHW. Hardy-Weinberg probability test: *P<0.05; **P<0.01; F_{IS}. Fixation indices; n.s. Not-significant; CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

Locus	Parameter	CHD	KH	CH	S
CtoF-172	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.82	0.75	0.77	0.79
	No	8	5	5	6
	Ne	5.34	3.92	4.27	4.50
	PHW	**	**	**	**
	F_{IS}	0.230	0.342	0.305	0.285
BL1-2b	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.87	0.80	0.82	0.78
	No	9	8	9	6
	Ne	7.14	4.73	5.14	4.32
	PHW	**	*	**	*
	F_{IS}	0.162	0.267	0.241	0.300
CnaB-030	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.78	0.79	0.75	0.79
	No	6	6	5	6
	Ne	4.39	4.56	3.82	4.50
	PHW	**	**	**	**
	F_{IS}	0.294	0.280	0.354	0.285
LleA-071	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.89	0.86	0.89	0.89
	No	12	10	10	11
	Ne	8.14	6.81	8.28	8.07
	PHW	n.s	**	n.s	**
	F_{IS}	0.140	0.171	0.137	0.141
Ca3	N	30	30	30	30
	Ho	0.96	1.00	0.86	0.90
	He	0.90	0.91	0.62	0.90
	No	13	14	14	14
	Ne	9.18	9.77	6.78	9.09
	PHW	n.s	n.s	**	n.s
	F_{IS}	0.084	0.113	0.272	0.011
Mean (all loci)	Ho	1.00	0.99	0.98	0.92
	He	0.82	0.85	0.83	0.82
	No	8.6	9.6	8.6	8.6
	Ne	5.96	6.84	6.9	5.66
	F_{IS}	0.234	0.182	0.204	0.153

CnaB-030 locus showed the lowest polymorphism regarding the number of observed allele (5.7), expected heterozygosity, He (0.777), polymorphism information content (PIC) up to 0.749 and the number of effective allele for each population was 4.31. On the other hand, Ca3 locus showed the highest polymorphism by calculating the number of observed allele, expected heterozygosity, PIC and the number of effective allele as 13.7, 0.892, 0.903 and 8.70, respectively. Genetic variations of all stations were high. The average of expected and observed heterozygosity measured as 0.849 and 0.978 respectively and the number of

effective and observed allele were 6.13 and 8.82 respectively (Table 2). Across the five loci and within the four populations, only six private (specific) alleles were observed, CtoF-172 and Ca3 showed 2 specific alleles for CHD and KH and LleA-071 and Ca3 showed only one specific allele for SB and CH, while, there were no specific alleles for Bl-12b and CnaB-030 loci at any populations.

Significant deviations from Hardy-Weinberg equilibrium at the locus level are shown in Table 3. From all combinations (station × locus) only CHD and CH station at LleA-071 and CHD, KH and SB at Ca3 locus were in Hardy-Weinberg equilibrium (Table 3). The other combinations showed significant deviation from Hardy-Weinberg equilibrium most of which due to heterozygosity excess except for CH at Ca3 locus which showed heterozygosity deficiency (Table 3).

Table 3. Deviation of Hardy-Weinberg equilibrium for five loci in four populations of *Petroleuciscus esfahani* (P<0.05). n.s. In Hardy-Weinberg equilibrium (P>0.05); HE. Heterozygosity excess; HD. Heterozygosity deficiency (P<0.001); CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

Population/locus	**CtoF-172**	**CnaB-030**	**BL1-2b**	**LleA-071**	**Ca3**
CHD	HE	HE	HE	n.s	n.s
KH	HE	HE	HE	HE	n.s
CH	HE	HE	HE	n.s	HD
SB	HE	HE	HE	HE	n.s

Genetic Variation among Sampling Regions

Pairwise F_{ST} values were ranging from 0.013 (between CHD and KH station) to 0.032 (between CHD and CH station) (Table 4). All values showed significant difference between each two populations, probabilities of F_{ST} determined by AMOVA tests, at $P \leq 0.0001$.

Table 4: Pairwise F_{ST} values between four populations based on five microsatellite loci, all combination between two populations were significant (P<0.01). CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

Stations*	CHD	KH	CH	SB
CHD	-----			
KH	0.013	-----		
CH	0.032	0.025	-----	
SB	0.019	0.014	0.026	-----

Genetic distance calculated between each pair of stations ranged from 0.141 (between CHD and KH) to 0.244 (between CHD and CH), while the genetic identity ranged from 0.756 (between CHD and CH) to 0.859 (between KH and CHD, Table 5).

Table 5. Pairwise population of genetic distance (below diagonal) and genetic identity (above diagonal) (Nei, 1972) detected at 5 loci in *Petroleuciscus esfahani* samples. CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

	Stations	**Genetic Identity**			
		CHD	**KH**	**CH**	**SB***
Genetic Distance	CHD	-	0.859	0.756	0.818
	KH	0.141	-	0.958	0.966
	CH	0.244	0.194	-	0.922
	SB	0.182	0.200	0.151	-

The genetic variations of all samples were separated into 3 different parts using molecular analysis of variance (AMOVA) including among populations, among individuals within population and within individuals which measured as 2.52, 18.49 and 78.99 %

respectively (Table 6). So, the highest amount of genetic variations was because of differences within individuals and only a small portion of the variations was assigned to the difference between populations (Table 6).

Table 6. AMOVA of data microsatellite for four populations. F_{IS}. 0.189**; F_{ST}. 0.025*; F_{IT}. 0.159**. **. P<0.01; *. P<0.05

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among population	3	14.258	0.052	2.52
Among individuals within population	116	189.300	0.381	18.49
Within individuals	119	287.500	2.39	78.99
Total	238	491.058	2.82	100

Gene flow was estimated at 4.49, 7.63, 12.14, 17.83 and 7.94 for CtoF-172, Bl1-2b, CnaB-030, Llea-071 and Ca3, respectively. The mean value of gene flow was as high as 8.18 for all population at all loci.

Discussion

The long-term persistence of a fish species can be investigated by allelic diversity, gene diversity, effective population size and population structure (Liu, 2007). Despite the importance of *Petroleuciscus esfahani* as an endemic fish in the Zayandeh Rood River, very little information regarding to this species is available.

In this study, we have employed 5 polymorphic microsatellite loci to assess the genetic relationship among populations of *P. esfahani* from four stations in the Zayandeh Rood River before and after the dam. According to the results, all stations had high number of alleles where KH station showed the highest (9.6 in average). Based on the expected hetrozygosity and number of alleles, the results of this study revealed that genetic diversity of four populations of *P. esfahani* was high. It is well-documented that artificial propagation and heavy fishing activities are two main reasons for reduction of aquatic genetic diversity (Beaumont and Hoare, 2003). Based on the available information, there have been no or very limited activities done on this species in the Zayandeh Rood basin. So, it could be expected that the populations have not been touched showing high level of genetic diversity in each station because of the high population effective size.

The deviations from Hardy-Weinberg equilibrium in most loci were observed. Some factors such as genetic drift, selection and small size of population can affect deviations from Hardy-Weinberg equilibrium (Birgitte *et al.*, 2005; Zhao *et al.*, 2005). Hardy-Weinberg equilibrium theory is based on distribution of genes and different genotypes in a population and thus, the accuracy of the theory is related to fulfilling some conditions such as no migration, large number of the population, no genetic drift, random reproduction, no selection and no mutation (Freeland, 2007). Among all 15 meaningful deviations from Hardy-Weinberg most of them showed heterozygosity excess maybe because of population mixing and sampling bias (Rousset and Raymond, 1995; Liu, 2007). Heterozygosity deficiency which was observed in CH population could arise from existence of substructured population of this species in this area. It is well-documented that an overall deficiency of heterozygosity is obvious in substructured stocks, the proportional magnitude depends on the nature of substructuring, i. e., the number of subpopulations, the time of divergence and the rate of gene flow amongst them (Chakraborty and Jin, 1992). The CH samples were collected from a relatively large man-made lake which may provide a pool of

different stocks in this area. However, it is necessary to do some other extensive studies to find out the main parameters affecting heterozygosity deficiency in this area.

In this study, all F_{ST} values showed significant difference between each two populations ($P<0.01$), suggesting that the four populations are genetically differentiated and don't represent a single panmictic population, meaning that there is some mating restrictions, either genetic or behavioural, upon the population (Freeland, 2007). The effects of different barriers such as hydroelectric dams or even agricultural weirs have been studied on different freshwater fish species mainly migratory forms such as bull trout, *Salvelinus confluentus* (Neraas and Spruell, 2001), white-spotted charr, *Salvelinus leucomaenis* (Yamamoto *et al*., 2004), asper, *Zingel asper* (Laroche and Durand, 2004), brown trout, *Salmo trutta* (Hansen *et al*., 2014) and some cyprinids such as chub, *Leuciscus cephalus* (Laroche *et al*., 1999) and roach, *Rutilus rutilus* (Hanfling *et al*., 2004). In most cases, it has been clearly stated that landscape fragmentation has caused a significant genetic differentiation and population isolations in freshwater fish. Recently, Blanchest *et al*. (2010) showed that response to fragmentation was highly species-specific and depended on different factors such as dispersal ability (body size) and effective population size. They stated that although, smaller fish such as *Phoxinus phoxinus* has lower dispersal ability, they are slightly affected by fragmentation because of the larger effective population size. On the contrary, fish species of intermediate body size such as *Leuciscus leuciscus* was highly affected, whereas the largest fish species, *Leuciscus cephalus* was moderately affected by fragmentation mainly because of their dispersal ability. Based on our finding, *P. esfahani* can be categorized as intermediate body size fish. So, its population structure, at least from genetic point of view, can be affected seriously by fragmentation.

The findings reported in this study nevertheless reveal important implications for stock conservation of *P. esfahani* in the Zayandeh Rood River. The scenario, however, is not complete and further samplings from additional sites, taken during different seasons of the year, and using more specific molecular markers should be taken into account.

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References

Beaumont, R. A. and Hoare, K. (2003) Biotechnology and Genetics in Fisheries and Aquaculture. Blackwell Publishing, Oxford.

Birgitte, J., Hansen, M. and Loeschcke, V. (2005) Microsatellite DNA analysis of northern pike (*Esox lucius*) population: Insights into the genetic structure and demographic history of a genetically depauperate species. Biological Journal of the Linnaean Society 84: 1-11.

Blanchet, S., Rey, O., Etienne, R., Lek, S. and Loot, G. (2010) Species-specific responses to landscape fragmentation: implications for management strategies. Evolutionary Applications 3: 291-304.

Chakraborty, R. and Jin, L. (1992) Heterozygote deficiency, population substructure and their implications in DNA fingerprinting. Human Genetics 88: 267-272.

Coad, B. W. and Bogutskaya, N. G. (2010) *Petroleuciscus esfahani*, a new species of fish from central Iran (Actinopterygii: Cyprinidae). Zootaxa 2534: 34-47.

Excoffier, L. G. L. and Schneider, S. (2005) ARLEQUIN ver. 3.1: An integrated software package for

population genetics data analysis. Evolutionary Bioinformatics 1: 47-50.

Freeland, J. R. (2007) Molecular ecology. John Wiley and Sons Ltd., Chichester.

Dubut, V., Martin, J. F., Costedoat, C., Chappaz, R. and Gilles, A. (2009a) Isolation and characterization of polymorphic microsatellite loci in the freshwater fishes *Telestes souffia* and *Telestes muticellus* (Teleostei: Cyprinidae). Molecular Ecology Recourses 9: 1001-1005.

Dubut, V., Martin, J. F., Gilles, A. E., Van Houdt, J., Chappaz, R. E. M. and Costedoat, C. (2009b) Isolation and characterization of polymorphic microsatellite loci for the dace complex: *Leuciscus leuciscus* (Teleostei: Cyprinidae). Molecular Ecology Resources 9: 1179-1183.

Dubut, V., Sinama, M., Martin, J. F., Meglécz, E., Fernandez, J., Chappaz, R., Gilles, A. and Costedoat, C. (2010) Cross-species amplification of 41 microsatellites in European cyprinids: A tool for evolutionary, population genetics and hybridization studies. Bio Med Central Research Notes 3(135): 1-9.

Ghasabshiran, Z., Dorafshan, S. and Keivany, Y. (2013) Population genetic structure of Iranian cichlid, Iranocichla hormuzensis as an only cichlidae family in Iran using microsatellite markers. Taxonomy and Biosystematics (5)14: 9-16. (in Persian)

Hanfling, B., Durka, W. and Brandl, R. (2004) Impact of habitat fragmentation on genetic population structure of roach, *Rutilus rutilus*, in a riparian ecosystem. Conservation Genetics 5: 247-257.

Hansen, M. M., Limborg, M. T., Ferchaud, A. L. and Pujolar, J. M. (2014) The effects of Medieval dams on genetic divergence and demographic history in brown trout populations. Bio Med Central Evolutionary Biology 14: 122-135.

Keivany, Y., Nasri, M., Abbasi, K. and Abdoli, A. (2015) Atlas of inland water fishes of Iran.1st edition, Iran Department of Environment Press, Tehran (in press).

Laroche, J. and Durand, J. D. (2004) Genetic structure of fragmented populations of a threatened endemic percid of the Rhone River: *Zingel asper*. Heredity 92: 329-334.

Laroche, J., Durand, J. D., Bouvet, Y., Guinand, B. and Brohon, B. (1999) Genetic structure and differentiation among populations of two cyprinids, *Leuciscus cephalus* and *Rutilus rutilus*, in a large European river. Canadian Journal of Fisheries and Aquatic Science 56: 1659-1667.

Liu, Z. (2007) Aquaculture genome technologies. Blackwell Publishing, Iowa.

Liu, Z. J. and Cordes, F. J. (2004) DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238: 1-37.

Liu, K. and Muse, S. V. (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128-2129.

May, B., Charles, C., Krueeger, C. and Kincaid, L. (1997) Genetic variation at microsatellite loci in sturgeon primer sequence homology in *Acipenser* and *Scaphirhincus*. Canadian Journal of Fisheries and Aquatic Sciences 54: 1542-1574.

Nadig, S. G., Lee, K. L. and Adams, S. M. (1997) Evaluating alterations of genetic diversity in sunfish populations exposed to contaminants using RAPD assay. Aquatic Toxicology 43: 163-178.

Neraas P. L. and Spruell, P. (2001) Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. Molecular Ecology 10: 1153–1164.

Nei, M. (1972) Genetic distance between populations. The American Naturalist 106: 283-292.

Nei, M., Tajima, F. and Tateno, Y. (1983) Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution 19: 153-170.

Okumus, I. and Ciftci, Y. (2003) Fish population genetics and molecular markers: II- molecular markers and their applications in fisheries and aquaculture. Turkish Journal of Fisheries and Aquatic Sciences 3: 51-79.

Raymond, M. and Rousset, F. (1995) GENEPOP (version 3.2): population genetic software for exact tests and ecumenicist. Heredity 86: 248-249.

Rezaei, M., Shabani, A., Shabanpour, B. and Kashiri, H. (2010) Genetic comparison of Caspian Sea, Rutilus frisii kutum (Kamenskii, 1901) in Gorganroud and Cheshmekile (Tonekabon) rivers using microsatellite markers. Taxonomy and Biosystematics 2 (1): 1-14. (in Persian)

Rousset, F. and Raymond, M. (1995) Testing heterozygote excess and deficiency. Genetics 140: 1413-1419.

Schneider, S., Kueffer, J. M., Roessli, D. and Excoffier, L. (2000) ARLEQUIN, Version 2.000: Software for Population Genetic Data Analysis. University of Geneva, Genetics and Biometry Laboratory. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Shafee, Z., Dorafshan, S., Keivany, Y. and Qasemi, S. A. (2013) Genetic structure of Mosul bleak (*Alburnus mossulensis* Heckel, 1843) using microsatellite marker in Tigris basin. Taxonomy and Biosystematics (5)17: 9-22. (in Persian)

Ward, R. D. and Grewe, P. M. (1995) Appraisal of molecular genetics techniques in fisheries. In: Molecular Genetics in Fisheries (Eds. Carvalho, G. R. and Pitcher, T. J.). Chapman and Hall, London.

Yamamoto, S., Morita, K., Koizumi, I. and Maekawa, K. (2004) Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: Spatial-temporal changes in gene frequencies. Conservation Genetics 5: 529-538.

Zhao, N. A., Shao, W., Zhu, Z., Brosse, B. and Chang, J. (2005) Microsatellite assessment of Chinese sturgeon (*Asipencer sinensis*). Journal of Applied Ichthyology 21: 7-13.

ژنتیک جمعیت عروس‌ماهی اصفهانی (*Petroleuciscus esfahani*) (ماهیان استخوانی، کپورماهیان) در رودخانه زاینده‌رود، ایران

ندا شجاعی [۱]، سالار درافشان [۱]*، نوراله میرغفاری [۱] و مجید طالبی [۲]
[۱] گروه شیلات، دانشکده منابع طبیعی، دانشگاه صنعتی اصفهان، اصفهان، ۸۴۱۵۶-۸۳۱۱۱، ایران
[۲] گروه بیوتکنولوژی کشاورزی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ایران

چکیده

جمعیت‌های عروس‌ماهی اصفهانی (*Petroleuciscus esfahani*) در رودخانه زاینده‌رود با استفاده از پنج جفت نشانگر مولکولی ریزماهواره بر روی ۱۲۰ نمونه بالغ صید شده از چهار ایستگاه: چشمه دیمه، خرسونک، چمگردان و پل صفائیه ارزیابی شد. تمامی جایگاه‌های بررسی شده چندشکل بودند. تعداد ۵۴ آلل برای پنج جایگاه تولید شد که در محدوده ۶ آلل در جایگاه CnaB-030 و ۱۷ آلل در جایگاه Ca3 قرار داشت. میانگین تعداد آلل مشاهده شده در جمعیت چشمه دیمه برابر با ۹/۶ عدد و در سایر جمعیت‌ها برابر با ۸/۶ عدد بود. میانگین هتروزیگوسیتی مشاهده شده برای ۵ جایگاه در گستره ۰/۹۲ تا ۱ قرار داشت که بیانگر سطح بالای تنوع ژنتیکی در هر جمعیت بود. در اغلب موارد، انحراف از تعادل هاردی-واینبرگ در آزمون جایگاه-جمعیت، عمدتا به علت افزایش هتروزیگوسیتی مشاهده شد. حداقل و حداکثر فاصله ژنتیکی به ترتیب بین جمعیت‌های چشمه دیمه-خرسونک و چشمه دیمه-چمگردان محاسبه شد. نتایج مقادیر اندک اما معنی‌دار F_{ST} را در مقایسه جفت جمعیت‌ها نشان داد. مطالعه حاضر، حضور چهار جمعیت متفاوت عروس‌ماهی اصفهانی را در رودخانه نشان داد که می‌تواند بیانگر اثر تکه‌تکه شدن زیستگاه بر جمعیت ژنتیکی عروس‌ماهی اصفهانی باشد.

واژه‌های کلیدی: ریزماهواره، عروس‌ماهی اصفهانی (*Petroleuciscus esfahani*)، جمعیت، رودخانه زاینده‌رود

* sdorafshan@cc.iut.ac.ir